IN VITRO REGENERATION POTENTIALITY OF POTATO (*Solanum tuberosum* L.) CULTIVARS UNDER DIFFERENT CHEMICAL MUTAGENS

BY

MD. NAYEEM MORSHAD

REGISTRATION NO: 07-02306

A Thesis
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
GENETICS AND PLANT BREEDING

SEMESTER : JANUARY-JUNE, 2014

Approved by:

Naheed Zeba

(Prof. Dr. Naheed Zeba)
Dept. of Genetics and Plant Breeding
Supervisor

(Prof. Dr. Md. Ekramul Hoque)
Dept. of Biotechnology
Co- Supervisor

(Prof. Dr. Md. Sarowar Hossain)
Chairman
Dept. of Genetics and Plant Breeding
Sher-e-Bangla Agricultural University, Dhaka

Prof. Dr. Naheed Zeba
Dept. of Genetics and Plant Breeding
Sher-e Bangla Agricultural University
Dhaka-1207, Bangladesh
Mob: +88 01913091772

CERTIFICATE

*This is to certify that thesis entitled, "**IN VITRO REGENERATION POTENTIALITY OF POTATO (Solanum tuberosum L.) CULTIVARS UNDER DIFFERENT CHEMICAL MUTAGENS**" 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 GENETICS AND PLANT BREEDING**, embodies the result of a piece of bona fide research work carried out by, **Registration No. 07-02306** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma in any institute.*

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

Naheed Zeba

.....

(Prof. Dr. Naheed Zeba)
Supervisor

Dated: June, 2014
Place: Dhaka, Bangladesh

DEDICATED TO

MY

BELOVED PARENTS

ACKNOWLEDGEMENTS

All praises are due to the "Almighty Allah" who enabled the author to pursue higher education in Genetics and Plant Breeding and to submit the thesis for the degree of Master of Science (M.S.) in Genetics and Plant Breeding.

The author wishes to express his profound sense of appreciation and heartiest gratitude to his Supervisor, Professor Dr. Naheed Zeba, Department of Genetics and Plant Breeding, Sher-e-Bangla Agricultural University, Dhaka for her help, scholastic supervision, continuous encouragement and constructive suggestion throughout the period of research and for taking immense care in preparing this manuscript.

The author expresses his immense gratitude to his Co-supervisor, Professor Dr. Md. Ekramul Hoque, Department of Biotechnology, Sher-e-Bangla Agricultural University, Dhaka for his valuable advice, cordial inspiration, guidance and continuous counseling during the research work in his Biotechnology Laboratory.

The author extends his greatful appreciation to the honorable chairman, Professor Dr. Md. Sorowar Hossain and Professor Dr. Md. Firoz Mahmud, Department of Genetics and Plant Breeding, Sher-e-Bangla Agricultural University, Dhaka, for their inspiration and encouragement throughout the whole period of the research work. There have been so many persons who showed their friendship and helped the author that he cannot mention them all by name here, but thanks to all of them .

The author express his gratitude and indebtedness to all the honorable course instractors of the Department of Genetics and Plant Breeding, Sher-e-Bangla Agricultural University, Dhaka for their kind help and co-operation in various stages towards completion of research work.

The author would like to thank all his friends and classmates for their support and inspiration through his study period in SAU. He extends his acknowledgements to his great friend and classmate Md. Arifur Rahman for making moments to be cherished for lifetime. Author wants to especially acknowledge his friend Md. Zohurul Kadir Roni for all his help.

.The author feels proud of expressing his sincere appreciation and gratitude to Ministry of Science and Technology for selecting him NST fellow and funding.

The author is also thankful to Sher-e-Bangla Agricultural University Research System (SAURES) for providing research support.

The author can never repay the debt of his beloved parents, uncle, aunty, sisters, brothers and well wishers for their inspiration, constant encouragement and sacrifice for his higher education.

The author expresses his immense gratefulness to all of them who assisted and inspired him to achieve higher education and regret for his inability for not to mention every one by name.

The Author

TABLE OF CONTENT

TABLE OF CONTENT (CONT'D)

TABLE OF CONTENT (CONT'D)

LIST OF TABLES

LIST OF TABLES (CONT'D)

LIST OF PLATES

IN VITRO REGENERATION POTENTIALITY OF POTATO (*Solanum tuberosum* L.) CULTIVARS UNDER DIFFERENT CHEMICAL MUTAGENS

ABSTRACT

By

NAYEEM MORSHAD

The present study was undertaken in the Genetics and Plant Breeding Laboratory and the Tissue Culture Laboratory of the Department of Biotechnology, Sher-e-Bangla Agricultural University (SAU), Dhaka, during the period of January 2013 to March 2014 , to investigate the *in vitro* regeneration potentiality of three potato cultivars viz. Diamant, Cardinal, and Granola under different chemical mutagen treatment. Five levels of GA_3 (100, 200, 300,400 and 500 ppm/L) were used to find out the best concentration for sprout initiation . It was revealed that 500 ppm/L GA_3 showed maximum sprouting within short period of time. The effect of concentration of 2,4-D , EMS and 5-BU on *in vitro* callus induction and plant regeneration were studied on 1mg/L, 3mg/L, 5mg/L concentration. Only 2, 4-D had the ability to induce callus. The highest callus size (2.30 cm) was observed at MS+3.0 mg/L 2,4-D with the variety Granola. The maximum number of shoot (13.67) were found in the treatment MS+3.0 mg/L 2,4-D in the variety Granola at 45 DAI and the highest number of root (14.0) were also found in the same treatment. The highest number of shoot/plantlet (7.30) was recorded in variety Granola at MS + 1.0 mg/L EMS and the highest number of leaves/plantlet were also recorded in the same variety (17.30 leaves/plantlet) at the concentration of MS+3.0 mg/L at 45 DAI. The highest number of roots/plantlet was noted in the variety Granola (11.00 roots/plantlet) at normal MS medium at 45 DAI. The higher concentration (5.0 mg/L) of EMS and BU showed huge abnormality on *in vitro* regeneration in all three varieties of potato. Thin stem, deformed shoot development and very less leaf formation were also observed in higher concentration. The mutagen treated variants were acclimatized in plastic tray and subsequently in the field condition. It was noticed that, the variety Granola survived at the high rate in plastic tray and in open atmosphere . Among all the varieties Granola showed the best performance on callus induction , plant regeneration and *in vivo* establishment under field condition.The mutagenic treated minituber were harvested and preserved for further study.

CHAPTER 1
INTRODUCTION

CHAPTER I

INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the prominent crop capable of nourishing the world's population. It is one of the most economically important annual vegetable crop in Solanaceae family. In respect to land under cultivation potato is the third major subsistence crop in Bangladesh. (Shaker *et al.*, 2002). It produces the largest quantity of carbohydrates per day per unit area among the food crops. It consists of 80% water, 2-3% protein and 18% carbohydrate. It supplies at least 12 essential vitamins, minerals, proteins, carbohydrates and iron (Gray and Hughes, 1978; Thornton and Sieczka, 1980). It has multipurpose use in daily consumption and also industrial purpose. It is used in a wide variety of table, processed, livestock feed and industrial uses . The total area under potato cultivation is near about 4.60 lack hectares and the average yield was 19.37 t/ha in Bangladesh, (BBS, 2014).

The average yield of potato in Bangladesh is low as compared to many potato growing countries like Netherlands (45.7 t/ha), the USA (46.6 t/ha) and Germany (43.3 t/ha) (FAO, 2014). Among the reasons of lower yield of potato, non-availability and higher price of quality seed are important. In Bangladesh, Bangladesh Agricultural Development Corporation (BADC) supplies about 7-8 thousand tones of high quality seed potato which is only 3.5 to 4.3% of total seed requirement of the country (BADC, 2014). Some private companies are also supplying few thousand tones of commercial grade seed tubers. The rest amount of tuber planted every year for the production of potato in the country is the tubers produced as table potatoes (Siddique, 1999). Its indicate that quality seed tuber is one of the major constrains of potato production.

The development of high yielding, starch enriched, disease resistant varieties is needed for sustainable potato production. Potato and many other vegetatively propagated crops are frequently characterized by their inability to produce seed due to presence of one or more factors : incompatibility, dichogamy, abnomal seed and seedling development, seed dormancy and environmental factors. These affect

flowering and seed setting. Presence of these factors possesses margins on the use of breeding techniques for improvement. Potato can be propagated sexually (by botanical seed, also called true potato seed) and asexually (vegetatively) by means of tubers (Otroshy, 2006). In spite of having problems in conventional breeding, most of the potato varieties available now a days have been developed through natural selection and conventional breeding which is very lengthy process. There are two major problems associated with conventional clonal multiplication of potato-seed stocks. Firstly, low multiplication rate in the field that takes as long as 7 to 12 years, causing a lack of flexibility to the changing needs of the end-users. Secondly, high susceptibility of potato to viral bacterial and fungal diseases (Dobranszki *et al.*, 2008). Routine production of disease free seed tubers is necessary to maintain adequate yields. That is why reliable and pathogen free propagation has been started all over the world . Among these, the important role is played by the *in vitro* vegetative micro propagation, since this method is pathogen free.

As potatoes are vegetatively propagated crop, the seed borne pathogens of previous year pose a serious threat to the potato production of the following year. Researchers showed that some viruses can decrease the yield by 40% singlely and in combination with other viruses, the loss is 90% (Siddiqui *et al.*, 1996). Plant tissue culture offers an efficient method for production and rapid propagation of pathogen-free material and germplasm preservation of plants to overcome this unwanted situation. *In vitro* plant regeneration has become a popular and useful technique and being applied to solve the problems of many agricultural crops. Creation of novel germplasm through techniques of tissue culture and gene transfer holds great potential for improving the quality, resistance to diseases and agronomic characters of potato (Jayasree *et al.*, 2001). A reproducible protocol for *in vitro* regeneration is a prerequisite for rapid micropopagaton . Type of explants, media compositions, growth conditions, and genotypes affect, callus induction are major factors for *in vitro* regeneration. Hence, it is necessary to identify which variety is more prone to *in vitro* culture. It is important to standardize the protocol of explants response for callus induction and plant regeneration of potato cultivars.

In vitro mutagenesis is an important tissue culture technique for crop improvement which provides an opportunity to increase the variability of economically important cultivars. *In vitro* mutagenesis offers the possibility of altering only one or a few traits of a cultivar, while preserving the overall characteristics. In addition, there is no loss of the mutants, as micropropagules are sub-cultured under sterile conditions. Different chemicals such as Ethyl Methane Sulphonate (EMS), 5-Bromo Uracil (BU) and 2,4-Dicholorophenoxyacetic acid (2,4-D) are most frequently used for mutation induction.

Genetic variability is one of the important parameter for crop improvement. Artificial variability can be created under *in vitro* condition through callus induction. Among different chemical, 2,4-D has high potentiality to induce callus on *in vitro* regeneration. Callus induction has addition advantage to produce huge number of plantlet by several time sub-culturing the explant. Hence, 2,4-D was used as chemical mutagen for creation of genetic variability in potato.

Therefore, the present investigation was carried out, to identify the chemical mutagen which has the ability to create mutation in potato cultivar. Hence, the experiment was designed for the following objectives –

1. To establish an efficient *in vitro* regeneration protocol of potato under different mutagenic treatment.
2. To investigate callus induction ability of potato under chemical mutagen.
3. To identify best chemical mutagen for mutation induction in potato.
4. To study the concentration of different mutagen for creation of variation in potato.
5. To study the acclimatization potentiality of *in vitro* regeneration mutant of potato.

CHAPTER 2

REVIEW OF LITERATURE

CHAPTER 2

REVIEW OF LITERATURER

Potato is one of the most economically important annual vegetable crops in Solanaceae family (Keeps, 1979). Now-a-days plant tissue culture techniques are being applied for rapid clonal propagation of potato. However, many research works on *in vitro* micro propagation of potato have been conducted in different countries of the world. There are various factors that effect *in vitro* callus proliferation and plant regeneration such as position of the explants, on the plant as well as size of explants, genotypes of the explants, physiological state of the donor plants and explants, concentration of the nutrients and plant growth regulators in the culture medium and environment under which cultures are grown i.e. light, temperature and humidity (Gregory and John, 1998). A lot of many studies have been made earlier too in view callus induction and regeneration of potato. In this chapter, the most pertinent literature relevant to the present study is reviewed here under separate headings.

2.1. Concept of potato tissue culture

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 their 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. *In vitro* callus induction and plant regeneration of potato

Shahab-ud-din *et al.* (2011) 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 Murashige and Skoog medium which was supplemented with different concentrations of 2,4-Dichlorophenoxy acetic acid (2,4-D), α-naphthalene Acetic Acid (NAA), Benzyl Adenine (BA), 2,4-D in combinations with BA and NAA in combination with BA for callus induction. Among the treatments 2, 4-D at different concentrations produced different degree of callus but comparatively a massive amount of callus was 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.

Kumar *et al.* (2014) studied on rapid callus induction and plant regeneration of potato. The leaf explants of two potato (*Solanum tuberosum L.*) cultivars viz. (Kufri Chipsona 3 and MP-97/644) were cultured for callus induction and plant regeneration. Best callus growth from both the cultivars was observed on Murashige and Skoog (MS) (1962) media containing 3.0 mg/l of 2,4-D (2,4-dichlorophenoxy acetic acid) and1.0 mg/l of kinetin. MS medium supplemented with different concentrations and combinations of BA, Kinetin and AdSO4 (Adenine sulphate) were employed for shoot regeneration. Best shoot regeneration from callus was observed on MS media containing 1.5 mg/l BA (6-benzyladenine) and 25.0 mg/l AdSO4 (Adenine sulphate).

Forooghian and Esfarayeni (2013) studied three types of potatoes (*Solanum tuberosum* L.) including Sante, Agria and Savalan in order to review the effect of plant growth regulators and light on callus induction. In this study, factorial test is used in terms of absolutely random design with 18 different treatments and three repeat in any treatment. Plantlets of these figures after reproducing by *in vitro*

method, were placed at MS culture environment under different hormonal treatments include 2,4-D at three levels (0, 3 and 5 mg per liter) and Kinetin in three levels (0, 5/0 and 2 mg/L) and different photoperiods (light and dark) to produce callus. Under study traits include fresh callus weight, callus diameter, seedling length and percentage of callus induction. Results showed that there is a very significant different between numbers and different hormonal treatments in terms of all traits studied. Also there is a very significant difference between different photoperiods in terms of all traits, except for callus fresh weight. Calluses produced under dark conditions and hormonal treatments of 3 mg/L 2,4-D and 2 mg/L Kinetin showed superiority among under study cultivars over other treatments. In under study cultivars, medium 2,4-D hormone concentration in cultivate environment cause to more cell divisions and producing larger calluses. Generally, results showed that the highest and lowest callus induction percent was seen in Santer and Agria cultivars, respectively.

Sherkar and Chavan (2014) conducted an experiment on callus induction and shoot regeneration in potato (*Solanum tuberosum* L.). The sprouts of potato tubers were used as starter explant to establish initial culture. The explants were cultured on basal Murashige and Skoog medium supplemented with different hormonal concentrations and combinations were studied. Ten explants were cultured in each combination. The highest degree of callus formation on MS media supplemented with 2.0-4.0 mg/l of 2,4-D was observed. The regeneration of shoots observed when calli were sub cultured on MS media supplemented with 1-4mg/l of TDZ and 1-4 mg/l of BAP.

Elaleem *et al.* (2009) conducted an experiment to investigate the effects of different concentrations and combinations of growth regulators on callus induction and plant regeneration of potato (*Solanum tuberosum* L.) cultivar Diamant. The tuber segments were used as explants and cultured on MS medium supplemented with different concentrations of NAA, 2,4-D, BA and Thidiazeron (TDZ) alone and 2,4-D in combinations with BA for callus induction. The best degree for callus formation (6,0) was obtained on MS

medium supplemented with 2,4-D alone at 3.0 mg/L or 2,4-D in combination with BA both at 2.0mg/L. MS medium containing 5.0mg/L TDZ was the best for days to shoot initiation, the highest percentage of callus with shoot (81%) and the highest number of shoot/callus (3.4). Callus derived shoots were rooted most effectively in half-strength MS medium containing 0.5 mg/L IBA.

Khatun *et al.* (2003) conducted an experiment with different concentrations of 2,4-D and kinetin showed highly significant differences for length and weight of callus formed except interaction of callus weight. Leaf explants appeared to be best of all for callus length and weight when 1.0 mg/L 2,4-D + 0.25 mg/L kinetin concentrations was used. Similarly, different explants versus different concentrations of BAP/GA_3'IAA showed significant differences for shoot length and leaf number/plantlet and also for toot length. However, interaction term confirmed node and node/internode explants produced better results in shoot length and number of leaves/plantlet when concentrations 1.0 mg/L BAP + 0.1 mg/L IBA. The regenerated shoots were rooted on MS and ½ MS medium containing different concentration of IBA and maximum rooting response was achieved in ½ MS + 1.0 mg/L IBA.

A study as conducted by Omidim and Shahpiri (2003) to determine the effects of growth regulators (2,4-D and kinetin), cultivar, explants and light on callus induction in potato. They found significant effect of 2,4-D combined kinetin and their interactions on the frequency of callus induction and roots on the callus. The effect of cultivar, explants and their interaction on frequency of callus induction was not significant, while the effects of these factors on the initiation time of callus induction was significant. Callus was mostly induced in leaf explants under dark conditions, but was induced in internode explants under both dark and light conditions.

An effective procedure has been developed by Jayasree *et al.* (2001) for including somatic embryogenesis from leaf cultures of potato cv. Jyothi. Leaf sections were initially cultured on 2.4-D+ Benzyl adenine (BA) and NAA+BAP supplemented Murashige and Skoog (MS) media. Nodular embryogenic callus developed form the cut ends of explants on media containing 2, 4-D and BA, whereas compact callus developed on media containing NAA and BA.

Shirin *et al.* (2007) was conducted an experiment with the internodal and leaf explants of four potato cultivars *viz* Diamant, Multa, Atlas and Lalpakri. Where callusing response of both types of explants and for Atlus intermodal esplants was the best in 3.0 mg/L 2,4-D containing MS media among 2,4-D, NAA alone and with BAP combinations. MS medium containing 4.0 mg/L KIN + 0.5 mg/L NAA was the best for maximum shoot regeneration from the internode and lea derived calli in most of the cultivars. The regenerated shoots were rooted in MS_0 medium and successfully transplanted to the field.

Effect of growth regulators for callus induction of potato (*Solanum tuberosum* L.) genotyped (Christian and Roclas) was studied by Andreea *et al.* (2009). It was revealed that the best callus induction was for MS medium supplemented with 1mg/L 2,4-D and 0,5 mg/I MAP for both the genotypes.

Callus formation of 5 potato genotypes with different genetic origin was analyzed by Dobranszki *et al.* (1999) on different media to select the optimal treatment. Both induction and the rate of callus growth were strongly influenced by genotype and medium and significant 2^{nd} order interactions were proved statistically. The best undifferentiated growth of friable calli on leaf explants was observed after 4 weeks on medium containing 0.25mg kinetin and 5.0mg 2,4-D/L.

2.3. Chemical mutagen treatment and plant regeneration :

Talebi *et al.* (2012) studied about the chemical and physical mutagenesis which has been used to increase genetic variability in crop plants. More than 430 new varieties have been derived as mutants of rice (*Oryza sativa* L.) via the application of different mutagenic agents. Chemical mutagens such as ethyl methane sulphonate (EMS), diepoxybutane-derived (DEB), sodium azide and irradiation (Gamma rays, X-rays and fast neutrons) have been widely used to induce a large number of functional variations in rice and others crops. Among chemical mutagens, the alkylating agent, ethyl methane sulfonate (EMS) is the most commonly used in plants as it causes a high frequency of nucleotide substitutions, as detected in different genomes. In this study, seeds of potential genotype of the popular variety, (*Oryza sativa* L. spp. Indica cv. MR219) were treated with EMS at concentrations of 0.25%, 0.50%, 0.75%, 1%, 1.25%, 1.5% and 2%. Sensitivity to EMS was determined by various measurements on the M1 generation. As concentration of applied EMS increased, will decrease in germination, seedling height, root length and emergence under field conditions was observed in M1 generation as compared to the non-treatment control. Plant height and root length also decreased with increases in EMS mutagenesis in an approximately linear fashion. The LD25 and LD50 values were observed based on growth reduction of seedlings after EMS treatment with 0.25% and 0.50% on the rice variety (*Oryza sativa* L. spp. Indica cv. MR219).

Sharma *et al.* (2013) conducted an experiment to determine the effect of different doses of physical mutagens i.e. gamma rays and chemical mutagens i.e. ethyl methane sulphonate (MMS) and methyl methane sulphonate (MMS) in seeds and epicotyls of rough lemon (*Citrus jambhiri* Lush.) on seed germination, plant regeneration and growth parameters. For physical mutagenesis seeds were irradiated with different doses of gamma rays (0, 40, 60, 80, 100 and 120 Gy) and different concentrations of chemical mutagens i.e. EMS (0.2, 0.3 and 0.4%), MMS (0.05, 0.1, 0.2 %). The seeds were germinated on MS basal media liquid as well as solid without mutagens (control). The epicotyls of *in vitro* grown 45 day old

seedlings of *Citrus jambhiri* were cut and treated with different doses of EMS (0, 0.2, 0.3 and 0.4%) and MMS (0.05, 0.1, 0.2 %) in regeneration media (MS+ BAP 0.5mg/L) for 4 hours at 26°C and 100 rpm, while in control epicotyls were cultured immediately on regeneration media. Based on the survival and the regeneration potential, 60 Gy were observed to be the optimum mutagenic dose of gamma rays for 40 seeds. For chemical (EMS and MMS) mutagenesis, 0.2 % each was the most suitable dose for 45 day old cultures.

Svetleva and Crino (2005) investigated on the Influence of ethyl methane sulfonate (EMS) and N-nitrose-N′-ethyl urea (ENU) mutagenic treatments on three time sub-cultured calli obtained from leaf petiole explants of 7-day old sterile plants. Calibrated sterile seeds of the common bean Bulgarian variety Plovdiv 11M were pre-cultivated on MS basal medium supplemented with 1 μ mM BAP. Then, both mutagens EMS and ENU were applied for different times such as 15, 30, 60 and 90 min on the explants at the concentrations of: 2.5 ×. 10^{-2} M and 6.2 × 10^{-3} M, respectively. Times of the mutagenic treatments influenced callus growth, calli from 30-min treatment with both mutagens showing the highest weights. In both cases, the 90-min mutagen application caused a too relevant effect either on callus browning or growth inhibition. In general, ENU showed a stronger effect than EMS. The effect of subcultures on callus growth was higher than mutagenic treatments. Interactions between these factors checked by correlation ratio (η%) were quite low.

Hofmann *et al.* (2004) studied that embryogenic suspension cultures of soybean (*Glycine max* L. cv. Iroquois) were subjected to mutagenesis using varying concentrations (1, 3, 10, and 30 mM) of ethyl methanesulfonate (EMS). Depending on the concentration of EMS used, the mean survival rate of embryogenic cultures decreased from 74 % (1 mM EMS) to 43 % after 30 mM EMS treatment. Random amplified polymorphic DNA (RAPD) analysis was used to determine whether induction of genetic variability in embryogenic cultures in response to the different EMS treatments may result in identification of polymorphic markers. Two of 35 'core' primers tested revealed polymorphisms. One of the primers, OPO-01/1150,

revealed polymorphism in tissue treated with 10 mM EMS, while the other primer, OPO-05/1200, revealed polymorphism in tissue treated with either 1 or 30 mM EMS.

Fang (2011) investigate the efficiency of the chemical mutagen ethyl methanesulphonate (EMS) to induce mutations in Saintpaulia. In vitro leaf sections of Saintpaulia cv. Crystobal were exposed to various EMS treatments at 0%, 0.2%, 0.4%, and 0.6% for 30, 60, 120, and 240 min after which adventitious shoots were recovered from the treated explants. Shoots producing at least six leaves were induced to root and the resulting plantlets were transplanted to soil. A total of 1838 plantlets was grown to flowering stage and 10 mutants were identified. Four of the mutants were variegated leaf chimeras and the remaining six presented variations at the level of flower color and/or fringe. Results in the present study showed the efficiency of EMS to induce *in vitro* mutation of Saintpaulia and the method can be used in the future to assist breeding in this popular ornamental plant.

Qin *et. al.* (2011) investigate the effects of ethyl methane sulfonate (EMS) as an *in vitro* mutation mutagen using the anther-derived embryos of loquat (*Eriobotrya japonica* Lindl) which had been newly developed in the laboratory for the first time in the world. The results showed that EMS treatment caused changes in vitality and conformation of the anther-derived embryos. All EMS treatments employed in the study resulted in lower vitality to various extents depending on the EMS concentration and duration. The death rate of loquat anther-derived embryos was over 50% while the EMS concentration ranged from 0.1 to 0.9% and the exposure time of treatment was 0.5, 1 and 2 h, respectively. Maximal survival rate (46.2%) was obtained in the treatment of 0.3% EMS for 0.5 h and minimal survival rate (2%) was obtained in the treatment of 0.7% EMS for 2 h. The induction ability of secondary embryos decreased after the EMS treatment. Both the maximal percentage (46.2%) of embryos producing new embryos and the maximal number (5.4) of newly formed embryos per embryo producing new embryos were obtained in the treatment of 0.3% EMS for 0.5 h. Minimal percentage (2.8%) of embryos producing new embryos was obtained in the treatment of 0.7% EMS for 2 h.

Minimal number (2.75) of newly formed embryos per embryo producing new embryos was obtained in the treatment of 0.9% EMS for 2 h. EMS treatment caused more morphological malformations in the secondary anther-derived embryos.

Jafri *et al*. (2011) conducted an experiment to study the effect of base analogue 5-bromouracil (5-BU) on the medicinal herb, *Cichorium intybus*. 5-BU induced miss pairing during the DNA replication. The seeds of *C. intybus* were treated with different concentrations of 5-BU. Variations in some parameters such as seed germination, seedling survival, seedling height, pollen fertility, days to flowering, days to maturity, number of leaves per plant, plant height, and chromosome behavior were studied in M1 generation. A positive correlation between increasing concentrations of mutagen and various cytomorphological characters of *C .intybus* was observed.

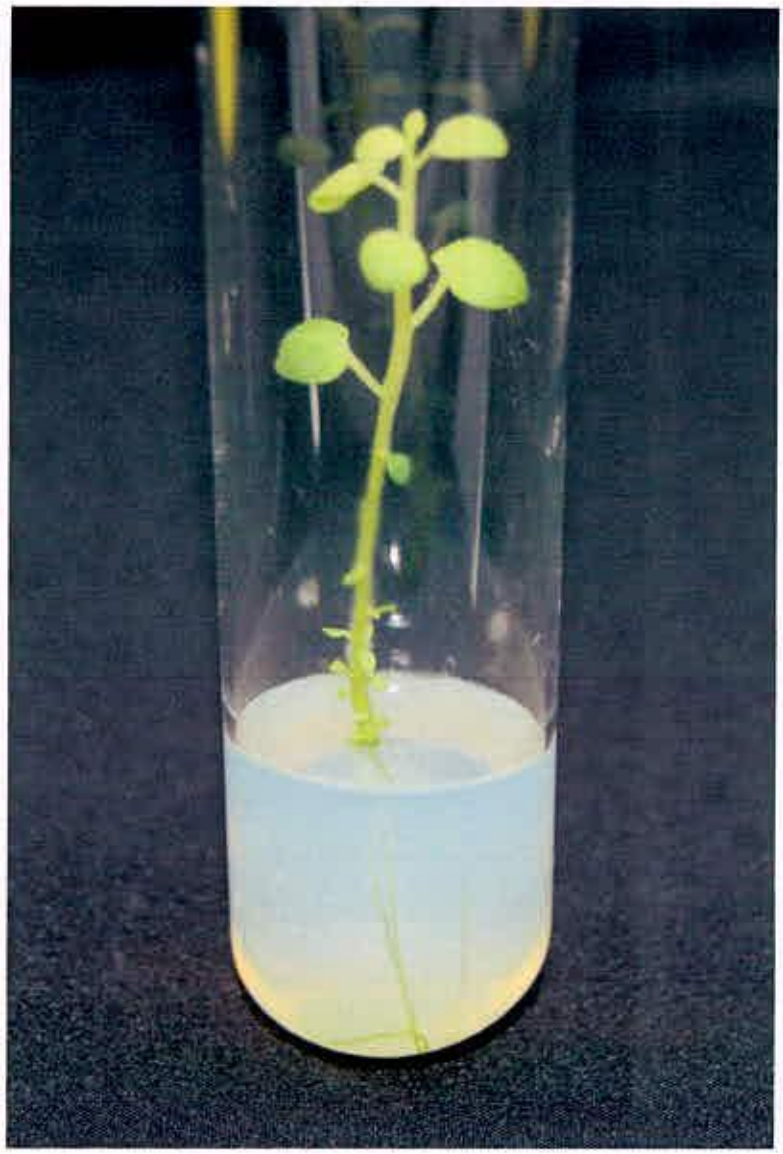
Kleinhofs *et al.* (1968) studied that hydroxylamine (HA), 2-aminopnrine (AP), 5-bromourani (BU), and ethyl methane sulfonate (EMS) were tested for mutagenic activity on seeds of *Melilotus alba annua.* Mutants were classified into the following categories: o-hydroxycinnamic acid (o-HCA) content and β-glucosidase category and the dwarf category were unreliable for determining mutation rates. Based on the chlorophyll-deficient and miscellaneous morphological categories, mutation rates for HA, AP, and BU treatments did not differ from the control rates, but EMS at 0.003M and 0.004M concentrations was highly effective in inducing mutations in these categories. Mutation rates based on chlorophyll deficiency were somewhat higher and more sensitive to EMS concentration than were mutation rates based on the miscellaneous categ.ory. Large numbers of chlorophyll, dwarf, and morphological variants, several more vigorous than the controls, were isolated from the EMS treatments, but no new o·HCA or β-glucosidase mutants were obtained.

Ehasanpour *et al.* (2007) reported that calli were obtained from *in vitro* grown potato stem or leaf segments cv. *Cosima* on MS medium containing 2,4-D, NAA, kinetin and yeast extract. After three subcultures, well grown calli were exposed to UV-C radiation with approximately 6.24 μ mol photon/m2/s for 30 min three times at an interval of 2 weeks. Somaclonal variation induced by UV-C radiation was revealed by 5 out of 28 random primers and also ISSR4 primer after PCR amplification of DNA. UV-C radiation changed DNA patterns as a source of genetic variation. However, somaclonal variation can be used for selection of potato calli toward desirable traits, such as salt or drought stress.

From the above review of literature it appears that both callus induction and in vitro plant regeneration of potato are controlled mainly by different factors such as explants, genotypes, culture media, culture condition, phytohormone combination etc. Very limited research works has been done on mutagenic treatment followed by *in vitro* regeneration on potato. The present study may be open new avenue of research for potato improvement under Bangladesh condition.

CHAPTER 3

MATERIALS AND METHODS



CHAPTER 3

MATERIALS AND METHODS

The effect of variable concentrations of 2,4-D (2,4-Dicholorophenoxyacetic acid), EMS (Ethyl Methane Sulphonate) , and 5-BU (5-Bromo Uracil) on *in vitro* plant regeneration using potato sprout and their multiplication was conducted in the Genetics and Plant Breeding Laboratory and the Tissue Culture Laboratory of the Department of Biotechnology, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka-1207, during the period January 2013 to March 2014. Three experiments were conducted to fulfill the objectives of the present study.

Experiment 1: Sprouting potentiality of potato cultivar under different concentrations of GA_3 treatment

Experiment 2: Study on chemical mutagenic treatment and subsequent *in vitro* regeneration of potato varieties.

Experiment 3: Acclimatization and establishment of mutagen treated plantlets of potato under field condition .

The major operational steps of three experiments are :

1. Sprout initiation of the potato tuber by application of different doses of GA_3 .
2. *In vitro* callus induction from sprout of potato varieties with the supplementation of different concentration of 2,4-D, EMS, and 5-BU .
3. *In vitro* plant regeneration of potato varieties under different mutagen concentration.
4. *In vitro* multiplication of the plantlets .
5. Transfer of the *In vitro* plantlet in the net house .
6. Acclimatization of the *in vitro* plantlet

3.1. Experimental materials

3.1.1. Plant materials

Experimental materials are sprouts of these BARI released three popular potato cultivars (Plate 1) namely :

1. Diamant
2 Cardinal
3. Granola

3.1.2. Sources of plant materials

All of these potato tubers were collected from Tuber Crop Research Center (TCRC), Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur. These disease free potato tubers were sprouted in the dark condition at room temperature with different concentrations of GA_3.

3.2. Plan of work

3.2.1. GA_3 treatment

- Different potato varieties were collected from TCRC (Tuber Crop Research Center) , BARI, Joydebpur, Gazipur.
- Washed them thoroughly with running tap water for several times to remove soil from the potato
- Then washed them with liquid detergent for cleaning purpose
- Washed several times with running tap water to remove detergent
- Then washed with distilled water
- Soaked the potato tuber into liquid GA_3 at the concentration of 500 ppm/L for 15 minutes
- Poured the GA_3 in spray container
- Regular spray of the tuber for get immediate result twice in a day
- To find out the best concentration of GA_3 different potato varieties were selected for applying five concentrations of GA3 treatments (0, 100, 200, 300 , 400, 500 ppm/L).

- After 4-5 days sprout was initiated from the tuber
- Of these GA_3 treatment, 500 ppm/L concentration was found the best for sproutinitiation, because it required less time for sprout initiation.

3.3. Culture media

Success of any experiment depends on the culture media, hormone combination, mutagen, tissue and employing cell. Murashige and Skoog (1962) medium were used supplemented with three chemical mutagen viz. 2,4-D, EMS and 5-BU as culture medium for callus induction, shoot and root regeneration. Mutagen were added to MS media as per treatment of the experiment. For the preparation of media, stock solutions were prepared at the beginning and stored in the refrigerator at 4±1°C. The respective media were prepared from stock solutions.

3.4. Preparation of the stock solutions

The first step in the preparation of the medium is the preparation of stock solutions of the various constituents of the MS median. 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 ready use.

3.4.1. Stock solution of macronutrients (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. Dissolve all the macronutrient one by one except $CaCl_2$. The stock solution of $CaCl_2$ should be prepared separately in order to avoid precipitation. And in this way, dissolved all the salts 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.

3.4.2. Stock solution of micronutrients (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 is preferable to first make a separate stock solution of those two salts (100*) and then an appropriate volume can be pipetted and put into the main micronutrient stock solution .This stock solution was also stored in refrigerator at 4°C.

3.4.3. Stock solution of iron (Fe-EDTA) (stock 3)

Iron-EDTA should be added fresh and it was made 100 times the final strength of the medium in one liter DW. Here, two constituents, $FeS0_4.7H_20$ 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.

3.4.4. Stock solution of vitamins (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 HCI (Vitamin B_6), Thiamine HCI (Vitamin B_1), Glycin.

Each of the vitamins except myo-inositol 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.

3.4.5. Preparation of other stook solutions

3.4.6. Preparation of 1N NaOH :

1. Fourty gm NaOH pellets were weighted and placed in 1 L volumetric flask

2. Slowly 900 mL distilled water was and stir until dissolved

3. The flask in a thermostat at 20°C and maintain for 1 hour

4. Added distilled water up to the 1 L mark and mix the closed bottle.

3.4.7. Preparation of 70% Ethanol

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

3.5. MS Media preparation

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

1. 500 ml double distilled water was taken into 1 liter beaker .

2. 100 ml of stock solution of macro-nutrients, 10 ml of stock solution of micro nutrient, 10 ml of stock solution of Fe-EDTA and 10 ml of stock solution of vitamins and growth regulators were added in this 500 ml double distilled water.

3. 30g of sucrose was dissolved in this solution with the help of magnetic stirrer .

4. Different concentrations of mutagen supplements as required were added to this solution and were mixed thoroughly .

5. Since each stock solution contained in 100 ml of solution, to make one liter of medium, addition of 1ml/L, 3ml/L, 5ml/L of 2,4-D , EMS and 5-BU singly was added to prepare 1 liter of medium .

7. The whole mixture was then made up to 1 liter with further addition of double distilled water.

3.6. pH of the medium

pH of the medium was adjusted to 5.8 by pH meter with the addition of I N NaOH or 0.1 N HCI whichever was necessary.

3.7. Agar

The media was gelled with 8 g/L agar and the whole mixture was gently heated on microwave oven at 250 °C Temperature for 8-10 minutes.

3.8. Sterilization

3.8.1. Sterilization of culture media

Fixed volume of medium was dispensed into test tube. The test tubes were plugged with non- absorbent cotton. After dispensing the test tubes were covered with aluminum foil paper and marked with different codes with the help of a permanent glass marker to indicate specific hormonal supplement. Then the test tubes were autoclaved at 15 psi pressure at 121°C for 20 minutes. The medium was then transfer into the culture room and cooled at 24°C temperature before used. Marking is also necessary.

3.8.2. Sterilization of glassware and instruments

Glassware, culture vessels, beakers, petri dishes, pipettes, slides, plastic caps, other instruments such as forceps, needles, scissor, spatula, surgical blades, brush, cotton, instrument stand and aluminum foil were sterilized in an autoclave at a temperature of 121°C for 20 minutes at 15psi pressure. Before this, all types of glassware instrument was washed properly by liquid detergent, cleaned with running tap water and finally washed with distilled water.

3.8.3. Sterilization of culture room and transfer area (Laminar air flow hood)

In 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 laminar air flow hood (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.

3.8.4. Precaution of ensure aseptic conditions

All inoculation and aseptic manipulations were carried out under laminar air flow cabinet. The cabinet was usually switched on with ultra violet light half an hour before use and wiped with 70% ethanol to reduce the chances of contamination. The instruments like scalpels, forceps, needles, surgical blades, scissor, pipettes, slides, plastic caps, spatula, brush, cotton etc. were presterilized by autoclaving and subsequent sterilization were done by dipping in 70% ethanol followed by flaming and cooling method inside the laminar flow cabinet. While not in use, the instruments were kept inside the laminar airflow cabinet into the instrument stand. Hands were also sterilized by 70% ethanol and wearing of hand gloves. It is also necessary to wear apron and mask to avoid contamination . Other required materials like distilled water, culture vessels, beakers, glass plates, petridishes etc. were sterilized in an autoclave following method of media sterilization. The neck of test tubes were flamed before open and also dipping with ethanol with the help of soaked cotton before closing it with the aluminum foil paper. Aseptic conditions were followed during each and every operation to avoid the contamination of cultures.

3.9. Culture methods

The following culture methods were in work in the present investigation.

I. Explant culture

2. Subculture

3.9.1. Preparation of explants

The sprout of potato were used as explants. The sprouts were seperated from the potato and washed thoroughly with double distilled water into the laminar airflow cabinet. For surface sterilization, sprouts were then sterilized with 70% (v/v) ethanol for one minute. After sterilization, the sprouts were then rinsed and washed with brush for three times with sterile distilled water into the petridish to remove the trace of alcohol. Afterwards the sprouts were transfer into another petridish and were again surface sterilized by immersing in 0.1% $HgCl_2$ solution supplement with three drops of Tween-20 mixtures and then finally rinsed and washed four times with sterilized distilled water. The surface sterilized disinfested sprouts were then cut into small segments and kept under sterilized distilled water into the sterilized petridishes to make the sprout alive. Now the explants were ready for inoculation.

3.9.2. Inoculation of culture

The explants were prepared carefully under aseptic condition inside the laminar airflow cabinet. One explant was directly inoculated to each test tubes and vials containing 10 ml of MS medium supplemented with different mutagen concentrations as per treatments. There were three replications for each treatment The test tubes and the culture vials were covered and sealed with aluminum foil paper. The total operation was done in the laminar airflow cabinet on the clean bench in sterile condition.

3.9.3. Placement of the sprout

The surface sterilized sprouts were transferred aseptically to the test tube and vials and flaming with lamp and covered with aluminum foil. Each and every test tube and vials was labeled with a code that indicates mutagen dose, replication number and date of culture. Then the test tubes and vials were incubating at 25± 2°C in dark condition under 24 hours dark periods into the growth chamber for 5-7 days or until callus induction. After initiation of callus or not, it was kept in light where it was 16/8 hours photoperiod. The observation of cultures was started from the 2nd day of inoculation and continued up to 28^{th} days.

3.9.4. Maintenance of calli

Callus initiated after 10-12 days of explant inoculation in the medium. The developed calli were also kept under 16 h photoperiod at 25±2 °C. The test tubes and the vials were checked daily to note the response and the development .

3.9.5. Culture incubation conditions

The prepared cultures were kept in a growth room on the shelves. All the cultures were kept at 25±2 °C illuminated with 1.83 m florescent tubes (4.83 ft). Those tubes broad sprectum of light, especially in the red wavelength. The room was illuminated 16 h daily with a light intensity of 3000 lux (Approximate) and monitored by using luxmeter..

3.10. Subculture

3.10.1. Subculture of the callus for shoot regeneration

Four weeks after incubation of explants, the calli attained convenient size. Those which does not produced any root or shoot, were then remove aseptically from the test tubes and the vials on a sterilized petridishes, and callus was cut into small pieces with the help of sharp sterilized blade and place again on fleshly prepared sterilized MS medium in a glass vials inside the laminar airflow cabinet for shoot, root initiation. The subcultured test tubes and vials were then incubated at 25±1°C with 16 h photoperiod. After shoot initiation, more light intensity was given for shoot elongation. The test tubes and the vials showing sign on contamination were discarded from the laboratory to reduce the rate of contamination. Repeated subculture was attended at regular interval of 21-25 days while incubated under the same temperature. The observations and data collections were noted regularly.

3.10.2. Subculture of the regenerated shoot for root initiation

The subculture calli contained proliferated and differentiated shoot and the microplants those which directly produce shoot from the calli was again need to subculture. When these shoots grew about 5-6 cm in length, were taken out from the flask, separated from each other and placed on a sterilized petridishes. Leaf, stem and internodal segments were cut aseptically into small pieces with the help of sharp sterilized blade or aseptic scissor and the small segments of stem were then used as explant. It was then again cultured in another culture vials with freshly prepared medium for root induction.

3.11. Transfer of plantlets to *in vivo* condition

When the plantlets fully matured well developed shoots, leaves and roots, then in vitro rooted plants were removed from culture vials very carefully and aseptically with the help of fine forceps. Attached medium to roots were smoothly washed out with the help of soft brush in the running tap water to remove adhering gel. Then the plantlets were transplanted to plastic pots containing autoclaved garden soil, sand and cowdung in the ratio of 1:2:1 and covered with plastic paper. Plants were kept under culture room conditions 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. Immediately after transplantation the plantlets were irrigated with a fine spray of water and the plantlets along with plastic trays were covered with transparent polythene bags to prevent desiccation. Initially plants are kept in high humidity and with low light intensity. The humidity is gradually decreased to the ambient level after 7-15 days and the light intensity is increased. It was irrigated regularly at an interval of 2 days.

3.12. Transfer of plantlets to the soil

Alter 7-10 days the plantlets were established and then the polythene bags were removed. Finally, the plantlets appeared too self-sustainable and then transfer to soil in open environment.

3.13. Experimental Factors

The experiment consisted of two factors.

A. Variety

B. Different concentrations of 2,4-D, EMS, 5-BU

3.13.1. Factor A:

Experimental materials are BARI released three popular potato cultivars viz.

1. Diamant
2. Cardinal
3. Granola

3.13.2. Factor B: Different concentrations of 2,4-D , EMS, 5-BU .

3.14. Treatments

In vitro clonal propagation techniques were applied for callus induction and potato plantlet regeneration and creation of somaclonal variation. Sprouted bud was used as explant. MS (Murashige and Skoog, 1962) medium supplemented with with three concentrations viz., 1.0, 3.0 and 5.0 mg/L of each chemical mutagen, EMS (Ethyl Methane Sulphonate), BU (5-Bromo-Uracil) and 2,4-D (2,4-dichlorophenoxy acetic acid).

3.15. Experimental design

The experiment was laid in Completely Randomized design (CRD) having two factors (Variety and Treatment) with three replications.

3.16. Collection of data

To investigate the effect of different treatments of this experiment, the following parameters were recorded:

3.16.1. GA_3 application and sprout initiation in tuber

I. Days to sprout initiation : After spraying with variable concentrations of GA_3, starting date of spraying was recorded for each treatment and each variety. As soon as any sprout was initiated, data were recorded and the number of days required to sprout initiation was noted.

2. Total sprouting time: Duration of individual variety were recorded for sprouting.

3. Sprout length (cm): Maximum length of sprout was recorded in cm with the help of plastic scale.

4. Number of sprouts/potato: Maximum number of sprout per potato was collected and recorded.

5. Node/sprout: The number of nodes per sprout was recorded.

3.16.2. *In vitro* callus induction

I. Callus colour : The colour of the callus was taken whether it is white, green, light green, deep green, brown, pink, yellow or in combination of those color by visual observation. And the color of the callus varies with the light intensity. It was white in color up to 5-7 days when it was kept in dark condition.

2. Texture of callus: Texture of the callus measured by either it is friable or non-friable/ compact for their physical characteristics.

3. Days to callus induction: Generally callus induction started after few days of explant incubation. Days to callus induction was recorded until callus was not able to induced plantlet. The mean value of the data provided the days to callus induction.

4. Size of callus: It was measured with a plastic scale not under test tube from base to apex of callus. Size of the callus was recorded at 15, 30, 45 days after inoculation (DAI) of callus. Callus length was measured horizontally and breadth was measured vertically. The formula (Thadavong *et al.*, 2002) used for estimating the size of callus is given below:

$$\text{Size of callus} = \frac{\text{Breadth} + \text{length}}{2}$$

3.16.3. *In vitro* plant regeneration

To investigate the effect of different mutagen treatment of this experiment, the following parameters were recorded.

1. Days to shoot initiation : Shoot initiation started after 9-12 days of incubation of explants. The mean value of the data provided the days required for shoot initiation.

2. Number of shoots/plantlet : The number of shoot proliferated was recorded at 15, 30 and 45 days after inoculation (DAI) and the number of shoots/explant was counted and recorded.

3. Length of shoots/plantlet : The length of shoots in cm was measured using a plastic scale in laminar airflow cabinet at an interval of 15, 30 and 45days after inoculation (DAI). The mean value of the data provided the shoot length.

4. Number of leaves/plantlet: Number of leaves/plantlet was recorded by visual observation at an interval of 15, 30 and 45 days after inoculation (DAI). The mean value of the data provided the number of leaves/plantlet.

5. Days to root initiation: Root formation was initiated within 10-12 days . The mean value of the data provided the days to root initiation.

6. Number of roots/plantlet : The number of roots/plantlet was recorded at an interval of 15, 30 and 45 days after inoculation (DAI) of explants inoculation and it was recorded and mean was calculated.

7. Length of roots/plantlet: The length of roots/plantlet was determined by using a plastic scale in side the laminar airflow cabinet by plotting the plant in a petridish. Length of root in cm was recorded at an interval of 15, 30 and 45 days after inoculation (DAI) of explants inoculation and it was recorded and mean was calculated.

3.17. The effect of mutagen

Different chemical mutagens were used in MS media to observed any morphological variation occurred in cultured plantlet of potato. Any abnormalities such as thin stem, heavy branching, less leaf formation, abnormal stem regeneration, curl or modified shoot tip development, died of regenerated plantlet were recorded in all the treatment combination for all three potato varieties.

3.18. Subculture of *in vitro* plantlet

Sub culture of *in vitro* regenerated plantlet was done at due time and the following parameter were recorded for further analysis.

I. Number of shoots/plantlet
2. Length of shoots/plantlet (cm)
3. Number of leaves/plantlet
4. Number of roots/plantlet
5. Length of roots/plantlet (cm)

3.19. Acclimatization

3.19.1. Transfer of plantlets from culture vessels to soil

During the in vivo acclimatization and the establishment of the previously regenerated and subcultured plantlets in to the soil, data were collected for the following parameter.

3.19.2. Survival rate (%) of plantlets

The survival rate of established plants was calculated based on the number of plants placed in the growth chamber and the number of plants finally survived. The survival rate of plantlets established were calculated by using the following formula:

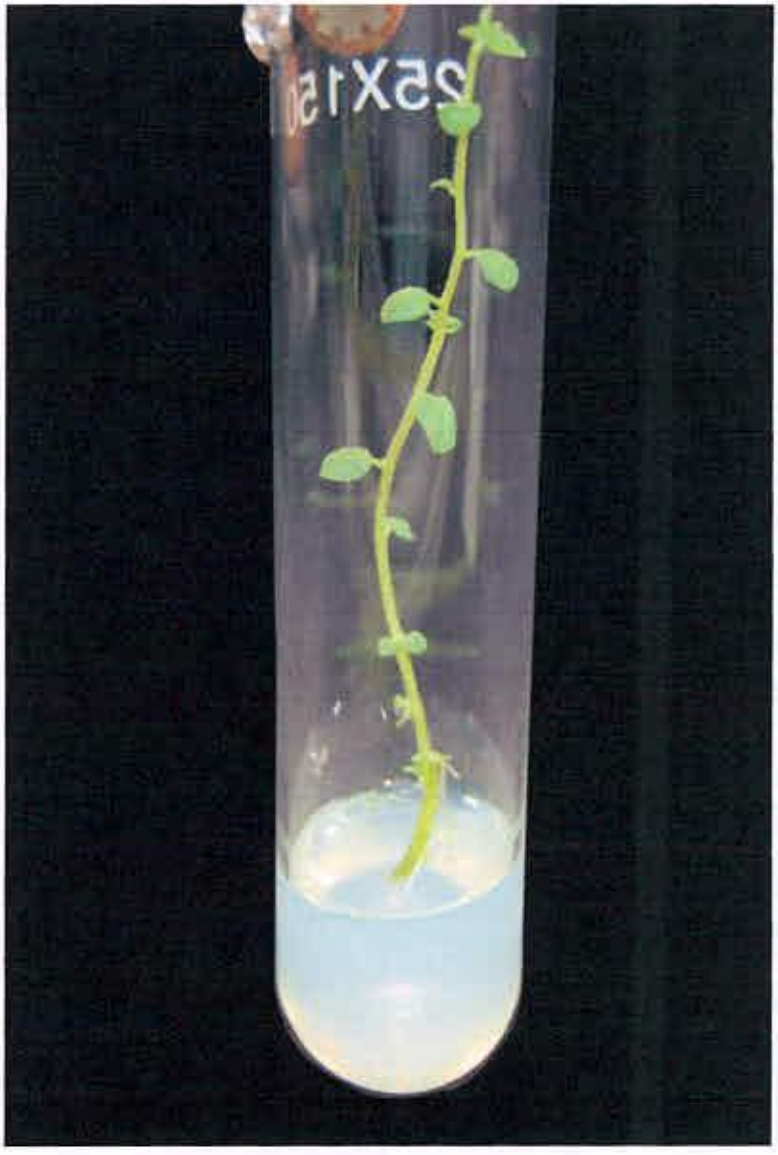
$$\text{Survival rate (\%) of plant} = \frac{\text{Number of established plantlets}}{\text{Total number of plantlets}} \times 100$$

3.20. Statistical analysis

The data for the characters under present study were statistically analyzes where applicable. The experiment was conducted in growth chamber and arranged in Completely Randomized Design (CRD). Data were analyzed using MSTAT-C statistical package programme. The analysis of variance was performed and differences among the means were compared by the Least Significant Different test at 5% level of significance.

CHAPTER 4

RESULTS AND DISCUSSION



CHAPTER 4

RESULTS AND DISCUSSION

Three separate experiments were conducted to study the performance of different popular potato varieties. The results obtained from these studies have been presented and discussed separately under different heading. Each of the parameter as influenced by varieties, treatments and their combinations were discussed.

4.1. Experiment 1: Sprouting potentiality of potato cultivar under different concentrations of GA_3 treatment

The experiment was conducted to study the effect of five different concentrations (100, 200, 300,400 and 500 ppm/L) of GA_3 on sprouting potentiality in off season (March- May) on BARI released three potato varieties. The experiment was carried out under laboratory condition. The results are presented in Table 1 .

4.1.1. Days to sprout initiation

The potato cultivars responded differently with different combination of GA_3 concentration. It was revealed that the variety Granola responded very quickly for sprouting. Days to sprout initiation was the minimum in 500 ppm/L of GA_3, application for all the three varieties (Table 1 and Plate 1). Within this concentration, Granola showed the minimum (5 days) days for sprouting and it was the maximum (7 days) in Diamant. Maximum days for sprout initiation was observed with 100 ppm of GA_3 in all the varieties, where Cardinal took the maximum (12 days) and the minimum (10 days) was in Granola. It revealed that days to sprout initiation gradually decreased with the increased concentration of GA_3.

Table 1 . Sprouting potentiality of potato under different concentration of GA_3 treatment

GA_3 Treatment (ppm/L)	Variety	Days to sprout initiation	Duration for sprouting	Length of sprout (cm)	Number of sprouts/ potato	Node / sprout
0	Diamant	-	-	-	-	-
	Cardinal	-	-	-	-	-
	Granola	-	-	-	-	-
100	Diamant	11	25	1.5-2	1	2
	Cardinal	12	25	2.5	1	2
	Granola	10	15	2-3	1	2
200	Diamant	12	20	2-2.5	1	2
	Cardinal	10	14	2.4	2	2
	Granola	9	16	2-2.5	4	3
300	Diamant	8	15	2.5	3	3
	Cardinal	7	10	2.4	3	3
	Granola	7	12	2-2.5	5	4
400	Diamant	8	12	2	3	4
	Cardinal	7	10	2.5	4	4
	Granola	6	10	2-2.5	6	4
500	Diamant	7	11	2.2	5	4
	Cardinal	6	10	2.6	5	4
	Granola	5	8	2.5-3	7	5

4.1.2. Duration of sprouting

Total sprouting time was also the minimum in 500 ppm/L of GA_3 treatment Granola took only 8 days for total sprouting and it was the maximum (25 days) in Diamant and Cardinal in 100 ppm.

4.1.3. Length of sprout

Average sprout length varied from 2-3 cm for all the treatments and varieties. Remarkable variation was not notice for this morphological trait among the experimental materials.

4.1.4. Number of sprouts/potato

Number of sprout/potato was minimum 1 and it was maximum 7 in 500 ppm/L GA_3 application. It was observed that lower concentration of GA_3 produced minimum sprout for all the varieties under study and it increased generally with higher concentration.

4.1.5. Node/sprout

The maximum sprouting (5 node/sprout) was found in 500 ppm/L of GA_3 application. Node/sprout ranged from 2-5 for all the treatments and varieties. No remarkable variation was responded for this trait.

The overall experimental findings revealed that the variety Granola was the most responsive to GA_3 application for sprouting. Higher concentration of GA_3 showed better performance to sprouting for all the materials under study. The treatment 500 ppm/L GA_3 concentration found the best for sprout initiation, because it required less time to get maximum number of sprout.A pictorial view of sprouting of different varieties of potato is presented in Plate no. 1.

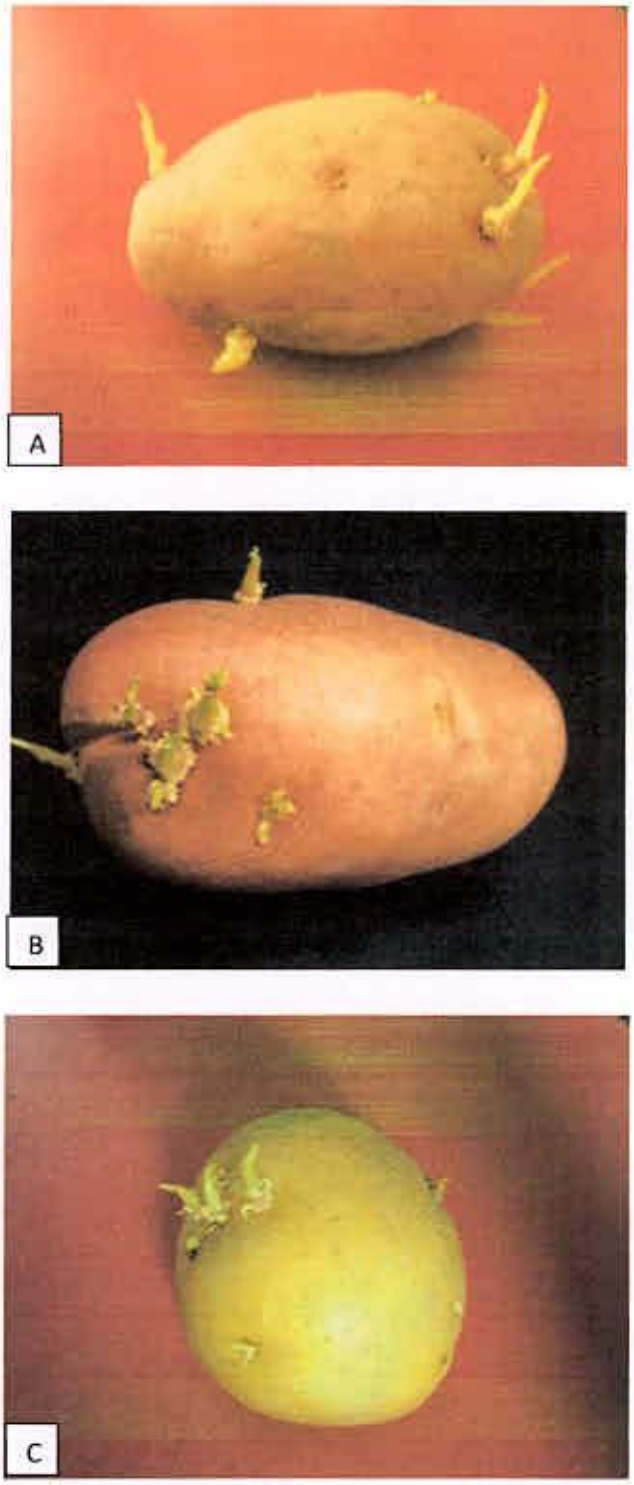


Plate 1. Maximum sprouting @ GA3 500 ppm/L concentration Sprout of A. Diamant, B. Cardinal and C. Granola

4. 2. Experiment 2: Study on chemical mutagenic treatment and subsequent *in vitro* regeneration of potato varieties.

The chemical mutagen viz. 2,4-D, EMS and 5-BU were used in three potato varieties – Diamant, Cardinal and Granola. It was observed that, out of them only 2,4-D had the ability to induce callus in different potato cultivars.

Effect of 2,4-D on *in vitro* regeneration :

4.2.1. Days to callus induction

The results of major effect of varieties on days to callus induction have been presented in Table 2. Callusing started within 11 days after inoculation up to 45 days. The size of calli increases but after 45 days it gradually lost its totipotency and died within 60 days after inoculation. The maximum days to callus induction (11.50 days) and the lowest (10.58 days) were noticed in Cardinal and Granola variety respectively.

There was significant influence of different concentrations of 2,4-D on days to callus induction. It was observed that simple MS medium had no ability to induce callus in all the three varieties. The maximum days (13.55 days) to callus induction was found at MS+5.0 mg/L 2,4-D and the minimum (12.44 days) was observed in MS+3.0 mg/L 2,4-D (Table 3). This result showed that medium concentrations of mutagen 2,4-D effective for callus formation. Forooghian and Esfarayeni (2013) observed the same result. They studied three types of potato (*Solanum tuberosum* L.) cultivars on fresh callus weight, callus diameter, seedling length and percentage of callus induction. They found that medium 2,4-D concentration give more cell divisions and producing larger calluses.

The combined effect of different potato varieties and mutagen 2,4-D showed significant variation on days to callus induction. The maximum (14.00 days) to callus induction was noticed in the treatment combination MS+5.0 mg/L 2,4-D in the variety Diamant. The minimum days (11.15 days) to callus induction was found in variety Granola with MS+3.0 mg/L 2,4-D (Table 4).

Table 2 . Effect of different varieties on days to callus induction and size of callus (cm)

Variety	Days to callus induction	Size of Callus (cm)		
		15 DAI	30 DAI	45 DAI
Diamant	11.16	0.38	0.86	1.15
Cardinal	11.50	0.64	1.18	1.35
Granola	10.58	0.88	1.19	1.44
SE±	1.028	0.015	0.050	0.027
LSD	0.85	0.067	0.037	0.138
Level of significance	*	*	*	*

DAI = Days after inoculation

Table 3 : Effect of mutagen 2,4-D on days to callus induction and size of callus (cm)

Treatments	Days to callus induction	Size of Callus (cm)		
		15 DAI	30 DAI	45 DAI
T1=Normal MS	-	-	-	-
T2=MS+1 2,4-D	13.33	0.76	1.42	1.67
T3=MS+3 2,4-D	12.44	0.70	1.51	1.90
T4=MS+5 2,4-D	13.55	0.73	1.39	1.77
SE±	1.028	0.015	0.050	0.027
LSD	0.98	0.123	0.043	0.159
Level of significance	*	*	*	*

DAI = Days after inoculation

Table 4 . Effect of different varieties and mutagen 2,4-D on days to callus induction and size of callus (cm)

Variety	Treatments (mg/L)	Days to callus induction	Size of Callus (cm)		
			15 DAI	30 DAI	45 DAI
Diamant	T1= MS	-	-	-	-
	T2=MS+1 2,4-D	13.00	0.51	1.06	1.30
	T3=MS+3 2,4-D	12.66	0.71	1.42	1.73
	T4=MS+5 2,4-D	14.00	0.31	.99	1.58
Cardinal	T1= MS	-	-	-	-
	T2=MS+1 2,4-D	12.66	.77	1.35	1.54
	T3=MS+3 2,4-D	11.40	.75	1.52	1.95
	T4=MS+5 2,4-D	13.33	1.04	1.85	1.83
Granola	T1= MS	-	-	-	-
	T2=MS+1 2,4-D	13.39	.65	1.35	1.88
	T3=MS+3 2,4-D	11.15	0.66	1.58	2.30
	T4=MS+5 2,4-D	13.43	0.85	1.35	2.05
	SE±	1.028	0.015	0.050	0.027
	LSD	1.70	0.213	0.073	0.276
	Level of significance	*	*	*	*

4.2.2. Size of callus

The size of callus was recorded after 15, 30 and 45 days of cultured on MS media containing different concentrations of 2,4-D. The results have been presented in Table 2- Table 4 and Plate 2-3.

The main effect of varieties showed a size of callus at different DAI in Table 2. The highest size of callus was found in the variety Granola (0.88, 1.19 and 1.44 cm at 15, 30, 45 DAI, respectively). The minimum size of callus was found in Diamant (1.15 cm at 45 DAI).

The mutagen concentration of MS+3.0 mg/L 2,4-D showed the highest size of callus (0.70, 1.51 and 1.90 cm, respectively at 15, 30 and 45 DAI) and the lowest size of callus (1.67 cm at 45 DAI) was observed at the concentration of MS+1.0 mg/L 2,4-D. (Table 3). Sherkar and Chavan (2014) showed the similar result. They conducted an experiment on callus induction and shoot regeneration in potato (*Solanum tuberosum* L.). They used sprouts as explants which were cultured on basal Murashige and Skoog medium supplemented with different hormonal concentrations and combinations. The highest degree of callus formation on MS media supplemented with 2.0-4.0 mg/L of 2,4-D was observed.

The combined effect of varieties and different concentration of 2,4-D showed significant variation on size of callus at different DAI (Table 4). The highest callus size (2.30 cm) was observed at MS+3.0 mg/L 2,4-D with the variety Granola and the lowest size of callus (1.30 cm) was found at MS+1.0 mg/L 2,4-D with the variety Diamant at 45 DAI .

Plate 2. Callus induction on MS media supplemented with 3.0 mg/L 2,4-D at 20 DAI in A. Diamant, B. Cardinal, C. Granola .

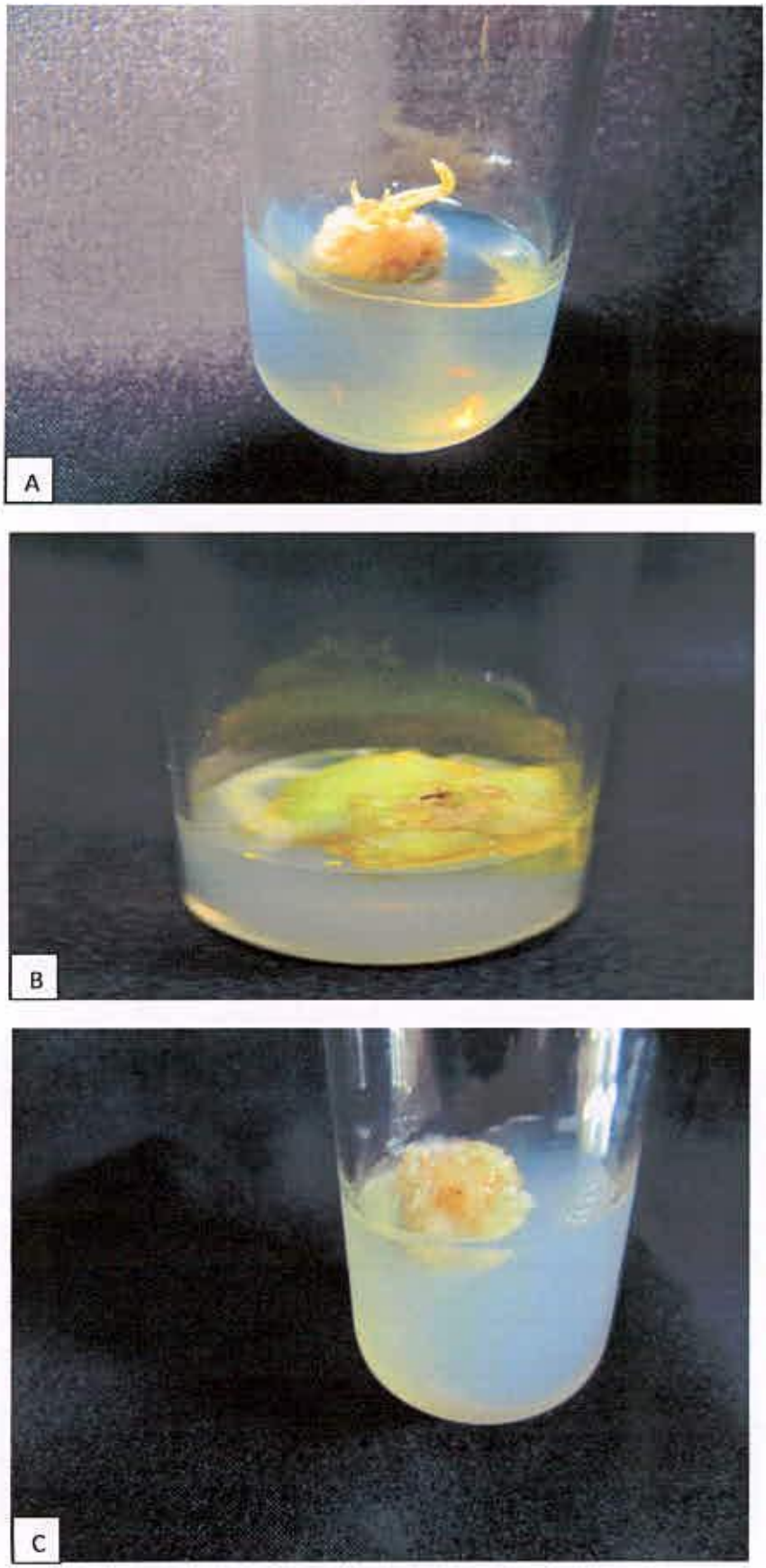


Plate 3 . Maximum size of callus on media supplemented with MS+ 3.0 mg/L 2,4-D at 45 DAI in A. Diamant, B. Cardinal, C. Granola.

4.2.3. No. of shoots/plantlet

The main effect of potato varieties exhibit significant variation in respect of number of shoot/plantlet at 15, 30 and 45 DAI. The variety Granola produced the highest number of shoot (5.33 at 45 DAI) whereas the variety Diamant produced the lowest number of shoots (4.41 at 45 DAI) (Table 5).

The effect of different concentration of 2,4-D in respect to number of shoots/planted are shown in Table 6. The maximum shoot/plantlet (10.44) was observed in the treatment combination of MS+3.0 mg/L 2,4-D at 45 DAI . The minimum shoot/plantlet (1.00) was observed in the treatment combination of MS+5.0mg/L 2,4-D also at 45 DAI .

Shoot per plantlet increased gradually in 30 days and 45 days but abnormal and deformed shoot formation were observed in the higher concentration (MS + 5.0 mg/L 2,4-D) shown in Table 7. The maximum number of shoot (13.67) were found in the treatment MS+3.0mg/L 2,4-D in the variety Granola at 45 DAI (Plate 4), where as treatment MS+5.0mg/L 2,4-D in the variety Cardinal give lowest result (1.00 shoot) at 45 DAI . This result is almost similar with Shahab-ud-din *et al.* (2011). They investigate the effects of different concentrations of plant growth regulators and their combinations on callus induction of potato (*Solanum tuberosum* L.). Among the treatments 2, 4-D at different concentrations produced different degree of callus but comparatively a massive amount of callus was formed on MS medium supplemented with 2, 4-D alone at 3.0 mg/L.

4.2.4. Length of shoot/plantlet

The length of shoot were recorded at 15, 30 and 45 DAI. The highest length of shoot was found in the variety Granola (8.54 cm) and lowest was found in the variety Cardinal (7.50 cm) at 45 DAI. (Table 5).

The effect of different concentration of 2,4-D in respect to length of shoot/plantlet shown in Table 6. The highest (9.28 cm) was obtained from the treatment of MS+3.0 mg/L 2,4-D at 45 DAI and lowest (2.05 cm) with MS+5.0mg/L 2,4-D.

The combination effect of different varieties and 2,4-D concentration were showed significant differences. The highest length of shoot (10.03 cm) were found in the treatment MS+1.0 mg/L 2,4-D in the variety Granola at 45 DAI (Plate 5) where as treatment MS+5.0 mg/L 2,4-D in the variety Cardinal showed lowest result (1.48 cm) . (Table 7).

4.2.5. Number of roots/Plantlet

The number of roots/plantlet was recorded after 15, 30 and 45 DAI. A significant variation was found on number of roots in different potato varieties. The variety Granola produced maximum number of roots/plantlet (6.00) and variety Diamant produced minimum number of roots/plantlet (5.50) at 45 DAI .(Table 5).

The effect of different concentration of 2,4-D in respect to number of roots per plantlet are shown in Table 6. The highest no of root (11.44) were found from the treatment MS+3.0 mg/L 2,4-D at 45 DAI and lowest number of root (5.20) were found from the treatment MS+5.0 mg/L 2,4-D at 45 DAI . Kumar *et al.* (2014) also give the similar result They used leaf as explants of two potato (*Solanum tuberosum L.*) cultivars which were cultured for callus induction and plant regeneration. Best callus growth from both the cultivars was observed on Murashige and Skoog (MS) media containing 3.0 mg/L of 2,4-D (2,4-dichlorophenoxy acetic acid).

The combination effect showed significant differences. The highest number of root (14.0) were found in the treatment MS+3.0 mg/L 2,4-D in the variety Granola at 45 DAI (Table 7 and Plate 6), and the lowest number of root (4.10) were found in the variety Diamant with treatment MS+ 5.0 mg/L 2,4-D at 45 DAI. Root per plantlet showed positive correlation with shoot per plantlet. Ehsanpour *et al.* (2007) obtained calli from *in vitro* grown potato leaf segment on MS medium containing 2,4-D, NAA, kinetin and yeast extract. They reported the changed DNA pattern as the source of genetic variation.

Table 5. Effect of different varieties on no. of shoot per plantlet, length of shoot per plantlet and no. of root per plantlet.

Variety	No. of shoot per plantlet			Length of shoot per plantlet (cm)			No. of root per plantlet		
	15 DAI	30 DAI	45 DAI	15 DAI	30 DAI	45 DAI	15 DAI	30 DAI	45 DAI
Diamant	11	2.83	4.41	1.89	4.55	7.71	2.08	3.91	5.50
Cardinal	2.16	3.66	5.16	1.85	4.30	7.50	2.08	3.58	5.58
Granola	2.50	3.66	5.33	1.60	4.07	8.54	2.66	4.16	6.00
SE±	0.25	0.47	0.52	0.02	0.03	0.78	0.25	0.25	0.63
LSD	0.42	0.57	0.61	0.12	0.16	0.74	0.42	0.42	0.67
Level of significance	*	*	*	*	*	*	*	*	*

Table 6. Effect of mutagen 2,4-D on number of shoot per plantlet, length of shoot per plantlet and no. of root per plantlet.

Treatments	No. of shoot per plantlet			Length of shoot per plantlet (cm)			No. of root per plantlet		
	15 DAI	30 DAI	45 DAI	15 DAI	30 DAI	45 DAI	15 DAI	30 DAI	45 DAI
T1= MS	1.66	2.66	3.66	2.06	5.40	8.61	2.00	4.11	7.56
T2=MS+1 2,4 D	2.22	3.55	4.77	2.30	5.30	9.05	2.77	4.33	8.77
T3=MS+3 2,4 D	3.33	6.33	10.44	2.08	5.14	9.28	4.33	7.11	11.44
T4=MS+5 2,4 D	1.00	1.00	1.00	0.67	1.32	2.05	2.90	3.75	5.20
SE±	0.25	0.47	0.52	0.02	0.036	0.78	0.25	0.25	0.63
LSD	0.48	.66	.70	0.14	0.18	0.85	0.48	0.48	0.77
Level of significance	*	*	*	*	*	*	*	*	*

Table 7. Effect of different varieties and mutagen 2,4-D on number of shoot per plantlet, length of shoot per plantlet and no. of root per plantlet

Variety	Treatments	No. of shoot per plantlet			Length of shoot per plantlet (cm)			No. of root per plantlet		
		15 DAI	30 DAI	45 DAI	15 DAI	30 DAI	45 DAI	15 DAI	30 DAI	45 DAI
Diamant	T1= MS	1.66	2.66	3.66	2.10	5.60	9.45	2.33	4.33	7.66
	T2=MS+ 1 2,4-D	1.66	3.55	4.66	1.93	4.83	7.40	2.33	4.33	6.67
	T3=MS+ 3 2,4-D	1.66	6.33	7.33	1.93	4.70	9.33	3.66	6.00	8.66
	T4=MS+ 5 2,4-D	1.00	1.00	2.00	1.60	3.10	4.66	2.40	3.53	4.10
Cardinal	T1= MS	1.66	0.22	4.33	2.06	5.20	8.90	1.33	3.33	6.33
	T2=MS+ 1 2,4-D	2.33	3.66	5.00	2.03	5.26	9.73	2.33	3.67	8.33
	T3=MS+ 3 2,4-D	3.66	3.00	10.33	2.86	5.86	9.90	4.66	7.33	12.6
	T4=MS+ 5 2,4-D	1.00	2.00	1.00	0.43	0.87	1.48	2.81	4.25	7.60
Granola	T1= MS	1.66	2.66	3.00	2.03	5.40	7.50	2.33	3.67	6.66
	T2=MS+ 1 2,4-D	2.66	3.33	4.67	2.93	5.80	10.03	3.66	5.00	8.33
	T3=MS+ 3 2,4-D	4.66	7.66	13.67	1.46	4.86	8.63	4.66	8.00	14.0
	T4=MS+ 5 2,4-D	1.00	1.00	1.20	1.50	3.81	5.52	2.01	4.42	5.20
	SE±	0.250	0.47	0.528	0.021	0.036	0.78	0.25	0.25	0.63
	LSD	0.84	1.15	1.22	0.24	0.31	1.48	0.84	0.84	1.34
	Level of significance	*	*	*	*	*	*	*	*	*

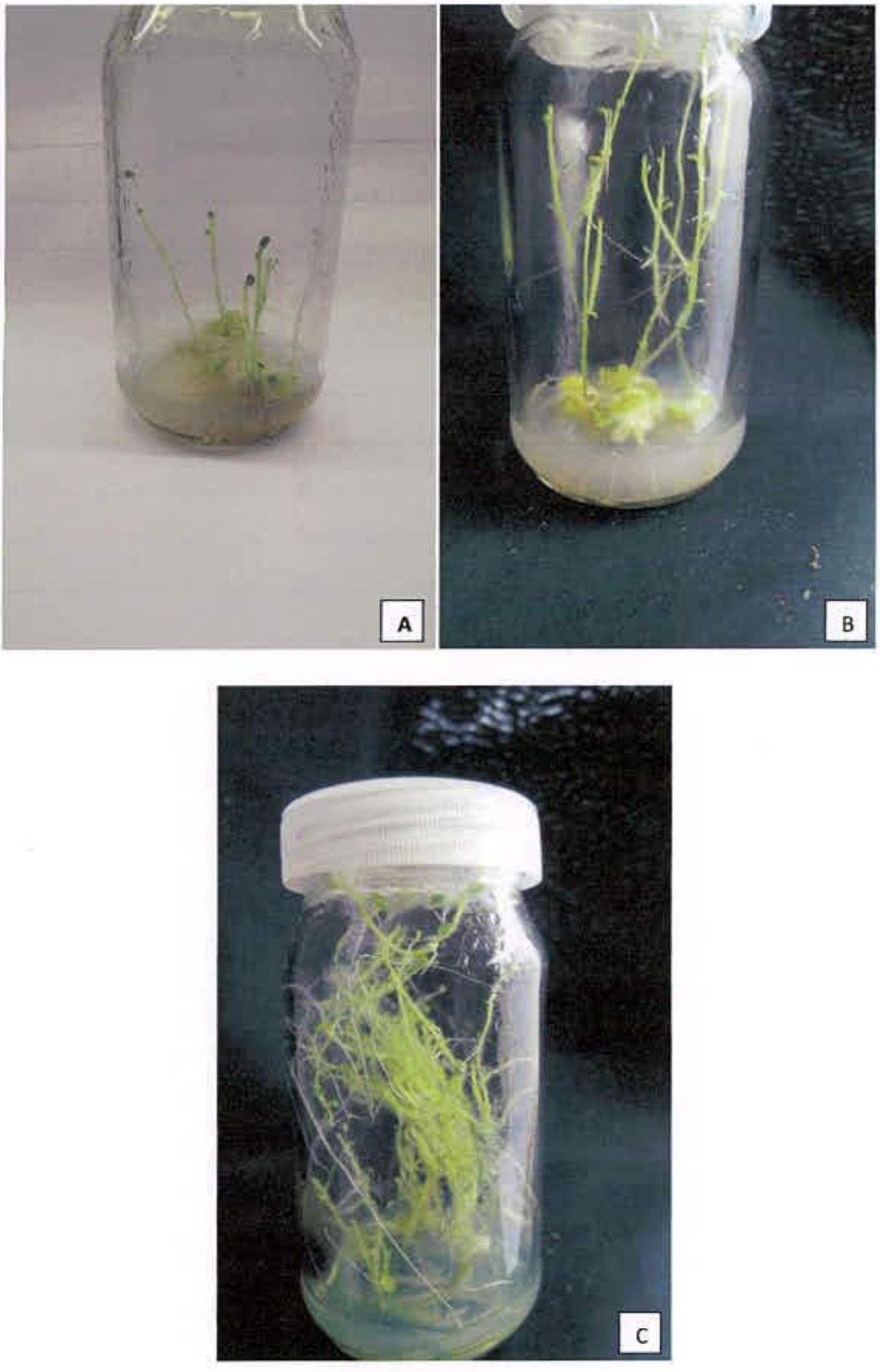


Plate 4. Number of shoot initiated on MS media supplemented with 3.0 mg/L 2,4-D at 45 DAI in A. Diamant, B. Cardinal, C. Granola

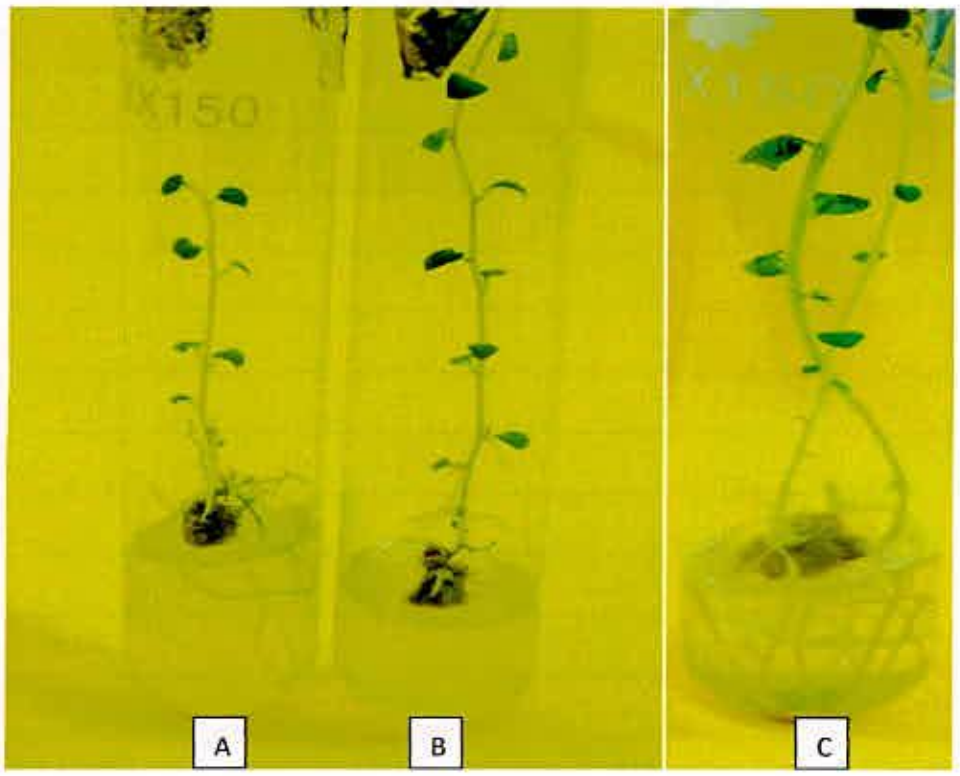


Plate 5. Shoot length at 45 DAI on MS media supplemented with 1 mg/L 2,4-D in A. Diamant, B. Cardinal, C. Granola

Plate 6. Maximum number of root at 45 DAI on MS media supplemented with 3 mg/ L 2,4- D in A. Diamant, B. Cardinal, C. Granola

Effect of EMS and 5-BU on *in vitro* regeneration:

The effect of chemical mutagen (EMS and 5-BU) on *in vitro* regeneration in potato are presented in Table 8-22. Direct shoots were developed from the explants treated with the mutagen. Toxic effect was observed in higher concentration of mutagens. Among the mutagens, BU had more corrosive effect on the plantlet. However, the individual effect of each treatment is given below

4.2.6. Days to shoot initiation:

Shoot initiation data were recorded 15, 30 and 45 days of culture on MS media containing different concentrations of EMS and 5-BU. After 45 days the plantlet were subcultured for better regeneration. The result have been presented in Table 8-10.

The maximum days to shoot initiation was observed in variety Diamant (11.28 days) whereas the minimum days was recorded in Granola (9.19 days) (Table 8)

There was significant influence of different concentration of EMS and 5-BU on days to shoot initiation. The maximum days to shoot initiation was recorded on MS+5.0 mg/L BU (12.84 days) and MS+1.0 mg/L EMS required minimum days (9.22 days) to shoot initiation (Table 9)

The combined effect of varieties and mutagens combinations on days to shoot initiation has been presented in the Table 10. Here the maximum days to shoot initiation was found in variety Diamant (13.67 days) with MS+5.0 mg/L BU and the minimum days to shoot initiation in variety Granola (8.20 days) at MS+1.0 mg/L EMS.

Table 8 : Effect of different varieties on days to shoot initiation and number of shoots/plantlet at different days after inoculation

Variety	Days to shoot initiation	Number of shoots/plantlet		
		15 DAI	30 DAI	45 DAI
Diamant	11.28	1.10	3.14	4.47
Cardinal	10.23	2.20	3.33	5.10
Granola	9.19	2.85	4.40	5.90
SE±	0.90	0.34	0.71	0.95
LSD	0.63	0.36	0.52	0.60
Level of significance	*	*	*	*

Table 9 : Effect of different Mutagens on days to shoot initiation and number of shoots/plantlet at different days after inoculation

Mutagen	Days to shoot initiation	Number of shoots/plantlet		
		15 DAI	30 DAI	45 DAI
T1= Normal MS	10.33	2.45	4.22	5.78
T2=MS+ 1 EMS	9.22	2.52	3.45	6.50
T3=MS+ 3 EMS	10.67	2.34	3.75	5.89
T4=MS+ 5 EMS	12.50	1.95	3.45	4.23
T5=MS+ 1 BU	11.45	1.80	3.89	5.88
T6=MS+ 3 BU	12.66	1.78	3.78	4.95
T7=MS+ 5 BU	12.84	1.72	2.56	2.95
SE±	0.90	0.34	0.71	0.95
LSD	0.97	0.56	0.80	0.92
Level of significance	*	*	*	*

Table 10 : Combined effect of different varieties and different mutagen days to shoot initiation and number of shoots/plantlet at different days after inoculation

Variety	Mutagen	Days to shoot initiation	Number of shoots/plantlet			Abnormal shoot regeneration (out of 10 plantlet)	Percentage of abnormality (%)
			15 DAI	30 DAI	45 DAI		
Diamant	T1= Normal MS	9.33	2.67	5.25	6.33	-	-
	T2= MS+ 1 EMS	10.29	2.00	3.25	6.25	-	-
	T3=MS+ 3 EMS	10.00	2.00	3.00	4.00	10 (1.25)	12.50
	T4=MS+ 5 EMS	11.25	1.66	2.33	3.10	10 (6.32)	63.20
	T5=MS+ 1 BU	11.34	1.00	1.68	3.66	-	
	T6=MS+ 3 BU	12.15	2.66	3.33	4.66	10 (2.56)	25.60
	T7=MS+ 5 BU	13.67	1.00	1.40	1.90	10 (8.00)	80.00
Cardinal	T1= Normal MS	10.35	2.34	3.67	5.36	-	-
	T2= MS+ 1 EMS	10.62	2.37	4.00	6.10	-	-
	T3=MS+ 3 EMS	10.33	2.00	4.72	5.95	10 (1.63)	16.30
	T4=MS+ 5 EMS	11.72	1.95	3.45	5.33	10 (6.63)	66.30
	T5=MS+ 1 BU	12.10	1.25	2.00	2.56	-	-
	T6=MS+ 3 BU	12.32	1.33	2.33	2.67	10 (2.20)	22.00
	T7=MS+ 5 BU	12.66	1.36	1.69	1.80	10 (8.80)	88.00
Granola	T1= Normal MS	9.80	2.33	3.67	6.65	-	-
	T2= MS+ 1 EMS	8.20	2.50	4.45	7.30	-	-
	T3=MS+ 3 EMS	10.65	2.00	3.66	6.00	10 (2.21)	22.10
	T4=MS+ 5 EMS	11.00	2.66	3.85	4.33	10 (6.52)	65.20
	T5=MS+ 1 BU	10.34	1.00	2.00	3.33	-	-
	T6=MS+ 3 BU	13.00	1.33	2.66	3.66	10 (2.12)	21.20
	T7=MS+ 5 BU	12.10	1.56	2.10	2.90	10 (7.75)	77.50
	SE±	0.90	0.34	0.71	0.95	0.88	
	LSD	1.78	0.97	1.39	1.60	1.25	
	Level of significance	*	*	*	*	*	

4.2.7. Number of shoots/Plantlet :

The main effect of potato varieties exhibit variation in respect of number of shoots/plantlet at 15, 30 and 45 DAI. The variety Granola produce highest number of shoots (5.90) at 45 DAI whereas the variety Diamant produced the lowest number of shoots (4.47) at 45 DAI (Table 8).

The effect of different concentration of EMS and 5-BU gave the significant variation in respect of number of shoots/plantlet (Table 9) at 15, 30 and 45 DAI. It was observed the maximum no. of shoot (6.50) was produced by MS+1.0 mg/L EMS at 45 DAI and minimum number of slots (2.95) was produced by MS+5.0 mg/L BU at 45 DAI.

Combined effect of varieties and different concentrations of mutagen showed significant variation at different days on number of shoot/plantlet. The highest number of shoot/plantlet (7.30) was recorded in variety Granola at MS + 1.0 mg/L EMS and the lowest number of shoot/plantlet (1.80) was generated in variety Cardinal in the treatment MS+5.0 mg/L BU at 45 DAI (Table 10) .

4.2.8. Abnormal shoot regeneration :

Abnormal shoot regeneration data were presented in Table 10. It was noticed that, all the three varieties showed some abnormal shoot at higher concentration (MS + 5.0 mg/L) of chemical mutagens. Very thin stem, huge branching of stem was observed in both the mutagen EMS and 5-BU.

Highest deform plantlet was noticed in the variety Cardinal at the treatment MS + 5 mg/L 5-BU. Among the two mutagen , 5-Bromo Uracil (5-BU) showed more corrosive effect than Ethyle Methane Sulphonate (EMS). At least 80% abnormality was observed in all the varieties when treated with MS + 5 mg/L of BU, where as the unusual plantlet regeneration was 60% in MS+5.0 mg/L of EMS (Table 10 and Plate 9 & Plate 10) .

The lower concentration (1 mg/L) of BU & EMS did not show any abnormality on plantlet regeneration. The deform shoot regeneration was observed at 3.0 mg/L of BU & EMS but the deformity rate is less than 30% .The leaf development, leaf number, shoot length were not clear on the higher concentration (5mg/L) of BU & EMS (Plate 9 & Plate 10). The present findings indicated that, the higher concentration of BU & EMS may has the ability to create new varient in potato genotype.

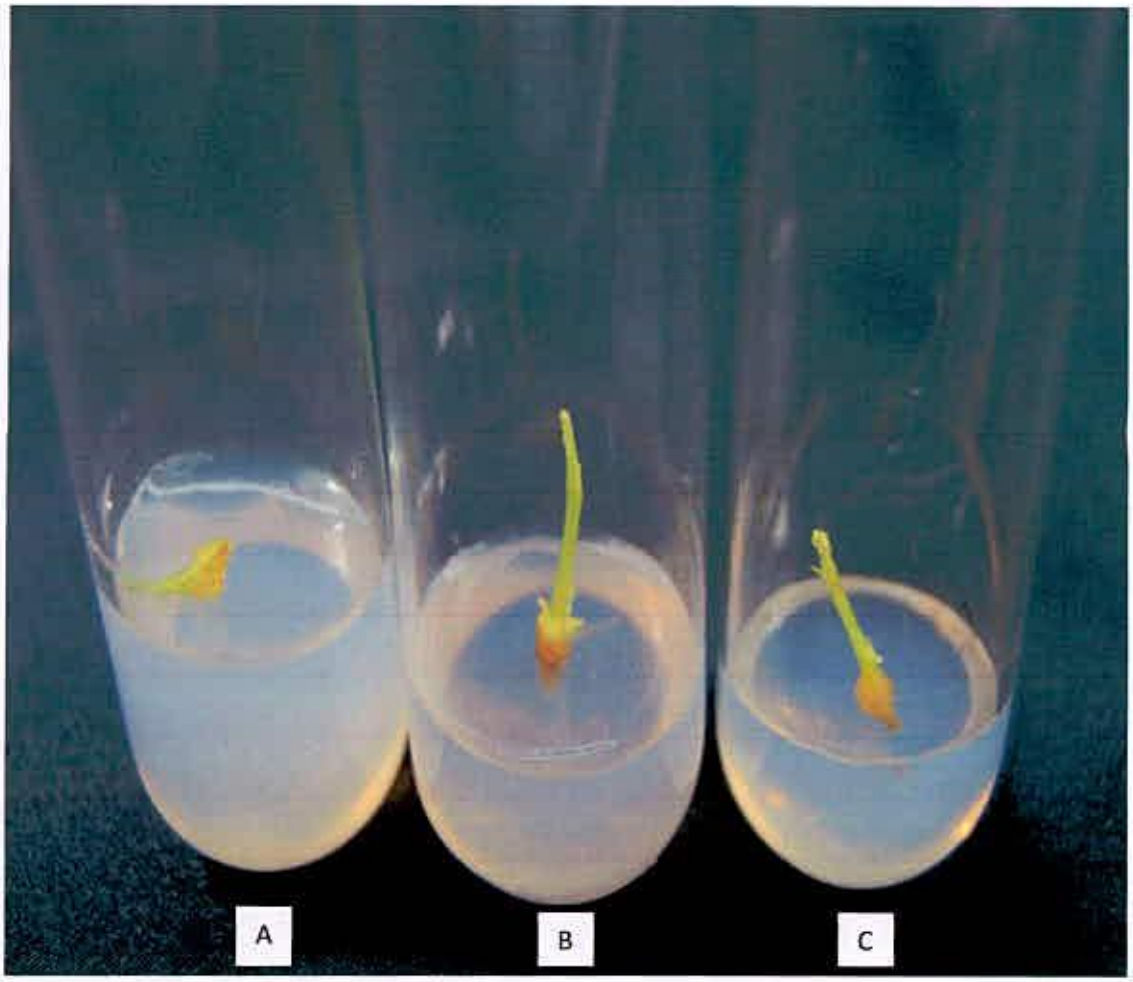


Plate 7. Shoot initiated on MS media supplemented with 1.0 mg/L EMS at 20 DAI in A. Diamant, B. Cardinal, C. Granola

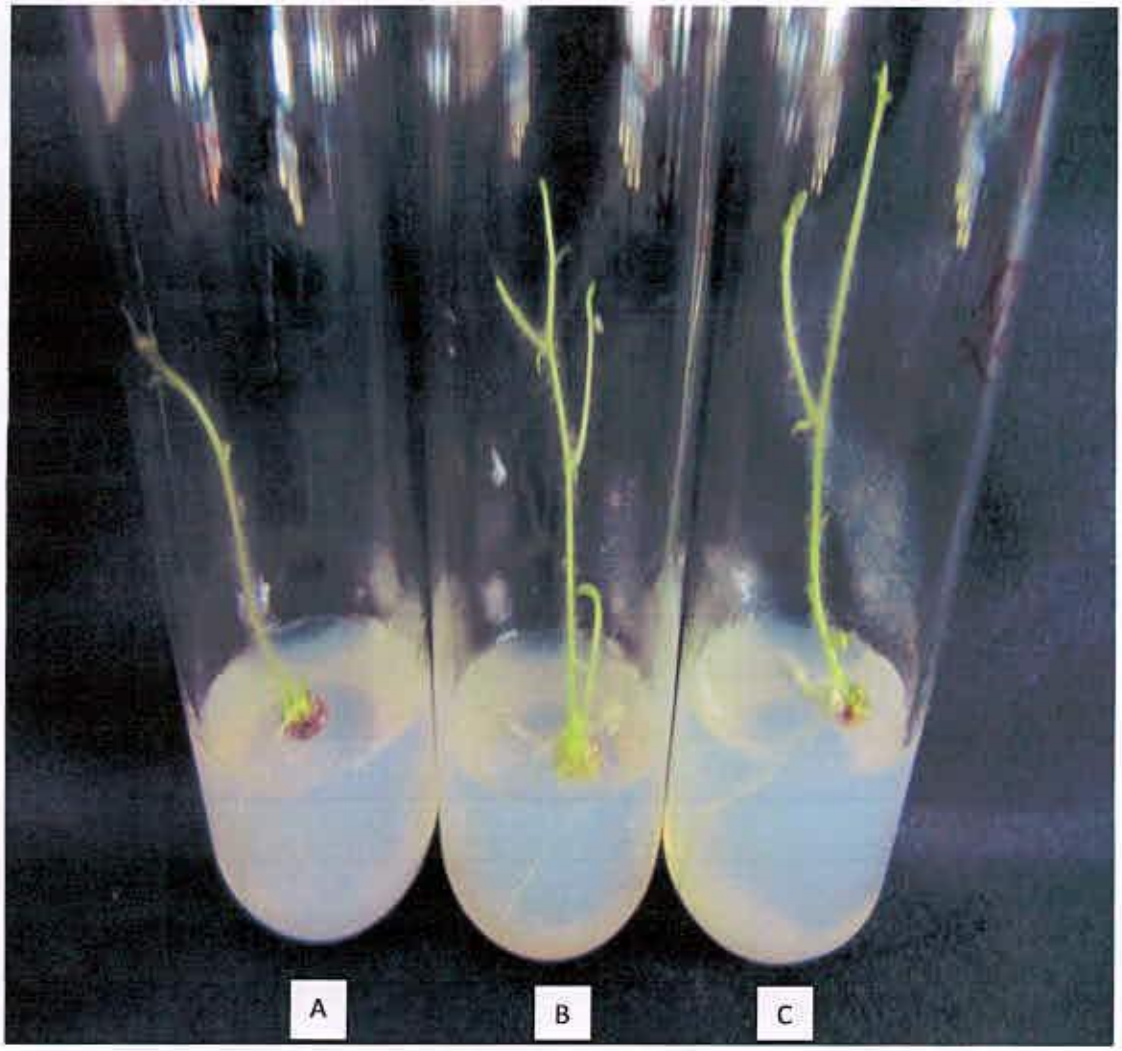


Plate 8. Number of shoot initiated on MS media supplemented with 3.0 mg/L EMS at 45 DAI in A. Diamant, B. Cardinal, C. Granola

Plate 9 . Abnormal plantlet regeneration occurred at 45 DAI on MS media supplemented with 5.0 mg/L EMS in A. Diamant, B. Cardinal, C. Granola

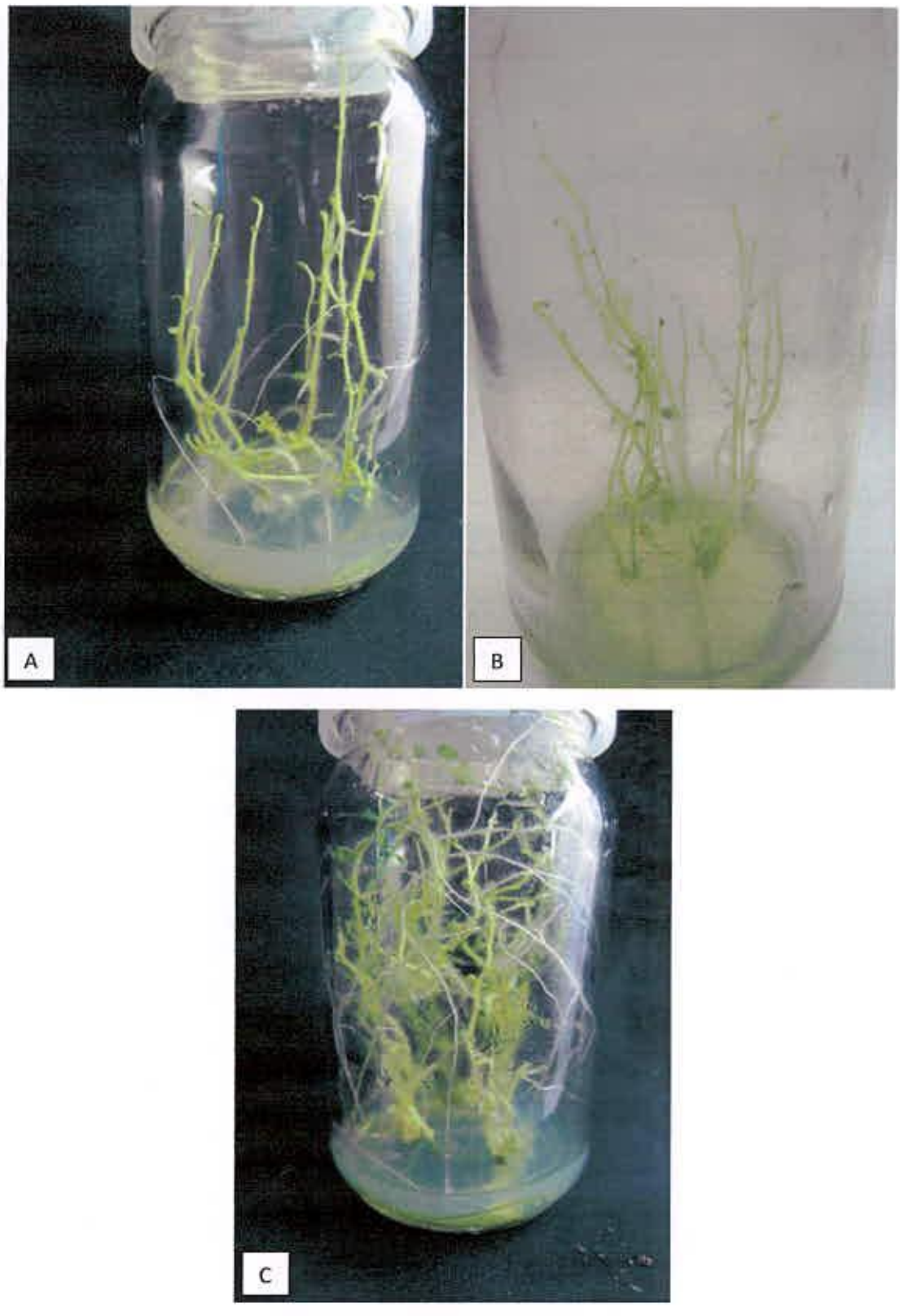


Plate 10. Abnormal plantlet regeneration occurred at 45 DAI on MS media supplemented with 5.0 mg/L 5-BU in A. Diamant, B. Cardinal, C. Granola

4.2.9. Length of shoots/Plantlet

Length of shoots of different varieties was significantly increased at 15, 30 and 45 DAI. The highest length of shoots was found in the variety Granola (8.77cm) at 45 DAI and lowest length of shoot was found in the variety Cardinal (7.72 cm) at 45 DAI (Table 11).

The effect of different concentration of EMS and 5-BU was significantly influenced on length of shoots/plantlet, which has been presented in Table 12. Among the treatments, it was observed that length of shoots increased gradually with the advancement of time. The largest shoot (9.35cm) were obtained from the treatment MS+3.0 mg/L EMS at 45 DAI and shortest shoot were obtained from the treatment (6.98 cm) with MS+5.0 mg/L BU (Table 12 and Plate 11).

The combined effect of different varieties and different concentrations of EMS and 5-BU also showed significant differences. The longest shoot (9.83 cm) was produced by the treatment MS+3.0 mg/L EMS with variety Granola at 45 DAI and shortest shoot (4.63 cm) was produced by the treatment MS+5.0 mg/L BU with variety Cardinal at 45 DAI (Table 13).

Table 11 . Effect of different varieties on length of shoots/plantlet at different days after inoculation

Variety	length of shoots/plantlet (cm)		
	15 DAI	30 DAI	45 DAI
Diamant	2.10	5.60	8.20
Cardinal	2.80	5.14	7.72
Granola	2.85	5.70	8.77
SE±	0.142	0.390	1.102
LSD	0.234	0.388	0.653
Level of significance	*	*	*

Table 12 . Effect of different Mutagens on length of shoots/plantlet at different days after inoculation

Mutagen	length of shoots/plantlet (cm)		
	15 DAI	30 DAI	45 DAI
T1= Normal MS	2.38	6.11	8.70
T2= MS+ 1 EMS	2.53	5.70	9.04
T3=MS+ 3 EMS	3.14	6.41	9.35
T4=MS+ 5 EMS	2.87	6.06	8.75
T5=MS+ 1 BU	2.12	4.46	7.73
T6=MS+ 3 BU	2.56	4.77	7.06
T7=MS+ 5 BU	2.47	4.85	6.98
SE±	0.142	0.390	1.102
LSD	0.358	0.594	0.998
Level of significance	*	*	*

Table 13 . Combined effect of different varieties and different mutagen length of shoots/plantlet at different days after inoculation

Variety	Mutagen	length of shoots/plantlet (cm)		
		15 DAI	30 DAI	45 DAI
Diamant	T1= Normal MS	1.93	5.80	8.70
	T2= MS+ 1 EMS	1.96	5.30	8.73
	T3=MS+ 3 EMS	2.00	6.30	8.83
	T4=MS+ 5 EMS	1.63	5.20	7.00
	T5=MS+ 1 BU	2.03	4.56	7.33
	T6=MS+ 3 BU	2.53	6.03	8.20
	T7=MS+ 5 BU	2.63	6.00	8.60
Cardinal	T1= Normal MS	3.03	5.50	8.26
	T2= MS+ 1 EMS	3.36	6.63	9.20
	T3=MS+ 3 EMS	4.10	7.03	9.73
	T4=MS+ 5 EMS	3.86	6.93	9.43
	T5=MS+ 1 BU	1.83	3.36	5.73
	T6=MS+ 3 BU	1.56	2.86	5.10
	T7=MS+ 5 BU	1.86	2.00	4.63
Granola	T1= Normal MS	2.20	7.03	9.13
	T2= MS+ 1 EMS	2.26	5.16	9.20
	T3=MS+ 3 EMS	3.33	5.90	9.83
	T4=MS+ 5 EMS	3.13	6.06	9.50
	T5=MS+ 1 BU	2.50	5.46	7.90
	T6=MS+ 3 BU	3.60	5.43	7.90
	T7=MS+ 5 BU	2.93	4.86	7.96
	SE±	0.142	0.390	1.102
	LSD	0.620	1.029	1.730
	Level of significance	*	*	*

Plate 11. Length of shoot at 45 DAI on MS media supplemented with 3.0 mg/L EMS in A. Diamant, B. Cardinal, C. Granola.

4.2.10. Number of leaves/plantlet:

The number of leaves/plantlet was recorded 15, 30 and 45 days of cultured on MS media containing different concentration of EMS and 5-BU. The results have been presented in Table (14-16)

The significant effect of three potato varieties was found in respect of number of leaves per plantlet. At 15, 30 and 45 DAI the maximum number of leaves/plantlet was showed in variety Granola (14.67 leaves/plantlet.) and the minimum number of leaves/plantlet was found in Diamant (12.81 leaves/plantlet) at 45 DAI (Table 14).

Different concentrations of EMS and 5-BU on number of leaves also statistically different days. The highest number of leaves/plantlet (16.00 leaves/plantlet) was produced with the concentration of MS+3.0 mg/L EMS at 45 DAI and the lowest number of leaves/plant (11.44 leaves/plantlet) was produced with the concentration of MS+5.0 mg/L BU at 45 DAI (Table 15).

The combined effect of varieties and different concentration of EMS and 5-BU on number of leaves/plantlet was statistically significant at 15, 30 and 45 DAI. Highest number of leaves/plantlet were recorded in variety Granola (17.30 leaves/plantlet) at the concentration of MS+3.0 mg/L at 45 DAI. The lowest number of leaves/plantlet was showed in variety Diamant (8.67 leaves/plant) with MS+5.0 mg/L BU at 45 DAI (Table 16).

Table 14 . Effect of different varieties on number of leaves/plantlet at different days after inoculation

Variety	Number of leaves/plantlet		
	15 DAI	30 DAI	45 DAI
Diamant	3.33	7.81	12.81
Cardinal	4.95	9.28	14.05
Granola	4.19	10.24	14.67
SE±	0.778	1.683	2.873
LSD	0.549	0.808	1.056
Level of significance	*	*	

Table 15 . Effect of different Mutagens on number of leaves/plantlet at different days after inoculation

Mutagen	Number of leaves/plantlet		
	15 DAI	30 DAI	45 DAI
T1= Normal MS	4.00	8.77	12.56
T2= MS+ 1 EMS	5.11	10.56	15.33
T3=MS+ 3 EMS	5.22	10.56	16.00
T4=MS+ 5 EMS	5.55	11.11	15.67
T5=MS+ 1 BU	2.55	7.11	12.67
T6=MS+ 3 BU	3.22	7.33	12.22
T7=MS+ 5 BU	3.44	8.33	11.44
SE±	0.778	1.683	2.873
LSD	0.839	1.234	1.613
Level of significance	*	*	*

Table 16 . Combined effect of different varieties and different mutagen number of leaves/ plantlet at different days after inoculation

Variety	Mutagen	Number of leaves/plantlet		
		15 DAI	30 DAI	45 DAI
Diamant	T1= Normal MS	2.66	6.66	10.33
	T2= MS+ 1 EMS	3.33	8.33	12.67
	T3=MS+ 3 EMS	4.33	8.66	13.67
	T4=MS+ 5 EMS	4.66	10.67	15.67
	T5=MS+ 1 BU	2.00	6.00	14.00
	T6=MS+ 3 BU	3.33	7.00	12.67
	T7=MS+ 5 BU	3.00	7.33	8.67
Cardinal	T1= Normal MS	4.00	7.66	12.00
	T2= MS+ 1 EMS	7.33	12.00	16.67
	T3=MS+ 3 EMS	6.66	11.00	16.33
	T4=MS+ 5 EMS	6.66	11.00	15.33
	T5=MS+ 1 BU	3.00	7.66	12.67
	T6=MS+ 3 BU	3.33	6.66	11.33
	T7=MS+ 5 BU	3.66	4.00	10.00
Granola	T1= Normal MS	5.33	12.00	15.33
	T2= MS+ 1 EMS	4.66	11.33	16.67
	T3=MS+ 3 EMS	4.66	12.00	17.30
	T4=MS+ 5 EMS	5.33	11.67	17.00
	T5=MS+ 1 BU	2.66	7.66	11.33
	T6=MS+ 3 BU	3.00	8.33	12.67
	T7=MS+ 5 BU	3.66	8.66	12.67
	SE±	0.778	1.683	2.873
	LSD	1.453	2.138	2.793
	Level of significance	*	*	*

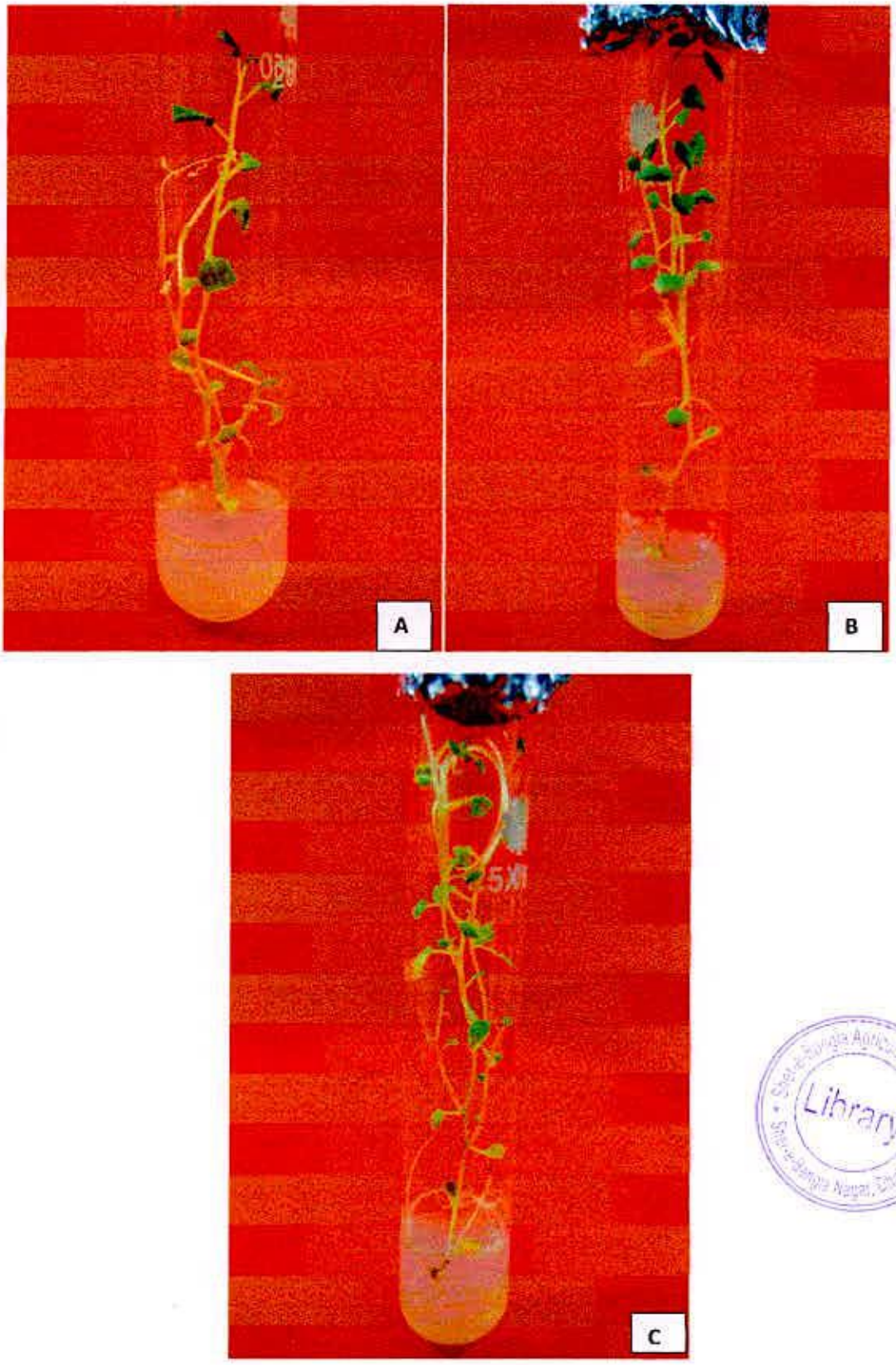


Plate 12. Maximum number of Leaf initiated at 45 DAI on MS media supplemented with 3.0 mg/L EMS in A. Diamant , B. Cardinal, C. Granola.

4.2. 11. Days to root initiation

Days to root initiation was recorded after 15, 30 and 45 days to cultured on MS media containing different concentrations of EMS and 5-BU. The results have been presented in Table 17-19.

The results of major effect of varieties on days to root initiation have been presented in Table 17. The days to root initiation varied significant among the three varieties. The maximum days was recorded in variety Granola (12.19 days) where as the minimum days was noticed in variety Diamant (9.81 days).

There was significant influence of different mutagen concentrations of EMS and 5-BU on the days to root initiation (Table 18). The minimum days (9.55 days) was observed with MS+1.0 mg/L EMS and the maximum days (13.89 days) was observed at the concentration of MS+1.0mg/L BU.

The potato varieties and different level of mutagen showed significant interaction in relation to the days to root initiation. The maximum days (15.67 days) to root initiation on Cardinal variety with MS+1.0 mg/L BU and the minimum days was observed in the variety Granola (9.0 days) on simple MS medium (Table 19).

Table 17 . Effect of different varieties on days to root initiation and number of roots/plantlet at different days after inoculation

Variety	Days to root initiation	Number of roots/plantlet		
		15 DAI	30 DAI	45 DAI
Diamant	9.81	2.28	5.04	7.76
Cardinal	11.52	1.61	4.19	6.81
Granola	12.19	1.95	4.66	7.00
SE±	1.152	0.381	1.032	1.603
LSD	0.887	0.384	0.632	0.788
Level of significance	*	*	*	*

Table 18 . Effect of different Mutagens on days to root initiation and number of roots/plantlet at different days after inoculation

Mutagen	Days to root initiation	Number of roots/plantlet		
		15 DAI	30 DAI	45 DAI
T1= Normal MS	10.67	1.66	3.44	6.00
T2= MS+ 1 EMS	9.55	2.44	5.66	8.88
T3=MS+ 3 EMS	9.88	2.44	5.11	8.22
T4=MS+ 5 EMS	9.88	2.77	5.11	8.77
T5=MS+ 1 BU	13.89	0.77	3.44	5.22
T6=MS+ 3 BU	12.22	1.77	4.00	6.44
T7=MS+ 5 BU	12.11	1.77	4.66	6.77
SE±	1.152	0.381	1.032	1.603
LSD	1.356	0.587	0.966	1.204
Level of significance	*	*	*	*

Table 19. Combined effect of different varieties and different mutagen days to root initiation and number of shoots/plantlet at different days after inoculation

Variety	Mutagen	Days to root initiation	Number of roots/plantlet		
			15 DAI	30 DAI	45 DAI
Diamant	T1= Normal MS	9.86	1.66	3.66	7.00
	T2= MS+ 1 EMS	9.66	2.00	4.66	9.00
	T3=MS+ 3 EMS	9.33	2.66	6.00	8.66
	T4=MS+ 5 EMS	9.66	2.33	4.66	7.33
	T5=MS+ 1 BU	10.67	1.33	4.33	6.33
	T6=MS+ 3 BU	10.67	3.33	6.00	8.00
	T7=MS+ 5 BU	10.00	2.66	6.00	8.00
Cardinal	T1= Normal MS	10.67	2.33	4.33	7.33
	T2= MS+ 1 EMS	9.66	2.00	4.66	8.00
	T3=MS+ 3 EMS	9.66	2.00	5.33	9.00
	T4=MS+ 5 EMS	10.00	2.33	5.66	8.33
	T5=MS+ 1 BU	15.67	0.33	2.66	4.00
	T6=MS+ 3 BU	12.67	1.00	2.66	5.33
	T7=MS+ 5 BU	12.33	1.33	4.00	5.66
Granola	T1= Normal MS	9.00	3.45	7.85	11.00
	T2= MS+ 1 EMS	10.33	3.33	7.66	9.33
	T3=MS+ 3 EMS	10.67	2.66	4.00	7.00
	T4=MS+ 5 EMS	12.67	3.66	6.00	8.50
	T5=MS+ 1 BU	15.33	0.66	3.33	5.33
	T6=MS+ 3 BU	13.33	1.00	3.33	6.00
	T7=MS+ 5 BU	14.00	1.33	4.00	6.66
	SE±	1.152	0.381	1.032	1.603
	LSD	2.34	1.017	1.674	2.086
	Level of significance	*	*	*	*

Plate 13. Root initiated on MS media supplemented with 1.0 mg/L EMS at 45 DAI in Diamant , B. Cardinal , C. Granola.

4.2.12. No. of roots/plantlet :

The number of roots/plantlet was recorded after 15, 30 and 45 days to culture. Roots per plantlet showed the positive correlation with shoot per plantlet.

The variety Diamant produced maximum number of roots per plant (7.76 roots/plantlet) at 45 DAI and the variety Cardinal produced minimum number of roots per plant let (6.81 roots/plantlet) (Table 17).

The effect of different concentrations of EMS and 5-BU on number of roots/plantlet was statistically significant. The highest number of roots (8.88 roots/plantlet) was observed in treatment MS+1.0 mg/L EMS at 45 DAI. Where as the minimum number of roots (5.22 roots/plant) was observed in treatment MS+1.0 mg/L BU at 45 DAI (Table 18).

The combined effect of variety and different concentrations of EMS and 5-BU on number of roots/plantlet showed statistically significant results at 15, 30 and 45 DAI (Table 19). At 45 DAI, the highest number of roots/plantlet was noted in the variety Granola (11.00 roots/plantlet) at normal MS medium and at 45 DAI (Plate 14), the lowest number of roots/plantlet (4.00 roots/plantlet) was recorded in the variety Cardinal on MS+1.0 mg/L BU.

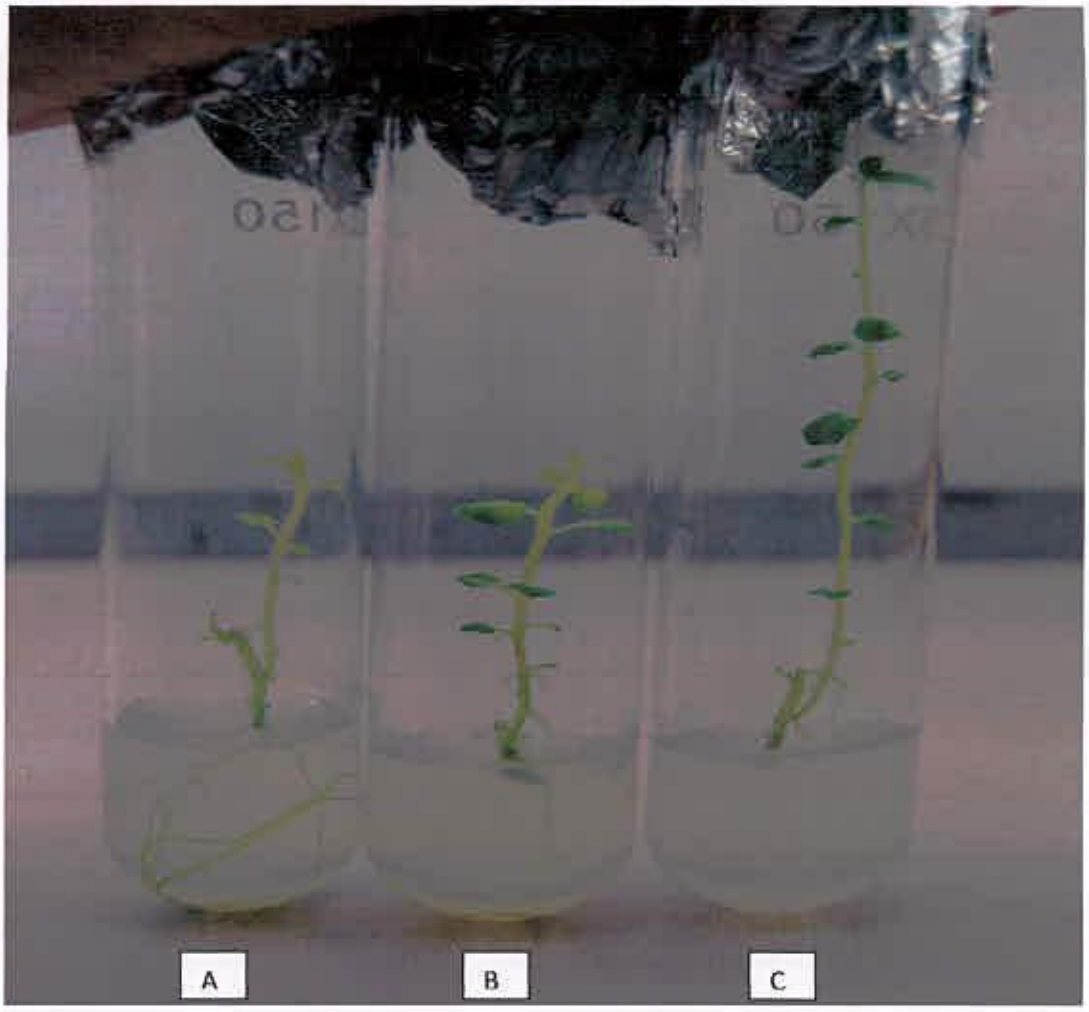


Plate 14. Number of root with 30 DAI on MS media supplemented with 1.0 mg/L EMS in A. Granola , B. Cardinal, C. Diamant.

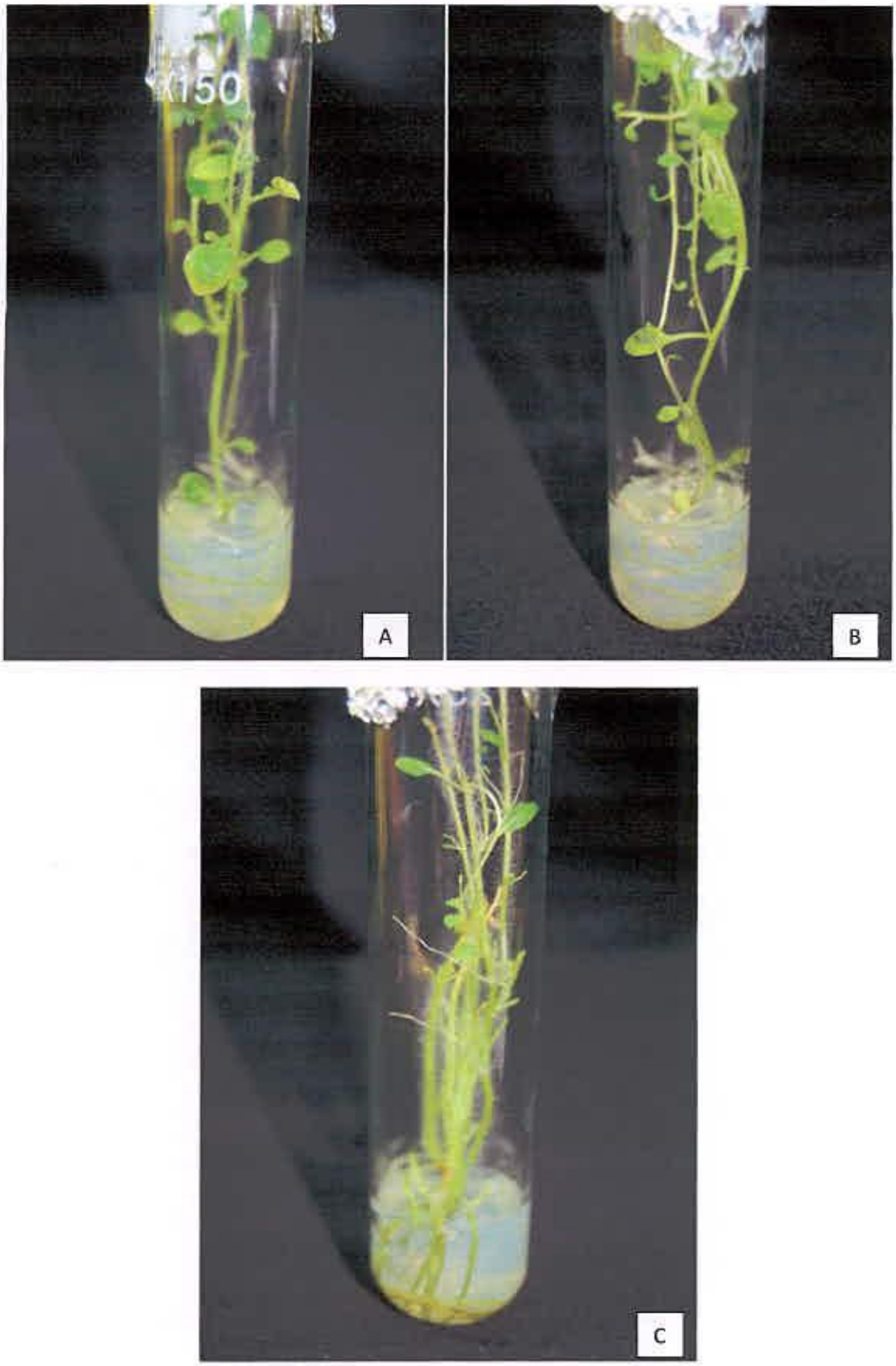


Plate 15. Maximum number of root at 45 DAI on normal MS media in A. Granola , B. Cardinal, C. Diamant.

4.2.13 . Length of roots/plantlet

The length of roots/plantlet was recorded 15, 30 and 45 days of cultured on MS media containing different concentrations of EMS and 5-BU. The results have been presented in Table 20-22.

The influence of these potato varieties was significance in respect of length of roots at 15, 30 and 45 DAI. The maximum length of roots at 45 days of culture was recorded variety Cardinal (9.53cm) and the minimum length of roots was found in the variety Granola (6.59 cm) (Table 20).

Length of root was also differed significantly due to the main effect of different concentration of EMS and 5-BU. The maximum length of roots (11.32 cm) was recorded at the concentration of MS+3.0 mg/L BU at 45 days of culture (Plate 15), where as minimum length of roots (4.93 cm) was produced on normal MS media (Table 21).

The results of the present experiment showed that there was significant effect on root length due to the combined effect of varieties and different concentrations of EMS and 5-BU at 15, 30 and 45 days to culture (Table 22). The maximum length of root (13.17cm) was recorded in the variety Diamant at the concentration of MS+3.0mg/L BU at 45 days to culture. The minimum length of root (3.06cm) found in Granola variety with Normal MS media at 45 days to culture.

Table 20. Effect of different varieties on length of root/plantlet at different days after inoculation

Variety	length of roots/plant (cm)		
	15 DAI	30 DAI	45 DAI
Diamant	3.03	6.37	9.35
Cardinal	2.44	5.67	9.53
Granola	2.16	4.46	6.59
SE±	0.413	0.479	2.464
LSD	0.400	0.431	0.977
Level of significance	*	*	*

Table 21. Effect of different Mutagens on length of roots/plantlet at different days after inoculation

Mutagen	length of roots/plant (cm)		
	15 DAI	30 DAI	45 DAI
T1= Normal MS	2.04	3.74	4.93
T2= MS+ 1 EMS	3.56	6.05	10.90
T3=MS+ 3 EMS	3.06	6.22	9.52
T4=MS+ 5 EMS	3.26	6.44	8.73
T5=MS+ 1 BU	1.16	3.24	5.48
T6=MS+ 3 BU	2.17	7.07	11.32
T7=MS+ 5 BU	2.54	5.73	8.55
SE±	0.413	0.479	2.464
LSD	0.611	0.658	1.493
Level of significance	*	*	*

Table 22 . Combined effect of different varieties and different mutagen length of roots/plantlet at different days after inoculation

Variety	Mutagen	length of roots/plant (cm)		
		15 DAI	30 DAI	45 DAI
Diamant	T1= Normal MS	2.60	4.60	6.40
	T2= MS+ 1 EMS	3.36	7.20	12.73
	T3=MS+ 3 EMS	4.50	7.90	10.07
	T4=MS+ 5 EMS	3.80	6.06	6.63
	T5=MS+ 1 BU	1.86	3.80	6.10
	T6=MS+ 3 BU	2.13	8.33	13.17
	T7=MS+ 5 BU	2.96	6.73	10.40
Cardinal	T1= Normal MS	2.30	4.10	5.33
	T2= MS+ 1 EMS	3.73	5.60	10.30
	T3=MS+ 3 EMS	2.30	6.46	11.70
	T4=MS+ 5 EMS	2.06	5.63	10.63
	T5=MS+ 1 BU	0.96	4.16	7.167
	T6=MS+ 3 BU	2.30	6.66	12.40
	T7=MS+ 5 BU	3.46	7.06	9.20
Granola	T1= Normal MS	1.23	2.53	3.06
	T2= MS+ 1 EMS	3.60	5.36	9.66
	T3=MS+ 3 EMS	2.40	4.30	6.80
	T4=MS+ 5 EMS	3.93	7.63	8.93
	T5=MS+ 1 BU	0.66	1.76	3.20
	T6=MS+ 3 BU	2.10	6.23	8.40
	T7=MS+ 5 BU	1.20	3.40	6.06
	SE±	0.413	0.479	2.464
	LSD	1.059	1.140	2.587
	Level of significance	*	*	*

Plate 16. Length of root initiated at 45 DAI on MS media supplemented with 3.0 mg/L BU in A. Diamant , B. Cardinal , C. Granola.

4.3. Experiment 3: Acclimatization and establishment of mutagen treated plantlets of potato under field condition .

Rate of survival of regenerated varieties after transplanted were presented in Table 23 and Plate 17. Acclimatization efficiency of the regenerated variants was recorded under natural field condition.

Preparation of plot and transplantation :

Potting mixture containing garden soil, sand and cowdung in the ratio 1:2:1 was mixed properly and autoclaved one hour in 121^0 C for 20 minutes at 1.61 kg/cm^2. After cooling the 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 roots. 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. Immediately after transplantation the plants along with the pots were covered with moist polythene bag to prevent desiccation. To reduce sudden shock the pots were kept in the growth room for 7-15 days under controlled environment. After 2-3 days, polythene bags were gradually perforated to expose the plants to natural environment. The polythene bags were completely removed after 10-15 days when the plantlets, appeared to be self-sustainable. At this stage, the plantlet were placed in natural environment for 3-10 hours daily. The highest survival rate found in Granola (43.33%) and the lowest survival rate found in Diamant (30.83%) (Table. 23).

Finally, after 15-20 days, they were transferred to the net house (under mosari) for hardening and after hardening the plantlets were transplanted to the soil (Plate18(A), 18(B) & 19). As soon as new leaves started to initiate, plants were watered with ordinary tap water. Gradually the plantlets were adapted to the soil. In open atmosphere plantlets of cv. Granola gave the highest survival rate 48.07 % and the lowest was 37.83% in cv. Diamant (Table. 23).

Table 23. 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 plastic tray at growth chamber	Diamant	120	37	30.83
	Cardinal	120	44	36.66
	Granola	120	52	43.33
In natural field condition under netting	Diamant	37	14	37.83
	Cardinal	44	19	43.18
	Granola	52	25	48.07

Plate 17. Acclimatization of regenerated plantlets in growth chamber.

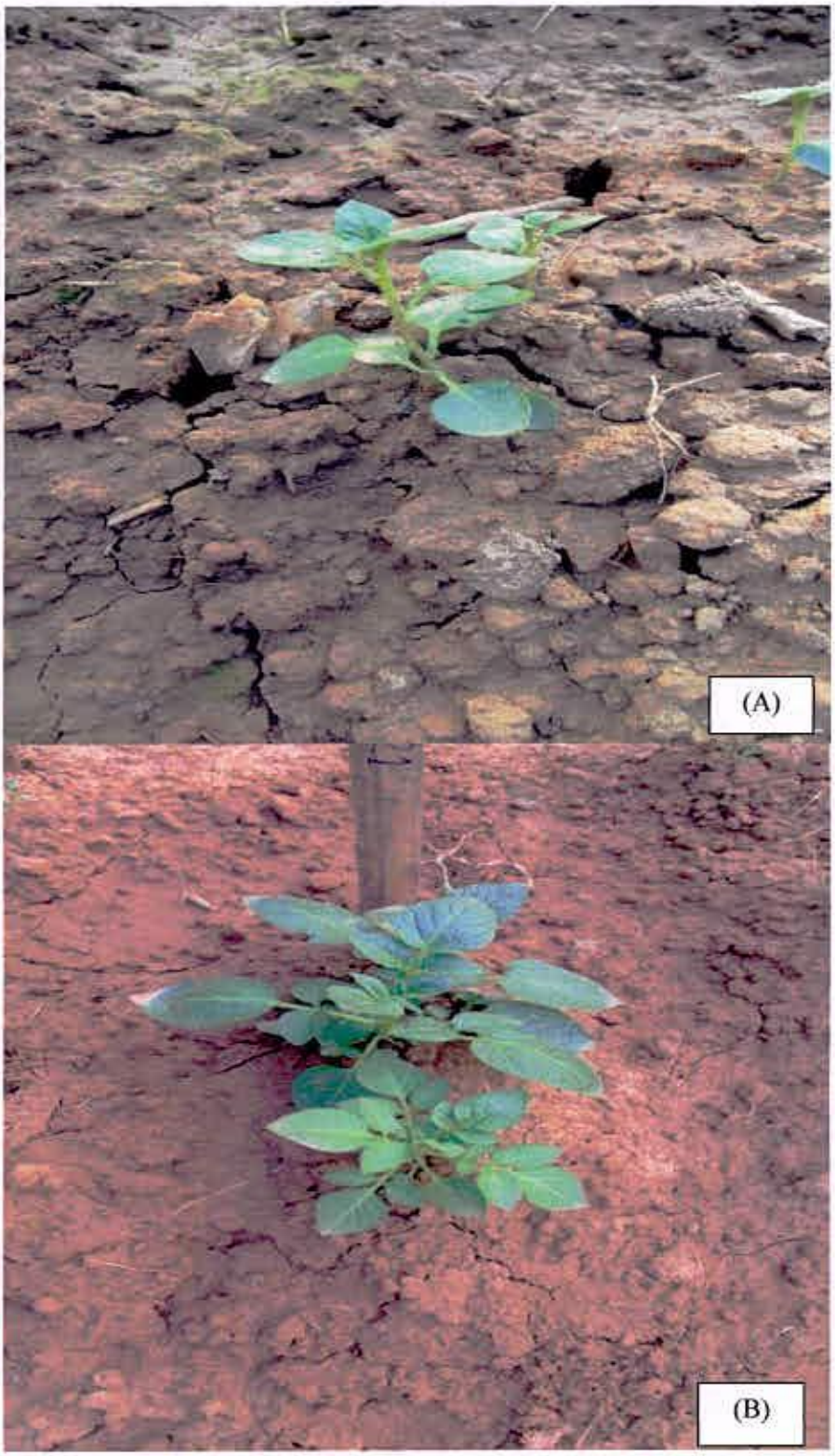


Plate 18 (A). Mutagenic treated plantlet at 30 days of transplanting .
(B). Mutagenic treated plantlet at 45 days of transplanting .

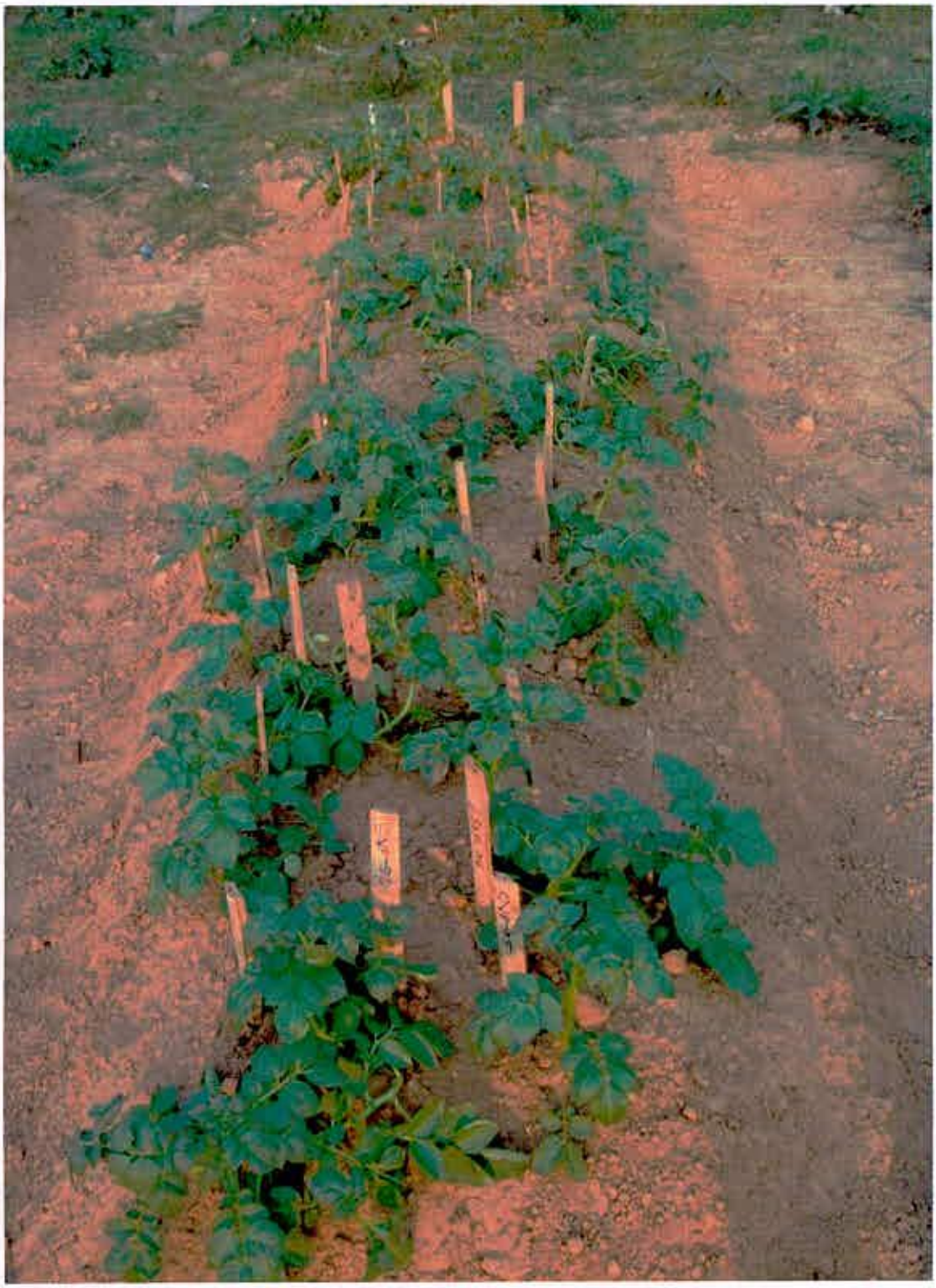


Plate 19. Field view of mutagenic treated plantlet at vegetative stage .

CHAPTER 5

SUMMARY AND CONCLUSION

CHAPTER 5

SUMMARY AND CONCLUSION

The present experiment was conducted in the Genetics and Plant Breeding laboratory and the Tissue culture laboratory of the Department of Biotechnology, Sher-e-Bangla Agricultural University (SAU), Dhaka, during the period of January 2013 to March 2014 from sprout of three potato varieties namely Diamant, Cardinal and Granola to establish *in vitro* callus induction and plant regeneration using different concentration of 2, 4-D EMS and 5-BU. The experiment was conducted at Completely Randomized Design (CRD) with 3 replications. The concentrations of 2, 4-D, EMS and 5-BU were 1mg/L, 3mg/L and 5mg/L. To investigate the effect of different treatments of this experiment, the parameters were recorded on days to callus induction, callus size, days to shoot initiation, number of shoots/plantlet, days to root initiation, number of roots/plantlet and length of roots/plantlet.

It was observed that only 2, 4-D had the ability to induce callus. It revealed that days to callus induction and size of callus were significantly influenced by different varieties and different concentrations of 2, 4-D. Callusing started within 11 days after inoculation up to 45 days. The size of calli increases but after 45 days it gradually lost its totipotency and died within 60 days after inoculation.The maximum (14.00 days) to callus induction was noticed in the treatment combination MS+5.0 mg/L 2,4-D in the variety Diamant. The minimum days (11.15 days) to callus induction was found in variety Granola with MS+3.0 mg/L 2,4-D. The highest callus size (2.30 cm) was observed at MS+3.0 mg/L 2,4-D with the variety Granola and the lowest size of callus (1.30 cm) was found at MS+1.0 mg/L 2,4-D with the variety Diamant.

The different concentrations of 2, 4-D significantly influenced the no of shoots/ plantlet, length of shoot per plantlet number of roots per plantlet. The maximum number of shoots (13.67) were found in the treatment MS+3.0mg/L 2,4-D in the variety Granola at 45 DAI, where as treatment MS+5.0 mg/L 2,4-D in the variety Cardinal give the lowest result (1.00 shoot) at 45 DAI . The highest length of

shoot (10.03 cm) were found in the treatment MS+1.0 mg/L 2,4-D in the variety Granola at 45 DAI where as treatment MS+5.0 mg/L 2,4-D in the variety Cardinal showed lowest result (1.48 cm). The highest number of root (14.0) were found in the treatment MS+3.0 mg/L 2,4-D in the variety Granola at 45 DAI

.

The different concentrations of EMS and 5-BU significantly influenced the days to shoot initiation , no of shoots/ plantlet, length of shoot / plantlet, number of leaves per plantlet, number of roots per plantlet and length of roots/plantlet. The highest number of shoots/plantlet (7.30) was recorded in variety Granola at MS + 1.0 mg/L EMS and the lowest number of shoots/plantlet (1.80) was generated in variety Cardinal in the treatment MS+5.0 mg/L BU. The longest shoot (9.83 cm) was produced by the treatment MS+3.0 mg/L EMS with variety Granola at 45 DAI and the shortest shoot (4.63 cm) was produced by the treatment MS+5.0 mg/L BU with variety Cardinal at 45 DAI. The highest number of leaves/plantlet were recorded in variety Granola (17.30 leaves/plantlet) at the concentration of MS+3.0 mg/L at 45 DAI. The lowest number of leaves/plantlet was showed in variety Diamant (8.67 leaves/plant) with MS+5.0 mg/L BU at 45 DAI. At 45 DAI the highest number of roots/plantlet was noted in the variety Granola (11.00 roots/plantlet) at normal MS medium and at 45 DAI, and the lowest number of roots/plantlet (4.00 roots/plantlet) was recorded in the variety Cardinal on MS+1.0 mg/L BU.

The overall experiment findings revealed that the variety Granola was the most responsive to GA_3 application for sprouting and days to sprout initiation . The result of the present investigation also indicated that potato cultivars Diamant, Cardinal and Granola could be successfully micro propagated using MS+3.0 mg/L 2,4-D for rapid callus induction and shoot regeneration. It was also revealed that simple MS media, EMS and 5-BU do not produce any callus .

Huge abnormality were noticed at higher concentration (5.0 mg/L) of 5-BU and EMS on all the three varieties under investigation. Thin stem, more branching, deform leaf size and shape were observed in both the mutagenic treatment. Its

shoot (10.03 cm) were found in the treatment MS+1.0 mg/L 2,4-D in the variety Granola at 45 DAI where as treatment MS+5.0 mg/L 2,4-D in the variety Cardinal showed lowest result (1.48 cm). The highest number of root (14.0) were found in the treatment MS+3.0 mg/L 2,4-D in the variety Granola at 45 DAI

The different concentrations of EMS and 5-BU significantly influenced the days to shoot initiation , no of shoots/ plantlet, length of shoot / plantlet, number of leaves per plantlet, number of roots per plantlet and length of roots/plantlet. The highest number of shoots/plantlet (7.30) was recorded in variety Granola at MS + 1.0 mg/L EMS and the lowest number of shoots/plantlet (1.80) was generated in variety Cardinal in the treatment MS+5.0 mg/L BU. The longest shoot (9.83 cm) was produced by the treatment MS+3.0 mg/L EMS with variety Granola at 45 DAI and the shortest shoot (4.63 cm) was produced by the treatment MS+5.0 mg/L BU with variety Cardinal at 45 DAI. The highest number of leaves/plantlet were recorded in variety Granola (17.30 leaves/plantlet) at the concentration of MS+3.0 mg/L at 45 DAI. The lowest number of leaves/plantlet was showed in variety Diamant (8.67 leaves/plant) with MS+5.0 mg/L BU at 45 DAI. At 45 DAI the highest number of roots/plantlet was noted in the variety Granola (11.00 roots/plantlet) at normal MS medium and at 45 DAI, and the lowest number of roots/plantlet (4.00 roots/plantlet) was recorded in the variety Cardinal on MS+1.0 mg/L BU.

The overall experiment findings revealed that the variety Granola was the most responsive to GA_3 application for sprouting and days to sprout initiation . The result of the present investigation also indicated that potato cultivars Diamant, Cardinal and Granola could be successfully micro propagated using MS+3.0 mg/L 2,4-D for rapid callus induction and shoot regeneration. It was also revealed that simple MS media, EMS and 5-BU do not produce any callus .

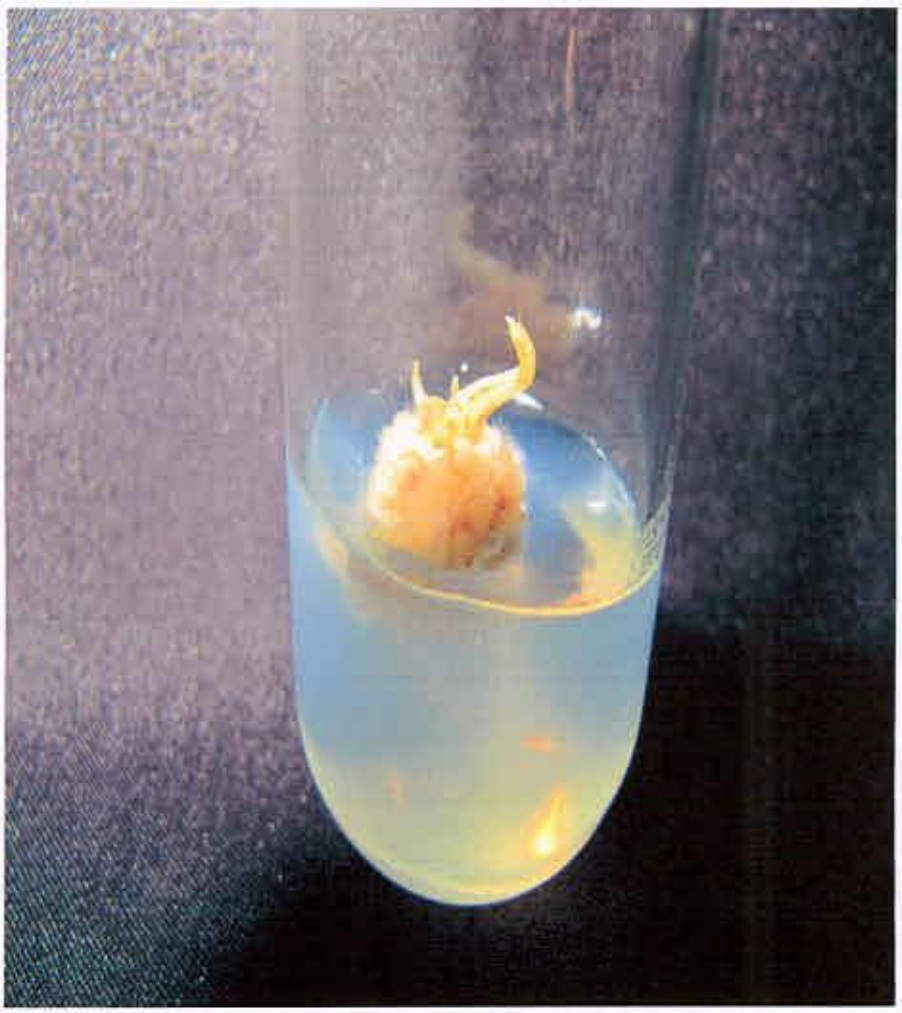
Huge abnormality were noticed at higher concentration (5.0 mg/L) of 5-BU and EMS on all the three varieties under investigation. Thin stem, more branching, deform leaf size and shape were observed in both the mutagenic treatment. Its

indicated that, the mutagen were interact with chromosome at the time of cell division and differentiation, which finally effect on plantlet regeneration.

For acclimatization, plantlets were transplanted from culture media to soil in tray with small hole in the growth chamber, where percentage of survival was the highest (43.33%) in cv. Granola and minimum percentage of survival (30.83%) was in Diamant. All cultural practices were done in the field condition and minitubers were collected from the mutagenic treated plantlet for further research.

CHAPTER 6

REFERENCES



CHAPTER 6
REFERENCE

Andreea, N., Campeanu, G., Chiru, N. and Karacsonyi, D. (2009). Effect of Auxin and Cytokinin on Callus induction in Potato (*Solanum tuberosum L.*). Asian network of scientific information. *Biotechnology*. **3(2):** 194-199.

BADC. (2014). Bangladesh Agricultural Development Corporation, Bangladesh, Dhaka.

BBS. (2014). Statistical Year Book. Bangladesh Bureau of Statistics, Ministry of Planning. The People's Republic of Bangladesh, Dhaka.

Dobranszki, J., Magyar, T.K. and Hudak, I. (2008). *In vitro* tuberization in hormone-free systems on solidified medium and dormancy of potato microtubers. *Fruit, Vegetable Cereal Sci. Biotech.* **2(1)**: 82-94.

Dobranszki, J., Takacs, H.A., Magyar, T.k. and Ferenczy, A. (1999). Effect of medium on the callus forming capacity of different potato genotypes. *Acta Agronomica Hungarica*. **47(1)**: 59-61.

Ehsanpour, A. A., Madani, S. and Hoseini, M. (2007). Detection of somaclonal variation in potato callus induced by UV-C radiation using RAPD-PCR.. *General and Applied Plant Physiology*. **33 (1-2):** 3-11.

Elaleem, K.G.A., Modawi, R.S. and Khalafalla, M.M. (2009). Effect of plant growth regulators on callus induction and plant regeneration in tuber segment culture of potato cultiver Diamant. *African J. Biot.* **8 (11)**: 2529-2534.

Fang, J. (2011). In Vitro Mutation Induction of Saintpaulia Using Ethyl Methanesulfonate. *Hort science* . **46(7)**: 981–984.

FAO. (2014). Potato. http:// www. Potato 2014.org

Forooghian, S. and Esfarayeni, S. (2013). An Evaluation of Effects of Plant Growth Regulators and Light on Callus Induction for Varieties of Potatoes. *American-Eurasian J. Agric. Environ. Sci.* **13(8)**: 1129-1134.

Gray, D. and Hughes, J.C., (1978). Tuber Quality. *In*: Harris, P.M., (*Ed.*), The Potato Crop Halsted Press, New York, pp: 511.

Gregory, C.P. and John, F.H. (1998). Plant Regeneration by Organogenesis From Callus and Cell Suspension Cultures, Plant Tissue and Organ Culture, Fundamental Methods. *Hort. Science*. **20(3)**: 419-422.

Hofmann, N.E., Raja, R., Nelson, R.L. and Korban, S.S. (2004). Mutagenesis of embryogenic cultures of soybean and detecting polymorphisms using RAPD markers. *Biologia Plantarum* .**48 (2)**: 173-177.

Jafri, I.M., Khan, A.H., Gulfishan, M. (2011). Genotoxic effects of 5-bromouracil on cytomorphological characters of *Cichorium intybus L. African Journal of Biotechnology*. **10 (52)**: 10595-10599.

Jayasree, T., Pavan, U., Ramesh, M., Rao, A.V., Reddy, K.J.M. and Sadanandam, A. (2001). Somatic embryogenesis of leaf cultures of potato. *Plant Cell Tiss. Org. Cult.* **64(1)**:13-17.

Keeps, M.S. (1979). Production of Field Crops. 6th Edn. Tata McGraw Hill Publ. Co. Ltd. New Delhi. pp.369.

Khatun, N., Bari, M.A., Islam, R., Huda, S., Siddiqe, N.A. and Mollah, M.U. (2003). Callus induction and regeneration from nadal segments of potato cultivar Diamnt. *J. Biol.Sci.* **3(12)**: 1101-1106.

Kleinhofs, A., Herman, J. and Francis, A. (1968). Mutation Induction in *Melilotus alba annua* by Chemical Mutagens. Agronomy & Horticulture -- Faculty Publications. University of Nebraska – Lincoln. January, 1968.

Kumar, V., Rashma, D. and Banerjee, M. (2014). Callus Induction and Plant Regeneration in *Solanum tuberosum* L. cultivars (Kufri Chipsona 3 and MP-97/644) via Leaf Explants. *Int. Res. J. Biological Sci.* **3(6)**: 66-72.

Murashige, T and Skoog, F. (1992). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* **15**:473-497.

Naik, P.S. and Sarker, D. (2000). *In vitro* propagation and conservation of genetic resources in potato. *In*: K.L. Chandha, P.N. Ravindran and S. Lella (eds) Biotechnology Crops, Malhotra Publishing House, New Delhi, pp. 369-406.

Omidim, M., and Shahpiri, A. (2003). Callus induction and plant regeneration *in vitro* in potato (*Solanum tuberosum L.*). Potatoes, Healthy Food for Huminity: International Development in Breeding, Production , Protection and Utilization . A Proceeding of the XXVI International Horticultural Congress. *Acta Hort.* **619**: 315-322.

Otroshy, M. (2006). Utilization of tissue culture techtiques in a seed potato tuber production scheme. Doctoral thesis, Wageninngen University, Wageninngen, The Netherlands.

Qin, H., Wang. Y.Q. and Hou, C. H. (2011). Effect of ethyl methane sulfonate (EMS) in *in vitro* mutation on anther-derived embryos in loquat (*Eriobotrya japonica* Lindl.) . *African J. Agril. Rese.* **6(11)**: 2450-2455.

Shahab-ud-din, I.N., Sultan, M.A., kakar, A., Yousafzai, F.A., Sattar,F., Ahmmad, S.M., Ibrahim, M., Hassanullah and Arif, B. (2011). The Effects of Different Concentrations and Combinations of Growth Regulators on the Callus Formation of Potato (*Solanum tubrosum*) Explants. *Curr. Res. J. Biol. Sci* . **3(5)**: 499-503.

Sharker, R.H. and Mustafa, B.M. (2002). Regeneration and Agrobacterium-mediated Genetic Transformation of Low Indigenous Potato Varieties of Bangladesh. *Plant Tiss. Cult.* **12**: 69-77.

Sharma, L.K., Manisha, k., Gill, M.I.S. and Bali, S.K. (2013). Germination and Survival of Citrus Jambhiri Seeds and Epicotyls after Treating with Different Mutagens under *in vitro* Conditions. *Middle-East Journal of Scientific Research* .**16 (2)**: 250-255.

Sherkar, H.D. and Chavan, A. M. (2014). Effect of 2,4-D; BAP and TDZ on Callus Induction and Shoot regeneration in Potato. *Sci. Res. Repo.* **4(1):**101-105.

Shirin, F., Hossain, M., Kabir, M.F., Roy, M. and Sarker, S.R. (2007). Callus induction and Plant regeneration from Internodal and Leaf explants of four potato (*Solanum tuberosum L.*). cultivers. *World J. Agric. Sci.* **3(1)**: 01-06.

Siddique, M.A. (1999). Prospect of TPS in National Seed Potato System. Paper Presented in second National Workshop on Tubers Crops Held at BADC, Dhaka .

Siddiqui, S.U., Chaudharay, M.F. and Joshi, S.D. (1996). *In vitro* preservation of patato (*Solanum tuberosum* L.) germplasm. *Pakistan J. Bot.* **28**: 37-40.

Svetleva, D.L. and Crino, P. (2005). Effect of ethyl methanesulfonate (EMS) and n-nitrose-n.-ethyl urea (ENU) on callus growth of common bean. *Central European of Agriculture*. **6(1):** 59-64.

Talebi, A.B., Talebi, B. and Ahahrokhifar, B. (2012). Ethyl Methane Sulphonate (EMS) Induced Mutagenesis in Malaysian Rice (cv. MR219) for Lethal Dose Determination. *American. J. Plant Sci.* **3**: 1661-1665.

Thornton , R.E. and Sieczka, J.B., (1980). Commercial potato production in North America. *Am. Pot. J.* **57**: 534-536.