IN VITRO PROPAGATION AND NUCLEUS SEED PRODUCTION EFFICIENCY OF BARI DEVELOPED CLONE POTATO VARIETIES

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This is to certify that the thesis entitled "In vitro Propagation And Nucleus Seed Production Efficiency of BARI Developed Clone Potato Varieties" submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of ***MASTER OF SCIENCE IN BIOTECHNOLOGY,*** *research work carried out by Naznin Sultana, Registration No.10- 4168, under my supervision and guidance. No part of this thesis has been submitted for any other degree or diploma.*

I further certify that any help or sources of information as has been availed of during the course of this work has been duly acknowledged & style of the thesis have been approved and recommended for submission.

Dated:

Dhaka, Bangladesh

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List of Abbreviation

AEZ	=	Agro Ecological Zone
BARI	=	Bangladesh Agricultural Research Institute
BBS	=	Bangladesh Bureau of Statistics
LAI	=	Leaf area index
Ppm	=	Parts per million
et al.	=	And others
N	=	Nitrogen
TSP	=	Triple Super Phosphate
MP	=	Muriate of Potash
RCBD	=	Randomized complete block design
DAS	=	Days after sowing
ha^{-1}	=	Per hectare
G	=	gram (s)
Kg	=	Kilogram
µg	=	Micro gram
SAU	=	Sher-e-Bangla Agricultural University
SRDI	=	Soil Resources and Development Institute
HI	=	Harvest Index
No.	=	Number
Wt.	=	Weight
LSD	=	Least Significant Difference
^0C	=	Degree Celsius
mm	=	Millimeter
Max	=	Maximum
Min	=	Minimum
%	=	Percent
cv.	=	Cultivar
NPK	=	Nitrogen, Phosphorus and Potassium
CV%	=	Percentage of coefficient of variance
Hr	=	Hour
T	=	Ton
viz.	=	Videlicet (namely)

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***IN VITRO* PROPAGATION AND NUCLEUS SEED PRODUCTION EFFICIENCY OF BARI DEVELOPED CLONE POTATO VARIETIES**

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Abstract

An experiment was conducted at Tissue Culture Lab and Net House of Tuber Crops Research Centre, Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur from April 2016 to February 2017 to investigate *In vitro* propagation and nucleus seed production efficiency of BARI developed clone potato varieties. *In vitro* shoots of potato varieties BARI Alu-35, BARI Alu-36, BARI Alu-37, BARI Alu-40, BARI Alu-46 and BARI Alu-62 were used as planting materials in this experiment. BARI Alu-7 was used as check variety. Significant difference were found among the growth parameters of *in vitro* plantlets of the potato varieties. The maximum number of leaves (14.80), length of shoot (99.98cm), weight of fresh shoot (1.66 mg) were observed in BARI Alu-36. Length of root of plantlets varied significantly among the varieties. The highest length of root (5.54 cm) was recorded in BARI Alu-36. The maximum number of primary root (17.00), number of secondary root (28.20) and fresh weight of root (0.359 mg) were observed in BARI Alu-36. BARI Alu-36 showed better performance on most of the morphological traits under *in vitro* condition. On the other hand, survival percentage and nucleus seed (G_1 generation) production of potato have been calculated from virus free *in vitro* plantlets of potato at net house of TCRC, BARI during November 2016 to February 2017. Number of G_1 seed tuber per plant 21.2, 16.0, 18.6, 19.4, 30.4, 37.4 and 52.4 were recorded in BARI Alu -7, BARI Alu -36, BARI Alu-37, BARI Alu-62, BARI Alu-40, BARI Alu-35, and BARI Alu-46 respectively. The maximum weight of G_1 seeds per plant were found from the variety BARI Alu-46 (358.0 g) followed by BARI Alu-7 (306g), BARI Alu -62 (256.6g), BARI Alu- 36 (194.2g), BARI Alu- 40 (182.4g), BARI Alu-35(142g) and BARI Alu-37(113.4g).

Chapter I
INTRODUCTION

1.1 General

Potato (*Solanum tuberosum* L.) is the most important vegetable crop of Bangladesh. It alone contributes to about 71.25% of the total annual vegetable production in Bangladesh (BBS 2014-15). It is a prominent crop in consideration of its production and demand in Bangladesh. Potato cultivation has been getting popular over the last many years. Total area under potato cultivation is 4,71,023 hectares and total potato production has been estimated 92,54,285 metric tons. Average yield of potato has been estimated 19.65 metric tons per hectare (BBS, 2014-15).

Potato is an annual plant belonging to a genus *Solanum* in the family Solanaceae (Acquaah, 2007). The cultivated potato, *Solanum tuberosum* is an autotetraploid having a genome size estimated at 840 million base pairs (Pasare, 2012). It is an herbaceous, freely branching dicotyledonous perennial plant. It is assumed that at the beginning of the 17^{th} century, the Portuguese navigators first brought potato to Indo-Bangladesh sub-continent (Islam, 2009). Now potato stands as a short duration winter crop in sub-tropical countries like Bangladesh (Hossain, 2011).

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).

All conventional potato seed production systems are characterized by low multiplication rate and progressive accumulation of degenerative viral diseases during clonal propagations. Availability of pathogen free starting material is a prerequisite for any seed potato production system. Using aseptic culture, the pathogen free plants can be maintained indefinitely in tissue culture and a constant flow of disease free plants from micro propagation is easily possible. Mini tuber is an intermediate stage of potato seed production between *In vitro* propagation and field multiplication. Multiplication method consists of *In vitro* propagation and multiplication rooted *In* vitro and micro tuber production at a very high density into beds in net house with an appropriate organic mixture (Dodds, 1989). Micro tubers are small (1-25 g wt.) and high quality commonly known as mini tuber or nucleus seed. Production of mini tuber or nucleus seed potatoes using tissue cultured plantlets is an important work. These seeds are used for breeder seed production in next year.

1.2 Background of the Study

Over the past 50 years, the application of cell and tissue culture techniques has been most conspicuous in potato *(Solanum tuberosulI1* L.) than any other crop species. The first successful establishment of tissue culture from potato tubers was reported as early as in 1951 (Steward and Caplin, 1951), and since then *in vitro* cultures in potato were developed from different

plant parts such as leaves, petioles, ovaries, anthers, stems, roots, and shoot tips (Bajaj, 1987). Due to its high amenability to *in vitro* manipulations, a range of techniques has been perfected in this crop over the years. These techniques are of differing degrees of complexity forming a complete spectrum of technologies. While some of these technologies have been applied to improve potato production by means of micropropagation and pathogen elimination, others are still being refined and improved. The use of *in vitro* techniques for virus elimination (meristem culture) and clonal mass propagation (micropropagation) is the most prominent application in potato.

In vitro-produced disease-free potato clones combined with conventional multiplication methods have become an integral part of seed production in many countries (Naik and Sarkar, 2000). Intermediate level technologies such as embryo and anther cultures have been applied on germplasm enhancement *vis-a-vis* potato improvement. Since storage of botanical seed is not feasible in this tetraploid crop due to heterozygocity and various degrees of sterility, maintenance of differentiated plantlets and meristematic explants in tissue culture has been successfully used to conserve its vast genetic resources. The developments in the fields of cellular selections, somaconal variations, somatic hybridization and genetic transformation have not only improved the present-day potato, but also generated novel genetic variability for the synthesis of future potatoes. Although some of the basic techniques like *in vitro* selection, microspore and protoplast regeneration and somatic fusion have been available since the late 1970s, the challenge is to make these procedures reproducible, universal and economic so that these can be integrated into practical potato improvement programmes.

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). Very few reports are available regarding the varietal effect and explants on plantlet multiplication under *in vitro* condition and nucleus seed production from clonal developed potato varieties. The present study has been undertaken with the following objectives:

1. To observe the *in vitro* propagation efficiency of BARI developed clone potato varieties.
2. To evaluate the physiomorphology of the varieties at net house conditions.
3. To evaluate the performance of nucleus seed (G_1) production at net house conditions.

Chapter II

REVIEW OF LITERATURE

Plant tissue culture is the backbone of plant biotechnology, which is comprised of micropropagation, induction of somaclones, somatic hybridization, cryopreservation and regeneration of transgenic plants. Plant tissue culture is a technique through which any plant part is cultured on a sterile nutrient medium in controlled light and temperature with the purpose of obtaining growth. The idea of plant tissue culture originated from the cell theory that was formulated by Schwann in 1839. Tissue culture techniques have been played a great role in the micropropagation of horticultural and ornamental plants. These techniques have been widely used in disease elimination and vegetative propagation (Husseyg, 1979). Now-a-days, it is very common practice all over the world to explore different aspects about potato using this technology. But unfortunately, it is very limited in Bangladesh. However, some related works already performed by different institutes in home and abroad have been reviewed and some of the most relevant literatures have been cited below.

2.1 Concept of potato tissue culture

Over the past 50 years, the application of cell and tissue culture techniques has been most conspicuous in potato *(Solanum tuberosum* L.) than any other crop species. The first successful establishment of tissue culture from potato tubers was reported as early as in 1951 (Steward and Caplin, 1951), and since then *in vitro* cultures in potato were developed from different plant parts such as leaves, petioles, ovaries, anthers, stems, roots, and shoot tips (Bajaj, 1987). Due to its high amenability to *in vitro* manipulations, a range of techniques has been perfected in this crop over the years. These techniques are of differing degrees of complexity forming a complete spectrum of technologies. Intermediate level technologies such as embryo and anther cultures have been applied on germplasm enhancement *vis-a.-vis* potato improvement. Since storage of

botanical seed is not feasible in this tetraploid crop due to heterozygocity and various degrees of sterility, maintenance of differentiated plantlets and meristematic explants in tissue culture has been successfully used to conserve its vast genetic resources. The developments in the fields of cellular selections, somaclonal variations, somatic hybridization and genetic transformation have not only improved the present-day in potato, but also generated novel genetic variability for the synthesis of future potatoes. Although some of the basic techniques like *in vitro* selection, microspore and protoplast regeneration and somatic fusion have been available since the late 1970s, the challenge is to make these procedures reproducible, universal and economic so that these can be integrated into practical potato improvement programmes.

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 research 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 Explants

The propagation of potato (*Solanum tuberosum* L.) by shoot is commonly used in the production of disease free seed tuber, germplasm exchange, and conservation. . *In vitro* cultured internode produce shoots when incubated under suitable conditions. Shoot culture is the basic technique of potato biotechnology. It enables efficient and first establishment *In vitro* cultures using shoot tip as starting material. Once establishment comes, regenerated shoots can provide requirement of explants for other techniques including cell, tissue and organ cultures, protoplast culture, somatic embryogenesis and *Agrobacterium*-mediated-transformation. The main application shoot cultures have is in clonal propagation. They can be used for production of plants (micropropagation) tubers (microtubers), or both. Genotype stoppage (gene banks) is another important application achieved by sustain (minimal, slow) growth of shoot cultures. In order to regenerate shoot node were cut into small pieces 0.3-0.5cm, containing one axillary bud in each explant and were cultured on MS media containing 0.5ml/L IBA (conc. 0.1mg/ml) . The cultures were incubated at 25^0C, under 16h light photoperiod. In cryopreservation, growth is completely arrested at the temperature of liquid nitrogen. Meristem tip culture is an important component in virus eradication used either alone or in combination with thermotherapy.

2.3 Callus induction and plantlet regeneration

In vitro micropropagation is an alternative to conventional (vegetative) propagation of potatoes whereas aseptically meristem cultures were used which gave pathogen free plants. However, three concentrations of disinfectant bleach (Clorox) 15, 20, 25% with two exposure time 15 and 20 min were used for disinfecting the isolated potato sprouts from four potato genotypes named Lady Rosetta, Jaerla, Cara and Hermis. It was found that, as simplest disinfection protocol, concentration 20% Clorox was the suitable one at 20 min of exposure

time giving high percentages of survived individuals with low percentage of dead and contaminated individuals. The sterilized sprouts were cut and cultured on shoot induction medium containing solidified MS salts with vitamins and free of exogenous plant growth regulators and incubated in a growth chamber at optimized culture conditions in room culture. The initiated shootlets from the aseptic cultures were cut to nodal cuttings which were culture on the previous MS medium for mass propagation of potato plantlets *in vitro*. The results cleared that MS medium with vitamins and solidified by agar without exogenous plant growth regulators can be used for mass propagation of free-pathogen true to type of potato genotype *in vitro* under the optimized culture conditions (Ebad *et al*. 2015).

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±1ºC 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 to establish a rapid disease free seed production system in potato. MS medium supplemented with 4 mg/L 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 mg/L kinetin combination of treatment was the best for *in vitro* tuberization among the parameters under study.

Hussain, (2005) investigated i*n vitro* response and its relationship with different varieties, explants and medium in potato (*Solanum tuberosum*). Direct *In vitro* regeneration protocol from diverse explant source is a prerequisite for transformation studies. Three potato cultivars *viz.*, Cardinal, Atlash and Diamond 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 Diamond 14.3 and Atlash 9.0. Shoot apices also resulted in shoot regeneration comparatively better than leaf discs 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, enape and Niska exhibited a regeneration rate of 98%).

2.4 Effect of Growth Regulators

Initially potato *in vitro* culture was started from nodal cuttings and maintained on a hormone free media at 23±2 ºC for 2-weeks. It is clearly evident from the data that direct shoot regeneration was remarkably influenced by type and concentrations of the auxins, cytokinines and GA_3 used and no organogenesis was recorded in the basal MS media i.e., T_0 media. Both the varieties exhibited fairly high direct plantlet regeneration, when internodes explants were cultured on T_2 medium i.e. MS+GA_3 (1.0 mg/L) + IAA (0.01 mg/L)+Zeatin (2.0 mg/L). Maximum number of shoots per explants after 30 days were recorded in cultivar Diamond followed by Cardinal i.e. 18 and 16, respectively in comparison to control (T_0) i.e. 2 and 1. Moreover, either increase or decrease in the concentrations of growth regulators declined number of shoots. The replacement of IAA with another auxin i.e. NAA and replacement of zeatin with another cytokinin i.e. BAP increased the number of shoots considerably in both the cultivars in comparison to control. After replacement, the growth regulators maximum number of shoot was observed in T_6 treatment but the number was less than T_2 treatment. Although the T_2 are several reports for the use of hormone free MS medium during potato propagation. However, the growth of explants is slow in such hormones free, cost effective media. Otherwise, the growth rate of explant can be improved by supplementing medium with growth regulators. This data shows that for direct shoot regeneration IAA and Zeatin was more effective than NAA and BAP. Further, increase in the concentration of IAA i.e. up to 0.015 mg/L and zeatin up to 3.0 mg/L was not effective for direct shoot regeneration from internodes. Cytokinine at high concentrations increased chlorophyll content and compactness of the tissues. The higher levels of cytokinins might have resulted in chlorophyll development rather than shoot regeneration.

Anoop Badoni and Chauhan (2009) studied meristem of potato (*Solanum tuberosum)* on Murashige and Skoog (MS) medium, supplemented with different

hormonal combinations i.e. MSGN1 (0.25 mg/L GA_3 and 0.01 mg/L NAA), $MSGN_2$ (0.25 mg/L GA_3 and 0.03 mg/L NAA), $MSGN_3$ (0.25 mg/L GA_3 and 0.04 mg/L NAA), $MSKN_1$ (0.01 mg/L Kinetin and 0.1 mg/L NAA), $MSKN_2$ (0.001 mg/L Kinetin and 0.1 mg/L NAA) and $MSKN_3$ (1 mg/L Kinetin and 0.1 mg/L NAA), which affected *In vitro* propagation of potato. After 35-40 days of culture shoot height, number of nods, root length, shoot and root fresh weight were measured. Shoot height in MS medium with GA_3 and NAA combination showed better result in comparison to MS medium with Kinetin and NAA. Shoot height in $MSGN_1$ combination reached 8.28 (±0.5) cm. with 11.9 (±1.1) cm. root length and 9.4 (±1.0) nodes while in $MSKN_1$ shoot height reached 6.4 cm. (±0.6) with 8.2 (±0.5) cm. root length and 5.0 (±0.7) nods. $MSKN_2$ and $MSKN_3$ reached low shoot height respectively 5.3 cm. (±1.2) with 4.2 (±0.8) nods and 4.0 cm. (±0.6) with 2.7 (±0.7) nods in comparison to all combinations. $MSGN_2$ and $MSGN_3$ combinations reached respectively 7.15 (±0.5) cm. with 8.2 (±1.0) nodes and 6.15 (±0.6) cm. with 6.3 (±0.9) nodes. Result showed that lower concentration of auxin (0.01 mg/l NAA) with Gibberelic Acid (0.25 mg/l GA_3) is best for development of complete plantlets and multiplication from meristem tips.

From the review of literature illustrated above, it may be concluded that the growth and development of plantlets greatly influenced by variety to variety. BARI developed clone potato varieties are recently released. It is essential to observe the *in vitro* propagation and nucleus seed production efficiency which will help to boost up the production of potato.

Chapter III

MATERIALS AND METHODS

The materials and methods of the experiment have been presented in this chapter. It includes a brief description of materials and methods used for the study, design of the experiment, data collection procedure and procedure of data analysis. Consequently following two experiments were conducted to fulfill the present objectives:

Experiment I: *In vitro* propagation of BARI developed clone potato varieties

Experiment II: Nucleus seed (G_1) production efficiency of BARI developed clone potato varieties

3.1 Experimental period

The experiment was conducted during the period from April 2016 to February 2017 to investigate the *in vitro* propagation and nucleus seed (G_1) production efficiency of BARI developed clone potato varieties.

3.2 Description of experimental site

The experiments were conducted at tissue culture laboratory and net house of TCRC, Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur.

3.3 Climatic condition

The climate of experimental site was the monsoon period from May to October and winter from November to February. The monthly average temperature, humidity and rainfall during the crop growing period were collected from Weather Yard, Bangladesh Meteorological Department, and presented in the Appendix I.

3.4 Experimental materials

Experiment I: *In vitro* propagation of BARI developed clone potato varieties

3.4.1 Source of plant materials

The tuber of potato varieties BARI Alu-7, BARI Alu-35, BARI Alu-36, BARI Alu-37, BARI Alu-40, BARI Alu-46 and BARI Alu-62 were used as plant materials for the experiment. BARI Alu-7 has been used as check variety. All of the varieties were collected from the Tuber Crops Research Center (TCRC), BARI, Gazipur-1701.

3.4.2 Plant materials

In vitro potato plantlets of these above mentioned varieties were developed from source materials. Stem segments having 2-3 nodes from *in vitro* plantlets were used for micropropagation and determining IBA effect on root production.

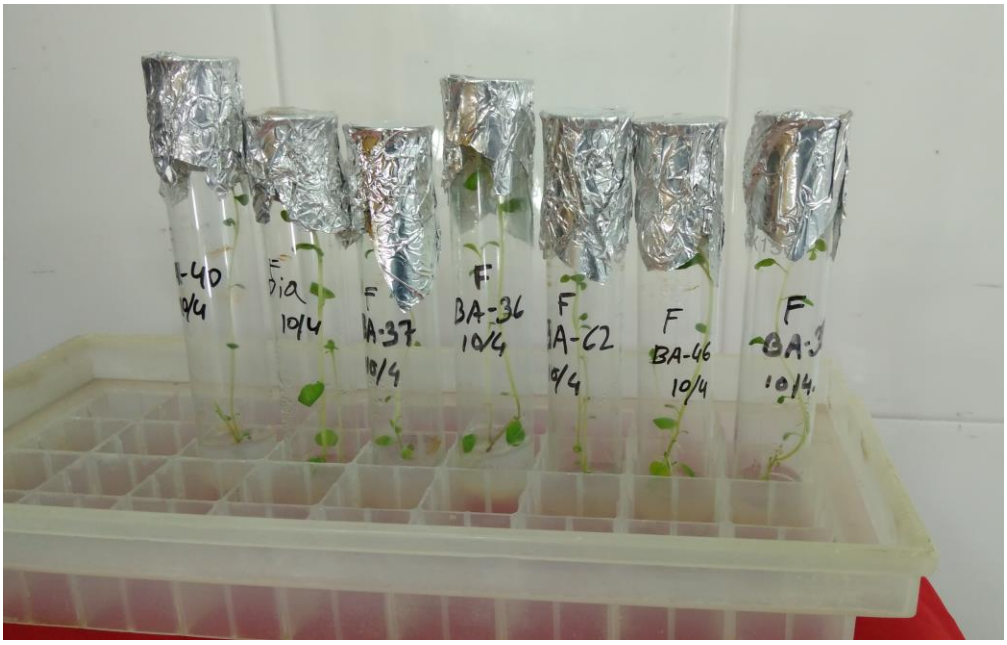


Figure 3.1: Explant collection

3.5 Laboratory preparation

Laboratory preparation was started on April 2016 by collecting list of chemicals and instruments used in this study are given in Table 3.1.

3.5.1 Chemicals and instruments

The chemicals and instruments used in this experiment are listed below:

Table 3.1. List of the chemicals and instruments used in the experiment

Chemicals		Instruments	
1	a) MS medium (Duchefa, Netherlands) MS medium ingredients	1	Autoclave
2	Sterilizing chemicals a. Sodium hypo chloride b. Tween-20	2	Hotplate with magnetic stirrer
3	Sucrose	3	Digited drying oven
4	Agar	4	Freeze (4^0c)
5	NaOH (1.0 N/ 0.1N)	5	Incubators
6	KCl (3M), IBA	6	Laminar Air Flow Chamber
7	Sterilized distiled water	7	Microwave oven
8	Absolute ethanol	8	Pipettes
9	Ethanol (70%)	9	Plant growth chamber
10	HCl	10	Safety cabinets
11	Methilated spirit	11	Shakers
12	Water Purification System	12	pH meter
13	Culture vials (petridishes, test tube etc.)	13	Scalpel, forceps, scissors etc.
		14	Electric balance

3.5.2 Culture media

Success of any experiment depends on the culture media, hormone combination, tissue and employing cell.

Murashige and Skoog medium, Liquid medium were used as culture medium for *in* vitro propagation.

3.5.3 Stock solutions preparation

The first step in the preparation of the medium is the preparation of stock solutions of macro and micro salt and vitamins of the MS medium. As different media constituents were required in different concentrations, separate stock solutions for the macronutrients, micronutrients, Fe-EDTA (Iron stock), vitamins and growth regulators were prepared separately for instant use.

3.5.3.1 Macronutrients stock solution (stock 1)

Stock solution of macronutrients was prepared with 10 times the final strength of the medium in one liter of distilled water (DW). Ten times the weight of the salts required for one liter of medium weighted accurately and was dissolved one by one except $CaCl_2$. The stock solution of $CaCl_2$ was prepared separately in order to avoid precipitation. And in this way, all the salts were dissolved thoroughly in 750 ml of distilled water and final volume was made up to one liter by further addition of DW. The stock solution was poured into a clean sterilized glass container and stored in a refrigerator at 4°C for ready use.

3.5.3.2 Micronutrients stock solution (stock 2)

A stock solution of all the micronutrients with 100x concentration is generally prepared. Since copper and cobalt are required in very small quantities, it was prepared first to make a separate stock solution of those two salts (100) and then an appropriate volume was pipetted and put into the main micronutrient stock solution. This stock solution was also stored in refrigerator at 4°C.

3.5.3.3 Iron (Fe-EDTA) stock solution (stock 3)

Iron-EDTA was added freshly and it was made 100 times the final strength of the medium in one liter DW. Here, two constituents, $FeSO_4.7H_2O$ and Na_2EDTA, were dissolved in 750 ml of DW in a conical flask by heating in a water bath until the salts dissolved completely and final volume was made up to one liter by further addition of DW. This stock should be stored in an amber color bottle or a bottle covered with an aluminum foil and stored in refrigerator at 4°C.

3.5.3.4 Vitamins stock solution (stock 4)

The following vitamins were used in the present study for the preparation of MS medium. Myo-inositol (Inositol), Nicotinic acid (Vitamin B_3), Pyridoxin HCl (Vitamin B_6), Thiamine HCl (Vitamin B_1) and Glycin. Each of the vitamins were taken at 100 times of their final strength in measuring cylinder and dissolved in 400 ml of distilled water. The final volume was made up to 1000 ml by further addition of distilled water. This stock solution was also labeled and stored in a refrigerator at 4°C.

3.5.3.5 Other stock solutions preparation

Preparation of 1N 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.

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. The solution was stored in a sterilized glass bottle. This solution was made fresh each time before use.

3.5.4 Preparation of MS media

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. 50 ml MS I solution , 5.0 ml MS II, 5.0 ml MS III, 5.0 ml MS IV media (20X) and 30 gm of sucrose were added and gently stirred to dissolved these ingredients completely with the help of a hot plate magnetic stirrer.
3. 0.5ml/L IBA (conc. 0.1mg/ml) was added to the mixture.
4. The whole mixture was then made up to 1 liter with further addition of double distilled water.
5. pH of the medium was adjusted to 5.80±0.1 by pH meter with the addition of 1 N NaOH or 0.1 N HCl whichever was necessary.
6. Finally, 8 g agar was added to the mixture and heated for 10 minutes in an electric oven for melting of agar (In case of liquid media agar was not added to the mixture).

3.5.5 Agar

The media was gelled with 8 g/L agar and the whole mixture was gently heated on microwave oven for 8-10 minutes.

3.5.6 Sterilization

Sterilization of glassware and instruments:

All types of glassware instrument was 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, surgical blades, brush, cotton, instrument stand were sterilized in an autoclave at a temperature of 121°C for 45 minutes at 15 psi pressure.

Sterilization of culture media:

One liter of MS medium were dispersed into culture vials. Then the vials were autoclaved at 15 psi pressure at 121^0C for 20 minutes. The medium was then transfer into the culture room and cooled at 20^0C temperature. The media was was dispersed into culture vial. After dispensing the vial were covered with thin polythene cap and marked with different codes with the help of a permanent glass marker to indicate specific varieties.

Sterilization of culture room and transfer area:

At the beginning, the culture room was spray with formaldehyde and then the room was kept closed for one day. Then the room was cleaned through gently washing the floors walls and rakes with a detergent. This is followed by careful wiping them 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 usually sterilized by switching on the cabinet. The ultra-violate ray kills the microbes inside the laminar airflow. It switches on 30 minutes before working in empty condition and for 20 minutes with all the instruments. The working

surface was wiping with 70% ethanol, 30 minutes before starting the transfer work.

3.5.7 Preparation of explants

The shoot of potato was used as explants. The shoot was separated and disinfected shoots were then cut into small segments and kept under sterilized distilled water into sterilized petridishes to keep the shoot alive. Then the explants were ready for inoculation.

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 liquid medium. The vials were capped with the polythene and kept at growth room to growth and development of plant under white florescent light (2500- 3000 Lux).

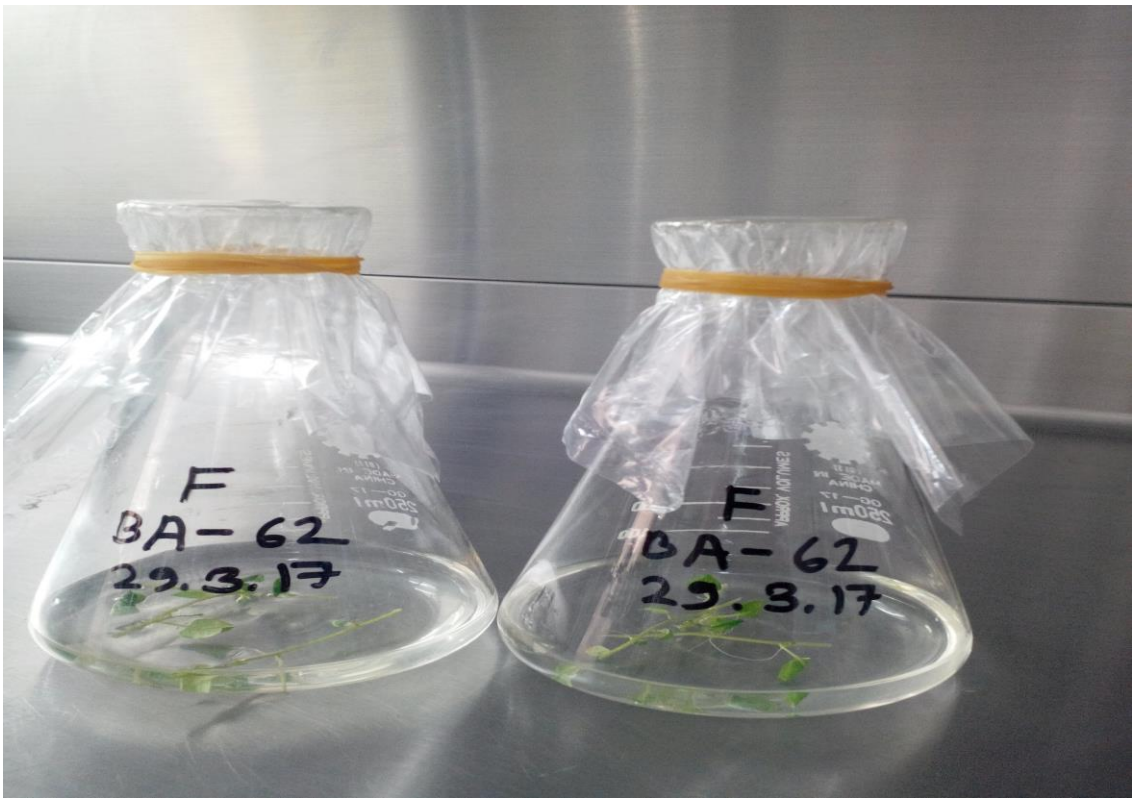


Figure 3.2: Explant preparation in liquid media

3.5.9 Subculture

The regenerated plantlets were sub-cultured after 4 week of inoculation. The shoot was cut into small pieces and placed on prepared sterilized MS medium. The subcultured vials were then inoculated at 25 ± 1^{0}C with 16 h photo period. Repeated subculture was attended at regular interval of 28 days. The observations and data collection were noted regularly.

Figure 3.3: The regenerated plant in liquid media

Figure 3.4: Root and shoot development in solid media

3.6 Experimental design

In laboratory condition, Completely Randomized Design (CRD) with five replications and in field condition Randomized Complete Block Design (RCBD) having three replications were used.

3.7 Data collection

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

01. **Days required for shoot and root appearance:** The cultures were observed at alternate days starting from 3rd day of inoculation and continued up to 28th day for shooting. Any change or development in culture when observed was recorded as days to shoot initiation or

appearance and any development or outcome of root was recorded as days to root initiation.

2. **Days to well-developed shoot and root:** Days required for vigorous growth of shoot bearing buds and leaves were considered as days to well-developed shoot. Days required for vigorous growth of root having 1 or 2 secondary roots were considered as days to well-developed root.

3. **Length of the shoot or height of the plant (cm):** The length or height of the plant was measured against a ruler in cm. at 28th days after culture. The length from the base of plantlet to the tip of the plantlet was considered as height of the plant. In case of multiple shoot, the length of the tallest plant was considered as plant height and measured in cm.

4. **Length of the root (cm.):** Root length of the plantlet was measured against a ruler in mm. at 28th day after culture. The length from the base of plantlet to the tip of the root was considered as length of the plant. In case of multiple root, the length of the longest root was considered as root length and measured in mm.

5. **Number of leaves per explant:** The number of leaves of plant was counted at 28^{th} day after culture. In case of single stem plantlet, all the leaves were counted from base to tip of the plantlet as 1 to 15 and in case of multiple stem plantlet, all the leaves counted in plantlet were divided by the total number of stems of the plantlet.

6. **Fresh weight of the shoot and root (mg):** The fresh weight of root and shoot were measured in mm. by a digital balance at 28th day after culture using following formula:

$$\textbf{Fresh weight of root / shoot (mg)} = \frac{\text{Total weight of shoot/root}}{\text{Total number of shoot/root measured}}$$

7. **Dry weight of the shoot and root (mg):** Shoot and root were collected at 28th day after culture and dried in an oven at 60^0 C for 48 hours. Then

dry weight of the shoot and root was measured in mg by a digital balance using following formula:

$$\textbf{Dry weight of root / shoot (mg)} = \frac{\text{Total weight of shoot/ root}}{\text{Total number of shoot/root measured}}$$

All the data were collected following plant destructive methods

Experiment II: Nucleus seed (G_1) production efficiency of BARI developed clone potato varieties

The plantlets (grown *in vitro*) about 28 days were transferred to the net house and were kept in a shady condition without removing the lids. The beds of the net house were properly ploughed and were covered with a mixture of sand, decomposed cow dung, burnt rice husk and coconut dust (1:1:1:1 v/v). The mixture was sterilized properly to avoid damping off disease caused by soil borne pathogens of the transplant. After two weeks, the plantlets were transplanted in beds under net house. Beds were drenched with fungicides before planting. Three to four water sprays were given daily with a sprayer to keep the soil moist and maintained humidity for initial one week. Additional soil substrate was added on the nursery beds to bury lower nodes. The crop was allowed to mature and mini tubers were harvested. Data on yield and yield contributing characters were recorded. G_1 generation seeds were kept in the cold storage for using as a planting material in the next crop season for breeder seed production.

3.8 Statistical analysis

The data obtained for different characteristics were statistically analyzed to find out the significance difference among the treatments. The mean values of all the recorded characteristics were evaluated and analysis of variance was performed 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.

Chapter IV

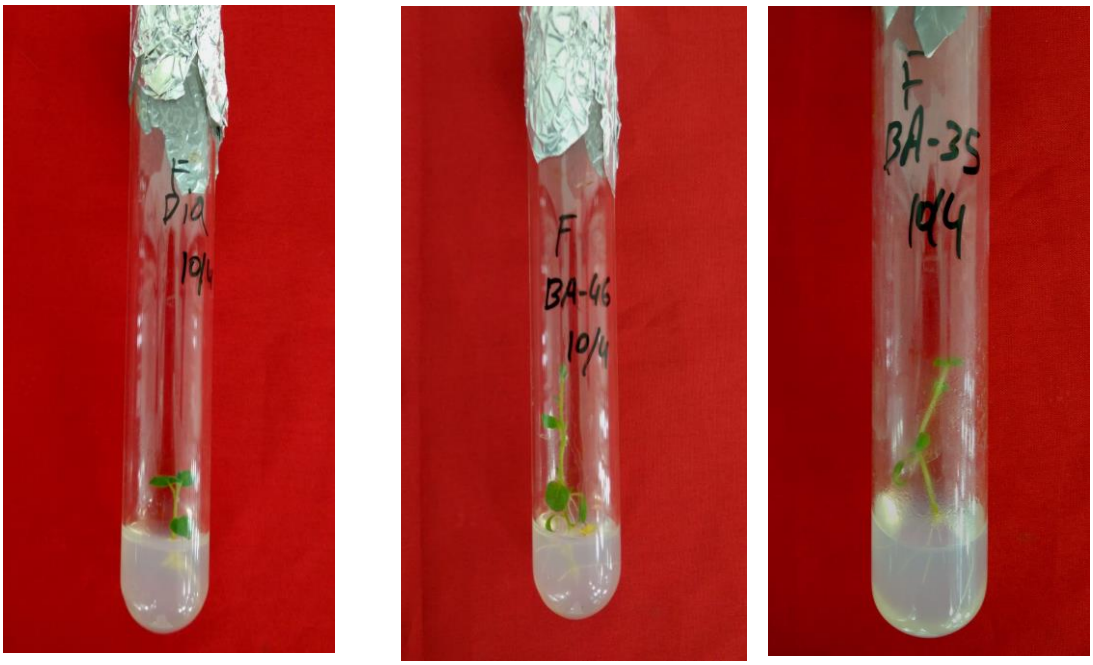
RESULTS AND DISCUSSION

Two experiments were conducted to achieve the objectives. In vitro experiment was conducted under the laboratory conditions and the nucleus seed production experiment was conducted at net house condition. The results have been presented and discussed under the following headings:

4.1 Experiment I: *In vitro* propagation of BARI developed clone potato varieties

4.1.1 Days to shoot initiation

Significant variation was observed among the different varieties of potato in respect of days to shoot initiation (Table 4.1). The maximum days to shoot initiation were recorded in BARI Alu- 7 (6.40 days) and BARI Alu- 35, BARI Alu- 36 and BARI Alu- 37 required minimum 6.00 days.



4A **4B** **4C**

Figure 4.1: Shoot proliferation on MS media (A) BARI Alu-7 (B) BARI Alu-46 (C) BARI Alu-35 at 14 days

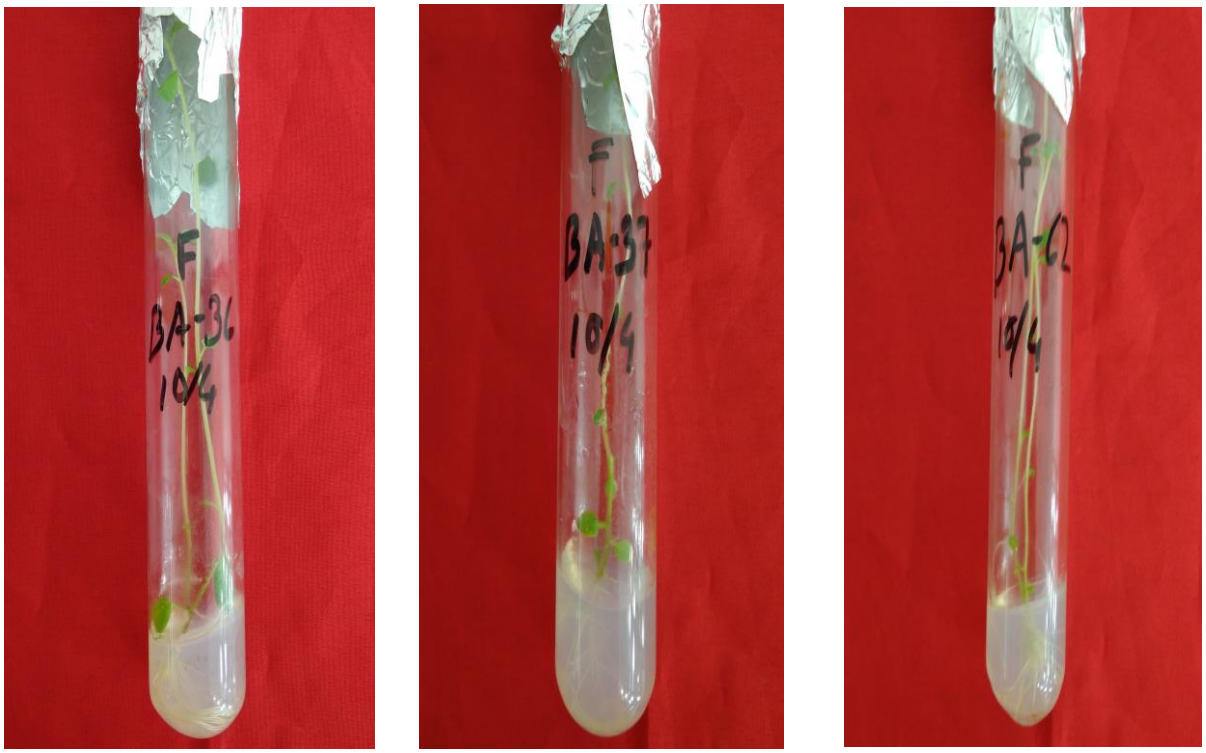


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Figure 4.2: Shoot proliferation on MS media - (A) BARI Alu- 36, (B) BARI Alu-37, and (C) BARI Alu- 62 at 28 days

Table 4.1. Days to shoot initiation as influenced by variety

Treatment	Days to initiation of shoot	
BARI Alu- 7	6.80	a
BARI Alu- 35	6.00	b
BARI Alu- 36	6.00	b
BARI Alu- 37	6.00	b
BARI Alu- 40	6.60	a
BARI Alu- 46	6.40	ab
BARI Alu- 62	6.40	ab
LSD (0.05)	0.53	
CV (%)	6.47	

4.1.2 Number of leaf per plant

Number of leaf of potato varieties under study varied significantly among each other (Table 4.2). The maximum number of leaves (14.80) were recorded in BARI Alu- 36, which was statistically identical with BARI Alu- 37. The minimum number of leaf (6.20) was observed from BARI Alu- 7.

4.1.3 Length of shoot (cm)

Length of shoot of potato plantlet varied significantly among the varieties under study (Table 4.2). The highest length of shoot (9.98 cm) was recorded from BARI Alu- 36, while the lowest length of shoot (3.76 cm) was observed from BARI Alu- 7 which was statistically similar to all variety except BARI Alu- 36.

Fig. 4.3: Shoot length of BARI Alu-36

Fig 4.4: Shoot length of BARI Alu-7

4.1.4 Fresh weight of shoot (mg)

Fresh weight of shoot were influenced by potato varieties. The maximum weight of fresh shoot (1.669 mg) was observed in BARI Alu- 36 and the minimum weight (0.096 mg) was found in BARI Alu- 7 (Table 4.3).

4.1.5 Dry weight per plant (mg)

Dry weight of shoot was not significantly influenced by variety of potato. The maximum dry weight per plant (0.00922 mg) was observed in BARI Alu- 36 and the minimum dry weight (0.00572 mg) was observed in BARI Alu- 7 (Table 4.3).

Table 4.2. Effect of varieties on number of leaf per plant and shoot length of potato

Treatment	Number of leaf per plant	Shoot length (cm)
BARI Alu- 7	6.20 d	3.76 b
BARI Alu- 35	10.20 bc	4.70 b
BARI Alu- 36	14.80 a	9.98 a
BARI Alu- 37	13.20 ab	4.56 b
BARI Alu- 40	9.80 c	4.22 b
BARI Alu- 46	12.00 abc	4.20 b
BARI Alu- 62	12.00 abc	4.24 b
LSD (0.05)	3.04	1.33
CV (%)	10.85	7.84

Table 4.3. Effect of varieties on average fresh weight and dry weight of shoot of potato

Treatment	Average fresh weight of shoot (mg)	Average Dry weight of shoot (mg)
BARI Alu- 7	0.09552 b	0.00572 a
BARI Alu- 35	0.79900 b	0.00624 a
BARI Alu- 36	1.66900 a	0.00922 a
BARI Alu- 37	0.67110 b	0.00822 a
BARI Alu- 40	0.12160 b	0.00842 a
BARI Alu- 46	0.68270 b	0.00908 a
BARI Alu- 62	0.20310 b	0.00642 a
LSD (0.05)	0.71140	0.00413
CV (%)	9.86000	9.98000

4.1.6 Days to root initiation

Significant variation was observed among different variety of potato on days to first root initiation (Table 4.4). The maximum days to root initiation were recorded in BARI Alu- 7 (5.8 days) and BARI Alu- 36 required minimum 3.80 days.

Figure 4.5: Well develop root and shoot development

Table 4.4. Days to root initiation as influenced by Variety

Treatment	Days to initiation of root	
BARI Alu- 7	5.80	a
BARI Alu- 35	4.20	bc
BARI Alu- 36	3.80	c
BARI Alu- 37	4.00	c
BARI Alu- 40	5.00	ab
BARI Alu- 46	5.80	a
BARI Alu- 62	4.00	c
LSD (0.05)	0.8619	
CV (%)	14.7	

4.1.7 Length of root (cm)

Length of root of potato plantlet varied significantly among the varieties under study (Table 4.5). The highest length of root (5.54 cm) was recorded from BARI Alu- 36, while the lowest length of shoot (1.50 cm) was observed from BARI Alu- 7 which was statistically similar with BARI Alu- 35.

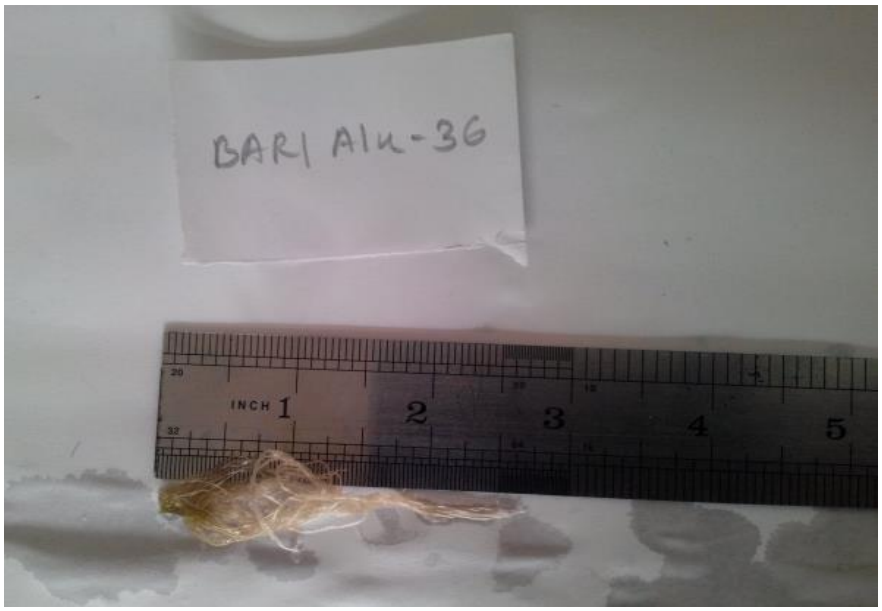


Figure 4.6: **The root length of BARI Alu- 36**

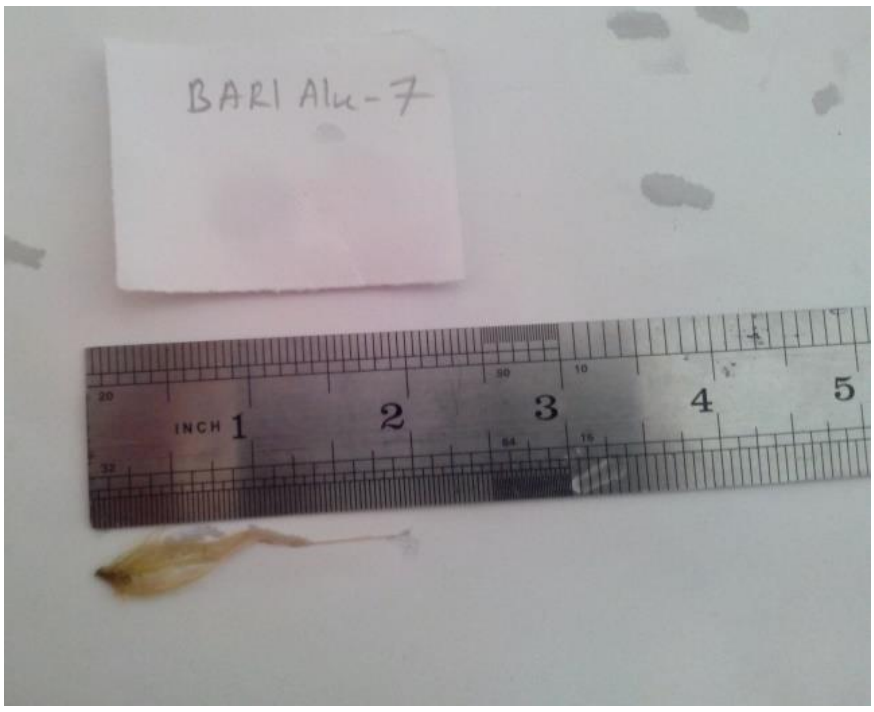


Figure 4.7: **The root length of BARI Alu-7**

4.1.8 Number of primary root

Number of primary root was significantly influenced by varieties under study (Table 4.5). The maximum number of primary root (17.00) was recorded from BARI Alu- 36, which was statistically identical with BARI Alu- 35, BARI Alu- 37, BARI Alu- 40, BARI Alu- 46, and BARI Alu- 62. The minimum number of primary root (10.40) was observed in BARI Alu- 7.

4.1.9 Number of secondary root

Number of secondary root varied significantly in different varieties (Table 4.5). The highest number of secondary root (28.20) was recorded from BARI Alu- 36, whereas the lowest number of secondary root (0.6) was found from BARI Alu- 7.

Table 4.5. Effect of varieties on Root length, number of primary root, secondary root per plant of potato

Treatment	Root length (cm)	Number of primary root per plant	Number of Secondary root per plant
BARI Alu- 7	1.80 c	10.40 b	0.60 c
BARI Alu- 35	1.82 c	12.80 ab	7.80 b
BARI Alu- 36	5.54 a	17.00 a	28.20 a
BARI Alu- 37	3.02 b	13.80 ab	3.60 c
BARI Alu- 40	2.36 bc	14.00 ab	6.60 b
BARI Alu- 46	2.12 bc	12.80 ab	2.60 c
BARI Alu- 62	2.94 b	15.00 ab	0.80 c
LSD (0.05)	1.018	5.276	2.862
CV (%)	4.79	9.53	10.61

4.1.10 Fresh weight of root (mg)

Fresh weight of root significantly influencedby the variety. The maximum weight of fresh root (0.359 mg) was observed in BARI Alu- 36 and the minimum weight (0.089mg) was found in BARI Alu- 7 (Table 4.6).

4.1.11 Dry weight of root per plant (mg)

The dry weight of root per plant was significantly influenced by variety of potato. The maximum dry weight of root per plant (0.00112mg) was observed in BARI Alu- 36 and the minimum dry weight of root (0.00402mg) was observed in BARI Alu- 7 (Table 4.6).

Table 4.6. Effect of varieties on average fresh weight and dry weight of root of potato

Treatment	Average fresh weight of root (mg)		Average Dry weight of root (mg)	
BARI Alu- 7	0.0895	b	0.0040	c
BARI Alu- 35	0.1941	ab	0.0073	abc
BARI Alu- 36	0.3591	a	0.0112	a
BARI Alu- 37	0.2874	ab	0.0090	ab
BARI Alu- 40	0.1282	ab	0.0046	bc
BARI Alu- 46	0.1064	ab	0.0063	bc
BARI Alu- 62	0.2864	ab	0.0033	c
LSD (0.05)	0.2261		0.0041	
CV (%)	8.7500		10.0900	

Experiment II: Nucleus seed production of BARI developed clone potato varieties

BARI developed six clone potato varieties BARI Alu-35, BARI Alu-36, BARI Alu-37, BARI Alu-40, BARI Alu-46 and BARI Alu-62 have been studied under net house conditions for their yield and yield contributing character for nucleus seed production. BARI Alu- 7 used as check variety. Result and discussion are given below.

4.2.1 Plant height (cm)

Plant height of seven potato varieties varied significantly under net house conditions (Table 4.7). The tallest plants (86.73 cm) was recorded in BARI Alu-46 followed by BARI Alu-40 (77.43). There was no significan difference between the variety BARI Alu- 7 and BARI Alu- 35. The shortest plant (51.53 cm) was observed in BARI Alu- 35.

4.2.2 Number of shoot per plant

Number of shoot per plant of potato varied significantly due to different varieties (Table 4.7). The highest number of shoot per plant (9.00) was recorded from the variety BARI Alu- 35. The lowest number of shoot per plant (1.20) was found from BARI Alu- 37. Number of shoot per plant produced by BARI Alu- 7 and BARI Alu- 62 was statistically identical (Table 4.7).

4.2.3 Number of leaf per plant

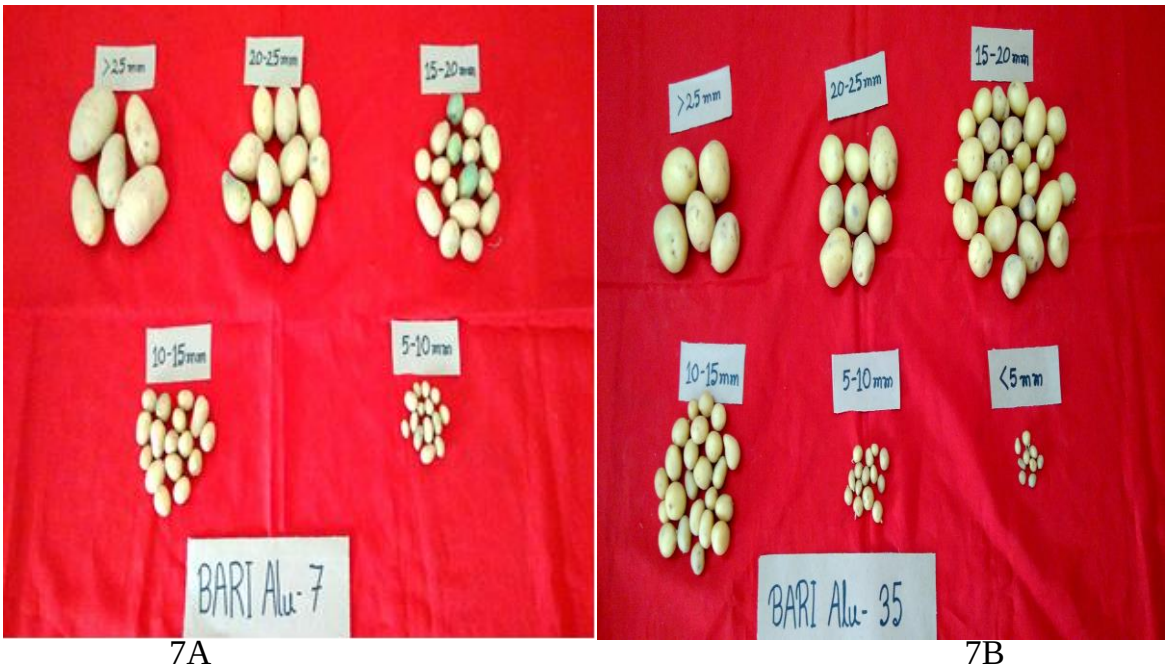
Significant variation was observed among the different varieties of potato on number of leaf per plant (Table 4.7). The maximum number of leaves per plant (134.50) was recorded from potato variety BARI Alu- 35. The lowest number of leaf per plant (23.13) was found from BARI Alu- 36 which was statistically similar with BARI Alu- 37.

Table 4.7. Effect of varieties on plant height, number of shoot and leaf per plant

Treatment	Plant height (cm)		Number of shoot per plant		Number of leaf per plant	
BARI Alu- 7	65.33	d	4.20	d	74.73	d
BARI Alu- 35	65.53	d	9.00	a	134.50	a
BARI Alu- 36	51.53	f	2.40	e	23.13	f
BARI Alu- 37	57.73	e	1.20	f	23.33	f
BARI Alu- 40	77.53	b	5.00	c	88.13	b
BARI Alu- 46	86.73	a	5.40	b	85.53	c
BARI Alu- 62	68.40	c	4.00	d	59.13	e
LSD (0.05)	1.98		0.23		1.97	
CV(%)	7.65		8.81		7.59	

4.2.4 Number of tubers per plant

Number of tuber per plant varied significantly in different varieties under the study (Table 4.8). The highest number of tuber (52.40) was recorded from BARI Alu- 46 followed by 37.40 in BARI Alu-40.The lowest number of tuber (18.60) was found from BARI Alu- 37.



7A 7B

Figure 4.8: Different size of minituber - (A) BARI Alu- 7 & (B) BARI Alu- 35

Figure 4.9: Production of minituber in a single plant

4.2.5 Weight of tuber

Average weight of tuber varied significantly for different in varieties at net house condition (Table 4.8). The maximum weight of tuber the plant (358.30g) was recorded from BARI Alu- 46. The minimum average weight of tuber (142.30 g) was found from BARI Alu- 35.

Table 4.8. Effect of varieties on number of tuber per plant and weight of tuber per plant

Treatment	Number of tuber per plant		Weight of tuber per plant (g)	
BARI Alu- 7	21.20	d	307.30	b
BARI Alu- 35	37.40	b	142.30	f
BARI Alu- 36	16.00	g	195.50	d
BARI Alu- 37	18.60	f	114.70	g
BARI Alu- 40	30.40	c	182.70	e
BARI Alu- 46	52.40	a	358.30	a
BARI Alu- 62	19.40	e	256.90	c
LSD (0.05)	0.22		2.83	
CV(%)	6.45		5.71	

Chapter V
CONCLUTIONS AND RECOMMENDATIONS

5.1 Conclusions

The study was conducted at the Laboratory and net house of TCRC, Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur from April 2016 to February 2017 to see the *In vitro* propagate and Nucleus Seed Production efficiency of BARI developed clone potato varieties. BARI Alu-7, BARI Alu-35, BARI Alu-36, BARI Alu-37, BARI Alu-40, BARI Alu-46 and BARI Alu-62 were used as planting materials for this experiment. Data on different growth parameters in laboratory conditions were recorded.

Significant variation was observed among the different varieties of potato on days to shoot initiation. The maximum day to shoot initiation was recorded in BARI Alu-7 (6.40 days where as maximum days to shoot initiation was recorded in BARI Alu-7 (10.10 days). Number of leaf of potato varieties under the study varied significantly among each other. The maximum number of leaf (14.80) was recorded from BARI Alu-36. Length of shoot of potato plantlet varied significantly among the varieties. The highest length of shoot (9.98 cm) was recorded from BARI Alu-36. Potato Variety significantly influenced the fresh weight of shoot. The maximum weight of fresh shoot (1.669 mg) was observed in BARI Alu-36. The dry weight of shoot was not significantly influenced by the variety. The maximum dry weight per plant (0.0112 mg) was observed in BARI Alu-36.

Significant variation was observed among the different varieties of potato on days to first root initiation. The maximum days to root initiation were recorded in BARI Alu-7 (5.8 days). Days for well develop root initiation were significantly influenced by different varieties of potato. The maximum days to well develop root initiation were recorded in BARI Alu-7 (5.8 days). Length of root of potato plantlet varied significantly among the varieties under study. The

highest length of root (5.54 cm) was recorded from BARI Alu-36. Number of primary root was significantly influenced by varieties under study. The maximum number of primary root (17.00) was recorded from BARI Alu-36. Number of secondary root varied significantly for different variety. The highest number of secondary root (28.20) was recorded from BARI Alu-36. Potato Variety significantly influenced the fresh weight of root. The maximum weight of fresh root (0.359 mg) was observed in BARI Alu-36. The dry weight of root per plant was significantly influenced by variety of potato. The maximum dry weight of root per plant (0.00112mg) was observed in BARI Alu-36 and the minimum dry weight of root (0.00402mg) was observed in BARI Alu-7. Number of G_1 seed tuber per plant 21.2, 16.0, 18.6, 19.4, 30.4, 37.4 and 52.4 was recorded from the varieties BARI Alu -7, BARI Alu -36, BARI Alu-37, BARI Alu-62, BARI Alu-40, BARI Alu-35, BARI Alu-46 respectively. The maximum weight of G_1 seeds per plant was found from the variety BARI Alu-46 (358.0 g) followed by BARI Alu-7 (306g), BARI Alu -62 (256.6g), BARI Alu-36 (194.2g), BARI Alu- 40 (182.4g), BARI Alu-35(142g) and BARI Alu-37(113.4g). Based on the results and discussion it can be concluded as followed:

1. The variety BARI Alu-36 showed better performance (Number of leaf, length of shoot, weight of fresh root, length of root, maximum number of primary root, number of secondary root and fresh weight of root) under *in vitro* condition.

2. Growth, development and yield of G_1 seeds/minituber/plant was found better in BARI Alu- 46 followed by BARI Alu- 7 and BARI Alu- 62.

5.2 Recommendations of the study

Further research may be carried out on the following mentioned points:

1. A total of 14 BARI developed clone potato varieties should be studied to observe *in vitro* regeneration efficiency.
2. Growth development and performance of G_1 seed production/minituber production should be studied.
3. Physiomorphological characteristics and tuberization behavior of clone varieties should be studied.

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APPENDICES

Appendix I. Composition of Duchefa Biochemic MS (Murashige and Skoog, 1962) medium including vitamins

Components	Concentrations (mg/L)	Concentrations
Micro Elements	**mg/L**	**µM**
$CoCl_2.6H_2O$	0.025	0.11
$CuSO_4.5H_2O$	0.025	0.10
Fe Na EDTA	36.70	100.00
H_3BO_3	6.20	100.27
KI	0.83	5.00
$MnSO_4.H_2O$	16.90	100.00
$Na_2MoO_4.2H_2O$	0.25	1.03
$ZnSO_4.7H_2O$	8.60	29.91
Macro Elements	**mg/L**	**mM**
$CaCl_2$	332.02	2.99
KH_2PO_4	170.00	1.25
KNO_3	1900.00	18.79
$MgSO_4$	180.54	1.50
NH_4NO_3	1650.00	20.61
Vitamins	**mg/L**	**µM**
Glycine	2.00	26.64
Myo-Inositol	100.00	554.94
Nicotinic acid	0.50	4.06
Pyridoxine HCl	0.50	2.43
Thiamine HCl	0.10	0.30

Total concentration of Micro and Macro elements including vitamins: 4405.19 mg/L

Manufacturing Company: Duchefa Biochem

Appendix II. Analysis of variance on Days to first and last initiation of shoot

Source	Degrees of Freedom	Mean square	
		Days to first initiation of shoot	**Days to last initiation shoot**
Replication	4	0.1	0.071
Factor A	6	0.524	1.029
Error	24	0.167	0.171

Appendix III. Analysis of variance on Number of leaf per plant and Shoot length

Source	Degrees of Freedom	Mean square	
		Number of leaf per plant	Shoot length
Replication	4	3.957	33.185
Factor A	6	38.495	23.654
Error	24	5.424	36.038

Appendix IV. Analysis of variance on Average fresh and Dry weight of shoot

Source	Degrees of Freedom	Mean square	
		Average fresh weight of shoot	Average Dry weight of shoot
Replication	4	0.578	0.034
Factor A	6	1.529	0.027
Error	24	0.297	0.028

Appendix V. Analysis of variance on Days to first and last initiation of root

Source	Degrees of Freedom	Mean square	
		Days to first initiation of root	Days to last initiation of root
Replication	4	0.186	0.757
Factor A	6	3.781	3.362
Error	24	0.436	0.54

Appendix VI. Analysis of variance on average root length, Number of primary and secondary root

Source	Degrees of Freedom	Mean square		
		average root length	Number of primary root	Number of Secondary root
Replication	4	6.13	3.386	14.957
Factor A	6	8.493	20.99	466.96
Error	24	8.608	16.336	74.807

Appendix VII. Analysis of variance on average fresh and Dry weight of root

Source	Degrees of Freedom	Mean square	
		Average fresh weight of root	average Dry weight of root
Replication	4	0.036	0.032
Factor A	6	0.055	0.032
Error	24	0.03	0.031

Appendix VIII. Analysis of variance on Plant height, Number of shoot per plant and Number of leaf per plant of potato

Source	Degrees of Freedom	Mean square		
		Plant height(cm)	Number of shoot per plant	Number of leaf per plant
Replication	2	72.905	3.716	72.905
Factor A	6	415.47	18.469	4624.5
Error	12	1.238	0.016	1.238

Appendix IX. Analysis of variance on Number of tuber per plant Weight of tuber per plant of potato

Source	Degrees of Freedom	Mean square	
		Number of tuber per plant	Weight of tuber per plant(g)
Replication	2	3.716	107.19
Factor A	6	520.99	23591
Error	12	0.016	2.524