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Improvement of Potato through Parasexual Methods of Breeding

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IMPROVEMENT OF POTATO THROUGH PARASEXUAL METHODS OF BREEDING

Ph.D Thesis

This Ph.D Thesis submitted to the Department of Botany, University of Rajshahi, as partial Fulfillment of the Requirement for the Degree of Ph.D.

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DECLARATION

I do hereby declare that the Ph.D thesis entitled "**IMPROVEMENT OF POTATO THROUGH PARASEXUAL METHODS OF BREEDING**" has been carried out by me in the Plant Biotechnology and Microbiology Laboratory under the guidance and supervision of Dr. M. Firoz Alam, Professor, Department of Botany, Rajshahi University, Rajshahi is now submitted as a thesis towards the partial fulfillment for the degree of Doctor of philosophy in the Department of Botany, University of Rajshahi, Rajshahi, Bangladesh. I also declare that, I have never submitted the thesis or any part of this thesis for any degree or diploma elsewhere. This is the original work of mine.

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CERTIFICATE

It is my pleasure to certify that the research work (Thesis) presented in this dissertation entitled "**IMPROVEMENT OF POTATO THROUGH PARASEXUAL METHODS OF BREEDING"** is submitted by Molina Begum in partial fulfillment for the requirements of the degree of Doctor of Philosophy in Botany in the field of Biotechnology, to the Department of Botany, University of Rajshahi, Bangladesh. The work or part of it has not been submitted before as candidature for any other.

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ABSTRACT

An efficient *in vitro* technique on meristem culture, micro propagation and plant regeneration via callus induction was developed for potato (*Solanum tuberosum* L.) using three varieties as called Atlanta, Japanese red and Kenne bec. In the present study meristem tips were cultured on Murashige and Skoog (MS) medium, supplemented with different hormonal conditions, i.e. 0.3, 0.4, 0.5 mg/l GA_3; 0.1, 0.5, 1 mg/l BAP; 0.3, 0.4, 0.5 mg/l KIN; BAP1+0.3KIN, 1BAP+0.4KIN, 1BAP+0.5KIN, BAP1+0.3GA_3 BAP1+0.4GA_3, BAP1+ 0.5GA_3 as showed differential effect on in *vitro* propagation of potato. Meristm culture was found to be the best technique for virus elimination among tested potato varieties. Shoot multiplication into MS semisolid basal medium supplemented with different cytokinins, auxins and GA_3 either singly or in combination resulted that media formulation with 0.3 mg/l GA_3 is best for development of complete plantlets and multiplication from meristem tips. Best response was observed in variety Atlanta.

For callus culture and plant regeneration two explants (internodes and leaf segments) were used and 2,4-D (3, 3.5 and 4) NAA(1.5, 2 and 2.5) and NAA+BAP (1+0.5, 0.5+1 and 1+1) levels were applied to MS media for callus induction as well as plantlet regeneration. Ten explants were cultured in each combination. Internode showed better performance in callus induction and plantlet regeneration. The highest percentage (88.88%) of callus was induced with 3mg/l 2,4-D in Atlanta and highest no. of shoot per callus was 3.417 in Atlanta.

The success of plant tissue culture for *in vitro* culture of potato was encouraged by acclimatization of the plantlets in the green house conditions. Regenerated plants were morphologically uniform with normal leaf shape and growth pattern. For callus induction among the three genotypes Atlanta was found to be best. Regeneration of plantlets from internodes derived calli was optimum

when they were transferred on MS medium supplemented BAP 3mg/l+1mg/l NAA and was found most effective in production of shoot.

In vitro evaluations of salinity (NaCl) effects on three potato varieties (Atlanta, Japanese red and Kenne bec) were investigated with four NaCl level (0,25,50 and 75 mM) by using single node explants. The results revealed that the growth of all genotypes was significantly inhibited by increasing salt treatments under *in vitro* condition. Salinity stress gradually depressed plant growth and root development with increased NaCl concentration in MS media. The result indicates that Kenne bec performed better in shoot length and root length than Atlanta and Japanese red. Highest salinity level drastically inhibits root growth in all the cultivars tested. The control and 25 mM NaCl containing MS media did not affect the growth traits of *in vitro* potato plantlets. The control was found superior in growth characterized than rest of the tested NaCl levels.

The result is replicable for tropical, subtropical and for winter conditions specially it can be useful for the researchers, teachers of the different Universities and specially for the ministry of agriculture of Bangladesh.

CONTENTS

CHAPTER IV

LIST OF TABLES

LIST OF FIGURES

ABBREVIATION

BAP	: 6 – Benzyl amino purine
%	: Percentage
µ	: Micron
µM	: Micro mole
2,4-D	: 2,4-dichlorophenoxy acitic acid
0.1 N	: 0.1 Normal
^{0}C	: Degree Celsius
cm	: Centimeter
Cv	: Cultivar (s)
etc.	: Etcetera = And others
e.g.	: Exampli gratia, for example
et al.	: et alile = Other people
Fig.	: Figure
gm	: Gram
GA_3	: Gibberelic acid
HCl	: Hydrochloric acid
$HgCl_2$	: Mercuric chlorid
IBA	: Indole-3 butyric acid
IAA	: Indol-3 acitic acid
i.e.	: Id est = that is
KIN	: 6-furfuryl amino purine (Kinetin)
KOH	: Potassium hydroxide
m	: Meter
mg	: Milligram
mg/l	: Milligram per liter
ml	: Milliliter
mM	: Milli mole
mm	: Millimeter
MS	: Murashige and Skoog (1962) medium

MS_0	: MS Medium without plant growth regulators
min	: Minute
NAA	: α-napthalene acetic acid
No.	: Number
Na_2EDTA	: Sodium salt of ferric ethylene diamine tetra acetate
NaOH	: Sodium hydroxide
NaOCl	Sodium hypochlorite
PGR	: Plant Growth regulator
pH	: Negative logarithm of hydrogen ion (H^+) concentration
TDZ	: Thidiazuron
Var.	: Variety
Viz.	: Videlicet = namely
wt.	: Weight

CHAPTER 1
INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the most important food crops in the world. It is grown in large areas around the world (Haliliglu *et al*., 2002) and ranks the fourth in importance after rice, wheat and maize (Uranbey *et al*., 2004). Potato is a herbaceous dicotyledonous plant that propagated vegetative through tubers; it is a native of South America. Potato is used for human consumption, animal feed and as source of starch and alcohol (Miassar *et al*., 2011).

The estimated requirements of seed potatoes for Bangladesh is about 0.81million tones per annum (Hossain and Hossain, 1999). The average yield of Bangladesh is 11.26t/ha which is much lower than that of many potato growing countries of the world such as Scandinavian countries (49.09t/ha), the Netherlands (44.81t/ha) and USA (46.23t/ha) (FAO, 1999).

Virus and viroid diseases are among the major significant diseases in potato seed production and seed certification. They include: potato leaf roll virus (PLRV, potato virus A (PVA), potato virus M (PVM), virus S (PVS), potato virus X (PVX), potato virus Y (PVY) and potato spindle tuber viroid (PSTVd) (Abou- jawdah *et al.*, 2001). The most common viruses affecting potato throughout the world were (PVY), (PVX) and (PLRV) (Shalaby *et al*., 2002 and Khalid *et al.,* 2000). The presence of viral disease is an important reason attributed to low yield of potato varieties; the yield reduction may be up to 75% caused by the infection of some varieties. As such PVX alone may cause yield reduction of 15-30%; PLRV and some strains of PVY frequently reduce tuber yield by 50- 80% (Mellor *et al.*, 1987).

Plant tissue culture offers an efficient method for production and rapid propagation of pathogen free material and germplasm preservation of plants to overcome this unwanted situation. Tissue culture has a potential value in plant

breeding. It is widely recognized and generally used as an experimental tool for crop improvement (Ali *et al*., 2007).

Diseases free good quality seeds and pathogen free planting materials are produced through tissue culture in the world. But in tropical and subtropical areas like Bangladesh, it is difficult to produce seed tubers of potato due to lack of appropriate storage facilities and transport, as well as the presence of virus diseases (Omidi *et al.,* 2003). Great progress has been made in potatoes for plant regeneration in recent years (Ehsanpour and Jones, 2000; Fiegert *et al*., 2000; Ahan *et al.,* 2001).

Tissue culture techniques have several advantages over traditional propagation methods that are new for our culture which can be transferred into our society. The regeneration of plants by tissue culture method is an important and essential component of biotechnological research and also required for genetic manipulation. A breeding program associated to biotechnological tools depends upon the development of an efficient *in vitro* plant regeneration system (Hossain, 1994).

Plant regeneration become a useful technique and in applied to solve the problems of much agriculture crops. Creation of novel germplasm through techniques of tissue culture. Plant biotechnology has approached an efficient and rapid way for creating new varieties and their reproduction (Dai *et al*., 2000).

Meristem culture has been successfully applied in potato for development of virus free plants and appeared a new venture in obliging virus free potato tuber seeds (Wang *et al*., 2011); an increasing of 40% in potato yield could be obtained after virus free tubers were used. Potato virus free clones with meristem culture methods were also practiced by the different researches (Ebadi *et al.,* 2007; Nagib *et al*., 2003) in the world.

Meristem culture along with thermotherapy has become a powerful and successful tool for virus elimination such as PVX, PVY and PLRV from infected plants (Zaman *et al*., 2001; Faccioli, 2001).

So far limited research has been done for improvement of agronomic practiced in Bangladesh. Now a day in many countries of the world virus free potato plantlets are produced by means of meristem culture and combination of heat treatment and meristem culture to improve the yield (Dhingra and Sangar, 1983). Very little information on improving the potato variety through making them virus free by means of meristem culture is available in Bangladesh. Therefore, it would be logical to apply meristem culture technique to improve the potato varieties.

To date very little work has been reported on the field performance of potato varieties after cleaning from viruses. However, the author Hossain *et al.* (1989) and Hossain (1991) reported, that the indigenous potato varieties after cleaning from viruses became viney and bushy with a tendency of producing on tuber or numerous small sized tubers. This raises the question of urgency of field studies on virus free *in vitro* produced plantlets.

Callus tissue means unorganized proliferative mass cells produced from isolated plant cells, tissues or organs when growth aseptically on artificial nutrient medium in glass vials under controlled experimental conditions. Callus induction is one of the ways to generate somaclonal variation. To generate somaclonal variability, induction and establishment of fast growing embryonic calli are prerequisite. Because, during cell division in artificial condition, different types of abnormalities occur in the genetic constituents, which ultimately contributed to the regenerated plants (Larkin and Scowcroft, 1981; Khatun *et al.,* 2003; Shamima *et al.,* 2003). Plants derived from tissue culture; has been variously referred as somaclones or calliclones or protoclones and variations displayed by such plants are simply called somaclonal variation.

Somaclonal variation commonly appears in plant after tissue culture involving a callus state (Shepard *et al.,* 1980; Larkin and Scowcroft, 1981). As a result a lot of variation may be found in the plants regenerated from single callus population.

This study was undertaken to standardize the protocol for callus induction and plant regeneration for the potato variety of potato, which would be efficient and suitable for the investigation of induced somaclonal variation. Based on the study the influence of auxins and cytokinins on the callus induction and subsequent plant regeneration was observed.

Potato production is hampered due to the attack of virus and bacterial diseases causing huge loss in crop during the cultivation and storage. Biotechnology can contribute to solution of these problems and realized great benefit to potato farmers. The regeneration of plants from cell and tissue culture represent an essential component of biotechnology and have the potential not only to improve the existing cultivars, but also for the generation of novel plants in a comparatively short time compared to conventional breeding.

Salinity tolerance evaluations can be performed during seed germination and early seedling growth stage. Salinity stress decreases the seed germination percentage as well as seedling early rates. Salinity tolerance may be defined as the ability of a plant to grow and complete its life cycle under stressful salt conditions like NaCl or with association of other salts. Soil salinity stress is a critical environmental constraint to crop productivity. Six of the 14 billion ha of arable land available in the world are located in arid and semi –arid areas and of this; about 1 billion ha are affected by excess salt. Although potato has been classified as moderately susceptible to salinity stress (Maas *et al.,* 1977). Plant salt tolerance may vary among cultivars (Bilski *et al*., 1988a; Bilski *et al*., 1988b). Little information is available on salt tolerance of different cultivars, particularly, in potato true seeds (Jefferies, 1996).

The investigation of the reaction to salinity *in vitro* culture condition can prepare the convenient ground for introducing the new cultivars of potatoes (*Solanum tuberosum* L.) that are tolerant to salinity.

Aim and objectives

Author of the research has realized the different development of tissue culture in the world as well as it was studied by her very well about the issue and felt that such development is totally poor in South Asian Resign. So, the interest brought her here to work out gradually and finally it has worked by her with new enthusiasm on it. The study is scientifically new in Bangladesh, which can be drawn important role in our native culture to develop and fulfill the demand of Bangladesh community. Its maximum culture can attract the wider audience scientists as well as the consumers towards the community in time.

Under the current scenario, the present research is conducted with following objectives:

1. Standardization of culture media for primary *in vitro* establishment of isolated meristems from different potato genotypes.
2. Standardization of suitable nutrient media composition and selection of suitable hormonal supplements for rapid shoot induction, elongation and multiplication from the cultured meristems.
3. Development of standard method for the production of virus –free plantlets through meristem culture.
4. Callus induction for developing seasonal variants through plant regeneration.
5. To find the effect of different levels of NaCl on *in vitro* growth traits of 3 potato varieties by using single node cuttings and evaluate salt tolerant varieties effective for further use under salinity conditions.
6. Acclimatization and transplantation of *in vitro* grown plantlets and sowing of micro tuber into soil under field condition.
7. To evaluate several genotypes on their performance and
8. Field evaluation of virus free plantlets.

CHAPTER-II
REVIEW OF LITERATURE

2.1 ORIGIN AND DISTRIBUTION

Potato was unknown most of the areas of the world before 400 years. The potato originated in South America (since the Latin American Civilization was emerged, but was even unknown in the Europe and Asia), likely somewhere in present day Peru, Bolivia and Chile. Potatoes from an important part of Andean culture and the farmers grow many different varieties possessing a remarkable diversity of colors and shapes.

Potatoes were first cultured 14, 000 years ago by the native American population. They were introduced to Europe (Spain) in 1565.In Russia potatoes have been known since the late 17^{th} century. The second part of the 18^{th} century witnessed widely spread cultivation of potatoes in the fields, by the end of the 19^{th} century potatoes were cultivated on over 1.5 000000 (crore) hectares. In St. Petersburg (recently it was called Leningrad) pharmaceutical vegetable Garden potatoes were first cultivated in 1786). The cultivation of potatoes in Russia was great promoted by the decree of the Senate of 1765. The Poland, Germany and the USA have the largest areas sown with potatoes. In the Russian Federation potatoes are cultivated mainly in the Nonchernozem Belt, the Central Black Earth Belt, the Volga Region, Siberia, the Urals and the Far East. In the former Republics of the USSR potatoes are mainly grown in Belorus and in the North and West of the Ukraine. In 2004 the state Register of Breeding Achievements Approved for practical application listed about 180 potato cultivars including 125 varieties for human consumption and 54 general purpose varieties (interactive Agricultural Economical Atlas of Countries).

2.2 BOTANICAL ASPECTS OF POTATO

Basically, Potato is the most productive, common and multi use horticultural vegetable crop. It is also a short duration crop that produces more protein and calories per unit time and per unit of water than any other major plant food crops. The crop has high nutritional value and great yield potential. It provides roughly half of the world's annual production of all root and tuber based foods, making it the leading non cereal crop. It is a part of the diet of half a billion consumers in the developing countries (Ghislain *et al*., 1999).

Indeed, potato belongs to the family solanaceae, a large group containing many species, which typically can produce underground tuber as means propagation (Turner and Evans, 1998). The family solanaceae contains 84 genus and almost 3, 000 species that occur on every vegetated continent of the world. The genus *Solanum* has over 230 wild species, many of which are diploid. Practically, most wild species are self-incompatible, but can from fertile f_1 hybrids between other wild species and with *Solanum tuberosum*. Potato was originated from the cross of cultivated diploid variety *Solanum stenotorun* and wild type diploid variety *Solanum spnsipilum.* It is segmental polyploids. Most (73%) of the species are diploids, with 15% tetraploids, 2% pentaploids and 6% are natural hexaploids (Hawkes, 1992). A basic number of 6 have also been suggested there are five label of poliploidy in the tuber bearing *Solanum.* The commercially cultivated species *Solanum tuberosum* L. is a tetraploid with the chrosome number 24=48 (Poehlman and Borttarkar, 1977).

2.3 IMPORTANCE OF POTATO

In Bangladesh, potato is primarily used as a vegetable, although in many countries of the world it constitutes the staple food and contributes more than 90% of the carbohydrate food source. Millions of tons of potatoes are processed annually in Europe into starch, alcohol, potato meal, flour, dextrose and other products. Some are processed into potato chips, dehydrated mashed

potatoes, French fries and canned potatoes. Large quantities of potatoes in the Netherlands, Ireland, Germany and other countries of Europe are grown specifically for manufacture of alcohol, starch, potato meal or flour, soup and for livestock feeding. Asian countries consume more rice than potato for carbohydrate foods (Das and Khair, 2006).

In Bangladesh, although the principal use of potatoes is to make potato curry along with fish, meat, and eggs, there exists a great diversity in the consumption of potatoes. Notable among potato-based food items are the boiled potato, fried potato, mashed potato, baked potato, potato chop, potato vegetable mix, potato singara, potato chips, french fry etc. In recent years, bakeries and fast food shops have started preparing a wide variety of potato-based food delicacies.

In Bangladesh potato is grown in an area of about 3, 36, 740 acres land. For this purpose about 1, 50, 000 m tons of seed potatoes are necessary. Most of the used seeds were not of high quality. The farmers generally use the tubers they keep for their own consumption as seeds. This results in poor yield in the following season.

Table 1. Area and production of potato during 2007-08 to 2008-09 Potato stat. 2007-08 Potato stat. 2008-09

Potato stat. 2007-08	**Potato stat. 2008-09**
Total Area	Total Area
5, 20, 447 hac.	4, 64, 035 hac
Yield : 17.75 t/hac.	Yield : 14.86 t/hac.
Total Production : 92, 36, 838 ton	Total Production : 68, 94018 ton

Source: DAE, 2009; Uddin *et al.,* 2010

2.4 IMPORTANT DISEASES OF POTATO

Naturally, potato is susceptible to a variety of diseases caused by bacteria, fungi and viruses. Further damage can be inflicted by insects such as 7 aphids, cutworm, Colorado potato beetles and leaf hoppers. Various wild *solanum* species including *S. chacoense* are found to have insect/disease resistances. Disease affects a plant in four main ways; either by causing the premature death of foliage; by stunting growth or reduced growth of foliage; by causing a breakdown in the transport of photosynthates to the tubers; or by causing tubers to rot either during tuber growth or during harvesting (Struik and Wiersema, 1999). Some of the economically significant diseases of potato are describe below:

2.4.1 Potato Virus X (PVX)

Potato virus X is known under a variety of names, including mild mosaic virus, common mosaic virus and potato interveinal mosaic virus (Gleadle, 1992). It is the most widespread of potato viruses and can cause reduced yields, even if the plants appear healthy. This virus is transmitted by infectious sap from leaves and tubers. The infection can then be carried on mechanical planters, cultivating equipment by animals and by contact of sprouts, leaves or roots (Salazar, 1996). Infected cells die very quickly and since the virus can only exist and multiply within living cells, it dies before spreading through the remainder of the plant. If virus does spread within the plant, however, necrotic spots appear on the leaves around the growing point. Necrosis spreads to the stem and the plant dies back from the top. Death from top necrosis takes a few weeks. The tubers from infected, necrotic plants have necrosed eyes or may produce plants that show a general necrosis. The necrotic offspring soon die, though some healthy plants may survive by escape (Gleadle, 1992).Virus can be collected by the application of mineral oil for virus inactivation; use of high quality virus free starting material; use of virus resistant varieties elimination of infection sources within and outside the field and using isolated growing sites (Caldiz *et al.*, 1999).

2.4.2 Potato Virus Y (PVY)

Potato virus Y disease is caused by the virus; potato virus Y (PVY).This virus can cause disease on members of the potato family, pepper, tobacco, datura, dahlia, henbane and goosefoot. The virus may kill plants in severe cases, but may only cause a loss of yield. It is transmitted by aphids in a non-persistent manner (Ruize de Galarreta *et al*., 1998). Symptoms of PVY are chronic and may cause the following changes in the behavior of potato plant (Salazar, 1996):

1) Macroscopic alterations in stems and leaves; colour deviations (vein clearing, mosaic, yellowing); deviations in shape, size or texture (leaf roll, crinkle, leaf deformation, enations), deviations in general appearance of plant (dwarfing, stunting, bunching).

2) Macroscopic alterations in tubers; deviations in shape, texture (tuber elongation, overgrowth, cracking).

These alterations are also associated with small yield loss. If infection occurs late in the season, plants may even show hardly any symptoms at all. Yet a certain proportion of the daughter tubers of virus and when used as seed tubers, they may cause severe secondary symptoms. Viruses are usually not transmitted from one generation to the next when sexual reproduction occurs. control measures for PVY include the use of virus free seed potatoes; use of resistant varieties roughing out of having diseased plants early in the season and disruption of landing behavior of aphids (by using aluminum foil or polypropylene fleece) (Harrewijn *et al*., 1991).

2.4.3 Late Blight {Phytophthors Infedtans (Mont.) De Bary}

It is one of the most serious potato fungal diseases that earned a place in history during the Irish famine. It may spread exceptionally quickly both within a field and from field to field and can destroy a susceptible potato crop in only a few weeks (Ruize de Galarreta *et al.,* 1998). All parts of the plant can be attacked. The first signs are water soaked lesions on the leaves in cool, wet weather. Lesions appear between 3and 7 days after infection, are initially light green,

and then turn brown. These spread to the petioles and stems and in severe cases, whole plants and field may be killed. Infected tubers are irregular and develop lumpy areas of various sizes with a purple to brown skin. The contribution of infected seed tubers to its spread is usually minor, since other sources of inoculums are abundant. The fungus is air-borne, seed borne and soil-borne. It survives on plant debris, refuse heaps or in store (Andersson *et al.,* 1998). Due to the rapid multiplication and spread however, every source of inoculums must be controlled. The spread of blight can be collected by spraying with suitable fungicides (copper based); by sanitation (Destruction of diseased foliage by spraying) preventing spores being washed down to tubers, thus reducing levels of tuber infection and by growing resistant cvs.

2.4.4 Potato Virus S

Potato virus S is a disease cause by potato virus S (PVS). In temperate regions, the only host in potato, although there are other hosts in more tropical climates .This disease was not discovered until the early1950's because the symptoms are very inconspicuous. The disease may cause a slight decrease in yield. It is transmitted by aphids in a non- persistent manner. Some strains of this virus are transmitted only by planting infected tubers.

The plant as must be infected early in the season, because most potatoes become naturally immune as they mature. Most of the potato cultivars show no symptoms. On the others, a slight deepening of the veins occurs. Practically the leaves may be slightly roughtened. The growth of the plant may be more open than normal. Some plants may have mild mottling or bronzing of the leaves, and may have tiny dead spot on the upper surfaces of the leaves. When the leaves are older and shaded, greenish spots may remain as the rest of the leaf turns yellow. Tubers may be smaller in size.

Biotechnology applications deriving from cell biology, molecular biology and molecular genetics differ among crops these are influenced by relative

amenability to cell manipulation and by the nature of their reproduction systems. Potato breeding is slow when compared to other major food crops. Therefore, it is not surprising that several applications of biotechnology have found a particular fit with the potato crop (Ghislain *et al.,* 1999).

Major techniques of potato biotechnology can be divided in to two categories:

1) The propagation, storage and dissemination of virus free plants.
2) The widening of genetic variability and the introduction of novel traits into potato by somaclonal and protoclonal variation, ploidy manipulations, embryo rescue, genetic transformation and somatic hybridization. While progress in the area of biotechnology provides new opportunities for potato breeding, most experts agree that biotechnology based methods will always supplement, but not replace traditional breeding methods.

The idea of plant tissue culture has been derived from the demonstration of cellular totipotency (Schwann, 1839) and (Haberlandt, 1902) first attempted to regenerate plant from single cell on the basis of Schwaan's concept. It has opened a tremendous potentiality for the application of tissue culture. Tissue culture techniques are becoming significantly popular as an alternative means of plant vegetative propagation. During the last three decades plant cell, tissue and organ culture have developed rapidly and become a major biotechnological tool in agriculture, horticulture forestry and industry.

Plant tissue culture is the regeneration of plants from bacteria free plant parts. These are grown on artificial nutritive media under controlled environmental conditions. Tissue culture is essentially vegetative or asexual reproduction. Healthy plants can be grown in the biotechnology which has potentially to deliver virus free plants, enhance propagation rates and enrich genetic diversity in reduced time spans.

2.5 MERISTEM CULTURE

The meristem is a dome of actively dividing cells; about 0.1 mm in diameter and 0.25 mm long and together with one to three youngest leaf primordial (Ramawat, 2000).A plant meristem culture is a unique technique to produce virus free plant. Various reasons attributed to the escape of the meristem invasion are:

- Viruses move readily in a plant body through the vascular system which in meristem is absent,
- A high merabolite activity dividing meristematic cells does not allow virus replication and
- A high endogenous auxin level in shoot apices may inhibit virus multiplication.

In 1952, Morel and Martin isolated 100μm long shoot meristems and cultures them to obtain virus free shoots. Since then the advances made in elimination of virus by meristem culture has become a popular horticultural practice .Meristem tip culture has also enabled plants to be free from other pathogens including viroids, mycoplasma, bacteria and fungi (Walkey, 1978; Bhojawani and Razdan, 1983; Pierik, 1987).

In attempt to recover pathogen-free plants through tissue culture, horticulturist and pathologist have designated the explants (isolate meristem) used for initiating cultures as shoot tips. The isolated meristem can be cultures aseptically on Paper bridge dipping into liquid media and under the appropriate condition it will grow out directly into a small leafy shoot. This *in vitro* technique is called meristem tip culture (Razdan, 1993) or plant meristem culture (Wang and Hu, 1980). Meristem is thus the more accurate term to describe the explants used in most culture is without serious consequence. Some scientists use a more restricted spense to meristem culture on the culturing of the apical dome only (Prakash and Pierik, 1993).

Generally to establish a virus free plant, one can culture the apical dome plus 2 or 4 subjacent primordial leaves. This technique is also valuable for the maintenance of carefully defined stock of specific varieties is disease Free State. Alternately, Quak (1977) postulated that the absence of vascular elements in the meristem is a possible reason, as why the virus concentration is so low in meristem. Mellor and Smith (1977) proposed that are normally available to the cells near the meristematic dome and when such tips are exercised their growth process are temporarily disorganized and that enzymes that are required for one or more steps of viral replication become unavailable thus, interrupting the production of infective virus.

Badoni *et al*. (2009) reported that lower concentration of auxin (0.01mg/l NAA) with Gibberelic acid (0.25mg/lGA_3) is the best for development of complete plantlets and multiplication from meristem tips. Higher concentration of NAA inhibit root and shoot growth (Pennazio and Vecchiati, 1976).

The application of meristem culture either to eliminate virus infection in clonal plants or large scale production of asexual seeling and other horticulture benefits have been discussed in many different publications (Djurdjina *et al*., 1997; Islam and Chowdhury, 1998; Zamman *et al*., 2001).

Huda and Sikdar (2006) observed the growth of meristem was on semisolid MS medium supplemented with 0.05mg/l Kn +1mg/l GA_3. After three weeks, they transferred meristems to MS supplemented with BA, Kn IBA, NAA and IAA singly or in combination for shoot elongation and root initiation. Among different treatments for shoot initiation with elongation were obtained in MS supplemented with 1.0 mg/l BA+0.1mg/lIBA +0.3 mg/l GA_3 .On the other hand, good rooting was observed when 0.5mg/l IBA and 0.1mg/l NAA were used to fortify MS semisolid medium.

Ghaffoor *et al.* (2003) conducted an experiment with three different growth regulators viz. NAA, IAA and IBA with five concentration levels (0, 0.05,

0.15. 0.25 and 0.35 mg/1) on meristem culture of potato for production of virus free plantlets. Maximum plant height, number of nodes and number of leaves per plant was recorded from 0 .15 mg/l NAA, 0.35 mg/1 IBA and 0. 25 mg/l IAA, respectively.

Miassar *et al.* (2011) observed that meristem culture has become a powerful and successful tool for virus elimination from infected plants and has been successfully applied in potato. A total of 70 potato (*Solanum tuberosum*) tubers for each of three cultivars that grown in Jordan were obtained from the Ministry of Agriculture and Subjected to (ELISA) test for detection of virus infection. The percentage of infection with potato virus Y (PVY) was 21.4%, 15.7%, 12.8% for Spunta, Alaska and Safrane cultivars, respectively. Meristem culture was applied on infected potato tubers for all the 3 cultivars. Results of shoots and roots development indicated that medium supplemented with 0.5 mg/l Indole buteric acid (IBA) was the best for shoots length with mean (7.71cm), roots length with mean (9.41cm), number of shoots with mean (2.60) and number of leaves with mean (15.40), ELISA results for *in vitro* produced plantlets showed that all tested plantlets were PVY free. The acclimatization of the plantlets that generated from *in vitro* shoots and roots multiplication stage revealed 90% of successful with Spunta cultivars.

The developed meristems were supplemented with 0.5 mg/l NAA and 0.5 mg/l IBA and on hormone free MS medium. The results of this study showed that the medium supplemented with 0.5 mg/l IBA was the best medium for shoots length with mean (7.71cm), roots length with mean (9.41cm), no of shoots with mean (2.60) and number of leaves with mean (15.40). Using of 0.5 mg/l IBA was most effective for proper shoot and root development from primary developed meristem (Ghaffor *et al.,* 2003).

Elaleen *et al.* (2009) were reported different response of different potato varieties due to genetic make up towards *in vitro* shoot multiplication and their development.

Zaman *et al.* (2001) was evaluated the effect of three different auxins viz. naphthalene acetic acid (NAA), indole acetic acid (IAA) and indole butyric acid (IBA) each of four levels (0, 0.1, 0.5 and 1mgl^{-1}) on meristem culture of potato (*Solanum tuberosum* L.) for production of virus free plantlets. Maximum plantlet height (8.3cm), largest number of nodes/plantlets (7.3) and highest number of leaves/plantlet (8.9) were recorded at 0.5 mgl^{-1} of NAA followed by IBA 1 mgl^{-1} where as extensive number of roots/plantlet (23.7) as well as the earliest micro tuber formation (17days after transplantation) were recorded at 1 mgl^{-1} of IBA followed by NAA at 0.1 mgl^{-1} largest root (4.2cm) was observed for IAA a1 mgl^{-1} concentration. Enzyme Linked Immunosorbent Assay (ELISA) test proved that all plants were free of potato virus X (PVX), potato virus Y (PVY)and potato leaf role virus (PLRV) except one which was positive for potato virus X (PVX).

Xiangsheng (1998) and Yousaf *et al*. (1997) also reported that MS medium supplemented with NAA would be better for maximum number of nodes/plantlet and rapid propagation of virus free seedlings of potato.

Rosenberg *et al*. (2010) observed that regenerated plantlets obtained through virus eradication procedure were preserved as meristem clones *in vitro*. The progeny of each meristem was the basis for meristem clone. The results showed that meristem clones differed in yield, number and weight of tubers and late blight resistance. The research provided new information about the effect of thermotherapy. It was detected deviation from true to type in morphological characteristics of meristem clones.

Pasco *et al.* (2006) studied the variability of potato genotypes was under detailed observation from the disease-resistance point of view. The resistance

to bacterial and fungal diseases enables to produce potato with environmentally friendly methods. Therefore the genotypic reaction of breeding material and old varieties to diseases was studied.

Danci *et al*. (2011**)** were observed that technique of meristem tip culture is one of the most used for *in vitro* culture initiation. It is one of the most used techniques to obtain virus free plantlets. Four Romanian potato cultivars and ten artificial nutritive medium variants were used in this study, in order to establish the best protocol for potato shoots regeneration starting from meristems. The best results have been obtained on PM medium added with 1 mg/l indolyl acetic acid, 1 mg/l indolyl butyric acid, and 0.3 mg/l giberelic acid for the genotype Nicoleta when the meristem was constituted of meristematic dome and four leaf primordia

Ebadi *et al.* (2007) demonstrated that the best media for meristem culture was MS containing 0.1 mgl^{-1} NAA + 0.25 mgl^{-1} GA_3.

Nagib *et al*. (2003) stated that the best media for meristem culture is the MS liquid containing 0.5$mgl^{-1}GA_3$ +0.04 mgl^{-1} KIN and subculture phase the large number of stem, root and the larger stem length was achieved in MS semi-solid, containing 0.5mgl^{-1}IBA+0.5mgl^{-1}BA.

Vanaei and Coworkers (2008) showed that the best medium for meristem culture and in subculture phase was MS containing GA_3 and KIN.

Yasmin *et al*. (2011) evaluated that the meristem tips of two potato cultivars (Desiree and patrones) were excised under aseptic conditions and cultured on Murashige and Skoog (MS) basal medium with nine different treatments of BAP (0.5, 1.0 and 2.0 mg L-1) with NAA (0.5, 1.0 and 2.0 mg L-1) and six different treatments of GA3 (0.25, 0.5, 1.0 mg L-1) with pantothenic acid (1.0 and 2.0 mg L-1). The best regeneration of meristem tips was obtained when MS medium was supplemented with 1.0 mg L-1 pantothenic acid plus 0.5 mg

L-1 gibberellic acid. This combination took minimum time for regeneration of multiple shoots and roots on meristem tips of variety Desiree. It is interesting to report that the combinations of BAP and NAA did not result in complete plantlets formation; most of the explants formed shoots and calli at the base without regenerating roots.

2.6 CALLUS INDUCTION

Callus culture is also one of the ways of *in vitro* propagation; cell and tissue culture are being explored as innovative breeding method for the genetic modification and improvement of plants. These techniques included *in vitro* propagation of desirable genotypes through anther culture, callus culture, protoplast, protoplast fusion and transformation. Callus induction is the way to generate somaclonal variation.

Haque *et al.* (2009) reported *in vitro* callus induction and regeneration from different explants (such as leaf, node, internodes and shoot tip) using various concentrations of 2, 4-D and Kinetin (Kn). Leaf explants appeared to be best callus production when1.0 mg /I 2, 4-D + 0.25 mg/I Kn was used.

Omidi *et al.* (2003) was observed the effect of cultivar and explants on callus induction in leaf and internodes explants of six potato cultivars (viz. Agria, Cosmos Sante, Con cord, Ajix and Diamant. Cultivars, explants and their interaction on frequency of callus induction were significant on MS medium supplemented with 5.0 mg/l 2, 4 -D and 0.25 mg/I Kn.

Many workers have been on potato to enhance callus induction, improve the frequency of plant regeneration from the callus and investigate the factors affection plant regeneration. Both callus induction and plant regeneration from explants require the presence of appropriate concentrations and combinations of plant growth regenerators in the culture media. Many researchers work to standardize the optimum concentrations of growth regulators for regeneration

of potato and consequently great prigress has been made in potato callus induction and plant regeneration (Dobranszki *et al.,* 1999; Hansen *et al.,* 1999; Ehsanpour and jomes, 2000; Fiegert *et al.,* 2000; Khatun *et al*., 2003; Yasmin *et al.,* 2003; Shirin *et al.,* 2007).

Potato is easily regenerated from different explants in MS medium supplemented with different auxin and cytokinin (Yadav and Sticklen, 1995; Cearly and Bolyard; 1997, Rabbani *et al.,* 2001). Improvement of species through genetic engineering depends on *in vitro* regeneration system and high regeneration frequency of plants from tissues and cells. Both callus induction and plants regeneration from explants require the presence of appropriate combination and concentration of plant growth regulators in the culture media (Eapen *et al.,* 1998; Ehsanpor *et al.,* 2000; Fiegert *et al.,* 2000; Ahan *et al.,* 2001).

It may be mention here that several attempts have been taken to establish *in vitro* regeneration protocol for potato. During these attempts a wide variety of explants have been used with the application of several growth regulators to regulate plantlets without intervention of callus. For potato regeneration protocol, many scientists reported that direct shoot regeneration of potato from various explants, such as, shoot apices, leaf discs, nodes and internodes. Hossain *et al.* (2005) studied all of these explants, Juan *et al.* (2004) using leaf explants, Shahpiri *et al.* (2004) used internodes and leaf discs as explants from *in vitro* grown plantlets to investigate the effect of growth regulator concentrations, cultivars and explants on callus induction and shoot regeneration.

Elaleem *et al.* (2009) among all the growth regulators used 2, 4-D was found to be the most effective growth regulator for potato callus induction either when used alone or in combinations with cytokinons (BA). The best result (6.0) for degree of callus formation was obtained when 2, 4-D at 2.0 mg/l was used in combination with BA at the same concentration.

Cytokinins, such as BA and Kinetin, at low concentrations in combination with auxins were often used in plant species to promote callus initiation (Chai and Marian, 1998).

Shirin *et al*. (2007) who used 2,4-D for callus iniduction from intermodal and leaf explants obtained from four potato cultivars including Diamant and found that among all concentrations and combinations of 2,4-D at 3.0 mg/l was found to be the most effective auxin concentration for callus induction in all cultivars.

Badoni *et al.* (2009) reported that direct regeneration of shoots from callus was observed with 60% response on medium containing the combination of BAP and kinetin (0.6+0.6mg/l). Concentrations of 0.6 mg/l BAP induced good amount of compact, light green callus with 40% shoot regeneration, kinetin with 0.4 mg/l concentration was also sowed very good compact callus but poor shoot regeneration was occurred in this concentration.

Doo and Boe (2001) reported leaf discs regeneration on MS medium with various combinations of IAA and Zeatine riboside (ZR). The medium containing 3.5 mg/l IAA and 4.0 mg/l ZR produced the most plantlets.

Potato is easily regenerated from different explants in MS medium supplemented with different auxin and cytokinin (Yadav and Sticklen, 1995; Cearly and Bolyard, 1997; Rabbani *et al.,* 2001). Improvement of species through genetic engineering depends on *in vitro* regeneration system and high regeneration frequency of plants from tissues and cells. Both callus induction and plants regeneration from explants require the presence of appropriate combination and concentration of plant growth regulators in the culture media (Eapen *et al.,* 1998; Ehsanpor *et al.,* 2000; Fiegert *et al.;* 2000; Ahan *et al.,* 2001).

Zeatine riboside (ZR) reduces callus phage and accelerates bud formation (Beaujean *et al.,* 1998). However, BAP and thidiazoron (TDZ) are also used as growth regulator (Ashari and Villiers, 1998). TDZ a substituted N-phenyl urea

(Ricci *et al.,* 2001) has been established as an important regulator for morphogenic responses in a large number of species as well as diverse experimental systems. These responses includes somatic embryogenesis (Saxena *et al.,* 1992; Visser *et al.,* 1992; Murthy *et al.,* 1995), micropropagation (Murch *et al.,* 2000; Fratini and Ruiz 2002; Jones *et al.,* 2007), regeneration and multiple shoot formation (Eapen *et al.,* 1998; Li *et al.,* 2000; Chengalrayan *et al.,* 2001).

Within different concentrations of 2, 4-D when used alone, the highest degree (6.0) for friable yellow callus formation from the tuber segment was recorded in the MS media supplemented with 3mg/l. The result is in agreement with Shirin *et al.* (2007) who used 2, 4-D for callus induction from intermodal and leaf explants obtaining from 4 potato concentrations and combinations 2, 4-D at 3 mg/l was found to be the most effective auxin concentration for callus induction in all cultivars.

2.7 SALINITY EFFECTS

Salinity in agricultural term is the excess of salts above level plant require. The most of the crop plants is to intolerable to high salinity conditions resulting decrease yield.

Homayoun *et al*. (2011) Agria cultivar is less sensitive to salinity stress in comparison with Marphona cultivar. The effect of different salinity concentrations (0, 50, 100, 150 and 200mg/l NaCl *in vitro* culture condition investigated in this research to evaluate their yields to salinity stress. These observations consist of node number, stem diameter, lateral branch number, the number of leaves with less than 0.5 mm, with 0.5-1mm size, with more than 1mm size and the number of micro tuber. With respect to less sensitivity to salinity in Agria cultivar, there is positive and significant correlation between the number of micro tuber and the number of leaves with more than 1mm size that can affect tolerance to salinity stress. In other words, it is possible that the

adjustment of the micro tuber number is related to the number of leaves and nodes in Agria cultivar for tolerant mechanism to salinity.

Tal (1996) during the trail proved that very few scientific studies into the glass on the cultures failed to produce potato plants are resistant to salt. Salt tolerant lines of potatoes grown in environments containing 45 to 60 mm mol sodium chloride by Ochatt *et al*. (1999) were selected.

Sasikala *et al*. (1994) observed that shoot height was more affected than the dry matter accumulation and more proline accumulated in the stem than in the root and leaf. Proline accumulation was either positively (CFL, Desiree, Kennebec, Mariva, Spunta) or negatively correlated) rest of the cvs with protein content, suggesting that the additional proline accumulation was not always due to breakdown of existion protein or at the expense of normal protein synthesis. Accumulation of high levels of sodium and potassium due to salinity in many cultivars suggest the possibility of unregulated ion flow due to the damages to or non development of the root system and membrane destruction in other tissues. Based on both morphological and physiological characters reasonable growth up to 8 gm/l salt, levels of Proline and carbohydrate levels and relatively. Low levels of sodium and potassium accumulation, the commercially used cvs Desiree and Kenne bec are classified as moderately salt tolerant.

Potluri and Prasad (1993) used axillary bud cultures for studing the physiology of salt tolerance in six specially developed at the international potato center (CIP), Lima Peru lowland tropical cultivar of potato like the field grown plants the *in vitro* plantlets can also be sub cultured by nodal cuttings and there was no problem with regeneration after the exposure to stress. The six lowland cultivar of potato exhibited three different types of adjustment and response to salinity. Study on lowland tropical cultivars, proline accumulation in response to salinity under *in vitro* conditions.

Levy *et al*. (1988) in potato, proline accumulation was shown in response to salinity under field conditions in cultivars Alpha, Cara, Desiree, Blanka and Idit and in Multa and Atom alue (Ahmed and Abdallah, 1982). He also reported on greenhouse pot traits of potato cvs.Alpha, Banca, Cara and Desiree growing under NaCl stress (20.5, 34.2 and 51.3 mM) that NaCl stress reduced tuber yield, water and osmotic potentials in leaves and tubers but increased the total tuber dry matter. Cv. Alpha was moderately tolerant, with a 15% tuber dry weight reduction at 34.2mM NaCl.

Jefferies (1996) selecting salt-tolerance plants in potato on the basis of germination and survival of seedlings under 75 or 150 mM NaCl demonstrated that genetic variation exists in salt-tolerance in potato and showed that salinity reduces the germination of potato true seeds.

Asghari-Zakaria *et al*. (2008) reported that increasing salt concentrations reduced the germination percentage as well as germination rate. Germination of most genotypes inhibited after 50 mM salt concentration. True seeds from Picasso cultivar were able to germinate at100 mM, while germination of other genotypes was less than 25% after 50 mM salt concentration. True seeds from Picasso cultivar were able to germinate at 100 mM while germination of other genotypes was less than 25% after 50mM salt concentration.True seeds from Picasso cultivar had the highest germination index and coefficient of velocity of germination time among genotypes at all of salt concentrations. Significant differences were also found in radical and hypocotyls lengths depending on genotype and salt concentration. Enhancemmment of radical length was observed at 50mM salt concentration. Overall, true seeds from Picasso cultivar showed high salt tolerance among other genotypes. Genetic differences in salt-tolerance were apparent in true seeds of potato cultivars.

Sasikala *et al*. (1993) tropical solanum for the response to sea salt was studied.8% of its high concentration and decreases with the growth of shoot tip

necrosis was soluble carbohydrate in the shoot increased with increasing salt. With increasing salt concentration, increasing amount of sodium and potassium in roots were observed in the branches. Positive relationship between performance and micro tuber dry weight ratio of potassium there.

Caldiz (1994) proved that the experimental potato is a plant that Glycovit is very sensitive to increased salinity.

Patricia and Maria (2001) reported potato plants at three concentrations 25, 50 and 100 mmol sodium chloride were grown in hydroponic. After 28 days, differences in their relative growth were observed so that the relative growth rate and accumulation of stored water depended on the amount of potassium and sodium.

Mahmood *et al*. (2002) showed that salinity concentrations 4, 3, 2, 1 percent in four varieties of potato seedlings grown *in vitro* a reduced survival percentage, seedling fresh weight and height and the number is and length of roots has been observed to increase significantly.

The potato plants also respond to other stresses, including heat (Tang *et al*., 2006) and frost (Martinez *et al*., 1996), by activation of their antioxidant enzymes.

Rahname and Ebrahimzadeh (2004) reported that under salt stress, relatively salt-tolerant potato cultivars accumulate more shoot fresh and dry weights than do salt-sensitive cultivars.

Turhan (2005) reported that four independent transgenic genotypes obtained from at 3 levels of NaCl (0%, 0.25%, 0.50%, 0.75% and 1.00% w/v) under glasshouse conditions (*in vivo*). The results revealed that the growth of all genotypes was significantly inhibited by increasing salt treatments under both *in vitro* and in vivo conditions.

Aghaei *et al.* (2008) reported that in two potato cultivtars Kenne bec and Concord the lengths of roots were less severely affected than those of the in shoots by increasing salt treatment.

Aghaei *et al.* (2009) reported that nodal cuttings of the tolerant cultivar, Kenne bec and the sensitive cultivar, Concord were exposed to media without and with 30, 60, 90 or 120mmol/lNaCl, the length and fresh and dry weight of both shoots and roots of Concord showed greater decrease than those of Kenne bec. The decrease in shoot growth was more severe than that of the root for both cultivars.

CHAPTER-III
MATERIALS AND METHODS

3.1 MATERIALS

The present investigation was planned under three main heads: (1) meristem culture for disease elimination (2) callus culture for induction of somaclonal variation (3) effect of different levels of NaCl on *in vitro* growth traits of 3 potato varieties by using single node cuttings and evaluate salt tolerant varieties effective for further use under salinity condition. To conduct the present investigation following materials were used.

3.1.1 Plant Materials

Shoot tips of three potato (*Solanum tuberosum* L.) varieties (Atlanta, Japanese red and Kenne bec) were used in the present investigation. Shoot tips of these potato varieties were collected from 25-30 days old field grown seedlings (A.H.Z. Bioteck Seeds Ltd.nursery, Namo bhadra, Rajshahi, Bangladesh) for meristem isolation and shoot induction. Nodal segments were collected from meristem-derived plantlets for massive plantlet propagation and micro tuber formation. Besides, these internodes and leaves were cultured for callus induction followed by somatic embryogenesis, plantlet regeneration and plantlet formation.

3.1.2 Chemicals

3.1.2.1 Sterilent and surfactant

In the present investigation 95% ethyl alcohol and $HgCl_2$ were used as surface sterilizing agents. Additionally, tween -20 and savlon were used as detergent and surfactant.

All chemical compounds including macro and micro nutrients, organic and inorganic acids, sugar, agar, KOH, $HgCl_2$, 70% ethyl alcohol etc. used in the present study which were the reagent grade products of either BDH, England

or E-mark Germany. The vitamins, amino acids and different growth regulators were the products of Sigma chemical Co.USA.

3.1.2.2 Nutrient medium

In the present investigation MS (Murashige and Skoog, 1962) basal salt formulation was used to conduct all of the experiments.

3.1.2.3 Plant growth regulators

The basal medium was supplemented with various concentrations of different plant growth regulators .The following plant growth regulators were employed for the present investigation.

Auxins: Auxins promote cell enlargement, root induction and callus induction

α -napthalene acetic acid (NAA)

Indole -3-butyric acid (IBA)

Indole-3-acetic acid (IAA)

2,4-Dichlorophenoxy acetic acid (2,4-D)

Cytokinins: Cytokinins promote cell division and shoot initiation

6-benzyl amino purine (BAP)

6-furfuryl amino purine (KIN)

Gibberellin:

Gibberellic acid-3 (GA_3)

3.1.2.4 Equipments

The culture vessels such as test tubes (150x25mm), bottles (12x5cm), measuring cylinders, conical flasks (250ml, 500ml, 1000ml), separating funnels, pipettes, pipette pump, parafilm, cotton plugs , rubber bands, aluminum foils, cotton, fire box, marker pen, tiles, spirit lamp, needle, sharp blade, stereo microscope, scissors , various size of forceps, electronic balance, magnetic stirrer, auto clave, P^H meter, laminar airflow machine etc were used. On the other hand, field materials such as plough, spade, smoothing plane, hoe, ploughshare etc. were also used in the experiment.

3.2 METHODS

Experimental methods used for carrying out this investigation were accomplished through the following steps:

3.2.1 Preparation of Stock Solution for Culture Media

The first step of the preparation of MS (Murashige and Skoog, 1962) culture medium was the preparation of the stock solutions. The various constituents of the respective nutrient medium were prepared into stock solutions for ready use during the preparation of media for different experiments. As different constituents were required in different concentrations, separate stock solutions of macronutrients, micronutrients, organic compounds (vitamins and amino acids) and growth regulators (cytokinin and auxin) etc. were prepared separately.

3.2.1.1 Stock solution A (macronutrients)

The stock solution A was made up to 10 times of final strength of the medium in 1000 ml of distilled water. At first 10 times the weight of the salts present per liter of the medium was weighed accurately, dissolved once at a time and sequentially in 500 ml of distilled water and then made up to 1000 ml stock solution was stored in deep freeze.

3.2.1.2 Stock solution B (micronutrients)

Stock solution B was made up to 10 times the final strength of the medium in 100 ml of distilled water as described for the stock solution A. This stock solution was filtered and stored in a refrigerator at 0ºC(temperature).

3.2.1.3 Stock solution C (iron stock: Fe-EDTA)

It was made 10 times the final strength of the medium in 100 ml of distilled water. Here, two constituents, FeSO4 and NA_2-EDTA, were dissolved separately in distilled water and were cheated for 24 hours at 58ºC by placing it in an incubator. Then the two solutions were mixed and volume was made up

to 100 ml by adding distilled water. The pH of the solution was adjusted at 5.6 and after filtering it was stored at 4ºC in refrigerator.

3.2.1.4 Stock solution D (vitamins and amino acids)

The following vitamins and amino acids were used in the present investigation

Pyridoxine HCl (vitaminB_6)

Thiamine HCl (vitamin B_4)

Nicotinic acid (VitaminB_3)

Glycine

Myoinositol (inositol)

Ten times each of the above mentioned vitamins and amino acids were dissolved separately in distilled water for stock preparation. They were mixed and the volume was made to 100 ml by additional distilled water. The stock solution was stored in a refrigerator at 4ºC.

3.2.1.5 Stock solution E

In addition to the nutrients, it is generally necessary to add one or more growth regulators such as auxin, cytokinin and gibberellic acid to the media to support good growth of tissues and organs (Bhojwani and Razdan, 1983). These growth regulators were dissolved in appropriate solvent as shown against each of them.

Table 2: Preparation of stock solution for growth regulators.

Plant growth regulators	Amount taken (mg)	Dissolving solvent (ml)	Final volume of the of the stock solution with DDW (ml)	Strength of the stock solution (mg/ml)
Auxins				
IAA	10	80% ethanol 1 ml	100	0.1
IBA	10	70% ethanol 1ml	100	0.1
2,4-D	10	70% ethanol 1ml	100	0.1
NAA	10	0.1N KOH 1ml	100	0.1
Cytokinins				
BAP	10	0.1N KOH 1ml	100	0.1
KIN	10	0.1N NaOH 1ml	100	0.1

To prepare stock solution 10 mg of any of the growth regulators was taken in a clean test tube and dissolved in required volume of appropriate solvent. Final volume of the solution was made up to 100 ml by adding double distilled water. Thus stock solutions of all the growth regulators were prepared and stored at 4ºC in refrigerator.

3.2.1.6 Preparation of culture media

The following steps were followed to prepare one liter MS medium according to Appendix 1.

(i) Assembling of the media components

For preparing 1liter MS medium, 20 ml of stock solution 1, 20ml of stock solution 11, 20 ml of stock solution 111, 20 ml of stock solution 1V, 20 ml of stock solution V, 2 ml of stock solution V1 and 2 ml of stock solution V11 in 1 liter flask containing 500 ml distilled water and mixed well.

(ii) Addition of growth regulators

Different concentrations of hormonal supplements were added either singly or in different combinations to the above solution and were mixed thoroughly. Since each hormonal stock solution contained 10g of the chemical in 10ml of solution, the addition of 10 ml of any hormonal stock solution to made one liter of medium results in 1.0 mg/l concentrations of that hormonal supplement. For preparing hormonless MS_0 medium no growth regulators were added.

(iii) pH of the medium

The pH of the medium was generally adjusted 5.8 using p^H meter with the help of 0.1 N KOH or 0.1N HCl whichever as necessary.

(iv) Sucrose

After adjustment of pH, 30 g sucrose was added and the final volume of the mixture was made 1liter by adding distilled water.

(v) Agar

The nutrient media used were of two types, liquid and semi solid. The liquid nutrient medium was made up without addition of any agar and using filter paper bridge in the test tube for supporting the plant material (specially in meristem culture) .The semisolid media were prepared with agar for shoot multiplications, root induction and callus formation. In the present investigation 7-8 g of agar (Carolina Biological supply Co.) was added for one liter of medium. Then the whole mixture was gently heated in micro-oven for 5 minutes, the agar was melted completely and making a clear solution. Care was taken at the time of heating that the solution not be evaporated any way.

(vi) Medium dispensing to culture vessels

Fixed volume of above hot melted medium was transferred into culture vessels like test tubes (150×25mm) and conical flask (250ml) through separating funnel. The culture vessels were plugged with non-absorbent cotton, which

were inserted tightly at the mouth of the culture vessels. The culture vessels were marked to designate specific hormonal supplements.

(vii) Sterilization

Finally the culture vessels containing medium were autoclaves at 15 Ib /sq inch pressure and at the temperature 120-121ºC for 20 minutes to ensure sterilization. Then the vessels with the medium were allowed to cool as vertically and marked with a glass marker pen to indicate specific hormonal supplements and stored in the culture room for ready use.

3.3 CULTURE TECHNIQUE

The following methods were employed in the present investigation for
i) meristem culture and micro propagation of meristem derived plantlets, ii) callus culture followed by plantlet regeneration iii) field establishment of plantlets derived from meristem culture and callus culture iv) Evaluation of salinity effects of three potato varieties.

3.3.1 Explants Collection

Apical shoot tips (1-3cm) of six potato cultivars were excised with the help of sharp blade and collected separately in reagent bottle containing distilled water with few drops of savlon and few drop of tween -20 (poly –oxyetheline sorbitan, monolaurate) and quickly brought to laboratory. The shoot tips were collected from 20-25 days old field grown plants. Then the explants were washed for 3-5 times with gradual change of distilled water.

3.3.2 Surface Sterilization of Explants (Shoot Tips)

The materials were transferred to 250 ml sterilized conical flask. Surface sterilization was carried out by dipping the materials in 0.1% $HgCl_2$ solution with gentle shaking for 1, 2 or 3 minutes. Then the sterilized shoot tips were washed at least 5 times with sterile distilled water immediately to remove all traces of $HgCl_2$.

3.3.3 Inoculation Technique

All inoculation and aseptic manipulations were carried out in a laminar air flow cabinet. The cabinet was switched on for half an hour before use and cleaned with 70% ethyl alcohol to reduce the changes of contamination. All instruments like scalpels, needle, forceps, tiles, Petri dish etc. were covered with aluminium foil and sterilized by steam sterilization method. During working time, these were again sterilized by 70% ethyl alcohol dip and flaming method inside the inoculation chamber. To ensure complete aseptic condition, both hands were also wiped by 70% ethyl alcohol.

3.3.4 Isolation of Meristem and Culture

After surface sterilization, explants materials were laid on the sterile tiles using sterile forceps. Shoot tip was hold in one hand under the stereo- microscope with the help of a pair of forceps and the immature leaves and leaf primordial were snapped with slight pressure from the needle. Then the exposed meristem tips that appeared as a shiny dome were gently isolated with a sharp blade. After deplugging of culture tubes and a singly excised meristem tips (0.1-0.3mm) were carefully placed on the ‘M” shaped filter paper bridge of the culture tubes containing liquid MS medium supplemented with different kinds of growth regulators for the establishment of primary meristem culture. The neck of the tubes were flamed with spirit lamp and then plugged.

3.3.5 Culture Incubation

The inoculated culture tubes were incubated in a growth chamber providing a special culture environment. The tubes were placed on the shelves of a culture rack in the growth chamber. It may be mentioned specially that, all cultures were grown in the growth chamber illuminated by 40 watts white fluorescent tubes. The cultures were maintained a 25º ± 2ºC with light intensity varied from 2000-3000 lux. The photoperiod was maintained generally 16 hours light and 8 hours dark. The culture tubes were checked daily to note the

morphogenic responses of cultured explants for different experiments conducted in the present investigation.

3.3.6 Subculture

After 4 weeks of inoculation of meristem, the primary established meristems those showed morphogenic response were removed aseptically from the culture tubes and transferred into tubes containing agar gelled MS medium supplemented with different growth regulators. During inoculation special care was taken so that the explants must touch the medium equally and do not deep into medium. After 30-35 of culture initiation, the primary established meristem formed shoot and root and well established in the meristem.

3.3.7 Virus Indexing: ELISA Test

The use of various serological methods for rapid detection of plant viruses in *in vitro* grown meristem derived plantlets in quick common now a day in clinical plant virology. It is popularly known as serodiagnosis (Akanda *et al*., 1991a; 1991b; Purcifull and Hiebert 1979; Regenmortel, 1978). Among the various serological methods so far developed, the double antibody sandwich enzyme linked immuno- sorbent assay (DAS-ELISA) is extensively applied for its various merits like high sensitivity, rapidness and reliability over the other methods as reported by Clark and Bar- Joseph (1984). Different samples representing each symptom were tested by DAS-ELISA.

The ELISA test were performed by following steps

1. Each of the polystyrene micro titer plates were coated with γ-globulin (2µg/ml, 200µl/well) in 0.05M carbonate buffer, pH 9.6 containing 0.02% sodium azide and incubated at 37º C for 3hours in an incubator.
2. After then the micro titer plates were kept out of the incubator and washed 3 times with washing buffer. Washing buffer was of phosphate buffer saline (PBS), pH 7.1 with few drops of T-20.

3. Sample plantlets (*in vitro* grown meristem derived plantlets) were taken in a polythene bag and were macerated well with the help of PBS and T-20 in 1:3 ratios. Then the mixture solutions were kept on every grove of the polystyrene micro liter plates and kept in 4ºC temperature for overnight.
4. After 24 hours the micro titer plates were kept out of the refrigerator and the plates were washed for 3 times with PBS-T-20.Then every grove of the plates were block with the 1% carnation non fat milk in 200 µl/well and kept in room temperature for 1hour.Then again washed for 3 times with PBS-T-20.
5. The enzyme conjugated γ- globulin solution (200µl/well) was added in the plate and the plate was then incubated at 37ºC temperature for 4 hours. After then the plate was again washed for 3 times with PBS-T-20.
6. An amount of 200 µl of substrate (P-nitrophenylphosphate, 1mg/ml) in 10% Diethanolamine buffer containing 0.02% sodium azide (pH 9.8) per well was applied following incubation for 3 hours at 30ºC. After 10-30 minutes reaction was held and the positive reaction was visualized by making eyes observing yellow colour development that proved the presence of specified virus and the white or no colour proved the negative result that proved no virus in the sample plants.

3.3.8 Micro Propagation

After 4 weeks of incubation when the plantlets attained a height of 7-9 cm, micro propagation was started. The plantlets of four potato varieties identified as disease-free (checked by ELISA) were used for massive micro propagation. Nodal segments 5-6 mm long were isolated from disease free plantlets having auxiliary bud. Isolated nodes were transferred into 250 ml flasks or test tubes containing agar-solidified medium. The node cutting was placed in such a way that it was in good contact with the surface of the medium but it was not pushed below the surface of the medium. Node cuttings were incubated at

22±2ºC with a 16 hours photoperiod. The developed plantlets were sub cultured as required after every 5-6 weeks.

3.3.9 Field Preparation

The first condition of potato cultivation, that the land should be plain enough. The field was ploughed well four to five times and during ploughing, the soil was pulverized with manure, fertilizer and sand mixed with soil according to need. The bed was prepared after the field was well pulverized. The length of each bed was 3mm and the width was 1mm. The distance between two beds was 50 cm. Before transplanting the plantlets, the field was treated with 1% formaldehyde solution to prevent the soil borne pathogen and covered with polythene sheets for 3-5 days. After 5-6 days, polythene sheets were removed. The entire field was covered with nylon nets in order to prevent viral vectors.

3.3.10 Acclimatization of *in vitro* Grown Plantlets

Well-rooted plantlets from meristem with 3-5 cm height were ready for transplanting into soil. The plantlets grown inside the flask or test tubes were brought out from the growth chamber. After deplugging the culture tubes were kept in the room temperature for 4-5 days to bring them in contact to normal temperature. After 3-5 days of hardening the plantlets were taken out very carefully from the test tubes. Agar attached to the root was gently and carefully washed out under running tap water. Then the plantlets were ready for field.

3.3.11 Transplantation of Meristem Derived Plantlets in Field

The plantlets were transplanted in rows keeping 12 cm space between two plants. The distance between two rows was 20 cm. Care was taken to avoid damage to the roots and to ensure good contact between roots and soil. The plantlets were firstly transferred to small Polly bags or pots containing soil and sand in ratio of 1:1. The pots with the plantlets were kept in sandy place and covered with polythene sheet to prevent sudden desiccation. The soil in pots was sprayed with water everyday to maintain high humidity. The polythene bag

was gradually perforated to expose the plants to outer environment. After 15-25 days plantlets were finally transplanted to field, where they eventually developed into mature plants.

3.4 CALLUS CULTURE AND PLANTLET REGENERATION

3.4.1 Choice of Explants

Explant is a great factor for callus induction. For callus induction. Internodes and leaves of *in vitro* grown plants (developed through meristem culture) were used for induction of callus.

3.4.2 Induction of Callus

To induce callus, explants (internode, leaf) were cultured in MS medium supplemented with different callus inducing substances and incubated in light of 25±2ºC for 3-6 weeks. MS medium was supplemented with different concentrations of 2, 4-D alone or in combination of 2,4-D with KIN and 2,4-D with BA or in combination of NAA with BA. After 21-30 days of incubation in the dark the callus induction frequency was determined. The natures, type, colour of callus were recorded after 3-6 weeks of culture.

3.4.3 Plant Regeneration from Callus

Plants were regenerated by transferring the selected calli in MS semisolid medium supplemented with two kinds of cytokinin alone or in combination with NAA for shoot initiation and multiplication.

The cultures are incubated at 22 ± 2ºC under white light for 16h photoperiod. After 3-6 weeks differentiation shoots and roots differentiation are observed. The number of calli producing shoots and total number of shoots are counted for each treatment. The shoots from selected callus were excised and transferred on MS_0 medium for further growth. The plantlets from each of individual callus were further multiplied by internodes culture using MS_0 medium.

3.5 DATA COLLECTION AND ANALYSIS

3.5.1 Data Collection for *in vitro* Grown Meristem Derived Plantlets

Data were collected using the following parameters and the methods of data collection are given below.

i) Days to shoot initiation.

ii) Percentage of response: Percentage of explants responded was collected using following formula

$$\% \text{ of explants responded} = \frac{\text{Number of explants responded}}{\text{Total number of explants cultured}} \times 100$$

iii) Number of shoots/explants: Number of shoots was counted for each culture after 28 days of incubation and average shoot number was calculated and noted.

iv) Shoot length: After 28 days of culture shoot length was measured in cm scales for each plantlets and average shoot length was collected and recorded.

3.5.1.1 *Data collection for field grown plants*

Data on the following characters were recorded from ten randomly selected plants of each variety.

i) % of survivability
ii) Plant height
iii) Number of leaves/ plant
iv) Tuber weight/plant

The number of plantlets that survived in the field was expressed as percentage. At the time of harvest plant height, numbers of leaves/plant, tuber weight/plant were recorded.

3.5.1.2 Data analysis

LSD analysis and subsequent significant test were performed using software MSTAT (version2.10; Russell, D.Freed, Michigan State University, USA) and expressed as mean ± SEM.

3.5.2 Data Recorded for Callus Derived Plantlets

Data were collected using the following parameters and the methods for data collection given below.

3.5.2.1 For callus induction

i) Percentages of explants induced callus: Explants were cultured in 25×150 mm culture tubes containing media with different concentrations of plant growth regulators for callus induction. After required days of culture percentage of callus induction was calculated using the following formula.

$$\%\ \text{of callus induction} = \frac{\text{Number of explants induced callus}}{\text{Number of cultured explants}} \times 100$$

ii) Quality assessment of callus: After few days of culture the explants started to develop callus in medium with proper growth regulators but colour of callus varied in respect of growth regulators supplements. For this reason, different symbols were used to denote the different colour of callus as given below:

Description of callus colour	Symbols
Yellowish white	YW
Cream	Cr
Creamish white	CrW

3.5.2.2 For shoot induction

a) The percentage of calli induced to develop adventitious shoots were calculated using the following formula:

$$\% \text{ of calli induced shoots} = \frac{\text{Number of calli induced shoots}}{\text{Total no. of calli cultured}} \times 100$$

b) Number of shoots/callus: Number of shoots per callus was computed after required days of culture. Mean number of adventitious shoots per callus was calculated using following formula.

$$\bar{X} = \frac{\sum Xi}{N}$$

Where $\bar{X}$ = Average number of shoots

$\sum$ = Summation

Xi= Total number of shoots

N= Number of observation

c) Length of longest shoots: Length of longest shoot was measured in cm scales for each explant after 30 days of inoculation. Average length of the longest shoot was calculated by using above mentioned formula.

3.5.2.3 Data analysis

LSD analysis and subsequent significant test were performed using software MSTAT (version 2.10; Russell, D. Freed, Michigan State University, USA) and expressed as mean ±SEM.

3.6 EFFECT OF SALINITY

Single node cuttings of potato plantlets derived from meristem culture were subjected to initiate this experiment. Four weeks grown *in vitro* plantlets of Atlanta, Japanese red and Kenne bec (Bangladeshi Indigenous cultivar) were prepared with shoot apex and single nodes (1 cm) was separated and cultured in MS medium having 3% sucrose.0.8% agar with a range of NaCl (0, 25, 50, 75mM) treatments. Potato plantlets grown in high NaCl (75mM) media were recultured in the same concentration NaCl media and NaCl free MS

(Murashige and Skoog 1962) media to check their growth consistence and their tolerance potential *in vitro*.

The cultures were maintained and sub-cultured in a growth chamber under 16:8 h light: dark photoperiod with 15µmol/m per s^{-1} illumination at 25±1°C. *In vitro* grown plants were prepared by sub-culturing with an interval of 3weeks. Single node was transferred to MS media containing 0.25, 50, 5 mM /L NaCl. Three nodal segments were implanted in each culture vessel and four replications of each genotype at each salinity level were employed. After 4 weeks, the physiological parameters including shoot and root length, shoot and root fresh weight were measured. The experiments were repeated threefold.

The data were analyzed by IRRISTAT program and the differences among the treatments were tested with least square difference (LSD) at 5% level of significance.

CHAPTER IV
RESULTS

The present investigation was carried out towards the production of disease free clones through meristem culture, development of somaclonal varients through callus culture and evaluation of salt tolerant genotypes by node cutting of the potato (*Solanum tuberosum* L). The results of the experiments are described under the following heads:

4.1 THE ESTABLISHMENT OF MERISTEM CULTURE OF THREE POTATO VARIETIES

For primary establishment of *in vitro* cultures from field grown explants surface sterilization was essential before inoculation of explants because of the presence of loose contaminants attached to the surface of shoot tips. The surface sterilization was carried out by a trial and error experiments using 0.1% $HgCl_2$ as surface sterilant for disinfection.

4.1.1 Effect of Different Concentrations of GA_3 Singly

This experiment was conducted to observe the effect of different concentrations of GA_3 singly on shoot formation from the cultured meristems. The excised meristems (from sterile shoot tips) were cultured in MS liquid medium supplemented with different concentrations of gibberellins singly.

The data on percentage of meristems responded, number of meristems responded, morphogenic response were recorded on 21 days after initiation of culture and are presented in bellow in the table as follow.

The culture meristems started their initial growth by increasing in size and gradually changed to light green in color within 4 to 10 days.

The statistical results of the isolated meristems of three potato varieties are showed significant differences ($P<0.05$) which were observed among the

varieties and concentrations (Table 3). P values is less than 0.05 ($P<0.05$) were consider as significant.

In all formulations the meristems produced primary shoot but none of the root formation was observed in any formulations used. Among all the treatments most of the cultured meristems showed excellent shoot growth but in few media formulations showed moderate shoot growth and these media were 0.4mg/l GA_3 and 0.5mg/l GA_3 (Table 4). The highest percentages (46.29%) of meristems among the three varieties were recorded in Atlanta followed by 26.85% in Japanese red and the lowest response (22.22%) was noted in Kenne bec (Table 4).

Among the different treatments, the highest 43.51% of explants responded and the highest 5.222 no. of meristem was recorded in GA_3 0.3mg/l followed by 27.77% and 3.333 no. of meristem in 0.4 mg/l GA_3 and the lowest 24.07% of explants responded and 2.889 no. of meristem was noted in 0.5mg/l GA_3.

No significant differences were observed among 0.4 mg/l GA_3 and 0 .5mg/l GA_3 but 0.3mg/l GA_3 and 0.4 mg/l GA_3, 0.5mg/l GA_3 were significantly different from others.

Like different concentrations, mean separation (Table 4) for different varieties shows that the mean no. of explants of Atlanta (5.556) in the top and significantly expressed difference from others. Rest of the varieties shows no .significant differences among themselves. For the percentage of the explants the mean value of Atlanta (46.29%) was significantly different from Japanese red (26.85%) and Kenne bec (22.22%) but no difference was observed between Japanese red and Kenne bec.

Table 3. Statistical results (ANOVA) of survival no. of isolated meristems of three potato varieties at different concentrations of GA_3.

Source of variation	Degree of Freedom	Sum of squares	Mean Square	F Value	Prob.
Replication	2	3.630	1.815	3.4690	0.0560
Variety (V)	2	42.296	21.148	40.4248	0.0000
Concentration (C)	2	27.630	13.815	26.4071	0.0000
V×C	4	4.148	1.037	1.9823	0.1459
Error	16	8.370	0.523	-	-
Total	26	86.074	-	-	-

Table 4. Mean separation on survival of plantlets at different concentrations of GA_3 of three potato varieties.

Source	No. of explants responded	% of explants responded	Morphogenic response	
			Shooting	Rooting
Atlanta	5.556A	46.29A	+++	-
Japanese red	3.222B	26.85B	+++	-
Kenne bec	2.667B	22.22B	++	-
LSD at 5% level	1.252	10.43		
Concentration				
$GA_3$0.3mg/l	5.222A	43.51A	+++	-
$GA_3$0.4mg/l	3.333B	27.77B	++	-
$GA_3$0.5mg/l	2.889B	24.07B	++	-
LSD at 5%level	1.252	10.43		

4.1.2 Effect of Different Concentrations of Cytokinin Singly

This experiment was conducted to evaluate the effect of different concentrations of cytokinins singly on shoot formation from the cultured meristems. The excised meristems (from sterile shoot tips) were cultured in MS liquid medium supplemented with different concentrations of cytokinin only alone (0.1mg/lBAP, 0.5mg/ lBAP and 1mg/l BAP) and (0.3mg/l KIN, 0.4 mg/l KIN and 0.5mg/l KIN). The data on number of meristem and percentage of meristems were recorded after 30 days of culture and are presented in Table 6, Table 8.

The frequency of response and shoot growth was influenced by different types of cytokinins as well as their different concentrations used. The cultured meristems started their initial growth by increasing in size and gradually changed to light green in color within 10-15 days.

The statistical results of isolated meristems of three potato varieties showed the significant differences ($P<0.05$) among the varieties and concentrations (Table 5). P values is less than 0.05($P<0.05$) were consider as significant.

When the media was supplemented with different concentrations of BAP the highest 30.55% of explants responded and the highest 3.667 number of meristem was recorded in 0.1 mg/l BAP followed by 23.14 % of explants and 2.778 number of explants were responded in 0.5 mg/l BAP and the lowest 12.03% of explants and 1.444 number of meristems were noted 1mg/l BAP (Table 6).

Among different varieties the highest percentage (33.33%) of explants responded and the highest 4.00 no. of meristems were recorded in Atlanta, when the media were employed with BAP concentrations followed by 21.29% and 2.556 no. of meristems in Japanese red and the lowest (11.11%) of explants responded and 1.333 number of meristems were recorded in Kenne bec (table-6).

The statistical results of isolated meristems of three potato varieties showed significant differences ($P<0.05$) among the varieties, concentrations and in interaction - V×C (Table 7).

Among the different concentrations of KIN, the highest 31.48% of explants and 3.778 number of meristems were recorded in 0.4 mg/l KIN followed by 30.55% of explants and 3.667 number of meristems were recorded in 0.3mg/l KIN and the lowest 25% explants and 3.00 number of meristems were noted in 0.5 mg/l KIN (Table 8).

When the media were employed with KIN concentrations the highest 42.59% of explants and 5.111 numbers of meristems were recorded in Atlanta followed by 29.63% and 3.556 number of meristems were recorded in Japanese red and the lowest 14.81% and 1.778 numbers of meristems were recorded in Kenne bec (Table 8).

No significant differences were observed among 0.1mg/l BAP, 0.5 mg/l BAP and 0.5 mg/l BAP, 1mg/l BAP for the number of explants and percentage of explants responded (Table 6) but significant differences were observed in 0.1mg/ l BAP and 1mg/l BAP for both parameters. But no differences were in KIN for the number of explants and % of explants (Table 8).

Like different concentrations three varieties shown significant differences among Atlanta and Kenne bec for the number of explants and percentage of explants in BAP. But there were no significant differences among the Atlanta, Japanese red and Japanese red, kenne bec in different concentrations of BAP (Table 6). When the media were supplemented with KIN, significant differences were observed in Atlanta, Japanese red and Kenne bec for the number of explants and percentage of explants (Table 8).

In all the formulations of BAP and KIN the meristems produced primary shoot but no root formation were observed. Among all the treatments most of the

cultured meristems shown excellent shoot growth but in few media formulations being shown moderate shoot growth and these media were 0.5mg/l BAP and 1mg/l BAP. For different concentrations of KIN moderate shoot growth was observed in 0.5mg/l KIN (Table 6, Table 8).

Table 5. Statistical results (ANOVA) of survival no. of isolated meristems of three potato varieties at different concentrations of BAP.

Source of variation	Degree of freedom	Sum of square	Mean square	F value	Prob
Replication	2	1.407	0.704	0.9441	-
Variety (V)	2	32.074	16.037	21.5155	0.0000
Concentration (C)	2	22.519	11.259	15.1056	0.0002
V ×C	4	0.370	0.093	0.1242	-
Error	16	11.926	-	-	-
Total	26	68.296			

Table 6. Mean separation on survival of plantlets at different concentrations of BAP of three potato varieties.

Source	No. of explants responded	% of explants responded	Morphogenic response	
			Shooting	Rooting
Atlanta	4.000A	33.33A	+++	-
Japanese red	2.556AB	21.29AB	+++	-
Kenne bec	1.333B	11.11B	++	-
LSD at 5% level	1.494	12.45		
Concentration				
BAP 0.1 mg/l	3.667A	30.55A	+++	-
BAP 0.5 mg/l	2.778AB	23.14AB	++	-
BAP 1mg/l	1.444B	12.03B	++	-
LSD at 5% level	1.494	12.45		

Table 7. Statistical results (ANOVA) of survival no. of isolated meristems of three potato varieties at different concentrations of KIN.

Source of variation	Degree of freedom	Sum of square	Mean square	F value	Prob
Replication	2	7.630	3.815	16.4800	0.0001
Variety (V)	2	50.074	25.037	108.1600	0.0000
Concentration (C)	2	3.185	1.593	6.8800	0.0070
V ×C	4	4.148	1.037	4.48800	0.0128
Error	16	3.704	0.231	-	-
Total	26	68.741			

Table 8. Mean separation on survival of plantlets at different concentrations of KIN of three potato varieties.

Source	No. of explants responded	% of explants responded	Morphogenic response	
			Shooting	Rooting
Atlanta	5.111A	42.59A	+++	-
Japanese red	3.556B	29.63B	++	-
Kenne bec	1.778C	14.81C	++	-
LSD at 5% level	0.8319	6.939		-
Concentration				-
KIN 0.3 mg/l	3.667A	30.55A	+++	-
KIN 0.4 mg/l	3.778A	31.48A	+++	-
KIN0. 5mg/l	3.000A	25.00A	++	-
LSD at 5% level	0.8319	6.939		

4.1.3 Effect of Different Concentrations and Combinations of Cytokinin and Gibberilin.

In this experiment isolated meristems of three potato varieties viz. Atlanta, Japanese red and Kenne bec were cultured separately on M shaped filter paper bridge in culture tubes containing liquid MS medium supplemented with selected combinations 1mg/l BAP+0.3mg/l GA_3, 1mg/lBAP+0.4 mg/l GA_3 and 1mg/lBAP+0.5mg/l GA_3 and 1mg/l BAP+0.3mg/l KIN, 1mg/lBAP+0.4 mg/l KIN and 1mg/lBAP+0.5mg/l KIN of Plant growth regulators to find out the response of 3 potato varieties. The data on percentage of meristems responded, morphogenic response and degree of shoot growth was recorded after 21 days of culture and are presented in Table 10, table -12.

The statistical results of isolated meristems of three potato varieties showed significant differences ($P<0.05$) were observed among variety and concentration, but in replication ($P>.05$) and interaction cases - V×C, ($P>.05$) non significant differences were observed (Table - 9).

In different concentrations and combinations of BAP and KIN, the highest 36.11% of explants and 4.333 number of meristem were observed in 1mg/lBAP+0.3mg/l KIN followed by 27.77% and 3.333 number of meristem in 1mg/l BAP+ 0.4mg/l KIN and 25% of explants and 3.00 number of meristem were observed in 1mg/lBAP and 0.5 mg/l KIN (Table -10).

No significant difference was observed between 1mg/lBAP+0.3mg/l KIN, 1mg/lBAP+0.4mg/l KIN and 1mg/lBAP+ 0.4mg/lKIN, 1mg/lBAP+0.5mg/l KIN for both parameters. But the significant differences were observed in 1mg/lBAP+0.3mg/l KIN, 1mg/lBAP+0.5mg/l KIN (Table - 10).

Like different concentrations, no significant differences were observed in Atlanta, Japanese red and Kenne bec, Japanese red in different concentrations of BAP +KIN. On the other hand, significant differences were observed in Atlanta and Kenne bec (Table - 10).

For different concentrations BAP+KIN (Table 10) the highest 37.96% of explants and 4.556 number of meristems were noted in Atlanta followed by 30.55% of explants and 3.667 number of meristem in Japanese red and the lowest 20.37% of explants and 2.444 number of meristem was recorded in Kenne bec.

The statistical results of isolated meristems of three potato varieties showed significant differences ($P<0.05$) were observed among variety and concentration (Table -11).

Among the different varieties, the highest 45.37% and 5.444 number of meristem was recorded in Atlanta followed by 30.55% and 3.667 number of meristems in Japanese red and 17.59% and 2.111 number of meristem was noted in Kenne bec for different concentrations of BAP+$GA_{3.}$ (Table -12).

Means of different concentrations, 1mg/l BAP+0.3mg/l $GA_{3,}$ 1mg/lBAP+0.4 mg/l GA_3 and 1mg/lBAP+0.5mg/l GA_3 and 1mg/lBAP+0.4mg/l GA_3 were observed no significant difference but significant differences were observed in 1mg/l BAP+0.3mg/l $GA_{3,}$ /lBAP+0.5 mg/l GA_3 (Table - 12).

Like different concentrations, three varieties showed significant differences among Atlanta, Japanese red and Kenne bec for the number of meristem and percentage of meristem in different concentrations of BAP + GA_3 (Table -12).

Table 9. Statistical results (ANOVA) of survival no. of isolated meristems of three potato varieties at different concentrations and combinations of BAP+ KIN.

Source of variation	Degree of freedom	Sum of square	Mean square	F value	Prob
Replication	2	2.667	1.333	2.4615	0.1169
Variety (V)	2	20.222	10.111	18.6667	0.0001
Concentration (C)	2	8.667	4.333	8.0000	0.0039
V ×C	4	4.444	1.111	2.0513	0.1353
Error	16	8.667	0.542		
Total	26	44.667			

Table 10. Mean separation on survival of plantlets at different concentrations of BAP+KIN of three potato varieties.

Sources	No. of explants responded	% of explants responded	Morphogenic response	
			Shooting	Rooting
Atlanta	4.556A	37.96A	+++	-
Japanese red	3.667AB	30.55AB	++	-
Kenne bec	2.444B	20.37B	++	-
LSD at 5% level	1.274	10.61		
Concentration				
BAP 1 mg/l +0.3mg/lKIN	4.333A	36.11A	+++	-
BAP 1 mg/l+0.4mg/lKIN	3.333AB	27.77AB	+++	-
BAP 1mg/l+0.5mg/lKIN	3.000B	25.00B	++	-
LSD at 5% level	1.274	10.61		

Table 11. Statistical results (ANOVA) of survival no. of isolated meristems of three potato varieties at different concentrations and combinations of BAP+ GA_3.

Source of variation	Degree of freedom	Sum of square	Mean square	F value	Prob
Replication	2	0.296	0.148	0.3368	-
Variety (V)	2	50.074	25.037	56.9263	0.0000
Concentration (C)	2	16.074	8.037	18.2737	0.0001
V ×C	4	1.704	0.426	0.9684	-
Error	16	7.037	0.440		
Total	26	75.185			

Table 12. Mean separation on survival of plantlets at different concentrations of BAP+GA_3 of three potato varieties.

Source	No. of explants responded	% of explants responded	Morphogenic Response	
			Shooting	Rooting
Atlanta	5.444A	45.37A	+++	-
Japanese red	3.667B	30.55B	++	-
Kenne bec	2.111C	17.59C	++	-
LSD at 5% level	1.148	9.564		
Concentration				
BAP 1 mg/l+0.3mg/lGA_3	4.667A	38.89A	+++	-
BAP 1 mg/+0.4mg/lGA_3	3.778AB	31.48AB	+++	-
BAP 1mg/l+0.5mg/lGA_3	2.778B	23.14B	++	-
LSD at 5% level	1.148	9.564		

4.1.4 Response of different varieties in MS_0

In this experiment number of meristem and the percentage of meristem were also cultured on MS_0 medium and no significant differences were observed. The results were shown in the table -13.

In the Atlanta, 4.667 numbers of meristem and 38.88% of meristem was responded when MS_0 medium supplemented.For Japanese red, 36.11% of meristem responded when MS_0 medium were supplemented. The numbers of meristems were 4.333.In the Kenne bec, number of meristem was recorded 3.667 and 30.55% of meristem responded when MS_0 medium were supplemented.

The Shoot multiplications from established meristems of three potato varieties were highly influenced by MS_0 medium. In all the varieties of potato, the meristems of Atlanta produced excellent shoots and Japanese red produced moderate shoots but Kenne bec showed very poor shoots. No root formation was observed in MS_0 medium.

Table 13. Primary establishment of meristems cultured on filter paper bridge at different varieties of potato in MS liquid medium with MS_0.

Variety	No. of explants responded	% of explants responded	Morphogenic response	
			Shooting	Rooting
Atlanta	4.667A	38.88A	+++	-
Japanese red	4.333A	36.11A	++	-
Kenne bec	3.667A	30.55A	+	-
LSD at 5% level	1.511	12.59		

A. Atlanta **B. Japanese Red** **C. Kenne bec**

Figures 1: Meristem Cultures on filter paper bridge in MS liquid medium supplemented with GA_3 0.3 mg/L of three potato varieties- A. Atlanta, B. Japanese red C. Kenne bac 15 days after inoculation.

4.2 SHOOT MULTIPLICATION FROM PRIMARY ESTABLISHED MERISTEM FOR THREE POTATO VARIETIES

In this experiment the primary established meristems were rescued aseptically and sub cultured onto MS semisolid basal media supplemented with different types of growth regulators (cytokinins, auxins) either singly or in various combination in order to find out a suitable culture media for rapid shoot multiplication. Shoot multiplication was highly influenced by the type of cytokinin with or without Gibberellin as well as their different concentrations used. Details of the experiments are described under the following heads.

4.2.1 Effect of Different Concentrations of Gibberellins Singly.

The Primary established meristems were sub cultured in MS semisolid media supplemented with three different concentrations of gibberellins for multiplication of shoots. The number of nodes per culture and shoot length per culture were considered as parameters for evaluation this experiment. The data of these parameters from different treatments were recorded after four weeks of subculture and are presented in the following table.

The statistical results of number of nodes of survival plantlets from meristems of three potato varieties showed significant differences ($P<0.05$) among

replication and variety but in interaction cases - V×C, no differences (P>.36) were observed (Table 14). The shoot length of survival plantlets from meristems of three potato varieties showed significant differences (P<0.05) among replication, variety and in interaction - V×C, (Table -15).

When the media was supplemented with GA_3 alone, the highest 4.277 number of nodes were recorded in media with 0.3 mg/l GA_3 followed by 4.231 number of nodes in MS media supplemented with 0.4mg/l GA_3. The lowest number of nodes (4.077) was formed in MS media with 0.5 mg/l GA_3. The highest shoot length (4.761cm) was recorded in media with 0.5mg/l GA_3 followed by 4.702cm shoot length in 0.4 mg/l GA_3.and the lowest shoot length (4.661cm) was observed in media having 0 .3mg/l GA_3 (Table -16). No significant differences were observed in different concentrations of GA_3. (Table - 16).

The Shoot multiplication from established meristems of three potato varieties was highly influenced by different concentrations and combinations of growth regulators. In all the tested combinations, the meristems of Atlanta and Japanese red produced excellent shoots with roots but Kenne bec showed very poor shoot and root growth (Table -16).

Among different varieties the 4.544 number of nodes and 5.018 cm shoot length was observed in Atlanta followed by 4.247 numbers of nodes and shoot length (4.850cm) in Japanese red. The lowest shoot length (4.257cm) and 3.793 numbers of nodes were noted in Kenne bec (Table -16).

No significant differences were observed in Japanese red and Kenne bec but significant differences were observed in Atlanta and Kenne bec for shoot length. There was no significant difference between different varieties for the number of nodes.

Table 14. Statistical results (ANOVA) of no. of nodes of survival plantlets from meristem of three potato varieties at different concentrations GA_3.

Source of variation	Degree of freedom	Sum of square	Mean square	F value	Prob
Replication	2	1.958	0.979	3.6601	0.0491
Variety (V)	2	2.575	1.288	4.8132	0.0231
Concentration (C)	2	0.198	0.099	0.3697	-
V×C	4	1.126	0.282	1.0526	0.4114
Error	16	4.280	0.267		
Total	26	10.137			

Table 15. Statistical results (ANOVA) of shoot length of survival plantlets from meristem of three potato varieties at different concentrations GA_3.

Source of variation	Degree of freedom	Sum of square	Mean square	F value	Prob
Replication	2	1.431	0.716	5.0950	0.0194
Variety (V)	2	2.878	1.439	10.2463	0.0014
Concentration (C)	2	0.045	0.023	0.1619	-
V ×C	4	2.360	0.590	4.2010	0.0163
Error	16	2.247	0.140		
Total	26	8.963			

Table 16. Mean separation of survival plantlets from meristems at different concentrations of GA_3 of three potato varieties.

Source	No. of nodes/explants	Shoot length after 30 days	Morphogenic response	
			Shooting	Rooting
Atlanta	4.544A	5.018A	+++	+++
Japanese red	4.247A	4.850AB	+++	+++
Kenne bec	3.793A	4.257B	+	+
LSD	0.8944	0.6476		
Concentration				
GA_3 0.3 mg/l	4.277A	4.661A	+++	+++
$GA_3$0.4 mg/l	4.231A	4.702A	+++	+++
$GA_3$0.5 mg/l	4.077A	4.761A	++	+
LSD	0.8944	0.6476		

A. Atlanta **B. Japanese Red** **C. Kenne bec**

Figures 2: Meristem Culture on filter paper bridge in MS liquid medium supplemented with GA_3 0.3 mg/L of three potato varieties- A. Atlanta, B. Japanese red C. Kenne bac 60 days after inoculation.

4.2.2 Effect of Different Concentrations of Cytokinin Singly

The statistical results of no. of nodes and shoot length of survival plantlets from meristems of three potato varieties showed significant differences ($P<0.05$) among variety, concentration and in interaction - V×C, (Table -17, 18).

When the media was supplemented with different concentrations of BAP the highest 4.258 number of nodes was recorded in media 0.3 mg/l BAP followed by 3.968 number of nodes was recorded in 0.4 mg/l BAP and the lowest 2.583 number of nodes was noted 0.5 mg/l BAP. On the other hand, the highest shoot length (4.071cm) was recorded in media 0.4mg/l BAP followed by 3.911cm in 0.3mg/l BAP and the lowest shoot length (2.720cm) was observed in media having 0.5mg/l BAP (Table -19).

For the different varieties the highest 4.323 number of nodes and 4.662 cm shoot length were recorded in Atlanta when the media were employed with BAP concentrations followed by 4.067 no of nodes and 3.729 cm shoot length in Japanese red and the lowest 2.419 number of nodes and 2.311 cm shoot length were recorded in Kenne bec (Table -19).

No significant differences were observed among 0.3 mg/l BAP and 0.4 mg/l BAP (table -19) but significant differences were observed between 0.3mg/l BAP, 0.5 mg/l BAP and 0.4 mg/l BAP, 0.5 mg/l BAP for both parameters.

Like the different concentrations, three varieties showed significant differences among Atlanta and Kenne bec and Japanese red, Kenne bec for the number of nodes and shoot length in BAP. But there were no significant differences among Atlanta and Japanese red in different concentrations of BAP (Table - 19).

The statistical results of no. of nodes and shoot length of survival plantlets from meristems of three potato varieties showed significant differences ($P<0.05$) among variety, but in concentration and in interaction - V×C, no differences were observed (Table -20, 21).

The different concentrations of KIN, the highest 3.873 number of nodes was recorded in 0.4 mg/l KIN followed by 3.808 number of nodes in 0.5 mg/l KIN and the lowest 3.707 number of nodes was noted in 0.3mg/l KIN. The highest shoot length (4.080cm) was recorded in media 0.4mg/l KIN followed by 3.831cm in 0.3mg/l KIN and the lowest shoot length (3.816cm) was observed in media having 0.5mg/l KIN (Table -22).

When the media was employed with KIN concentrations the highest 4.180 number of nodes and 4.424 cm shoot length were recorded in Atlanta followed by 4.066 number of nodes and 4.072 cm shoot length were recorded in Japanese red and the lowest 3.142 number of nodes and 3.230 cm shoot length were recorded in Kenne bec (Table - 22).

No significant differences were observed in three varieties for the number of nodes, when the media was supplemented with KIN But significant differences were observed in Atlanta, Kenne bec and Japanese red, Kenne bec (Table -22). No differences were in KIN for the number of nodes and shoot length (Table - 22).

In all the formulations of BAP and KIN meristems produced primary shoots and roots. For all the treatments most of the cultured meristems showed excellent shoot growth but 0.5mg/l BAP media formulations showed moderate shoot and root growth and BAP 1mg/l showed poor root and shoot growth. Kenne bec showed poor shoot and root growth and Japanese red showed moderate shoot and root growth in all treatments of KIN. But the degree of shoot and root growth was found highly satisfactory for the variety of Atlanta for all formulations of BAP and KIN. For different concentrations of KIN moderate root growth was observed in 0.4 mg/l KIN and poor shoot and root growth showed in 0.5mg/l KIN (Table -19, Table - 22).

Table 17. Statistical results (ANOVA) of no. of nodes of survival plantlets from meristem of three potato varieties at different concentrations of BAP.

Source of variation	Degree of freedom	Sum of square	Mean square	F value	Prob
Replication	2	0.080	0.040	0.1839	-
Variety (V)	2	19.224	9.612	44.2772	0.0000
Concentration (C)	2	14.414	7.207	33.1981	0.0000
V ×C	4	13.595	3.399	15.6563	0.0000
Error	16	3.473	0.217		
Total	26	50.786			

Table 18. Statistical results (ANOVA) of shoot length of survival plantlets from meristem of three potato varieties at different concentrations of BAP.

Source of variation	Degree of freedom	Sum of square	Mean square	F value	Prob
Replication	2	0.154	0.077	0.2504	-
Variety (V)	2	25.227	12.613	40.9770	0.0000
Concentration (C)	2	9.810	4.905	15.9341	0.0002
V ×C	4	16.243	4.061	13.1921	0.0001
Error	16	4.925	0.308		
Total	26	56.359			

Table 19. Mean separations of survival plantlets at different concentrations of BAP of three potato varieties.

Source	No. of nodes/explants	Shoot length after 30 days	Morphogenic response	
			Shooting	Rooting
Atlanta	4.323A	4.662A	+++	+++
Japanese red	4.067A	3.729A	++	+++
Kenne bec	2.419B	2.311B	++	++
LSD at 5% level	0.8063	0.9606		
Concentration				
BAP0 .3 mg/l	4.258A	3.911A	+++	+++
BAP 0.4 mg/l	3.968A	4.071A	++	++
BAP 0.5mg/l	2.583B	2.720B	+	+
LSD at 5% level	0.8063	0.9606		

Table 20. Statistical results (ANOVA) of no of nodes of survival plantlets from meristem of three potato varieties at different concentrations of KIN.

Source of variation	Degree of freedom	Sum of square	Mean square	F value	Prob
Replication	2	6.052	3.026	5.9395	0.0118
Variety (V)	2	5.828	2.914	5.7197	0.0134
Concentration (C)	2	0.127	0.063	0.1245	
V ×C	4	5.064	1.266	2.4849	0.0852
Error	16	8.151	0.509		
Total	26	25.222			

Table 21. Statistical results (ANOVA) of shoot length of survival plantlets from meristem of three potato varieties at different concentrations of KIN.

Source of variation	Degree of freedom	Sum of square	Mean square	F value	Prob
Replication	2	4.105	2.052	14.0317	0.0003
Variety (V)	2	6.780	3.390	23.1781	0.0000
Concentration (C)	2	0.396	0.198	1.3549	0.2860
V ×C	4	0.264	0.066	0.4521	
Error	16	2.340	0.146		
Total	26	13.886			

Table 22. Mean separations of survival plantlets of meristems at different concentrations of KIN of three potato varieties.

Source	No. of nodes/ explants	Shoot length after 30 days	Morphogenic response	
			Shooting	Rooting
Atlanta	4.180A	4.424A	+++	+++
Japanese red	4.066A	4.072A	++	++
Kenne bec	3.142A	3.230B	+	+
LSD at 5% level	1.235	.6614		
Concentration				
KIN 0.3 mg/l	3.707A	3.831A	+++	+++
KIN0.4 mg/l	3.873A	4.080A	+++	++
KIN0.5 mg/l	3.808A	3.816A	+	+
LSD at 5% level	1.235	.6614		

4.2.3 Effect of Different Concentrations and Combinations of Cytokinin and Gibberilin

In this experiment, isolated meristems of three potato varieties viz. Atlanta, Japanese red and Kenne bec were cultured separately on 'M' shaped filter paper bridge in culture tubes containing liquid MS medium supplemented with selected combinations 1mg/lBAP+0.3mg/l KIN, 1mg/lBAP+0.4mg/l KIN, 1mg/lBAP+0.5mg/l KIN 1mg/l BAP+0.3mg/l GA_3, 1mg/lBAP+0.4 mg/l GA_3 and 1mg/lBAP+0.5mg/l GA_3 of plant growth regulators to find out the response of 3 potato varieties. The data on percentage of meristems responded, morphogenic response and degree of shoot growth was recorded after 21 days of culture and are presented in Table 25, table -28.

The statistical results of no. of nodes of survival plantlets of three potato varieties shown significant differences ($P<0.05$) in variety. But in replication, non significant differences ($P>.1$) were observed (Table -23).

In case of shoot length of survival plantlets of three potato varieties showed significant differences in variety ($P<.05$). But in concentration, non significant differences ($P>.28$) were observed (Table -24). From the results it was observed that the highest 4.344 number of nodes and 4.278 cm shoot length was responded in Atlanta followed by 3.809 number of nodes and 4.013 cm shoot length in Japanese red and 3.174 number of nodes and 3.348 cm shoot length was observed in Kenne bec for different concentrations of BAP+KIN (Table. 25).

The different concentrations and combinations of BAP+KIN, the highest 3.840 number of nodes and 4.042 cm shoot length were recorded in1mg/lBAP+.4 mg/l KIN followed by 3.814 number of nodes and 3.838 cm shoot length in1mg/lBAP+0.3 mg/l KIN and the lowest 3.673 number of nodes and 3.759 cm shoot length were noted in1mg/lBAP+0.5mg/l KIN (Table -25).

No significant difference were observed among 1mg/lBAP+.3mg/l KIN, 1mg/lBAP+.4mg/l KIN, 1mg/lBAP+.5mg/l KIN for both parameters (Table -25).

Three varieties showed significant differences among Atlanta, and Kenne bec for the number of nodes and shoot length in different concentrations of BAP + KIN. But no significant differences were observed in Atlanta Japanese red and Kenne bec, Japanese red in different concentrations of BAP + KIN (Table -25).

In all the formulations of BAP and KIN meristems produced primary shoots and roots. Among all the treatments most of the cultured meristems showed excellent shoot growth but BAP1mg/l+KIN0.3mg/l media formulations showed moderate shoot and poor root growth and BAP1+KIN0.5 media showed only poor shoot growth. The Kenne bec showed moderate shoot growth in all treatments of BAP+KIN. The degree of shoot and root growth was found highly satisfactory for the variety of Atlanta and Japanese red for all formulations of BAP+KIN (Table - 25).

The statistical results of no. of nodes and shoot length of survival plantlets from meristems of three potato varieties showed significant differences ($P<0.05$) among variety, But in replication, concentration and in interaction - V×C, non significant differences ($P>.1$) were observed (Table -26, 27).

All the treatments, excellent shoot growth showed in media 1mg/lBAP+0. 3mg/lGA_3 and 1mg/lBAP+0.4mg/lGA_3 but in media 1mg/lBAP+0.5mg/lGA_3 showed only poor shoot growth. The degree of shoot and root growth was found highly satisfactory for the variety of Atlanta. Japanese red showed moderate shoot and root and Kenne bec showed poor shoot and root growth (Table - 28).

When the media was employed with BAP+ GA_3 concentrations the highest 4.199 number of nodes and 4.199 cm shoot length were recorded in Atlanta followed by 3.902 number of nodes and 3.793 cm shoot length were recorded

in Japanese red and the lowest 2.607 number of nodes and 3.501cm shoot length were recorded in Kenne bec (Table -28).

For different concentrations and combinations of BAP+ GA_3 the highest 3.684 number of nodes and 3.990 cm shoot length then were observed in 1mg/lBAP+.3mg/l GA_3 followed by 3.512 number of nodes in 1mg/lBAP+ .4mg/l GA_3 and 3.511 number of nodes was observed in 1mg/lBAP and .5 mg/l GA_3 (Table 28).

Means of different concentrations, no significant difference were observed among and 1mg/l BAP+.3mg/l GA_3, 1mg/lBAP+.4 mg/l GA_3 and 1mg/lBAP+.5mg/l GA_3 for both parameters (Table - 28) and significant differences were observed between Atlanta, Kenne bec and Japanese red, kenne bec in different concentrations of BAP+GA_3 for the number of nodes. But no significant differences were observed in Atlanta and Japanese red in this parameter (Table -28).

Table 23. Statistical results (ANOVA) of number of nodes of survival plantlets from meristem of three potato varieties at different concentrations and combinations of BAP+ KIN.

Source of variation	Degree of freedom	Sum of square	Mean square	F value	Prob
Replication	2	1.161	0.580	2.0915	0.1560
Variety (V)	2	6.175	3.087	11.1251	0.0009
Concentration (C)	2	0.145	0.073	0.2613	
V ×C	4	0.720	0.180	0.6482	
Error	16	4.440	0.278		
Total	26	12.640			

Table 24. Statistical results (ANOVA) of shoot length of survival plantlets from meristem of three potato varieties at different concentrations and combinations of BAP+ KIN.

Source of variation	Degree of freedom	Sum of square	Mean square	F value	Prob
Replication	2	1.558	0.779	5.5671	0.0146
Variety (V)	2	4.133	2.067	14.7662	0.0002
Concentration (C)	2	0.385	0.192	1.3750	0.2812
V ×C	4	0.174	0.044	0.3117	-
Error	16	2.239	0.140		
Total	26	8.490			

Table 25. Mean separations on survival plantlets at different concentrations and combinations of BAP+ KIN of three potato varieties.

Source	No. of nodes/explants	Shoot length after 30 days	Morphogenic response	
			Shooting	Rooting
Atlanta	4.344A	4.278A	+++	+++
Japanese red	3.809AB	4.013A	+++	+++
Kenne bec	3.174B	3.348B	++	-
LSD at 5%level	0.9126	0.6476		
Concentration				
BAP1mg/l+KIN0.3mg/l	3.814A	3.838A	++	+
BAP1mg/l+KIN0.4mg/l	3.840A	4.042A	+++	++
BAP1mg/l+KIN0.5mg/l	3.673A	3.759A	+	-
LSD at 5% level	0.9126	0.6476		

Table 26. Statistical results (ANOVA) of no of nodes from survival plantlets of three potato varieties at different concentrations and combinations of BAP+ GA_3.

Source of variation	Degree of freedom	Sum of square	Mean square	F value	Prob
Replication	2	0.769	0.384	1.7545	0.2047
Variety (V)	2	12.905	6.452	29.4518	0.0000
Concentration (C)	2	0.179	0.090	0.4088	
V ×C	4	0.359	0.090	0.4101	
Error	16	3.505	0.219		
Total	26	17.718			

Table 27. Statistical results (ANOVA) of shoot length from survival plantlets of three potato varieties at different concentrations and combinations of BAP+ GA_3.

Source of variation	Degree of freedom	Sum of square	Mean square	F value	Prob
Replication	2	0.321	0.161	0.8509	
Variety (V)	2	2.214	1.107	5.8611	0.0123
Concentration (C)	2	0.341	0.170	0.9021	
V ×C	4	0.365	0.091	0.4835	
Error	16	3.022	0.189		
Total	26	6.263			

Table 28. Mean separations on survival plantlets at different concentrations and combinations of BAP+ GA_3 of three potato varieties.

Source	No. of nodes/ explants	Shoot length after 30 days	Morphogenic response	
			Shooting	Rooting
Atlanta	4.199A	4.199A	+++	+++
Japanese red	3.902A	3.793A	++	++
Kenne bec	2.607B	3.501A	+	+
LSD at 5% level	.8100	0.7525		
Concentration				
BAP1mg/l+0.3GA_3 mg/l	3.684A	3.990A	+++	+++
BAP1mg/l+0.4GA_3 mg/l	3.512A	3.746A	+++	-
BAP1mg/l+0.5GA_3 mg/l	3.511A	3.758A	+	-
LSD at5% level	.8100	0.7525		

4.2.4 Response of Different Varieties in MS_0

In this experiment the primary shoots developed from meristem culture that were aseptically taken out and cut into nodal segments. The individual nodal segments were cultured on MS semisolid medium without any growth regulators. The data were recorded on number of nodes and shoot length and the results were presented in table - 29.

The results show that shoots length of potato varieties increased with the advancement of time and within one month the shoots attained 3.103-4.430 cm. The table which shows that the variety of Atlanta produced the longest shoot (4.430cm) which, the highest number of nodes (4.847). The variety Japanese red also showed moderate performance in their shoot length (3.823cm) and

number of nodes (3.580) were recorded after 30 days of culture in Kenne bec (Table -29).

The shoot multiplications from established meristems of three potato varieties were highly influenced by MS_0 medium. In all varieties of potato, the meristems of Atlanta produced excellent shoots and roots, Japanese red produced moderate shoots and excellent roots but Kenne bec showed very poor shoots and moderate roots (Table -29).

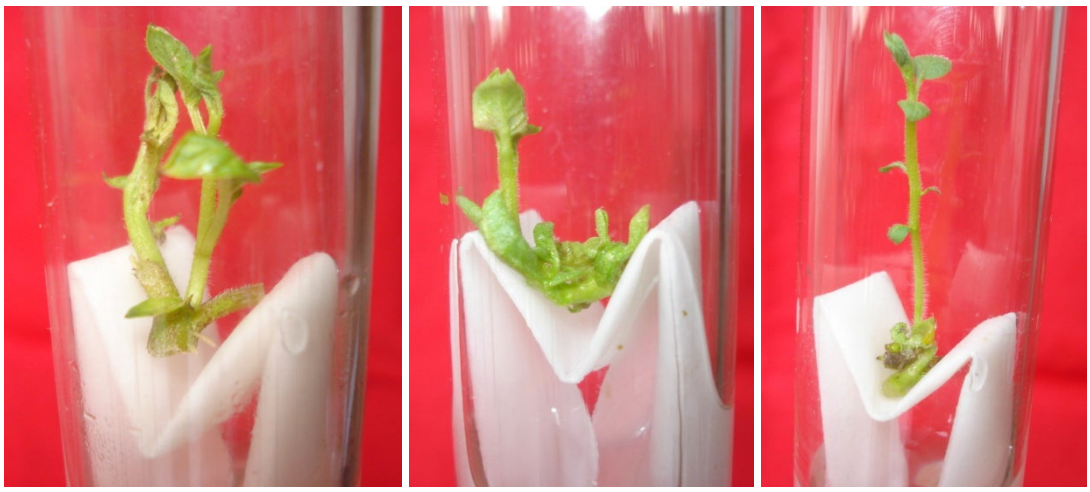
The three varieties showed significant differences among Atlanta, and Kenne bec for the number of nodes. But no significant differences were observed between Atlanta and Japanese red in this parameter (Table -29).

For shoot length, significant differences were observed between Atlanta and Kenne bec. But no significant differences were observed between Atlanta, Japanese red and Kenne bec, Japanese red (Table -29).

In this table it was observed that the highest number of nodes and shoot length was observed in Atlanta and MS_0 was also performed well (Table -29).

Table 29. Shoot induction on nodal segments obtained from primary established shoot cultures of potato in MS_0 (without hormone).

Variety	No. of nodes/ explants	Shoot length (cm)	Morphogenic response	
			Shooting	Rooting
Atlanta	4.847A	4.430A	+++	+++
Japanese red	4.277A	3.823AB	++	+++
Kenne bec	3.580B	3.103B	+	++
LSD at 5% level	0.5955	0.8267		

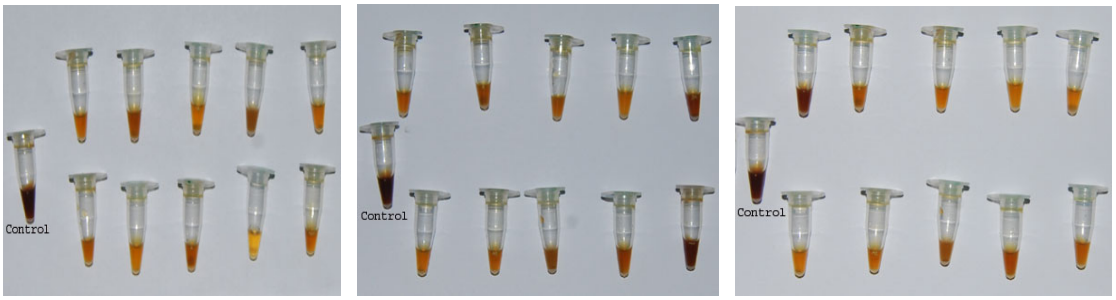


A. Atlanta **B. Japanese Red** **C. Kenne bec**

Figures 3: Meristem Culture on filter paper bridge in MS liquid medium supplemented with GA_3 0.3 mg/L of three potato varieties- A. Atlanta, B. Japanese red C. Kenne bec 6 months after inoculation.

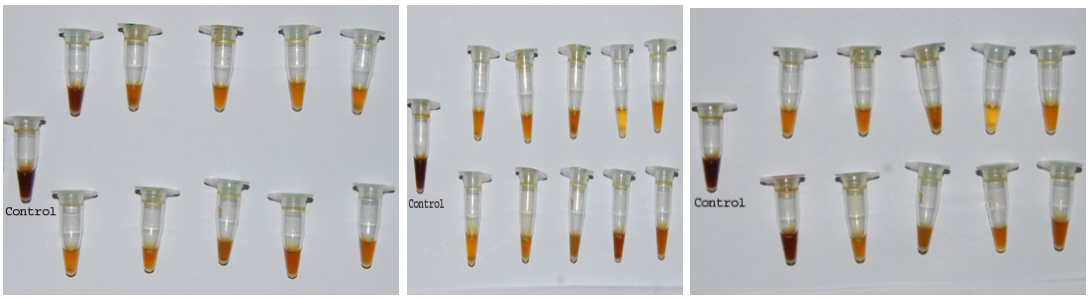
4.3 DAS-ELISA TEST FOR VIRUS DETECTION FROM PRIMARY ESTABLISHED MERISTEM DERIVED PLANTLETS

For testing of meristem derived plantlets to detect virus, a serological DAS-ELISA test was carried out before micro propagation of meristem derived plantlets. In this experiment, no positive color was found in the micro titer plates when the *in vitro* grown meristem derived plantlets were used as samples. On the other hand, color precipitation was appeared when field grown plants were used as samples. So, from the experiment it was proved that no virus (PVX, PVY, PLRV) were present in the *in vitro* grown meristem derived plantlets (table-30).



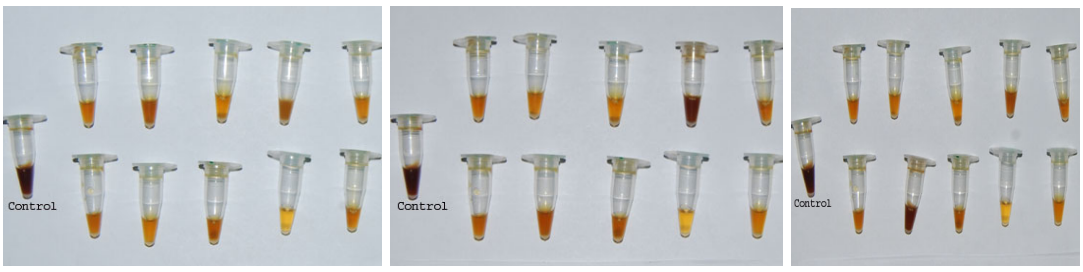
A. Atlanta **B. Japanese red** **C. Kenne bec**

Figure 4: Detection of PLRV virus through ELISA test from heat treated *in vitro* established culture of three potato varieties; A. Atlanta B.Japanese red C.kenne bec



A. Atlanta **B. Japanese red** **C. Kenne bec**

Figure 5: Detection of PVX virus through ELISA test from heat treated *in vitro* established culture of three potato varieties; A. Atlanta B.Japanese red C.kenne bec.



A. Atlanta **B. Japanese red** **C. Kenne bec**

Figure 6: Detection of PVY virus through ELISA test from heat treated *in vitro* established culture of three potato varieties; A. Atlanta B. Japanese red C. kenne bec.

Table 30. Field Evaluation of meristem derived plants.

Varieties	Plant source	No. of samples tested	Disease index					
			PLRV		PVX		PVY	
			No. of samples infected	% of infection	No. of samples infected	% of infection	No. of samples infected	% of infection
Meristem culture derived plants (MP)								
Atlanta	MP	10	1	10	-	-	-	-
Japanese red	MP	10	1	10	1	10	1	10
Kenne bec	MP	10	-	-	1	10	1	10
Control plants (CP)								
Atlanta	CP	10	-	-	1	10	1	10
Japanese red	CP	10	3	30	2	20	1	10
Kenne bec	CP	10	1	10	3	30	1	10

MP = Meristem culture derived plants
CP = Control plants
- = No disease symptom

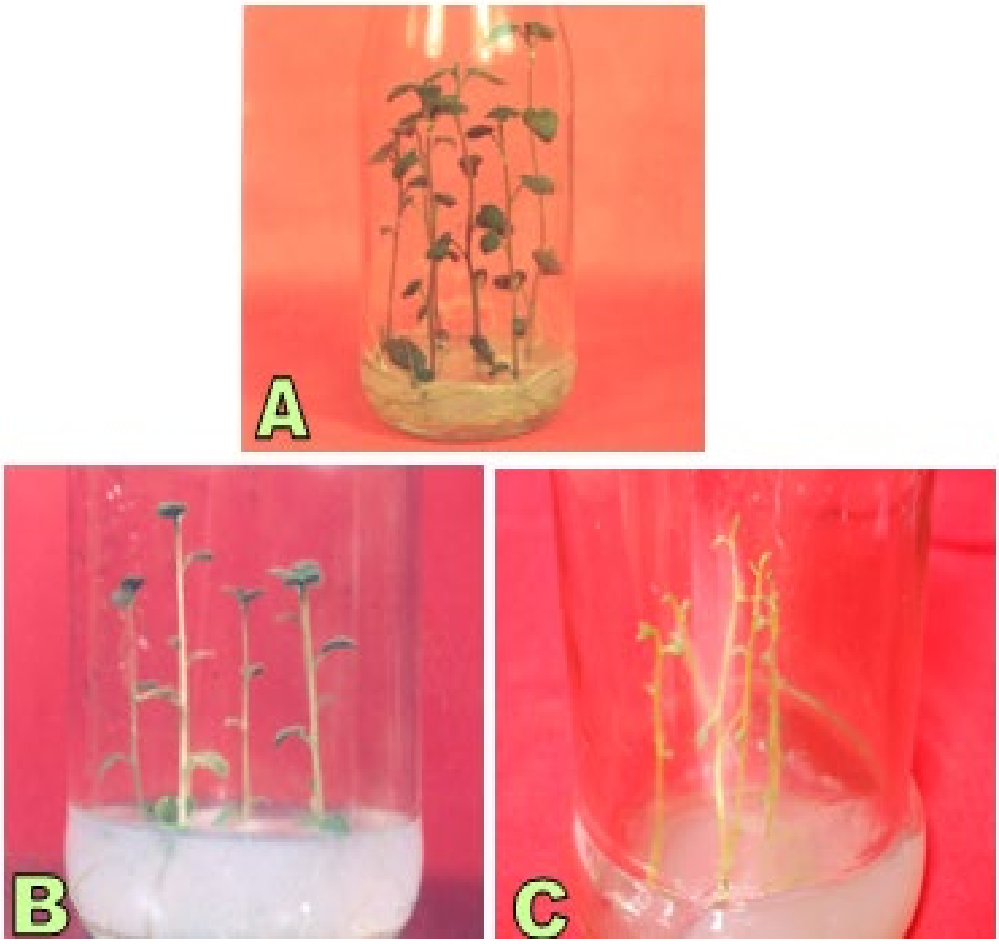


Figure 7: A, B, C: multiplication of virus free plantlets from nodal segments of different potato varieties in MS semi-solid medium having 0.3mg/L GA_3. .

A. Plantlet development from nodal segments of Atlanta after six weeks of culture.

B. Plantlet development from nodal segments of Japanese red after six weeks of culture.

C. Plantlet development from nodal segments of Kenne bec after six weeks of culture.

4.4 FIELD PERFORMANCE OF *IN VITRO* PRODUCED MERISTEM DERIVED PLANTLETS

Well developed *in vitro* produced meristem derived plantlets for each cultivar separately was excised and cultured. The acclimatized well in out door condition and grew maturity and developed tubers. The field performances of *in vitro* produced meristem derived plantlets were evaluated and the data on plant height, no of leaves/plants, no. of branches/plant, and no. of tubers/plant were recorded. The results are presented in tables below:

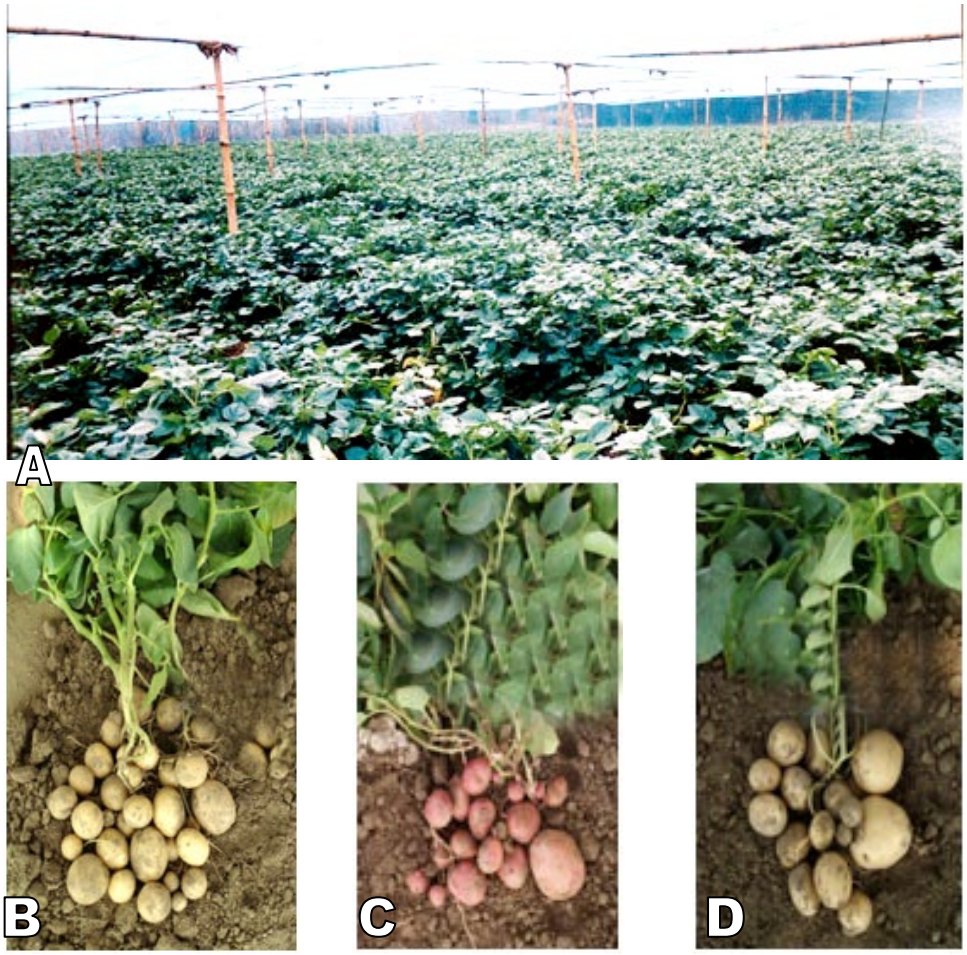


Figure 8: A-D: Field establishment of meristem derived plantlets.

A: The field of plantlets after 90 days of plantation.
B: The plant from plantlets Atlanta after 90 days of plantation with minitubers.
C: The plant from plantlets Japanese red after 90 days of plantation with minitubers.
D: The plant from plantlets Kenne bec after 90 days of plantation with minitubers.

The statistical results of different morphological characters of meristem derived plants of three potato varieties showed highly significant differences for plant height, no. of tuber, tuber wt. highly among themselves and non-significant differences were observed in replication(table-31,32,33,34,35).

Table 31. Statistical results (ANOVA) of Plant height of meristem derived plants of three potato varieties.

Source of variation	Sum of square	Degree of freedom	Mean square	F-value	Table value
Variety	214.3322	2	107.1661	80.41981	6.94
Replication	11.24327	2	5.621633	4.218598	
Error	5.330333	4	1.332583		
Total	230.9058	8			

Table 32. Statistical results (ANOVA) of no of leaf of meristem derived plants of three potato varieties.

Source of variation	Sum of square	Degree of freedom	Mean square	F-value	Table value
Variety	120.6251	2	60.31254	15.26979	6.94
Replication	5.831622	2	2.915811	0.738218	
Error	15.79918	4	3.949794		
Total	142.2559	8			

Table 33. Statistical results (ANOVA) of no of branches of meristem derived plants of three potato varieties.

Source of variation	Sum of square	Degree of freedom	Mean square	F-value	table value
Variety	26.72222	2	13.36111	10.20001	6.94
Replication	0.515356	2	0.257678	0.196714	
Error	5.239644	4	1.309911		
Total	32.47722	8			

Table 34. Statistical results (ANOVA) of no of tuber of meristem derived plants of three potato varieties.

Source of variation	Sum of square	Degree of freedom	Mean square	F-value	Table value
Variety	406.9142	2	203.4571	44.4615	6.94
Replication	12.00889	2	6.004444	1.312152	
Error	18.30411	4	4.576028		
Total	437.2272	8			

Table 35. Statistical results (ANOVA) of tuber wt. of meristem derived plants of three potato varieties.

Source of variation	Sum of square	Degree of freedom	Mean square	F-value	Table value
Variety	42673.56	2	21336.78	24.4797	6.94
Replication	489.5556	2	244.7778	0.280834	
Error	3486.444	4	871.6111		
Total	46649.56	8			

In Table. 36 the variety Atlanta showed the highest height (39cm) of plantlets that survived in the field and its height was significantly difference from the rest of the varieties. The lowest height (27.51cm) was recorded in Japanese red.

According to the (Table 36) plant height, significant differences were observed among three varieties. The highest no. of leaves/plant (21.12) was observed in Atlanta which was significantly different from Japanese red. The second highest no. leaves/1plant (17.54) was observed in Kenne bec and no significant differences were observed in Atlanta and kenne bec.The highest no of branches/plant (8.923) was recorded in Atlanta and was closely followed by Kenne bec. The lowest no. of branches/plant (4.756) was observed in Japanese red which was significantly different from other varieties. Atlanta produced the highest number of tubers/plant (32.33) and was closely followed by Kenne bec and the lowest no. of tubers/plant was 16.05. All varieties were significantly different from others. The highest tuber weight/plant was recorded in Atlanta which was significantly higher than rest of the varieties. The lowest weight of tubers/plant was (75.66g) recorded in Japanese red which was significantly different from others.

Table 36. Field performance of plantlets of different potato varieties. Data were taken from 10 randomly selected plants of each replication. The mean values were calculated from three replications.

Variety	Plant height	No. of leaves/ plant	No. of branches/ plant	No. of tubers/ plant	Tuber weight/ plant
Atlanta	39.00a	21.12a	8.923a	32.33a	237.66a
Japanese red	27.51c	12.21b	4.756b	16.05c	75.66b
Kenne bec	30.40b	17.54a	7.423a	26.37b	197.33a
LSD	2.61	4.504	2.59	4.848	66.916

4.5 CALLUS INDUCTION

The present investigation was carried out to induce indirect regeneration through callus culture of using different types of explants viz. leaf, internode from three potato varieties. To achieve the objectives of this study, the leaf, internode explants was cultured stepwise for callus induction, for somatic embryogenesis from the callus and subsequently plant regeneration from callus. Finally, the regenerated plants of experiments were conducted for optimizing the process of plant regeneration through callus culture and the results are described below.

4.5.1 Induction of Callus from Different Explants

Internode and leaf segments (5×3 mm) from *in vitro* plantlets were cultured separately onto agar gelled MS nutrient medium supplemented with different concentrations of auxins (2,4-D, NAA) or in combination with auxin and cytokinin (NAA+BAP). The explants were induced to develop callus in many of the culture media formulations. Callus development was not observed in MS medium without plant growth regulators. Explants showed first response by increasing their size and changing their color and callus proliferation was observed to start from the cut surface of the explants. The data were collected on % of callus induction, callus type and callus color. The results are described below under different headlines as per culture media formulations.

4.5.1.1 Effect of 2, 4-D on induction of callus

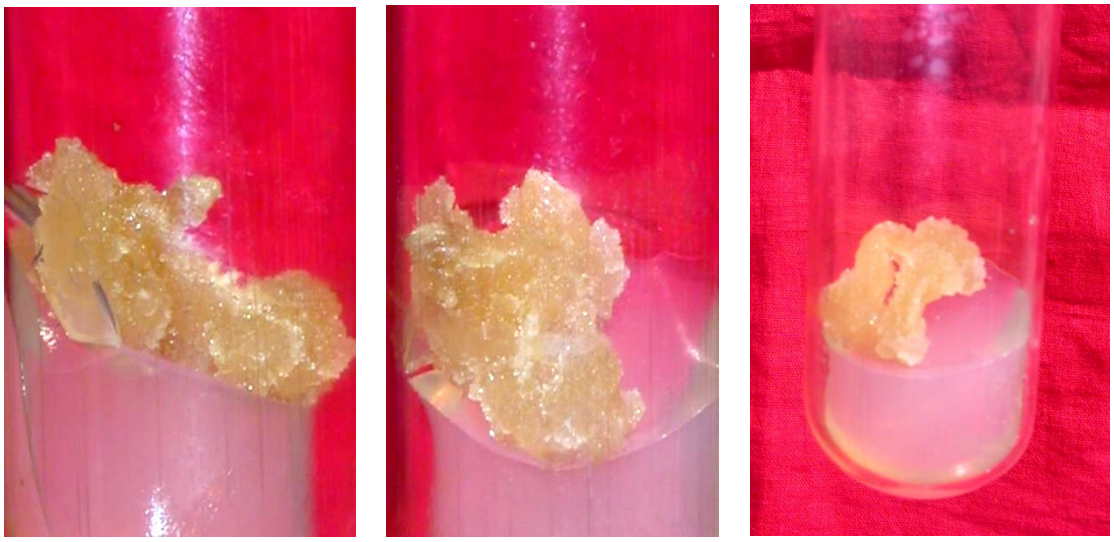
Internode and leaf segments were cultured onto MS medium supplemented with different concentrations of 2, 4-D (3, 2.5, 4 mg/l) for callus induction. The data was recorded after 2-6 weeks of culture and the results are presented in Table 37, Table 38. In general, it was observed that 2, 4-D (3mg/l) was more effective on callus induction and proliferation. The calli proliferated from internode explants were two types; friable and compact. Friable calli were cream and white and wet or dry types whereas, the compact calli were dry, yellowish cream coloured.

In Atlanta, variations were noted for the percentage of callus induction and ranged from 63.88- 88.88% in internode explants. The highest 88.88% of explants induced callus was obtained from internode explants in medium 3mg/l 2, 4-D. The calli proliferated in this culture medium were friable and cream in color. The lowest 63.88% of explants induced calli was recorded in medium 4 mg/l 2,4-D. In this medium callus color was white and friable in texture (Table 37).

In case of leaf, the highest 77.78 % of callus induction was recorded in medium containing 3 mg/l followed by 66.66% in medium with 3.5 mg/l 2,4-D and 52.78 % in medium with 4mg/l 2,4-D. Among different levels of 2,4-D, 3mg/l 2,4-D , 4 mg/l 2,4-D and 3.5mg/l 2,4-D, 4mg/l 2,4-D showed significantly different (Table 38).

In JPR, the best result in terms of callus proliferation was recorded for internode from medium having 3mg/l 2,4-D. In this culture medium, the highest 72.22% explants were induced to develop callus. The second highest 58.33% callus formation was recorded in MS medium containing 3.5 mg/l 2, 4-D. The lowest frequency (38.89%) of callus formation was recorded in medium containing 4 mg/l 2,4-D. Among different levels of 2,4-D, 3mg/l 2,4-D, 4 mg/l 2,4-D and 3.5mg/l 2,4-D, 4mg/l 2,4-D showed significantly different (Table 37).

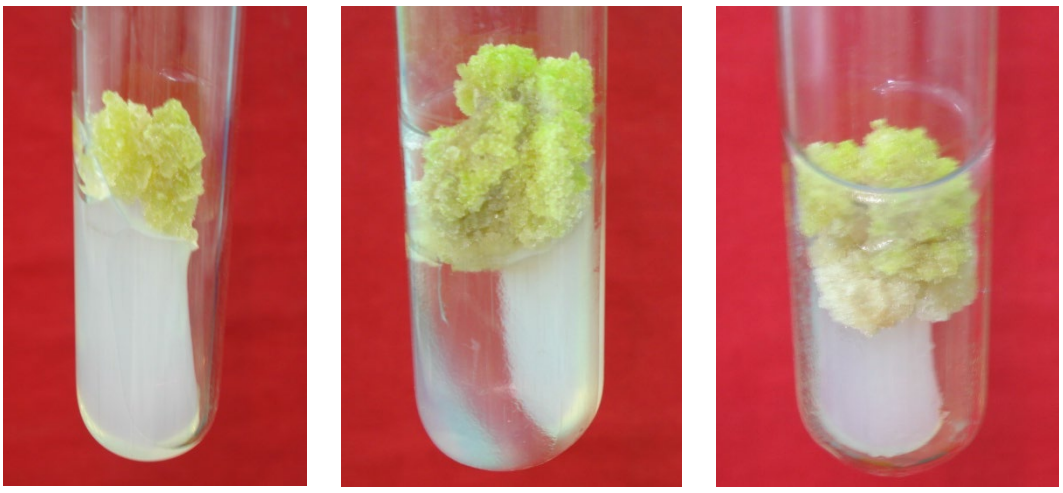
In case of leaf, variations were noted for the percentage of callus induction , the highest 66.66% of callus induction was recorded in medium containing 3 mg/l 2,4-D followed by 50% in medium with 3.5 mg/l 2,4-D and 27.78% in medium with 4mg/l 2,4-D (Table 38).



A.Atlanta **B. Japanese red** **C. Kenne bec**

Figure 9: Callus induction from A-C.

A: Development of callus in Atlanta after 8 weeks of inoculation on 3.0mg/l 2, 4-D.
B: Development of callus in Japanese red after 8 weeks of inoculation on 3.0mg/l 2, 4-D.
C: Development of callus in Kenne bec after 8 weeks of inoculation on 3.0mg/l 2, 4-D.



A. Atlanta **B. Japanese red** **C. Kenne bec**

Figure10: Shoot bud proliferation from internode in MS media supplemented with 3.0 mg//l 2, 4-D.

A. Atlanta B. Japanese red and C. Kenne bec

In kenne bec, no variation was observed for the percentage of explants induced calli and ranged from 36.11-61.11% in internode explants. The highest 61.11% of explants induced callus was obtained from internode explants in medium 3mg/l 2,4-D. The calli proliferated in this culture medium were friable and brown in color. The lowest 36.11%of explants induced calli was recorded in medium 3.5 mg/l 2, 4-D. In this medium callus color was cream and friable in texture (Table 37).

The leaf explants were also incubated in MS supplemented medium with different concentrations of 2, 4-D for callus induction. The observations were made 3-6 weeks after culture and the results were presented in Table 37.The highest 44.44% explants were induced to develop friable, cream callus with 3mg/l2, 4-D followed by 33.33% in medium with 3.5 mg/l 2, 4-D. The lowest percentage (30.55%) of explants induced calli was recorded in medium 4 mg/l 2, 4-D. The calli developed in this genotype were brown in color and compact in texture (Table 38).

4.5.1.2 Effect of NAA on induction of callus

Internode and leaf explants of the three potato varieties were incubated in MS supplemented medium with different concentrations (1.5, 2, and 2.5) of NAA for callus induction. The observations were made 3-6 weeks after culture and results are presented in Table 37, 38.

In Atlanta, the percentage of explants induced calli ranged from 27.78- 52.78% in internode explants. The highest 52.78% of explants induced callus was obtained from internode explants in medium 1.5 mg/l NAA. The calli proliferated in this culture medium were friable and white in colour. The lowest 27.78%of explants induced calli was recorded in medium 2.5 mg/l NAA. In this medium callus colour was white and friable in texture. Among different levels of NAA, 1.5mg/l NAA, 2.5 mg/l NAA and 2mg/l NAA, 2.5mg/l NAA showed significantly different (Table 37).

The leaf explants were also incubated in MS supplemented medium with different concentrations of NAA for callus induction. The observations were made 3-6 weeks after culture and the results were presented in Table 38.The highest 38.89% explants were induced to develop compact, white callus with 1.5mg/l NAA followed by 36.11% in medium with 2 mg/l NAA. The lowest percentage (19.44%) of explants induced calli was recorded in medium 2.5 mg/l NAA. The calli developed in this genotype were cream in color and friable in texture. Among different levels of NAA, 1.5mg/l NAA, 2.5 mg/l NAA and 2mg/l NAA, 2.5mg/l NAA showed significantly different (Table 38).

In JPR, no variation was observed for the percentage of explants induced calli. The highest 47.22% explants were induced to develop friable callus in case of internode for MS medium having 1.5mg/lNAA.The second highest 41.66% explants induced calli was recorded in MS medium containing 2mg/l NAA. The lowest frequency 36.11% of callus induction was noted in medium with 2.5 mg/l NAA (Table 37).

In case of leaf, the highest 33.33% of compact callus formation was recorded in media supplemented with 1.5 mg/l NAA. But cream coloured friable 27.78% calli were formed both with 2mg/l NAA and 2.5 mg/l NAA (Table 38).

In Kenne bec, the maximum 41.66% callus formation was recorded on 1.5 mg/l NAA from the internode explants followed by 36.11% explants induced callus formation was observed on 2mg/l NAA. Callus formation was not observed in media having 2.5 mg/l NAA (Table 37).

In case of leaf, 27.78% callus formed in medium having both in 1.5 mg/l NAA and 2mg/lNAA. These calli were friable, cream in color. Callus formation was not observed in medium having 2.5 mg/l NAA (Table 38).

4.5.1.3 Effect of NAA+ BAP on induction of callus

The three different concentrations and combinations of NAA+BAP were tested for callus induction from internode and leaf explants of potato. The data on % of callus induction, callus color and callus type were recorded after 6 weeks of culture and the results are shown in Table 37, 38.

The cultured explants produced callus in all treatments but the response of callus induction varied greatly with different concentrations and combinations of growth regulator formulations.

In Atlanta, the highest 44.44 % explants were induced to develop compact callus in case of internode for MS medium having 1mg/lNAA+0.5mg/l BAP. The second highest 41.66 % explants induced calli was recorded in MS medium containing 0.5mg/l NAA+1mg/l BAP. The lowest frequency 33.33% of callus induction was noted in medium with 1mg/l NAA+1mg/l BAP (Table 37).

In case of leaf, the highest 36.11% yellowish cream callus were formed both in media supplemented with 1mg/lNAA+0.5mg/l BAP and 0.5mg/lNAA+1mg/l BAP But cream coloured compact 22.22 % calli were formed in media supplemented with 1mg/l NAA +1 mg/l BAP (Table 38).

In JPR, the maximum 41.66% explants were induced to develop compact callus with 1 mg/l NAA+.5mg/l BAP in case of internode. Cream color calli was formed with MS medium containing 1mg/l NAA+1mg/lBAP. On the other hand, callus induction was not observed in media fortified with.5mg/lNAAwith 1mg/lBAP from the internode explants (Table 37).

In case of leaf, the maximum 27.78% of compact callus formation was recorded in media supplemented with .5 mg/l NAA+1mg/l BAP and the callus continued their proliferation upon sub culturing onto same medium. These callis were white in color. But cream coloured friable 25.00% calli were

formed with 1mg/l NAA and 1 mg/l BAP. Callus formation was not observed in media having .5 mg/l NAA+1mg/l BAP (Table 38).

In Kenne bec, no variation was observed for the percentage of explants induced calli. The maximum 41.66% callus formation was recorded on 1mg/l NAA+.5 mg/l BAP from the internode explants followed by 36.11% explants induced callus formation was observed on .5mg/l NAA+1mg/l BAP. These calli were friable and cream in color. Only 22.22% explants induced to develop friable and white color callus in 1 mg/l NAA+1mg/l BAP (Table 37).

In case of leaf, 25.00% callus formed in medium having both in 1mg/l NAA+0.5mg/lBAP and .5mg/lNAA1mg/lBAP. These calli were friable, cream in color. Only 16.66% explants induced to develop friable and cream colour callus in 1 mg/l NAA+1mg/l BAP (Table 38).

Table 37. Effect of different concentrations of 2, 4-D or NAA singly or NAA in combination with BAP in MS medium on callus induction from internode explants of three varieties of potato. In each treatment 10 explants were inoculated. Data were recorded after eight weeks of culture.

PGRS (mg/l)	Variety Atlanta			Variety Japanese red			Variety Kenne bec		
	% of callus induction	Callus colour	Callus type	% of callus induc-tion	Callus colour	Callus type	% of callus induction	Callus colour	Callus type
2,4-D									
3	88.88A	Cr	Fri	72.22A	Cr	Fri	61.11A	W	Fri
3.5	72.22B	Cr	Fri	58.33AB	W	Fri	36.11AB	Cr	Fri
4	63.88C	W	Fri	38.89C	YCr	Com	41.66AB	Ycr	Fri
NAA									
1.5	52.78D	W	Fri	47.22BC	Cr	Fri	41.66AB	Cr	Com
2	47.22DE	W	Fri	41.66C	Cr	Fri	36.11AB	Cr	Fri
2.5	27.78F	W	Fri	36.11C	W	Fri	0.0000C	-	-
NAA+BAP									
1+.5	44.44DE	Cr	Com	41.66C	W	Com	41.66AB	Cr	Com
.5+1	41.66E	Cr	Com	.0000D	-	-	36.11AB	Cr	Fri
1+1	33.33F	Cr	Fri	38.88C	Cr	Fri	22.22BC	W	Fri

Cr = Creamy
W = White
YCr = Yellowish Creamy
Fri = Friable
Com = Compact

Table 38. Effect of different concentrations of 2, 4-D or NAA singly or NAA in combination with BAP in MS medium on callus induction from leaf explants of three varieties of potato. In each treatment 10 explants were inoculated. Data were recorded after eight weeks of culture.

PGRS (mg/l)	Varieties			Variety			Variety		
	Atlanta			Japanese red			Kenne bec		
	% of callusinduction	Callus colour	Callus type	% of callus induction	Callus colour	Callus type	% of callus induction	Callus colour	Callus type
2,4-D									
3	77.78A	Cr	Fri	66.66A	W	Com. Dry	44.44A	YCr	Com.Dry
3.5	66.66A	Cr	Fri	50.00B	W	Com	33.33AB	Cr	Dry
4	52.78B	Cr	Fri	27.78C	Cr	Fri	30.55AB	W	Fri
NAA									
1.5	38.89C	W	Com	33.33C	Cr	Fri	27.78AB	Cr	Fri
2	36.11CD	Cr	Fri	27.78C	Cr	Fri	27.78AB	Cr	Fri
2.5	19.44E	Cr	Fri	27.78C	Cr	Com	0.0000C	-	-
NAA+BAP									
1+0.5	36.11CD	YCr	Fri	27.78C	W	Com	25.00AB	Cr	Fri
0.5+1	36.11CD	YCr	Com	0.0000D	-	-	25.00AB	Cr	Fri
1+1	22.22DE	Cr	Com	25.00C	Cr	Fri	16.66BC	Cr	Fri

Cr = Creamy
W = White
YCr = Yellowish Creamy
Fri = Friable
Com = Compact

4.6 INDUCTION OF SOMATIC EMBRYO

The calli developed from different explants of potato varieties in MS media containing different concentrations of phytohormones were aseptically taken out and cut into small pieces and sub cultured onto fresh basal media for somatic embryo induction.

Two experiments were conducted to induce somatic embryos and the results obtained are explained below.

4.6.1 Effect of Explants Source

Internode, leaf explants derived compact or friable, dry, cream coloured calli were selected for somatic embryogenesis. The selected calli were sub-cultured at every 2-3 weeks interval in the corresponding same media compositions for further callus growth. After 2-3 sub-cultures when the calli were turned to light yellow in colour and more friable texture they were transferred to MS media supplemented with different concentrations and combinations of auxins (2,4-D and NAA) and cytokinin (KIN+BAP) and incubated in dark for the induction of somatic embryo. Somatic embryo formation was observed over the surface of the callus in same of the tested media formulations within 1-5 weeks after transfer.

The no. of somatic embryos was counted with the help of stereo microscope. Data on days of embryo initiation, % of calli induced somatic embryo and no. of somatic embryo was recorded. The results on the effect of different culture media formulations on the induction of somatic embryos are described under the following heads.

4.6.2 Effect of BAP+NAA on Somatic Embryogenesis

The internode derived calli were sub-cultured onto MS media supplemented with different concentrations of BAP+NAA and incubated in dark for the induction of somatic embryo. Data were collected on the effect of morphogenic

differentiation of cultured calli to somatic embryogenesis and results are presented in Table 39.

Somatic embryo formation from the internode derived calli was noticed in all BAP+NAA and KIN+NAA formulations. However response of the calli to embryo development was found to vary significantly with plant growth regulator formulations. Among the all formulations, 3mg/L KIN in combination with 1 mg/L was found to be the most effective formulations for somatic embryo induction from leaf and internode derived callus. The highest 52% of the calli incubated in this medium formulation were induced to develop somatic embryo for internode explants.33.89% calli when cultured onto 3mg/L BAP+0.5mg/L NAA underwent somatic embryogenesis (Table 39).

Time taken to somatic embryo initiation in different plant growth regulator formulations was not so different. The stages of somatic embryo differentiation were clearly distinguishable. All stages of developing embryos such as proembryogenic cell mass, globular, apparently heart shape embryo were visible in mass on the same callus.

The no. of somatic embryogenic calli was found to be different depending upon plant growth regulator formulations. The highest 29.66 somatic embryogenic callus was recorded onto 3mg/L KIN with 1mg/L NAA supplemented medium. The 2nd highest no of somatic embryos was recorded in 3mg/L BAP+1 mg/L NAA. The no. of somatic embryos was the lowest in 3mg/L BAP+0.5 mg/L NAA supplemented culture medium (table. 39).

In case of leaf, the percentage of calli formed somatic embryo ranged from 24.56-44.56%. Among the different media formulations however, 3mg/L KIN in combination with 1mg/L NAA was found to be the most effective media formulation where maximum 44.56% calli underwent somatic embryogenesis. The lowest 24.56% of the calli induced to develop somatic embryos in media supplemented with 4mg/LKIN+1mg/L NAA (Table 39).

Days of somatic embryo initiation and no's of somatic embryos were varied. The highest no. of somatic embryo was 25.76 recorded onto 3mg/L KIN with 1mg/L NAA supplemented medium, whereas no. of somatic embryo was the lowest in 4mg/LKIN with 1mg/L NAA supplemented medium.

From the experiment it is observed that internode was found to be the best explant sources for somatic embryogenesis. It further is clear that KIN+NAA (3+1) mg/l was the best for induction of somatic embryos from internode and leaf explants.

Table. 39. Effect of different concentrations of BA+NAA or combination of KIN+NAA in MS medium on somatic embryo development from leaves and internodes explants derived callus of different potato varieties. In each experiment 12 explants were inoculated, data were recorded after 4 weeks of culture.

PGR	Source	Days of embryo initiation	% of calli induced Somatic embryogenesis	No. of somatic embryo
BAP+NAA	**Internode**			
3+1		17.92B	49.22AB	28.34A
3+1.5		25.27A	40BC	21.73B
3+0.5		25.09A	33.89C	17.57B
KIN+NAA				
4+1		23.53AB	39.33BC	19.89B
4+1.5		22.21AB	39.44BC	20.12B
3+1		17.91B	52.00A	29.66A
LSD at 5% level		5.387	9.464	5.760
BAP+NAA	**Leaf**			
3+1		19.10A	41.56AB	24.60A
3+1.5		21.33A	30.89BC	15.47B
3+0.5		20.58A	31.00ABC	16.22B
KIN+NAA				
4+1		22.19A	24.56C	12.20B
4+1.5		22.90A	28.33BC	13.14B
3+1		19.22A	44.56A	25.76A
LSD at 5% level		5.743	12.40	6.025

4.6.3 Effect of Genotypes

This experiment was conducted with internode and leaf explants from 3 potato varieties. Objective of this investigation was to find out genotypic potentiality of somatic embryogenesis from different explants derived calli of 3 potato varieties. The % of calli formed somatic embryos, no. of embryos was recorded after 6 weeks of subculture and the results are presented in table 40.

From internode, the percentage of calli induce somatic embryo ranged from 31.33-52.56%. In Atlanta, the highest percentage (52.56%) of calli induced somatic embryo was obtained from internode explants. The lowest (31.33%) percentage of calli induced somatic embryo was noted in Kenne bec (Table 40).

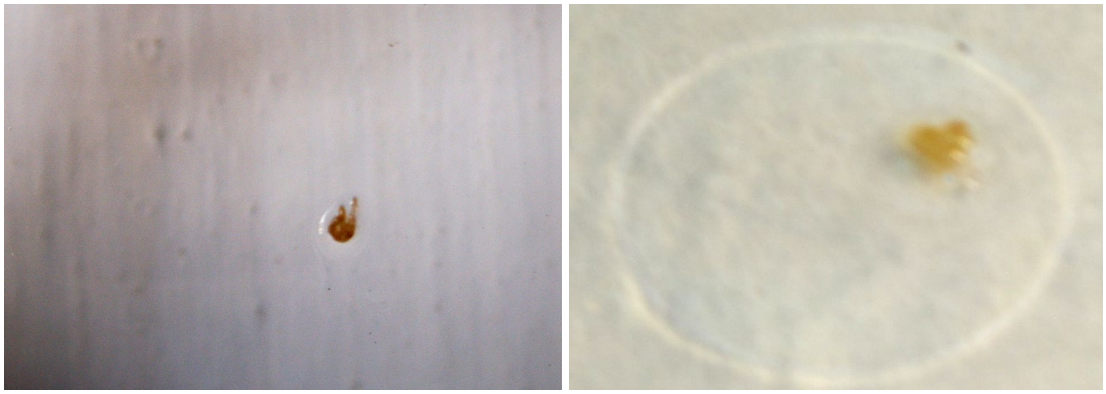
The no. of somatic embryos ranged from 14.78-30.85. The highest no. of somatic embryos was noted in Atlanta followed by 23.02 in Japanese red. The lowest no. of embryos was noted in Kenne bec. All varieties are significantly different from each other for the percentage of somatic embryo and no. of somatic embryo (Table 40).

Variations were noted for the days of embryo initiation with the culture media formations. In Japanese red, the cultured calli took comparatively shorter lag period to undergo somatic embryogenesis. In this variety, the days to embryo initiation was recorded 20.15.On the other hand; somatic embryogenesis took place in the calli after a longer period of time (24.34) in Kenne bec (Table 40).

From leaf explants, the percentage of calli formed somatic embryo ranged from 24.89-44.83%. The highest (44.83%) of calli induced somatic embryo was obtained from leaf explants in Atlanta followed by 30.72% in Japanese red. The lowest percentage (24.89%) of calli induced somatic embryo was recorded in Kenne bec. In this parameter, Atlanta was significantly different from others. In this parameter, Atlanta was significantly different from others (Table 40).

Days of embryo initiation ranged from 19.47-22.48. In Japanese red, cultured calli took comparatively shorter lag period (19.47) to undergo somatic embryogenesis. On the other hand, somatic embryogenesis took place in the calli after a longer period of time (22.48) in Kenne bec.

Significantly variations were also observed on no. of somatic embryos. Numerous somatic embryos at different stages of development were appeared over the surface of the embryogenic callus. The highest 26.95 somatic embryos callus was observed in Atlanta. The lowest no. of somatic embryos (11.38) was noted in Kenne bec. It was observed that Atlanta was significantly different from other varieties.



A. Atlanta **B. Japanese red**

Figure 11: Somatic embryo development from embryogenic callus in MS medium supplemented with MS+BAP 3mg/l+ NAA 1mg/l of different potato varieties.

A. Torpedo shaper embryo in Atlanta.

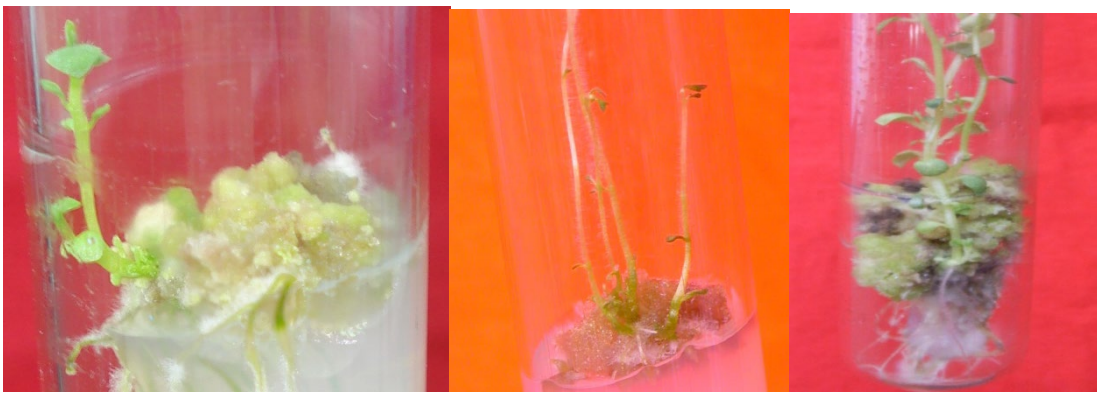
B. Heart shaped embryo in Japanese red.

Table. 40. Effect on somatic embryo development from leaves and internodes explants derived callus of different potato varieties. In each experiment 12 explants were inoculated, data were recorded after 4 weeks of culture.

Variety	Source	Days of embryo initiation	% of calli induced somatic embryogenesis	No of somatic embryo
	Internode			
Atlanta		21.47AB	52.56A	30.85A
Japanese red		20.15B	43.06B	23.02B
Kenne bec		24.34A	31.33C	14.78C
LSD at 5% level		3.809	6.692	4.073
	Leaf			
Atlanta		20.71A	**44.83A**	**26.95A**
Japanese red		19.47A	30.72B	15.37B
Kenne bec		22.48A	24.89B	11.38B
LSD at 5% level		4.061	9.472	4.260

4.7 SHOOT REGENERATION FROM CALLUS

The calli from internode and leaf explants were further transferred to fresh MS basal media with different types of growth regulators to form shoot and root of different potato varieties. The callus formed shoot 2-3 weeks after transfer to MS media with different concentrations of kinetin alone or in combination with BAP+NAA and KIN+NAA. Here green shoots were evidently observed after 4 weeks of culture. The culture for another 4 weeks led to the formation of complete plantlets. These plantlets could easily be separated from each other and also from the original callus. Not all the somatic embryos develop into plantlets, some of them disappeared while others formed root only.



A Atlanta **B. Japanese red** **C. Kenne bec**

Figure 12: Shoot regeneration from internode derived calli.

A. In MS +BA 3mg/l + NAA 1mg//l after four weeks of subculture in Atlanta.
B. In MS +BA 3mg/l + NAA 1mg//l after four weeks of subculture in Japanese red.
C. In MS +BA 3mg/l + NAA 1mg//l after four weeks of subculture in Kenne bec.

4.7.1 Shoot Regeneration from Internode Derived Callus

The culture of internode derived calli is described under the following heads.

4.7.1.1 Effect of different concentrations and combinations of BAP+NAA on shoot and root regeneration from internode derived calli

Here the Calli derived from internodal explants of different potato varieties were sub cultured on semisolid MS medium supplemented with different concentrations and combinations of BAP with NAA in order to find out the most suitable culture media formulation to format shoot. The percentage of shooting and rooting, number of shoot and root per callus were considered as parameters for evaluating with experiment. Data on these parameters from different treatments were recorded after 8 weeks of culture and are presented in Table 41.

In Atlanta, the highest 47.22% of shooting was obtained from internode explants in MS media supplemented with different concentrations and combinations of 3 mg/l BAP+1 mg/l NAA followed by 27.78% of shooting in

media 3mg/l BAP+.5mg/l NAA. The lowest 22.22% of shooting was recorded in medium containing 3mg/l BAP+1.5 mg/l NAA (Table 41).

The percentage of rooting per callus ranged from 63.89 to 86.11%.The highest 86.11% of rooting per callus was obtained in medium having 3 mg/l BAP+1 mg/l NAA. The lowest 63.89% of rooting was recorded in medium containing 3mg/l BAP+1.5 mg/l NAA (Table 41).

Number of shoots per callus ranged from 1.567 to 3.417. The highest (3.417) number of shoots per callus was observed in media having 3 mg/l BAP+1 mg/l NAA. In culture media supplemented with 3mg/l BAP+1.5 mg/l NAA, the lowest 1.567 number of shoots was observed (Table 41).

The highest 4.933 number of roots was formed in MS media supplemented with different concentrations and combinations of 3 mg/l BAP+1 mg/l NAA. This combination was found to be most effective for root and shoot formation. In the culture medium supplemented with 3mg/l BAP+1.5 mg/l NAA the lowest 2.800 number of roots were formed (Table 41).

In Japanese red, it was observed that the percentage of *in vitro* shoot formation ranged from11.11- 30.55 %.The highest 30.55% calli induced *in vitro* shooting was obtained from internode explants in MS media supplemented with different concentrations and combinations of 3 mg/l BAP+1 mg/l NAA followed by 16.66% of shooting in media 3mg/l BAP+.5mg/l NAA. The lowest 11.11% of shooting was recorded in medium containing 3mg/l BAP+1.5 mg/l NAA (Table 41).

The percentage of rooting per callus ranged from 47.22 to 77.78%. The highest 77.78 % of rooting per callus was found in medium having 3 mg/l BAP+1 mg/l NAA. The lowest 47.22 % of rooting was recorded in medium containing 3mg/l BAP+1.5 mg/l NAA (Table 41).

The number of shoots per callus ranged from 1.167 to 1.933. The highest (1.933) number of shoots per callus was observed in media having 3 mg/l BAP+1 mg/l NAA followed by 1.267 in internode derived calli and the lowest 1.167 number of shoots was observed in culture media supplemented with 3mg/l BAP+1.5 mg/l NAA (Table 41).

The highest 3.713 number of roots was formed in MS media supplemented with different concentrations and combinations of 3 mg/l BAP+1 mg/l NAA. This combination was found to be most effective for root and shoot formation. In the culture medium supplemented with 3mg/l BAP+.5 mg/l NAA the lowest 2.400 number of roots were formed (Table 41).

For the calli derived from internodal segments of Kenne bec, the highest 36.11% of shooting was obtained from internode explants in MS media supplemented with different concentrations and combinations of 3 mg/l BAP+1 mg/l NAA followed by 13.88% of shooting in media 3mg/l BAP+1.5mg/l NAA. The lowest 11.11 % of shooting was recorded in medium containing 3mg/l BAP+0.5 mg/l NAA.

Here the percentage of rooting per callus ranged from 49.99- 83.33 %.The highest 83.33% of rooting per callus was found in medium having 3 mg/l BAP+1 mg/l NAA. The lowest 49.99% of rooting was recorded in medium containing 3mg/l BAP+1.5 mg/l NAA (Table 41).

The number of shoots per callus also ranged from 1.383-2.233. The highest (2.233) number of shoots per callus was observed in media having 3 mg/l BAP+1 mg/l NAA. In culture media supplemented with 3mg/l BAP+0.5 mg/l NAA, the lowest 1. 3 83 number of shoots was observed.

The highest 3.933 number of roots was formed in MS media supplemented with different concentrations and combinations of 3 mg/l BAP+1 mg/l NAA. This combination was found to be most effective for root and shoot formation.

On the other hand, 3mg/l BAP+1.5 mg/l NAA and 3mg/l BAP+0.5 mg/l NAA showed the similar number of roots.

4.7.1.2 Effect of different concentrations and combinations of KIN+NAA on shoot and root regeneration from internode derived calli

Internode derived calli developed in MS medium containing different concentrations and combinations of KIN+NAA for shoot and root regeneration. Subcultures were done at three weeks interval and morphogenic differentiation of the subcultured calli was observed periodically. The results of the effect of KIN+NAA on shoot and root regeneration on shoot and root regeneration are shown in Table 41.

In Atlanta, the highest 33.33% of shooting was obtained in MS media supplemented with different concentrations and combinations of 3 mg/l KIN+1 mg/l NAA followed by 27.77% of shooting in media 4mg/l KIN+1mg/l NAA. The lowest 13.89% of shooting was recorded in medium containing 4mg/l KIN+1.5 mg/l NAA. Among the different levels of KIN+NAA, 4mg/l KIN+1.5 mg/l NAA and 3 mg/l KIN+1 mg/l NAA showed significantly different (Table 41).

The percentage of rooting per callus was found to be different between 4mg/l KIN+1mg/l NAA and 3 mg/l KIN+1 mg/l NAA and ranged from 58.33 to 77.78% in the research. The highest 77.78% of rooting per callus was obtained in medium having 3 mg/l KIN+1 mg/l NAA. The percentage of roots was the lowest 58.33% recorded in 3mg/l KIN +1.5 mg/l NAA supplemented culture medium.

The variations were observed between 3 mg/l KIN+1 mg/l NAA , 4mg/lKIN+1mg/l NAA and 3 mg/l KIN+1 mg/l NAA, 4mg/l KIN+1.5 NAA for the number of shoots per callus and ranged from 2.433- 3.167. Among the different media formulations however, 3 mg/l KIN in combination with1 mg/l NAA was found to be most effective medium formulation, where callus underwent maximum 3.167 number of shoots. In culture media supplemented

with 4 mg/l KIN+1mg/l NAA, the lowest 2.433 number of shoots was observed.

Variations were noted between 4mg/l KIN+1mg/l NAA and 3 mg/l KIN+1 mg/l NAA. The highest 4.923 number of roots was formed in MS media supplemented with different concentrations and combinations of 3 mg/l KIN+1 mg/l NAA. Whereas, number of roots was the lowest in 4mg/l KIN+1 mg/l NAA supplemented medium.

In Japanese red, percentage of shooting in different combinations of KIN+NAA was not so different. The highest 27.78% of shooting was recorded from internode explants onto 3 mg/l KIN+1 mg/l NAA supplemented medium. followed by 25% of shooting in media 4mg/l KIN+1mg/l NAA. The lowest 22.22% of shooting was recorded in medium containing 4mg/l KIN+1.5 mg/l NAA (Table 41).

The percentage of rooting per callus ranged from 44.44- 58.33 %.The highest 58.33% of rooting per callus was recorded onto 3 mg/l KIN+1 mg/l NAA medium. On the other hand, 44.44% of rooting was recorded when cultured onto 4mg/l KIN+1.5 mg/l NAA.

The number of shoots per callus was not varied with different formulations of KIN+NAA and ranged from 1.567-1.933. The highest (1.933) number of shoots per callus was recorded onto 3 mg/l KIN+1 mg/l NAA supplemented medium. Whereas, number of shoots was the lowest in 4mg/l KIN+1.5 mg/l NAA, supplemented medium.

Number of roots per callus was found to be different between 4mg/l KIN+1mg/l NAA and 3 mg/l KIN+1 mg/l NAA and ranged from 2.987 to 4.433. The highest 4.433 number of roots was formed in MS media supplemented with different concentrations and combinations of 3 mg/l KIN+1 mg/l NAA. Results indicate that addition of 3mg/l KIN with 1mg/l NAA to the

culture medium gave better effect on shoot and root formation. In the culture medium supplemented with 4mg/l KIN+1.5 mg/l NAA the lowest 2.987 number of roots were formed.

In Kenne bec, variation was not observed in the percentage of shooting. The highest 25.00 % calli induced to form *in vitro* shooting was obtained both in MS medim having 3 mg/l KIN+1 mg/l NAA and 4mg/l KIN+1mg/l NAA. The lowest 22.22 % of shooting was recorded in medium containing 4mg/l KIN+1.5 mg/l NAA (Table 41).

The variations were noted for percentage of rooting per callus and ranged from 47.22 to 80.55%.The highest 80.55 % of rooting per callus was found in medium having 3 mg/l KIN+1 mg/l NAA. The lowest 47.22 % of rooting was recorded in medium containing 4mg/l KIN+1.5 mg/l NAA.

The number of shoots per callus ranged from 1.767 to 2.067 and no variations were observed in this parameter. The highest (2.067) number of shoots per callus was observed in media having 3 mg/l KIN+1 mg/l NAA and the lowest 1.767 number of shoots was observed in culture media supplemented with 4mg/l KIN+1.5 mg/l NAA.

The highest 3.567number of roots was formed in MS media supplemented with different concentrations and combinations of 3 mg/l BAP+1 mg/l NAA. This combination was found to be most effective for root and shoot formation. In the culture medium supplemented with 4mg/l KIN+1 mg/l NAA the lowest 3.043 number of roots were formed.

4.7.2 Shoot Regeneration from Leaf Derived Callus

4.7.2.1 Effect of different concentrations and combinations of BAP+NAA on shoot and root regeneration from leaf derived calli

The calli derived from leaf explants of different potato varieties were sub cultured on semisolid MS medium supplemented with different concentrations

and combinations of BAP with NAA in order to find out the most suitable culture media formulation to format shoot. Percentage of shooting and rooting, number of shoot and root per callus were considered as parameters for evaluating with experiment. Data on these parameters from different treatments were recorded after 8 weeks of culture and are presented in Table 41.

In Atlanta, the highest 25.00 % of shooting was obtained from leaf explants in MS media supplemented with different concentrations and combinations of 3 mg/l BAP+1 mg/l NAA followed by 22.22 % of shooting in media 3mg/l BAP+0.5mg/l NAA. The lowest 13.88% of shooting was recorded in medium containing 3mg/l BAP+1.5 mg/l NAA (Table 41).

The variation was noted for the percentage of rooting per callus and ranged from 41.66 to 77.77%. Among the three treatments of BAP and NAA the highest 77.77% of rooting per callus was obtained in medium having 3 mg/l BAP+1 mg/l NAA. The lowest 41.66 % of rooting was recorded in medium containing 3mg/l BAP+.5 mg/l NAA.

The highest (2.333) number of shoots per callus was observed in media having 3 mg/l BAP+1 mg/l NAA. On the other hand, number of shoots per callus was similar noted in other BAP and NAA media formulations.

The highest (3.333) number of roots per callus was observed in media having 3 mg/l BAP+1.5 mg/l NAA. On the other hand, the number of roots per callus was almost similar noted in other media formulations of BAP and NAA.

In Japanese red, no variation was observed for the percentage of *in vitro* shoot formation. Percentage of shoot (16.66%) per callus was similar both in 3 mg/l BAP+1 mg/l NAA and 3mg/l BAP+1.5mg/l NAA. The lowest 13.89% of shooting was recorded in medium containing 3mg/l BAP+.5 mg/l NAA (Table 41).

Variation was noted for the percentage of rooting per callus and ranged from 38.89 to 63.89 %. The highest 63.89% of rooting per callus was found in medium having 3 mg/l BAP+1 mg/l NAA. The lowest 38.89 % of rooting was recorded in medium containing 3mg/l BAP+1.5 mg/l NAA.

Number of shoots per callus ranged from 1.200 to1.733. The highest (1.733) number of shoots per callus was observed in media having 3 mg/l BAP+1 mg/l NAA and the lowest 1.200 number of shoots was observed in culture media supplemented with 3mg/l BAP+1.5 mg/l NAA.

The highest 3.407number of roots was formed in MS media supplemented with different concentrations and combinations of 3 mg/l BAP+1.5 mg/l NAA. . In the culture medium supplemented with 3mg/l BAP+0.5 mg/l NAA the lowest 2.800 number of roots were formed.

For the calli derived from leaf segments of Kenne bec, the highest 22.22% of shooting was obtained from leaf explants in MS media supplemented with different concentrations and combinations of 3 mg/l BAP+1 mg/l NAA. The lowest 8.330% of shooting was recorded in medium containing 3mg/l BAP+1.5 mg/l NAA. Variations were observed in both medium formulations.

Percentage of rooting per callus ranged from 25.00 - 63.89%. The highest 63.89% of rooting per callus was found in medium having 3 mg/l BAP+1 mg/l NAA followed by 30.55% of rooting in media 3mg/l BAP+1mg/l NAA. The lowest 25.00 % of rooting was recorded in medium containing 3mg/l BAP+.5 mg/l NAA. In this parameter variation was observed between 3mg/l BAP+1mg/l NAA and 3mg/l BAP+1.5 mg/l NAA and 3mg/l BAP+1mg/l NAA, 3mg/l BAP+.5mg/l NAA

Number of shoots per callus ranged from1.267 -2.233. Highest (2.233) number of shoots per callus was observed in media having 3 mg/l BAP+1 mg/l NAA. In culture media supplemented with 3mg/l BAP+.5 mg/l NAA, lowest 1.267

numbers of shoots was observed. In this parameter variation was observed between 3mg/l BAP +.5 mg/l NAA and 3mg/l BAP+1.5 mg/l NAA.

The highest number of roots (3.467) was formed in MS media supplemented with different concentrations and combinations of 3 mg/l BAP+1 mg/l NAA. On the other hand, the lowest 1. 267 numbers of roots were observed in 3mg/l BAP+1.5 mg/l NAA. No variation was observed in this parameter.

4.7.2.2 Effect of different concentrations and combinations of KIN+NAA on shoot and root regeneration from leaf derived calli

Leaf derived calli developed in MS medium containing different concentrations and combinations of KIN+NAA for shoot and root regeneration. Subcultures were done at three weeks interval and morphogenic differentiation of the sub cultured calli was observed periodically. The results of the effect of KIN+NAA on shoot and root regeneration on shoot and root regeneration are shown in Table 31.

In Atlanta, highest 19.44 % of shooting was obtained in MS media supplemented with different concentrations and combinations of 3 mg/l KIN+1 mg/l NAA followed by 13.88% of shooting in media 4mg/l KIN+1.5mg/l NAA. Lowest 11.11% of shooting was recorded in medium containing 4mg/l KIN+1mg/l NAA (Table 41).

The percentage of rooting per callus ranged from 33.33 to 47.22%. Highest 47.22% of rooting per callus was obtained in medium having 3 mg/l KIN+1 mg/l NAA. The percentage of roots was lowest 33.33 % recorded in 4mg/l KIN+1.5 mg/l NAA supplemented culture medium.

Variations were observed for the number of shoots per callus and ranged from 1.433 - 2.567. Among the different media formulations however, 3 mg/l KIN in combination with 1 mg/l NAA was found to be most effective medium formulation, where callus underwent maximum 2.567 number of shoots. In

culture media supplemented with 4 mg/l KIN+1mg/l NAA, the lowest 1.433 number of shoots was observed.

No variation was noted for the number of roots per callus. The highest 3.833 number of roots was formed in MS media supplemented with different concentrations and combinations of 3 mg/l KIN+1 mg/l NAA. Whereas, number of roots was the lowest 3.033 in 4mg/l KIN+1 mg/l NAA supplemented medium.

In Japanese red, percentage of shooting in different combinations of KIN+NAA was not so different. The highest 22.22% of shooting was recorded from leaf explants onto 3 mg/l KIN+1 mg/l NAA supplemented medium. The lowest 16.66% of shooting was recorded in both medium containing 4mg/l KIN+1.5 mg/l NAA and 4mg/l KIN+1mg/l NAA (Table 41).

Percentage of rooting per callus ranged from 36.11 -49.99 %. The highest 49.99% of rooting per callus was recorded onto 3 mg/l KIN+1 mg/l NAA medium. On the other hand, 36.11 % of rooting was recorded when cultured onto 4mg/l KIN+1.5 mg/l NAA and 4mg/l KIN+1mg/l NAA. Variation was observed between 3mg/l KIN+1mg/l NAA , 4mg/lKIN+1mg/l NAA and 3mg/l KIN+1mg/l NAA, 4mg/l KIN+1.5 mg/l NAA.

Number of shoots per callus was not varied with different formulations of KIN+NAA and ranged from 1.200-1.633. The highest (1.633) number of shoots per callus was recorded onto 3 mg/l KIN+1 mg/l NAA supplemented medium. Whereas, number of shoots was the lowest (1.200) in 4mg/l KIN+1.5 mg/l NAA, supplemented medium.

Number of roots per callus ranged from 2.883 to 3.933. Highest 3.933 number of roots was formed in MS media supplemented with different concentrations and combinations of 3 mg/l KIN+1 mg/l NAA. Results indicate that addition of 3mg/l KIN with 1mg/l NAA to the culture medium gave better effect on shoot

and root formation. In the culture medium supplemented with 4mg/l KIN+1.5 mg/l NAA the lowest 2.883 number of roots were formed.

In Kenne bec, variation was not observed in the percentage of shooting. The highest 22.22 % calli induced to form *in vitro* shooting was obtained both in MS medium having 3 mg/l KIN+1 mg/l NAA and 4mg/l KIN+1mg/l NAA. The lowest 13.88 % of shooting was recorded in medium containing 4mg/l KIN+1.5 mg/l NAA (Table 41).

The percentage of rooting per callus ranged from 41.66 to 47.22%.The highest 47.22% of rooting per callus was found in medium having 3 mg/l KIN+1 mg/l NAA. The lowest 41.66 % of rooting was recorded in medium containing 4mg/l KIN+1.5 mg/l NAA.

Number of shoots per callus ranged from 1.267 to1.617 and no variations were observed in this parameter. The highest (1.617) number of shoots per callus was observed in media having 3 mg/l KIN+1 mg/l NAA and the lowest 1.267 number of shoots was observed in culture media supplemented with 4mg/l KIN+1.5 mg/l NAA.

Highest 2.933 number of roots was formed in MS media supplemented with different concentrations and combinations of 3 mg/l KIN+1 mg/l NAA. This combination was found to be most effective for root and shoot formation. In the culture medium supplemented with 4mg/l KIN+1 mg/l NAA the lowest 2.760 number of roots were formed.

Table 41. Effect of different concentrations and combinations of BAP with NAA and KIN with NAA in MS medium on shoot regeneration from internode and leaf explants- derived calli of different potato varieties. In each treatment 10 calli were inoculated. Data were recorded after 30 days of culture.

PGRS (mg/l)	Callus source	% of morphogenic response		No of shoot/ callus	No of root/ callus
		Shooting	Rooting		
Atlanta					
BAP+NAA					
3+1	Internode	47.22A	86.11A	3.417A	4.933A
	Leaf	25.00A	77.77A	2.333AB	3.033A
3+1.5	Internode	22.22BC	77.78AB	1.567C	3.033B
	Leaf	13.88AB	61.11B	1.633BC	3.333A
3+.5	Internode	27.78BC	63.89BC	1.700BC	2.800B
	Leaf	22.22AB	41.66C	1.633BC	3.033A
KIN+NAA					
4+1	Internode	27.77BC	58.33C	2.433ABC	2.600B
	Leaf	11.11B	33.33C	1.433C	3.033A
4+1.5	Internode	13.89C	72.22ABC	2.700AB	3.800AB
	Leaf	13.88AB	44.44C	1.683BC	3.533A
3+1	Internode	33.33AB	77.78AB	3.167A	4.923A
	Leaf	19.44AB	47.22BC	2.567A	3.833A
Japanese red					
BAP+NAA					
3+1	Internode	30.55A	77.78A	1.933A	3.713AB
	Leaf	16.66A	63.89A	1.733A	3.233A
3+1.5	Internode	11.11B	47.22B	1.167B	3.283BC
	Leaf	16.66A	38.89C	1.200A	3.407A
3+.5	Internode	16.66AB	50.00B	1.267AB	2.400C
	Leaf	13.89A	47.22B	1.367A	2.800A

PGRS (mg/l)	Callus source	% of morphogenic response		No of shoot/ callus	No of root/ callus
		Shooting	Rooting		
KIN+NAA					
4+1	Internode	25.00AB	52.77B	1.750AB	3.567AB
	Leaf	16.66A	36.11C	1.383A	2.883A
4+1.5	Internode	22.22AB	44.44B	1.567AB	2.987BC
	Leaf	16.66A	36.11C	1.200A	3.567A
3+1	Internode	27.78A	58.33AB	1.933A	4.433A
	Leaf	22.22A	49.99B	1.633A	3.933A
Kenne bec					
BAP+NAA					
3+1	Internode	36.11A	83.33A	2.233A	3.933A
	Leaf	22.22A	63.89A	1.467AB	3.467A
3+1.5	Internode	13.88BC	49.99C	1.967A	3.067A
	Leaf	8.330B	30.55BC	2.233A	3.267A
3+.5	Internode	11.11C	58.33BC	1.383A	3.067A
	Leaf	13.88AB	25.00C	1.267B	2.800A
KIN+NAA					
4+1	Internode	25.00AB	63.89B	1.967A	3.043A
	Leaf	22.22A	44.44B	1.517AB	2.760A
4+1.5	Internode	22.22BC	47.22C	1.767A	3.133A
	Leaf	13.88AB	41.66BC	1.267B	2.833A
3+1	Internode	25.00AB	80.55A	2.067A	3.567A
	Leaf	22.22A	47.22AB	1.617AB	2.933A

4.7.2.3 Field evaluation of callus derived plantlets

Well developed plantlets from the selected calli for each of cultivars were excised / exercised and transplanted separately. Accession of somaclones was named and maintained as A_1-A_5, J_1-J_5, K_1-K_5 foe Atlanta, Japanese red and Kenne bec respectively. These plantlets grown inside the test tubes were brought out from the growth chamber. After deplugging the culture tubes were kept in the room temperature for 4-5 days to bring them in contact to normal temperature. After 3-5 days of hardening the plantlets were taken out from test tubes carefully. The acclimatized plantlets were transplanted on the specially prepared field. Data were collected from 5randomly selected plants of each somaclonal line and mean values were calculated.

For evaluation of somaclonal variations among the somaclones, data on plant height, no. of leaves/plant, no. of branches/plant, no. of tuber/ plant and tuber weight/plant were recorded and the results are presented in Table 42.

Considerable variation was noticed among the plants of the 5 callus lines of variety Atlanta for all the characters. Among the different somaclones A_5 showed the highest plant height (27.503cm) and the lowest plant height was recorded (22.373cm) in A_3 callus line. LSD value revealed that the variation observed for the plant height among some of callus lines were statistically significant. The highest number of leaves (17.857) was recorded in A2 callus line and the lowest (13.893) for the plants for A_3.Similarly the highest number of branches (7.483) was recorded in A_1 callus line and the lowest (4.863)for the plants for A_3. Highest number of tubers (29.880) was recorded for the plants belong to A_1 callus line and the lowest (17.037) for the plants of A_3. Tuber weight/ plant (234.870g) was recorded highest for callus line A_3 and lowest (187.240g) for the plants under the callus line A_1. Analysis of variance followed by subsequent LSD test for these attributes supported real difference among the different callus lines. In addition of these changes or modifications in some other characters were also observed. Plants with more green leaves, deformed leaves, dwarfism and modification in canopy structure were also noted in some of the plants.

Table 42. Somaclonal variation among randomly selected 5 somaclones of the potato variety Atlanta. Data on plant height, number of leaves/plant and number of branches/plant were recorded 60 days after plantation and number of tubers/plant and tuber weight/plant was recorded 90 days after plantation.

Sl.No.	ATLANTA					
	Plant accession	**Plant height (cm)**	**Number of leaves/ plant**	**Number of branches/ plant**	**Number of tubers/ plant**	**Tuber weight/ plant (g)**
01	A1	27.37	16.167	7.483	29.88	187.24
02	A2	25.467	17.857	6.777	25.61	223.877
03	A3	22.373	13.893	4.863	22.57	234.87
04	A4	22.497	14.51 7	5.737	29.063	203.64
05	A5	27.503	17.69	6.113	17.037	189.63
Mean		25.042	16.025	6.195	25	207.851
LSD at 5% level		1. 397	1.982	1.299	2.323	54.91

Somaclonal variation among different callus lines of variety Japanese red were also observed for all characters under study (Table 42) Plants developed from the callus line J_1 displayed the highest plant height (27.37cm).Highest number of the leaves per plant (17.927)was noted in J_4 callus line. The highest number of branches (6.143) and highest number of tuber (18.970) per plant was noted for the plants belonging to the callus lines J_3 , J_1 respectively. The highest weight of tuber (79.197g) per plant was found in callus line J_4.On the other hand, the lowest plant height (18.523cm), lowest number of leaves (17.927), lowest number of branches (6.143), lowest number of tuber (18.970) and lowest weight of tuber (79.197g) were recorded for the plants belonging to the callus lines J_3 , J_1 , J_2 , J_3 and J_4 respectively. Statistical analysis for this character also supported the presence of significant variation. .

Table 43. Somaclonal variation among randomly selected 5 somaclones of the potato variety Japanese red. Data on plant height, number of leaves/plant and number of branches/plant were recorded 60 days after plantation and number of tubers/plant and tuber weight/plant was recorded 90 days after plantation.

Sl. No.	JAPANESE RED					
	Plant accession	**Plant height (cm)**	**Number of leaves/ plant**	**Number of branches/ plant**	**Number of tubers/ plant**	**Tuber weight/ plant (g)**
01	J1	26.317	11.743	5.387	18.97	58.610
02	J2	20.06	15.52	5.36	14.273	57.423
03	J3	18.523	14.707	6.143	11.597	51.467
04	J4	24.367	17.927	5.407	12.24	79.197
05	J5	19.733	15.303	5.63	13.553	61.417
Mean		21.8	15.04	5.585	14	61.623
LSD at 5% level		4.385	2.231	1.527	2.285	4.7761

In Kenne bec variety variations among different callus lines were observed for all the characters under study. Plants developed from the callus line K_5 displayed the highest plant height (31.497cm), highest tuber weight (206.873g) per plants. The highest number of leaves (23.347), highest number of tuber (31.293) and highest number of branches (8.300) per plant were recorded for the plants belonging to the callus lines K_2 and K_1.On the other hand, lowest plant height (24.877cm), lowest number of leaves (10.730) were recorded in K_4. Lowest number of branches/plant (3.963), lowest tuber weight/plant (144.563g) and lowest number of tuber/plant (17.800) were observed for the plant belonging to the different callus lines K_3 and K_5.Statistical analysis of the individual character also supported the existence of significant variation observed among the different callus lines.

Table 44. Somaclonal variation among randomly selected 5 somaclones of the potato variety Kenne bec. Data on plant height, number of leaves/plant and number of branches/plant were recorded 60 days after plantation and number of tubers/plant and tuber weight/plant was recorded 90 days after plantation.

	Kenne bec					
Sl.No.	**Plant accession**	**Plant height (cm)**	**Number of leaves /plant**	**Number of branches/ plant**	**Number of tubers/ plant**	**Tuber weight /plant (g)**
01	K1	30.293	20.53	8.3	23.007	203.763
02	K2	25.34	23.347	6.07	31.293	153.830
03	K3	27.05	17.427	3.963	27.747	144.563
04	K4	24.877	10.73	6.467	20.833	159.217
05	K5	31.497	15.46	5.437	17.8	206.873
Mean		27.811	17.499	6.047	24	173.649
LSD at 5% level		3.37	2.009	0.9361	3.78	19.12

4.8 RESPONSE OF POTATO UNDER NaCl STRESS CONDITION

In the present investigation, changes in growth have been studied under NaCl stress condition. The results of these experiments are as follows:

From above ANOVA table the statistical results of different morphological characters of salt tolerance of 3 potato varieties showed significant differences among variety and treatment (table-45,46, 47, 48).

Table 45. Statistical results (ANOVA) on shoot length of salt tolerance of three potato varieties.

Source of variation	Sum of squares	Degree of freedom	Mean square	F-value	Table value
Variety	11.40727	2	5.703633	14.167	5.14
Treatment	32.68993	3	10.89664	27.065	4.76
Error	2.415667	6	0.402611		
Total	46.51	11			

Table 46. Statistical results (ANOVA) on shoot fresh mass of salt tolerance of three potato varieties.

Source of variation	Sum of squares	Degree of freedom	Mean square	F-value	Table value
Variety	3469.706	2	1734.853	9.260	5.14
Treatment	19907.07	3	6635.691	35.418	4.76
Error	1124.11	6	187.3516		
Total	24500.89	11			

Table 47. Statistical results (ANOVA) on root length of salt tolerance of three potato varieties.

Source of variation	Sum of squares	Degree of freedom	Mean square	F-value	Table value
Variety	11.58582	2	5.792908	17.232	5.14
Treat	31.02849	3	10.34283	30.767	4.76
Error	2.016983	6	0.336164		
Total	44.631293	11			

Table 48. Statistical results (ANOVA) on root fresh mass of salt tolerance of three potato varieties.

Source of variation	Sum of squares	Degree of freedom	Mean square	F-value	Table value
Varity	267.5357	2	133.7679	15.145	5.14
Treatment	546.4096	3	182.1365	20.621	4.76
Error	52.99635	6	8.832725		
Total	866.94165	11			

4.8.1 Effect of Salt Stress on Growth of Potato Plants

To evaluate the effects of salt stress on the growth of potato, the physiological differences between 3 potato varieties grown under saline conditions were determined. The length of shoots and roots in Atlanta, Japanese red and Kenne bec varieties were measured 4 weeks after exposure to 25mM/L, 50mM/L, 75mM/L NaCl. Sodium chloride treatment adversely affected the shoot and root length of three varieties but plantlet growth was not affected by 25mM NaCl containing MS media and generally it was almost similar to control levels where as 75 mM NaCl media significantly reduced plantlet growth compared with the control.

Four weeks after NaCl treatment, the fresh weight of shoots and roots were measured in the 3 potato varieties. Shoot length, shoot fresh mass, root length and root fresh mass was gradually decreased with the increase of NaCl level.

In shoot length significant differences were observed with different NaCl level. Significant differences were also observed between 25mM NaCl level and control plantlet in shoot fresh mass. For root length and root fresh fresh mass significant variations were observed in 75mM NaCl level and control planets. The control was always superior compared to the treatments employed for all growth traits (Table 49).

4.8.2 Effect of Salt Stress on Genotypes

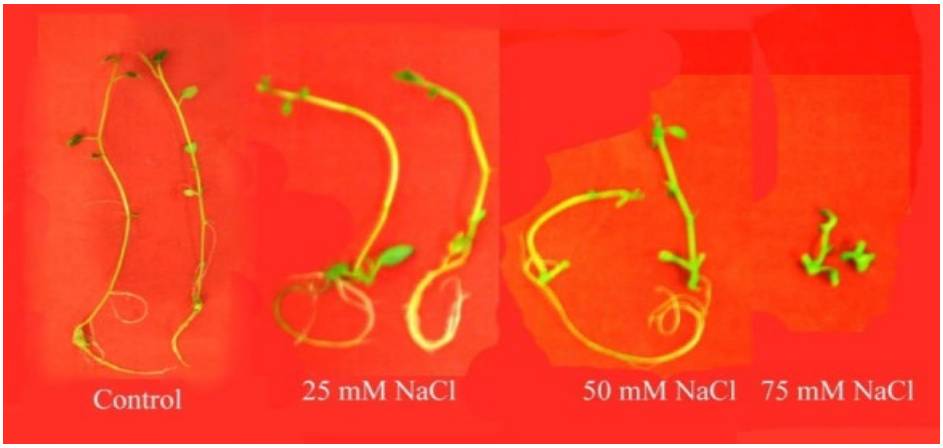
To evaluate the mechanisms of salt tolerance in potato varieties, the morphological changes were observed. The highest shoot length (5.695) and root length (6.345) was observed in Kenne bec followed by 4.395 and 4.65 in Atlanta. The lowest shoot and root length was 3.31 and 4.01 in Japanese red. The Kenne bec was significantly different from others for both parameters (Table 49).

Table 49. Analysis of mean data on shoot length, shoot fresh mass, root length and root fresh mass of *in vitro* potato plantlets as affected by NaCl and varieties after four weeks of incubation.

Variety	Shoot length	Shoot fresh mass	Root length	Root fresh mass
Atlanta	4.395b	77.72a	4.65b	30.35a
Japanese red	3.31b	45.61a	4.01b	20.24b
Kenne bec	5.695a	84.64b	6.345a	20.43b
LSD at 5% level	1.27	27.35	1.16	5.94
Treatment				
25mM	4.87b	68.35b	4.82b	33.22b
50mM	3.63c	41.7cd	4.44b	25.22b
75mM	2.45d	31.35cd	3.17c	21.81c
control	6.91a	135.88a	7.58a	14.45a
LSD at 5% level	1.097	23.68	1.003	5.14

From the Table 49 the shoot fresh mass was noted maximum in Kenne bec followed Atlanta and Japanese red. In this parameter Kenne bec was significantly different from others.

Maximum root fresh mass was noted in Atlanta (30.35) and it was about similar in Japanese red and Kenne bec. It was observed that Atlanta was significantly different from others.



A

B

C

Figure 13: Salinity stress gradually affects the plantlets growth of three potato cultivars: A) Atlanta, B) Japanese red and C) Kenne bec

CHAPTER-IV
DISCUSSION

5.1 MERISTEM CULTURE

The technique of meristem tip culture is one of the most use *in vitro* culture initiation (Danci *et al*., 2012).

Initially, meristems were isolated from field grown 20-25 days explants of potato varieties and cultures on MS (Murashige and Skoog, 1962) liquid media with different types of pgrs like BAP, GA3 KIN, NAA either singly or in combination in order to find out the best culture media formulation for primary established meristem culture.

DAS-ELISA methods conclusively proved that PVX, PVY and PLRV viruses were not present in the meristem derived plantlets. Many workers used this popular serological detection method for identification of different kinds of viruses in different crops (Alam, 1995; Akanda *et al*., 1991a, 1991b). Thus the results also support that the plantlets produced through meristem culture were free from these viruses.

The use of liquid medium for initial establishment of isolated meristem was also supported by other crops (Yee *et al*., 2001; Islam *et al*., 2003; Alam *et al*., 2003; Ayabe and Sumi, 2001).

Potatoes seem to harbor many varieties very often in mixed infection. For the purpose of this study tubers infected with PVY and PVY as demonstrate by serology/ELISA were used as a source of meristem culture. The correct diagnosis of any viral disease in a prerequisite of control, the more rapidly and accurately the causal organism in identified, the sooner the proper controls can be instituted. Apathy towards research on control of viral disease is due to large

of difficulties in identifying viruses. Therefore, the use of methods of viruses' detection including ELISA test was essentially needed.

Meristem of 3 cultivated varieties namely Atlanta, Japanese red and Kenne bec were established on with and without hormone in order to produce potato plant PVY free. The developed meristem were subculture on MS media with different types PGRS like BAP, GA_3, KIN, NAA either singly or in combination in order to find out the best culture media formulation for primary establishment meristem culture in potato. The media with 1 mg/l BAP+0.3mg/l GA_3 also showed better performance to enhance the growth of the meristem. Using of 0.5mg/l GA_3 was most effective for proper shoot development from primary developed meristem. Similarly, in combination of GA_3+KIN was found to be the best media for the primary establishment of meristem. It was also observe that GA_3 was found to be the best for the best media for shoot induction.

The variety Atlanta was more responsive for micro propagation of plantlets than Japanese red and Kenne bec. Different response of different potato varieties due to genetic makeup towards *in vitro* shoot multiplication and their development were reported by (Ghaffor *et al.*, 2003; Elaleem, 2009).

The results of this research showed that all potato plantlets samples from the three cultivars derived from meristem culture that Atlanta was free from PVX and PVY. Very little bit infection of PVX and PVY showed in Japanese red and Kenne bec.

This study also demonstrates the value of meristem culture to free potato from important virus PVX, PVY. Previous investigation of virus by tip culture could be due to the failure of virus to enter the meristem (Ostrolucka *et al.,* 2004; Donnelly, 2003; Helliot, 2002).

Potato is generally propagated and distributed as vegetative material. Several important vascular pathogens including viruses and virus like diseases are systemic in diseased plants and are difficult to detect unless special indexing tests are used. Because of the high risk of introducing new pathogens in the transfer of planting material, it may be possible, however, to promote the international exchange of potato by shipping pathogen-free plants in the form of meristem tip cultures.

Lower concentration of auxin (0.01mg/l NAA) with Gibberelic acid (0.25mg/lGA_3) is best for development of complete plantlets and multiplication from meristem tips (Badoni and Chauhan, 2009).Higher concentration of NAA inhibit root and shoot growth (Pennazio and Vecchiati, 1976).

Shibli *et al.* (2002) subcultured potato variety Spunta on liquid MS media containing benzyladinine (BA) and Kinetin. Significant reduction in stem and internode length was observed by increasing BA and kinetin concentrations.

Rabbani *et al*. (2001) studied the effects of different concentrations (1, 2, 3, 4 and 5mg/l) of GA_3 and benzylaminopurin (BAP) on the *in vitro* multiplication of potato cv. Desiree. The maximum shoot length (8.96cm) was obtained when 4mg/l GA_3 was applied. The maximum number of shoots (14) was obtained when 2mg/l BAP was applied.

Shoot induction and shoot elongation was found to be highly influenced by the type and concentration of growth regulators present proliferation media. Among all the combinations and concentrations GA_3 0.5mg/l showed the best performance for shoot multiplication. The result also demonstrated that the highest length (4.76cm) of the longest shoot were recorded in media having GA_3 0.5 mg/l.

Plant regeneration from tissue culture of potato has been successfully applied to breeding programmes by many workers. For many years tissue culture

techniques have been applied to improve potato production by means of micro propagation, pathogen elimination. Potato is usually vegetatively propagated and very much susceptible to the number of viral disease, which causes remarkable decrease in yield. However, meristem culture is one of the important methods to produced virus free stock plants (Wang and Hu, 1982). Whereas, micro propagation does not permit the micro clones to be free from viruses. The shoot of a apical meristem and first step to primordial leaves are generally not connected to the vascular system of the plant and therefore, are not contaminated by virus that travel through the vascular system. If this explants is carefully excised so as not to contaminate it with sap from more mature leaves or stem tissue and it is placed in culture tube a virus free plant can be established. Thermotherapy or heat treatment has proved to be very effective in virus elimination (Towill, 1981; De, 1992).Meristem culture in combination with heat treatment can be useful technique to eliminate different viruses. The present investigation was therefore undertaken to eliminate viruses by the application of meristem. In this study the primary explants produced shoots in media with cytokinin singly or in combinations with cytokinin and auxins but did not show any roots of the explants.

In order to find out genotypic response, 3 varieties Atlanta, Japanese red Kennebec were cultured separately in MS liquid medium supplemented with different concentrations of plant growth regulators. In the experiment it was observed that genotypic difference existed among 3 varieties and Atlanta showed the best response followed by Japanese red, Kenne bec. Kenne bec showed the poorest response.

For shoot multiplication, established meristems were subcultures onto MS semi-solid media supplemented with different concentrations of cytokinin and auxins either singly or in combination in order to find out suitable growth regulator formulations for maximum shoot proliferation. Multiple shoot induction and shoot elongation were found to be highly influenced by the type

and concentrations of growth regulators present in shoot proliferation media. Among all the concentrations and combinations of cytokinin and auxin, GA_3 0.3 mg/l showed the best performance for shoot multiplication. The highest number of shoots and length of the longest shoot were recorded in media having GA_3 0.3mg/l and GA3 0.5mg/l.

Generally, during shoot multiplication development of sufficient roots was also observed. Therefore, it was not necessary to conduct separate experiment for adventitious root induction on the regenerated shoots.

To find out the varietals performance of 3 potato varieties viz. Atlanta, Japanese red and Kenne bec an experiment was carried out and the results of this experiment proved that 3 varieties showed more or less equal performance with all combinations and concentrations of GA_3, KIN and BAP. The varieties showed brilliant performance in media with GA_3 0.3 mg/l for multiple shoot proliferation. The variety Atlanta was more responsive for massive micro propagation of plantlets than Japanese red and Kenne bec.

In the present study five agronomic traits were screened to detect variation of the meristem derived plants of different cultivars. Highest number of branches/ plant was observed in Atlanta which was significantly higher than rest of the varieties. Lowest no. of branches/plant was observed in Japanese red. The no. of leaves/plant varied with the variety. Highest number of leaves/plant was observed in Atlanta followed by Kenne bec that differed significantly than the other varieties.

It was also observed that the Atlanta produced higher number and weight of tuber/plant which was significantly higher than varieties followed by Kenne bec while the lowest no. and weight of tuber was observed in Japanese red.

5.2 CALLUS INDUCTION

In the present study *in vitro* response of three locally grown potato varieties were tested with respect of 4 different explants and various hormonal combinations. Considering the responses of two potato explants, leaf and internode segment from 3 potato varieties viz. Atlanta, Japanese red and Kenne bec, it may be concluded that internode was more responsive for induction of callus and showed better callusing than leaf segments. The main effect showed that the percentage of callus induction from internode was higher than leaf segments.

In Atlanta, Japanese red and Kenne bec among all the treatments of 2, 4-D the percentage of callus induction was found significantly higher in 3mg/l 2, 4-D from internode explants. In Atlanta, the highest callusing rate was recorded in media having 3mg/l 2, 4-D (88.88%) from intermodal explants followed by 3.5 mg/l 2,4-D (72.22%). The lowest callus was found in 4mg/l 2, 4-D (63.88%). The percentage of callus induction was the highest in MS media containing 3mg/l (77.78%) followed by 3.5 mg/l (66.66%) from leaf explants. In general 2,4-D formulations was found more effective in callus induction then those of NAA. The explants cultured without growth regulator did not produce any callus; these results are in support of the results which obtained by (Fiegert *et al*., 2000; Jayasree *et al*., 2001; Yasmin *et al*., 2003).

According to the results of the present study also indicated that the highest regeneration percentage was found in Atlanta in different concentrations and combinations of auxin and cytokinin (3mg/l BAP+1 mg/l NAA) from internode explants. The results were similar to Nasrin *et al*. (2003) in potato. Khatun *et al*. (2003) also found same result when using 1.0 mg/l BAP+1.0 mg/l NAA in potato callus induction. Similar results were also reported by Kathari and Chandra (1984) in African Merigold.

There were some results which was dissimilar with the previously reported studies. Yadav and Sticklen (1995); Alphonse *et al*. (1998); Hamdi *et al*. (1998) observed that leaf was the best explants for regeneration. It may be due to use of different genotypes.

From the above experimental results, it was clearly focused that MS media supplemental with 3.5mg/l and 3 mg/l 2, 4-D were the best formulation for callus induction in potato. The result is in agreement with Shirin *et al*. (2007) who used 2,4-D for callus induction from internodal and leaf explants obtained from four potato cultivars including Diamant, and found that among all concentrations and combinations 2,4-D at 3.00 mg/l was found to be the most effective auxin concentration for callus induction in all cultivars. Cytokinins, such as BAP with kinetin, at low concentrations in combination with auxins were often used in plant species to promote callus initiation (Chai and Marian, 1998). However, Mamun *et al.* (1996) reported that 2, 4-D proved less effective when use alone for other plant species. Sultana (2001) used 2, 4-D alone for callus induction from internode and leaf explants of potato. She also obtained same results like Mamun *et al.* (1996) in three potato varieties. However, Malamug *et al.* (1991) found better results when used 2, 4-D as a callus inducing plant growth regulator in potato callus induction. Khatun *et al*. (2003) and Nasrin *et al*. (2003) used 2, 4-D alone for callus induction and found better results in potato.

Castillo *et al*. (1998) reported that auxin 2,4-D by itself or in combination with cytokinins has been widely used to enhance callus induction and maintenance. Moreover many researchers observed 2, 4-D as the best auxin for callus induction as common as in monocot and even in dicot (Evans *et al*., 1981; Ho and Vasil, 1983; Jaiswal and Naryan, 1985; Chee, 1990; Mamun *et al*., 1996).

Calli derived from the two sources of explants (internode and leaf segments) were subcultured for shoot regeneration in MS medium supplemented with different concentrations and combinations of BAP with NAA and KIN with NAA both were found effective for shoot regeneration of 3 varieties from internode and leaf explants derived callus. Internode showed better performance in regeneration through callus. The percentage of regeneration was recorded higher in case of internode (47.22%) than leaf segment (25%) to develop shoot. The highest regeneration percentage was found in 3+1 BAP+NAA in Atlanta followed by 3+1mg/l BAP+NAA (36.11%) in Kenne bec from internode explants. But there is no regeneration without growth regulator. It was also observed that internode was also more responsive explants than leaf segments.

Somaclonal variation among plant as regenerated through callus culture was reported by Chandra *et al*. (1985); Nasrin *et al*. (2003); Sultana *et al*. (2005). They observed that plantlets regenerated through callus culture were not genetically stable. In the present study statistically significant variations regarding some morphological characters were also observed among the different calliclones of four potato cultivars, which support the findings of Chandra *et al*. (1985). Somaclonal variations was also observed and reported in many other crops by many earlier workers (Shepard *et al*., 1980; Larkin *et al*., 1984; Scowcroft, 1977).

Different concentrations of growth regulators showed significant differences in shoot length. The interaction effects between explants concentrations on shoot length had shown also significant differences. The longest shoot (5.28cm) was produced by the treatment combination of 1.0 mg/L BAP+0.1mg/L GA_3.This agree with Sarker and Mustafa (2002) and Asma *et al*. (2001) who also observed the organogenesis of potato with the BAP and GA_3. Martel *et al.* (1992) reported that both BAP and GA_3 with higher concentrations were necessary for shoot formation.

The auxin 2, 4-D by itself or in combination with cytokinins has been widely used to enhance callus induction and maintenance. The tuber segment explants induced callus on MS media supplemented with 2, 4-D. On the other hand, failed to produced callus on MS medium without 2, 4-D, this declared that, the presence of 2, 4-D was capable to inducing callus. Moreover, many researchers observed 2, 4-D as the best auxin for callus induction as common as in monocot and even in dicot. When the MS media were supplemented with the highest concentration (2.0- 5.0mg/l) of 2, 4-D, 100% of the explants were found yellow, watery callus and recorded the highest degree for callus formation. Among all concentrations, only 2, 4-D at high concentration (3.0mg/l) was found to be the most effective concentration for callus induction in all cultivars used (khadiga *et al*., 2009).

Bakul (2005) reported that regeneration of potato plantlets and microtuberization of cardinal, Diamant and patrones varieties. BAP has found to enhance plantlet regeneration markedly. All the varieties showed better response on plantlet regeneration with 1.0-2.0 mg/l BAP alone or 1mg/lBAP+0.01-0.1mg/L NAA.

Zhang *et al*. (2005) reported the effect of auxin; GA_3 and BAP on potato shoot growth and tuberization under *in vitro* condition. The shoot length of potato explants increased with the increasing of concentrations (0.5-10.0mg/dm^3 of IAA treatment especially with the addition of GA_3 (0.5mg/dm^3), but was inhibited by BAP (0.5 mg/dm^3).

Omidi *et al*. (2003) was observed the effect of cultivar and explants on callus induction in leaf and internodes explants of six potato cultivars (Viz.Agria, cosmos Santi, Concord, Ajix and Diamant cultivars), explants and their interaction on frequency of callus induction were significant on MS medium supplemented with 5 mg/l 2,4-D and 0.25 mg/l KIN.

Sarker and Mustafa (2002) reported regeneration of two indigenous potato varieties of Bangladesh, Lal Pakri and Jam Alu. They observed the effects of various combinations of BAP, KIN and GA_3 on multiple shoot regeneration from nodal segments of potato.

For internode and leaf explants, it may be mentioned that among the three varieties used in the present study, Atlanta was found to be the best genotype for *in vitro* direct regeneration and it was also found that internode explants showed better shoot regeneration, such as, explants producing roots and number of multiple shoots than leaf explants.

For direct shoot regeneration, the appropriate concentration of cytokinin was BAP+NAA (3+1mg/l) for internode and leaf explants. Highest 47.22% and 25% shoot was produce in Atlanta at BAP+NAA (3+1) mg/l concentration from internode and leaf explants.

Among the varieties, Atlanta showed the best response but other two varieties also produced shoot followed by *in vitro* rooting. The plantlets were successfully transplanted in soil. Therefore, the presently evaluated hormonal supplements gives not only a good evaluation of popular potato varieties, but also establish a genotype independent regeneration protocol can be used in future to develop transgenic crops in improve production.

5.3 SALINITY RESPONSE OF POTATO

Although potato is a commercially important crop, it cannot grow satisfactory in arid or semiarid areas of the world where salt stress is a major problem. Little is known about the salt tolerance or the physiological consequences of salt tolerance or the physiological consequences of salt stress in potato (Backhosen *et al.,* 2005).In field experiments, it has been shown that potato plants lose chlorophyll and protein and accumulate proline / praline when subjected of salt stress (Heure and Nadler, 1998). Concentrations of NaCl

above 50 mmol/L were sufficient to cause growth restrictions and decrease tuber yield in various field grown cultivars (Backhosen *et al*., 2005). These findings indicated that potato plants display the same salt sensitively as other glycophytic crops. However, all these results come from field experiments in which environmental factors cannot be satisfactorily controlled.

Salt stress severely decreased the growth of three cultivars that we examined, but the adverse effects of salt were much more severe in Japanese red than others in terms of all the physiological characteristics that we measured. This indicates that Kenne bec, Atlanta are relatively salt-tolerant cultivars compared with Japanese red, which is very sensitive to salt. This is in agreement with the findings of Jeffferies (1996), who proposed that genetic variations in salt tolerance exist in potato cultivars. The reduction in growth parameters under salt stress in potato cultivars in our experiments confirmed previous studies that potato was a relatively salt sensitive crop (Backhosen *et al*., 2005).In a manner consistent with our results, salt sensitive cultivars of pea (Hernandez *et al*., 2000) and lentil (Bandeoglu *et al*., 2005) have shown considerably the reduced growth compared with tolerant cultivars under NaCl treatment. It has also been reported that under salt stress, relatively salt tolerant potato cultivars (Rahman and Ebrahimzadeh, 2004).The addition of NaCl to the culture media decreased the osmotic potential of the media inducing salinity stress that a adversely affected the callus growth and *in vitro* regeneration capacity of potato cultivars. Several authors reported the use of NaCl for *in vitro* salinity screening in different plants (Vijayan *et al*., 2003; Zhao *et al.*, 2009).With increasing of NaCl, the proline/ praline content of all cultivars significantly increased. Marthirez *et al*. (1996) reported a positive relationship between proline accumulation and NaCl tolerance in potato (Mohamed *et al*., 2007; Aghaleh and Niknam, 2009).

The regeneration potential was decreased with increasing NaCl levels. A similar objective was found by Yusef *et al*. (1994), Cano *et al*. (1998) and

Mercado *et al.* (2000) in tomato using tissue culture techniques for *in vitro* selection for salinity tolerance Hamdy (2002); Liu and Li (1991) found that callus was formed on 5% NaCl, but was less vigorous and the shoot formation rate was decreased as compared as compared with the control treatment.

It was noticed that shoot length decreased with increased root length and root fresh mass. On the other hand, shoot fresh mass and root fresh masss increased with the decreased NaCl levels in media. The results indicate that Kenne bec performed better in shoot length, shoot fresh mass and root length than Atlanta and Japanese red. The Atlanta performed better in root fresh mass than Japanese red and Kenne bec at different NaCl media. Highest salinity level drastically inhibits root growth in all the cultivars tested.

Based on the physiological, biological and morphological characters observed in the present study, three varieties Atlanta, JPR and Kenne bec can be classified as moderately salinity tolerant.

From this experiment it can be recommended that taking three growth parameter measurements at 25, 50, 75mM NaCl for further micro propagation scheme and consequently it can be used as a source of salt tolerant line for field transplantation where salinity level is a major obstacle to potato production.

CHAPTER-VI

SUMMARY

Integrated biotechnological techniques such as meristem culture for producing pathogen free planting materials, plant generation from induced callus for producing somaclonal variants and *in vitro* salt tolerant lines for identifying genotypes useful for cultivation in salinity soil condition were studied on three potato varieties (Atlanta, Japanese red, Kenne bec).

To establish virus free plantlets, meristems were isolated from shoot tips of 20-25 days old field grown plants. For surface sterilization of shoot tips, $HgCl_2$ (0.1%) treatment for 3 minutes was found to most effective for complete killing of surface pathogens and getting healthy tissues of isolated meristems. Isolated meristems were placed on filter paper bridge in tube containing MS liquid medium supplemented with different concentrations and combination of growth regulators and MS_0 in order to find out suitable culture media for primary establishment of meristem. Among these media formulations both MS_0 and GA_3 (0.3 mg/L) were found most effective and suitable for primary establishment of isolated apical meristem.

Established meristems were sub cultured on semisolid medium of MS_0 and different concentrations and combinations of auxins, cytokinins and gibberellic acid for auxillary shoot proliferation. Among all these growth regulators, GA_3 of 0.3 mg/l was the best medium formulation for shoot proliferation. Among the studied 3 varieties, Atlanta was found most responsive for micro propagation using meristem culture.

Field performance of mericlones of 3 potato varieties was found encouraging for producing pathogen free planting materials. Atlanta was the best performing varieties in ex vitro condition. Few meristem mericlones were infected with pathogens, whereas most of the control plants were infected with

pathogens. So, therefore, meristem culture in potato can successfully be used for production of virus free stock plants for commercial cultivation.

Between two types of explants (internode and leaf), internode was found most responsive for callus induction where tested in MS medium supplemented with different concentrations and combinations of auxins and cytokinins. In this case, 2, 4-D (auxin) was found most effective plant hormone for callus induction. 2, 4-D was found the best concentration for producing effective callus in studied three genotypes. Calli derived from different types of explants and different media compositions were used for shoot regeneration. Comparing between BAP+NAA combination with KIN+NAA, maximum shoot induction from internode explants derived calli was noticed in BAP+NAA. Regarding concentration, 3mg/l BAP+1mg/l NAA was recorded the best combination for plant regeneration from induced callus. Here 47.22% of internode derived callus produced multiple shoots in Atlanta, 30.55% in Japanese red and 36.11% in Kenne bec. Highest number of shoots (3.4) was also recorded in the same medium. On the other hand, when leaf derived calli were tested for shoot initiation maximum calli (25%) induced to develop multiple shoots regeneration in the same media (3mg/l BAP+1mg/l NAA). In Atlanta also 3mg/l BAP+1mg/l combination of phytohormone can be recorded for multiple shoot formation for calli either induced from internode or leaf.

Plants regenerated through callus culture in potato showed distinct somaclonal variation for plant height, number of leaves/plant, Number of tubers/plant and tuber weight/plant. This variability can be useful to select improve potato lines.

In vitro evaluations of salinity (NaCl) effects on 3 potato varieties (Atlanta, Japanese red and Kenne bec) were investigated under three levels (0, 25, 50, 75mM) of NaCl using node culture. Significant differences were observed among the varieties as well as in NaCl levels of the studied traits. Salinity stress gradually hampered plant growth and root development with the increase

of NaCl concentration. Although the studied genotypes survived at high (75mM) concentration level of NaCl but exhibited different growth patterns. The results indicate that Kenne bec performed better at different NaCl media than other two genotypes. High salinity level drastically inhibits in all tested genotypes. Here it is noticed that 25mM NaCl did not affect mass the growth traits of *in vitro* potato plantlets.

CHAPTER VII
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APPENDIX

Preparation of stock solution of MS (Murashige and Skoog, 1962) basal medium.

Components	Amount (mg/l)	Strength of stock solution	Volume of stock solution (ml)	Amount for stock solution (mg)	Amount (ml) for 1 liter medium
Stock solution I					
1. $NH_4 NO_3$	1650	20x	1000	33000	20
2. KHO_2	1900	20x		38000	
3. KH_2PO_4	170	20x		3400	
Stock solution II					
1. $MgSO_4.7H_2O$	370	20x	1000	7400	20
Stock solution III					
1. $CaCl_2.2H_2o$	440	20x	1000	8800	20
Stock solution IV					
1. $FeSO_4.7H_2O$	27.8	20x	1000	556	20
2. $Na_2EDTA.2H_2O$	37.3	20x		746	
Stock solution V					
1. $MnSO_4.4H_2O$	22.3	20x	1000	446	20
2. H_3BO_4	6.2	20x		124	
3. $ZnSO_4.7H_2O$	8.6	20x		172	
Stock solution VI					
1. KI	0.83	1000x	1000	830	2
2. $CuSO_4.5H_2O$	0.025	1000x		25	
3. $Na_2MoO_4.2H_2O$	0.25	1000x		250	
4. $CoCl_2.6H_2O$	0.025	1000x		25	
Stock solution VII					
1. Myoinositol	100	100x	200	10000	2
2. Nicotinic acid	0.5	100x		50	
3. Pyridoxine-HCI	0.5	100x		50	
4. Thaimine-HCI	0.5	100x		50	
5. Glycine	2.0	100x		200	

$FeSO_4/7H_2O$ and Na_2EDTA $2H_2O$ were dissolved separately in 400 ml DW by heating and constant stirring. The two solutions were then mixed, the pH was adjusted to 5.5. DW was added to make the final volume to 1000 ml.