EFFECTS OF PLANT GROWTH REGULATORS AND CULTURE SYSTEMS ON *IN VITRO* REGENERATION AND MICROTUBERIZATION OF MERISTEM AND SHOOT TIP CULTURE OF SELECTED POTATO CULTIVARS

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The thesis attached hereto, entitle "**Effects of Plant Growth Regulators and Culture Systems on *In Vitro* Regeneration and Microtuberization of Meristem and Shoot Tip Culture of Selected Potato Cultivars**" was prepared under the direction of chairperson of the Candidate's Supervisory Committee and has been approved by all members of that committee as a requirement for the degree of **Master of Agricultural Science (Horticulture and Agricultural Biotechnology).**

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This thesis represents the original work of the author, except where otherwise stated. It has not been submitted previously for a degree at any University.

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DEDICATED TO MY BELOVED PARENTS,
U HLA DIN AND DAW KHIN LAY

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ABSTRACT

The experiment was conducted at the Plant Tissue Culture Laboratory, Department of Horticulture and Agricultural Biotechnology, YAU from 2015 to 2016. Three selected potato cultivars, Up-to-date, L-11 and Atlantic were used in this study. In initial culture, RCB design was used with 4 replications and treatments were three potato cultivars. There were two main experiments in this study: meristem culture and shoot tip culture.

In experiment I, two factors factorial arrangement in RCB design was used with 4 replications. The meristem explants, were used for shoots induction in three potato cultivars as factor A, using 9 different combinations of 6-benzyl aminopurine (BAP) 0.0, 1.5, 3.0 $mg.L^{-1}$ and gibberellic acid (GA_3) 0.0, 0.3, 0.6 $mg.L^{-1}$ as factor B. The meristem explants produced hundred percent survival shoots in medium both supplemented with 1.5 $mg.L^{-1}$BAP in combination with 0.6 $mg.L^{-1}GA_3$ and 3.0 $mg.L^{-1}$BAP in combination with 0.3 $mg.L^{-1}GA_3$ for all tested cultivars. From this experiment, the shoots induced by Up-to-date were used for shoot multiplication due to better response in meristem culture. For shoot multiplication, three different combinations of plant growth regulators (PGRs) 0.05 $mg.L^{-1}$Naphthalene acetic acid (NAA) in combinations with two levels of 1.0 and 0.5 $mg.L^{-1}$BAP, and PGRs free as control were used as treatments in 3 x 4 RCB design. Among all treatments the significantly longest shoot length was observed in shoots developed on PGRs free medium. Microtuberization was done to evaluate the effects of two different concentrations of sucrose 6%, 8% and two level of BAP 3 $mg.L^{-1}$, 5 $mg.L^{-1}$ using RCB design with 4 replications. Plantlets developed on medium supplemented with 8% sucrose and 3 $mg.L^{-1}$BAP showed 100% microtuberization and gave the largest sized tuber in meristem culture derived explants.

In experiment II, shoot multiplication of shoot tip culture derived three potato cultivars, 3 factor factorial in RCB arrangement was used with 4 replications, factor A was three potato cultivars, factor B was 3 different combinations of PGRs used in experiment I and factor C was 2 culture systems: solid and liquid culture. Liquid culture system was superior to solid MS in such growth parameter as shoot length, no. of nodes, no. of roots and fresh weight. PGRs free provided the best result in liquid medium. However, 0.05 $mg.L^{-1}$NAA and 0.5 $mg.L^{-1}$BAP combination was suitable to use in solid medium. For microtuberization of shoots derived from shoot tip culture, the same treatments of sucrose and PGRs applied in experiment I were used in three potato

cultivars. L-11 cultivar provided 100% microtuberization in medium supplemented with 8 % sucrose and 5 mg.L^{-1}BAP. Medium modified with 8% sucrose and 3 mg.L^{-1}BAP combinations gave 96.43% microtuberization in L-11 and 92.85% Up-to-date cultivar. In comparing meristem culture with shoot tip culture in Up-to-date cultivar, meristem explants provided better performance in shoot multiplication, microtuberization and size of microtuber. In confirmation of plantlets developed from meristem culture by using "POCKET DIAGNOSTIC", these plantlets were free from potato five multivirus and *Rhizotonia solanacearum.*

Keywords: PGRs, shoot multiplication, explants, meristem culture, microtuberization

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CHAPTER I
INTRODUCTION

Potato (*Solanum tuberosum* L.) is an important food and cash crop with a high nutritional value that gives better nutrients per unit area per unit time than any crop (Lemaga et al. 2009). It is the fourth largest food crop and one of the most widely distributed crops in the world (Zhang et al. 2004). According to the World Potato Center's research, worldwide demand for potato will exceed that of rice, wheat or corn by 2020. It is also regarded as a high potential food security crop because of its ability to provide a yield of high quality product per unit input with a shorter crop cycle (mostly<120 days) than major cereal crops like maize (Hirpa et al. 2010). Moreover, potato is a good cheap source of carbohydrate and provides vitamin, minerals, protein and most of the trace elements which can meet the energy requirements of the developing countries (Irfan 1992).

Asian countries occupy 40% of the world potato production area. In Myanmar, according to DAP (2015) the total potato growing area is 37,000 ha with an average yield of 15.11 t ha^{-1} that amounts to a total production of 551,000 t. Potato is also one of the main culinary crops and a promising profitable crop to Myanmar growers, so cultivation of potato is being extended to the arable lands in Myanmar. It can be grown all year round depending on season of planting. The hilly regions in Shan State are the most important potato production area with 60% of total national production whereas the central region of the country occupies 17%. In 2015, the total sowing areas in Shan State are 21874 ha with average yields of 15.27 t ha^{-1} (DOA Shan State 2015).

The most widely-grown cultivars in Shan State are Up-to-date (Sit Bo), L-11 (Shwe Heho) and Atlantic. Among them, Up-to-date cultivar is being grown all of the potato growing seasons. Because of its good eating quality and good storage quality, Up-to-date cultivar has been recommended as a good cultivar by the potato growing farmers. But it was introduced in 1915 and now subjected to degeneration. Moreover, it is highly susceptible to late blight, which is considered as one of the constraints for yield and production of potato (Myint 2001). Another late blight resistant cultivar is CIP-393371.157, serial number L-11, newly introduced clone from CIP (International Potato Research Center) since 2006. Because of its late blight disease resistance, the potato growers' preference and demand is increasing. Wiersema (1984) reported that the physiological quality and safety of seed tubers are one of the most important factors

influencing potato yield. Mass production of disease free, high quality seed tubers for potato growers is required. Moreover, it is needed to set up a suitable protocol for *in vitro* regeneration of potato cultivars to produce quality seed tubers (Pronk 2015).

In vitro propagation methods using meristem culture, nodal cuttings and microtubers are more relied for maintaining genetic integrity of the multiplied clones since de-differentiation and the subsequent organogenesis/ embryogenesis with the accompanying genetic changes have been reported by Wang and Hu 1982. Meristems are free from viruses, regeneration of virus free plants are possible through meristem culture (Jha and Ghosh 2005). Commercial production of disease free microtubers for the enhancement of potato yield through *in vitro* techniques along with traditional procedures can provide the excellent result (Faccioli and Colombarini 1996).

The availability of tissue culture technology for rapid multiplication of disease-free planting material has facilitated potato seed production to a great extent (Dodds 1992). Moreover, micro-tubers have tremendous advantages in terms of storage, transportation and mechanization. They can directly be sown into the soil and can be produced in bulk in any season. They also have similar morphological and biochemical characteristics to field produced tubers. Therefore, mass production of potato micro-tuber is likely to revolutionize the world potato production (Akita and Takayama 1994).

One of the problems in potato seed tuber production is degeneration, causing decrease in yield and quality of potato (Shibli et al. 2001). In general, increase sucrose concentration can increase the percentage and earliness of microtuberization. In our country, there are less technique for potato tissue culture and microtuber production.

Plant growth regulators (PGRs) are the most important factors in the regulation of shoot propagation. The previous findings indicated that the action of NAA, BAP and GA_3 improved growth and development of plantlets (Fufa and Diro 2013). Badoni and Chauhan (2009) found that MS medium supplemented with combination of GA_3 and NAA is good for shoot regeneration than the combination of Kinetin and NAA in potato cultivar Kufri Himalini. Tissue culture propagation method has high cost due to usage of hormones, media and other factors. Therefore, it is needed to reduce cost for tissue culture without compromising the quality of *in vitro* plantlets. Prakash (1993) classified *in vitro* culture media into two groups; liquid and solid media. Solid media is prepared by adding a solidifying agent into the liquid media. Use of liquid culture media showed better shoot and root growth in many plant species (Mehrotra et al. 2007). Pierik (1997)

pointed out that high shoot proliferation in liquid media could be due to availability for ease of uptake of water and nutrients and closer contact between explants and the medium. However, plantlet can suffer from hyperhydricity due to lower concentration of gelling agent in liquid media. Kuria et al. (2008) also reported that higher biomass accumulation is found in liquid media than in solid media. The growth of the nodal sections of potato plantlets in liquid and solid medium, the plantlet in liquid medium showed better and more vigorous growth than the other one (Qureshi et al. 2014).

It has been observed that low agar concentration in culture media increase the growth of certain potato species cultivated *in vitro* (Debergh 1983; Rossel et al. 1987), due to increased availability of water and dissolved substances to the explants which on the other hand also lower the cost of production. For these reason, the present study has been designed to observe the growth response of developed potato plantlets on solid (with agar) and liquid (without agar) media with different combination of PGRs and its effect on potato micropropagation and also microtuber production on different levels of sucrose and BAP for disease free seed tuber production were examined according to the following objectives:

1. To investigate the different responses of selected potato cultivars on PGRs combination in meristem culture
2. To examine suitable culture system, PGRs and sucrose combination for *in vitro* multiplication and microtuberization of the selected potato cultivar
3. To compare the responses of meristem and shoot tip culture on multiplication and microtuberization effiency
4. To confirm virus free plantlets developed from meristem culture

CHAPTER II
LITERATURE REVIEW

2.1 Potato Crop

Potato is a leading vegetable and the main cash crop of the world. It is a wholesome food and a good source of carbohydrates, vitamins, minerals and protein. It is originated in Andes of South America. The potato is a dicot plant belonging to the family Solanaceae and the genus *Solanum*. This is a large genus and contains 2000 species. According to the latest classification, the genus has been divided into two sub geneses, *Pachystemonum* and *Leptostemonum*. *Pachystemonum* has been further divided into five sections, of which section potato contains most of the tuber bearing species (Thamburaj and Singh 2001). Traditionally potato was adopted as a commercial food crop in temperate climate, mainly in Europe. As a crop of high biological value for its starch, protein and substantial amount of vitamins, minerals and trace elements, it is undoubtedly an important crop (Gebre et al. 2001).

2.1.1 Potato production in the world

The potato crop productivity average in Africa, Asia and Latin America has increased 44 percent, 25 percent and 71 percent, respectively, within a 30 year-old period (FAO 2001). Potato production and yield vary considerably among countries in Latin America. Bowen (2003) stated that potato is exceeded only by wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.), and maize (*Zea mays* L.) in the world production for human consumption. Potato provides nutritious food in diverse environments. The major world producers, in order of production, are China, India, Ukraine, United States. (FAO 2015).

2.1.2 Potato as a food crop and raw materials

Potato is an important human food with wider adaptability potential to fill the gap between food needs and cereals. The importance of vegetables in human nutrition is well known. In a country with limited resources, where the nutrition level of the population has to be maintained under unfriendly situations, the potato has a special value as food (Saini 2001).

2.2 Potato Tissue Culture

Potato is amenable to a number of tissue culture techniques ranging from *in vitro* propagation via shoot culture to regeneration of whole plants from protoplasts. In general these all involve the growth of plants, cells, tissues and organs in sterile condition,

supported by an appropriate culture medium. Tissue culture media normally contain a mixture of major and minor salts, vitamins, sugar (as a carbon source) and plant growth regulators. The most widely used media formulations are based on that of Murashige and Skoog (1962), which is available commercially (Karp 1987).

Potato is usually propagated asexually by means of tubers (Rahman et al. 2013). *In vitro* regeneration of potato is easily done from different explants on MS medium supplemented with different auxin and cytokinin for diseases free good quality seeds and pathogen free planting materials. Both callus induction and plant regeneration from explants require the appropriate combinations and concentrations of plant growth regulators in the culture media. BAP, zeatin or kinetin helps to produce adventitious shoots (Ehsanpour and Jones 2000).

Rapid multiplication is used to obtain large number of clonal plants while multiple shoot induction leads to production of disease free mother plants and seed tubers in large number. Tissue culture offers an excellent technique for rapid multiplication of potato plant (Tovar and Dodds 1986).

2.3 Potato Meristem and Shoot Tip Explants

Smith and Murashige (1970) accomplished the first true meristem culture of an isolated shoot angiosperm meristem into a complete plant. Meristem tip culture of cassava became popular and research was oriented towards elimination of virus disease for recovery of healthy clones in 1980s (Kartha and Gamborg 1975). Meristem culture technique has been applied to many crops, especially vegetative propagated crops such as potato, over the last 40 years to eliminate viruses from important cultivars (Bhojwani and Razdan 1983; Hartmann et al. 1990).

Shoots develop from a small group of cells known as shoot apical meristem. The apical meristem maintains itself, gives rise to new tissues and organs, and communicates signals to the rest of the plant (Medford 1992). Shoot-tips and meristem-tips are perhaps the most popular source of explants to initiate tissue cultures. The shoot apex explant measures between 100 to 500 μm and includes the apical meristem with 1 to 3 leaf primordia. The apical meristem of a shoot is the portion lying distal to the youngest leaf primordium (Cutter 1965), and is ca.100 μm in diameter and 250 μm in length with 800-1200 cells. In practice, shoot-tip explants between 100 to 1000 μm are cultured to free plants from viruses (Quak 1977).

Ebadi et al. (2007) demonstrated that the best medium for meristem culture was MS containing 0.1 $mg.L^{-1}$ NAA and 0.25 $mg.L^{-1}$ GA_3. Nagib et al. (2003) stated that the

best media for meristem culture is the MS liquid containing 0.5 mg.$L^{-1}GA_3$ and 0.04 mg.L^{-1}KIN, and in subculture phase the larger number of stem, root and the larger stem length was achieved in MS semi-solid, containing 0.5 mg.L^{-1}IBA and 0.5 mg.L^{-1}BA. The best medium for meristem culture and in subculture phase was MS containing GA_3 and KIN (Vanaei et al. 2008).

2.3.1 Potato shoots multiplication

In vitro shoot growth and multiplication is a central part of micropropagation technology. Lawrence (1981) stated that the manipulation of the growth regulator composition and balance is commonly the most successfully method of regulating *in vitro* shoot multiplication. The auxin-cytokinin balance in controlling root and shoot initiation appears to be a general phenomenon among plants (Murashige et al. 1974 and Murashige 1977).

2.4 Production of Microtubers and Minitubers

Microtubers are miniature tubers developed *in vitro* under tuber inducing conditions. They are very small (average weight 100-150 mg) and convenient for handling, storage and long distance transportation (Naik and Karihaloo. 2007). Microtubers are an ideal propagating material for producing high quality seed potatoes (Hussain et al. 2006).

Microtubers have become an important mode of rapid multiplication for seed tuber of potato. These micro-tubers are utilized for minitubers production in greenhouse or screen house. Wherever microtuber and minituber production technologies have been implemented, they have halved the field time necessary for conventional method to supply to the commercial growers (Zakaria et al. 2008). Differential response between potato cultivars dependence of microtuber production was influenced by genotype under the same environment conditions, these processes are regulated by specific gene expression patterns (Bachem et al. 2000; Gargantini et al. 2009).

The use of microtubers in storage and exchange of germplasm and seed potato production is advantageous (Seabrook et al. 1993; Rannali et al. 1994). Microtubers are the first generation of potato seed from tissue culture, being used to solve the problems of transplanting the plantlets from *in vitro* to *in vivo* conditions. They can be planted directly in the soil and they can be produced in any period of the year (Nistor et al. 2010). These from meristem grown and/or regenerated microplants are now produced and used in Australia, Brazil, Chile, China, Ecuador, India, Indonesia, Kenya, Korea, Peru,

Philippines, Taiwan, UK, Vietnam and even in Bangladesh as disease free seed (Hossain et al. 2005). The technique is controlled by various physical (light, temperature etc.) and chemical (growth regulators) factors. Among the media components, sucrose played an important role in the induction and development of potato microtubers on *in vitro* and BAP promoted initiation and growth of microtubers (Yong et al. 1996).

2.4.1 Effects of sucrose on microtuber production

Nistor et al. (2010) stated that sucrose is the most critical stimulus for inducing microtubers at high concentration. It is a cheap, save and superior agent for microtuber production (Hussain et al. 2006). Al-Abdallat and Suwwan (2002) proved that better microtuber yield was obtained when medium applied with 6% sucrose and 5 $mg.L^{-1}$BAP and kept under complete dark condition. The response of variety to microtuberization was highly dependent on genetic factors (Hossain and Sultana 1998).

Fufa and Diro (2014) described that microtubers were not produced at 4% sucrose. However, when 6% sucrose was added to growth media “Hunde” produced microtubers in 36 days, which is significantly earlier than that of “Ararse” 43 days. Increasing the amount of sucrose from 6% to 8% delay the microtuber formation in both varieties but more pronounced on “Ararse”. According to Fufa and Diro (2014), there was observed decreasing microtuber mean weight (g) in both varieties, as the level of sucrose increased. This might be due to the effect of high osmotic condition of the culture for water uptake that affects cell turgidity and microtuber weight (George et al. 2008).

Increasing sucrose concentrations are efficient to improve *in vitro* microtuber production as described by a number of investigators. Motallebi- Azar et al. (2013) reported that the high sucrose concentrations are essential for microtuber induction, influencing this process through the osmotic effect, and by serving as energy source. Sucrose converted to starch in microtubers developing, increased cell division and expansion of the stolon end are followed rapidly by a massive deposition of starch and storage protein as a result of coordinated expression of genes involved in starch and protein biosynthesis, accumulation of starch leads to increase in size and weight of microtubers (Prat et al. 1990; Visser et al. 1994).

2.4.2 Effects of light on microtuber production

Light has two main functions in plants. First, it is a free and unlimited source of energy of efficiently harnessed by photosynthesis. Second it provides information enabling plants to become oriented in space and time (Vanterhalter et al. 2008). Hossain et al. (2015) reported that the highest microtuber number, size and weight were at 8% sucrose kept under 10 days light duration before kept in continuous darkness in both tested cultivars. Differential response between varieties dependence of microtuber production agreed well with the results by other workers who reported that genotypes under the same cultural conditions showed a wide range of variations in their growth pattern (Gopal et al. 1998; Srivastava et al. 2012; Singh et al. 2001), these processes are regulated by specific gene expression patterns (Bachem et al. 2000; Gargantini et al. 2009).

Al-Hussaini et al. (2015) proved that modification of environmental factors, such as photoperiod is very important, darkness after light duration were reported to increase microtubers by enhanced tuberonic acid synthesis (chemically very similar to jasmonic acid) which plays an important role in tuber formation during *in vitro* condition.

Hussain et al. (2006) observed that the complete obscurity was an essential factor in tuber induction. Cultures kept under 16 hr, photoperiod were not able to produce microtubers. During incubation under light, GA_3 is synthesized which inhibits tuber induction while darkness enhanced tuberonic acid synthesis. Dobranszki et al. (1999) and Donnelly et al. (2003) demonstrated that microtuberization efficiency has been increased by short day's exposure or continuous darkness during culture condition. The potato shoots grown with 16 hr photoperiod when placed to under darkness induced tuber formation (Zaida and Elizabeth 1991).

2.5 Plant Growth Regulators (PGRs)

In the whole plants, auxins play a role in phototropism and geotropisim, apical dominance, root induction, and wounding responses. In general, auxin promote cell division, enlargement and root initiation. Natural and synthetic auxins most commonly used in tissue culture are Indole-3-acetic acid (IAA), Indole-3 butyric acid (IBA), 1-Naphthalenacetic acid (NAA), 2,4-Dichlorophenoxyacetic acid (2,4-D), and 4-amino-3,5,6-trichloropicolinic acid or picloram (PIC) (Gunan et al. 1997).

Cytokinins were discovered for their ability to promote cell growth *in vitro*. In the whole plant, cytokinin plays a role in a variety of processes including cell division and

differentiation, delay of senescence, development of chloroplasts, resource uptake and allocation, nodulation in legume species, vascular development, as well as initiation and development of shoots. In tissue culture they used to alleviate apical dormancy, allowing shoot proliferation, and to stimulate cell division often in concert with auxins. The relative amounts of auxins to cytokinins are important in determining whether cultures develop shoots (high cytokinin-to-auxin ratio), roots (low cytokinin-to-auxin ratio), or callus (relatively high levels of both) (Caula 2011). Plant growth regulator supplements into the nutrient media is one of the most influential factors affecting efficiency of *in vitro* adventitious shoot development (Mohamed et al. 2007).

Dixon and Gonzales (1994) recommended that the growth regulators are required in combination in the media as it is always the manipulation and variation of auxins and cytokinins levels that can successfully change the growth behavior of plant cultures. Among the BAP, CW, and Kin used for shoot proliferation of orchids, BAP influences shoot proliferation by quick cell divisions to induce large number of multiple shoots. Combination of 3.0 $mg.L^{-1}$BAP and 0.2 $mg.L^{-1}GA_3$ showed the highest survival and the lowest survivalist was observed when 1.5 $mg.L^{-1}$BAP and 0.0 $mg.L^{-1}GA_3$ was used (Yakimova et al. 2000).

Gibberellines (gibberellic acid, GA_3) are a group of naturally occurring substances that influence cell enlargement and stem elongation. In the whole plants, gibberellins exert powerful effects on stem elongation and sex expression. They also play a dominant role in dormancy and germination. Commercially, gibberellins are used to increase the size of seedless grapes. Gibberellins typically do not play a large role in regulation of *in vitro* development, although they have been used to enhance shoot elongation before rooting or to stimulate the conversion of buds into shoots (Gaba 2005).

On virus elimination through meristem culture of potatoes, cv. Cardinal with 1.0 $mg.L^{-1}$BAP give maximum number of shoot per explant, whereas, cv. Dheera with 1.5 $mg.L^{-1}$BAP give the tallest plantlet. On the other hand explants of Dheera and Cardinal with 1.5 $mg.L^{-1}$ of BAP and explants of Diamant, Granula and Carinal with 2.0 $mg.L^{-1}$ of BAP produced no roots (Hossain. et al. 2013).

MS medium supplemented with 0.5, 1.0, 1.5, 2.0, 4.0 $mg.L^{-1}$ of KIN and IAA in the combination of both 2.0 $mg.L^{-1}$KIN and IAA showed the best response to multiple shoot and root regeneration and minimum time for regeneration. Maximum number of shoots was regenerated due to the application of both shooting and rooting hormone but it was depended on genotype (Hoque 2010). The combine effects of cytokinin and auxin are

significantly affected between the treatments of potato meristem culture. However, it also depends on genotypes (Hossain et al. 2013).

2.6 Medium

The medium on which the plant cells or organ are cultured is known as culture or nutrient medium. The nutrient medium contains inorganic salts, trace elements, certain vitamins, a carbon source (generally sucrose) and, where needed growth regulators (Rahbar 1996). During the past decades, many types of media have been developed for *in vitro* plant culture (Pierik 1989; Torres 1989) with different formulations for the specific plants and tissues (Conger 1981). Some tissues respond much better on solid media while others on liquid media.

In general, the choice of medium is done by the purpose and the plant species or variety to be cultured. The most commonly used media for potato plant tissue culture is MS medium (Murashige and Skoog 1962). This basal medium is composed of different kinds and concentrations of organic and inorganic salts, vitamins, phytoharmones and carbon source. Agar is the most commonly used gelling agent and an important ingredient of tissue culture media. Agar quality could affect the plant developmental processes, especially the regeneration of adventitious shoots and roots (Scholten and Pierik 1998).

The growth of shoot culture in liquid and agar-solidified media was compared by these researchers Avila et al. (1996). They proved that liquid media was far superior increasing the shoot length, number of nodes, shoot dry weight and root dry weight in cultivars ‘Spunta’, ‘Kennebec’ and ‘Hunikal’. The effects of liquid medium was attributed to increased sucrose and introduction in liquid medium resulting from an improve contact rate. The authors also suggested a possibility that the presence of agar gel matrix physically reduced the diffusion of nutrients through the medium.

The phenotypic differences in growth rate were observed and the plantlets cultured on liquid media showed much better growth of shoot and root then solid media plantlets (Qureshi et al. 2014). In addition to their results, Qureshi et al. (2014) proved that comparative studies on growth of potato plantlets developed in liquid and solid medium revealed that the nodal sections cultured on liquid media showed better growth and are more vigorous as compared to solid media. The use of growth regulators in liquid cultures proved to be more effective and it is due to the direct contact of plant with the medium.

CHAPTER III
MATERIALS AND METHODS

The experiments were conducted at the plant tissue culture laboratory, Department of Horticulture and Agricultural Biotechnology, Yezin Agricultural University (YAU) from January, 2015 to June, 2016.

3.1 Plant Materials

The potato tubers of three commercially grown varieties Up-to-date (local name Sit-Bo), L-11 (CIP-393371.157/ Shwe Heho) and Atlantic were obtained from the Heho Seed Multiplication Farm, Department of Agriculture Southern Shan State.

3.2 Surface Sterilization of the Potato Tubers

Firstly, each variety of fresh tubers was washed with aqueous solution with a few drops of liquid soap followed by several times rinsed with tap water. Then the clean tubers were soaked in 1% NaOCl (Sodium Hypochloride) for 15 minutes followed by several times rinsed with sterilized water. After that the sterilized tubers were dried and placed in ambient and dark condition for 8-10 weeks till the sprouting started. As soon as the sprouting rate reached more than 75% the tubers were placed in light condition to prevent etiolation (Plate 1).

3.3 Initial Culture Media and Condition

Murashige and Skoog (MS) medium (Murashige and Skoog 1962) was used as basal medium. The basal medium was prepared by weighing the powder of MS 4.41 $g.L^{-1}$ of MS powder (Duchefa, Netherlands) containing B_5vitamins and also supplemented with 3% sucrose, 100 $mg.L^{-1}$ myo inositol, 200 $ml.L^{-1}$ coconut water and then added 1 liter of double distilled water to make up volume. Then the mixture was well shaken by using electric shaker. The media was adjusted to pH 5.8 and solidified with 0.6% agar (Difco Bacto agar). Each 150 x 25 mm test tube contained 10 ml of MS medium. Then the media was autoclaved at with 15 psi for 15 minutes. All cultures were maintained in culture room at 25 ± 2°C with 16/8 hr photoperiod and 30 $\mu mol.m^{-2}s^{-1}$ using white fluorescent lamps.

3.4 Initial Culture

3.4.1 Culturing of potato sprouts

The sterilized seed tubers free from disease and pest were selected and incubated in culture room for about 28-35 days. Carefully selected the healthy and vigorous mother

seed tubers and sprout of about 6-8 mm in length were cut carefully from these sterilized mother seed tubers (Plate. 1). The excised sprouts were surface sterilized in 1.0% Clorax (Sodium hypochloride NaOCl) for 10 minutes using with magnetic stirrer. After that these sprouts were washed with sterilized distilled water for 3 times and then washed sterilized distilled water with containing 2-3 drops of twin-20 for 5 times. After that the sprouts were soaked in 70% ethanol for 1 minute, followed by 3 times washing with sterilized distilled water. Finally, the sprouts were soaked in 3 $mg.ml^{-1}$ rimfampicim for 3 minutes. All of the sterilization steps were done in horizontal laminar air flow chamber. Finally the sterilized sprouts were used as explants, the bleached tissue were trimmed away and 3-5 mm length single explant was inoculated in (150 x 25 mm) test tubes which contained 10 ml of MS hormone free medium. All the cultures were maintained in a temperature controlled room at 25 ± 2˚C with 16/8 hr photoperiod and 30 $\mu mol.m^{-2}s^{-1}$ using white fluorescent lamps. After 4-6 weeks of inoculation, the sprout explants were developed into plantlets having 5-8 nodes. The *in vitro* shoots were used as explant source for nodal cuttings for shoot regeneration by culturing again in the same medium at about 4-6 weeks intervals to get enough numbers for next experiment (Plate 2).

Before sterilized sprout of 6 - 8 mm Inoculated sprout

Plate 1. Sprout for initial shoot tip culture

3.4.2 Experimental design

The experiment was conducted using Randomized Complete Block (RCB) design with four replications for each variety and each treatment consisted of 20 test tubes. The three potato cultivars were used as treatment to compare their growth.

3.4.3 Data Collection

Evaluations for each treatment were done 5 days after culture based on following data and the observations were recorded regularly until 45 days.

(1) Survival%

(2) Days to Shoot induction

(3) Shoot length (cm) per explant

(4) Number of leaves per explant

(5) Number of nodes per explant

(6) Number of shoots per explant

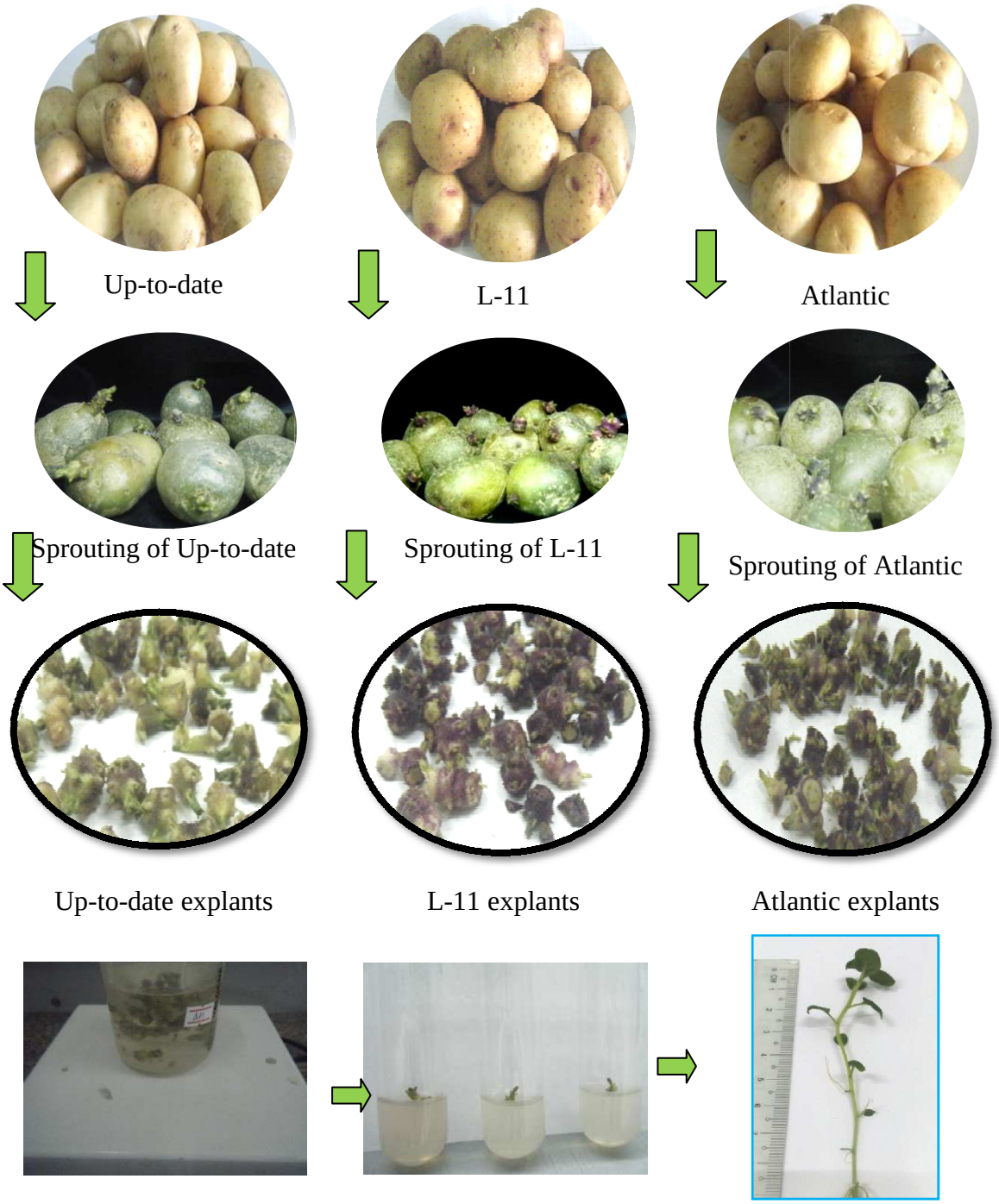


Plate 2. Procedure in initial shoot tip culture

3.5 Experiment 1: Effects of Plant Growth Regulators and Cultivars on Production of Potato Meristem Culture

The *in vitro* explants from shoot tip culture four weeks old plantlets were used as explants source for meristem culture.

The medium contained 4.41 $g.L^{-1}$ of MS powder (Duchefa, Netherlands), containing vitamins, and also supplemented with 3% sucrose, 100 $mg.L^{-1}$ myo-inositol. The MS basal medium was supplement with combinations of BAP and GA_3. The media was adjusted to pH 5.8 before autoclaving and solidified with 0.6% agar (Difco Bacto agar). Then the media was autoclaved at 15 psi for 15 minutes. Each 150 x 25 mm test tube contained 10 ml of MS medium.

The meristem were isolated under laminar horizontal air flow cabinet using a 50x magnification with binocular stereoscope microscope, the pedicel, immature leaves and the outer leaf primodia surrounding the meristem region were discarded one after another using sharp microsurgical blades and pointed forceps, until only the meristem region and one or two leaf primodia about 0.2-0.3 mm remain. At this stage, the manipulation was very delicate and care was taken not to damage the meristem region. Lastly, a single piece of isolated meristem was transferred immediately to each test tube containing MS medium supplemented with combinations of three different levels of BAP (0.0, 1.5 and 3.0 $mg.L^{-1}$) and three different levels of GA_3 (0.0, 0.3 and 0.6 $mg.L^{-1}$). Then the cultures were kept at 16/8 hr photoperiod with 25 ± 2 ˚C and 30 $\mu mol.m^{-1}s^{-1}$. Culture duration for this experiment was 24 weeks for shoot regeneration (Plate 3).

Data Collection

(1) Survival%
(2) Shoot length (cm) per explant
(3) Number of leaves per explant
(4) Number of nodes per explant
(5) Number of shoots per explant

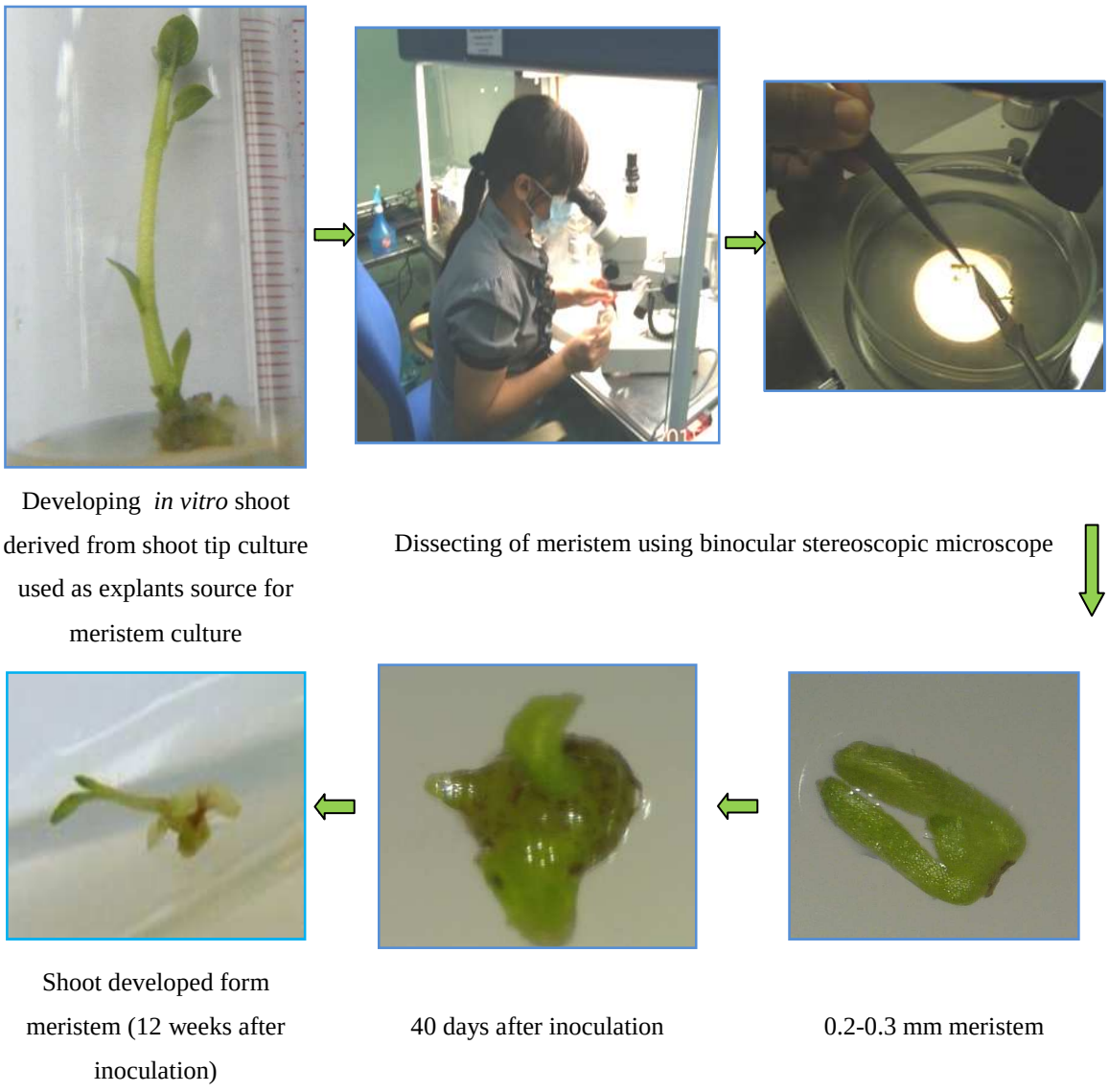


Plate 3. Procedure for meristem culture

3.5.1 Experiment 1.a: Effects of plant growth regulators on shoot multiplication of meristem derived nodal explants of Up-to-date cultivar

In that experiment, only shoots induced from Up-to-date cultivars were used as source explants for meristem shoot multiplication. Solid shoot multiplication medium was composed of MS medium (Murashige and Skoog 1962) supplemented with 3% (w/v) sucrose, 0.6% (w/v) agar, 20 $mg.L^{-1}$ coconut water and different PGRs combinations. After media preparation, pH was adjusted to 5.8.

The experimental design for this experiment was RCB with four replications. The MS medium supplemented with combinations of two levels of NAA (0.0, 0.05 $mg.L^{-1}$) and three different levels of BAP (0.0, 0.5, 1.0 $mg.L^{-1}$) were used as treatments. There were three treatments and each treatment was replicated four times and each treatment

consists of four culture vessels per treatment. There were four explants in each culture vessel. Each vessel consisted of 40 ml solid media. The culture condition for explants were 16/8 hr photoperiod with temperature of 25 ± 2 ˚C and light intensity of 30 µmol.m^{-1}.s^{-1}. Culture duration for this experiment was 6 - 8 weeks.

Data collection

The following data were collected.

(1) Shoot length (cm)
(2) Number of leaves per explant
(3) Number of nodes per explant
(4) Number of shoots per explant
(5) Shoot fresh weight per explant (g)

3.5.2 Experiment 1.b: Combination effects of different levels of Sucrose and BAP for *in vitro* microtuberization of meristem derived nodal explants of Up-to-date cultivar

Single node cutting of 8-10 mm in length consisting of one axillary bud from the middle portion of 5-6 weeks old plantlets were used as explants source for microtuberization. The microtuberization medium was composed of MS medium (Murashige and Skoog 1962) supplemented with 0.6% (w/v) agar, 20% (w/v) coconut water and different concentration of sucrose in combination of various levels PGRs. After that the culture media was adjusted pH to 5.8 and dispensed to each magenta box. Each vessel consisted of 50 ml media. Then, all the culture vessels were autoclaved at 15 psi for 20 minutes.

The experiment was conducted by using RCB design. Two different concentrations of sucrose (6 and 8%) and two different levels of BAP (3, 5 mg.L^{-1}) were used as treatment and supplemented to the basal MS medium. There were four treatments and each treatment was replicated four times and each treatment consists of four culture vessels per treatment. There were four explants in each culture vessel. Culture condition for this experiment was 16/8 hr photoperiod with temperature of 18±2 ˚C and light intensity of 30 µmol.$m^{-2}$$s^{-1}$ for 15 days and kept in complete dark period for 60 days. Culture duration for this experiment was 12 weeks (Plate. 4).

Data collection

The following data were collected at 12 weeks after culture in this experiment.

(1) Microtuberization %
(2) Number of microtubers per plantlet
(3) Length of micreotuber (mm)
(4) Microtuber weight (gm)
(5) Microtuber diameter (mm)

Dormancy breaking, acclimatization and detection of Virus

After harvest, normal seed tubers show dormancy for about 1-15 weeks depending on cultivar, tuber size, conditions before harvest and storage conditions. Small tubers, such as mini-tubers, have longer period of dormancy (Lommen 1993). Struik and Lommen (1999) suggested that microtubers are more sensitive to adverse conditions during storage. Microtubers harvested from meristem derived plantlets were immersed for 30 minutes by 40 ppm gibberellic acid treatment solution and microtubers were placed into plastic petri-dish and kept in ambient conditions until sprouting (Hassani et al. 2014) (Appendix - 3).

The sprouted microtubers were grown in sterilized soil and kept in culture room transplanted to plastic trays 4 weeks after planting.

Detection of virus by using pocket diagnostic

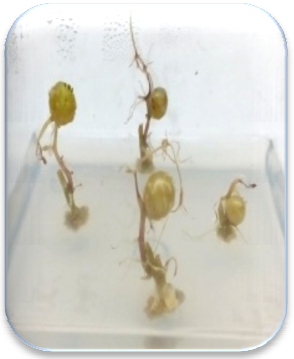
The potato viruses were checked by using “Pocket Diagnostic kits” which was manufactured in UK by Forsite Diagnostics, Sand Hutton, York YO41 1LZ, UK. That virus test kits was kindly given by Heho Seed Farm, Department of Agriculture (Appendix - 4).

5 weeks old shoot developed from PGRs free treatment of shoot multiplication stage was used as explants source for microtuberization

Inoculation of nodal explant with one axillary bud

Complete dark period for 60 days

Covered with black cloth to induced microtubers
(15 days after inoculation)



Microtuberization (8 weeks after inoculation)

Plate 4. Procedure for microtuberization

3.6 Experiment 2: Shoot Tip Culture of Three Selected Potato Cultivar

3.6.1 Experiment 2.a: Effects of different culture systems, different combinations of PGRs on shoot multiplication of shoot tip culture derived explants of three selected potato cultivars

Single node stem explants of 8-10 mm in length with one axillary bud were used as explants for this experiment. The shoots from initial shoot tip culture of 5 weeks old plantlets were used as explants source for microtuberization. There were two different culture systems, solid and liquid culture MS medium. Although the other chemical compositions and nutrients were the same for both solid and liquid culture system, liquid culture system was prepared without addition of solidifying agent, plant agar. Each media contained 3% (w/v) sucrose, 0.6% (w/v) agar supplemented with combinations of two different levels of NAA (0.0, 0.05 $mg.L^{-1}$) and three different concentrations of BAP (0.0, 0.5 and 1.0 $mg.L^{-1}$). The pH of the medium was kept at 5.8 before autoclaving and 40 ml of media was dispensed in each culture vessels. These magenta culture vessels were autoclaved at 15 psi for 20 minutes.

The experimental design for this experiment was three factors factorial in RCB arrangement with four replications. Factor A was two different types of culture systems, solid and liquid MS medium, factor B was three different PGRs combination of 0.05 $mg.L^{-1}$NAA and 1.0 $mg.L^{-1}$BAP, 0.05 $mg.L^{-1}$NAA and 0.5 $mg.L^{-1}$BAP, and PGRs free medium. Factor C was assigned for three different selected potato cultivars, Up-to-date, L-11 and Atlantic respectively.

There were 18 treatments for each variety and each treatment was replicated 4 times and each treatment consists of 4 culture vessels per replication. There were 4 explants in each culture vessel. For solid media, the culture vessel consisted of 40 ml solid media. But for liquid medium, 150 ml conical flasks were used with containing 20 ml of liquid media and before autoclaving these conical flasks were capped with aluminium foil. For multiple shoot induction, liquid medium was shaken on horizontal electric shaker at 100 rpm. The culture condition for explants were 16/8 hr photoperiod with temperature of 25 ± 2 ˚C and light intensity of 30 $\mu mol.m^{-1}s^{-1}$. Culture duration for this experiment was 6-8 weeks.

Data collection

The following data were collected in this experiment.

(1) Shoot length (cm)
(2) Number of leaves per explant
(3) Number of nodes per shoot
(4) Number of roots per shoot
(5) Number of shoots per explant
(6) Shoot fresh weight (g)

3.6.2 Experiment 2.b: Effects of Sucrose and BAP for *in vitro* microtuberization of shoot tip culture derived three potato cultivars

Single node cutting of 8-10 mm in length consisting of one axillary bud from the middle portion of 5 weeks old plantlets were used as explants for microtuberization. The explants sources for this experiment were the plantlets developed on the PGRs free medium of previous experiment and multiplied to sufficient amount by using single node explants on PGRs free medium.

The experimental design for this experiment was two factors factorial in RCB arrangement with four replications. Factor A was three selected potato cultivars, Up-to-date, L-11 and Atlantic and factor B was MS basal medium supplemented with two different levels of sucrose 6% and 8% and two different concentrations of BAP (3 and 5 $mg.L^{-1}$).After preparing the media, pH of the media was adjusted to 5.8 and dispensed 15 ml to each test tube according to the treatments. Then the test tubes were autoclaved at 15 psi for 20 minutes. Each treatment consisted of 7 test tubes per replication. Only single node cutting was culture in a (150 x 25 mm) size test tube containing 10 ml media. Culture condition for this experiment was 16/8 hr photoperiod with 18 ± 2 ˚C and 30 $\mu mol.m^{-2}s^{-1}$ for 15 days and kept in complete dark period for 60 days. Culture duration for this experiment was 12 weeks.

Data collection

The following data were collected in this experiment.

(1) Microtuberization %
(2) Number of microtubers per plantlet
(3) Length of micreotuber (mm)
(4) Microtuber weight (mg)
(5) Microtuber diameter (mm)

3.7 Data Analysis

The collected data were subjected to analysis of variance (ANOVA) using statistix (version 8.0). Treatment means were compared at 5% least significant difference (LSD) level.

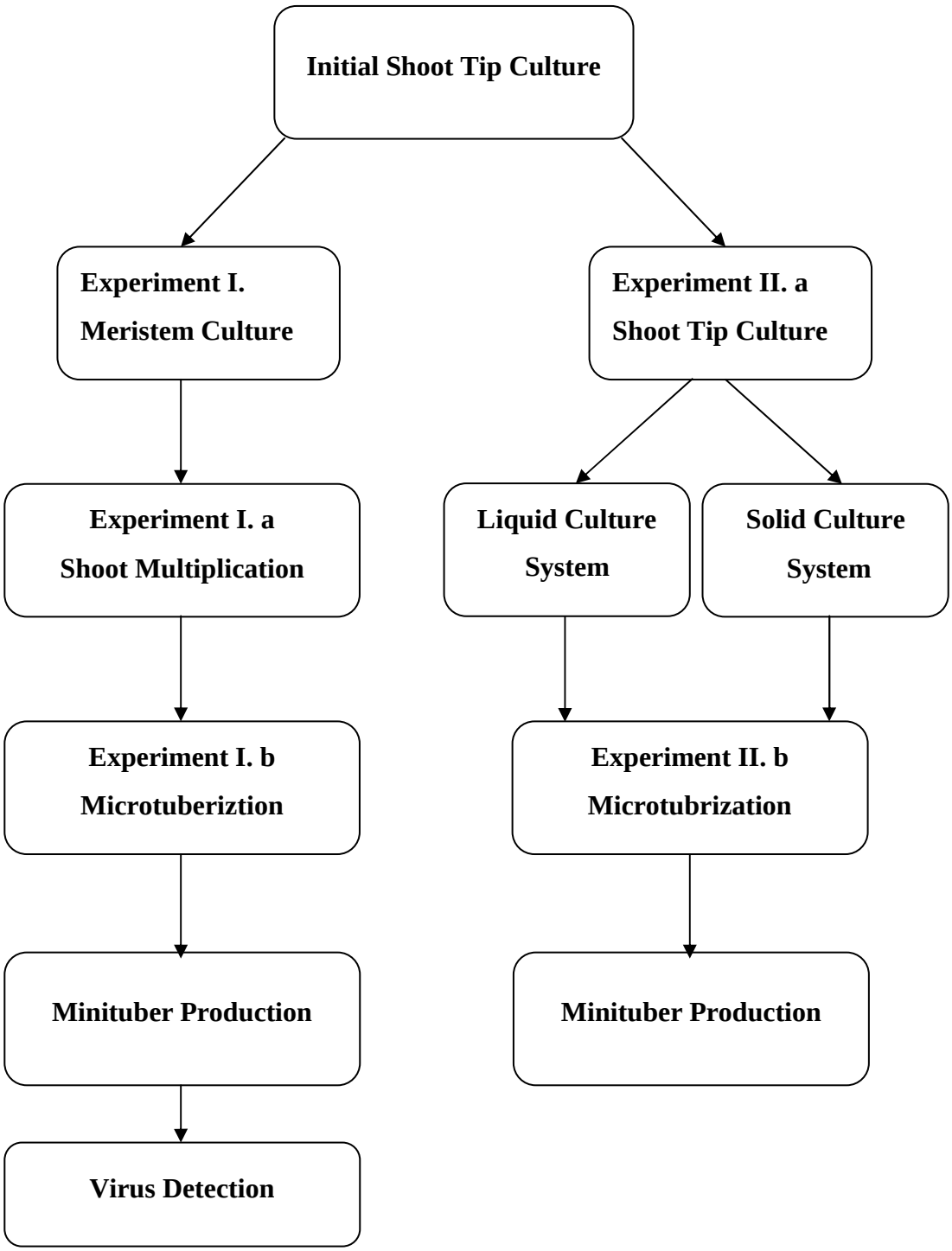


Figure 3.1 Flow diagram for *in vitro* regeneration of meristem and shoot tip culture of selected potato cultivars.

CHAPTER IV
RESULTS AND DISCUSSION

4.1 Initial Culture: Growth response of three selected potato cultivars on hormone free MS medium

4.1.1 Different growth response of three selected potato cultivars

The different growth responses of three potato cultivars are described in Table 4.1. The least days to shoot induction (5.00) days were found in Up-to-date cultivar followed by Atlantic (5.50) and the longest days to shoot induction (6.25) in L-11.There were highly significant differences in shoot length per explant (cm) between tested potato cultivars (Pr>F = 0.01) and the data were described in Table 4.1. Up-to-date cultivar provided the maximum shoot length (4.59 cm), Atlantic cultivar (3.07 cm) and in L-11 (2.73 cm) respectively. All of the cultivar gradually increased in shoot length 5 days after inoculation but a dramatic increase was found in Up-to-date cultivar 30 days after inoculation (Figure 4.1). The maximum number of nodes per explant was recorded 9.02 in Up-to-date cultivar. L-11 and Atlantic cultivars showed number of nodes (6.76 and 7.35). Number of nodes produced by initial shoot tip was uniformly increased 5 days after inoculation to 35 days after inoculation (Figure 4.2).

This finding was agreed with the following researchers, Abe and Futsuhara (1986) reported that the regeneration potential of micropropagated plants is genotype dependent. Moreover, Yasmin et al. (2011) reported that the slow growth rate was found during potato proliferation stage in such hormone free, cost effective media. For this study, the growth of culture shoot tips on MS media supplemented with 20% coconut water (cw) was satisfactory for regeneration. This result agreed with Moeinil et al. (2011) who reported that best medium for rooting and shooting was solid MS medium without PGRs supplement.

Table 4.1 Difference growth responses in initial shoot tip culture of three selected potato cultivars at 6 weeks after inoculation

Treatment	Survival %	Days to shoot induction	Shoot length (cm)	No. of leaves	No. of nodes	No. of shoots
Up-to-date	75.00 a	5.00 b	4.59 a	10.06 a	9.02 a	1.40 a
L-11	87.50 a	6.25 a	2.73 b	7.79 b	6.76 b	0.97 b
Atlantic	81.25 a	5.50 ab	3.07 b	8.40 ab	7.35 ab	1.00 b
$LSD_{(0.05)}$	16.12	1.03	1.07	2.23	2.12	0.38
CV%	11.47	10.76	17.86	14.78	15.93	19.79
Pr>F	0.24	0.06	0.01	0.10	0.09	0.05

*Mean value in each column having the same letter are not significantly different at 5% level.
Data were collected 6 weeks after culture.

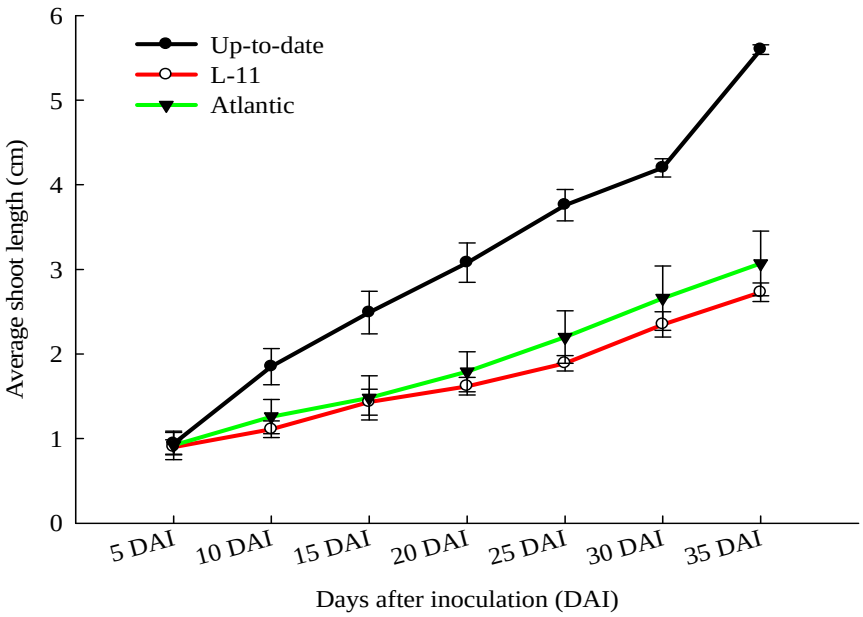


Figure 4.1 Different responses in shoot elongation per explant of three selected potato cultivars at 5 days interval in initial shoot tip culture

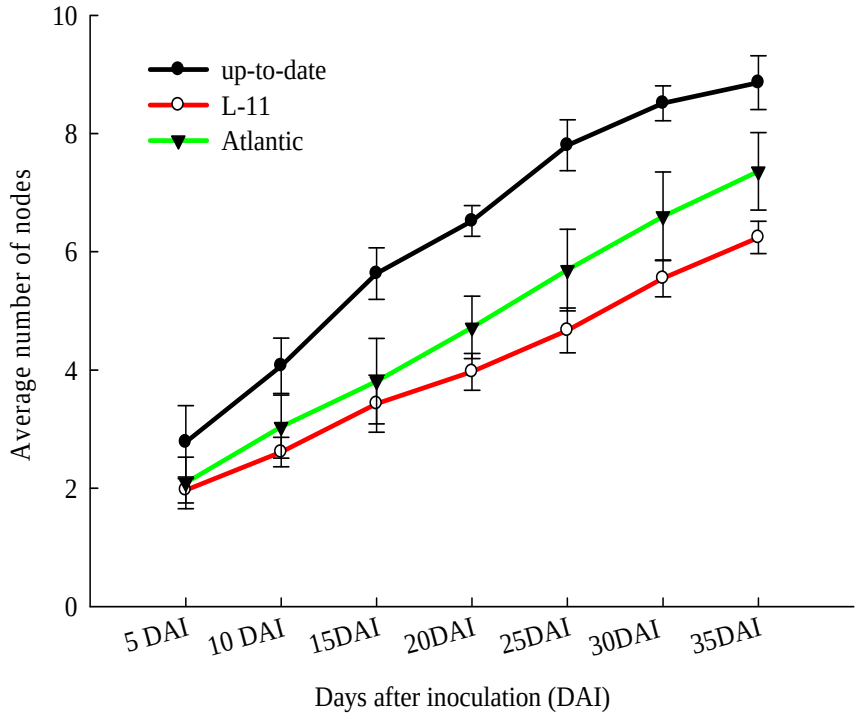


Figure 4.2 Different responses in number of nodes production per explant of three selected potato cultivars at 5 days interval in initial shoot tip culture

4.2 Experiment I: Effects of Plant Growth Regulators and Cultivars on Production of Potato Meristem Culture

4.2.1 Survival percent

The combination effects of plant growth regulators on survival percent of meristem on three potato cultivars are shown in Table 4.2. In the analysis of variance, there was no significant difference in survival percent per meristem among the different PGRs combinations (Pr>F = 0.09). There was no variation on survival among various combinations of PGRs.

However, the significant difference was found between the tested cultivars (Pr>F = 0.01). The hundred percent survivals were recorded in Up-to-date cultivar in all PGRs combinations and PGRs free, except in MS medium supplemented with 0.0 $mg.L^{-1}$BAP and 0.3 $mg.L^{-1}GA_3$ and 0.0 $mg.L^{-1}$BAP in combination with 0.6 $mg.L^{-1}$ GA_3. This finding is in lined with Ali et al. (2013) they stated that BAP was responsible for growth and survival of meristem explants.

In L-11 cultivar, hundred percent survival was recorded except in MS medium supplemented with 0.0 $mg.L^{-1}$BAP and 0.3 $mg.L^{-1}GA_3$, 1.5 $mg.L^{-1}$BAP in combined with 0.0 $mg.L^{-1}GA_3$, 1.5 $mg.L^{-1}$BAP in combined with 0.3 $mg.L^{-1}GA_3$ and 3.0 $mg.L^{-1}$BAP in combination with 0.0 $mg.L^{-1}GA_3$. Among all the tested cultivars Atlantic cultivar showed the lowest survival rate except the MS medium supplemented with 1.5 $mg.L^{-1}$BAP and 0.6 $mg.L^{-1}GA_3$, 3.0 $mg.L^{-1}$BAP and 0.0 $mg.L^{-1}GA_3$, and 3.0 $mg.L^{-1}$BAP and 0.3 $mg.L^{-1}$ GA_3. Therefore, Atlantic variety is sensitive in survival rate. According to this result, MS basal medium modified with 0.0 $mg.L^{-1}$BAP in combined with 0.3 $mg.L^{-1}GA_3$ gave less survive for all tested cultivars. Therefore, only 0.3 $mg.L^{-1}GA_3$ in MS base medium should not be used in *in vitro* meristem culture for these tested potato cultivars.

There was interaction between PGRs combinations treatment and tested cultivars (Pr>F = 0.01) in survival percent of *in vitro* meristem culture (Table 4.3). However, Atlantic cultivar gave 25% survivals on PGRs free medium, 0.0 $mg.L^{-1}$BAP and 0.3 GA_3, and 0.0 $mg.L^{-1}$BAP and 0.6 $mg.L^{-1}GA_3$ supplemented medium. Regeneration of shoot from meristem culture, MS medium modified with 1.5 $mg.L^{-1}$BAP in combined with 0.6 $mg.L^{-1}GA_3$ and MS medium supplemented with 3 $mg.L^{-1}$BAP in combination with 0.3 $mg.L^{-1}$ GA_3 gave good response. These combinations were suitable for shoot regeneration in meristem culture of the selected potato cultivars.

Wide variation on obtaining *in vitro* plantlet through meristem culture was observed at different treatments (Table 4.3). However, as it appears, the higher survival

percentage of meristem derived plantlets may not be a varietal character rather it depends on many other factors such as size of meristems, method of excision of meristem, degree of temperature and precautions followed during inoculation. Virus-free rate depends on meristem size in regeneration, the highest percentage of plants free of viruses were obtained from meristems with the smallest size of which were free of viruses (Danci et al. 2011).

4.2.2 Shoot length per explant

The shoots induced from meristem are significant difference in shoot length (cm) among 9 levels of PGRs combinations (Pr>F = 0.01) and the data were described in Table 4.2. The maximum shoot length 4.67 cm was recorded in the medium modified with 1.5 mg.L^{-1}BAP and 0.6 mg.$L^{-1}GA_3$ followed by (4.18cm) in the medium applied with 3.0 mg.L^{-1}BAP and 0.3 mg.$L^{-1}GA_3$, the minimum shoot length (0.32 cm) was recorded in the medium combination with 1.5 mg.L^{-1}BAP and 0.0 mg.$L^{-1}GA_3$, respectively. These results are in lined with Yasmin et al. (2011) and that *in vitro* growth responses depended on different genotypes on the same combination of plant growth regulators. These results are also agreed with Alam et al. (2010) that effective primary shoot induction from sweet potato meristem was found in MS medium supplemented with 2.0 mg.L^{-1}Kn and 0.5 mg.$L^{-1}GA_3$. Shoot length (cm) per explant as affected by the different cultivars was significantly difference among them (Pr>F = <0.001) (Table 4.2). L-11 cultivar provided longest shoot length 4.88 cm, followed by Up-to-date (1.93 cm) and the shortest (1.05 cm) was found in Atlantic cultivar.

Shoot length (cm) developed from meristem explants as affected by different PGRs combinations and selected potato cultivars was significantly different (Pr>F = <0.001). The data are described in Table 4.3. Atlantic cultivar did not produced shoot in MS medium supplemented with 0.0 mg.L^{-1}BAP and 0.6 mg.L^{-1} GA_3. Pereira and Fortes (2003) reported that MS liquid medium supplemented with 0.25 mg.L^{-1} gibberellic acid and 5.0 mg.L^{-1} pantothenic acid as the most suitable regime for potato micropropagation. Águila et al. (2001) cultured potato meristems on MS media supplemented with 1.0 mg.$L^{-1}GA_3$ in solid MS media. There was no interaction effect between PGRs combination and selected potato cultivars (Pr>F = 0.12).

4.2.3 Number of leaves per explant

The effects of PGRs combination on number of leaves produced by explant in meristem culture were significantly different (Pr>F = <0.001) and data are presented in Table 4.2. Between the PGRs combinations, the maximum number of leaves per explant (7.5) was found on the meristem developed on the medium combined with 3 mg.L^{-1}BAP and 0.6 mg.$L^{-1}GA_3$, the second (6.5) on 3 mg.L^{-1}BAP and 0.3 mg.$L^{-1}GA_3$ supplemented medium and third (4.33) on PGRs free treatment. This finding agreed with Roodbar-shojaei et al. (2010) and proved that GA_3 has a major effect on meristem culture and meristem growth in all cultivars. The larger growth was achieved in presence of high density of GA_3 single or in combination with low density of Auxin and cytokinin.

The number of leaves produced by meristem explant was significantly different among the cultivars (Pr>F = <0.001). L-11 cultivar gave maximum number of leaves 6.09, followed by Up-to-date cultivar and in Atlantic respectively. L-11 cultivar showed more response to PGRs than the rest. There was no interaction effect between PGRs combination and cultivars for number of leaves per explant.

4.2.4 Number of nodes per explant

Number of nodes per explant as affected by PGRs combinations and varieties is described in Table 4.2. There was significant difference in number of nodes per meristem explants between different PGRs combination treatments (Pr>F = 0.001) and among the cultivars (Pr>F = <0.001). The largest number of nodes per explant 6.50 was noted in the medium supplemented with 3.0 mg.L^{-1}BAP and 0.3 mg.$L^{-1}GA_3$, which was followed by 5.45 on 1.5 mg.L^{-1}BAP in combination with 0.6 mg.$L^{-1}GA_3$. In contrast number of nodes provided by meristem explant 3.66 was resulted from PGRs free treatment. This result in line with Nagib et al. (2003) and that use of growth regulators was found essential for quick development and high percentage of growth response of meristem was obtained when cultured in MS medium supplemented with GA_3 along with kinetin. Regardless of other PGR combinations, L-11 cultivar provided maximum nodes per explant 5.27, followed by 3.55 in Up-to-date and (1.69) for Atlantic in PGRs free treatment, respectively (Table 4.2).

Interaction between PGRs combinations and selected potato cultivars are shown in Table 4.3. Between the tested cultivars, Atlantic not only induced short nodes in the medium applied with 0.0 mg.L^{-1}BAP and 0.6 mg.$L^{-1}GA_3$ but also did in MS medium supplemented with combination of 0.0 mg.L^{-1}BAP and 0.6 mg.$L^{-1}GA_3$, also in PGRs free

supplemented MS medium. In contract, 2.25 in Up-to-date and 5.00 in L-11 cultivars were recorded nodes on the same medium PGRs supplemented with 0.0 $mg.L^{-1}$BAP and 0.6 $mg.L^{-1}GA_3$, and also in 0.0 $mg.L^{-1}$BAP with 0.6 $mg.L^{-1}GA_3$ combination medium and PGRs free medium.

According to the results shown in Table 4.3, to get more nodes on shoot it is needed to use PGRs with suitable combinations of BAP and GA_3. Moreover, L-11 cultivar provided maximum number of nodes even in PGRs free medium and gave more nodes on the medium supplemented with BAP and GA_3.This finding was in line with the following researchers. Roodbar-shojaei et al. (2010), they discussed that responses of different cultivars to the same culture were different, because of differences of genotypes, and similarities were because of growth regulators and genotypes similarities.

Table 4.2 Mean value in combine effects of BAP and GA_3 on survival, shoot length, no. of leaves, no. of nodes per explant of three selected potato cultivars in meristem culture at 24 weeks after inoculation

Treatment	Survival%	Shoot length (cm)	No. of leaves	No. of nodes
PGRs free	75 ab	3.87 ab	4.33 bc	3.66 bc
0.0 BAP + 0.3 GA_3	66 b	2.83 ab	3.37 c	2.95 c
0.0 BAP + 0.6 GA_3	66 b	1.78 bc	2.95 c	2.41 c
1.5 BAP + 0.0 GA_3	83 ab	0.32 c	2.62 c	1.66 c
1.5 BAP + 0.3 GA_3	75 ab	1.72 bc	3.20 c	2.54 c
1.5 BAP + 0.6 GA_3	100 a	4.67 a	2.62 c	5.45 ab
3.0 BAP + 0.0 GA_3	91 ab	1.73 bc	3.20 c	3.16 c
3.0 BAP + 0.3 GA_3	100 a	4.18 ab	6.5 ab	6.50 a
3.0 BAP + 0.6 GA_3	83 ab	2.48 abc	7.5 a	3.20 bc
$LSD_{(0.05)}$	0.27	2.51	2.38	2.25
Variety				
Up-to date	91 a	1.93 b	4.54 b	3.55 b
L-11	86 a	4.88 a	6.09 a	5.27 a
Atlantic	69 b	1.05 b	2.26 c	1.69 c
$LSD_{(0.05)}$	0.15	1.45	1.37	1.30
Pr>F				
(PGRs)	0.09	0.01	<0.001	0.001
(Var)	0.01	<0.001	<0.001	<0.001
(PGRs * Var)	0.01	0.12	0.45	0.58
CV%	40.54	117.74	68.25	79.07

*Means followed by the same letter in each column are not significantly different at 5% level.
Data were collected 24 weeks after culture.

Table 4.3 Combination effects of PGRs on average survival%, shoot length, no. of leaves, no. of nodes on meristem culture of three potato cultivars

Treatments		Survival %	Shoot length (cm)	No. of leaves	No. of nodes
BAP + GA_3 ($mg.L^{-1}$)	Potato varieties				
PGRs free	Up-to-date	100 a	2.42 d-f	4.5 b-f	3.5 b-i
	L-11	100 a	8.92 ab	8.00 ab	7.25 a-c
	Atlantic	25 c	0.27 f	0.5 fg	0.25 hi
0.0 BAP + 0.3 GA_3	Up-to-date	50 bc	4.80 b-e	4.87 b-e	4.37 b-f
	L-11	50 bc	3.65 c-f	4.75 b-e	4.25 b-g
	Atlantic	25 c	0.05 f	0.50 fg	0.25 hi
0.0 BAP + 0.6 GA_3	Up-to-date	75 ab	2.22 d-f	3.00 c-g	2.25 d-i
	L-11	100 a	3.12 c-f	2.37 d-g	5.00 a-e
	Atlantic	25 c	-	-	-
1.5 BAP + 0.0 GA_3	Up-to-date	100 a	0.25 f	3.00 c-g	1.75 e-i
	L-11	75 ab	0.48 ef	2.37 d-g	1.75 e-i
	Atlantic	75 ab	0.22 f	2.50 d-g	1.50 e-i
1.5 BAP + 0.3GA_3	Up-to-date	100 a	2.32 d-f	4.75 b-e	3.75 c-i
	L-11	75 ab	2.48 d-f	3.62 c-g	2.28 d-i
	Atlantic	50 bc	0.37 f	1.25 efg	1.00 f-i
1.5 BAP + 0.6 GA_3	Up-to-date	100 a	0.92 ef	4.75 b-e	3.75 c-i
	L-11	100 a	9.21 a	9.25 a	8.12 ab
	Atlantic	100 a	3.9 c-f	5.5 abcd	4.5 d-i
3.0 BAP + 0.0 GA_3	Up-to-date	100 a	0.83 ef	4.37 b-f	3.25 d-i
	L-11	75 ab	3.56 c-f	4.87 b-e	4.12 c-h
	Atlantic	100 a	0.80 ef	3.12 c-g	2.12 d-i
3.0 BAP + 0.3 GA_3	Up-to-date	100 a	1.97 d-f	6.87 a-c	5.87 a-d
	L-11	100 a	6.93 a-c	9.37 a	8.37 a
	Atlantic	100 a	3.63 c-f	6.25 a-d	5.25 a-e
3.0 BAP + 0.6 GA_3	Up-to-date	100 a	1.68 d-f	4.75 b-e	3.5 c-i
	L-11	100 a	5.56 a-d	6.75 a-d	5.75 a-d
	Atlantic	50 bc	0.20 f	0.75 e-g	0.37 g-i
$LSD_{0.05}$		0.47	4.35	4.13	3.90
CV%		40.54	117.74	68.25	79.07
Pr>F (Variety)		0.01	<0.001	<0.001	<0.001
Pr>F (Treatment)		0.09	0.01	<0.001	0.001
Pr>F (Treatment * Variety)		0.01	0.12	0.45	0.58

*Means followed by the same letter in each column are not significantly different at 5% level.
Data were collected 24 weeks after culture.

4.3 Experiment 1.a Effects of PGRs on Shoot Multiplication of Meristem Derived Nodal Explants of Up-to-date cultivar

4.3.1 Shoot length

Effects of different combinations of PGRs on shoot multiplication of meristem derived Up-to-date cultivar are showed in Table 4.4. There was a significant difference in shoot length among the treatments (Pr>F = 0.005). Maximum shoot length 7.10 cm was recorded in PGRs free supplemented medium. The second longest shoot 4.14 cm was found in medium applied with 0.05 mg.L^{-1}NAA in combination of 0.5 mg.L^{-1}BAP. In the medium modified with combination of 0.05 mg.L^{-1}NAA and 1.0 mg.L^{-1}BAP gave the shortest shoot length 3.45 cm (Plate 5). According to George et al. (2008), they suggested that the production of plants from nodal explants has proven the most generally applicable and reliable method of regenerating true-to-type *in vitro* plants. Hussey and Stacey (1981) reported that PGRs are not required for successful propagation of potato by shoot culture.

4.3.2 Number of leaves per explant

There was no significant difference in number of leaves per explant in three different levels of PGRs combinations. This mean there was no variation in shoot length due to different PGRs combinations (Table 4.4). The explants culture on the medium with PGRs free treatment gave the maximum number of leaves per explant 8.97, followed by the medium supplemented with 0.05 mg.L^{-1}NAA and 0.5 mg.L^{-1}BAP combination.

4.3.3 Number of nodes per explant

Effects of PGRs combinations on number of nodes per explant was not significantly different between the treatments (Pr>F = 0.16) due to no variation in PGRs combinations (Table 4.4). The maximum nodes per explant 7.91 were found in PGRs free medium, (7.35) in medium supplemented with 0.05 mg.L^{-1}NAA and 0.05 mg.L^{-1}BAP and 6.22 in 0.05 mg.L^{-1}NAA and 1.0 mg.L^{-1}BAP supplemented medium.

Although, the explants developed on both of medium containing 0.05 mg.L^{-1}NAA and 1.0, 0.5 mg.L^{-1}BAP provided shorter shoot length (3.45 and 4.15) compare to that of in PGRs free medium (7.10), gave similar numbers of nodes per explant. The longer plantlets were suitable to use as explants source for shoot multiplication or microtuberization. This is due to shorter inter-nodes length in respond to different PGRs combination. These observations are contradicted with Yousef et al. (2001) and that

longest main shoot and highest node numbers were found in medium containing NAA and BAP. BAP suppress apical dominance and stimulate the growth of lateral buds, whereas NAA decreases single nodes growth and rooting of potato plantlets.

4.3.4 Number of shoots per explant

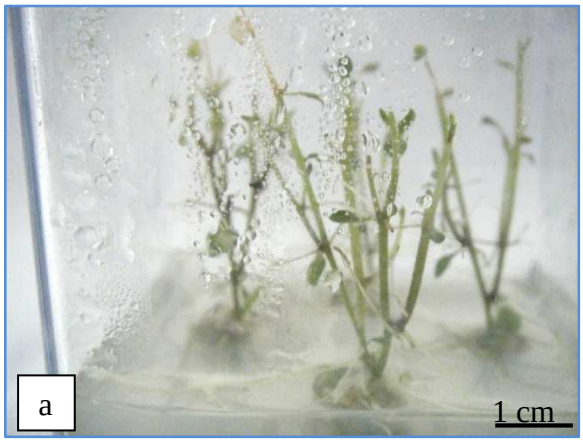
Number of side shoots produced per explant as affected by different combinations of PGRs and PGRs free treatments was significantly different (Pr>F = 0.01) and the data is described in Table 4.4. Medium supplemented with 0.05 $mg.L^{-1}$NAA and 1.0 $mg.L^{-1}$ BAP gave the maximum side shoots produced per explant (4.82), followed by (3.66) in PGRs free treated medium and lowest in 0.05 $mg.L^{-1}$NAA and 0.05 $mg.L^{-1}$BAP supplemented medium (2.35) (Plate 5).

It can be assumed that different responses to cytokinin and auxin in meristem derived explants. This finding was consistent with Elaleem et.al (2009) who concluded that the result of different concentrations of BAP and TDZ were varied depended on cultivars. According to Plate 4, explants cultured on medium supplemented with 0.05 $mg.L^{-1}$NAA and 1.0 $mg.L^{-1}$BAP produced more branching and it was noticeable that side shoots consist of 1 or 2 nodes. Further experiment is necessary to address the regeneration of these nodes for microtuberization or shoot multiplication.

4.3.5 Shoot fresh weight per explant

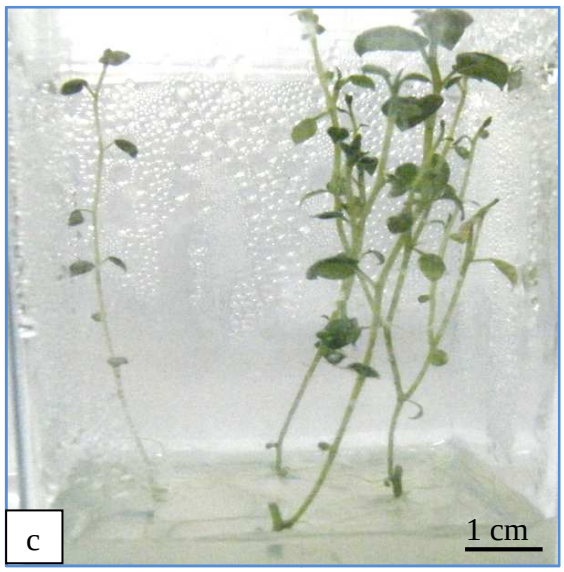
Effects of PGRs combination influenced on fresh weight per explant of meristem derived Up-to-date cultivar in shoot multiplication stage are described in Table 4.4. There was a significantly different among the PGRs combinations (Pr>F = <0.001). The explants developed in the medium applied with 0.05 $mg.L^{-1}$NAA and 1.0 $mg.L^{-1}$BAP produced the largest fresh weight per explant 0.67 g, the second in 0.05 $mg.L^{-1}$NAA and 0.05 $mg.L^{-1}$BAP supplemented medium 0.54 g and the lowest fresh weight 0.16 g in PGRs free treated medium. In this research, the explants developed in PGRs free supplemented medium gave the lowest weight without callus formation at the base of plantlets. The explants cultured on the medium supplemented with combination of 0.05 $mg.L^{-1}$NAA and 1.0 $mg.L^{-1}$BAP produced more branches than the other treatments and maximum shoot fresh weight was recorded from this treatment. Further investigation should be tested these branches on efficiency of shoot regeneration and microtuberization stage.

It was observed that meristem derived explants were more adaptable to the PGRs free medium, and it was cost effective for potato tissue culture.



(a)Meristem derived nodal explants developed in the MS medium supplemented with 0.05 mg.L^{-1}NAA and 1.0 mg.L^{-1}BAP

(b)Meristem derived nodal explants developed in the MS medium supplemented with 0.05 mg.L^{-1}NAA and 0.5 mg.L^{-1}BAP



(c)Meristem derived nodal explants developed in the MS medium supplemented without PGRs

Plate 5. Effects of different concentrations and combinations of PGRs in shoot multiplication stage of meristem derived nodal explants at 6 weeks after culture

Table 4.4 Effects of different combinations of PGRs on shoot length (cm), number of leaves, number of nodes, number of roots, number of shoots and shoot fresh weight (mg) on meristem derived nodal explants of Up-to-date cultivar

Treatment	Shoot length (cm)	Number of leaves	Number of nodes	Number of shoots	Fresh weight (mg)
0.05 mg.L^{-1}NAA +1.0 mg.L^{-1}BAP	3.45 b	7.25 a	6.22 a	4.82 a	0.67 a
0.05 mg.L^{-1}NAA + 0.5 mg.L^{-1}BAP	4.15 b	8.53 a	7.35 a	2.35 b	0.54 b
PGRs Free	7.10 a	8.97 a	7.91 a	3.66 ab	0.16 c
$LSD_{(0.05)}$	1.74	1.96	1.89	1.35	0.09
Pr>F	0.005	0.16	0.16	0.01	<0.001
CV	20.07	13.78	15.27	21.62	12.19

*Means followed by the same letter in each column are not significantly different at 5% level.
Data were collected 6 weeks after culture.

4.4 Experiment 1.b Effects of Two Different Levels of Sucrose in Combination with Two Different Levels of BAP on Microtuberization of Meristem Derived Nodal Explant of Up-to-date Cultivar

4.4.1 Microtuberization percent

Effects of different levels of sucrose and BAP on microtuberization percent is described in Table 4.5. There was a significantly difference between combination of different sucrose levels and different BAP concentrations. Maximum number (100%) microtuberization was found in MS medium supplemented with 8% sucrose in combined with 3 mg.L^{-1}BAP followed by MS basal medium supplemented with 6% sucrose in combined with 3 mg.L^{-1}BAP (93.75%). In 6% sucrose and 5 mg.L^{-1}BAP modified medium 87.50% microtuberization was recorded (Fig.4.3).

The highest response of microtuberization was given in MS medium modified with 8% sucrose in combined with 3 mg.L^{-1}BAP and the lowest response was found in MS medium applied with 8% Sucrose and 5 mg.L^{-1}BAP. According to this result, MS medium supplement with 8% sucrose and 3 mg.L^{-1}BAP can be recommended for microtuberization in meristem derived Up-to-date cultivar. The other researchers Haque (1996), Amma and Maity (1998) and Rodrigues-otube et al. (1999) they obtained maximum number of microtubers in different cultivars in medium supplemented with cytokinins, although at high concentrations 4 to 5 mg.L^{-1}. AL-Hussaini et al. (2015) stated that microtuberization in potato is influenced by many factors including sucrose, light and genotypes. Also they found that microtubers should be induced in medium supplemented with high concentration of 8% sucrose under 10 days light duration before darkness. Hossain et al. (2015) stated that microtuber initiation was affected by different levels of sucrose and 6-benzyl aminopurine (BAP).

Result from Virus test

The result of “Pocket Diagnostic Kits” produced from *in vitro* plantlets revealed the absence of potato viruses-5; namely potato Virus A, potato Virus S, potato Virus X, potato Virus Y, potato Virus V and *Rhizotonia solanaecium* (Appendix 5).

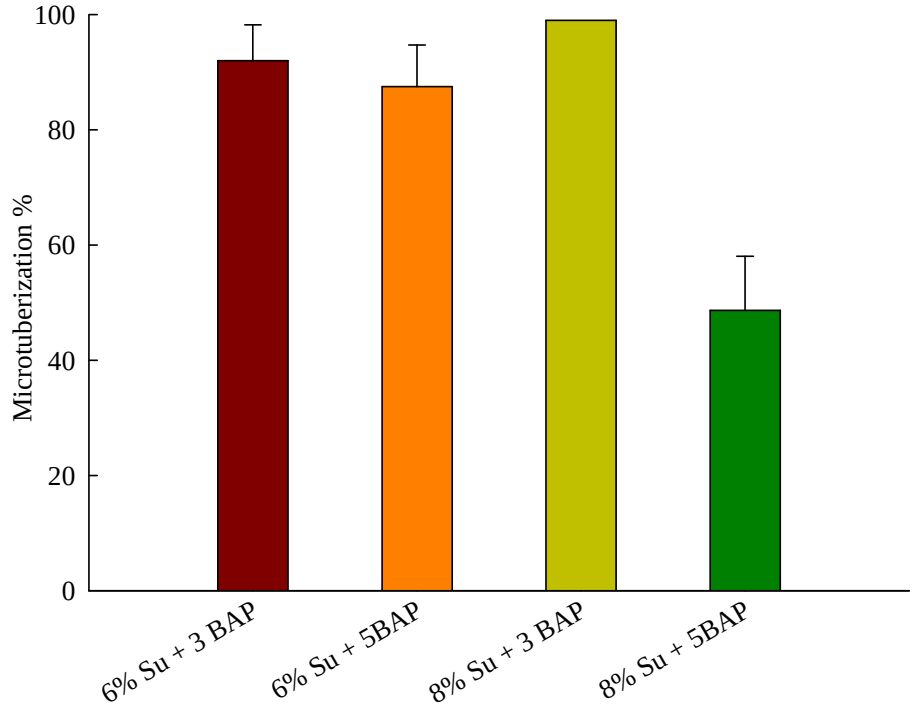


Figure 4.3 Effects of different levels of sucrose and BAP on microtuberization percent of meristem derived nodal explants of Up-to-date cultivar

Su = Sucrose

3 BAP = 3 mg.L^{-1}BAP

6 BAP = 5 mg.L^{-1}BAP

4.4.2 Number of microtubers per explant

Number of microtubers per explant as affected by treatment is described in Table 4.5. There is no significant effect of sucrose and PGRs combination in number of microtubers per explant. The higher number of microtubers per explant was found in the medium containing 6% sucrose and 5 mg.L^{-1}BAP. It may be assumed that the efficiency of microtuberization in potato was varied due to different PGRs concentrations in the MS based medium. This variation may be due to the degree of cell sensitivity towards the regulators, which depends on origin of explants and endogenous levels of plant growth regulators (Uranbey 2005).Variation in microtuber formation were also recorded by Al-Abdallat and Suwwan (2002) that sucrose and 5 mg.L^{-1}BAP were used and better microtuberization obtained by 6% sucrose and 5 mg.L^{-1}BAP under complete dark condition.

4.4.3 Height of microtuber

Height of microtuber as affected by different combinations of sucrose and PGRs is presented in Table 4.5. There were significant differences among the treatments. The highest microtuber height 4.88 mm was obtained from the explants developed on the MS medium supplemented with 8% sucrose and 3 mg.L^{-1}BAP. The shortest microtuber height 3.90 mm was recorded in the treatment of 6% sucrose and 3 mg.L^{-1}BAP supplemented MS medium. This may be due to high sucrose concentration gives high carbon source to explants. There are a number of investigators who reported that raising sucrose concentrations are efficient to improve *in vitro* microtuber production. Motallebi-Azar et al. (2013) stated that high sucrose concentrations are essential for microtuber induction, through the osmotic effect and by serving as energy source.

4.4.4 Fresh weight of microtuber

There is no significant difference among the treatments on fresh weight of microtuber developed from plantlets because there was no variation among the treatments on fresh weight of microtubers due to various sucrose concentrations (Table 4.5). The maximum microtuber weight 60.00 mg was provided from the plantlets developed on the medium containing 8% sucrose and 3 mg.L^{-1}BAP and followed by 50.00 mg on medium modified with 8% sucrose and 5 mg.L^{-1}BAP combinations. The minimum 40.00 mg was found not only in the MS medium supplemented with 6% sucrose and 3 mg.L^{-1}BAP but also 6% sucrose and 5 mg.L^{-1}BAP combination.

This can be assumed that sucrose converted to starch during microtuber development and accumulation of starch leads to increase in size and weight of microtubers. Prat et al. (1990) and Visser et al. (1994) reported that when sucrose convert to starch, increase cell division and rapid expansion of stolon end as a result of coordinate genes expression due to starch and protein biosynthesis. Therefore high concentration of sucrose supported to increase in microtuberization in potato. Garner and Bleak (1989) proved that used of MS medium with 8% sucrose compared to 4% or 12% sucrose gave early in initiation of tuberization and larger tuber size. Martine and Mario (1992) also reported that cytokinins can promote potato microtuberization as tuber inducing factors.

4.4.5 Diameter of microtuber

There is no significant difference among diameter of tuber developed on the media supplemented with different level of sucrose and BAP concentrations. This is due to no variation on combination of different levels of sucrose and different concentrations of BAP on diameter of microtuber (Table 4.5). The greatest microtuber diameter (4.25 mm) was recorded in the MS medium containing 8% sucrose and 3 $mg.L^{-1}$BAP, followed by (3.83 mm) in 8% sucrose and 5 $mg.L^{-1}$BAP combination. The minimum (3.63 mm and 3.68 mm) were found in MS media containing 6% sucrose and 3 $mg.L^{-1}$ BAP and 6% sucrose and 5 $mg.L^{-1}$BAP respectively (Plate 6).

The similar findings were reported by Hossain et al. (2015) that the highest number and weight of microtuber was in MS medium supplemented with 9% sucrose and 5 $mg.L^{-1}$BAP. And concomitantly, the lowest average weight were found in Cardinal cultivar cultured with 3% sucrose media at 7.5 $mg.L^{-1}$BAP and at 2.5 $mg.L^{-1}$BAP, respectively. Therefore, it can be assumed that, microtuber production can be promoted effectively by supplemented with high level of sucrose with proper 6-aminopurine (BAP).

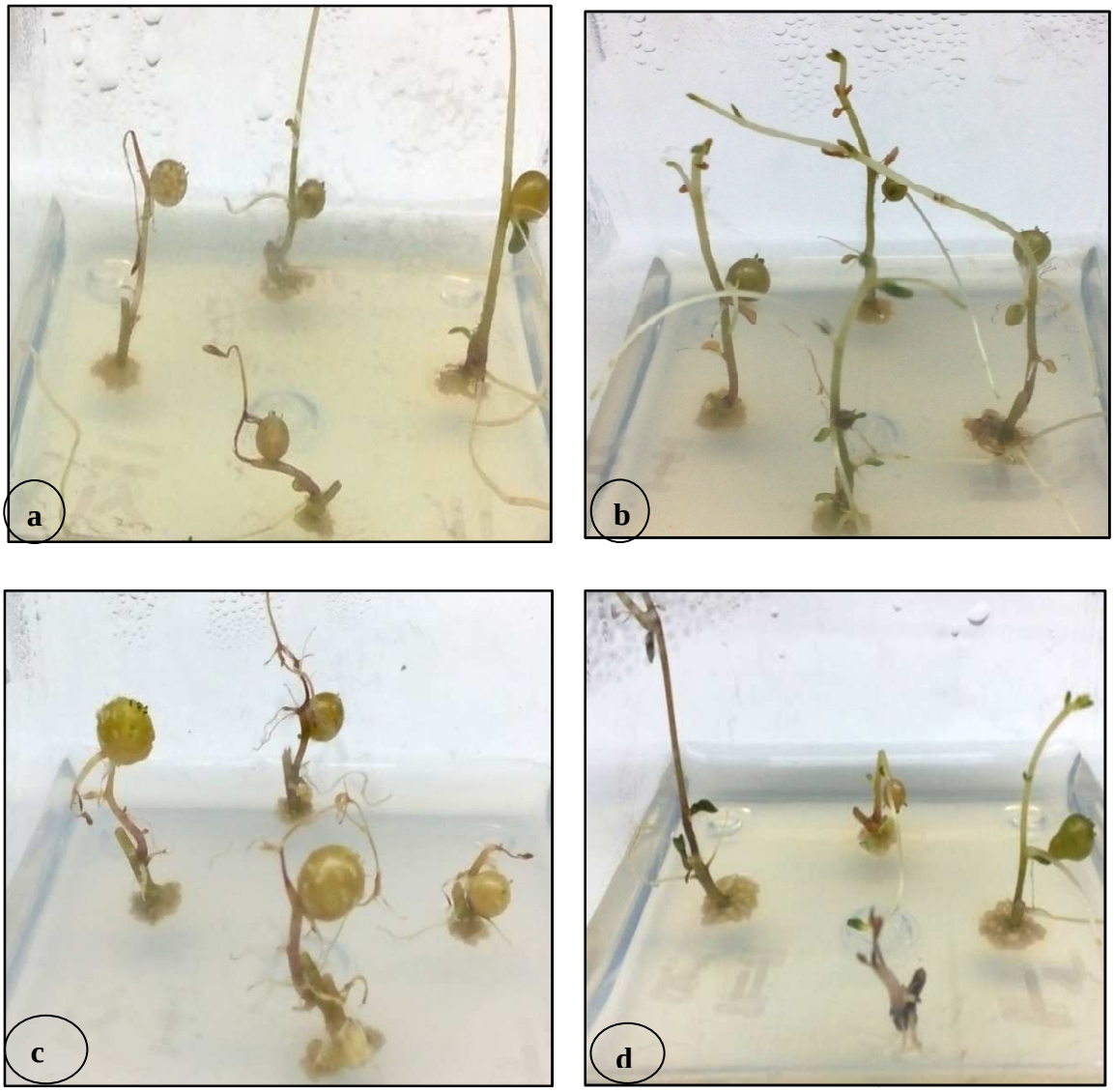


Plate 6. Microtuberization as affected by different combination of sucrose and BAP on the MS medium of meristem derived nodal explants of Up-to-date cultivar

(a) 6% sucrose + 3 mg.L^{-1}BAP

(b) 6% sucrose + 5 mg.L^{-1}BAP

(c) 8% sucrose + 3 mg.L^{-1}BAP

(d) 8% sucrose + 5 mg.L^{-1}BAP

Table 4.5 Combination effects of different levels of sucrose and BAP on microtuberization of meristem derived Up-to-date cultivar

Sucrose%	PGRs	Microtuberization %	No. of microtubers/ plantles	Height of microtuber (mm)	Fresh wt. of microtuber (mg)	Diameter of microtuber (mm)
6%	3 mg.L^{-1}BAP	93.75 a ± 6.25	1.03 ab ± 0.03	3.90 b ± 0.14	40.00 b ± 0.00	3.63 b ± 0.14
	5 mg.L^{-1}BAP	87.50 a ± 7.22	1.11 a ± 0.04	4.17 b ± 0.26	40.00 b ± 0.01	3.68 b ± 0.19
8%	3 mg.L^{-1}BAP	100 a ± 0.00	1.03 ab ±0.03	4.88 a ± 0.23	60.00 a ± 0.01	4.25 a ± 0.19
	5 mg.L^{-1}BAP	46.88 b ± 9.38	1.00 a ± 0.00	4.44 ab ± 0.28	50.00 ab ± 0.01	3.83 ab ± 0.37
$LSD_{(0.05)}$		17.71	0.10	0.58	0.01	0.56
Pr>F		<0.001	0.14	0.02	0.07	0.12
CV%		13.50	6.37	6.37	20.31	9.10

*Means followed by the same letter in each column are not significantly different at 5% level.
Data were collected 12 weeks after culture.

4.5 Experiment 2.a: Effects of Different Culture Systems, Different Combinations of PGRs on Shoot Multiplication of Shoot Tip Culture Derived Explants of Three Selected Potato Cultivars

4.5.1 Shoot length

Effects of different culture systems and different combinations of PGRs on shoot length (cm) of three selected potato cultivars are described in Table 4.6. There was a statistically significant difference on shoot length between the two culture systems (Pr>F = <0.001).The longest shoot length 9.01 cm was resulted from the explants cultured in liquid culture system. Pierik (1997) proved that high shoot proliferation in liquid media could have been due to availability, ease of uptake of nutrients and closer contact between explants and medium. Similar result obtained in this study.

Table 4.7 describes the effects of culture systems and PGRs on shoot length of the potato explants. There were no significant differences on mean shoot length among the PGRs treatments (Pr>F = 0.38). This revealed that different combinations of PGRs gave similar results in this study. However, in term of value PGRs free medium gave longer shoot length 7.49 cm than the other treatment. Regardless of variety the interaction between the medium and PGRs, the same result came out from liquid culture system using free PGR medium for shoot multiplication stage.

Table 4.8 shows the combination effect of culture system on selected potato cultivars in shoot multiplication stage; all tested cultivars gave longer shoot length in liquid culture medium. The maximum shoot length 11.02 cm was observed in L-11, followed by 8.06 cm in Atlantic and 7.94 cm in Up-to-date cultivar (Plate 8).

Shoot length as affected by the PGRs and cultivar was shown in Table 4.9. The result showed no significant effect of PGRs combination and cultivars in shoot proliferation stage. That can be assumed that different PGRs combinations had similar effects on shoot length of three potato cultivars. The medium supplemented with PGRs free treatment gave better results in all explants of shoot tip culture derived three potato cultivars, followed by the medium supplemented with 0.05 $mg.L^{-1}$NAA and 1.0 $mg.L^{-1}$BAP treatment. Although L-11 cultivar gave maximum shoot length in all treatments as compared to the rest. All the combination effects of medium, PGRs and variety were not significantly different on shoot length.

4.5.2 Number of leaves per explant

Effects of different culture systems and different combinations of PGRs on number of leaves per explant are shown in Table 4.6. There was a significantly different on solid and liquid culture medium (Pr>F = 0.05). The higher value 7.14 was resulted in liquid culture medium and lower 6.58 in the solid culture medium. The maximum number of leaves 7.12 was recorded in explants of Up-to-date and Atlantic cultivars and the minimum value 6.32 was found in L-11 cultivar. These results are agreed with Abe and Futsuhara (1986). They stated that regeneration potential of micropropagated plants is genotype dependent.

Combination effect of PGRs and culture system on number of leaves per explant was significantly different between the treatments and the data is described in Table 4.7. In liquid culture system, explants developed on the PGRs free medium and medium supplemented with low level of PGRs combination 0.05 mg.L^{-1}NAA and 0.5 mg.L^{-1}BAP provided the large amount of leaves. However, on solid culture system, medium supplemented with combination of 0.05 mg.L^{-1}NAA and 1.0 mg.L^{-1}BAP gave the value of 7.03 leaves per explant.

The combinations effect of PGRs and potato cultivars on number of leaves per explant is described in Table 4.8. Irrespective of hormonal combination the interaction between the culture systems and three potato cultivars, the result showed no significant effect between the treatments (Pr>F = 0.96). The maximum mean value in number of leaves per explant of three potato cultivars were found in liquid MS medium as (7.45) in Up-to-date, (7.38) in Atlantic and (6.58) in L-11 respectively. In liquid culture, within the shake culture conditions, the growth and multiplication rate of the shoots is enhanced by forced aeration, since continuous shaking of the medium provides sufficient oxygen supply to the tissue, which ultimately leads to their faster growth (Mehrotra et al. 2007).

The combine effects of PGRs and variety was not significantly different on number of leaves per explant (Pr>F = 0.37) and the data is described in Table 4.9. In this study, although various concentrations and combinations of PGRs were applied, the similar results were observed. The maximum number of leaves per explant was found in Up-to-date cultivar except in MS medium modified with 0.05 mg.L^{-1}NAA and 1.0 mg.L^{-1} BAP.

4.5.3 Number of nodes per explant

The mean value of number of nodes per explant was significantly different between the two culture systems (Pr>F = 0.04) and the data are showed in Table 4.6. The number of nodes per explant developed on the liquid culture medium 6.10 was significantly higher than that of solid culture medium 5.55. This result is in line with finding of (Mbiyu et al. 2012) who proved that the liquid MS medium gave more roots, more nodes and more leaves per plantlet than the solid MS medium.

There was no significant difference in number of nodes per explant developed on different levels and combinations of PGRs in shoot multiplication stage (Pr>F = 0.82) (Table 4.6.).

Number of nodes per explant developed from three potato cultivars was significantly different among them (Pr>F= 0.05) (Table 4.6). The largest number of nodes per explant (6.06) were recorded in both of Up-to-date and Atlantic, but L-11 cultivar provided the lowest (5.34). There was no significant interaction effect on medium, PGRs and variety on shoot multiplication (Pr>F = 0.36).

The interaction between culture medium and PGRs was significantly affected on number of nodes per explant (Pr>F = 0.01) (Table 4.7). Irrespective of variety, solid culture medium supplemented with 0.05 mg.L^{-1}NAA and 1.0 mg.L^{-1}BAP provided nodes per explant (6.03) and followed by (5.56) in that of 0.05 mg.L^{-1}NAA and 0.5 mg.L^{-1}BAP, lastly, the lowest nodes number 5.05 in PGRs free solid medium. However, the maximum number of nodes per explant 6.74 was resulted in liquid culture MS medium in PGRs free treatment. In liquid culture medium, maximum value of nodes (5.85) was recorded from the explants developed in medium fortified with 0.05 mg.L^{-1}NAA and 0.5 mg.L^{-1}BAP and minimum 5.72 in 0.05 mg.L^{-1}NAA in combination of 1.0 mg.L^{-1}BAP respectively. This result is in lined with the following researchers. Alam et al. (2010) suggested that liquid culture medium can provide easier availability of water and dissolved nutrients to the entire surface of the explants.

There was no interaction effect between different culture medium and variety on number of nodes per explant in *in vitro* shoot tip culture derived three potato cultivars (Pr>F = 0.91) (Table 4.8).

There was no significant difference between PGRs combinations and potato cultivars (Pr>F = 0.32) (Table 4.9). The data were recorded as maximum number of nodes per explant 6.25 in Up-to-date cultivar on PGRs free MS medium and minimum 4.92 in L-11 cultivar in the MS medium supplemented with 0.05 mg.L^{-1}NAA and

1.0 mg.L^{-1}BAP. Regardless of medium, the maximum number of nodes per explant 6.65 was found in the explants developed from shoot tip culture derived Atlantic cultivar on the medium supplemented with 0.05 mg.L^{-1}NAA and 1.0 mg.L^{-1}BAP and lowest 4.92 in L-11 on the same PGRs combination MS medium. On PGRs free MS medium Up-to-date cultivar provided the largest number of nodes per explant 6.25, followed by 5.89 in Atlantic and lowest 5.54 in L-11, in turn. It can be assumed that regeneration efficiency of micropropagated plants is genotype dependent.

The explants cultured on liquid culture system with PGRs free treatments produced longer shoot length 10.09 cm than solid culture system (4.88 cm) and the data was described in Table (4.7).

4.5.4 Number of roots per explant

Number of roots produced per explant from shoot tip culture derived explants in shoot multiplication stage as affected by culture medium was significantly difference between solid and liquid culture medium (Pr>F = < 0.001) (Table 4.6). Number of roots per explant 4.64 was noted in explants developed in liquid culture medium and 2.03 was found in that of solid culture medium. Mbiyu et al. (2012) found that liquid culture medium gave more roots, nodes and leaves per plantlet than solid medium and they suggested that root proliferation in liquid medium could have been due to the ease at which the roots penetrated the liquid medium compared to the solid medium.

There was a significant difference in number of roots per explant among PGRs combination in shoot regeneration stage of three potato cultivars (Pr>F = <0.001) (Table 4.6). The maximum number of roots per explant was recorded in PGRs free medium as (3.86) and (3.76) in medium supplemented with 0.05 mg.L^{-1}NAA and 0.5 mg.L^{-1}BAP. The lowest number of roots per explant 2.39 was found in medium supplemented with 0.05 mg.L^{-1}NAA and 1.0 mg.L^{-1}BAP. There was no significant difference among the selected potato cultivars in number of roots per explant provided in shoot multiplication stage (Pr>F =0.71) (Table 4.6). The maximum average number of roots per explant 3.78 were recorded in L-11, followed by (3.17) in Atlantic and (3.06) in Up-to-date cultivar, respectively.

The significant difference was found in interaction between different culture medium and different level of PGRs combinations (Pr>F = <0.001) (Table 4.7). The largest number of roots per explant (5.88) was resulted from the explants developed on liquid culture system without PGRs treatment, followed by 4.35 in 0.05 mg.L^{-1}NAA and

0.5 mg.L^{-1}BAP containing medium and 3.69 in medium with combination of 0.05 mg.L^{-1} NAA 1.0 mg.L^{-1}BAP. This is agreed with (Mehrotra et al. 2007) who reported that liquid medium caused the better shoots and roots growth because of the close contact of the tissue with the medium stimulate and facilitate the uptake of the nutrients and phytohormone.

There was a significant difference in interaction between types of culture system and cultivars on number of roots developed from shoot tip culture explants in shoot multiplication stage (Pr>F = 0.04) (Table 4.8). Atlantic cultivar provided maximum number of roots per explant (5.00) in liquid medium, followed by (4.72) in L-11 and minimum (4.20) in Up-to-date cultivar, in turn. In contrast, the lowest number of roots per explant was found in solid culture system, 1.34 in Atlantic, 1.92 in Up-to-date and 2.83 in L-11 respectively (Plate 7).

Number of roots produced per explant as affected by different combination of PGRs and cultivars was not significantly different among them (Pr>F = 0.68). The PGRs combination showed similar effects on all tested potato cultivars (Table 4.9). L-11cultivar gave the greatest number of roots per explant 4.49 in PGRs free medium and (4.17) in medium supplemented with 0.05 mg.L^{-1}NAA and 0.5 mg.L^{-1}BAP. Similarly, Up-to-date cultivar provided highest number of roots per explant 3.79 in PGRs free, (3.26) in medium supplemented with 0.05 mg.L^{-1}NAA and 0.5 mg.L^{-1}BAP and the lowest (2.13) in MS medium modified with 0.05 mg.L^{-1}NAA and 1.0 mg.L^{-1}BAP. In contrast, explants of Atlantic cultivar provided maximum number of roots per plantlet 3.85 in medium supplemented with 0.05 mg.L^{-1}NAA and 0.5 mg.L^{-1}BAP, and 3.27 in that of PGRs free medium. It was found that the number of roots per explant was decrease as the concentration of cytokinins increases in all selected potato cultivars. These results coincide with the finding of Shibli et al. (2001) who proved that both 6-benzyl aminopurine and Kinetinin inhibited root formation.

4.5.5 Number of shoots per explant

The effect of different culture system on number of shoots produced per explant is described in Table 4.6. There was no statistically difference between two culture systems (Pr>F = 0.11). This mean that number of shoot induction was not vary due to different culture systems in this study. The greatest number of shoots 3.05 was recorded in solid culture system. However, this finding is not agreed with the result of Alam et al. (2010), liquid medium gave significant effects on shoot growth on sweet potato at multiplication stage.

There was a significant difference in number of shoots developed per explant between different levels of PGRs combinations and culture systems (Pr>F = 0.002) and the data is shown in Table 4.6. The best response of shoots 3.39 was recorded in MS medium supplemented with 0.05 mg.L^{-1}NAA and 0.5 mg.L^{-1}BAP. Therefore, the development of shoots per explant in MS medium at multiplication stage has been, generally, suggested that needed a low ratio of auxin and cytokinin.

Among the potato cultivars, average number of shoots produced per explant was significantly difference (Pr>F = <0.001) (Table 4.6). L-11 cultivar gave the maximum shoots 3.55.This might be due to genotype as described earlier, according to the respond of potato cultivars, culture systems and different PGRs combinations (Plate 7 and 8).

Number of shoots per plantlet as affected by types of medium and PGRs combination was significantly different between the treatments and the data is described in Table 4.7. The maximum number of shoots per explant 4.17 was resulted from solid medium supplemented with 0.05 mg.L^{-1}NAA and 0.5 mg.L^{-1}BAP.

4.5.6 Shoot fresh weight

Shoot fresh weight (g) as affected by different culture system were significantly different between them (Pr>F = <0.001) (Table 4.6). Liquid culture medium showed shoot fresh weight 0.71 g and 0.23 g in solid one.

Effect of PGRs combination on fresh weight of shoot was significantly different among them (Pr>F = 0.01). The best PGRs combination for shoot fresh weight was 0.05 mg.L^{-1}NAA and 0.5 mg.L^{-1}BAP. According to this result, as increased in BAP concentration the decreased in shoot fresh weight. This finding was consistent with (Elaleem et al. 2009 and Mohamed et al. 2007) and they proved that the result of different concentration of BAP and TDZ were varied depend upon the cultivars.

There was significant difference in shoot fresh weight produced per explant by different potato cultivars (Pr>F = 0.001). The highest shoot fresh weight 0.54 g was recorded in Atlantic cultivar and followed by 0.48 g in L-11 and the lowest in Up-to-date cultivar, respectively. Shoot fresh weight resulted from Atlantic cultivar was higher than that of L-11 and Up-to-date cultivar although number of shoots per plantlet in L-11 was more than that of Atlantic. This may be due to callus formation at the base of Atlantic cultivar.

Irrespective of cultivars, shoot fresh weight as affected by different combinations of PGRs and culture system is shown in Table (4.7). In solid culture system 0.05 mg.L^{-1}

NAA and 0.5 mg.L^{-1}BAP supplemented medium gave greater shoot fresh weight due to callus formation at the base of the plantlets. However, in liquid culture system, PGRs free treatment gave the maximum shoot fresh weight per plantlet due to explants in liquid medium produced better shoot length, numbers of roots and leaves. This result was in line with (Qureshi et al. 2014) and they concluded that plantlets cultured on liquid media showed better growth of shoot and roots as compared to solid media. Liquid media plantlets emerged earlier and having greater number of leaves and nodes per plantlet than solid MS medium.

There was an interaction effect between different culture systems, PGRs combinations and selected potato cultivars (Pr>F = 0.01) (Table 4.6). Responses of different cultivars to the same culture medium were different, because of differences in genotypes, and similarities were because of growth regulators and genotypes similarities.

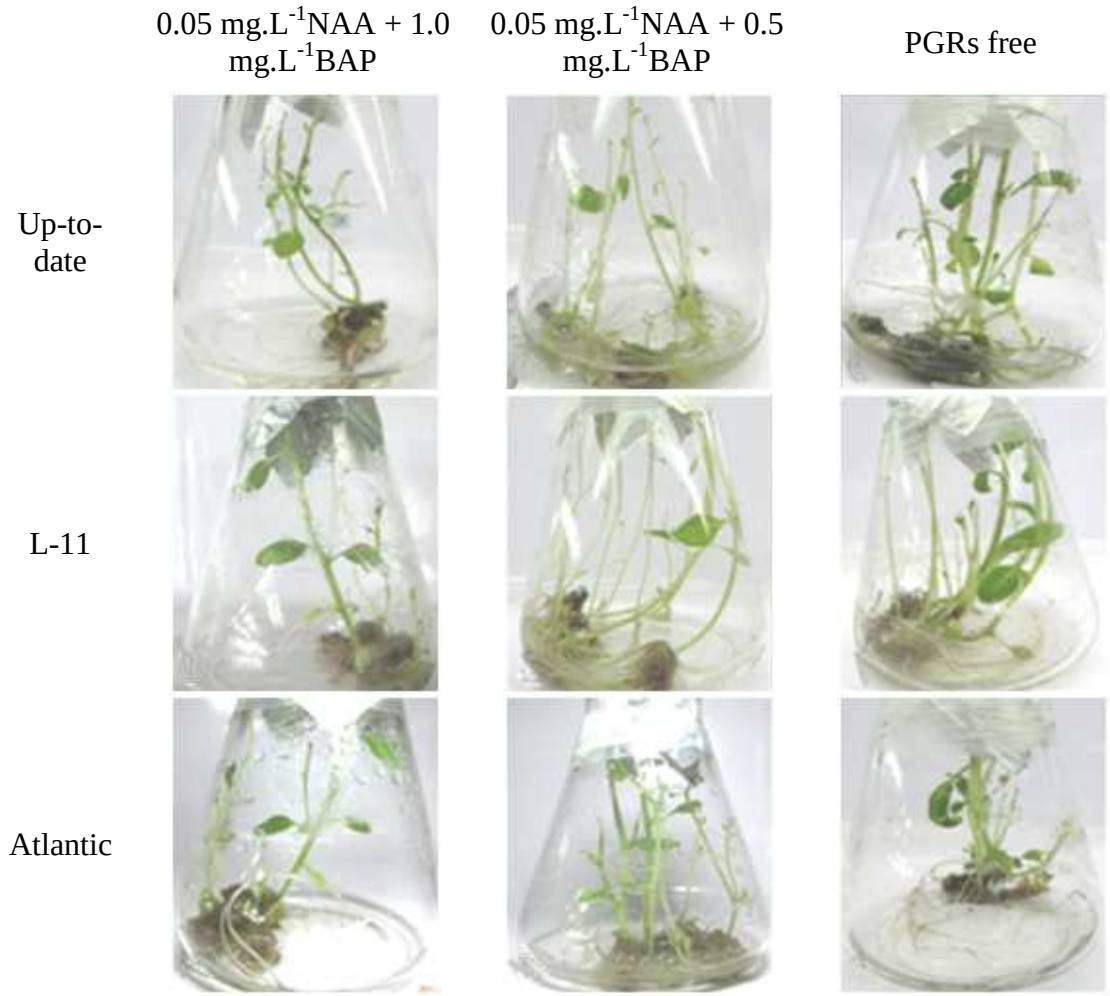


Plate 7. Effects of liquid culture system and PGRs combinations on Shoot length, No. of leaves, No. of nodes, No. of roots, No. of side shoots and shoot fresh weight of shoot tip culture derived three selected potato cultivars on shoot multiplication stage at 6 weeks after culture

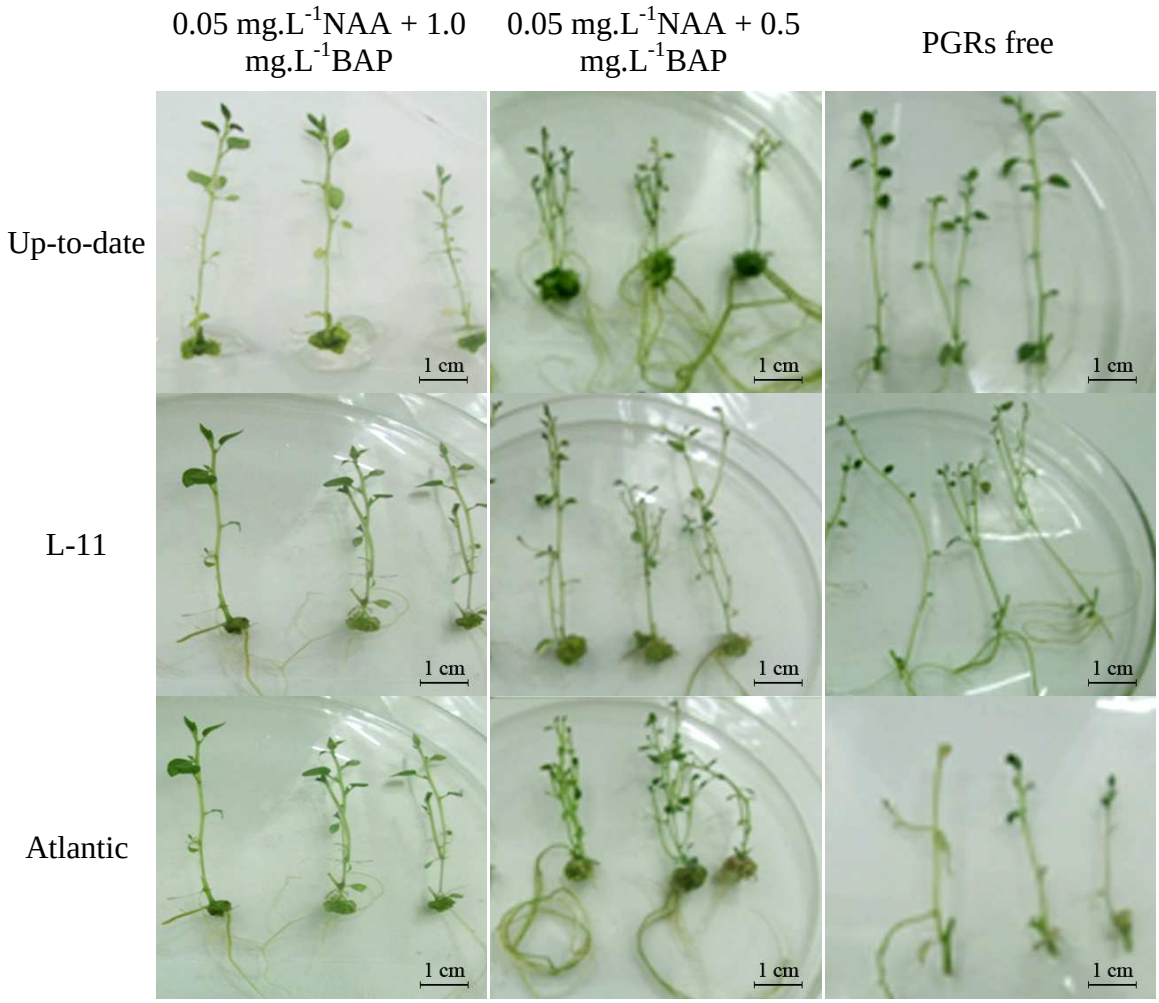


Plate 8. Effects of solid culture system and PGRs combinations on Shoot length, No. of leaves, No. of nodes, No. of roots, No. of side shoots and shoot fresh weight of shoot tip culture derived three selected potato cultivars on shoot multiplication stage at 6 weeks after culture.

Table 4.6 Effects of culture systems, variety and PGRs on shoot length (cm), number of leaves and number of nodes, number of roots, number of shoots and shoot fresh weight (mg) in shoot multiplication (6 weeks after culture)

Treatment	Shoot length (cm)	No. of leaves	No. of nodes	No. of roots	No. of shoots	Fresh weight (g)
Culture System						
Solid culture system	4.69 b	6.58 a	5.55 b	2.03 b	3.05 a	0.23 b
Liquid culture system	9.01 a	7.14 a	6.10 a	4.64 a	2.73 a	0.71 a
$LSD_{(0.05)}$	0.92	0.56	0.55	0.58	0.38	0.06
Pr>F(System)	<0.001	0.05	0.04	<0.001	0.11	<0.001
PGRs						
0.05 NAA + 1.0 BAP	6.36 a	6.85 a	5.88	2.39 b	2.67 b	0.41 b
0.05 NAA + 0.5 BAP	6.69 a	6.77 a	5.71	3.76 a	3.39 a	0.52 a
PGRs Free	7.49 a	6.95 a	5.89	3.86 a	2.61 b	0.47 a
$LSD_{(0.05)}$	1.14	0.68	0.67	0.71	0.47	0.07
Pr>F (PGRs)	0.13	0.88	0.82	<0.001	0.002	0.01
Variety						
Up-to date	6.14 b	7.12 a	6.06 a	3.06 b	2.78 b	0.38 b
L-11	8.46 a	6.32 b	5.34 b	3.78 a	3.55 a	0.48 a
Atlantic	5.94 b	7.12 a	6.06 a	3.17 ab	2.35 b	0.54 a
$LSD_{(0.05)}$	1.13	0.68	0.67	0.71	0.47	0.07
Pr>F(Variety)	<0.001	0.03	0.05	0.10	<0.001	0.001
Pr>F (System * PGRs * Var)	0.16	0.37	0.36	0.07	0.03	0.01
CV%	28.66	17.28	20.03	36.92	28.28	29.21

*Means followed by the same letter in each column are not significantly different at 5% level.

Table 4.7 Effects of culture systems and PGRs on shoot multiplication of single node explants of three potato cultivars (6 weeks after culture)

Type of medium	PGRs	Shoot length(cm)	Number of leaves	Number of nodes	Number of roots	Number of shoots	Fresh weight (g)
Solid	0.05 NAA + 1.0 BAP	4.38 c	7.03 ab	6.03 ab	1.10 d	2.56 b	0.20 d
	0.05 NAA + 0.5 BAP	4.84 c	6.59 b	5.56 bc	3.16 c	4.17 a	0.40 c
	PGRs free	4.88 c	6.11 b	5.05 c	1.83 d	2.41 b	0.08 e
Liquid	0.05 NAA + 1.0 BAP	8.35 b	6.69 b	5.72 bc	3.69 bc	2.79 b	0.62 b
	0.05 NAA + 0.5 BAP	8.57 ab	6.96 ab	5.85 abc	4.35 b	2.61 b	0.65 b
	PGRs free	10.09 a	7.79 a	6.74 a	5.88 a	2.82 b	0.85 a
	$LSD_{(0.05)}$	1.61	0.97	0.95	1.01	0.67	0.11
	Pr>F	0.38	0.01	0.01	<0.001	<0.001	<0.001
	CV%	28.66	17.28	20.03	36.92	28.28	29.21

*Means followed by the same letter in each column are not significantly different at 5% level.

Table 4.8 Effects of culture systems and variety on shoot multiplication of single node stem explants of three potato cultivars (6 weeks after culture)

Treatment	Variety	Shoot length(cm)	Number of leaves	Number of nodes	Number of roots	Number of shoots	Fresh weight (g)
Solid	Up-to-date	4.35 cd	6.97 ab	5.77 ab	1.92 bc	2.88 b	0.23 d
	L-11	5.91 c	6.06 b	5.08 b	2.83 b	4.19 a	0.28 d
	Atlantic	3.82 d	6.88 ab	5.79 ab	1.34 c	2.06 c	0.18 d
Liquid	Up-to-date	7.94 b	7.45 a	6.37 a	4.20 a	2.69 bc	0.55 c
	L-11	11.02 a	6.58 ab	5.60 ab	4.72 a	2.89 b	0.69 b
	Atlantic	8.06 b	7.38 a	6.34 a	5.00 a	2.63 bc	0.89 a
$LSD_{(0.05)}$		1.61	0.97	0.95	1.01	0.67	0.11
Pr>F		0.41	0.96	0.91	0.04	0.001	<0.001
CV%		28.66	17.28	20.03	36.92	28.28	29.21

*Means followed by the same letter in each column are not significantly different at 5% level.
Data were recorded 6 weeks after culture.

Table 4.9 Effects of PGRs and variety on shoot multiplication of single node stem explants of three potato cultivars (6 weeks after culture)

Treatment	Variety	Shoot length(cm)	Number of leaves	Number of nodes	Number of roots	Number of shoots	Fresh weight (g)
0.05 mg.L^{-1} NAA + 1.0 mg.L^{-1} BAP	Up-to-date	6.14 b	7.00 ab	6.06 ab	2.13 d	2.49 bc	0.38
	L-11	6.87 b	5.89 b	4.92 b	2.67 cd	3.58 a	0.44 bc
	Atlantic	6.09 b	7.65 a	6.65 a	2.39 d	1.94 c	0.41 c
0.05 mg.L^{-1} NAA + 0.5 mg.L^{-1} BAP	Up-to-date	5.51 b	7.12 a	5.89 ab	3.26 bcd	3.46 a	0.43 c
	L-11	8.84 a	6.56 ab	5.57 ab	4.17 ab	3.65 a	0.57 ab
	Atlantic	5.74 b	6.65 ab	5.65 ab	3.85 abc	3.06 ab	0.59 a
PGRs free	Up-to-date	6.78 b	7.24 a	6.25 a	3.79 abc	2.39 bc	0.35 c
	L-11	9.69 a	6.50 ab	5.54 ab	4.49 a	3.41 a	0.44 bc
	Atlantic	5.99 b	7.08 ab	5.89 ab	3.27 abcd	2.04 c	0.62 a
$LSD_{(0.05)}$		1.97	1.18	1.17	1.23	0.82	0.13
Pr>F		0.19	0.37	0.32	0.68	0.36	0.09
CV%		28.66	17.28	20.03	36.92	28.28	29.21

*Means followed by the same letter in each column are not significantly different at 5% level.

4.6 Experiment 2.b: Effects of different concentrations of sucrose and different levels of PGRs on microtuberization of three selected potato cultivars

4.6.1 Microtuberization percent

Microtuberization percent as affected by the two different concentrations of sucrose and two different levels of BAP are described in Table (4.10). There was a significant difference in microtuberization percent on the different levels of sucrose in combination of two BAP levels (Pr>F = 0.02). MS medium containing 8% sucrose and 3 mg.L^{-1}BAP gave the maximum microtuberization percent 80.95. The result revealed that the increase in BAP concentration decreased in microtuberization percent. In contrast the increased in sucrose level increased in microtuberization percent. This result is agreed with Saha et al. (2013). They suggested that microtuberization percentage was increased by increasing sugar level.

There was a significantly difference on microtuberization percent between three tested potato cultivars (Pr>F = <0.001). The data are described in Table (4.10). The higher microtuberization percent 85.71 in L-11 and 84.82 percent in Up-to-date cultivars were recorded and Atlantic cultivar gave minimum percent 46.42.

There was no interaction effect on microtuberization percent as affected by different levels of sucrose and different concentrations of BAP on three selected potato cultivars (Pr>F = 0.07). The data is described in Table 4.11. The higher microtuberization percent were recorded in 8% sucrose in combination with 3 and 5 mg.L^{-1}BAP supplemented media for both L-11 and Up-to-date cultivars. On sucrose 6% and 3mg.L^{-1} BAP supplemented medium, the microtuberization percent were recorded as 87.57 for L-11, 82.14 for Up-to-date and 60.71 for Atlantic cultivar, respectively. At higher levels of sucrose, the osmolarity of the medium increased and plants underwent stress. Due to this stress, plants behavior shifted towards maturity which leads to tuber formation. This result was agreed with the following researchers. Wang & Hu, (1982) and Khuri & Moorby (1995) who reported that sucrose as an essential osmoticum, energy source and at higher concentration may have a role as a signal for microtuber formation.

The cultivar L-11 provided 100% microtuberization and Up-to-date also gave 89.28% on the MS medium supplemented with 8% sucrose and 5 mg.L^{-1} BAP. However, Atlantic gave only 28.57% for microtuberization on the same medium. All of the selected cultivars provided better microtuberization percent on MS medium supplemented with

8% sucrose and 3 mg.L^{-1}BAP (Figure 4.4). It can be assumed that 3 mg.L^{-1}BAP was suitable for microtuberization in combination with high level of sucrose (Figure 4.4).

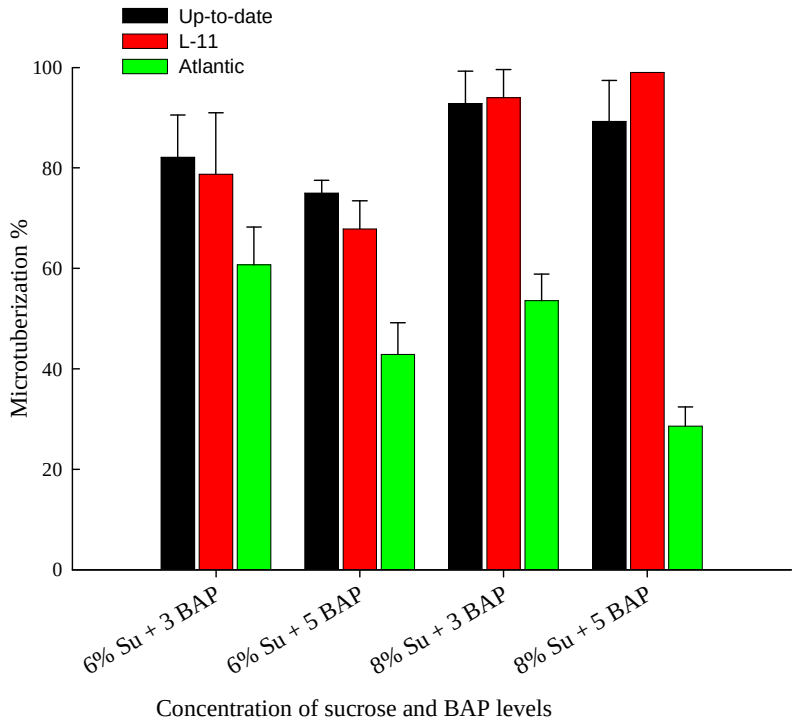


Figure 4.4 Effects of different concentrations of sucrose and different levels of BAP on microtuberization percent of shoot tip derived three potato cultivars

Su = Sucrose

3 BAP = 3 mg.L^{-1}BAP

5 BAP = 5 mg.L^{-1}BAP

4.6.2 Number of microtubers per explant

Number of microtubers per explant as affected by different concentrations of sucrose and BAP is described in Table (4.10). There was no significant difference effects on different concentrations of sucrose and different levels of BAP on number of microtubers produced per explant (Pr>F = 0.70). Sucrose converted to starch in microtubers developing, increased cell division and expension of the stolon end are followed rapidly by a massive deposition of starch and storage protein.

The number of microtubers produced per explant as affected by different concentrations of sucrose and different levels of BAP was not statistically different between cultivars (Pr>F = 0.82). Many researchers have shown that potato cultivar had a different potential in production of microtubers. Gopal et al. (1998) suggested that in the

same condition, genetic potential of cultivars have the greatest effect and will give the different yields because of initial capacity of the genotypes in production of endogenous levels of growth regulators.

There was no interaction effect between different levels of sucrose and BAP combinations and selected potato cultivars (Pr>F = 0.81).

4.6.3 Length of microtuber per explant

The length of microtuber per explant as affected by combination of different levels of sucrose and BAP was not significantly different among the treatments (Pr>F = 0.08) (Table 4.10). There was similar effect in different concentration of sucrose and PGRs on length of microtubers. Although in numerical value the greatest length of microtuber was found in 6% sucrose in combination with 5 $mg.L^{-1}$ BAP. Cytokinins affected in cell divisions and induction production of potato microtubers, the tubers size were increased the various concentrations. This was agreed with Liu and Xie. (2001) and they suggested that with an increased in cytokinin concentration, size and weight of microtuber was increased because of linear relation between these two hormones.

There was a significantly difference in length of microtuber per explant as affected by varieties (Pr>F = <0.001). Data is described in Table 4.10. Among the selected cultivars, Atlantic cultivar provided the maximum length of microtuber 4.09 mm. Atlantic cultivar produced the largest length of microtuber, although it gave the lowest microtuberization percent among the selected cultivars. Sucrose converted to starch during microtubers development, increased cell division and expansion of the stolon end are followed rapidly by a massive deposition of starch and storage protein as a result of genes involved in starch and protein biosynthesis. Then, the accumulation of starch leads to increase in size and weight of microtubers (Prat et al. 1990; Visser et al. 1994).

There was no interaction effect for length of microtuber (mm) among the different concentration of sucrose in combination with different levels of BAP and potato cultivars (Pr>F = 0.33) (Table 4.11). This means different levels of sucrose and BAP have similar effect on length of microtuber. Atlantic cultivar provided longest microtuber length 4.37 mm in media modified with 6% sucrose and both concentration of 3 and 5 $mg.L^{-1}$ BAP. According to Mashhadi and Moeini (2015), it was reported that the use of BAP and kinetin improved potato microtuber production by reduction the time required to start microtuberization, increasing the number of microtuber per explant and increase the weight and diameter of microtuber.

4.6.4 Weight of microtuber

Weight of microtuber (mg) as affected by different levels of sucrose and BAP is shown in Table 4.10. There was no significant difference in weight of microtuber (mg) among different concentrations of sucrose and BAP (Pr>F = 0.13). There was no variation on weight of microtuber (mg) by different concentrations and combinations of sucrose and BAP. Although it is not statistically different, in terms of value the larger weight of microtuber (45 mg) was found in the medium supplemented with 6% sucrose and 5 mg.L^{-1}BAP. Similar result was found in Hossain et al. (2015) and they pointed out that the larger size of microtuber was recorded in MS medium supplemented with 9% sucrose than the medium modified with 3% sucrose.

Weight of microtuber (mg) as affected by different concentration of sucrose and BAP among selected potato cultivars was significantly difference (Pr>F = <0.001) (Table 4.10). The greatest microtuber weight 52 mg was found in Atlantic cultivar and the lowest 27 mg was recorded in the rest Up-to-date and L-11 cultivars. This may due to genotypic differences between cultivars.

There was no statistically difference in interaction effect among combination of different sucrose concentration and BAP and varieties (Pr>F = 0.56) and the data is shown in Table 4.11. It represented that the combination of sucrose and BAP showed similar effect on weight of microtuber (mg). The maximum weight of microtuber 62.00 mg was recorded in Atlantic cultivar at 6% sucrose and 3 mg.L^{-1}BAP and a little bit decreased in weight of microtuber 60 mg when MS medium supplemented with 6% sucrose and 5 mg.L^{-1}BAP. Similarly, Atlantic cultivar gave (45 mg) of microtuber weight in 8% sucrose and 3 mg.L^{-1}BAP combination medium and that of 42 mg in 8% sucrose and 5 mg.L^{-1}BAP supplemented MS medium. The result of this study reveled that minimum concentration of BAP was suitable for larger microtuber sized and weight than high concentration of BAP. Aryaki and Hamidoghli (2010) suggested that BAP concentration of 0.75 and 1 mg.L^{-1} gave increasing microtuber weight and size more than others. Al-Taweel et al. (2004) proved that the highest percentage of microtuberization (70%) with higher weight of microtuber was recorded from the medium supplemented with 6% sucrose.

4.6.5 Diameter of microtuber

Diameter of microtuber (mm) as affected by different concentrations of sucrose and various levels of BAP is described in Table 4.10 and the data was not significantly difference between the average mean of treatments (Pr>F = 0.08). According to the results, 6% sucrose and 5 mg.L^{-1}BAP supplemented MS medium gave the largest diameter 3.61 mm, followed by (3.37 mm) diameter in medium supplemented with 6% sucrose and 3 mg. L^{-1}BAP. The lowest diameter of microtuber was found in (3.07 mm) in the medium applied in 8% sucrose and 3 mg.L^{-1}BAP and 3.14 mm in 8% sucrose and 6 mg.L^{-1}BAP. That result was contrasted with (Saha et al. 2013) they found that best survival rate and average diameter of microtuber per plantlet was found at 10% sucrose, in my study the greatest average diameter of tuber was found in 6% sucrose in combination of 3, 5 mg.L^{-1}BAP.

Diameter of microtuber as affected by various concentrations of sucrose and BAP was statistically significant different among the selected cultivars (Pr>F = <0.001). The data are showed in Table 4.10. The largest diameter of microtuber (3.93 mm) was recorded from Atlantic and (2.9 mm) in both Up-to-date and L-11 cultivar (Plate 9). It can be various diverted effect of cultivars on microtuberization, such as sucrose and PGRs. This is agreed with Kianmehr et al. (2012) they reported that the effects of PGRs on microtuberization are depending on the cultivar, environment and growth conditions. Moreover, El-Sawy et al. (2007) proved that sucrose is an important factor for microtuber formation.

There was no significant interaction effects on diameter of microtuber between different concentrations of sucrose, BAP and cultivars (Pr>F = 0.18) and the data is shown in Table (4.10). This can be assumed that different concentrations of sucrose and BAP shown similar effects on diameter of microtubers formation in tested potato cultivars. The largest microtuber diameter (4.46 mm) was found 6% sucrose and 3 mg.L^{-1} BAP, followed by (4.19 mm) in that of 6% sucrose and 5 mg.L^{-1}BAP. It was contrasted with the findings of Kanwal et al. (2006) and who reported that high level of sucrose was beneficial in producing larger microtubers. Although Atlantic cultivar produced fairly low microtuberization percent than the rest two cultivars, it provided larger size microtubers in low sucrose concentration compared to the rest cultivars, Up-to-date and L-11.

Present finding was in line with Imani et al. (2010) and who reported that maximum number and size of potato microtubers were achieved when sucrose at 6%.

While Iqbal et al. (2006) proved that the maximum microtuber induction was at 9% sucrose. The best effect of cytokinin is not only on cell division but also responsible for producing of nutritional sink (Hannapel 2004). Kianmehr et al. (2012) stated that microtuberization is accompanied by increasing endogenous cytokinin-like activity. Al-Hussaini et al. (2015) discussed that modification of environmental factors, such as photoperiod are very important, darkness after light duration were reported to increase microtubers by enhanced tuberonic acid synthesis which plays important role in tuber formation during *in vitro* condition. In addition to these facts, modification of environmental factors, such as low temperature and photoperiod are very important, darkness after light duration was needed to increase microtubers (Alisdair and Willmitzer. 2001).

Table 4.10 Combination effects of Sucrose and BAP on Microtuberization of Potato Varieties Up-to-date, L-11 and Atlantic

Treatment	Microtuberization %	No. of microtuber formation per explant	Lenght of microtuber (mm)	Weight of microtuber (mg)	Diameter of microtuber (mm)
Treatments					
6% Sucrose + 3 mg.L^{-1}BAP	73.81ab	1.19 a	3.35ab	36.00 ab	3.37 ab
6% Sucrose + 5 mg.L^{-1}BAP	61.89 b	1.18 a	3.89 a	45.00 a	3.61 a
8% Sucrose + 3 mg.L^{-1}BAP	80.95 a	1.09 a	3.43 b	30.00 b	3.07 b
8% Sucrose + 5 mg.L^{-1}BAP	72.62 ab	1.15 a	3.43 b	30.00 b	3.14 b
$LSD_{(0.05)}$	10.91	0.22	0.39	0.01	0.44
Pr > F	0.02	0.70	0.08	0.13	0.08
Variety					
Up-to-date	84.82 a	1.15 a	3.27 b	27.00 b	2.98 b
L-11	85.71 a	1.19 a	3.37 b	27.00 b	2.98 b
Atlantic	46.42 b	1.12 a	4.09 a	52.00 a	3.93 a
$LSD_{(0.05)}$	9.45	0.18	0.34	0.01	0.38
Pr > F (Var)	<0.001	0.82	<0.001	<0.001	<0.001
Pr > F (Suc+BAP) * (Var)	0.07	0.81	0.33	0.56	0.18
CV%	20.81	22.69	13.44	45.96	16.39

*Means followed by the same letter in each column are not significantly different at 5% level.
Data were recorded 14 weeks after culture.

Table 4.11 Combination effects of Sucrose, PGRs and variety on microtuberization stage of selected potato cultivars

Sucrose %	PGR	Variety	Microtuberization %	No. of microtubers/ explant	Lenght of microtuber (mm)	Fresh Weight of microtuber(mg)	Diameter of microtuber(mm)
6%	3 mg.L^{-1} BAP	Up-to-date	82.14 abcd	1.09 a	3.18 cd	25.00 bcd	2.82 de
		L-11	87.57 abcd	1.25 a	3.09 cd	22.00 bcd	2.82 de
		Atlantic	60.71 def	1.2 a	4.37 a	62.00 a	4.46 a
	5 mg.L^{-1} BAP	Up-to-date	74.99 bcde	1.22 a	3.60 bcd	40.00 abcd	3.43 bcd
		L-11	67.85 cde	1.13 a	3.70 bcd	35.00 bcd	3.19 cde
		Atlantic	42.85 fg	1.17 a	4.37 a	60.00 a	4.19 ab
8%	3 mg.L^{-1} BAP	Up-to-date	92.85 ab	1.03 a	3.38 bcd	27.00 bcd	3.04 cde
		L-11	96.43 ab	1.24 a	3.22 bcd	20.00 cd	2.73 de
		Atlantic	53.57 ef	1.00 a	3.70 abc	45.00 ab	3.44 bcd
	5 mg.L^{-1} BAP	Up-to-date	89.28 abc	1.23 a	2.91 d	17.00 d	2.60 e
		L-11	100 a	1.14 a	3.50 bcd	32.00 bcd	3.17 cde
		Atlantic	28.57 g	1.08 a	3.89 ab	42.00 abc	3.64 bc
$LSD_{(0.05)}$			18.90	0.37	0.69	0.02	0.77
Pr>F (Treat* Var)			0.07	0.81	0.33	0.56	0.18
CV%			20.81	22.69	13.44	45.96	16.39

*Means followed by the same letter in each column are not significantly different at 5% level.
Data were recorded 14 weeks after culture.

Plate 9. Microtuber formation as affected by different concentration of sucrose and different levels of BAP on shoot tip culture derived nodal explants of Up-to-date, L-11 and Atlantic cultivar at 14 weeks after culture

Scale bar = 5 mm

4.7 Comparison between Two Different Explants Source of Up-to-date Cultivar at Shoot Multiplication on the Same PGRs Combinations

4.7.1 Shoot length per explant (cm)

Effect of different concentration and combination of PGRs on shoot multiplication stage of two different sources of explants; meristem derived and shoot tip culture derived explants of Up-to-date cultivar are showed in Table 4.12. In meristem derived explants, there was significantly difference between shoot lengths response to different PGRs combinations. The maximum shoot length (7.10 cm) was recorded in medistem derived explants in PGRs free medium. No significant difference was observed in shoot tip culture derived explants for different combinations of PGRs, although the highest number of shoot length 4.43 cm was recorded in the MS medium supplemented with 0.05 mg.L^{-1} NAA and 0.5 mg.L^{-1}BAP. According to these results, it can be suggested that the meristem derived explants are more suitable to PGRs free medium and shoot tip derived explants need additional PGRs combination for *in vitro* shoot multiplication.

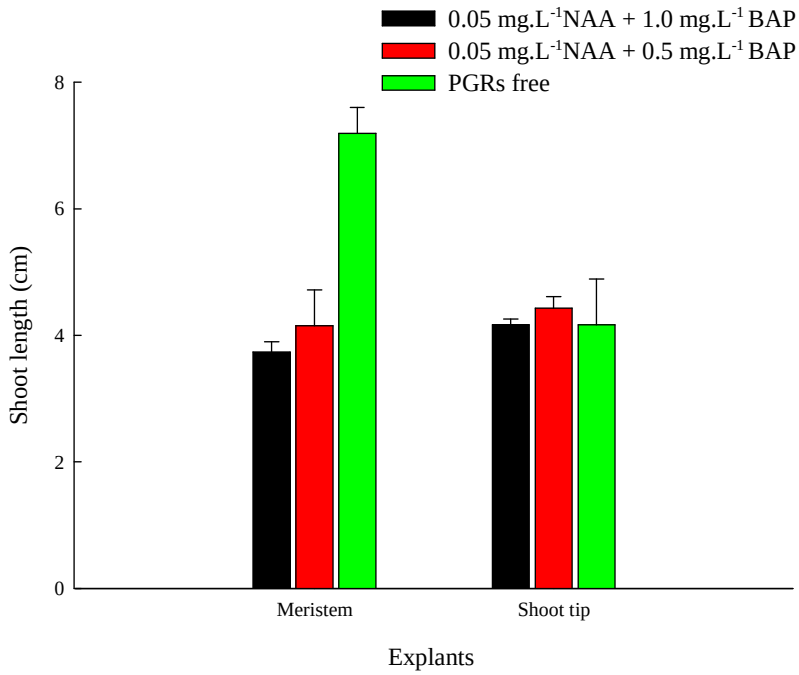


Figure 4.5 Comparison between two different sources of explants of Up-to-date cultivar on shoot length as affected by different combination of PGRs at shoot multiplication

4.7.2 Number of nodes per explant

Table 4.12 and figure 4.6 show the effects of different combinations and concentrations of PGRs between two different sources of explants in shoot multiplication. There was no significant effect of different PGRs combinations on number of nodes per plantlet of meristem derived explant. However, the maximum number of nodes per plantlet 7.91 was observed in PGRs free medium. Similar result, 7.35 nodes was provided from the plantlets developed in the medium containing with 0.05 mg.L^{-1}NAA and 0.05 mg.L^{-1}BAP.

In shoot tip derived explants, no significant effect of PGRs combination was found but the largest number of nodes per plantlet (5.97) was recorded in the MS medium modified with 0.05 mg.L^{-1}NAA and 1.0 mg.L^{-1}BAP (Figure 4.6).

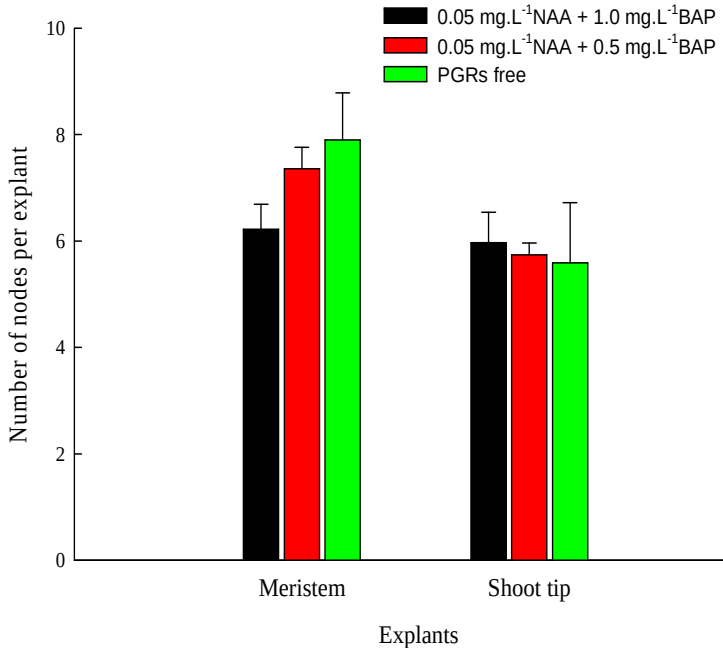


Figure 4.6 Comparison between two different sources of explants of Up-to-date cultivar on number of nodes per explant as affected by different combination of PGRs at shoot multiplication

4.7.3 Number of shoots per explant

Number of shoots produced per explant as affected by the different concentrations and combination of PGRs in meristem derived Up-to-date cultivar was statistically different (Table 4.12 and Figure 4.7). The maximum number of side shoots 4.82 was recorded in the medium applied with 0.05 mg.L^{-1}NAA and 1.0 mg.L^{-1}BAP.

There was significant difference between number of shoots produced per plantlet as affected by the different concentrations and combination of PGRs in shoot tip derived explant. The greatest number of side shoots (4.66) was found in the medium containing 0.05 mg.L^{-1}NAA and 0.50 mg.L^{-1}BAP. This is due to cytokinin increases the sensivity of tissue to auxin stimulation. This increased sensitivity favors sieve-tube differentiation resulting in the highest ratio of phloem/xylem under the optimal level of cytokinin under the optimal level of cytokinin. It can be suggested that the differentiation of phloem strands with no xylem in young internodes results from optimal cytokinin levels in the young part of the shoot.

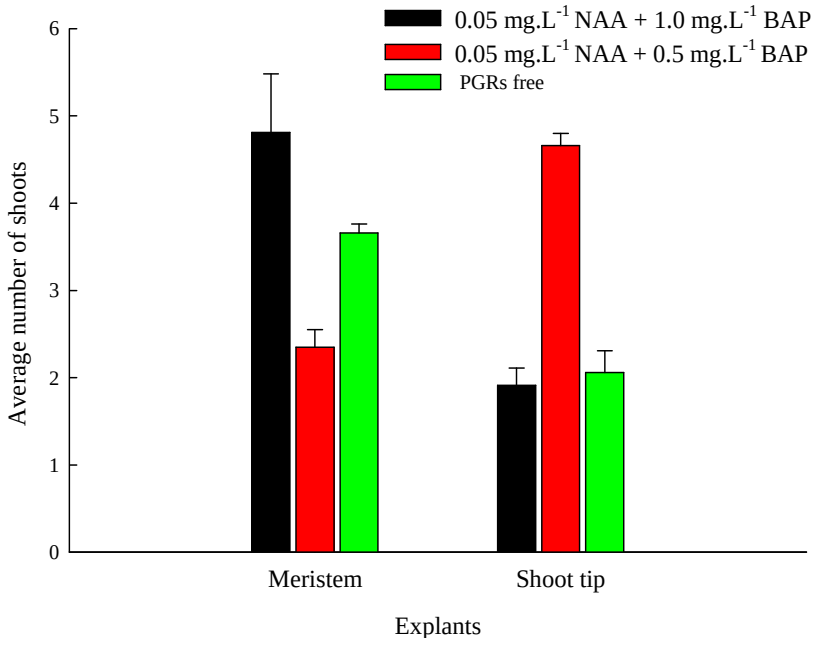


Figure 4.7 Comparison between two different sources of explants of Up-to-date cultivar on number of shoots per explant as affected by different combination of PGRs at shoot multiplication

4.7.4 Number of leaves per explant

Meristem derived explants provided more leaves per explant than shoot tip derived explants both in PGRs free medium and medium supplemented with 0.05 mg.L^{-1} NAA in combination of 0.5 mg.L^{-1}BAP (Figure 4.8).

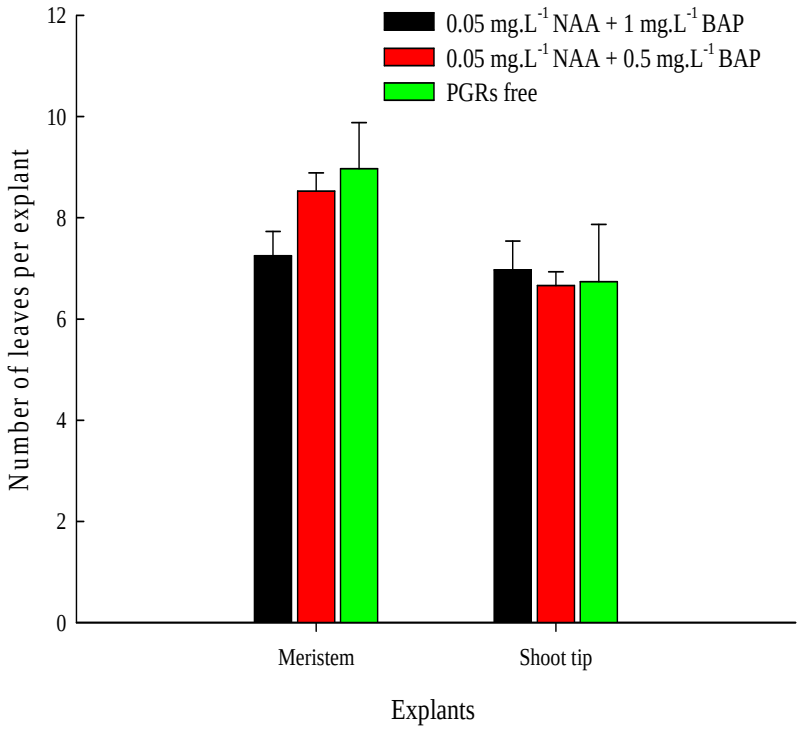


Figure 4.8 Comparison between two different sources of explants of Up-to-date cultivar on number of leaves per explant as affected by different combination of PGRs at shoot multiplication

4.7.6 Shoot fresh weight

Similarly the other parameters, meristem derived explants gave maximum shoot fresh weight in all PGRs combinations medium than shoot tip derived explant. Greater weight were recorded in both medium supplemented with PGRs as the callus formation at the base of the plantlets (Figure 4.8). The NAA and BAP ratio used in shoot multiplication favored the callus formation.

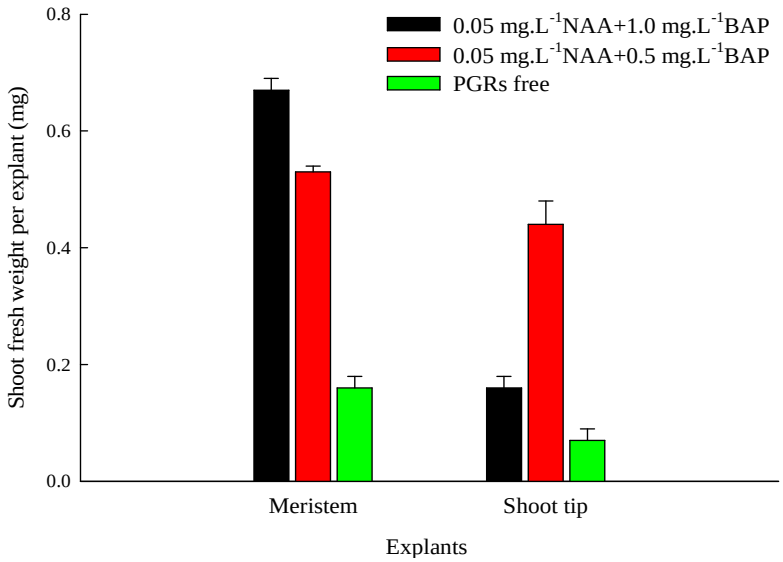


Figure 4.9 Comparison between two different sources of explants of Up-to-date cultivar on shoot fresh weight per explant as affected by different combination of PGRs at shoot multiplication

Table 4.12 Comparison between Shoot length, Number of nodes, Number of side shoots per explant of two different sources of explants in Up-to-date cultivar

Treatment	Shoot length (cm)		Number of nodes		Number shoots	
	Meristem	Shoot tip	Meristem	Shoot tip	Meristem	Shoot tip
0.05 mg.L^{-1}NAA +1.0 mg.L^{-1}BAP	3.45 b	4.17 a	6.22 a	5.97 a	4.82 a	1.91 b
0.05 mg.L^{-1}NAA + 0.5 mg.L^{-1}BAP	4.15 b	4.43 a	7.35a	5.74 a	2.35 b	4.66 a
PGRs free	7.10 a	4.17 a	7.91a	5.59 a	3.66 ab	2.06 b
$LSD_{(0.05)}$	1.74	1.55	1.89	2.08	1.35	0.56
Pr > F	0.005	0.89	0.16	0.63	0.01	<0.001
CV%	20.07	20.67	15.27	28.11	21.62	11.24

*Means followed by the same letter in each column are not significantly different at 5% level.
The data were recorded 6 weeks after culture.

4.8 Comparison between Two Different Explants Source of Up-to-date Cultivar on Microtuberization as Affected by Different Combinations of Sucrose and BAP

4.8.1 Microtuberization percent

There was significantly difference between microtuberization percent of meristem derived up-to-date cultivar among different concentration of sucrose and BAP combinations (Table 4.13). A hundred percent microtuberization was resulted from the explants developed on the medium supplemented with 8% sucrose in combination with 3 mg.L^{-1}BAP.

In shoot tip derived explants there was no significant effect of sucrose and BAP combination. However the greatest microtuber formation (92.85%) was resulted from the explants inoculated in the medium supplemented with 8% sucrose and 3 mg.L^{-1}BAP. Similarly, the MS medium containing 6% sucrose and 3 mg.L^{-1}BAP provided suitable response to microtuberization percent in both meristem and shoot tip derived explants.

4.8.2 Fresh weight of microtuber

Fresh weight of microtuber as affected by different concentrations and combinations of sucrose and BAP was not significantly different not only in meristem derived explants but also in shoot tip derived explants. The data were described in table 4.13. However, the maximum value of microtuber weight 60.00 mg was recorded from the meristem derived explants in the medium supplemented with 8% sucrose and 3 mg.L^{-1} BAP followed by 50.00 mg in the medium modified with 8% sucrose and 5 mg.L^{-1}BAP. Yu et al. (2000) stated that the effect of carbon source is more influential than other factors in promoting microtuber induction.

4.8.3 Number of microtuber per explant

There was no significant effect of sucrose and PGRs combination on number of microtuber produced per meristem derived explant. The data was described in Table 4.14. The larger number of microtubers 1.11 was found in the medium modified with 6% sucrose and 5 mg.L^{-1}BAP. However, in shoot tip derived explants no significant effect was resulted. Although, in term of value (1.23 and 1.22) were found in the media supplemented with 8% sucrose and 5 mg.L^{-1}BAP and 6% sucrose and 5 mg.L^{-1}BAP. Hussain et al. (2006) observed that complete obscurity was an essential factor in tuber induction. Cultures kept under 16 hrs, photoperiod were not able to produce microtubers. During incubation under light, GA_3 is synthesized which inhibits tuber induction while

darkness enhanced tuberonic acid synthesis, which plays important role in tuber formation.

4.8.4 Length of microtuber

Length of microtuber as affected by different levels of sucrose and different concentration of BAP was significantly different for the meristem derived explants. The greatest length of microtuber 4.88 mm was resulted in the medium supplemented with 8% sucrose and 3 $mg.L^{-1}$BAP. Similarly, the explants in the medium contain 8% sucrose and 5 $mg.L^{-1}$BAP provided larger length of microtuber 4.44 mm. In shoot tip culture dived explants, there was no significant effect between the treatment for length of microtuber. However, the larger value (3.38 mm) was recorded in the medium applied with 8% sucrose and 3 $mg.L^{-1}$BAP.

According to this result, it can be assumed that higher sucrose concentration in combination with BAP can produce lager size tubers. This is in line with the reports of Wang and Hu (1982) and Khuri & Moorby (1995) stated that sucrose seems to be the most critical stimulus for tuber formation. It may be essential as an osmoticum, as an energy source and at higher concentration may have a role as a signal for microtuber formation.

Table 4.13 Comparison between microtuberization%, no. of microtubers per explant, length of microtuber, fresh weight of microtuber and diameter of microtubers developed from two different sources of explants in Up-to-date cultivar

Sucrose	PGRs	Microtuberization %		Fresh Weight of Microtuber(mg)	
		Meristem	Shoot tip	Meristem	Shoot tip
6%	3 mg.L^{-1}BAP	93.75 a	82.14 a	40 b	20 a
	5 mg.L^{-1}BAP	87.50 a	74.99 a	40 b	40 a
8%	3 mg.L^{-1}BAP	100 a	92.85 a	60 a	20 a
	5 mg.L^{-1}BAP	46.88 b	89.28 a	50 ab	10 a
$LSD_{(0.05)}$		17.71	22.93	22.93	0.02
Pr>F		<0.001	0.35	0.35	0.32
CV%		13.50	16.90	16.90	59.38

*Means followed by the same letter in each column are not significantly different at 5% level.

Table 4.14 Comparison between microtuberization%, no. of microtubers per explant, length of microtuber, fresh weight of microtuber and diameter of microtubers developed from two different sources of explants in Up-to-date cultivar

Sucrose	PGRs	No. of Microtubers/ Explant		Length of Microtuber (mm)	
		Meristem	Shoot tip	Meristem	Shoot tip
6%	3 mg.L^{-1}BAP	1.03 ab	1.09 a	3.90 b	3.18 a
	5 mg.L^{-1}BAP	1.11a	1.22 a	4.17 b	3.06 a
8%	3 mg.L^{-1}BAP	1.03 ab	1.03 a	4. 88 a	3.38 a
	5 mg.L^{-1}BAP	1.00 a	1.23 a	4.44 ab	2.91 a
$LSD_{(0.05)}$		0.10	0.35	0.58	0.80
Pr>F		0.14	0.52	0.02	0.31
CV%		6.37	19.14	8.40	15.37

*Means followed by the same letter in each column are not significantly different at 5% level.

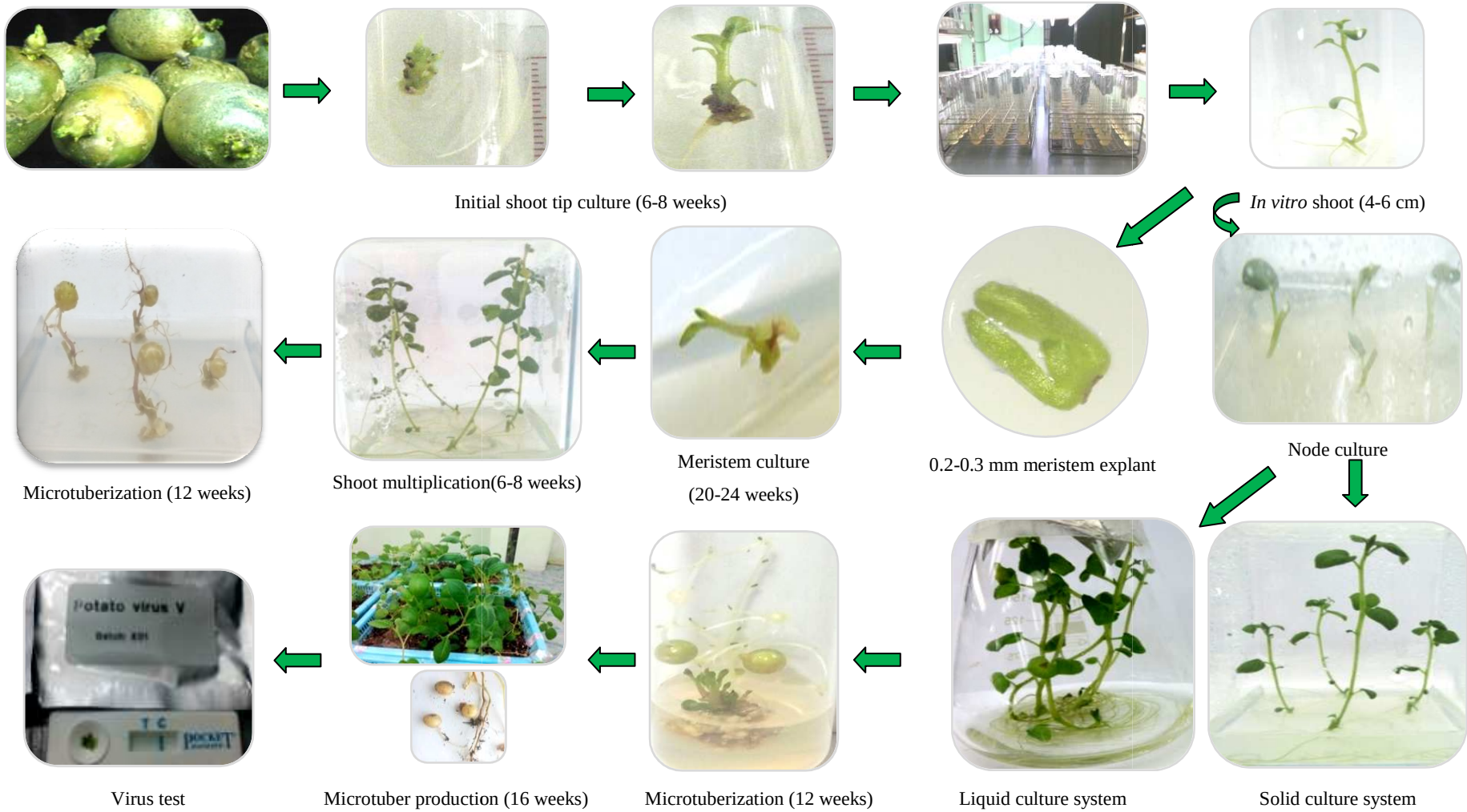


Plate 10. Protocol for *in vitro* regeneration of meristem and shoot tip culture in potato (*Solanum tuberosum*)

CHAPTER V
CONCLUSION

According to the results in this study, among the hormone free initial culture of three selected potato cultivars, Up-to-date cultivar provided maximum shoot length, number of leaves, number of nodes and number of side shoots.

In the initial stage of meristem culture of three selected potato cultivars, the meristem explants produced the hundred percent survival shoots in the medium both supplemented with 1.5 $mg.L^{-1}$BAP in combination of 0.6 $mg.L^{-1}GA_3$ and 3 $mg.L^{-1}$BAP in combination with 0.3 $mg.L^{-1}GA_3$ for all cultivars. Among the potato cultivars, Up-to-date cultivar gave higher survival percent than other two cultivars.

In shoot multiplication of meristem derived Up-to-date cultivar, longest shoot length, greater number of leaves and nodes per explant were resulted from the explants developed in PGRs free medium. However, maximum number of side shoots, roots and shoot fresh weight were influenced by the PGRs combination of 0.05 $mg.L^{-1}$NAA and 1.0 $mg.L^{-1}$BAP. In microtuberization, shoot plantlets from meristem derived Up-to-date cultivar, showed 100% microtuberization, and larger size tuber on MS medium modified with 8% sucrose and 3 $mg.L^{-1}$BAP.

In evaluation of culture systems and PGRs combinations on three selected potato cultivars on shoot multiplication stage of shoot tip derived explants, liquid culture system was superior to solid culture system. Among the PGRs combinations, maximum shoot length, number of leaves, number of nodes and shoot fresh were resulted from the PGRs free liquid culture system. However, in solid culture system, supplementation of 0.05 $mg.L^{-1}$NAA and 0.5 $mg.L^{-1}$BAP showed the highest number of shoots and roots. For the microtuberization stage of shoot tip culture derived three selected potato cultivars, the same result was observed as meristem culture. The best response came from the medium supplemented with 8% sucrose and 3 $mg.L^{-1}$BAP. Moreover, Up-to-date and L-11 were recorded as good for microtuberization response.

In the comparison between meristem and shoot tip culture derived explants of Up-to-date cultivar in shoot multiplication and microtuberization stages, meristem explants provided better performance in shoot multiplication, microtuberization and produced larger size tuber than shoot tip explants.

In detection of virus test for the plantlets from microtubers, meristem culture can be assumed as the one it can be the virus free protocol.

Further investigations should be carried out to overcome the problems for this crop. These are:

- meristem culture should be carried out with low concentration of various PGRs
- microtuberization of potato using liquid culture with multiple nodes explants should be tested

REFERENCES

Abe, T. and Y. Futsuhara. 1986. Plant regeneration from suspension culture of rice (*Oryza sativa* L.) Japanese Journal of Breeding, 36 : 1-6.

Águila, G. L., S. Z. Hernández, P. T. Moya and B. P. Mederos. 2001. Meristem culture for the elimination of the virus S of the potato in plants cultivated. Biotecnología Vegetal., 1(2) : 117-119.

Akita, M. and S. Takayama. 1994. Induction and development of potato tubers in a jar fermentor. Plant Cell Tissue and Organ Culture, 36 : 177-182.

Al-Abdallat, A. K. and M. A. Suwwan. 2002. Interactive effects of explant, sucrose and CCC on microtuberization of 'Spunta' potato. Dirasat. Agriculture Science, 29: 19-27.

Alam, I., S. K. Sharmin, M. K. Naher, M. J. Alam, M. Anisuzzaman and M. F. Alam. 2010. Effect of growth regulators on meristem culture and plantlet establishment in sweet potato (*Ipomoea batatas* (L.) Lam.), POJ, 3(2) : 35-39.

Ali, S., M. S. Kadian, O. Ortiz, B. p. Singh, V. K. Chandla and M. Akhtar. 2013. Degeneration of potato seed in Meghalaya an Nagaland States in North-Easten Hills of India. Potato Journal, 40(2) : 122-127.

Alisdair, R. and L. Willmitzer. 2001. Molecular and biochemical triggers of potato tuber development. Plant Physiology, 127 : 1459-1465.

AL-Hussaini, Z. A., SH. A.Yousif and AL-Ajeely. 2015. The role of sucrose and light duration on *in vitro* tuberization for two cultivars of potato *Solanum tuberosum* L. International Journal of Current Microbiology and Applied Sciences. ISSN: 2319-7706. 4 (2) : 277-283. http://www.ijcmas.com.

Al-Taweel, K., K. Al-Maarri, M. Kheeti and A. Abdul-Kader. 2004. Effects of some factors influencing on *in vitro* tuberization of potato cv. «Draga». Damascus University, Journal of Agriculture Science. 2(20) : 265-280.

Amma, K and S. Maity. 1998. Role of nodal position and hormones on microtuber production in potati (*Solanum tuberosum* L.). Journal of Horticulture, 11 : 65-7.

Aryakia, E. and Y. Hamidoghli. 2010. Comparison of kinetin and 6-benzyl aminopurine effect on *in vitro* microtuberization of two cultivars of potato (*Solanum tuberosum* L.). American-Eurasian Journal of Agriculture And Environmental Science, 8(6) : 710-714.

Avila, A. de L., S. M. Pereyra and J. A. Arguello. 1996. Potato micropropagation: Growth of cultivars in solid and liquid media. Potato Research, 39 : 253-258.

Bachem, C., R. V. D. Hoeven, J. Lucker, R. Oomen, E. Casarini, E. Jacobsen, and R. Visser. 2000. Functional genomic analysis of potato tuber life-cycle. Potato Res., 43 : 297-312.

Badoni, A. and J. S. Chauhan. 2009. A note on micro tuber seed production of Potato: Necessitate step for Uttarakhand Hills. Report and Opinion. 1(5) : 9-11.

Bhojwani, S. S. and Razdan, M. K. 1983. Plant Tissue Culture: Theory and Practice, Chapt. 14, Elsevier, Amsterdam. 287-312.

Bowen, W.T. 2003. Water productivity and potato cultivation .p 229-238. In J.W. Kijine, R. Barker and D. Molden (eds.) Water productivity in Agriculture: Limits and opportunities for improvement. CAB International 2003. Available on line at http://www.iwmi.cgiar.org/pubs/CA CABI Series/ Water Productivity/ Protected/

Caula, A. B. 2011. PGRs and their use in micropropagation. In N. T. Robert and J.G. Dennis (ed.) Plant Tissue culture, Development and Biotechnology. Publ., Taylor and Francis group, India.

Conger, B. Y. 1981. Cloning agricultural plants via *in vitro* techniques.CRC press, Boca Raton, Fl.

Cutter, E. G. 1965. Recent experimental studies of the shoot apex and shoot morphogenesis. Bot. Rev. 31:7-113.

Danci O., M. Danci., and F. Berbentea. 2011. Studies regarding potato micropropagation by single node culture, Research Journal of Agricultural Science, 40 (3) : 21-24.

DAP. 2015. *Myanmar Agriculture at a Glance 2015*. Department of Agricultural Planning, Ministry of Agriculture and Irrigation, Nay Pyi Taw, Myanmar.

Debergh, P. C. 1983. Effects of agar brand and concentration on tissue culture medium. Physiology. Plant. 59 : 270-276.

Dixon, R. A. and R. A. Gonzales. 1994. "*Plant Cell Culture: A Practical Approach*," 2nd Edition, Oxford University Press, Oxford.

DOA 2015. Annual report of Department of Agriculture, Shan State.

Dobranszki, J., K.M. Tabori and A. Ferenczy. 1999. Light and genotype effects *in vitro* tuberization of potato plantlets. Potato Research Journal. 42 : 483-488.

Dodds, J. H., D. Silva-Rodriguez and P. Tovar. 1992. Micropropagation of potato. In: YPR Bajaj (ed), Biotechnology in Agriculture and Forestry 19-High-Tech and Micropropagation III. Springer-Ver-lag, Berlin : 92-106.

Donnelly, J.D., W.K. Coleman and S.E. Coleman. 2003. Potato microtuber production and performance: A review. American Journal of Potato Research, 80 : 103-115.

Ebadi, M., A. Iranbakhsh and G. Bakhshi-Khaniki. 2007. Shoot micriopropagation and microtuberization in potato (*Solanum tuberosum* L.) by the semi-continues bioreactor. Pakistan Journal of Biological Sciences, 10(6) : 861-867.

Ehsanpour, A. A. and M. G. K. Jones. 2000. Evaluation of direct shoot regeneration from stem explants of potato (*Solanum tuberosum* L.) cv. *Delaware* by Thidiazuron (TDZ). Journal of Science and Technical Agriculture and Natural Resources, Isf. Univ. Tech., Iran. 4(3) : 47-54.

Elaleem, K. G.Abd., R. S. Modawi and M. M. Kalafalla. 2009. Effect of cultivar and growth regulator on *in vitro* Micropropagation of Potato (*Solanum tuberosum* L.). American-Eurasian Journal of Sustainable Agriculture, 3(3) : 487-492.

Faccioli, G. and A. Colombarini. 1996. Correlation of potato meristem tips with the percentage of virus free plantlets produced *in vitro*. Potato Research Journal, 39 : 129-140.

FAO. 2001. Food and Agriculture Organization, FAOSTATAgricultural Data. Agricultural,crops,primary.http://faostat.fao.org/ subset agriculture

FAO. 2015. Food and Agriculture Organization, IFAD and WFP. Meeting the international hunger targets: taking stock of uneven progress.Rome,The State of Food Insecurity in the World 2015.

Fufa, M. and M. Diro. 2013. Effect of BAP and NAA on *in vitro* multiplication of potato cultivars, Wudpecker . Journal of Agricultural Research, 2(10) : 283-285.

Fufa, M. and M. Diro. 2014. Microtuber Induction of Two Potato (*Solanum tuberosum* L.) Varieties. Advenced Crop Science and Technology. 2: 122.

Gaba, V. P. 2005. PGRs in plant tissue culture and development. In Plant Tissue Culture and Development, R. N. Trigano and D. J. Gray, eds.,CRC press, Boca Raton, FL : 87-99.

Garner, N. and J. Blake. 1989. The induction and development of potato microtubers *in vitro* on media free of growth regulating substances. Annual Report of Botany, 63 : 663-674.

Gargantini, P., V. Giammaria., Grandellis, C. Feingold, S. Maldonado, and S. R. Ulloa. 2009. Genomic and functional characterization of StCDPK1. Plant Molecular and Biology,70 : 153-172.

Gebre, Endale. and B. N. Sathyanarayana. 2001. Tapioca-A new and cheaper alternative to agar for direct *In vitro* shoot regeneration and microtuber production from nodal cultures of potato, African Crop Science Journal, 9(1) : 1-8.

George, E. F., M. A. Hall, J. De-Klerk. 2008. Plant propagation by tissue culture.3rd Edition: The Background, 1: 1-105.

Gopal, J., J. Minocha and S. Ghosal. 1998. Variability in response of potato genotypes to *in vitro* propagation. Potato Journal, 25 : 119-124.

Gunan, H. Y., P. Huisman and G. –J. De Klerk. 1997. Rooting of apple stem slides *in vitro* is affected by indoleacetic-acid depletion of the medeium. Angewandte Botanik, 71 : 80-84.

Hannapel, D. J., H. Chen, F. M. Rosin, A. K. Banerjeeand and P. J. Davies. 2004. Molecular control of tuberization . American Journal of Potato Research, 81 : 236-274.

Haque, M. I. 1996. *In vitro* micotuberization. Bangladesh Journal of Botany, 25 : 87-93.

Hassani, F., A. Iareian and E. Rezvani. 2014. Effects of chemical treatments on dormancy breaking and some sprouting characteristic of two potato cultivars in different sizes. European Journal of Experimental Biology, 4(4) : 98-102.

Hartmann, H. T., Kester, T. T., Davies, T.T. 1990. Plant propagation, 5th Edn. Pentice Hall.

Hirpa A., M. P. M. Meuwissen, A. Tesfaye, A. Q. Lommen, A. Lansink, Tsegaye and P. C. Struik. 2010. Analysis of seed potato systems in Ethiopia. American Journal of Potato Research, 87 : 573-552.

Hossain, M. J. and N. Sultana. 1998. Effects of benzylaminopurine (BAP) and chloro choline chloride (CCC) on *in vitro* tuberization of potato. Bangladesh Journal of Agricultural Research, 23 : 685-690.

Hossain, M. A., H. M. Faruquee, N, Islam, M. A. S. Miah and K. M. Nasiruddin. 2005. Agrobacterium-mediated transfer of PsCIPK salt tolerance gene in sugarcane variety Isd 35. Molecular biology and Biotechnology Journal, 3(1&2) : 273-283.

Hossain, M. A., K. M. Nasiruddin and Md. A. Kawochar. 2013. Effect of 6-benzyl aminopurine (BAP) on meristem culture for virus free seed production of some popular potato varities in Bangladesh. African Journal of Biotechnology. 12(18) : 2406-2413.

Hossain, Md. A, Md. A. Kawochar, A. Al. Mahmud, E. H. Md.S. Rahaman, Md. Al. Hossain and K. Md. Nasiruddin. 2015. Standardization of sucrose and 6-benzyl aminopurine for *in vitro* microtuberization of potato. American Journal of Agriculture and Foresty, 3(2) : 25-50.

Hoque, M. E. 2010. *In vitro* regeneration potentiality of potato under different hormonal combination. World Journal of Agricultural Science, 6(6) : 660-663.

Hussain, I., Z. Chaudhry, A. Muhammad, R. Asghar, S. M. S. Naqvi, and H. Rashid. 2006. Effect of chloroline chloride, sucrose and BAP on *in vitro* tuberization in potato (*Solanum tuberosum* L. Cardinal). Pakistan Journal of Botany, 38(2) : 275-282.

Hussey, G. and N. J. Stacey. 1981. *In vitro* propagation of potato (*Solanum tuberosum* L.) Annals of Botany 48 : 787- 796.

Imani, A., R. Gharemanzadeh, J. Azimi and J. Janpoor. 2010. The effect of various concentration of 6-benzyl aminopurine (BAP) and sucrose on *in vitro* potato (*Solanum tuberosum*.L) microtuber induction. American-Eurasian Journal of Agriculture & Environmental Science, 8 : 457-459.

Iqbal, H., C. H. Zubeda, M. Aish, A. Rehana, S. Saqlan, and R. Hamid. 2006. Effect of chlorocholine chloride, sucrose and BAP on *in vitro* tuberization potato (*Solanum tuberosum* L. cv. Cardinal). Pakistan Journal of Botany, 38 : 275-282.

Irfan, A. 1992. *Proceeding of the workshop on seed potato health*. Seed potato laboratory project, Sahiwal. Federal Seed Certification Department. Ministry of Food, Agriculture and Coperation : 1-2. Islam, M. S. 1995. Indigenous potato varieties of Bangladesh: Characterization by RAPD markers and production of virus free stock. Ph. D. Thesis, Dept. of Horticulture, Bangabandhu Sheikh Mujibur Rahman Agril. University, Gazipur, Bangladesh.

Jha, T. B. and B. Ghosh. 2005. *Plant Tissue Culture*: Applied and basic. Universities Press (India) pvt. Lit.

Kanwal, A., A. Amir and K. Shoaib. 2006. *In vitro* microtuberization of potato (*Sloanum tuberosum* L.) cultivar Kuroda new variety in Pakistan. International Journal of Agriculture and Biology, 8(3) : 1560-8350.

Karp, A., M. G. K. Jones, G. Ooms and S. W. J. Bright. 1987. Potato protoplast and tissue culture in crop improvement. Biotechnology and Genetic Engineering Reviews.5.

Kartha, K. K. and O. L. Gamborg, O. L. 1975. Elimination of Cassava mosaic disease by meristem culture. Phytopathology, 65: 826-828.

Khuri, S. and J. Moorby. 1995. Investigations into the role of sucrose in potato *cv.* Estima microtubers production *in vitro*. Annals of Botany, 75 : 295-303.

Kianmehr, B., M. Parsa, M. Otroshy, M. N. Mohallati and K. Moradi. 2012. Effect of plant growth regulators during *in vitro* phase of potato microtuberization. Journal of Agricultural Technology, 8(5) : 1745-1759.

Kuria p., Demo P, A. B. Nyende, E. M. Kahangi. 2008. Cassava starch as alternative cheap gelling agent for the *in vitro* micro-propagation of potato (*Solanum tuberosum* L.). African Journal of Biotechnology, 7(3) : 301-307.

Lawrence, R. H. 1981. *In vitro* plant cloning system. pp:289-300. In. Propagation of higher plants through tissue culture. Emerging technologies and strategis. M.J. Constantin, R.R. Henke, K.W. Hughes and B. W. Longer (eds.). Environmental Experiment Botany, 21 : 269-452.

Lemaga, B., R. Kakuhenzire, P. Gildmacher, D. Borus, W. G. Gebremedhin, B.J. Low and O. Ortiz. 2009. Current status and opportunities for improving the access to quality potato seed by small farmers in Easten Africa. Symposium 15th triennial of the Symposium of the International Society for Tropical Root Crops : 2-6.

Liu, J. and C. Xie. 2001. Correlation of cell division and cell expansion to potato microtuber growth *in vitro.* Plant Cell, Tissue and Organ Culture, 67 : 159-164.

Lommen, W. J. M. 1993. Phost-harvest characteristic of potato mini-tubers with different fresh weights and from different harvest. I. Dry-matter concentration and dormancy. Potato Research, 36 : 265-272.

Martine, J and C. Mario. 1992. Effects of some growth regulator on *in vitro* tuberization in *Dioscorea Alata* L. *Brazo Fuerte* and D. *abyssinica Hoch*. Plant Cell Report, 11(1) : 34-38.

Mashhadi, S. and M. J. Moeini. 2015. The effect of cytokinin and coumarin on *in vitro* micrrotuberization of potato (*Solanum tuberosum* L.) cv. Marfona. Ludus Vitalis, 11(1) : 165-170.

Mbiyu, M., J. Muthoni, J. Kabira, C. Muchira, P. Pwaipwai, J. Ngaruiya, J. Onditi and S. Otieno. 2012. Comparing liquid and solid media on the growth of plantlets from three Kenyan potato cultivars. American Journal of Experimental Agriculture, 2(1) : 81-89.

Medford, J. I. 1992. Vegetative apical meristems. Plant Cell. 4:1029-1039.

Mehrotra, S. M. K. Goel, A. K. Kukreja and B. N. Mishra. 2007. Efficiency of liquid culture systems over conventional micropropagation: A progress towards commercialization, African Journal of Biotechnology, 6(13) : 1484 1492.

Moeinil, M. J., M. Armin, M. R. Asgharipair and S. K. Yazdi. 2011. Effects of different plant growth regulators and potting mixes on micro-propagation and mini-tuberization of potato plantlets. Advanced Environmental Biology, 5(4) : 631-638.

Mohamed, M. F., M. A. A. Mohamed and A. M. Ahmed. 2007. Different axillary bud proliferation response of two sweet potato cultivars to benzyl adenine and thidizuron. Assiut University Bulletin Environment Research, 10(2) : 21-30.

Motallebi-Azar, A., S. Kazemiani and F. Yarmohamadi. 2013. Effect of sugar/osmotic levels on *in vitro* microtuberization of potato (*Solanum tuberosum* L.) Russian Agricultural Science,39 : 112-116.

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bio assay with tobacco tissue cultures. Physiol. Plantarum, 15 : 473-497.

Murashige, T., M. Serpa and J. B. Jones. 1974. Clonal multiplication of gerberra through tissue culture. Horticulture Science, 9 : 175-180.

Murashige, T. 1977. Clonal crops through tissue culture: 392-403. In: Plant tissue culture and its biotechnological application. W. Barn, E. Reinhard and M.H. Lenk (eds.) Springer-Verlag. New York.

Myint, M. M. 2001. Growing late blight resistant hybrid TPS. (In Myanmar). The Agri-Business News 221. Suppl.

Nagib, A., M. F. Hossain, M. M. Alam, R. Islam and R. S. Sultana. 2003. Virus free potato seed tuber production through meristem culture in tropical Asia. Asian Journal of Plant Sciences, 2(8) : 616-622.

Naik, P. S. and J. L. Karihaloo. 2007. Micropropagation for production of quality potato seed in Asia-Pacific. Asia-Pacific Consortium on Agricultural Biotechnology, New Delhi, India: 44+ viii.

Nistor, A., G. Campeanu, N. Atanasiu, N. Chiru, D. Karacsonyi. 2010. Influence of potato genotypes on "*in vitro*" production of microtubers. Romanian Biotechnological Letters.15:1-8.

Pereira, J. E. S. and G. R. Fortes. 2003. Protocol for potato propagative material production in liquid medium. Pesquisa Agropecuária Brasileira, 38(9) : 1035-1043.

Pierik, R. L. M. 1997. *In vitro* culture of higher plants. Kluwer academic publishers, Dordrecht, Netherlands, : 21-146.

Pierik, R. L. M. 1989. *In vitro* culture of higher plants. Martinus Nijhoff, Dordecht, Netherlands.

Pronk, A. A. 2015. Report for the potato mission of the Netherlands industry and knowledge institutions to Myanmar. Plant Research International, Wegning UR, Business Unit Agrosystem Research.

Prakash, S. 1993. Production of ginger and turmeric through tissue culture methods and investigation into making tissue culture propagation less expensive. Ph. D thesis. Bangalore University, Bangalore, India.

Prat, S, W. Frommer, R. Hofgenm, M. Keil, J. Kossmann, M. Koster-Topfer, X. Liu, B. Muller, H. Pena-Cortes, U. Sonnewald and L. Willmitzer. 1990. Gene expression during tuber development in potato plants. FEBS Lett, 268 : 334-338.

Quak, F. 1977. Meristem culture and virus free plants. In: Applied and Fundamental aspects of Plant Cell, Tissue and Organ Culture. Reinert, J. and Bajaj, Y.P.S. (Eds.*)* Springer- Verlag, Berlin : 598-615.

Qureshi, M. A. Z., S. Gul, S. Ali and A. R. Khan. 2014. Comparative growth response of potato plantlets developed on liquid vs solidified (MS) medium using tissue culture technology. International Journal of Recent Scientific Research, 5(4) : 736-739. **Available Online at http://www.recent scientific. Com.**

Rahber, A. 1996. Response of Various Explants Source of Bougainvillea (B.spectailis var. Veriegata) to Different Levels of BAP. A special problem submitted to the Department of Horticulture, Agricultural University Peshawar : 23.

Rahman, M., S. Haider, M. Hossain and R. Islam. 2013. Involvement of jasmonic acid and light periods on potato microtuber growth response. International Journal of Bioscience, 3 : 87- 94.

Rannali, P., M. Bizzari, L. Borghi, M. Mari. 1994. Genotypic influence on *in vitro* induction, dormancy length, advancing age and agronomical performance of potato microtubers (*Solanum tuberosum* L.). Annals of Apply Biology. 125 : 161-172 http://dx.doi.org.

Rodrigues-otubo, B. R., J. A. Usbertifilho, W.J. Siqueira, E. T. Domingues, N. P. Granja and H. D. S. Mirander. 1999. Responses of potato genotypes for *in vitro* tuberization. Brangantia Companas, 58 : 227-33.

Rossel, G., F. D. de. Bertoldi and R. Tizio. 1987. *In vitro* mass tuberization as a contribution to potato micropropagation. Potato Research, 30 : 111-116.

Saini, G. S., 2001. A text book of Vegetable Production. Aman Publishing House, Meerut, India.

Saha, S., M. Ahmed, M. M. Islam, R. N. Remme and M. R. Ali. 2013. Effect of different levels of sucrose on microtuberization and different subtrates on minituber production resulted from potato meristem culture. IOSR Journal of Agriculture and Veterinary Science, 4(6) : 58-62. www.iosrjournals.org

Scholten, H. J, and R. L. M. Pierik. 1998. Agar as a gelling agent: differential biological effect *in vitro*. Scientia Horticulture, 77(2) : 109 -116.

Seabrook, J., S. Coleman., D. Levy. 1993. Effect of photoperiod on *in vitro* tuberization of potato (*Solanumtuberosum* L.). Plant Cell Tissue Org. Cult. 34 : 43-51. http://dx.doi.org/.

Shibli, R., A. Abu-Ein and M. Ajouni. 2001. *In vitro* multiplication of virus free 'Spunta' potato. Pakistan Journal of Botany, 33: 35-41.

Singh, D., I. Chaubey and S. Singh. 2001. Rapid seed multiplication in potatoes through micro-tubers. Journal Indian Potato Association, 28 : 103-104.

Smith, R. H., and T. A. Murashige. 1970. *In vitro* development of the isolated shoot apical meristem of angiosperms. American Journal Botany, 57 : 186-197.

Srivastava, A., L. Diengdoh, T. Bag, B. Singh. 2012. *In Vitro* micropropagation and micro-tuberization potential of selected potato varieties. Indian Journal of Hill Farming, 25 : 14-17.

Struik, P. C., W. J. M. Lommen. 1999. Improving the field performance of micro and mini-tubers. Potato Research, 42 : 559-568.

Thamburaj, S. and Singh. 2001. Text book of vegetables, tuber crops and spices. Indian Council of Agriculture Research, New Delhi.

Torres, K. C. 1989. Tissue culture techniques for horticultural crops. Van Nostrand Reinhold, New York : 285.

Tovar, P. and J. H. Dodds. 1986. *Tissue Culture Propagation of Potato*. CIP slide Training series 1-5 Int. Potato center, Dept. of training and communications, P. O. Box. 5659, Lima, Peru.

Uranbey, S. 2005. Comparison of kinetin and 6-benzyladene (BA) on *in vitro* microtuberization of potato under short days conditions, Tarim Bilimleri Dergisi. Journal Agricultural Science, 15(1) : 39-41.

Vanaei, H., D. Kahrizi, M. Chaichi, G. Shabani and K. Zarafshani. 2008. Effect of Genotype, Substrate Combination and Pot Size on Minituber Yield in Potato (*Solanum tuberosum* L.). American-Eurasian Journal of Agricultural and Environmental Sciences, 3(6): 818-821.

Vanterhalter, Dragon. I. Dragicevic., and B. Vinterhalter 2008. Potato *in vitro* culture technique and biotechnology. Fruit, Vegetable and Cereal Science and Biotechnology. Global Science Books.

Visser, R., D. Vreugdenhil, T. Hendriks, and E. Jacobsen. 1994. Gene expression and carbohydrate content during stolon to tuber transition in potatoes (*Solanum tubrosum*) Physiol. Plant., 90 : 285-292.

Wang, P. and C. Hu. 1982. *In vitro* tuberization and virus free seed-potato production in Taiwan. American Journal of Potato Research, 59 : 33-37.

Wiersema, S. G. 1984. The Production and Utilization of Seed Tubers Derived from True Potato Seed. Ph. D thesis, University of Reading, Department of Agriculture and Horticulture, Reading, UK : 229.

Yakimova, E., V. K. Toteva, L. Groshkoff and G. Invaaova. 2000. Effects of BA and CPPU on protease and ɑ- amylase activity of *in vitro* culture explants of Rosa hybrid L. Plant Physiology, 26 : 39-47.

Yousef, A., A. R., M. A. Suwwan, A. M. Musa and H. A. Abu-Qaoud. 2001. *In vitro* culture and microtuberization of spunta potato(*Solanum tuberosum*). Dirasat Agric. Sci., 24: 173-181.

Yasmin, A., A. A. Jalbani and S. Raza. 2011. Effect of plant growth regulators on meristem tip culture of local potato cvs Desiree and Patrones. *Pakistan* Journal of Agriculture, Agricultural Engineering and Veterinary Science, 27(2) : 143-149.

Yong, L., D. Huiruo, X. Xin, Y. Hongfu, J. Liping, L. Huan, and Z. Ying. 1996. Changes in several endogenous phytohormones during *in vitro* tuberization in potato. In: E. T. Rasco and F. B. Aromin (Eds.). Asian Sweet Potato and Potato Research and Development, Manila, 1 : 30-37.

Yu, W. C., P. J. Joyce, D. C. Cameron, B. H. McCown. 2000. Sucrose utilization during potato microtuber growth in bioreactors. Plant Cell Reports, 19 : 407-413.

Zaida, L. and D. E. Elizabeth. 1991. *In vitro* tuberization of potato clones from different maturity groups. Plant Cell Rep., 9 : 691-695.

Zakaria, M., M. M. Hossain, M. A. K. Mian, T. Hossain and M. Z. Uddin. 2008. *In vitro* tuberization of potato influenced by benzyl adenine and chlorochline chloride. Bangladesh Journal of Agricultural Rersearch, 33(3) : 419-425.

Zhang, Zh. N., W. He, K. Q. Cao, Z. Yang and X. Gui. 2004. General situation of occurrence. and control of potato late blight in ESEAA : 11-16. *In: Proceeding of the Regional Workshop on Potato late Blight for East and Southeast Asia and the Pacific*, Yezin, Myanmar. GILB, International Potato Cent, Peru er (CIP), Lima.

APPENDICES

Appendix 1. The chemicals, MS powder, sucrose and equipments used in this study

Plant Agar, MS powder, Myo Inositol

Sucrose

Paraflim

Paraflim

Micropipette and pH meter

Digital slide clipper

Appendix 2. Nutritional value per 100 gm of potato

Nutritional value per 100 g (3.5 oz)	
Energy	321 kJ (77 kcal)
Carbohydrates	17.47 g
Starch	15.44 g
Dietary fiber	2.2 g
Fat	0.1 g
Protein	2 g
Vitamins	
Thiamine (B1)	(7%) 0.08 mg
Riboflavin (B2)	(3%) 0.03 mg
Niacin (B3)	(7%) 1.05 mg
Pantothenic acid (B5)	(6%) 0.296 mg
Vitamin B6	(23%) 0.295 mg
Folate (B9)	(4%) 16 µg
Vitamin C	(24%)19.7 mg
Vitamin E	(0%) 0.01 mg
Vitamin K	(2%) 1.9 µg
Minerals	
Calcium	(1%)12 mg
Iron	(6%)0.78 mg
Magnesium	(6%)23 mg
Manganese	(7%)0.153 mg
Phosphorus	(8%)57 mg
Potassium	(9%)421 mg
Sodium	(0%)6 mg
Zinc	(3%)0.29 mg
Other constituents	
Water	75 g

Link to USDA Database entry

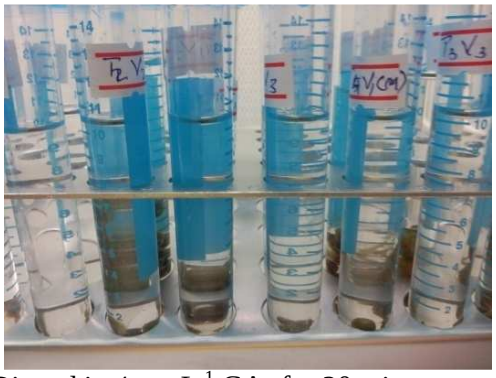
Units

µg = micrograms •
mg = milligrams
IU = International units

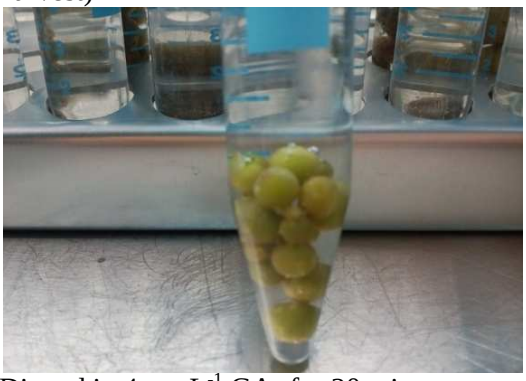
Source: USDA Nutrient Database

Appendix 3. Dormancy breaking and sprouting of harvested microtubers four weeks after harvesting

Sterilization of microtubers (4 weeks after harvest)



Dipped in 4 mg.L^{-1} GA_3 for 20 minutes



Dipped in 4 mg.L^{-1} GA_3 for 20 minutes

Kept in ambient condition to induce sprouts

Sprouting and germination

Sprouting and germination

Germination (2 weeks)

4 weeks after germination

Appendix 4. Acclimatization and Virus detection of harvested microtubers 8 weeks after planting.

Transplanting (4 weeks after germination)

Healthy plants in plastic trays (8 weeks)

Pocket Diagnostic (from UK)

Test kits for potato virus and fungi

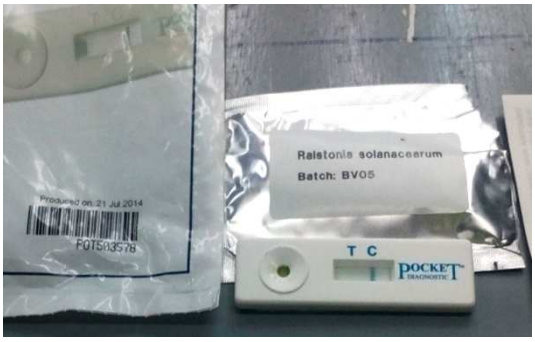
Test kits for potato virus and fungi

Extracting the leaves from selected plant

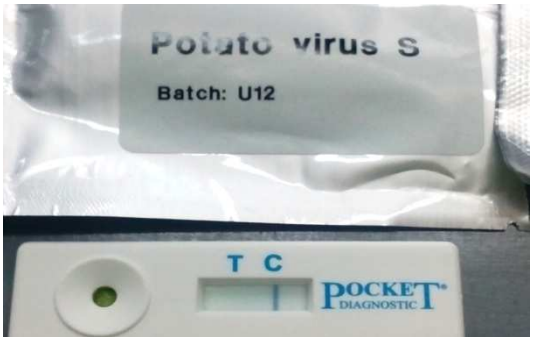
Gently squeezed 2-3 drops of sample liquid

Blue dye appeared in the viewing window

Appendix 5. Microtubers developed from meristem culture were induced germination four weeks after harvesting and checked for viruses 10 weeks after planting. According to results from test kits these plants materials were free from viruses and disease of *Rhizotonia Solancerum.*

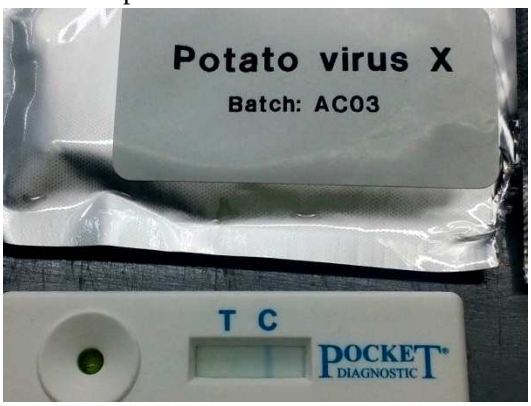


Free from *Rhizotonia Solancerum*



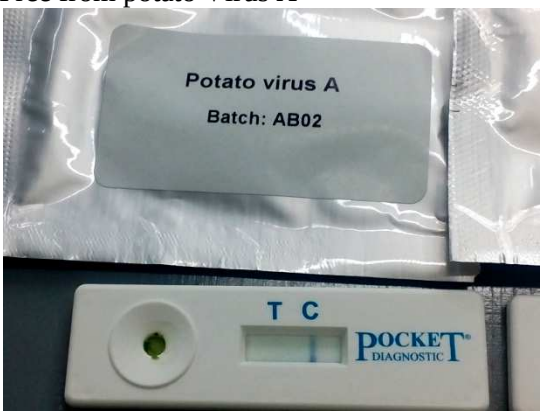
Free from potato Virus S

Free from potato virus V



Free from potato Virus X

Free from potato Virus Y



Free from potato Virus A