EFFECT OF PLANT GROWTH REGULATORS ON *IN VITRO* MULTIPLICATION OF POTATO

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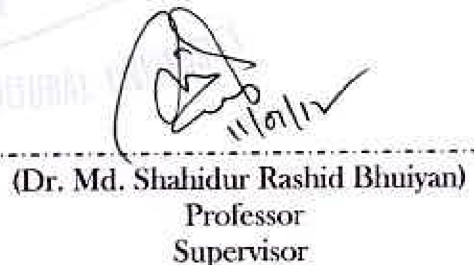
This is to certify that thesis entitled, "**EFFECT OF PLANT GROWTH REGULATORS ON *IN VITRO* MULTIPLICATION OF POTATO** submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE** in **GENETICS AND PLANT BREEDING**, embodies the result of a piece of bona fide research work carried out by **MD. KAMRUL HASAN**, Registration No. **04-01342** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

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.



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SOME COMMONLY USED ABBREVIATIONS AND SYMBOLS

The following abbreviations have been used through in this thesis:

Abbreviation		Full word
°C	:	Degree Celsius
%	:	Percentage
1N	:	1 Normal
2, 4-D	:	2,4-Dichlorophenoxy acetic acid
a.i	:	Active ingredient
BARI	:	Bangladesh Agricultural Research Institute
uM	:	Micro mole
Agric.	:	Agriculture
BAP	:	6-benzyl aminopurine
BBS	:	Bangladesh Bureau of Statistics
Cm	:	Centimeter
Contd.	:	Continued
CRD	:	Completely Randomized Design
cv.	:	Cultivar
CIP	:	International Potato Centre
Conc.	:	Concentration
DAI	:	Days after inoculation
dw	:	Distilled Water
DMRT	:	Duncan Multiple Range Taste
et al	:	And others
etc.	:	Etcetera
FAO	:	Food and Agricultural Organization
Fig.	:	Figure
g	:	Gram
g/l	:	Gram per litre
ha	:	Hectare
ha^{-1}	:	Per hectare
hr.	:	Hours
HgCl		Mercuric Chloride
i.e.	:	id est (That is)
IAA	:	Indole -3- Acetic Acid
NAA	:	Napthalene Acetic Acid
NaCl	:	Sodium Chloride
Int.	:	International
J.	:	Journal
mg	:	Milligram (s)
mg/l	:	Milligram per liter
ml	:	Milliliter
MS	:	Murashige and Skoog
GA	:	Gibbreline Acetic Acid
NaOH	:	Sodium Hydroxide
No.	:	Number
NS	:	Non Significant
pH	:	Negative logarithm of hydrogen ion Concentration (- $\log[H^+]$)
PRGs	:	Plant Growth Regulators
SAU	:	Sher-e-Bangla Agricultural University
Sci.	:	Science
Univ.	:	University
Viz	:	Namely
w/v	:	Weight/volume
ZR	:	Zeatin Riboside

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EFFECT OF PLANT GROWTH REGULATORS ON *IN VITRO* MULTIPLICATION OF POTATO

ABSTRATCT

By

Md. Kamrul Hasan

An investigation was carried out in the tissue culture laboratory, Biotechnology Division, Sher-e-Bangla Agricultural University (SAU), Dhaka, during the period from January, 2009 to December 2009 to study the effect of plant growth regulators (NAA, IAA, BAP & GA_3) on *in vitro* multiplication of potato through callus culture and to find out the regeneration potentiality of three potato varieties Diamant, Cardinal and Granola using stem segment as explants. Different concentrations and combinations of NAA and BAP were used on MS media for callus initiation and proliferation. Different concentrations and combinations of BAP and GA_3 were used with MS media for shoot induction and different concentrations and combinations of IAA and GA_3 were used for rooting.

The performance of cv. Cardinal was better for callus initiation. Minimum number of days required for callus initiation was recorded in cv. Cardinal produced on MS media supplemented with 3.0 mg/l NAA and 1.0 mg/l BAP. But the highest fresh weight of callus was found in cv. Diamant with the same concentration and combination of hormones at 60 DAI. In case of number of shoots/callus, it was found that maximum number of shoots per callus produced on MS media supplemented with 1.0 mg/l BAP and 0.1 mg/l GA_3 in cv. Diamant and Granulla at 60 DAI. But in case of days required for shoot induction, cv. Cardinal required minimum number of days. Highest shoot length found in cv. Diamant with same combinations and concentrations of hormones at 60 DAI. Highest number of leaves per plantlets found in cv. Diamant and Cardinal produced on MS media supplemented with 1.0 mg/l BAP and 0.1 mg/l GA_3 at 60 DAI and minimum number of leaves per plantlets found in cv. Granulla produced on MS media without any hormone at 60 DAI. Maximum number of days required for root formation was noticed in cv. Granulla with 1.00 mg/ IAA and 0.1 mg/l GA_3 and minimum number of days required for root formation was noticed in cv. Cardinal with 1.0 mg/l IAA and 0.05 mg/l GA_3. Highest number of roots found in cv. Diamant with 1.0 mg/l IAA and 0.05 mg/l GA_3 and minimum number of roots found in cv. Diamant and Granulla with 1.0 mg/l IAA and 0.0 mg/l GA_3 at 60 DAI. Highest root length found in cv. Diamant with 1.0 mg/l IAA and 0.05 mg/L GA_3 minimum root length found in cv. Granulla with the same concentration and combinations of the same hormones at 60 DAI.

The plantlets with sufficient number of well developed roots were successfully transferred in small holes of plastic tray in growth chamber and subsequently to soil in open atmosphere. Regenerated plantlets from cv. Diamant survived easily at high rate in growth chamber and in open atmosphere.

CHAPTER I

INTRODUCTION

CHAPTER I

INTRODUCTION

Potato *(Solanum tuberosum* L.), belongs to the family Solanaceae, is one of the most important food crops worldwide and is consumed as staple food in more than forty countries in the world. It seems to be originated in the central Andean areas of South America. Potato ranks as the world's fourth most important food crop, after maize, wheat and rice (FAO, 2008). Potato tubers give an exceptionally high yield per acre many times that of any grain crop (Burton, 1969) and are used in a wide variety of table, processed, livestock feed, and industrial uses (Feustel, 1987). In Bangladesh, Potato represents about 53% of the total edible vegetables (Rashid, 1999).

In 2007, Bangladeshi farmers harvested 12 times more than in 1961, which placed the country at No. 14 among the world's potato producers and No. 4 in Asia (FAO, 2008). Potato can be a substitute of rice and wheat, especially for the low income groups. With the consistent population increase, pressure on agricultural lands thereby available foods impart increased cost of rice and that directly affect low income population who are already deficient in calories. Among the tuber crops, Potato has become an important dietary source for many countries including Bangladesh. Several high yielding varieties (HYV) are now cultivated in Bangladesh although traditional varieties still occupy about 35% of the total area (Ilangantileke *et al*., 2001). Expansion of cultivated area and improved cultivar are two important factors pertaining potato production. In Bangladesh 5167 thousands M. tones of potato were produced from 345 thousands hectare of land during 2006-2007 (BBS, 2008). The average yield of potato in Bangladesh is 19.69 t/ha which is very low compared to many potato growing countries like the Netherlands (44.7 t/ha), the USA (44.6 t/ha) and Germany (42.3 t/ha) (FAO, 2008). However potato production has to be increased, even with the current rate of demand.

Cultivated potatoes are tetraploid species which is highly heterozygous and have tetrasomic inheritance. The use of botanical seeds in commercial cultivation is thus precluded by low germinability and large variability in the segregant generations (Gardner and Snustad, 1986). 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, abnormal seed and seedling

development, seed dormancy and environmental factors. These affect flowering and seed setting. Presence of these factors poses margins on the use of breeding techniques for improvement. 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. This propagation is characterized by low ratio of multiplication that ranges from 1:4 to 1:15. Tissue culture offers an excellent technique for rapid multiplication of potato plant (Tovar and Dodds, 1986). Recently plant genetic engineering have opened a new avenue in the area of crop improvement. An efficient tissue culture system is thought to be crucial to the success of plant genetic engineering, providing new tools to the plant breeder for crop improvement. The technique can be used to add desirable traits from the wild relatives to the existing cultivars. However, a prerequisite for transferring genes into plant is the availability of an efficient transformation and regeneration system (Hamrick *et al*., 2000).

Usually tuber is the means of potato propagation. Potato production is limited due to some important factors such as unavailability of compatible varieties and the disease free plant materials particularly in outlying regions not connected with dependable transport. As potatoes are propagated vegetatively, the tuber born pathogens of previous year can be perennated over generations with symptom expressed of latent. Consequently, much attention has been focused over *in vitro* production of virus free potato plantlets (Djurdjina *et al*., 1997).

Vegetative propagation through tissue culture is important for multiplication of potato plants. The growth regulators are used in tissue culture media. Plant growth regulators are the critical media components in determining the developmental pathway of plant cells. The most commonly used are plant growth hormones or their synthetic analogs. There are several classes of plant growth regulators- auxins, cytokinins, gibberellins, ethylene, and absciscic acid. They all play a distinct role in development of plant. For plant cells to develop into a callus it is essential that the nutrient medium contain a balanced ratio of plant hormones, i.e. auxins, cytokinins and gibberellins. The phytohormone balance varies for different tissue explants from different parts of the same plant. Thus, there is no ideal media. Relatively high ratio of auxin to cytokinin is required for root induction and high ratio of cytokinin to auxin is required for shoot induction from callus. After number of subculture, cells show differentiation of

tissues. The shoot differentiation has taken place from callus culture (Padmanabhan *et al*., 1974;Delanghe *et al*., 1976; Kartha *et al*., 1976). The process of transferring in vitro plantlets on plant regenerating media from test tube to the field is much more complex (Schilde and Schmiediche, 1984).

Many researchers work to standardize the optimum concentrations of growth regulators for regeneration of potato and consequently great progress has been made in potato callus induction and plant regeneration (Ahloowalia, 1982; Dobranszki *et al*., 1999; Hansen *et al*., 1999; Ehsanpour and Jones, 2000; Fiegert *et al*., 2000; Khutan *et al*., 2003; Yasmin *et al*., 2003; Shirin *et al*., 2007).

There are various factors that affect *in vitro* callus proliferation and plant regeneration such as position of the explants on the plant as well as size of explants, genotype of the explants, physiological state of the donor plant and explants, concentration of nutrients and phytohormones / plant growth regulators in the culture medium and environment under which cultures are grown i.e. light, temperature, and humidity (Phillips and Hubstenberger, 1998).

Farmers usually use previous harvest seed tubers, this favors seasonal build up of the tissue borne pathogens, and such pests lead to significant loss of yield and tuber quality via seed degeneration (Haverkort *et al*. 1991). Potatoes are found to be infected more than 20 viral diseases, of them PVX and PVY depress tuber yield up to 80% and may destroy whole crop in combination with others. The frequency of PVX free plants of potato reported to be much higher in plants regenerated from the meristem tip callus (46%) than those directly derived from meristem tips (Wang and Haung, 1980). So far, no method has been employed for cleaning up the virus infected plants for generation of virus free stock. However, it is now well known fact that through meristem culture it is possible to develop virus free potato planting stocks in a mass scale. More importantly, tissue culture systems are capable of creating genetic variability and producing plants with novel characters. This suggests, tissue culture application could be the viable alternative in developing new cultivars apart from generating virus free planting stocks and ameliorating heterozygous segregants.

Based on the above information the present piece of work was under taken with the following objectives:

I. To establish a protocol for regeneration of potato (*Solanum tuberosum*)

II. To investigate *in vitro* callus initiation ability from potato explant (stem segment) using different combination and concentration of plant growth regulators

III. To develop an *in vitro* regeneration system for Diamant, Cardinal and Granulla cultivars from stem segment

CHAPTER II
REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

Generally, Micro propagation of plants through *in vitro* culture is done to obtain healthy plants which depend on various factors like composition of media, explants and environmental conditions e.g. temperature, light, humidity etc. Very few works have been on these aspects. Potato is one of the most important vegetable crops throughout the world. It appears to be the number one vegetable of Bangladesh. Reports on *in vitro* regeneration of potato are very limited in our country. However, many workers have carried out research on micro propagation of potato in different countries of the world. In the present chapter, an attempt has been made to review the literature related to the present research work.

2.1 Concept of potato tissue culture

Conventional techniques of crop improvement are lengthy process. The techniques of plant tissue culture has been developed as a rapid and powerful tool for crop improvement (Carlson, 1975; Razdan and Cocking, 1981) and received wide attention of modern scientists (D. Amato, 1978; Larkin and Scowcroft, 1982).

Callus is an amorphous mass of loosely arranged thin walled parenchyma cells arising from the proliferating cells of parent tissue (Dodds and Roberts, 1990). Callus induction and regeneration from different explants of various potato varieties in different combination of growth regulators were reported by several workers. Murashige and Skoog (1962) reported that the nutritional requirement for optimal growth of *in vitro* tissues may vary with varieties. Even tissue from different parts of plants may show different requirements for satisfactory growth. The most relevant literature on terms of callus proliferation, shoot induction and regeneration of potato have been reviewed under the following sub-headings.

2.1.1 Effect of growth regulators on callus proliferation

Pacheco *et al.* (2003) assessed the three concentrations of IAA 0.0, 0.2, and 1.0 mg/L; BA 0.0, 1.0 and 3.0 mg/L; and their respective combination on callus induction. They reported that the most efficient combination of IAA was at 1.0 mg/L and BA at 1.0 mg/L and that resulted in 27.5% calli. Moreover, they had also observed that 0.05 mg IAA/L; 0.05 mg BA/L; and 0.1 mg GA_3/L being the best combination medium that induced roots 40% of the explants and different plantlets.

Omidi *et al.* (2003) from their study to assess effect of growth regulators (2,4-D and Kinetin), explants on callus induction in potato observed significant effect of 2,4-D combined Kinetin concentration, and their interactions, on the frequency of callus induction and roots on the callus. The effect of explants and their interaction on frequency of callus induction was not significant although the effect of these factors on the initiation time of callus induction was significant.

An efficient procedure has been developed by Jayasree *et al.* (2001) for including somatic embryogenesis from leaf culture of potato cv. Joythi. For this, leaf sections were initially cultured on 2,4-D + BA and NAA + BA supplemented Murashige and Skoog (MS) media.

More *et al.* (2001) evaluated to MH5 and Hiboras-6 to determine their effect on embryogenic callus formation of potato cv. Desire. They reported from their studies appropriate doses (0.0001-0.25 mg a.i/L) of MH5 and B6 as possible substitutes or hormonal complements. Furthermore, they observed best callus formation from medium-I supplemented with 0.1 mg MH5 + 0.01 mg BB-6 /L and from medium-II supplemented with 0.001 mg MH5 and 0.01 mg BB-6/L.

Fiegert *et al.* (2000) studied callus induction of potato (*Solanum tuberosum* cv. Tomensa). It was observed that maximum calli were induced from leaf on medium supplemented with 3.0 mg/L NAA and 0.25 mg/L BA. After cultivation of the resulting calli in medium supplemented with 3.0 mg/L NAA and 1.0 mg/L BA for 15-20 days, the hard green calli were transferred to embryo formation supplemented with

0.1 mg/L GA_3 –nd 0.05 mg/L zeatin. Embryo formation was detected after 30-35 days.

Fomenko *et al.* (2000) studied the dynamic of callus formation in potatoes. MS medium with NAA was selected for tuber explants and a medium with 2,4-D and Kinetin for leaf and stem explants. Protein and nucleic acids were analyzed during callus development.

An experiment was conducted by Zel *et al.* (1999) where leaf explants of potato in combination with different growth regulators, especially different concentrations of Zeatin Riboside (ZR) were tested and the great callus was obtained with 2.5 mg/L ZR, 0.2 mg/L NAA and 0.02 mg/L Gibberelic Acid (GA_3).

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

An experiment was conducted by Esna and Villiers (1998) where shoots, roots and calli were formed from potato cv. Desiree when grown *in vitro* on Murashige and Skoog (MS) medium supplemented with 2,4-D and/or BAP.

In vitro regeneration was assessed by Hamidi *et al.* (1998) in potato cv. Nagore, Desiree and Superior. Different explants such as leaves, tubers and different regeneration media varying in their hormonal composition were tested to increase the efficiency of the process. Callus induction rates were higher using an MS media containing glucose (30 g/L), NAA (0.02 mg/L), zeatin riboside (2.0 mg/L) and GA (0.02 mg/L).

Callus formation was investigated by Fomenko *et al.* (1998) in potato in potato hybrid 78563-76 and cultivars Rosinka and Otrada on MS medium supplemented with various growth regulators. They reported that different genotypes responded

differently to the growth regulators in the medium in terms callus formation. Protein and nucleic acid metabolism during callus formation also differed with genotype.

An experiment was conducted by Garcia *et al.* (1995) where somatic embryos were obtained from stem nodal section of *in vitro* cultured potato plants, 4-8 mm long. Calli were initiated in MS salts supplemented with 4 mg 2,4-D. After 90 days, calli were placed in media devoid of 2,4-D supplemented with 0.1 mg GA_3 or 1.0 mg BA/L. Embryos formation was detected after 60 days of tissue incubation. Histological studies confirmed the embryogenic nature of the calli. The embryos were produced at high frequency. They also isolated and recultivated in the same medium, and the developments of plantlets was observed 45 days after embryo formation.

An experiment was conducted by Kollist and Tikk (1994) to study the callus formation ability of 21 potato varieties on MS medium with different concentrations of growth regulators and sugars. They reported the callus induction ability was strongly dependent on variety and the composition of the medium. The most effective media contained auxin at low concentration and cytokinin at high concentrations (01.mg/L IAA and 6.0 mg/L BA)

An experiment was conducted by Martel and Garcia (1992) cultured potato cv. Sebago discs on MS medium with 30 gm/L sucrose where 0-0.1 mg/L NAA + 0-4.0 mg/L BA were added for the initial medium. NAA was essential for callus formation. The amount of callus formation was increased with increasing concentration of both NAA and BA with the highest concentrations of both growth regulators.

Tikk *et al.* (1992) from their experiments evaluated callus induction from leaf, stem and tuber explants of 11 varieties together with the 7 modifications of MS medium, and 12 modifications of the medium with supplements. They observed all the varieties had showed callus-forming ability in the optimum medium although there were differences in rate and stability of callus formation.

Martel *et al.* (1992) conducted an experiment leaf discs, were cultured on Murashige and Skoog medium. Thy observed NAA essential for callus formation and that

increased with increasing concentrations of both NAA and BA. They recorded the best results from the highest concentrations of both the growth regulators.

Somlenskya (1989) reported that the primary callus of Yantarnyi and Pigozhii was produced by culturing leaf and stem explants on medium with various supplements. Explants of the primary callus were cultured on the same medium but with BAP, NAA or both of these growth regulators. Shoot formation was successful on medium with GA_3

Pett and Tiemann (1987) scored the callus induction ability and the number of shoots regenerated per 100 explants in potato. Stem pieces, petioles and tissue from leaves were cultured on medium containing different combinations of cytokinin, auxin and Gibberelic acid. Stem pieces gave higher callus induction and regeneration rates than petiole and leaf explants and zeatin was more effective cytokinin than benzyl adenine.

Song *et al* (1987) reported from their studies the effect of various growth substances on callus induction and plantlet regeneration. Callus was induced on medium supplemented with 2,4-D and Kinetin or BAP. Induction was further improved by addition of GA_3 to the medium with 2,4-D + BAP. Callus proliferation was more active when transferred to a medium with higher concentration of 2,4-D, despite the GA_3 concentration had little effect. No shoot differentiation occurred in many treatment using tuber explants. Leaf explants had formed callus on medium with 2,4-D + BAP, and roots and shoots formed after transfer to medium in which 2,4-D was replaced by IAA and GA_3.

An experiment was conducted by Resende and Paiva (1986) for plantlet regeneration from explants of 5 potato cultivars. During the stage of callus initiation, MS medium supplemented with 0.01 mg/L NAA, 0.1 mg/L GA and 1.0 mg/L BA gave better result than MS medium supplemented with 0.4 mg/L Kn and 1.0 mg/L GA.

Gynheung *et al.* (1986) carried out an experiment to determine the best medium composition and concentration of phytohormone with a view to plant regeneration as well as *Agrobacterium* mediated transformation on potato. The reported result

indicated that the best medium for callus induction was 2.0 mg/L NAA, 0.5 mg/L BAP and 15.0 mg/L Glutamine.

Berjalk *et al.*(1985) reported that higher concentration of auxin and cytokinin were needed to achieve regeneration from tuber callus than from internode callus.

Khvikovskaya and Chwikowska (1982) obtain callus from leaves, stem and root explants, cultured on Murashige and Skoog medium with 1 mg/L 2,4-D. 0.2 mg/L Kinetin and 0.2 mg/L NAA. Callus formation began earlier, but morphogenesis from the calli occurred at the same time in both type of explant. Regenerated plantlets were obtained from callus originating from stem internodes and leaves of both monohaploid and dihaploid plants.

Novak (1980) studied the callus formation and differentiation with plantlet regeneration through potato stem internodes, leaf and root cultured (4 cv.) on Murashige and Skoog medium with addition of various growth substances alone and in combination. Callus formation was best on medium with 1mμ M 2,4-D + 5 mμ M IAA + mμ M Kunetin and was in the order stem > leaf > root.

2.1.2 Effect of growth regulators on shoot induction

Modarres and Moeini (2003) conducted an experiment with solid MS medium with 0.25 mg/L GA_3 and 0.01 mg/L NAA that sowed significant difference between different pH levels in respect of it s ability to induction of rooting and shooting in plantlets produced from the single node of two potato (*Solanum tuberosum* L.) varieties after subjecting them with thermotherapy. Overall pH 5.5 was the best for all the traits. Low and high level of pH from that 5.5 were found to reduce the growth and rooting of single nodes. The reduction was more pronounced at low levels than high levels pH.

Sidkou *et al.* (2003) in their study observed potato genotypes micropropagated *in vitro* in medium with sucrose at 2-12% and BA at 0-5 mg/L. Shoots were formed after one week. All cultivars showed 100% regeneration. Regeneration frequency increased with increasing concentration of BA and sucrose.

An experiment was conducted by Sarker and Mustafa (2002) where maximum shoot induction in two indigenous potato varieties was observed on MS semi-solid medium supplemented with 1.0 mg/L BAP and 0.1 mg/L GA. Among the two varieties, namely Lal Pakri and Jam Alu, the former showed the best response in terms of number of shoots/explants, nodes/shoot and shoot length.

Gebre and Sathyananaryana (2001) compared the efficiency of cheap alternating gelling agents, tapioca (from the tuber of *Manihor esculenta*) and sago (from the stem pith of *Mentroxylon sago*) and agar for *in vitro* shoot regeneration using internodal explants of potato. For shoot induction, agar maintained at 8.0 mg/L, while tapioca and sago varied between 9-18% and 10-14% (w/v). The medium supplemented with 3% sucrose, 0.03 mg/L NAA, 0.25mg/L GA_3 and 2.5 mg/L Ca-pantopthanate.

Ahn *et al.* (2001) conducted an experiment for the regeneration of potato through protoplast culture. Suitable plant growth regulators combination for shoot regeneration was Zeatin and IAA in both cultivars Dejima and superior and the optimum concentrations were 2.0 mg/L zeatin and 0.1 mg/L IAA for cv. Superior.

Asma *et al.* (2001) studied the effect of different concentrations (1.0, 2.0, 3.0, 4.0, 4.0 mg/L) of GA_3 and BAP on the *in vitro* multiplication of nodal fragments and stem segments of potato cv. Desiree. The maximum shoot length was obtained when 4.0 mg/L GA_3 was applied. The number of nodes was no significantly by any of the GA_3 concentrations used in this study. The maximum number of shoots (14) was obtained when 2.0 mg/L BAP was applied.

A study was conducted by Gebre and Sathyanarayana (2001) *in vitro* shoot regeneration by using nodal explants of potato. The regeneration medium was supplemented with, 0.03 mg NAA/L and 0.25 mg GA_3/L. Highest number of shoot (3.11) was obtained with the high concentration of NAA and in low concentration of GA_3.

Jatinder *et al.* (2000) observed that the *in vitro* propagation of four potato cultivars (Khufri Badshah, Khufri Chandramukhi, Khufri Jyoti, and Khufri Sinduri). Shoot nodal segments were excised, cultured on solid half-strength medium and full strength

MS medium. The shoots were rooted in half-strength MS medium with IBA (1.0 mg/L) resulting in 99% rooting with well developed roots. The percent shoot regeneration from cultured nodal segments was highest (98%) in Kufri Jyoti. Shoot establishment was better in half-strength than in full strength solid MS medium in all 4 cultivars. Kufri Sinduri exhibited the best shoot multiplication with the highest number of nodes (5.58) per shoot in liquid MS medium while on solid MS medium, Kufri Jyoti exhibited the highest number of nodes.

One, two and three step methods of plan regeneration from stem culture of potato cv. Delaware were tested by Ehsanpour and Jones (2000). Results showed that the one step procedure using a synthetic cytokinin was the best rapid plant regeneration. In this culture medium, several buds and shoots were regenerated from stem culture while other methods using z culture medium supplemented with combination of GA, NAA, Zeatin and 2-iP and IAA.

Internodes and leaf explants of potato in combinations with different plant growth regulators, especially different concentrations of Zeatin riboside (ZR), were tested, shoot induction was most successful on callus derived from internodes tissue cultured on induction medium supplemented with 2.5 mg/L ZR, 0.2 mg/L NAA, 0.02 mg/L GA for two weeks and then transferred to a shoot induction medium containing 2.5 mg/L ZR (Zel *et al.*, 1999).

Hasan *et al.* (1999) investigated shoot regeneration on explants from different leaves and leaflets of potatoes cvs, Posmo, Folva and Oleva. Explants were excised and on callus induction medium with IAA or 2, 4-D. Then, those transferred to auxin free shoot regeneration medium with GA_3 and BAP or Zeatin. By using the optimum combinations and concentrations of plant growth regulators and by excision of explants from particular regions of proximal leaflets from newly unfolded leaves, shoots regeneration frequencies of 44.6% were obtained for cv. Posmo and 32.1% from cv. Folva.

Zel *et al.* (1999) studied petioles, internodes and leaf explants in combination with different plant growth regulators, especially different concentrations of Zeatin

Riboside (ZR). Shoot regeneration was most successful on callus derived from internode tissues cultured on induction medium. In a comparison of the regenerative potential of Igor with that of Desiree, Igor had poorer and slower regeneration and produced fewer and shorter shoots.

Esna-Ashari and Villiers (1998) observed that shoot, root and callus were formed from tuber discs of potato cv. Desiree when grown *in vitro* on MS basal medium supplemented with 2, 4-D or BAP or both. Shoots were formed directly on MS medium with 1.0 mg/L BAP without the addition of 2,4-D.

In vitro regeneration was assessed by Hamidi *et al.* (1998) in potato cvs. Nagore, Desiree and Superior. Leaf explants in different regeneration media differing in the hormonal composition were employed to increase the efficiency of the process. Callus induction rates, number of shoots and number of regenerated plants were determined for the explant. The best observations were in regeneration rate, using an MS media containing NAA (0.02 mg/L) and GA_3 (0.03 mg/L).

An experiment was conducted by Alphnose *et al.* (1998) with leaflet explants, and callus tissues from micropropagated plantlets of potato cultured on a variety of medium supplemented with different growth regulators. Spunta showed the highest regeneration from leaflets, followed by Desiree. Supplementation of Murashige and Skoog medium with 1 mg BA, 1 mg IAA and 10 mg GA_3/L was the best combination of growth regulators for regeneration from leaflets, while 0.4 mg IAA 0.4 mg GA_3 and 0.8 mg Kinetin. Medium containing 5 mg 2, 4-D and 2 mg Kinetin/L was best for callus induction. Cara had the highest ability for callus induction.

Martel and Garcia (1992) cultured potato cv. Sebago discs on MS medium with 30 g/L sucrose, to which 0-4 mg/L BA + 1.0-5.0 mg/L GA_3 were added for the shoot proliferation. Both BA and GA_3 were necessary for shoot formation. Shoot formation occurred more rapidly with higher BA concentration.

Amezqueta *et al.* (1989) studied sub apical meristematic shoot tips, shoot tips from axillary buds and nodal sprouts through cultures in solutions containing various concentration and combination of NAA, IAA Kinetin or GA_3 They observed higher

number of regenerated plants and increased rate of explant growth increased with initial size of explant. The most regenerated plants and fastest growth rates were in MS medium supplemented with 0.5 mg IAA + 0.1 mg GA_3/L.

Smolenskaya (1989) reported that the shoot appears on the fourth day after culturing in Yantarnyi and on the fourteenth day in Prigozhi. Presence of BAP (3 mg/L) in the medium inhibited shoot formation, while presence of GA_3 alone (0.05 mg/L) or in combination with NAA (0.03 mg/L) stimulated shoot formation and also rooting, were occurred a month after subculturing.

Different media for plantlet regeneration were investigated by Resende and Paiva (1986). They recorded that a MS media supplemented with 0.01 mg NAA, 0.1 mg GA and 1 mg BA/L gave better results than MS medium supplemented with 0.4 mg kinetin and 1 mg GA/L. MS medium supplemented with 5 mg BA and 0.5 mg GA/L appeared best shoot growth amongst 5 media assessed. During proliferation and rooting the only medium tested was MS supplemented with 0.01 mg NAA, 0.1 mg GA and 1 mg Kinetin/L and this gave prolific and healthy shoots and good roots development. The best survival rates of cv. were 72.9% for Baronesa and 60% for Santo Amor.

Kwiatkowski *et al.* (1988) reported that trans-zeatin and zeatin riboside enhanced shoot regeneration from stem, tuber and leaf tissues of potato with or without BAP in the media. BAP generally did not induce shoot regeneration unless tissues were transferred to a BAP free medium following initial BAP exposure. This effect was overcome when zeatin was added.

The effect various growth substances on plantlet regeneration from potato explants were studied (Song *et al.,* 1987). When callus was transferred to media with IAA and GA, shoots were formed successfully.

Resende and Paiva (1986) studied 5 potato cultivars for plantlets regeneration and observed that during shoot formation, MS media supplemented with 0.5 mg/L GA and 5.0 mg/L BAP gave better results.

2.1.3 Effect of growth regulators on root formation and regeneration

An experiment was conducted by Sanavy *et al.* (2003) with different concentration of NAA and BAP to observe the best concentrations of auxin and cytokinin for root formation. They observed that the lower concentrations of auxin and cytokinin influenced the formation of root but at higher concentrations (more than 0.5 mg/L) showed reversed tends.

Modarres and Moeini (2003) conducted an experiment with solid MS medium with 0.25 mg/L GA_3 and 0.01 mg/L NAA that sowed significant difference between different pH levels in respect of it s ability to induction of rooting and shooting in plantlets produced from the single node of two potato (*Solanum tuberosum* L.) varieties after subjecting them with thermotherapy. Overall pH 5.5 was the best for all the traits. Low and high level of pH from that 5.5 were found to reduce the growth and rooting of single nodes. The reduction was more pronounced at low levels than high levels pH.

Sidkou *et al.* (2003) in their study observed potato genotypes micropropagated *in vitro* in medium with sucrose at 2-12% and BA at 0-5 mg/L. All cultivars showed 100% regeneration. Regeneration frequency increased with increasing concentration of BA and sucrose.

Sarker and Mustafa (2002) observed that half strength of MS containing 0.1 mg/L IAA, appeared to be the best for root induction from the excised shoots in two indigenous variety namely Lal Pakri and Jam Alu.

Shibili *et al.* (2001) stated that cuttings of 3 cm from glass house grown plantlets were successfully rooted by treating with 1.0 mg/L IBA + 0.5 mg/L IAA for five seconds.

Garcia *et al.* (2001) conducted an experiment with potato meristems cultured in MS media, supplemented with 1 mg GA_3/L. The plant regenerated and transferred in multiplication medium. The regeneration of plants decreased with the reduction in size of the meristems used. Plants regenerated from 0.06 to 1.00 mm meristems were 96% virus free.

Plant regeneration from stem culture of potato cv. Delaware was tested by Ehsanpour and Jones (2000). Results showed that the procedure using thidiazuron, a synthetic cytokinin, was the best for rapid plant regeneration. In these culture medium, several shoots and roots were formed from stem culture, while the other method using a culture medium supplemented with combinations of GA, BAP, NAA, Zeatin, 2ip (isopentyleadenine) and IAA produced white and green calli. Morphology and chromosome number of all regenerated plants were similar to the original plant. Results also showed that the regeneration system was suitable for cv. Delaware and that the culture conditions prevented genetic variation of the regenerated plants.

Rdriguez *et al.* (2000) described a one step regeneration system, using leaf explants of potato cultivars Diacol Capira (DC) and Parda Pastusa (PP). The effect of different ratios of auxins and cytokinins added to MS basal medium (supplemented with 30 g/L sucrose, 0.5 mg/L thiamine, 1.0 mg/L GA_3 40.0 mg/L ascorbic acid and 1.7 mg/L phytagel at pH of 5.7) was investigated. All leaf explants from DC treated with zeatin riboside (3.0 mg/L) and indole 3 acetic acid + IAA (1.00 mg/L) and PP treated with the zeatin riboside (3.0 mg/L) induced regeneration, producing green and morphologically normal plants.

Jatinder *et al.* (2000) observed that the *in vitro* propagation of four potato cultivars (Khufri Badshah, Khufri chandramukhi, Khufri Jyoti, and Khufri Sinduri). Shoot nodal segments were excised, cultured on solid half-strength medium and full strength MS medium. The shoots were rooted in half-strength MS medium with IBA (1.0 mg/L) resulting in 99% rooting with well developed roots. The percent shoot regeneration from cultured nodal segments was highest (98%) in Kufri Jyoti. Shoot establishment was better in half-strength than in full strength solid MS medium in all 4 cultivars. Kufri Sinduri exhibited the best shoot multiplication with the highest number of nodes (5.58) per shoot in liquid MS medium while on solid MS medium, Kufri Jyoti exhibited the highest number of nodes.

Esna-Ashari and Villiers (1998) investigated *in vitro* regeneration ability of the explants of potato cv. Desiree using different combinations and concentrations of

different growth regulators. After 9 weeks callus were transferred in MS media with 1.0 mg /L BAP with addition of 2, 4-D and roots were formed successfully.

Alphonse *et al.* (1998) cultured leaflet explants, tuber discs and callus tissues from micropropagated plantlets of potatoes cv. Cara, Desiree, Diamant and Spunta on MS media supplemented with different growth regulators. Supplementation of MS media with 1.0 mg/L BA, 1.0 mg/L IAA and 10.0 mg/L GA_3 was the best combination of growth regulators for regeneration from leaflets.

In vitro regeneration of plants of potato cv. Aistes from stem segments was studied by Pereyra *et al.* (1998). Explants were cultured on V. Rosenberg media (1) without cytokinins, (2) with 0.001 mg/L kn or (3) with kartolin-2 and kartolin-4 (0.04, 0.08, 0.12, 0.16, or 0.2 mg/L) instead of kinetin. Medium pH was 5.6 in all treatments and cultures were maintained at 20°C, HR 60%, 3500 lux and 16 h photoperiod. Measuremet of stem length and number of roots and leaves were carried out 10, 20 and 30 days after culture initiation. Kinetin affected stem lengthening, but had little effect on root and leaf formation. Better regeneration was observed with kartolin-4, the optimum concentration being 0.04-0.08 mg/L. Plantlet regeneration was best using apical portions of the stem as explants.

Potato plantlets do not require exogenous hormone for rooting. In fact, they can be propagated, elongated and rooted on single hormone-free medium (Vinterhalter *et al.* 1997). As soon as rooted plantlets are obtained, these were transferred to pot in green house.

An experimented was conducted by Badawi *et al.* (1996) to study the effect of 2.0 mg/L IBA, 1.0 mg/L IBA + 1.0 mg/L NAA or 1.0 mg/L IBA + 160 mg/L floroginical on rooting of nodal cuttings. It was observed that supplementing MS media with 2.0 mg/L IBA produced higher number of roots followed by 1.0 mg/L IBA + 1.0 mg/L NAA.

Ao and Liu (1991) conducted an experiment where leaf and stem explants from 6 cultivars were cultured on MS media supplemented with various combinations of growth regulators. Plant regeneration frequency average 60.1-88.7% for the 6 cultivars reaching 162.3% under the most favorable combination of genotype and growth regulators.

Song *et al.* (1987) studied the effect of various growth substances on plant regeneration. Callus from leaf explants on MS medium with 2, 4-D + BAP were transferred to media with IAA and GA_3 and roots were formed successfully.

Media for plant regeneration from explants of 5 potato cultivars were investigated by Resende and Paiva (1986). They observed that during proliferation and rooting MS medium supplemented with 0.01 mg/L NAA, 0.1 mg/L GA_3 and 1.0 mg/L kn gave good root development.

From the above reviews, it appears that different media, culture condition and different concentrations and combinations of growth regulators have remarkable influence on *in vitro* regeneration of potato.

CHAPTER III

MATERIALS AND METHODS

CHAPTET III

MATERIALS AND METHODS

An experiment on potato *In Vitro* regeneration using stem segments and the effect of plant growth regulators (PGRs) NAA, IAA, BAP and GA_3 on regeneration was conducted in the tissue culture laboratory, Biotechnology Department, Sher-e-Bangla Agricultural University (SAU), Dhaka, During the period from January, 2009 to December 2009. Studies were carried out in the following four stages of potato regeneration.

I. *In Vitro* callus initiation from stem segment of three potato varieties (Diamant, Cardinal and Granola) with the supplementation of different concentrations and combinations of NAA and BAP

II. *In Vitro* shoot induction with the supplementation of BAP and GA_3 at different concentrations and combinations.

III. *In Vitro* root formation and regeneration of plantlets through stem segment of three potato varieties (Diamant, Cardinal and Granola)

IV. *Ex vitro* acclimatization and transfer of plantlets to soil

The materials used and the methods followed to carry out these studies are as follows:

3.1 Experimental materials

3.1.1 Plant materials

Shoot tip of the plantlets of potato cv. Diamant, Cardinal and Granola grown *In Vitro* is used as plant material in this experiment.

3.1.2 Sources plant materials

Tubers of *Solanum tuberosum* L. cv. Diamant, Cardinal and Granola were obtained from the Tuber Crop Research Center (TCRC) of Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur.

3.1.3 Culture media

The degree of success of any technology employing cell, tissue and organ culture is related to relatively few major factors. Murashige and Skoog (MS) (1962) medium

were used with the supplementation different plant growth regulators as culture medium for callus initiation, shoot induction, root formation and regeneration of potato plants. The composition of MS medium has been presented in Appendix I. Similar compositions together with concentration of different salt were followed to prepare the medium for the present experiment. Moreover, different plant growth regulators were added separately to the different media according to the treatment combinations. Stock solutions were prepared and stored in the refrigerator at 4±1°C. The respective medium was prepared from the stock solutions. The detail procedures of preparation of media have been furnished bellow.

3.2 Methods

The following culture media were used for the present investigation, each for specific purpose. They were:

1) For sprout germination: Fresh Murashige and Skoog (MS) (1962) medium.
2) For callus initiation: MS medium was supplemented with NAA and BAP.
3) For shoot initiation: MS medium was supplemented with BAP and GA_3.
4) For root initiation: MS medium was supplemented with IAA and GA_3

3.3 Preparation of the stock solutions

The first step in the preparation of stock medium was the preparation of stock solution of the various constituents of the medium. As different media constituents are required in different concentrations, separate stock solutions, for the macronutrients, micronutrients, Fe-EDTA, vitamins, amino acids and growth regulators etc. were prepared separately for just before use.

3.3.1 Stock solution of Macronutrients (Soln. A)

Stock solution of macronutrients was prepared up to 10 folds (10x) of the final strength of the medium in 1000 ml of distilled water (DW). Ten times of the weight of salt required for 1 L of medium were weighed accurately and dissolved thoroughly in 750 ml of distilled water and final volume was made up to o1 L by further addition of distilled water. The stock solution was filtered through Whatmann no. 1 lifter paper to remove all the solid contaminants and solid particles like cellulose, dust, cotton etc. if any. The stock solution was poured in to a clean plastic container and stored in a refrigerator at 4±1°C for instant use.

3.3.2 Stock solution of Micronutrients (Soln. B)

Stock solution of micronutrients was made up to 100 folds (100x) higher the strength of that required concentration of the medium in 1 L of distilled water as described earlier for the stock solution A . The stock solution was filtered, labeled and stored in a refrigerator at 4±1°C for instant use.

3.3.2 Stock solution of Fe-EDTA (Soln. C)

It was made up to 100 folds (100x) the final strength of the medium in 1 L of distilled water. Here, two constituents $FeSO_4.7H_2O$ and Na_2-EDTA were dissolved in 750 ml of distilled water in a conical flask by heating on heater cum magnetic stirrer until salts dissolved completely. Then the volume was made up to 1 L by further addition of distilled water. Finally the stock solution was filtered, labeled and stored by wrapping with aluminum foils in a refrigerator at 4±1°C for later use.

3.3.3 Stock solution of vitamins and amino acids (Soln. D)

The following vitamins and amino acids were used in the present investigation for the preparation of MS medium:

Pyridoxine HCL (Vitamin B_6)

Thiamine HCL (Vitamin B_2)

Myoinositol (Inositol)

Glysine

Nicotinic acid (Vitamin B_3)

Each of the vitamins and amino acids except myoinositol were taken at 100 folds (100x) 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. Finally the stock solution was filtered and stored by wrapping with aluminum foils in a refrigerator at 4±1°C for later use. But the myoinositol was made up separately 100 folds (100x) the final strength of the medium in 1000 ml of distilled water. This stock solution was also filtered, labeled and stored in a refrigerator at 4±1°C for instant use.

3.3.4 Stock solution of growth regulators (Soln. E)

In addition to the nutrients, it is generally necessary to add growth regulators (hormones) such as auxin and cytokinin to the medium to support growth of tissues and organs. Separate stock solutions of hormones were prepared by dissolving the desired quantity of ingredients to the appropriate solvent and made the final volume with distilled water. The following growth regulators were used in the present investigation.

Auxin: α-naphthalene acetic acid (NAA) & Indole acetic acid (IAA)

Cytokinin: 6-benzyle amino purine (BAP)

The hormones were dissolved in proper solvent as shown against them

Plant growth regulators (solute)	**Solvent**
NAA	0.1N NaOH
IAA	0.1N NaOH
BAP	0.1N NaOH
GA_3	01N NaOH

To prepare the stock solutions of any of these hormones, 10 mg of hormone was placed in a small beaker and dissolved in 1 ml of respective solvent for that hormone. Finally the volume of the hormone solution was made 100 ml by adding distilled water using a measuring cylinder. The prepared hormone solution was labeled and stored in a refrigerator at 4±1°C and used for a maximum period of two month.

3.4 Step followed for the preparation of MS medium

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

I. 100 ml of macronutrients, 10 ml of Fe-EDAT, 10 ml of vitamins and 10 ml of myoinositol were taken from each of these stock solutions in to a 2-ltre beaker on a hot plate magnetic stirrer.

II. Five hundred ml of distilled water was added in to the beaker.

III. Thirty gram of sucrose was added to this solution and gently agitated to dissolve completely.

IV. Different concentrations of hormonal supplements as required were added to this solution and were mixed were thoroughly.

V. Since each of hormonal stock solution contained 10 mg of the chemical in 100 ml of the solution, to make 1L of medium addition of 10 ml of stocks of any of the given hormones should result in 1 mg/l concentration of the hormonal supplement. Similarly, addition 0.5, 1, 2, 3, 4, 5, 6, 7, 8 ml of specific hormonal stock solution resulted into 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8 mg/l of the specific hormonal supplement.

VI. The whole mixture was then taken into one litre of measuring cylinder to make the exact volume with the further addition of distilled water and poured back to a one litre conical flask and mixed well with the help of magnetic stirrer.

VII. The pH of the medium was adjusted to 5.8 by pH meter with the help of 0.1N NaOH or 0.1N HCL whichever was necessary.

VIII. To solidify the medium 8gm/l agar was added to the solution. The mixture was then heated in an electric heater cum stirrer with continuous stirring till complete dissolution of agar.

IX. Required volume of hot medium was dispensed into culture vessels or test tubes. After dispensing the medium the test tubes were covered with aluminum foil and marked with different codes by the help of glass marker to indicate specific hormonal supplement.

3.5 Sterilization

3.5.1 Sterilization of culture media

The culture tube containing media was sterilized in an autoclave at temperature of 121°C for 20 minutes at 15psi pressure. The medium was then cooled at room temperature before use. Marking is also necessary.

3.5.2 Sterilization of glassware and instruments

Glassware, culture vessel, beakers, pipettes, plastic caps, other instruments such as forceps, scalpels, needles, spatulas, were wrapped in aluminum foil and sterilized in an autoclave at temperature of 121°C for 20 minutes at 15psi pressure.

3.5.3 Sterilization of culture media and transfer area

Initially, the culture room was cleaned by gentle washing all floors and walls with detergent. This was followed by carefully wiped them with 70% ethyl alcohol. The process of sterilization of culture room was repeated at regular intervals. Laminar Airflow Cabinet was usually sterilized by switching on UV light of the cabinet for 30 minutes and wiping the working surface with 70% ethyl alcohol, 30 minutes before starting the transfer work.

3.5.4 Precautions to ensure aseptic condition

All inoculation and aseptic manipulations were performed out laminar airflow cabinet. The cabinet was usually switched on half an hour before use with ultraviolet light (UV) and wiped with 70 % ethyl alcohol to reduce the chance of contamination. The instruments like scalpels, forceps, needles etc. were pre sterilized by autoclaving and subsequent sterilization was done by dipping on 70% ethyl alcohol followed by flaming and cooling method inside the laminar airflow cabinet while not in use , the instruments were kept inside the laminar airflow cabinet. Hands were also sterilized by wiping with 70% ethyl alcohol. Other material like distilled water, glass plate, Petri dishes etc. were sterilized in an autoclave following method of media sterilization. The neck of culture vessels or test tubes flamed before closing it with the cap. Aseptic conditions were followed during each and every operation to avoid the contamination of cultures.

3.5.5 Preparation of microplants

The surface sterilization of the sprouts of *Solanum tuberosum* L. was carried out under laminar airflow cabinet. For surface sterilization, sprouts were first sterilized with 70% (v/v) ethanol for few seconds. After sterilization, the sprouts were surface sterilized by immersing in 0.1% $HgCl_2$ solution for 15 minutes and then washed several times with sterilized distilled water. The surfaced sterilized sprouts were then kept under sterilized water, which were ready for placement in to the medium.

3.6 Culture method

The following culture methods were employed in the present investigation-

I. Explant culture, II. Subculture and III. Transfer.

3.6.1 Explant culture

3.6.1.1 Preparation of explants

Under aseptic condition, one month old microplants (4 to 5 cm in height) (Plate-1) were taken out from test tubes and placed on a sterilized tile. Leaf and stem segments were cut aseptically into small pieces with the help of sharp aseptic knife and the small segments of stem were used as explant.

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3.3.15

Plate 1. Preparation of micro plant originated from tuber sprouts of potato on MS medium at 30 days after inoculation.

3.6.1.2 Preparation of culture

The explants were prepared carefully under aseptic condition inside the laminar airflow cabinet. Three to five segment of explants were directly inoculated into each test tube containing 10 ml of MS medium supplemented with different callus induction hormonal combinations of NAA and BAP.

3.6.1.3 Culture incubation condition

The expalnt were transferred aseptically to the test tubes containing MS medium with 3% sucrose. The prepared cultures were kept in a growth/culture room on the shelves. All the cultures were kept at 25±2°C illuminated with 1.83m fluorescent tubes (4.33 ft C 84 TDEL/Phillips). These tubes gaves broad spectrum of light, especially the red wave length. The culture room was illuminated 16 h daily with a light intensity of 1500 lux.

3.6.1.4 Maintenance of callus

Callus initiation started after 10-14 days of explant incubation. The development of calli was maintained at 16 h photoperiod at 25±2°C. After four weeks of culture, callus tissues were sub cultured at 21 days interval and finally transferred into the shoot induction media. Plantlets developed from calli in this medium after 14 to 21 days.

3.7 Subculture

3.7.1 Subculture of the callus for shoot regeneration

Four weeks after incubation of explants, the calli attained convenient size. They were then remove aseptically from the test tubes on a sterilized glass vials inside the laminar airflow cabinet and were placed again on freshly prepared sterilized medium containing appropriate hormonal supplements of GA_3 with fixed concentrations of BAP and GA_3 for shoot initiation. The subculturing media used in this present investigation were: MS media containing different combinations of BAP (1.0mg/l) and GA_3 (0.0, 0.05, 0.1, 0.2, 0.4mg/l). The sub cultured test tubes were then incubated at 22±2°C with 16 h photoperiod. After shoot induction more light intensity was given for shoot elongation. The test tubes showing sign on contamination were discarded. Repeated subculture was attended at an interval of

21 days while incubated under the same temperature. The observations were noted regularly.

3.7.2 Subculture of the regenerated shoot for root induction

The subculture calli contained proliferated and differentiated shoots. When these shoots grew about 3-4 cm in length, rescued aseptically from the test tubes, separated from each other and again cultured on another culture vessels with freshly prepared root induction medium (IAA with GA_3) to induce root. The culture vessels containing plantlets were incubated under continuous light. Day to day intended observations were recorded in response.

3.8 Transfer of plantlets to *In Vivo* condition

When the plantlets attained 60 days old with well developed shoots and roots, removed from culture vessels with the help of fine forceps. Attached medium to roots were gently washed out with running tap water. Then the plantlets were kept into the potting mixture containing garden soil and cowdung in the ratio of 1:2:1. 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. To reduce sudden shock, the trays were kept in the controlled laboratory environment and irrigated regularly at an interval of 2 days. The plantlets were established within 5 to 7 days and then polythene bags were removed. Finally, the plantlets appeared to self-sustainable and then transfer to soil in open environment

3.9 Experimental design

The experiments were laid in Completely Randomized Design (CRD) with three replications.

3.10 Collection of Data

3.10.1 *In Vitro* callus initiation and proliferation from stem segment using NAA and BAP at different concentration and combination

To investigate the effect of different level of hormones (NAA and BAP) treatments on callus initiation from stem segments of three potato varieties, the following parameters were recorded.

A. Number of days required for callus initiation

Generally callus initiation started after few days of explants incubation. The mean value of the data provided the days required for callus initiation.

B. Percentage of callus initiation

The percentage of callus initiation was noted after 28 days of inoculation by using the following formula:

$$\text{Percent callus initiation} = \frac{\text{Number of explants induced calli}}{\text{No of explants inoculated}} \times 100$$

C. Weight of callus

Callus weights in grams (g) were recorded at 20, 40 and 60 days after inoculation (DAI) of explants with the help of electrical balance in the laminar flow with proper precaution.

3.10.2 Effect of different concentration of BAP+GA_3 on *In Vitro* shoots induction of three potato varieties.

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

A. Number of days required for shoot induction

Shoot induction started after 14 to 28 days of incubation of explants. The mean value of the data provided the days required for shoot induction.

B. Number of Shoot

The number of shoot proliferated was recorded at 20, 40 and 60 days after inoculation (DAI) and the mean value of the data provided the number of shoot. The mean value was calculated using the following formula:

$$X = \sum X_i/n$$

Where,

$\overline{X}$ = Mean of shoot/explant

$\sum$ = Summation

X_i = Number of shoots/explant

n = Number of observations

C. Shoot length

The length of shoots proliferated was recorded at 20, 40 and 60 days after inoculation (DAI). The mean value of the data provided the shoot length

D. Number of leaves per plantlet

Number of leaves per plantlet was recorded at 20, 40 and 60 days after inoculation (DAI) of explants inoculation. The mean value of the data provided the number of leaves per plantlet.

3.10.3 Effect of different concentration of IAA+GA_3 on *In Vitro* root formation and plant regeneration of three potato varieties.

To investigate the effect of IAA+GA_3 at various concentrations in different varieties for root formation of aseptic plants, data were recorded for the following parameters:-

A. Number of days required for root formation

Root formation was initiated within one week. The mean value of the data provided the days required for root formation.

B. Number of roots per plantlet

Number of roots per plantlet was recorded at 20, 40 and 60 days after inoculation (DAI) of explants inoculation. The mean value of the data provided the number of roots per plantlet.

C. Length of roots

Length of root in cm was recorded at 20, 40 and 60 days after inoculation (DAI) of explants inoculation. The mean value of the data provided the length of roots.

3.10.4 Transfer of plantlets from culture vessels on soil

During the *ex vitro* acclimatization and the establishment of the previously regenerated and sub-cultured plantlets in to the soil, data were collected for the following parameter.

A. Per cent of plantlet establishment

The percentages of established plants were calculated based on the number of plants placed in the cubicles and the number of plants finally established or survived.

The percentages of plantlets established were calculated by using the following formula:

$$\text{Per cent of plantlet establishment} = \frac{\text{Number of established plantlets}}{\text{Total number of plantlets}} \times 100$$

3.11 Statistical analysis of data

The data for the characters under present study were statistically analyzed where applicable. The experiment was conducted in growth chamber and arranged in Completely Randomized Design (CRD). The analyses of variances for different characters were performed and means were compared by the Duncan's Multiple Range Test (DMRT).

CHAPTER IV
RESULTS AND DISCUSSION

CHAPTER IV

RESULTS AND DISCUSSION

Studies were conducted with three cultivated potato varieties Diamant, Cardinal and Granola to examine the effect of NAA, BAP, IAA and GA_3 on plant regeneration (sprout germination, callus initiation, shoot initiation, root initiation) using stem segment as explants. The results obtained from these studies have been presented and discussed separately under the title given below:

4.1 *In Vitro* callus initiation and proliferation from shoot tips of three potato varieties with the supplementation of different concentrations of NAA and BAP

Three potato varieties Diamant, Cardinal and Granola were cultured on MS media supplemented with different concentrations of NAA and BAP. The effect of NAA and BAP for callus initiation and proliferation are presented in Tables 1 – 3 and Plate 2 -7.

4.1.1 Number of days required for callus initiation

The results of major effect of varieties on the number of days required for callus initiation have been presented in Table 1. The number of days required for callus initiation varied significantly among the three varieties. The maximum number of days required for callus initiation was recorded in Granola (9.167 days) where as the minimum number of days required for callus initiation was noticed in Cardinal (8.600 days) and it was also found that there was no significant difference between the varieties Diamant (8.750 days) and Cardinal (8.600 days).

There was significant influence of different hormone combinations and concentrations of NAA and BAP on the number of days required for callus initiation (Table-2). The minimum days (5.444 days) required for callus initiation was observed on MS media with 3.0 mg/l NAA and 1.0 mg/l BAP. The maximum days (16.69 days) were observed for callus initiation on fresh MS media but from that callus no explants was found. So it was observed that MS media with 1.0 mg/l NAA and 0.25 mg/l BAP required maximum days (7.111 days) for callus initiation (Table 2).

The potato varieties and different levels of hormones showed significant interaction in relation to the number of days required for callus initiation. The maximum days (18.33 days) required for callus initiation was noticed on fresh MS media in cv. Granola (Table-3). Among the treatments maximum days (7.333 days) required for callus initiation was noticed at 1.0 mg/l NAA and 0.25 mg/l BAP in cv. Diamant. The minimum number of days required for callus initiation was observed in variety Granola (5.333 days) with 3.0 mg/l NAA and 1.0 mg/l BAP (Table 3).

Table 1: Effect of different varieties on callus initiation and proliferation at different days after inoculation

Variety	Days required for callus initiation	Fresh weight of callus (g)		
		20 DAI	40 DAI	60 DAI
Diamant	8.750b	0.225b	0.925ab	1.642b
Cardinal	8.600b	0.208b	0.858b	1.500c
Granola	9.167a	0.329a	0.991a	1.742a

In column, figure with same letter(s) do not differ significantly at 5% level of significance according to DMRT

Table 2: Effect of plant growth regulators (NAA and BAP) on callus initiation and proliferation at different days after inoculation

Hormone	Days required for callus initiation	Fresh weight of callus (g)		
		20 DAI	40 DAI	60 DAI
Fresh MS media	16.69a	0.000d	0.000d	0.000d
1.0 NAA + 0.25 BAP	7.111b	0.244c	0.911c	1.811c
2.0 NAA + 0.5 BAP	6.111c	0.311b	1.100b	2.189b
3.00 NAA + 1.0 BAP	5.444d	0.461a	1.689 a	2.511a

In column, figure with same letter(s) do not differ significantly at 5% level of significance according to DMRT

Table 3: Combined effect of different varieties and plant growth regulators (NAA and BAP) on callus initiation and proliferation at different days after inoculation

Variety	Hormone	Days required for callus initiation	Fresh Weight of callus (g)		
			20 DAI	40 DAI	60 DAI
Diamant	Fresh MS media	15.33c	0.000e	0.000f	0.000g
	1.0 NAA + 0.25 BAP	7.333d	0.200d	0.833e	1.767e
	2.0 NAA + 0.5 BAP	6.333ef	0.300bc	1.233c	2.200c
	3.00 NAA + 1.0 BAP	6.000fg	0.400b	1.633b	2.600a
Cardinal	Fresh MS media	16.40b	0.000e	0.000f	0.0000g
	1.0 NAA + 0.25 BAP	7.000de	0.200cd	0.900de	1.633f
	2.0 NAA + 0.5 BAP	6.000fg	0.233cd	0.900de	2.000d
	3.00 NAA + 1.0BAP	5.000h	0.400b	1.633b	2.367b
Granola	Fresh MS media	18.33a	0.000e	0.000f	0.000g
	1.0 NAA + 0.25 BAP	7.000de	0.333b	1.000d	2.033d
	2.0 NAA + 0.5 BAP	6.000fg	0.400b	1.167c	2.367b
	3.00 NAA + 1.0BAP	5.333gh	0.583a	1.800a	2.567a

In column, figure with same letter(s) do not differ significantly at 5% level of significance according to DMRT

4.1.2 Weight of callus

There was significant difference in callus weight among the varieties at 20, 40 and 60 DAI. The maximum weight of callus was observed in cv. Granola (0.3292, 0.9917 and 1,742 g at 20, 40 and 60 DAI respectively) whereas the minimum weight of callus was recorded in variety Cardinal (0.208, 0.858 and 1.500 g at 20, 40 and 60 DAI respectively) (Table 1).

The weight of callus was observed to be increased significantly due to the increased level of hormones. The maximum weight of callus (0.461, 1.689 and 2.511 g at 20, 40 and 60 DAI respectively) was recorded at 3.0 mg/l NAA and 1.0 mg/l BAP. On the other hand, 1.0 mg/l NAA and 0.25 mg/l BAP produced the minimum weight of callus (0.244, 0.911 and 1.811 g at 20, 40 and 60 DAI respectively) (Table 2). This result is similar with Gynheung *et al.* (1986) and partially agrees the result of Fiegert *et al.* (2000). But the results of Kollist *et al.* (1994) do not agree with this result. He found that low concentrations of auxin and high concentrations of cytokinin were effective for callus formation.

There was significant interaction effect between the varieties and hormone concentrations on callus weight at 20, 40 and 60 DAI. The highest weight of callus (0.583, 1.800 and 2.567 g at 20, 40 and 60 DAI) was recorded in cv. Granola on MS media supplemented with 3.0 mg/l NAA and 1.0 mg/l BAP and the lowest was (0.2, 0.833 and 1.633 g at 20, 40 and 60 DAI) found in variety Diamant with 1.0 mg/l NAA and .025 mg/l BAP (Table 3). Fomenko *et al.* (1998) also showed that different genotypes reacted differently to the growth regulators in terms of callus formation.

Plate 2. Callus induction of cv. Diamant at 40 DAI on MS media supplemented with 3.0 mg/l NAA and 1.0 mg/l BAP

Plate 3. Callus induction of cv. Diamant at 60 DAI on MS media supplemented with 3.0 mg/l NAA and 1.0 mg/l BAP

Plate 4. Callus induction of cv. Cardinal at 40 DAI on MS media supplemented with 3.0 mg/l NAA and 1.0 mg/l BAP

Plate 5. Callus induction of cv. Cardinal at 60 DAI on MS media supplemented with 3.0 mg/l NAA and 1.0 mg/l BAP

Plate 6. Callus induction of cv. Granulla at 40 DAI on MS media supplemented with 3.0 mg/l NAA and 1.0 mg/l BAP

Plate 7. Callus induction of cv. Granulla at 60 DAI on MS media supplemented with 3.0 mg/l NAA and 1.0 mg/l

4.2. *In Vitro* shoot induction with the supplementation of BAP and GA_3 at different concentrations

For shoot induction three potato varieties Diamant, Cardinal and Granola were cultured on shoot induction media containing different concentrations of BAP and GA_3. The results have been presented in Tables 4-6 and Plate 8-13.

4.2.1 Number of days required for shoot induction

Potato varieties differed significantly in the number of days required for their shoot induction. The maximum number of days required for their shoot induction was observed in variety Granola (16.33 days) where as the minimum number of days required for shoot induction was recorded in variety Cardinal (14.33 days) (Table 4). Ao and Liu (1991) observed the varietal differences of shoot regeneration in potato. Hamdi *et al.* (1998) also reported the similar results.

There was also significant influence of different concentrations of BAP and GA_3 on the number of days required for shoot induction. The maximum number of days required for shoot induction was recorded on fresh MS media (17.63 days) and 1.0 mg/l BAP and 0.1 mg/l GA_3 required minimum number of days (13.11 days) for shoot induction (Table 5).

The combined effect of varieties and hormone combinations on the number of days required for shoot induction has been presented in the Table 6. Here the maximum number of days required for shoot induction was found in variety Granola (18.67 days) on fresh MS media and the minimum number of days required for shoot induction in variety Cardinal (11.33 days) with 1.0 mg/l BAP and 0.1 mg/l GA_3.

Table 4: Effect of different varieties on shoot induction at different days

Variety	Days required for shoot induction	Number of shoot / Callus		
		20 DAI	40 DAI	60 DAI
Diamant	15.26b	1.283a	1.611a	2.056a
Cardinal	14.33c	0.888c	1.172b	1.500b
Granola	16.33a	1.111b	1.661a	2.056a

In column, figure with same letter(s) do not differ significantly at 5% level of significance according to DMRT

Table 5: Effect of different concentrations of BAP and GA_3 on shoot induction at different days

Hormone	Days required for shoot induction	Number of shoot / Callus		
		20 DAI	40 DAI	60 DAI
Fresh MS media	17.63a	0.555d	0.777c	1.111d
1.0 BAP + 0.0 GA_3	15.33c	1.111bc	1.556b	1.889c
1.0 BAP + 0.05 GA_3	14.22d	1.344ab	1.778a	2.222b
1.0 BAP + 0.1 GA_3	13.11e	1.556a	1.889a	2.667a
1.0 BAP + 0.2 GA_3	16.78b	1.000c	1.444b	1.667c
1.0 BAP + 0.4 GA_3	15.78c	1.000c	1.444b	1.667c

In column, figure with same letter(s) do not differ significantly at 5% level of significance according to DMRT

Table 6: Combined effect of different varieties and different concentrations of BAP and GA_3 on shoot induction at different days

		Days required for shoot induction	Number of shoot / Callus		
Variety	**Hormone**		**20 DAI**	**40 DAI**	**60 DAI**
Diamant	Fresh MS media	17.73b	0.666cd	0.666e	1.000f
	1.0 BAP + 0.0 GA_3	16.00d	1.333ab	1.667bc	2.000cd
	1.0 BAP + 0.05 GA_3	15.00ef	1.700a	2.000ab	2.666ab
	1.0 BAP + 0.1 GA_3	15.00ef	1.667a	2.333a	3.000a
	1.0 BAP + 0.2 GA_3	17.67bc	1.000bc	1.333cd	1.667de
	1.0 BAP + 0.4 GA_3	15.67de	1.333 ab	1.667bc	2.000cd
Cardinal	Fresh MS media	16.84c	0.3333d	0.6667e	1.000f
	1.0 BAP + 0.0 GA_3	14.00g	1.000bc	1.333 cd	1.667de
	1.0 BAP + 0.05 GA_3	13.00h	1.000bc	1.333cd	1.667de
	1.0 BAP + 0.1 GA_3	11.33i	1.333ab	1.333cd	2.000cd
	1.0 BAP + 0.2 GA_3	15.67de	0.6667cd	1.000de	1.333ef
	1.0 BAP + 0.4 GA_3	16.00d	1.000bc	1.367cd	1.333ef
Granola	Fresh MS media	18.67a	0.666cd	1.000de	1.333ef
	1.0 BAP + 0.0 GA_3	16.00d	1.000bc	1.667bc	2.000cd
	1.0 BAP + 0.05 GA_3	14.67fg	1.333ab	2.000ab	2.333bc
	1.0 BAP + 0.1 GA_3	16.00d	1.667a	2.000ab	3.000a
	1.0 BAP + 0.2 GA_3	16.00d	1.333ab	2.000ab	2.000cd
	1.0 BAP + 0.4 GA_3	15.67de	0.6667cd	1.300cd	1.667de

In column, figure with same letter(s) do not differ significantly at 5% level of significance according to DMRT

4.2.2 Number of shoots per callus

The number of shoot developed per callus varied significantly among the three potato varieties at 20, 40 and 60 DAI. The highest number of shoot per callus was found in variety Granola (1.238, 1.661 and 2.056 shoots/callus at 20, 40 and 60 DAI respectively) and the lowest number of shoot per callus was recorded in variety

Cardinal (0.889, 1.172 and 1.5 shoots/callus at 20, 40 and 60 DAI respectively). There was no significant difference found for number (2.056 shoots/callus) of shoots per callus in varieties Granola and Diamant at 60 DAI (Table 4).

The number of shoots per callus was significantly influenced at 20, 40 and 60 DAI in different concentrations of BAP and GA3. It was observed that the highest (1.556, 1.889 and 2.667 shoots/callus at 20, 40 and 60 DAI respectively) number of shoots per callus was generated on MS media supplemented with 1.0 BAP mg/l and 0.1 mg/l GA3. The minimum number (0.556, 0.778 and 1.111 shoots/callus at 20, 40 and 60 DAI respectively) of shoots per callus was noted in fresh MS media (Table 5).

The combined effect of varieties and different concentrations of hormones showed significant variation at different days on the number of shoots per callus. The highest number of shoots per callus (1.667, 2.000 and 3.000 shoots/callus at 20, 40 and 60 DAI respectively) was recorded in variety Granola at 1.0 mg/l BAP and 0.1 mg/l GA3 and lowest number of shoot per callus (0.667, 0.667 and 1.000 shoots/callus at 20, 40 and 60 DAI respectively) was generated in variety Diamant on fresh MS media (Table 6).

4.2.3 Shoot length with the supplementation of BAP and GA_3 at different concentrations

For shoot length measurement three potato varieties Diamant, Cardinal and Granola were cultured on shoot induction media containing different concentrations of BAP and GA_3. The results have been presented in Tables 7 – 9 and Figures 14 – 18.

Table 7: Effect of different varieties on shoot length at different days

Variety	Shoot length (cm)		
	20 DAI	**40 DAI**	**60 DAI**
Diamant	2.367a	2.689a	3.156a
Cardinal	2.139b	2.383b	2.933b
Granola	2.100b	2.450b	2.606c

In column, figure with same letter(s) do not differ significantly at 5% level of significance according to DMRT

Three varieties showed significant differences in shoot length. The variety Diamant showed the highest shoot length (2.367, 2.689 and 2.956 cm at 20, 40 and 60 DAI respectively). There were no significant differences of shoot length between varieties Cardinal (2.139 cm and 2.383 cm) and Granulla (2.200 cm and 2.450 cm) at 20 and 40 DAI but at 60 DAI varieties Cardinal and Granola showed significant difference in shoot length. Lowest shoot length (2.100, 2.450 and 2.606 cm at 20, 40 and 60 DAI respectively) was recorded in variety Granulla (Table 7).

Table 8: Effect of different concentrations of BAP and GA_3 on shoot length at different days

Hormone	Shoot length (cm)		
	20 DAI	40 DAI	60 DAI
Fresh MS media	0.488e	0.755d	1.378d
1.0 BAP + 0.0 GA_3	1.967d	2.467c	2.556c
1.0 BAP + 0.05 GA_3	2.611b	2.778b	3.022b
1.0 BAP + 0.1 GA_3	3.267a	3.811a	4.189a
1.0 BAP + 0.2 GA_3	2.400c	2.389c	2.833b
1.0 BAP + 0.4 GA_3	2.678b	2.844b	3.011b

In column, figure with same letter(s) do not differ significantly at 5% level of significance according to DMRT

Different concentrations of growth regulators showed significant differences in shoot length. Concentration 1.0 mg/l BAP and 0.1 mg/l GA_3 along with MS media produced significantly the highest shoot length (3.267, 3.811 and 4.189 cm at 20, 40 and 60 DAI respectively) and lowest value (0.4889, 0.7556 and 1.378 cm at 20, 40 and 60 DAI respectively) recorded on fresh MS media (Table 8). Martel *et al.* (1992) observed that both BAP and GA_3 are necessary for shoot formation occurred more rapidly with the higher concentration of BAP and GA_3.

Table 9: Combined effect of different varieties and different concentrations of BAP and GA_3 on shoot length at different days

		Shoot length (cm)		
Variety	**Hormone**	**20 DAI**	**40 DAI**	**60 DAI**
Diamant	Fresh MS media	0.600i	1.000f	1.500g
	1.0 BAP + 0.0 GA_3	2.100fg	2.600cde	2.833de
	1.0 BAP + 0.05 GA_3	2.833bcd	2.800cd	2.900de
	1.0 BAP + 0.1 GA_3	3.833a	4.432a	4.667a
	1.0 BAP + 0.2 GA_3	2.367ef	2.267e	2.800de
	1.0 BAP + 0.4 GA_3	2.467de	3.033c	3.033de
Cardinal	Fresh MS media	0.533i	0.633f	1.633g
	1.0 BAP + 0.0 GA_3	2.167efg	2.567de	2.700e
	1.0 BAP + 0.05 GA_3	2.500cde	2.600cde	3.167d
	1.0 BAP + 0.1 GA_3	2.867bc	3.500b	4.200b
	1.0 BAP + 0.2 GA_3	2.000g	2.233e	2.900de
	1.0 BAP + 0.4 GA_3	2.767bcd	2.767cd	3.000de
Granola	Fresh MS media	0.333i	0.633f	1.000h
	1.0 BAP + 0.0 GA_3	1.633h	2.233e	2.133f
	1.0 BAP + 0.05 GA_3	2.500cde	2.933cd	3.000de
	1.0 BAP + 0.1 GA_3	3.100b	3.500b	3.700c
	1.0 BAP + 0.2 GA_3	2.833bcd	2.667cde	2.800de
	1.0 BAP + 0.4 GA_3	2.800bcd	2.733cd	3.000de

In column, figure with same letter(s) do not differ significantly at 5% level of significance according to DMRT

The combined effect of different varieties and different concentrations of BAP and GA_3 also showed significant differences. The longest shoot (3.833, 4.433 and 4.667 cm at 20, 40 and 60 DAI respectively) was produced with the treatment concentration of 1.0 mg/l BAP and 0.1mg/l GA_3 supplemented with MS media with the variety Cardinal and the shortest (0.333, 0.633 and 1.000 cm at 20, 40 and 60 DAI respectively) shoot was produced on fresh MS media with the variety Granola.

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Plate 8. Shoot induction from cv. Diamant at 40 DAI on MS media supplemented with 1.0 mg/l BAP and 0.1 mg/l GA_3.

Plate 9. Shoot induction from cv. Diamant at 60 DAI on MS media supplemented with 1.0 mg/l BAP and 0.1 mg/l GA_3

Plate 10. Shoot induction from cv. Cardinal at 40 DAI on MS media supplemented with 1.0 mg/l BAP and 0.1 mg/l GA_3.

Plate 11. Shoot induction from cv. Cardinal at 60 DAI on MS media supplemented with 1.0 mg/l BAP and 0.1 mg/l GA_3.

Plate 12. Shoot induction from cv. Granola at 40 DAI on MS media supplemented with 1.0 mg/l BAP and 0.1 mg/l GA_3.

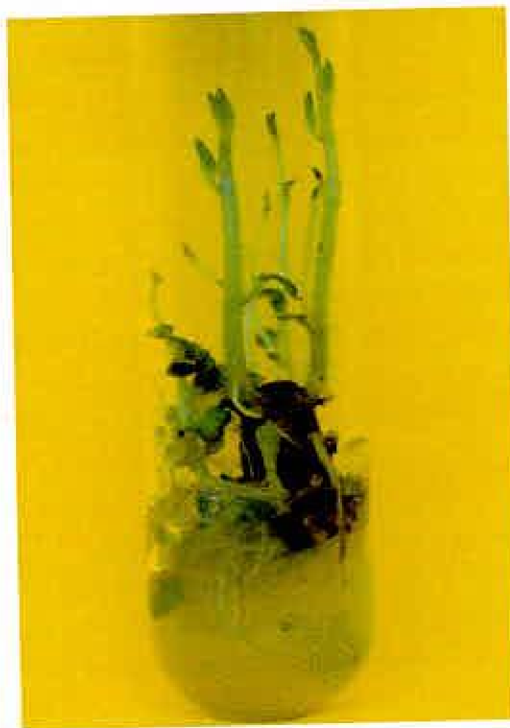

Plate 13. Shoot induction from cv. Granola at 60 DAI on MS media supplemented with 1.0 mg/l BAP and 0.1 mg/l GA

4.3 Number of leaves per plantlet with the supplementation of BAP and GA_3 at different concentrations

Three potato varieties Diamant, Cardinal and Granola were cultured on shoot induction media containing different concentrations of BAP and GA_3 for leaf initiation. The results have been presented in Tables 10-12.

Table 10: Effect of different varieties on Number of leaves per plantlet at different days

Variety	Number of leaves per plantlets		
	20 DAI	40 DAI	60 DAI
Diamant	1.389 a	1.944 a	2.278 b
Cardinal	1.389 a	2.000 a	2.568 a
Granola	1.144 b	1.678 b	2.000 c

In column, figure with same letter(s) do not differ significantly at 5% level of significance according to DMRT

The significant effect of three potato varieties was found in respect of the number of leaves per plantlets at 20, 40 and 60 DAI. The maximum number of leaves per plantlet was showed in variety Cardinal (1.389, 2.000 and 2.568 leaves/plantlets at 20, 40 and 60 DAI respectively) and the minimum number of leaves per plantlet was found in variety Granola (1.444, 1.678 and 0.188 leaves/plantlets at 20, 40 and 60 DAI respectively) (Table 10).

Table 11: Effect of different concentrations of BAP and GA_3 on number of leaves per plantlet at different days

Hormone	Number of leaves per plantlets		
	20 DAI	40 DAI	60 DAI
Fresh MS media	0.555d	0.888c	1.000d
1.0 BAP + 0.0 GA_3	1.444bc	1.889b	2.333bc
1.0 BAP + 0.05 GA_3	1.556bc	1.889b	2.222c
1.0 BAP + 0.1 GA_3	1.889a	2.444a	2.778a
1.0 BAP + 0.2 GA_3	1.667ab	2.444a	2.556ab
1.0 BAP + 0.4 GA_3	1.333c	1.889b	2.222c

In column, figure with same letter(s) do not differ significantly at 5% level of significance according to DMRT

Different concentrations of BAP and GA_3on number of leave also statistically different at different days. The highest number of leaves per plantlet (1.889, 2.444 and 2.778 leaves/plantlet at 20, 40 and 60 DAI respectively) was produced with the concentration of 1.0 mg/l BAP and 0.1 mg/l GA_3. Fresh MS media produced the lowest number of leaves per plantlet (0.556, 0.889 and 1.000 leaves/plantlet at 20, 40 and 60 DAI respectively) (Table 11).

Table 12: Combined effect of different varieties and different concentrations of BAP and GA_3 on Number of leaves per plantlets at different days

		Number of leaves per plantlets		
Variety	**Hormone**	**20 DAI**	**40 DAI**	**60 DAI**
Diamant	Fresh MS media	0.666d	1.000	1.000
	1.0 BAP + 0.0 GA_3	1.333c	1.667	2.333
	1.0 BAP + 0.05 GA_3	1.667bc	2.000	2.333
	1.0 BAP + 0.1 GA_3	2.000ab	2.667	3.000
	1.0 BAP + 0.2 GA_3	1.333c	2.333	2.667
	1.0 BAP + 0.4 GA_3	1.333c	2.000	2.333
Cardinal	Fresh MS media	0.666d	1.000	1.000
	1.0 BAP + 0.0 GA_3	1.333c	2.000	2.333
	1.0 BAP + 0.05 GA_3	1.667bc	2.000	2.333
	1.0 BAP + 0.1 GA_3	2.000ab	2.667	3.000
	1.0 BAP + 0.2 GA_3	1.333c	2.333	2.667
	1.0 BAP + 0.4 GA_3	1.333c	2.000	2.333
Granola	Fresh MS media	0.333d	0.6667	1.000
	1.0 BAP + 0.0 GA_3	1.667bc	2.000	2.333
	1.0 BAP + 0.05 GA_3	1.333c	1.667	2.000
	1.0 BAP + 0.1 GA_3	1.667bc	2.000	2.333
	1.0 BAP + 0.2 GA_3	2.333a	2.667	2.333
	1.0 BAP + 0.4 GA_3	1.333c	1.667	2.000

In column, figure with same letter(s) do not differ significantly at 5% level of significance according to DMRT

The combined effect of varieties and different concentrations of BAP and GA_3 on the Number of leaves per plantlet was statistically significant at 20 DAI but there was no significant difference at 40 and 60 DAI. At 20 DAI the highest numbers of leaves per plantlet were recorded in variety Granola (2.333 leaves/ plantlet) at the concentration of 1.0 mg/l BAP and 0.2 mg/l GA_3. The lowest number of leaves per plantlet was showed in every variety on fresh MS media at 20 DAI (Table 12).

4.4 *In Vitro* root formation and proliferation from shoot tips of three potato varieties with the supplementation of different concentrations of IAA and GA_3

In Vitro plantlet production was promoted by media combination. For the healthy plantlet production three potato varieties Diamant, Cardinal and Granola were cultured on MS media supplemented with different concentrations of IAA and GA3. The effect of IAA and GA_3 for root formation and proliferation are presented in Tables 13 – 15 and Plate 14-16.

4.4.1 Number of days required for Root formation

The results of major effect of varieties on the number of days required for root formation have been presented in Table 13. The number of days required for root formation varied significantly among the three varieties. The maximum number of days required for root formation was recorded in Granola (9.167 days) where as the minimum number of days required for root formation was noticed in Cardinal (17.83 days) and it was also found that there was no significant difference was found among the varieties Diamant (16.89 days) and Cardinal (16.56 days) (Table 13).

There was significant influence of different hormone combinations and concentrations of IAA and GA_3 on the number of days required for root formation. The minimum days (15.33 days) required for root formation was observed on MS media with 1.0 mg/l IAA and 0.05 mg/l GA_3. The maximum days (19.44 days) were observed for root formation on fresh MS media treatment. So it was observed that MS media with 1.0 mg/l IAA and 0.1 or 0.2 mg/l GA_3 requires maximum days (17.44 days) for root formation (Table 14).

The potato varieties and different levels of hormones showed significant interaction in relation to the number of days required for root formation. The maximum days (21.00

days) required for root formation on fresh MS media in cv. Granola. Another two varieties (Cardinal and Diamant) showed the same result. Among the treatment maximum days (17.67 days) required for root formation at 1.0 mg/l IAA and 0.02 mg/l GA_3 in cv. Diamant. The minimum number of days required for root formation was observed in variety Cardinal (14.33 days) with 1.0 mg/l IAA and 0.05 mg/l GA_3 (Table 15).

Table 13: Effect of different varieties on root formation and proliferation at different days after inoculation

Variety	Days required to root formation	Number of roots per plantlets		
		20 DAI	40 DAI	60 DAI
Diamant	16.89 b	1.389 a	1.651 b	1.944 b
Cardinal	16.56 b	1.222 b	1.556 b	1.833 b
Granola	17.83 a	1.389 a	1.978 a	2.111 a

In column, figure with same letter(s) do not differ significantly at 5% level of significance according to DMRT

Table 14: Effect of plant growth regulators IAA and GA_3 on root formation and proliferation at different days after inoculation

Hormone	Days required to root formation	Number of roots per plantlets		
		20 DAI	40 DAI	60 DAI
Fresh MS media	19.44a	0.666d	1.111e	1.333d
1.0 IAA + 0.0 GA_3	16.33c	1.333b	1.556cd	1.778c
1.0 IAA + 0.05 GA_3	15.33d	1.778a	1.889ab	2.444a
1.0 IAA + 0.1 GA_3	17.44b	1.778a	2.111a	2.444a
1.0 IAA + 0.2 GA_3	17.56b	1.444b	1.778bc	2.111b
1.0 IAA + 0.4 GA_3	16.44c	1.000c	1.444d	1.667c

In column, figure with same letter(s) do not differ significantly at 5% level of significance according to DMRT

Table 15: Combined effect of different varieties and different concentrations of IAA and GA_3 on root formation at different days

		Days	Number of roots per plantlets		
Variety	Hormone	required to	20 DAI	40 DAI	60 DAI
Diamant	Fresh MS media	18.33bc	1.000de	1.333c	1.333e
	1.0 IAA + 0.0 GA_3	17.68ef	1.333cd	1.333c	1.667de
	1.0 IAA + 0.05 GA_3	15.67f	2.000ab	2.000b	2.667b
	1.0 IAA + 0.1 GA_3	17.00de	1.667bc	2.000b	2.333bc
	1.0 IAA + 0.2 GA_3	17.67cd	1.667bc	1.667bc	2.000cd
	1.0 IAA + 0.4 GA_3	16.67def	0.666ef	1.333c	1.667de
Cardinal	Fresh MS media	19.00b	0.6667ef	1.333c	1.333e
	1.0 IAA + 0.0 GA_3	17.00ef	1.333cd	1.667bc	2.000cd
	1.0 IAA + 0.05 GA_3	14.33g	1.667bc	1.667bc	2.333bc
	1.0 IAA + 0.1 GA_3	17.00de	1.333cd	1.666bc	1.667de
	1.0 IAA + 0.2 GA_3	16.67def	1.333cd	1.667bc	1.680cd
	1.0 IAA + 0.4 GA_3	16.33ef	1.000de	1.333c	1.667de
Granola	Fresh MS media	21.00a	0.3333f	0.6667d	1.333e
	1.0 IAA + 0.0 GA_3	17.00de	1.333cd	1.667bc	1.667de
	1.0 IAA + 0.05 GA_3	16.00ef	1.667bc	2.000b	2.333bc
	1.0 IAA + 0.1 GA_3	18.33bc	2.333a	2.667a	3.333a
	1.0 IAA + 0.2 GA_3	18.33bc	1.333cd	2.000b	2.333bc
	1.0 IAA + 0.4 GA_3	16.33ef	1.333cd	1.667bc	1.667de

In column, figure with same letter(s) do not differ significantly at 5% level of significance according to DMRT

4.4.2 Number of roots per plantlets

The number of roots per plantlet was recorded after 20, 40 and 60 days of culture, which differed significantly. At 20 days of culture the variety Diamant and Granulla produced maximum number of roots per plantlet (1.389 roots/plantlet) and variety Cardinal produced minimum number of roots per plantlet (1.222 roots/plantlets). With

the progress of time, the number of roots per plantlets increased gradually in all varieties and at 60 days of culture it also became maximum in variety Granola (2.111 roots/plantlet). While the minimum number of roots per plantlets was found in the variety Cardinal (1.833 roots/plantlet) at 60 days of culture.

The main effect of different concentrations of NAA and GA_3 on number roots per plantlet was significant. The highest number of roots (1.778, 2.111 and 2.444 roots/plantlet at 20, 40 and 60 DAI) was produced with 1.0 mg/l IAA and 0.1 mg/l GA_3. Fresh MS media produced the lowest number of roots per plantlet (0.667, 1.111 and 1.333 roots/plantlet at 20, 40 and 60 DAI) (Table 14). This result partially similar to the results of Badawi *et al.* (1996).

The combined effect of variety and different concentrations of IAA and GA3 on the number of root per plantlet showed statistically significant result at 20, 40 and 60 DAI. At 60 days of culture, the highest number of roots per plantlet was noted in the variety Granulla (3.333 roots/plantlet) at the concentration of 1.0 IAA and 0.1 GA3. The lowest number of roots per plantlet (0.333 roots/plantlet) was also recorded in the variety Granola on fresh MS media.

4.4.3 Length of root (cm)

In this study three potato varieties Diamant, *Cardinal* and Granola were cultured on shoot induction media containing different concentrations of IAA and GA_3 for observing the length of root at 20, 40 and 60 days of culture. The results have been presented in Tables 16 – 18.

Table 16: Effect of different varieties on length of root at different days after inoculation

Variety	Length of roots (cm)		
	20 DAI	**40 DAI**	**60 DAI**
Diamant	4.233 a	5.033 a	6.194 a
Cardinal	4.267 a	5.050 a	6.117 a
Granola	3.756 b	4.472 b	5.322 b

In column, figure with same letter(s) do not differ significantly at 5% level of significance according to DMRT

The influence of three potato varieties was significant in respect of length of root at 20, 40 and 60 days of culture. The maximum length of root at 60 days of culture was recorded in the variety Diamant (6.194 cm) but there were no statistical difference with the variety Cardinal (6.117 cm). The minimum root length was found in the variety Granulla (5.322 cm) (Table 16).

Table 17: Effect of plant growth regulators IAA and GA_3 on length of roots at different days after inoculation

Hormone	Length of roots (cm)		
	20 DAI	40 DAI	60 DAI
Fresh MS media	1.533e	1.789d	2.333e
1.0 IAA + 0.0 GA_3	3.878d	5.022c	6.411c
1.0 IAA + 0.05 GA_3	5.378a	6.233a	8.044a
1.0 IAA + 0.1 GA_3	4.989b	5.589b	6.756b
1.0 IAA + 0.2 GA_3	4.256c	4.956c	5.800d
1.0 IAA + 0.4 GA_3	4.478c	5.522b	5.922d

In column, figure with same letter(s) do not differ significantly at 5% level of significance according to DMRT

Length of root was recorded at 20, 40 and 60 days of culture, also differed significantly due to the main effect of different concentrations of IAA and GA_3. The maximum length of roots (8.044 cm) was recorded at the concentration of 1.0 mg/l IAA and 0.05 mg/l GA_3 at 60 days of culture whereas the minimum length of root (2.333 cm) was produced on fresh MS media (Table 17). Sanavy *et al.* (2003) reported that low concentrations of auxin positively influenced root length.

Table 18: Combined effect of different varieties and different concentrations of IAA and GA_3 on length of root at different days

		Length of roots (cm)		
Variety	Hormone	20 DAI	40 DAI	60 DAI
Diamant	Fresh MS media	1.600g	1.733h	2.000j
	1.0 IAA + 0.0 GA_3	4.000e	5.233bcdef	7.567b
	1.0 IAA + 0.05 GA_3	6.000a	7.000a	8.633a
	1.0 IAA + 0.1 GA_3	5.100b	5.667bc	6.767cde
	1.0 IAA + 0.2 GA_3	4.567cd	5.067cdef	6.000fg
	1.0 IAA + 0.4 GA_3	4.133de	5.500bcd	6.200efg
Cardinal	Fresh MS media	1.367g	1.633h	2.233ij
	1.0 IAA + 0.0 GA_3	4.233de	5.833b	6.900cd
	1.0 IAA + 0.05 GA_3	5.900a	6.833a	8.833a
	1.0 IAA + 0.1 GA_3	5.100b	5.667bc	7.100bc
	1.0 IAA + 0.2 GA_3	4.200de	5.000def	5.833fg
	1.0 IAA + 0.4 GA_3	4.800bc	5.333bcdef	5.800fg
Granola	Fresh MS media	1.633g	2.000h	2.767i
	1.0 IAA + 0.0 GA_3	3.400f	4.000g	4.767h
	1.0 IAA + 0.05 GA_3	4.233de	4.867ef	6.667cde
	1.0 IAA + 0.1 GA_3	4.767bc	5.433bcde	6.400def
	1.0 IAA + 0.2 GA_3	4.000e	4.800f	5.567g
	1.0 IAA + 0.4 GA_3	4.500cd	5.733b	5.767fg

In column, figure with same letter(s) do not differ significantly at 5% level of significance according to DMRT

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 IAA and GA_3 at 20, 40 and 60 days of culture. The maximum root length (5.900, 6.833 and 8.833 cm at 20, 40 and 60 DAI) was recorded in the variety *Cardinal* at the concentration of 1.0 mg/l IAA and 0.05 mg/l GA_3. The minimum length of root 1.60, 1.733 and 2.00 cm at 20, 40 and 60 days of culture respectively were found in the variety of Diamant on fresh MS media (Table 18).

Plate 14. Regenerated plantlet from cv. Diamant on MS media supplemented With1.0 mg/l IAA and 0.05 mg/l GA_3 at 60 DAI.

Plate 15. Regenerated plantlet from cv. Cardinal on MS media supplemented with1.0 mg/l IAA and 0.05 mg/l GA_3 at 60 DAI.

Plate 16. Regenerated plantlet from cv. Granola on MS media supplemented with1.0 mg/l IAA and 0.05 mg/l GA_3 at 60 DAI.

4.5 *Ex vitro* acclimatization and establishment of plantlets on soil

After sufficient shoot and root development at 60 days of culture, the small plantlets were taken out from the culture vessel carefully without damaging any roots; Excess agar around the root was washed off by running tape water to prevent further microbial infection. The plantlets were then transplanted in small hole of the plastic tray filled with sterilized soil: sand: cowdung (1:2:1). Immediately after transplantation the plantlets were irrigated with a fine spray of water and the trays along with plantlets were covered with transparent polythene to prevent desiccation and placed in to the plant growth chamber for proper hardening (Plate 17). The highest survival rate 80.0% found in cv. Diamant and lowest survival rate 66.66% found in cv. Granulla. But the variety *Cardinal* does no show significant difference with the variety Diamant. (Table 19).

Table 19: Survival rate of *In Vitro* regenerated plants of three potato varieties

Acclimatization	Variety	No. plants transplanted.	No. of plants survived	Survival rate (%)
Initially small hole of plastic tray at growth chamber	Diamant	30	24	80.00
	Cardinal	30	23	76.66
	Granola	30	20	66.66
Subsequently when moved to soil in open atmosphere	Diamant	24	23	95.83
	Cardinal	23	21	91.30
	Granola	20	15	75.00

After hardening the plantlets were transplanted to soil (Plate 18). As soon as new leaves started to initiate, plants were watered with ordinary tap water. Gradually the plantlets were adapted to the soil. In the open atmosphere plantlets of cv. Diamant gave the highest survival rate 85.83% and lowest were 75.0% in cv. Granola and the variety *Cardinal* does not show any significant difference with the variety Diamant. (Table 19).

Plate 17. *Ex vitro* acclimatization of regenerated plantlets in growth chamber

Plate 18. *In Vivo* establishment of regenerated plantlets in open atmosphere.

CHAPTER V

SUMMARY AND CONCLUSION

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The present experiment was conducted in the tissue culture laboratory, Biotechnology Division, Sher-e-Bangla Agricultural University (SAU), Dhaka, during the period from January, 2009 to December 2009 to regenerate potato plants from stem segments of three potato varieties namely Diamant, Cardinal and Granulla.

The effect of different concentration and combination of NAA and BAP were studied on callus initiation and proliferation of the varieties Diamant, Cardinal and Granola using stem segments as explants in this experiment. The results of this experiment reveals that the number of days required for callus initiation and weight of callus were significantly influenced by varieties and different concentration and combination of NAA and BAP. The maximum number of days required for callus initiation was recorded in Granola (9.167 days) where as the minimum number of days required for callus initiation was noticed in cardinal (8.600 days) and it was also found that there was no significant difference was found among the varieties Diamant (8.750 days) and Cardinal (8.600 days). Among the hormone supplementation or the treatments maximum days (7.333 days) required for callus initiation was noticed at 1.0 mg/l NAA and 0.25 mg/l BAP in cv. Diamant. The minimum number of days required for callus initiation was observed in variety Granola (5.333 days) with 3.0 mg/l NAA and 1.0 mg/l BAP.

The fresh weight of callus increased gradually with increasing DAI in all varieties. The maximum weight of callus was observed in cv. Granola (0.3292, 0.9917 and 1,742 g at 20, 40 and 60 DAI respectively) whereas the minimum weight of callus was recorded in variety Cardinal (0.208, 0.858 and 1.500 g at 20, 40 and 60 DAI respectively. Among the hormone supplementation the maximum weight of callus (0.461, 1.689 and 2.511 g at 20, 40 and 60 DAI respectively) was recorded at 3.0 mg/l NAA and 1.0 mg/l BAP. On the other hand, 1.0 mg/l NAA and 0.25 mg/l BAP produced the minimum weight of callus (0.244, 0.911 and 1.811 g at 20, 40 and 60 DAI respectively. Fresh MS media without hormone did not induce survivable callus initiation.

In order to induce shoot regeneration, calli derived from stem segments of three potato varieties (Diamant, Cardinal and Granola) were cultured on shoot induction medium containing BAP and GA_3 at different concentrations and combinations. When varieties were taken under consideration, all the parameters are influenced. Granola required the maximum time (16.33 days) for shoot induction and cardinal required the minimum time (14.33 days) for shoot induction. In hormonal supplementation the maximum number of days (17.63) required for shoot induction without any hormone and the minimum number of days (13.11) for shoot induction in 1.0 mg/l BAP and 0.1 mg/l GA_3 hormonal combination. In combination the maximum number of days required for shoot induction was found in variety Granola (18.67 days) on Fresh MS media and the minimum number of days required for shoot induction in variety cardinal (11.33 days) with 1.0 mg/l BAP and 0.1 mg/l GA_3.

In case of number of shoots per callus, the highest number of shoot per callus was found in variety Granulla (1.238, 1.661 and 2.056 shoots/callus at 20, 40 and 60 DAI respectively) and the lowest number of shoot per callus was recorded in variety cardinal (0.889, 1.172 and 1.5 shoots/callus at 20, 40 and 60 DAI respectively). There was no significant difference found for number (2.056 shoots/callus) of shoots per callus in varieties Granola and Diamant at 60 DAI. In hormonal supplementation, the highest (1.556, 1.889 and 2.667 shoots/callus at 20, 40 and 60 DAI respectively) number of shoots per callus was generated in MS media supplemented with 1.0 BAP mg/l and 0.1 mg/l GA_3 and minimum number (0.556, 0.778 and 1.111 shoots/callus at 20, 40 and 60 DAI respectively) of shoots per callus was noted in the Fresh MS medias. In case of combined effect of varieties and different concentrations of hormone it is showed that the highest number of shoots per callus (1.667, 2.000 and 3.000 shoots/callus at 20, 40 and 60 DAI respectively) was recorded in variety Granulla at 1.0 mg/l BAP and 0.1 mg/l GA_3 and lowest number of shoot per callus (0.667, 0.667 and 1.000 shoots/callus at 20, 40 and 60 DAI respectively) was generated in variety Diamant on Fresh MS media.

In case of shoot length, highest shoot length (2.367, 2.689 and 2.956 cm at 20, 40 and 60 DAI respectively) found in Diamant and lowest shoot length (2.100, 2.450 and 2.606 cm at 20, 40 and 60 DAI respectively) was recorded in variety Granulla.

Among the treatments, 1.0 mg/l BAP and 0.1 mg/l GA_3 along with MS media produced the highest shoot length (3.267, 3.811 and 4.189 cm at 20, 40 and 60 DAI respectively) and lowest value (0.4889, 0.7556 and 1.378 cm at 20, 40 and 60 DAI respectively) recorded without any hormone in MS media. In combined effect of varieties and different concentration and combination of hormone, the longest shoot (3.833, 4.433 and 4.667 cm at 20, 40 and 60 DAI respectively) was produced with the treatment concentration of 1.0 mg/l BAP and 0.1mg/l GA_3 supplemented with MS media with the variety cardinal and the shortest (0.333, 0.633 and 1.000 cm at 20, 40 and 60 DAI respectively) shoot was produced on fresh MS media with the variety Granola.

In case of number of leaves per plant, the maximum number of leaves per plantlet was showed in variety cardinal (1.389, 2.000 and 2.568 leaves/plantlets at 20, 40 and 60 DAI respectively) and the minimum number of leaves per plantlet was found in variety Granola (1.444, 1.678 and 0.188 leaves/plantlets at 20, 40 and 60 DAI respectively). In hormonal supplementation, highest number of leaves per plantlet (1.889, 2.444 and 2.778 leaves/plantlet at 20, 40 and 60 DAI respectively) found with the concentration of 1.0 mg/l BAP and 0.1 mg/l GA3. Fresh MS media produced the lowest number of leaves per plantlet (0.556, 0.889 and 1.000 leaves/plantlet at 20, 40 and 60 DAI respectively). In case of combined effect, Number of leaves per plantlet was statistically significant at 20 DAI but there was no significant difference at 40 and 60 DAI. At 20 DAI the highest numbers of leaves per plantlet were recorded in variety Granulla (2.333 leaves/ plantlet) at the concentration of 1.0 mg/l BAP and 0.2 mg/l GA3. The lowest number of leaves per plantlet was showed in every variety on Fresh MS media at 20 DAI.

In case of number of days required for root initiation, it was found that maximum number of days required for root formation was recorded in Granola (9.167 days) where as the minimum number of days required for root formation was noticed in cardinal (17.83 days). In hormonal supplementation, maximum days (17.67 days) required for root formation at 1.0 mg/l NAA and 0.02 mg/l GA3 in cv. Diamant and minimum number of days required for callus initiation was observed in variety Granola (16.00 days) with 1.0 mg/l NAA and 0.05 mg/l GA_3. In combined effect, the

highest number of roots per plantlet was noted in the variety Granulla (3.333 roots/plantlet) at the concentration of 1.0 IAA and 0.1 GA_3 and the lowest number of roots per plantlet (0.333 roots/plantlet) was also recorded in the variety Granulla on MS media without hormone.

In case of root length, maximum length of root at 60 days of culture was recorded in the variety Diamant (6.194 cm) and minimum root length was found in the variety Granulla (5.322 cm). In hormonal supplementation, maximum length of roots (8.044 cm) was recorded at the concentration of 1.0 mg/l IAA and 0.05 mg/l GA_3 at 60 days of culture whereas the minimum length of root (2.333 cm) was produced on MS media without hormone. In combined effect, maximum root length (5.900, 6.833 and 8.833 cm at 20, 40 and 60 DAI) was recorded in the variety Cardinal at the concentration of 1.0 mg/l IAA and 0.05 mg/l GA_3 and the minimum length of root 1.60, 1.733 and 2.00 cm at 20, 40 and 60 days of culture respectively was found in the variety of Diamant on fresh MS media.

For acclimatization, plantlets were transplanted from culture media to soil in tray with small hole in the growth chamber, where percentage of survival was highest (80.0%) in cv. Diamant and minimum percentage of survival (66.66%) in Granulla. After transplanting the plantlets from small hole of tray to soil, 95.83% of *in vitro* regenerated plantlets in cv. Diamant and 75.0% in cv. Granola survived in the external garden environment. In case of the variety Cardinal there is no significant difference found in survivability with the variety Diamant.

The results of the present investigation indicated that potato cultivars Diamant, Cardinal and Granola could be successfully micropropagated using 3.0 mg/l NAA and 1.0 mg/l BAP for callus initiation and 1.0 mg/l BAP and 0.1 mg/l GA_3 for successive shoot regeneration and combination of the concentration 1.0 IAA and 0.1 GA_3 for rooting media.

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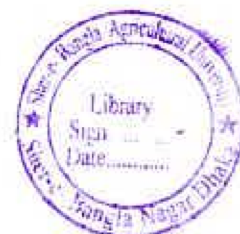
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APPENDICES

APPENDICES

Appendix I: Composition and concentrations used for the preparation of MS medium (Murashige and Skoog, 1962)

Components	Concentrations (mg/L)
Macronutrients	
KNO_3	1900.00
NH_4O_3	1650.00
$MgSO_4.7H_2O$	370.00
$CaCl_2.2H_2O$	440.00
KH_2PO_4	170.00
Micronutrients	
$MnSO_4.4H_2O$	22.30
H_3BO_3	6.20
$ZnSO_4.7H_2O$	8.60
$Na_2MoO_4.2H_2O$	0.25
$CuSO_4.5H_2O$	0.025
$CoCl_2.6H_2O$	0.025
KI	0.83
Iron Sources	
$FeSO_4.7H_2O$	27.80
$Na_2EDTA.2H_2O$	37.30
Vitamin and Organic Nutrients	
Nicotinic Acid	0.50
Pyrodoxin HCl	0.50
Thiamine HCl	0.50
Glycine	2.00
Myo inositol	100.00
Sucrose	3000.00
Agar	7000.00
pH adjusted to 5.8 before autoclaving	

AppendixII

Variety	df	Days required for callus initiation	Fresh Weight of callus (g)		
			20 DAI	40 DAI	60 DAI
Variety	2	1.034*	0.051**	0.053**	0.177**
Hormone	3	250.712**	0.332**	4.410**	11.335**
Interaction	6	2.301**	0.007*	0.030**	0.032**
Error	24	0.224	0.003	0.008	0.006
Total	35				

** Significant at 1% level of probability
* Significant at 5% level of probability

Appendix III

Variety	df	Days required for shoot induction	Number of shoot/Callus		
			20 DAI	40 DAI	60 DAI
Variety	2	23.165**	0.704**	1.302**	1.852**
Hormone	5	17.511**	1.051**	1.363**	2.552**
Interaction	10	2.351**	0.133*	0.207**	0.163*
Error	36	0.250	0.062	0.044	0.074
Total	53				

** Significant at 1% level of probability
* Significant at 5% level of probability

Appendix IV

Variety	df	Shoot length (cm)		
		20 DAI	40 DAI	60 DAI
Variety	2	0.250**	0.465**	0.691**
Hormone	5	8.190**	8.948**	7.381**
Interaction	10	0.312**	0.201**	0.165**
Error	36	0.039	0.055	0.048
Total	53			

** Significant at 1% level of probability
* Significant at 5% level of probability

Appendix V

Variety	df	Number of leaves per plantlets		
		20 DAI	40 DAI	60 DAI
Variety	2	0.019NS	0.241NS	0.463**
Hormone	5	1.896**	2.907**	3.452**
Interaction	10	0.285**	0.152NS	0.063NS
Error	36	0.077	0.137	0.078
Total	53			

** Significant at 1% level of probability
* Significant at 5% level of probability

Appendix VI

Variety	df	Days required to root formation	Number of roots per plantlets		
			20 DAI	40 DAI	60 DAI
Variety	2	7.907**	0.167*	0.241NS	0.352**
Hormone	5	17.930**	1.733**	1.130**	1.807**
Interaction	10	1.041**	0.300**	0.285**	0.419**
Error	36	0.377	0.050	0.106	0.042
Total	53				

** Significant at 1% level of probability
* Significant at 5% level of probability

Appendix VII

Variety	df	Length of roots (cm)		
		20 DAI	40 DAI	60 DAI
Variety	2	1.472**	1.947**	4.194**
Hormone	5	16.606**	22.181**	32.977**
Interaction	10	0.559**	1.048**	1.535**
Error	36	0.062	0.112	0.126
Total	53			

** Significant at 1% level of probability
* Significant at 5% level of probability

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