STUDY ON THE PRODUCTION AND DORMANCY BREAKING OF POTATO MICROTUBER

By

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Registration no. 03-01125

A Thesis

Submitted to the
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CERTIFICATE

This is to certify that the thesis entitled, "STUDY ON THE PRODUCTION AND DORMANCY BREAKING OF POTATO MICROTUBER" submitted to the Department of Horticulture and Postharvest Technology , Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of ***Master of Science in Horticulture*** *embodies the result of a piece of bona-fide research work carried out by* ***Freesia Farooque, Registration No. 03-01125*** *under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

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

Dated : 30.06.08

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June, 2008 ***The Author***

STUDY ON THE PRODUCTION AND DORMANCY BREAKING OF POTATO MICROTUBER

ABSTRACT

Experiments for the production and breaking of dormancy of microtubers of a potato cultivar "Diamant" have been conducted at the tissue culture laboratory of HRC, BARI, during January to June semester, 2008. Five levels of CCC (0,100, 300, 500 and 700 mg/l) and six levels of sucrose (0, 3, 6, 9, 12 and 15%) were used in two separate experiments for the production of microtuber. Microtubers from both the experiments were harvested at three different dates (75, 90 and 105 days after addition of tuberisation media). For breaking of dormancy, GA_3 solution (0, 0.1, 0.5 and 1.0 mg/l) was used to soak peeled microtuber for 1 and 5 minutes followed by suberisation at 85% RH and 15-18^{0}C for 48 hrs. CCC @ 300 mg/l produced microtuber earliest by 7.11 days in order to produced the maximum number (6.56) and weight of microtubers per flask (870.85 mg), and mean tuber weight by 500 mg/l CCC (137.24 mg). By the application of CCC the percentages of tuber of <100 mg, 100-200 mg and >200 mg sizes were in the ranges of 13.29-48.70%, 37.40-50.07% and 10.18-37.76%, respectively. A culture period of 105 days produced the maximum weight/flask (808.67 mg) and mean weight of microtuber (121.47 mg) compared to 75 days (556.81 mg and 105.13 mg respectively). Sucrose @ 9% and 12% gave the highest tuber weight/flask (782.12 mg and 683.34 mg) and mean weight (137.94 mg and 133.73 mg). No tuber was formed at 0% sucrose level. Sucrose levels of 9-15% produced the minimum and the maximum percentages of <100 mg (16.67-21.48) and >200 mg (32.22-34.81) size microtuber in number. GA_3 @ 0.1 mg/l, 5 min soaking time and suberised condition of tuber had the maximum 97.50% sprouted tuber within 7 days. CCC @ 300mg/l and 500 mg/l and sucrose level 9-15% for production and GA_3 0.1 mg/l for breaking dormancy showed the best result.

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ABBREVIATIONS

%	Percentage
°C	Degree Celsius
μM	Micromole
2, 4-D	2, 4-dichlorophenoxy acetic acid
ANOVA	Analysis of Variance
BA	Benzyl Adenine
BAP	6- benzyl amino purine
BARI	Bangladesh Agricultural Research Institute
BAU	Bangladesh Agricultural University
BBS	Bangladesh Bureau of Statistics
BINA	Bangladesh Institute of Nuclear Agriculture
BJRI	Bangladesh Jute Research Institute
BSMRAU	Bangladesh Sheikh Mujibur Rahman Agricultural University
BSRI	Bangladesh Sugarcane Research Institute
CCC	Chloro Choline Chloride
cm	Centimeter
CRD	Completely Randomized Design
df	Degrees of freedom
DU	Dhaka University
et al.	et alü = Associates
FAO	Food and Agriculture Organization
Fig	Figure
gm	Gram
hrs	Hours
IAA	Indole- 3 acetic acid
IBA	Indole- 3 butyric acid
ICRISAT	International Crop Research Institute for the Semi-Arid Tropics
J.	Journal
Kn	Kinetin
L	Linnaeus
mμ	Millimicron

mg/L	Milligram per litre
ml	Millilitre
MS	Murashige and Skoog
NAA	α- naphthalene acetic acid
No	Number
pH	Negative logarithm of hydrogen ion(H^+)
sp	Species
Var	Variety
Viz	Namely
Wt	Weight

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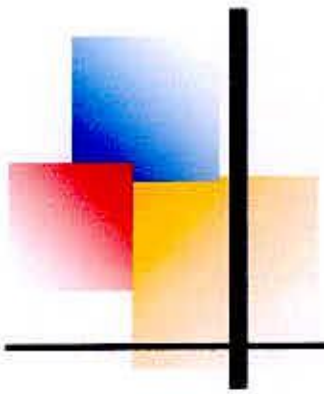


Chapter 1
Introduction

CHAPTER I

INTRODUCTION

Potato (*Solanum tuberosum* L) is an annual herbaceous dicotyledonous plant belonging to the family Solanaceae, section Tuberosum, which contains approximately 160 tuber bearing species. The potato has been originated from the Andes Mountains of South America, where it has served as a main item in the diet of native people for millennia.

Potato is one of the important vegetables worldwide. This crop ranks fourth amongst all global food crops (Horton, 1987) while ranking first both in area and production among the vegetable crops grown in Bangladesh (BBS, 2005). In Bangladesh, it is cultivated in 0.48 million hectares of land, and its production is 7.80 million tons with an average yield of 16.25 tons/ha (Hussain, 2008). Potato is an excellent crop in terms of dry matter production (2.2 t/ha), energy (216 MJ/ha/day) and nutrition (Beukema and Van der Zaag, 1990).

The yield of potato in Bangladesh is low compared to many other countries, like, the Netherlands (45 t/ha), Germany (46 t/ha), Scandinavian countries (48-52 t/ha) (FAO, 1999). (Lack of quality seed potatoes is one of the most important factors behind low yield of potato in Bangladesh. Bangladesh Agricultural Research Institute (BARI) produces pre-foundation seed potatoes via tissue culture techniques using microplants and microtubers. Bangladesh Agricultural Development Corporation (BADC) produced only about 4.5% and the private sector contributed about 7% of the total requirement of seed potatoes in this country (Dey, 2001).

The crop is usually propagated asexually by means of tubers, which are often prone to many pathogens, thereby resulting in poor quality and yields. Consequently much attention has been focused on the *in vitro* production of virus free potatoes (Djurdjina *et al.*, 1997).

The use of microtuber in seed potato production programme is becoming popular in many countries of the world, like South Korea (Hyouk *et al.* 1994), China (Jun *et al.*, 1994) and the Philippines (Paet and Zamora, 1994). Its production is negligible in Bangladesh due to a number of reasons, such as, lack of standard production technology and size. The microtubers produced in Bangladesh are often of small size, and small size microtubers are more vulnerable to storage damage (Naik *et al.*, 1998) and difficult for direct field planting (Jones, 1988). Several authors reported the production of microtubers in relation to different factors, like CCC (Al-Abdallat and Suwwan, 2002), sucrose (Naik and Sarker, 1997) and benzylaminopurine (Leclerc *et al.*, 1994).

At present, at least 20 commercial tissue culture laboratories are in operation in Bangladesh producing mostly microplants and microtubers in a limited scale as supplementary propagules. Since handling and establishment of microplants are cumbersome, most of the commercial laboratories are now interested to produce microtubers. The greatest problems are the production technology of microtubers and breaking of their dormancy.

Application of Cycocel at 500-1000 mg/l induce tuberisation. When both Cycocel and BAP are added to the culture media, the promotive effect of BAP is reinforced, tuber being formed even under less growth of stolons. In case of sucrose and BAP in culture media, both act independently, while plant growth regulators interact each other (Estrada *et al.*, 1986; Lillo, 1989).

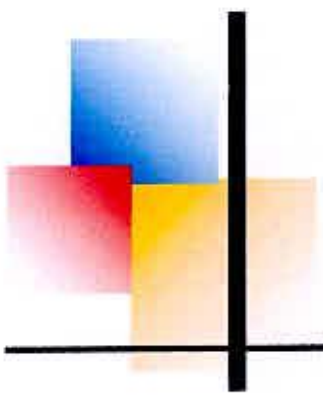
Microtubers could be produced throughout the year but the biggest limiting factor for their use is the dormancy period, which varies widely from 2-7 months (Tovar *et. al.*, 1985). Hossain and Sultana (1996) recorded a dormancy period of 45-85 days for 17 potato cultivars. In spite of many advantages, a strong microtuber production programme has not yet been started in the country due to low yield, small size and finally dormancy period of 60-70 days. All these aspects need to be standardised under Bangladesh context.

Considering above facts, a study for development of the technology of the production of potato microtubers in *vitro* and breaking of dormancy of the produced microtubers, was undertaken with the following objectives:

i) to increase the yield of potato microtubers in respect of number and weight;

ii) to optimize the size of microtubers; and

iii) to standardise a method of breaking the dormancy of microtubers for commercial production of minitubers (from microtubers) round the year under controlled condition.



Chapter 2
Review of Literature

CHAPTER 2

REVIEW OF LITERATURE

The potato (*Solanum tuberosum* L.) as a vegetatively propagated crop is prone to many infectious diseases like bacterial, fungal, viral and viroids, which cause degeneration of the crop over years. Virus diseases are the main concern to potato production worldwide. The successful production of potatoes particularly for seed purpose demand the control of virus diseases which can not be done by any physical or chemical agent. Tissue culture is the only method which is used for the production of virus free potato plant and has a potential to improve the quality and quantity of vegetatively propagated potato plants. Developing countries of the world now applying biotechnological approach in this field are not so fast. Therefore, literatures, which are most relevant and available to the present study, have been reviewed here under the following heads.

2.1 Concept of tissue culture

Reddy and Kishore, 1982 reported that, conventional techniques take more time for crop improvement, now-a- days, plant tissue culture techniques have been developed as a modern and worldwide accepted concept. It has been developed as a new and powerful tool for the improvement of different crops (Carlson, 1975) and received wide attention of modern scientists. Plant tissue culture can play an important role besides conventional method of plant improvement especially in the genetic manipulation of crop plant species.

Tissue culture technique is now used extensively in many National and International Organizations such as BARI, BSRI, BJRI, BAU, DU, BINA, CIP, IRRI, ICRISAT, where programs of crop improvement are in progress for the development of different crop.

2.2 Tissue culture of *Solanum tuberosum* L.

The *in vitro* culture of *Solanum tuberosum* L. was initiated by Chapman (1958) and since then gradually a large number of works have been done by many workers around the world on different aspects of tissue culture in potato like media, explants, growth regulators and others (Ahn *et al.*, 2001; Roy, 2004).

2.3 Production of microtuber

2.3.1 Scope and utilisation

Goodwin *et al.*, 1980; Struik and Wiersema, 1999 found that- *In vitro* propagation of potato by culture of single-node cutting has been used for more than two decades in the rapid multiplication of disease-free material in elite seed potato programmes . A plantlet is a propagule of choice, widely used by the industry for the production of minitubers, almost exclusively in greenhouses. Microtubers have a potential to be integrated into seed potato programme (Nasiruddin and Blake, 1994; Khuri and Moorby, 1995).Again Estrada *et al.*, 1986 repoted that, microtubers are convenient for handling, storage, and transport of germplasm and Coleman *et al.*, 2001 found that, unlike *in vitro* plantlets, do not need a hardening period in the greenhouse or in the field.

Pruski *et al.* (2003) suggested that microtubers could successfully be used in a commercial production of minitubers in the greenhouse. Several authors have reported that pre-conditioning of explants with jasmonic acid improved quality of microtubers and their field performance (Pruski *et al.*, 1993; Biondi *et al.*, 2000).

Many countries lacking isolated and vector-free growing areas that permit the production of quality potato seed tubers, can consider microtuber technology as a vital component of seed potato production system. These countries include Taiwan (Wang and Hu, 1982), South Korea (Joung *et al.*, 1994), Italy (Ranalli and Casarini, 1994), the Philippines (Rasco *et al.*, 1995), South Africa (Venter and Steyn, 1997), Bangladesh (Hossain and Zakaria, 2005) and many others. Microtubers may also provide a solution in countries where the availability of high quality seed tubers form a constraint due to explosive increase in new potato growing areas, such as China, India (Singh *et al.*, 1994) and other parts of Asia (Maldonado *et al.*, 1998).

Microtubers have been described as merely an alternative propagule to plantlets for which production technologies do not contribute, or contribute very little, to further multiplication (Struik and Wiersema, 1999). With increasing understanding of factors affecting tuberisation, both induction and subsequent weight accumulation, the overall productivity of both microtuber and minituber production systems is likely to increase.

2.3.2 Microtuberisation

Tovar *et al.*, 1985 suggested that- potato microtubers obtained through *in vitro* culture from single node cuttings are convenient for handling, storage and exchange of healthy germplasm. Benzylaminopurine proved to be a determing factor in increasing the rate of *in vitro* microtuberisation: the concentration of 2 mg/L appears to be the optimum. The addition of silver thiosulphate to the medium improved the explant growth but not the tuberisation (Nasiruddin and Blake, 1994). Genotypes are important sources

of variation. The day length plays a positive effect on the number and size of the micro-tubers produced.

2.3.3 Micropropagation of potato

Micropropagation is used mainly for seed potato production and for collection and distribution of germplasm throughout the world. For this purpose, *in vitro* virus-free plantlets are used as starting material.

2.3.4 Sucrose as a source of carbohydrate

Alix-Mj *et al.* (2001) found that microtuber initiation and growth in unventilated cultures was 100% at 8% sucrose falling to 40-50% at 4% sucrose and was absent at 1 or 2% with ventilation when rapid tuberisation (90-100%) occurred at initial sucrose concentrations of 2-8%.

Maretzki *et al.*, 1974 reported that- plants meet their energy requirements autotrophically by photosynthesis. However, in tissue culture, the explant lacks this autotrophic ability since the normal functions of the chloroplasts are either absent or blocked . Therefore, it is imperative to supply external carbon sources to produce enough carbohydrate in order to promote cell growth and subsequent regeneration (Butenko, 1968).

The best source of carbohydrate for plant tissue culture is sucrose (Barker, 1953) at a concentration of 2-5% (Butenko, 1968). Moreover, on autoclaving sucrose is partly hydrolysed to glucose and fructose (Ball, 1953).

2.3.5 Overview of tuberisation process

Microtubers are produced *in vitro* on complete plantlets or on plant organs by changing the nutrient medium and/or the external conditions. *In vitro* produced tubers generally weigh 0.2 g per tuber or less (Hussey and Stacey, 1984; Garner and Blake, 1989), though average weights of 0.4 g are reported when produced on liquid media containing growth regulators (Lillo, 1989).

The hormonal control of potato microtuberisation is a highly complex developmental process which is regulated by a number of plant growth regulators and inhibitors like benzylaminopurine (Hussey and Stacey, 1984; Leclerc *et al.*, 1994), 2-choroethyltrimethylammonium chloride (Tovar *et al.*, 1985; Al-Abdallat and Suwwan, 2002), coumarin (Dodds *et al.*, 1988) and jasmonic acid (Pelacho *et al.*, 1991).

Nitrogen is a regulatory factor for tuberisation in potato. Its optimisation is very important because a higher level is inhibitory to *in vitro* tuberisation. GA_3 acts as an inhibitor of tuberisation and tuber growth. Negative effect of GA_3 towards tuberisation in potato can be nullified partially with CCC (Rosell *et al.*, 1987). According to Okazawa and Chapman (1962) tuberisation is controlled by a balance between inhibitor (GA_3) and promoting substance (s), which is indicative to antigibberellins, like coumarin, abscisic acid, jasmonic acid, cytokinin, cycocel, triiodobenzoic acid and maleic hydrazide.

2.3.6 Factors affecting the tuberisation process

Tuberisation is a complex physiological process regulated by many factors. These include environmental factors, hormonal factors, nitrogen supply,

inoculation density, source of explant, potato cultivar and sucrose concentration.

2.3.6.1 Photoperiod

An experiment was conducted by Apichai *et al.*(1988) where lateral buds of explants were cultured in half-strength MS medium with 3% sucrose at 25°C and a 16 h photoperiod. In some cases healthy plantlets were developed with a multiplication rate of 10 times within 30 days. Microtubers were produced by cultivars Spunta, P3, DT02, (100% of cases), LT2 (85.7%) and DT03 (42.9%). Normal microtubers could be harvested at 90 days after culturing at 21°C in the dark.

Slimmon *et al.* (1989) conducted an experiment with single node leaf cuttings of potato cv. Red Pontiac, Shepody, Kennebec and Yukon Gold which were grown on Murashige and Skoog medium at 16°C in total darkness or under 8-h light/day photoperiod for 12 weeks. Percentage of tuberisation averaged over all 4 cultivars under the 8-h photoperiod was lower than under total darkness after 4 weeks but was similar at 8 and 12 weeks. Microtubers from all cultivars had a higher mean fresh weight (FW) when treated with an 8-h photoperiod compared with total darkness.

Lentini *et al.*1990 found that-*In vitro* tuberisation on shoot culture of early, mid season, late and very late potatoes was compared. Shoots were grown at 12, 16 or 20 h photoperiods; tuberisation was then induced at 0, 8 or 16 hr light. In the dark, shoots from early plants initially grown at 16 h consistently set tubers earlier than the other types, whereas the very late line tuberised later and produced significantly fewer tubers. Tuber setting of mid season plants could not be distinguished from the late type.

Yiem *et al.* (1990) studied the effects of culture medium temperature, frequency of subculturing, source of node used, light pretreatment, culture vessel and culture period on *in vitro* tuberisation of shoot nodes in potatoes cv. Dejima. More microtubers were produced from nodes at 20°C than at 15°C, culture media had no effect on the number of microtubers. Light pretreatment for 20 days gave more microtubers than for 14 days.

An experiment was conducted by Seabrook *et al.*(1993) where single node cuttings of potato cultivars Jemeg, Katahdin, Russet Burbank and Superior were cultured on a multiplication medium containing MS salts and no growth regulators. Cultures were exposed to 8-h (SD) and 16-h (LD) photopeirodic regimes. The subsequent plantlets were excised and single node cuttings from each photoperiodic regime were placed under SD or LD on a second medium containing growth regulators, which promoted tuberisation. Production of microtubers was strongly influenced by genotype and photoperiodic treatment. Compared with the other regimes, LD-SD photoperiod generally promoted formation of microtubers with larger diameters and significantly greater fresh weight.

An experiment was conducted by Gopal *et al.* (1998) where twenty two genotypes of potato (*Solanum tuberosum*) were induced to form microtubers under six *in vitro* culture conditions. Cultures maintained under a short photoperod (10 h of 6-12 μ E $m^{-2}S^{-1}$) and low temperature (Day 20°±2°C and night 18°±2°C) had both a higher yield (255 mg/plantlet) and a greater number (2/plantlet) of microtubers than those maintained under long days (16 h of 38-50 μE $m^{-2}S^{-1}$) combined with high temperatures (day 28°±2°C and night 25°±2°C) (yield 207 mg/plantlet; microtuber number, 0.9/plantlet), over a wide range of genotypes.

Early research works on tuberisation demonstrated that tuber formation is induced under short days (Chapman, 1958). Gregory (1954) reported that under short days, a tuber-inducing stimulus is produced in the leaves and translocated at the stolon tips. Similarly, in a day-length assay, Chapman (1958) further inferred that the stimulus is found throughout the potato plant and moves basipetally. Microtubers produced under an 8 hour photoperiod regime are larger with thicker epidermis and resistant to dehydration than those produced under total darkness (Forti *et al.*, 1991). Tuberisation is also favoured by high levels of irradiation (Menzel, 1985). It seems that high radiation increases the concentration of carbohydrate at the stolon tips (Madec, 1963).

2.3.7 Hormonal factors

The hormones are said to be produced under specific tuber inducing environmental conditions by the leaves and transported to the stolon tip (Gregory, 1956). The transmission of the tuberisation stimulus through grafts is a proof to this assumption (Chapman, 1958). The investigations carried out on the hormones identified in tuberisation are described below:

Gibberellins (Smith and Rappaport 1969), cytokinin (Vreugduenhil and Struik, 1989), Ethylene (Mingo-Castel *et al.*,1976), Abscisic acid (Koda and Okazawa, 1983) and Auxins (Harmey *et al.*, 1966) are reported to play important role in tuberisation

2.3.7.1 BAP/ Chlorocholine chloride/Coumarin on tuberisation

Chlorocholine chloride (CCC) has a positive effect on tuberisation of potato sprouts cultured on mineral medium without sucrose (De Stecco and Tizio, 1982). CCC induced tubers in a wide range of genotypes in 4 weeks (Estrada *et al.*, 1986). White-Nitsch-Morel Liquid medium supplemented with BAP, NAA and/or CCC was found better than MS liquid medium for rapid mass tuberisation (Rosell *et al.*, 1987). According to Rosell *et al.* (1987) CCC reduced internode length and turned the leaves to a darker green. Tuberisation was enhanced by CCC, more so in short than in long days.

CCC (Chlorocholine chloride), commonly known as Cycocel, is a commercial growth retardent used in potato to induce tuberisation under *in vivo* as well as *in vitro* conditions. The GA_3 induced delay in tuberisation can be overcome by the addition of Cycocel to the medium by inhibiting the synthesis and/or release of GA_3 like substances from potato sprout sections cultured. Cycocel probably stimulates tuberisation by reducing endogenous gibberellins in whole plants (Menzel, 1980).

The semi-early potato genotypes LH-85 was cultured by Simko (1991) on Murashige and Skoog medium and 25-day-old plants were given an 8% sucrose solution + 0.5 mg ABA, 100 mg Cycocel, 100 mg sodium 2,3-dichloroisobutyrate, 25 mg coumarin or 0.5 mg paclobutrzol/L. After 26-day of cultivation in the dark at 21°C, coumarin and paclobutrazol increased the percentage of tuberising plants, number of microtubers, average microtuber weight and total microtuber weight.

Wattimena *et al.* (1991) analysed the effects of different concentrations of 5 growth retardants on 5 characters of *in vitro* derived microtubers. They showed significant effects on earliness of the tuberisation, number and size of

microtubers and length of dormant periods. No retardation effects were observed for fresh and dry weights of microtuber.

An experiment was conducted by Choi *et al.* (1993) on enhancement of *in vitro* microtuberisation in potato. Microtuberisation was obtained within 30 days on a medium containing MS salts, 5 mg/L benzyladenine, 500 mg/L Cycocel ,6% sucrose and 0.2 gelrite.

An experiment was conducted by Vecchio *et al.*(1994) with nodal explants of the varieties Desiree, Monalisa and Spunta, cultured on MS basal medium with different concentrations of sucrose and Cycocel. Desiree showed better tuberisation than the other varieties under conditions of low Cycocel concentration or absence of Cycocel.

A study was conducted by Alsadon *et al.*(1998) to explore the possibility of estimating yielding ability by measuring *in vitro* microtuber production. Microtubers were induced on MS medium by adding 8% sucrose, 500 µl/L Cycocel and 5 µl BA (Benzyladenine)/L.

Effects of external BAP and Cycocel on the endogenous plant growth regulators GA_3, IAA, Kn, BAP and ABA during the *in vitro* formation of microtubers of potato cv. Favorita were investigated by Lian *et al.* (1998). Exogenous BAP promoted initiation and growth of microtubers, while Cycocel increased tuberisation but then retarded the development of microtubers.

An experiment was conducted by Al-Abdallat and Suwwan (2002) with 5 levels of sucrose (2.0, 4.0, 6.0, 8.0 and 10.0%), 4 levels of CCC (0, 250, 500

and 1000 mg/L) and 8 nodal segments (terminal, subteriminal, basal with no roots, above the basal, 2-most -terminal, 3-most -basal, 3-most -terminal and 3-most -basal) for microtuber production of potato cv. Spunta. The absence of Cycocel at the lowest sucrose level (2%) produced very long, thin and succulent stolons. Increasing the sucrose level in the absence of Cycocel tended to produce thicker and shorter stolons with more abundant aerial roots. The presence of Cycocel at all sucrose levels inhibited stolon formation and sessile tubers were formed. The 2 most basal node gave the highest number of microtuber at 8% sucrose. The 3-most-basal node gave the highest number of microtuber at 1000 mg CCC/L and 6% sucrose.

2.3.8 Nitrogen supply

Low supply of nitrogen induces tuber formation by increasing the carbohydrate/nitrogen ratio (Sattelmacher and Marschner, 1978). As far as *in vitro* studies are concerned, good tuberisation is enhanced by ammonium nitrate (Ewing, 1985).

2.3.9 Inoculation density

At low culture densities, the microtuber weight formed *in vitro* increases (Forti *et al.*, 1991). It must be pointed out that larger microtubers can be stored longer and are thus more cost effective.

2.3.10 Potato cultivar

The discrepancy of tuberisation among potato cultivars can be assigned to the difference in the critical photoperiod. Avetisov *et al.* (1989) investigated callus tissue of the varieties Domodedovskii and Yantarnyi subcultured on

MS medium with 2-7 mg/L 2, 4-D and obtained the best microtuberisation. They also observed that 10% sucrose gave best results.

Simko (1993) conducted an experiment with single nodal cuttings of 4 potato cultivars and *Solanum chacoense* were induced to produce *in vitro* microtubers on Murashige and Skoog (1962) medium supplemented with 80g/L sucrose and various concentrations of kinetin and paclobutrazol. The cultures were kept in darkness for 10 days and then transferred to a 14-h day length with 100 $\mu E\ m^{-2}\ S^{-1}$ light intensity at 21°C. Paclobutrazol alone stimulated early tuber initiation and inhibited stem growth.

Yildrim and Tugay (2002) conducted an experiment with the plantlets of 5 potato genotypes (cultivars: Agria, Resy and Sultan, and Clones 122 and 106) to study their microtuber production capacities. Agria and Resy produced higher percentages of microtuber (68 and 71% respectively).

Yoon *et al.*(2004) conducted a 2-stage *in vitro* tuberisation process comprising micropropagation of nodal explants followed by tuber induction of the potato cvs. Iwa and Daeji. The effects of MS medium supplemented with 3% each of sucrose or maltose on the growth of plantlets and subsequent *in vitro* tuberisation were investigated. Sucrose and maltose were equally effective in supporting the development of vigorous plantlets from the nodal explants of both potato cultivars. Upon transfer to a medium with an optimized level of sucrose (i.e. 8% W/V) for *in vitro* tuberisation, only the plantlets previously grown in the sucrose containing medium were capable of forming more microtubers of larger size (greater than 0.5g).

2.3.11 Different microtuberisation protocols

There are several published protocols for *in vitro* tuberisation in white potato (Kim, 1982; Tover *et al.*, 1985). In general, there are two basic stages in all protocols, the first stage aims to produce vegetative growth and the second stage to induce tuberisation and allow enlargement of the tuberlets.

Different authors recommended different media for vegetative multiplication and tuberisation. Tovar *et al.* (1985) recommended MS+0.5 mg/l BAP+0.4 mg/l GA_3 + 0.01 mg/l NAA for the propagation phase and MS +5 mg/l BAP+500 mg/l CCC + 8% sucrose for the tuberisation phase. On the average, about 10 tubers per flask were produced in 4 weeks dark condition on the tuberisation medium. The mean tuber weight was 49.8 to 143.5 mg.

Wang and Hu (1985) used stem of *in vitro* plantlets for an initial propagation on MS +0.005 mg/l NAA and tuberisation in MS + 10.0 mg/l BAP+8% sucrose. Tuberlets were formed in 16 weeks with an average weight of 250 mg per microtuber. Kim (1982) propagated the single nodes from *in vitro* plantlets on MS +0.1 mg/l GA_3 + 0.5 mg/l kin and induced tuberisation with MS + 5.0 mg/l BAP +6% sucrose. One node formed one tuberlet in 6-8 weeks under 8 hr light condition. The weight of microtuber was between 60 to 120 mg.

Paet and Zamora (1991) reported that *in vitro* tuberisation was improved with three noded stem cuttings by propagation on the MS nutrient formulation with the macronutrient component at twice the published concentration and further supplemented with 0.05 mg/l thiamine HCl, 0.05 mg/l nicotinic acid,. 0.10 mg/l pyridoxine HCl, 2.0 mg/l glycine, 10% coconut water and 4% sucrose (MP medium). Subsequent induction of tuberisation and enlargement of microtubers was done on MS with 1 mg/l

BAP, 50 mg/l coumarin, 10% coconut water and 8% sucrose (ZP medium) and induction at 18-22° C in continuous darkness.

2.4 Dormancy breaking of microtubers

2.4.1 Factors responsible for dormancy breaking of microtubers of potato

At harvest and for a finite period thereafter, potato tubers will not sprout and remain in a state of dormancy (Burton, 1989). Because tuber dormancy results from factors arising within the affected organ itself and not from external causes it is more properly classified as dormancy (Lang *et al.*, 1987). The duration of dormancy varies and is dependent on both the cultivars and, to some extent, the environmental conditions during tuber development (Burton, 1989). Within reasonable limits, postharvest environmental conditions have only a minimal effect on breaking dormancy of microtubers.

Information on dormancy breaking and sprouting of microtubers is lacking, although small tubers are known to posses a longer dormant period than larger tubers (Emilsson, 1949; Van Ittersum and Struik, 1992). They sprouted immediately (Ortiz-Montiel and Lozoya-Saldana, 1987) or after a dormant period varying from 60 to 210 days stored at 4°C depending on the conditions (Tovar *et al.*, 1985). Investigations by Ranalli *et al.* (1994a) showed that the length of dormancy of microtuber in inversely correlated with tuber size and that small tubers suffered more from dehydration when stored for a long time, with reduced vigour when planted directly in the field. Lommen (1995) reported that storage losses were higher in cv. Liseta, in minitubers with lower fresh weight. The dormant period was longer in minitubers with lower fresh weight than with higher weight.

The relationship between tuber inducing media and dormancy period of microtubers formed *in vitro* was studied by Young Whan *et al.* (1994). Plantlet culture conditions and sucrose concentrations in tuber-inducing medium did not affect the dormant period of microtubers. The length of dormant period was determined by the size of microtubers, larger tubers sprouted earlier. The percentage of sprouting was over 80 when microtubers were larger than 0.5 cm in diameter. Fifty percent of microtubers of cv. Dijet sprouted after 30 days of harvest. The dormancy period of microtubers from tuber induction media combined with 100 times strength of vitamins of MS basic medium and 9% sucrose (T1-5) was shorter than those from tuber induction medium combined with 100 times strength of vitamins of MS basic medium, 6% sucrose, 500 mg/l Cycocel and 5 mg/l BAP (T1-3). Dormancy period of microtubers was altered by tuber inducing media. Early sprouting and the highest sprouting rate of microtubers were observed with the microtubers of cv. Dejima from T1-5 medium. In cv. Irish Cobbler early sprouting was obtained with microtubers from T1-3 medium but the kinds of media did not affect the final percent of sprouting.

As with many aspects of plant development, plant hormones have been assigned a primary role in the regulation of tuber dormancy (Hemberg, 1958; Rappaport and Wolf, 1969). Four of the five principal classes of plant hormones (ABA, cytokinins, GA_3 and ethylene) have been implicated in dormancy regulation of potato tubers (Hemberg, 1958; Suttle, 1996a; Wiltshire and Cobb, 1996). In this situation, ABA was considered to be the principal dormacny-inducing agent, whereas both cytokinins and GA_3 were thought to regulate the termination of dormancy. However, much of the experimental evidences support this theory, which was based on the effects of exogenous growth regulators and on correlative studies comparing gross

changes in endogenous tuber hormone levels with sprouting behaviour (Suttle, 1996a).

Rosa (1925) was the first to report the effect of ethylene on shortening the dormancy period of tubers. Subsequent studies by Denny (1926a,b) failed to correlate these findings. Depending on the concentration and duration of exposure, exogenous ethylene can either hasten or delay tuber sprouting. Relatively short-term (less than 3 days) exposure to ethylene results in the premature termination of tuber dormancy, whereas long-term or continuous exposure to similar concentration inhibits subsequent sprout growth (Rylsky *et al.*, 1974). Temporary treatment with exogenous ethylene has also been reported to stimulate the sprouting of partially dormant tubers (Alam *et al.*, 1994).

Similarly, application of an ethylene-releasing agent, alone or in combination with GA_3, to dormant tubers has been reported to stimulate sprouting (Cvikrova *et al.*, 1994). Conversely, it has been reported that both preharvest and postharvest applications of the ethylene-releasing agent ethephon resulted in significant changes in dormancy (Korableva *et al.* 1998; Cvikrova *et al.*, 1994).

Microtuber dormancy can be regarded as extending from tuber initiation or induction *in vitro* to the resumption of active and continuous bud growth as noted by Burton (1957), and Wiltshire and Cobb (1996) for field grown tubers. However, it is unknown whether the mechanism of dormancy in microtubers is similar to field-grown tubers or not. Although microtuber dormancy duration has been positively and significantly correlated with dormancy duration in field grown tubers for a limited number of cultivars (Leclerc *et al.*, 1995), microtubers may exhibit a wide range of dormancy

responses. Microtubers may be non-dormant, or exhibited dormancy durations of up to 7 months depending on cultivars, and *in vitro* induction and growth environment (Rosell *et al.,* 1987; Harvey *et al.,* 1991; Vecchio *et al.,* 1994; Hoque *et al.,* 1996). Microtubers when were induced in compelete darkness and stored at 4°C, the average dormancy duration was 210 days. However, when induced under 8h-photoperiod, the average duration of dormancy was 60 days for the same cultivars (Estrada *et al.,* 1986). On the other hand, the date of top pulling may be very important for breaking of dormancy of tubers earlier (Coleman and King, 1984).

Park *et al.,* 1996 reported that- when stored at 6°C for six months dormancy of potato microtubers of variety "Superior" was broken and sucrose content decreased throughout the whole period of low temperature storage. On the otherhand, the contents of glucose and fructose increased at early stage of low temperature storage, and then became constant. Overall sugar contents were much in the sprout part than in the tuber part and especially the sucrose content of sprout part was very high compared with that of tuber part. These results appeared to be similar to the pattern of storage and sprouting behaviour of natural seed potatoes during low temperature storage.

Park *et al.* (1996) also investigated the storability and sprouting vigour in potato microtubers of six cultivars (Superior, Irish Cobbler, Dejima, Bintje, Clauster and Kennebec). The microtubers of potato cv. Kennebec showed only about 10% decrease in fresh weight even after 4-months storage at room temperature indicating that Kennebec microtubers had better storability. The potato microtubers of Dejima exhibited the earliest sprouting behaviour during the storage. Superior and Kennebec maintained a good vigour even after the longest dormant state.

Microtuber loss due to shrinkage and drying were observed during storage (Anon. 1988). Excessive biomass results in the determination of seed quality and poor sprout emergence (Singh and Naik, 1993). Tubers stored in diffused light performed better in terms of biomass loss and sprouting behaviour (Potts *et al.*, 1983).

Paet and Zamora (1994) stated desiccation, which was partially influenced by microtuber size that led to loss of microtubers after 8 months storage. The percentage of desiccated microtubers was the highest (55%) where 57% of the microtubers were <5 mm in diameter. Early sprouting was induced with storage in darkness and delayed by storage under diffused light.

Hossain *et al.* (1998) reported that weight loss of normal potato tubers was inversely proportional to tuber grades. Number and weight of sprouts per tubers were directly proportional to the tuber size. Sprouting was delayed in small size tubers. Hossain *et al.* (2002) tried to release dormancy of microtuber of four potato cultivars (Heera, Dheera, Cardinal and Diamant) using different physical and chemical treatments. Across the treatments, they recorded 30 days for first sprouting in Heera and most delayed by Diamant (57 days).

The dormancy of microtubers was found to be cultivar specific and significant correlation was between *in vitro* and *in vivo* dormant periods. Smaller microtubers (<250 mg) had longer dormant period than >250 mg size microtubers. Dormant periods were unaffected by addition of coumarin or Cycocel and BAP in the culture media or by the incubation period (28 and 56) days). Developmental age had no effect on the ability of individual buds to break dormancy and elongation. A positive correlation was observed

between tissue levels of abscisic acid and microtuber dormant periods (Leclerc *et al.*, 1995).

2.4.2 Effect of abscisic acid

Endogenous abscisic acid is primarily found in periderm tissues of potato tubers (Korableva *et al.*, 1998), considered initially as a seed dormancy inhibitor (Lang *et al.*, 1996). Abscisic acid concentration regulate tuber dormancy significantly in small (<250 mg FW) and large (>250 mg FW) microtubers (Leclerc *et al.*, 1995). Large microtubers sprouted earlier than the small ones under ambient conditions. This difference in dormancy may be due to tuber size or surface to volume ratios (Leclerc *et al.*, 1995). While ABA may play a vital role in dormancy induction and maintenence and there is a direct link between dormancy breaking and endogenous ABA levels of tubers and tuber dormancy release was related to the gradual depletion of ABA (Lang, 1996). In field-grown tubers, however, there was no clear relationship between tuber sprouting and endogenous levels of ABA at a storage temperature of 4°C (Coleman and King, 1984). A reversible decline in ABA content caused by controlled atmospheres composed of altered nitrogen, oxygen and carbon dioxide levels preceded dormancy release in field-grown tuber and greenhouse-produced minitubers (Coleman, 1998).

2.4.3 Effect of cytokinins

Cytokinins can enhance microtuberisation (Wang and Hu, 1985) as well as modify tuber dormancy depending on cultivars (Wattimena, 1983). High levels of N (56 mM) used in culture media enhanced sprouting in five potato cultivars when dormancy was measured from tuber initiation to sprouting (Wattimena, 1983). However, when measured from harvest to sprouting,

dormancy duration was only reduced in one (Russet Burbank) of the five cultivars. In field-grown tubers, exogenous cytokinins can break dormancy (Hemberg, 1970) with greatest efficacy when applied near the end of dormancy period (Turnbull and Hanke, 1985b; Sukhova *et al.*, 1993).

2.4.4 Effect of gibberellins

Numerous studies indicate that exogenous GA_3 enhances stolon formation, but is an effective inhibitor of microtuber induction (Palmer and Smith, 1970; Gracia-Torres and Gomez-Campo, 1973; Stallknecht and Farnsworth, 1982 a,b). This is also true for field grown tubers (Struik and Wiersema, *1999*). The use of ^{14}C lebelled GA_3 in a microtuberisation medium indicated limited degradation of GA_3 after 3 weeks of storage, suggesting that GA_3 was relatively long lived substance in microtubers (Couillerot, 1993).

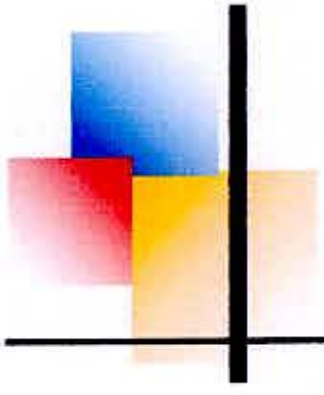
Treatments with promise for breaking dormancy of microtubers have included cutting with or without a subsequent brief dip for 5 min in 5 mg/l GA_3 solution (Ewing *et al.*, 1987). The GA_3 dip was of variable effectiveness, possibly due to lack of effect on dormant tubers (Turnbull and Hanke, 1985a; Wang *et al.*, 2000). or the dosage used. Soaking whole microtubers for 1 hr in 1 mg/l GA_3 was suggested as being effective for dormancy breaking (Choi *et al.*, 1994). In field-grown tubers, endogenous GA_3 increase during sprouting (Smith and Rappaport, 1969). GA_1 may be the active GA form in potatoes although its role in field-grown tubers or microtubers dormancy breaking is unknown (Suttle, 1996a). Exogenous GA_3 can promote tuber sprouting (Van Ittersum, 1992) but usually only after dormancy was completed (Turnbull and Hanke, 1985a; Couillerot, 1993).

4.4.5 Effect of ethylene

Endogenous ethylene is an important component of early dormancy development in microtubers (Suttle, 1998). Whether endogenous ethylene plays a role in dormancy release of microtubers remains to be demonstrated. In field tubers and microtubers, ethylene production increased during dormancy inception and then decreased to low levels until tuber sprouting (Cvikrova *et al.*, 1994; Suttle, 1998). However, exogenous ethylene can reduce dormancy duration (Rosa, 1925; Rylsky *et al.*, 1974; Alam *et al.*, 1994). Treatment of microtubers with ethylene antagonists, silver nitrate or 2,5-norbomadiene showed quick sprouting (Suttle, 1996b).

2.4.6 Conclusion

A number of exogenous chemicals can remove dormancy from field-grown tubers (Coleman, 1987; Wiltshire and Cobb, 1996), but similar evaluations with microtubers have been limited. Rindite (7 parts ethylene chlorohydrin:3 parts dichloroethane:1 part carbon tetrachloride) or carbon disulfide were effective dormancy releasing agent for microtubers (Wattimena, 1983; Kim *et al.*, 1997; Nasiruddin and Blake, 1997). However, their mutagenicity, carcinogenicity, and high toxicity make their commercial use unacceptable. The use of carbon dioxide, oxygen and ethylene were highly effective in dormancy breaking in microtubers, greenhouse- and field-grown tubers (Coleman *et al.*, 1992, Coleman, 1998; Coleman and Coleman, 2000). Moreover, the value of using a microtuber induction system for exploring the role of dormancy breaking agents as well as endogenous and exogenous plant growth regulators has been demonstrated (Suttle and Hulstrand, 1994; Suttle, 1995).



Chapter 3

Materials & Methods

CHAPTER 3

MATERIALS AND METHODS

Two experiments on *in vitro* tuberisation and one on dormancy breaking of microtuber of a standard potato variety "Diamant" were conducted at the tissue culture laboratory of Horticulture Research Centre (HRC), Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur during January to June,2008, in order to fulfill the objectives set in this research programme. The materials and methods of the research programme have been described under different sub-headings.

3.1 General activities

3.1.1 Preparation of MS stock solution

Chemical weighing: A digital chemical weighing balance of Shimadzu-220Y series (0.001 g) was used to weigh all the chemicals used in different experiments. Weighing boat of Sigma brand was used to weigh chemicals. To prepare MS medium, four different stock solutions were prepared (Table 3.1); (i) Major salts (10X concentrated), (ii) Minor salts (100X concentrated), (iii) Iron (100X concentrated) and (iv) Organic nutrients except sucrose (100X concentrated). For the preparation of stock solutions (i-iv) each component was separately dissolved to the last particle and then mixed with the others. Separate stock solutions were prepared for each growth regulators by dissolving it in a very small quantity of the appropriate solvent (if insoluble in water) and making up the final volume with distilled water. All the stock solutions were stored in glass bottles under refrigeration. The iron stock solution was stored in amber-colored bottle.

Table 3.1 Composition for MS (Murashige and Skoog, 1962) medium

Ingredient	Concentration	
	Stock(g/L)	Amount of stock solution used in 1L MS medium
Stock solution I		
NH_4NO_3	16.5	100 ml
KNO3	19.0	
$CaCl_2.2H_2O$	0.44	
$MgSO_4.7H_2O$	3.7	
KH_2PO_4	1.7	
Stock solution II		
KI	0.083	10 ml
H_3BO_3	0.62	
$MnSO_4.4H_2O$	2.23	
$ZnSO_4.7H_2O$	0.86	
$Na_2MoO_4.5H_2O$	0.025	
$CuSO_4.5H_2O$	0.0025	
$CoCl_2.6H_2O$	0.0025	
Stock solution III		
$FeSO_4.7H_2O$	0.278	10 ml
Na_2. EDTA. $2H_2O$	0.373	
Stock solution IV		
Inositol	10.0	10 ml
Nicotinic acid	0.05	
Pyridoxine HCl	0.05	
Thiamine HCl	0.05	
Glycine	0.2	
Sugar		30 g
Agar		6.50 g

Required quantities of $FeSO_4.7H_2O$ and Na_2. EDTA. $2H_2O$ were dissolved separately in 450 ml distilled water by heating and constant stirring. Two solutions were mixed together and the pH was adjusted to 5.8 and more distilled water was added to make up the final volume to 1 L.

3.1.2 Preparation of MS media

For normal micropropagation of the potato cultivar the sequence of steps involved in preparing a medium was as follows:

a. To prepare 1 L of MS medium 100 ml of stock solution I, 10 ml of stock solution II, 10 ml of stock solution III and 10 ml of stock solution IV. were taken in a 1 L beaker with 450 distilled water.
b. Seven gm of agar and 30 gm of sucrose were weighed and dissolved in water, about ¾ the final volume of the medium, by heating them in a water bath.
c. The final volume of the medium (1L) was made up with distilled water.
d. After mixing well, the pH of the medium was adjusted to 5.8 using 0.1N NaOH and 0.1N HCl.
e. The medium was poured into the desired culture flask. About 40 ml of the medium was dispensed in a 250 ml Erlenmeyer flask.
f. The flasks were plugged with autoclavable plastic cork.
g. The flasks containing media were transferred to appropriate baskets and sterilized by autoclaving at 15psi, 121°C for 20 minutes.
h. The media was allowed to cool at room temperature and was stored at 6°C.

3.1.3 Carbon source

The energy source for the growth of *in vitro* microplants and subsequent subculture of microplants was reagent grade sucrose. The rate of sucrose used for plantlet growth was 3%.

3.1.4 Brand of MS salts and growth regulators used

All the chemicals of MS salts including plant growth regulators used were of Sigma brand, USA.

3.1.5 Unit used

In all the experiments, the MS salts having the standard composition in mg/l for easy understanding including chloro choline chloride (CCC) and GA_3. Sucrose and agar were used in percentage.

3.1.6 P^H adjustment

The pH of culture media was adjusted after addition of the components except agar in case of semi-solid culture media but prior to autoclaving. A desk top pH meter of Hanna brand model no. HI 8013 was used. The pH of culture media was adjusted to 5.8 prior to autoclaving with the help of 0.1N KOH or 0.1N HCl.

3.1.7 Gelling agent

For solidification of culture media, agar (Agar fine powder of E. Merck, India) at the rate of 0.7% was used. After addition of agar, the media were dispensed in Erlenmeyer flask and the mouth was closed with heavy duty aluminum foil and then gently heated on magnetic stirrer heating plate until agar dissolved completely and the media looked almost transparent.

3.1.8 Volume of culture media used in culture jar

For normal propagation of microplants, 40 ml of culture media were dispensed in each of 250 ml Erlenmeyer flask. For microtuberisation, 40 ml liquid media were used in each flask

3.1.9 Storage of prepared media

After preparation, the media were autoclaved and then left for a while to reach an ambient temperature and then stored in refrigerator at 6°C.

3.1.10 Sterilisation of glasswares

The glassware (test tube, pipettee, petriplates, beaker, Erlenmeyer flask) used in different operations during the experiments were semi-sterilized in oven at 120°C for 24 hrs. While petriplates with filter paper used for excision of explants were sterilized in autoclave as stated above and then dried in incubator at 80°C for 1 hr before use.

3.1.11 Sterilisation of transfer hood

The transfer hood particularly used for transferring explants into culture jar was semi-sterilised by spraying 70% ethanol prior to starting work. Moreover, the UV light was kept on for 1-2 hrs before starting work as per laboratory's sanitary prescription.

3.1.12 Sterilisation of small tools

Small tools like forceps and scalpels used in different operations under the transfer hood were sterilized by direct heating in flame of spirit lamp.

3.1.13 Sterilization of growth chamber

The growth chamber which was used for incubation of cultures under dark or light conditions was semi-sterilized by spraying 2.5% formaldehyde weekly. Moreover, the floor of the room was wiped with commercial "Chlorox" @ 50ml/ L water at alternate days.

3.1.14 Subculturing

In vitro ready stock pathogen-free microplants of the potato cultivar under study were repeatedly subcultured on semisolid MS culture media for preparing sufficient materials for conducting the experiment on microtuberisation.

3.1.15 Light source for growth of microplants

Florescent light (40 W), Philips, Bangladesh were used to supply white light to the microplants. The microplants were supplied with 3000 lux light intensity for 16 hrs daily.

3.1.16 Temperature for the growth of microplants

A temperature range of 22±2°C was maintained in the growth chamber for 24 hrs.

3.2 Specific steps for microtuber production

The following experiments I and II were conducted for the production of potato microtuber and experiment III for breaking dormancy of the produced microtubers.

3.2.1 List of experiments

3.2.1.1 Expt. I. Effect of Chloro Choline Chloride (CCC) on the production of microtuber of potato cv. Diamant.

3.2.1.2 Expt. II. Effect of sucrose on the production of microtuber of potato cv. Diamant.

3.2.1.3 Expt. III. Effects of GA_3 concentration and soaking time on dormancy breaking of peeled and suberised microtubers under controlled temperature.

3.2.2 Cultivars used: One recommended potato cv. Diamant was used for the production and breaking of dormancy of microtubers.

3.2.3 Inducer used: For experiments conducted for microtuber production, ChloroCholineChloride (Cycocel) (CCC) and Benzylaminopurine (BAP) were used. CCC was used as in solid state and others in liquid form.

3.2.4 Preparation of solution of inducers/stimulators: Cycocel was usedin solid state directly in the culture media. BAP was prepared with the help of 0.1 N NaOH. BAP was first dissolved in separate beaker with few drops of 0.1 N NaOH and then distilled water was added to make the ultimate volume.

3.2.5 Explants used: In both the experiments of production of microtuber, 4-5 stem segments of 20-25 days old ready microplants of 5-6 cm tall were used.

3.2.6 Levels of factors: Five levels of CCC (0, 100, 300, 500 and 700 mg/l) were used in experiment I and six levels of sucrose (0, 3, 6, 9, 12 and 15%) in experiment 2. BAP @ 5 mg/l was used in both the experiments as stimulators.

3.2.7 Sucrose used for tuberisation: The rate of sucrose used for microtuber production was 80 g/l.

3.2.8 Explant and size of explant: For microtuberisation, 4-6 cm tall microplants were developed on liquid shaken culture. At the age of 30 days, the liquid culture media were replaced by 40 ml microtuberisation media under the clean hood as per treatment mentioned above.

3.2.9 Incubation environment: The cultures under experiment for the production of microtuber were incubated under complete dark at a temperature of 20±2°C.

3.2.10 Date of harvest: The developed microtubers were harvested in three different dates (75, 90 and 105 days) after addition of tuberisation media.

3.2.11 Harvesting of microtubers: The developed microtubers were harvested under septic conditions (in the laminar air flow cabinet). The microplants with the developed microtubers were taken out from the flask in a 150 mm autoclaved glass petriplates and all the microtubers were collected with the help of sterile forceps and BP blades no. 10.

3.2.12 Caring of microtubers: The microtubers harvested under aseptic condition were washed with distilled and autoclaved water and dried for 1 hr under natural environment and stored in germ-free glass petriplates under normal condition of refrigerator.

3.2.13 Handling of microtubers: Microtubers collected from different experiments were handled, preserved and observed very carefully.

3.2.14 Weighing of harvested microtubers: Microtubers after harvesting, washing and natural drying for 1 hr were immediately weighed by a digital balance of Shimadzu series 220 (0.000 g).

3.2.15 Grading of tubers: The harvested microtubers while weighed were recorded in three different grade or sizes, namely, <100, 100-200 and >200 mg. Tubers of each grade were packed, labeled properly indicating date of harvest and preserved separately.

3.3 Details of experiments of production of microtuber

3.3.1 Expt. I. Effect of Chloro Choline Chloride (CCC) on the production of microtuber of potato cv. Diamant.: The experiment was conducted with 5 levels of CCC (0,100, 300, 500 and 700 mg/l) and 3 levels of duration of

culture (75, 90 & 105 days) were used. The media were supplemented with 5 mg/l BAP and 8% sucrose. The experiment was replicated four times following CRD.

3.3.2 Expt. II. Effect of sucrose on the production of microtuber of potato cv. Diamant.: This experiment was setup with 6 levels of sucrose (0, 3, 6, 9, 12 and 15%) and 3 levels of duration of culture (75, 90 and 105 days). BAP at 5 mg/l was used. The experiment was replicated four times following CRD.

3.4 Activities on dormancy breaking of microtuber

3.4.1 Storage condition: The treated microtubers on moistened cotton pad containing in petridish were stored under controlled temperature (22±2°C).

3.4.2 Materials: The produced microtubers of the cv. Diamant were used in this experiment.

3.4.3 Selection of Materials: For the experiments, random samples of microtubers were used.

3.4.4 Preparation of materials for setting experiment: For the experiment, peeled (a small portion of the periderm of 2 mm^2 was peeled off) microtubers were used. The peeled microtubers were used under both suberised and nonsuberised conditions.

3.4.5 Chemical used: In order to break dormancy of microtuber, plant growth regulator (PGR), namely, gibberrelic acid (GA_3) at three levels (0.1, 0.5 and 1.0

mg/l) were used as stimulator for soaking the microtubers. The experiment was replicated four times following CRD.

3.4.6 Preparation of PGR solutions: Solutions of GA_3 were prepared with distilled water at higher concentrations and the required concentration was prepared by dilution factor like $\mathbf{V_1S_1 = V_2S_2}$.

Where, V_1= Volume of the initial solution to be taken,

S_1 =concentration of the initial solution,

V_2 =Volume of end solution to be prepared and

S_2=concentration of the end solution to be prepared.

3.4.7 Soaking of microtuber: Immediately after peeling of small portion of the periderm, the microtubers were soaked for different times (1 and 5 min) in solution of stimulator contained in a 250 ml Pyrex beaker.

3.4.8 Preparation of petriplates with water moistened cotton pad: Pyrex glass /Inotech Plastic petriplates of 150 mm diameter with moistened cotton pad were used to setup the experiment. Autoclaved absorbent cotton pads were prepared as per size of the petriplates and moistened with distilled water. Two pads, one at the bottom of petriplates and another to cover the microtuber were used. In case of experiments with aseptically collected microtubers, distilled and autoclaved water as well as petriplates and cotton pads were used.

3.4.9 Placement of treated microtubers on cotton pads in petriplates: The microtubers immediately after soaking for a definite time were taken out of the solution of stimulator with the help of forceps, placed on moistened cotton pad

and covered by another pad. Any excess water was syringed out. All preparations were done under clean hood. Filter sterilised (0.25 μM pore size) stimulator was used.

3.5 Details of experiment on dormancy breaking of microtubers

3.5.1 Expt. III. Effects of GA_3 concentration and soaking time on dormancy breaking of peeled and suberised microtubers under controlled temperature: Microtubers were randomly collected and peeled and soaked in GA_3 solutions (0, 0.1, 0.5 and 1.0 mg/l) for 1 and 5 min. After soaking, 50% tubers were immediately placed on moistened cotton pad (unsuberised tubers) and the rest 50% were suberised at 85 to 90% RH for 48 hrs and then placed on moistened cotton. The petriplates with the microtubers were kept under controlled temperature (22±2°C). The experiment was replicated 4 times following CRD.

3.6 Data collection

3.6.1 Microtuberisation

3.6.1.1 Days to tuber initiation

The cultures were observed at every alternate day starting from 3rd day after addition of CCC. Any swelling of buds or of sessile stolons was treated as microtuber initiation. For these parameters the cultures were observed for 30 days.

3.6.1.2 Number of microtuber per test tube

The number of microtubers per test tube was recorded after harvesting. Each and every microtuber was taken into consideration.

3.6.1.3 Weight of microtuber per test tube (mg)

After harvesting, all the microtubers of a test tube were weighed individually and summed them up to get the total weight.

3.6.1.4 Mean weight of microtuber

The mean weight of each microtuber was calculated using following formula.

$$\text{Mean wt. of each microtuber (mg)} = \frac{\text{Total wt. of microtuber of a particular culture jar(mg)}}{\text{Total number of microtuber of that culture jar}}$$

3.6.1.5 Grading of microtuber

After harvesting, each and every microtubers were weighed in a Mettler P-163 series digital balance and recorded as per size or grade, such as, >200, 150-200, 100-150, 50-100 and <50 mg. The tuber was graded in percentage both of number and weight using the following formula:

$$\textbf{\% microtuber by no. of a particular size} = \frac{\textbf{No. of microtubers of a particular size}}{\textbf{Total number of microtuber}} \times 100$$

3.6.1.6 Lenticels count

The harvested microtuber of <50 to >200 mg sizes were studied for lenticels present under stereobinocular microscope, which is shown Table 3.6.1.6.1.

Table 3.6.1.6.1 The number of lenticels in microtubers in relation to size

Size of microtuber (mg)	Lenticels number (Range)
<50	15-30
50-100	30-55
100-150	55-70
150-200	70-85
>200	85-100

3.7 Dormancy breaking of microtubers

3.7.1 Sprouted microtubers: The microtubers were observed regularly for recording the number of sprouted microtubers and any visible sprout in microtubers was recorded as sprouted microtubers. The percentage of sprouted microtubers was recorded on cumulative basis and calculated using the following formula.

$$\% \text{ Sprouted microtubers} = \frac{\text{No. of sprouted microtubers}}{\text{Total number of microtubers}} \times 100$$

3.7.2 Rot or unsprouted tubers: The tubers were observed daily starting from third day. The rotten tubers were recorded and discarded. Any change from normal was treated as rotten.

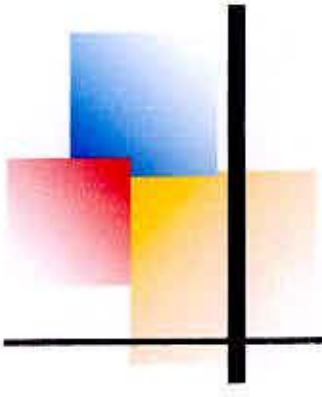
3.8 Experimental design and statistical analysis

All the three experiments regarding production and dormancy breaking of potato microtubers were done under laboratory condition. As such, the

experiments were conducted in the Completely Randomised Design (CRD). The experiment, each treatment was replicated four times. The collected data on different parameters were analysed following a MSTAT-5 package computer programme. Mean separation was also done using LSD values with the help of the same computer package.

3.9 Transformation

The percentage data were subjected to appropriate transformation such as square root wherever applicable according to Gomez and Gomez (1984).



Chapter 4
Results and Discussion

CHAPTER 4

RESULT AND DISCUSSION

Two experiments on *in vitro* production of microtuber and one on dormancy breaking of produced microtuber of a standard potato variety Diamant were conducted at the tissue culture laboratory of Horticulture Research Centre (HRC), Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur during January to June 2008. The results obtained in this research with discussion are presented below.

4.1 Production of microtuber

4.1.1 Effect of ChloroCholine Chloride (CCC) on the production of microtuber of potato cv. Diamant

4.1.1.1 Days to tuber initiation: The number of days required for tuber initiation varied significantly due to addition of Cycocel in the culture media (Appendix I &Table 1). Initiation of tuber was the earliest by 7.11 days under 300 mg/l CCC which was statistically similar to 500 mg/l CCC (7.33 days). The maximum number of days required for tuber initiation was 12.11 days under 700 mg/l CCC compared to 8.78 days under the control. Hossain and Sultana (1998) attempted to induce microtuber in three potato cultivars like, Chamak, Lalshil and Patrones using MS media supplemented with 5 and 10 mg/l BAP and 120, 160 and 200 mg/l CCC. They recorded 13-15 days for tuber initiation under dark condition and 20 days for the control which was rather late as compared to the results of the present study. Zakaria

(2003) used 0, 125, 250 and 500 mg/l CCC for induction of microtuber in potato varieties like Cardinal, Diamant and Heera and recorded 15.9 to 17.8 days for tuberisation, which was also later than that of the present findings.

The number of days required for initiation of tuber in relation to the duration of culture was also significant. Tuberisation occurred most early by 8.33 days for 105 days of duration of culture which was statistically similar to 75 days of duration (9.53 days). Tuber initiation was most delayed for 90 days of duration (9.8 days) (Fig. 2).

Table 1. Main effect of cycocel (CCC) on the production of microtuber of the potato cv. Diamant

CCC level (mg/l)	Days to tuber initiation	Number of tuber per flask
0	8.78	4.22
100	10.78	4.11
300	7.11	6.56
500	7.33	6.44
700	12.11	3.33
LSD 0.05	1.19	0.79
LSD 0.01	1.61	1.07

The combined effect of CCC and duration of culture for the number of days required for tuber initiation was significant. The minimum number of days required for tuber initiation was 5.67 when tubers were produced with 300 mg/l CCC and harvested after 105 days. The combined treatment was statistically similar to a number of treatments. However, the higher doses of CCC (500 and 700 mg/l), irrespective of duration of culture took maximum

days for tuber initiation. These values ranged from 6.33-12.67 days (Table 3).

4.1.1.2 Number of tuber per flask: The number of tubers per flask was highly influenced due to the addition of CCC in the culture media. The maximum number of microtuber per flask obtained was 6.56 when 300 mg/l CCC was added in the culture media, which was statistically similar to 500 mg/l CCC (6.44) (Plate 1). The number of tubers under other treatments was statistically similar, ranging from 3.33 under 700 mg/l CCC to 4.22 under the control (Table 1) (Plate 2). Bakul (2005) used 0, 300, 500 and 700 mg/l CCC for induction of microtuber in three potato cultivars like Cardinal, Diamant and Patrones and recorded the maximum 4.41 and the minimum 1.50 microtuber per tube under 300 and 0 mg/l CCC. Wang and Hu (1982) and Al-Abdallat and Sawwan (2002) used 0-1000 mg/l CCC and obtained no significant variation which was contradictory to the present results. In the present investigation, the control and 700 mg/l CCC gave statistically lower number of microtuber per flask. Whereas, Hossain and Sultana (1998) obtained the maximum 8 microtubers per flask and Zakaria (2003) harvested 9 microtuber from each flask. In the present investigation, maximum 6.56 microtuber were harvested from each flask, which seems to be similar to the results previously reported.

The number of tubers of per flask did not vary significantly in relation to duration of culture. However, the maximum number of tubers per flask was 5.13 under 75 days of culture period compared to minimum 4.80 under 90 days of culture duration (Fig. 2).

Table 2. Main effect of duration of culture on the production of microtuber of the potato cv. Diamant

Duration(Days)	Wt. of tubers per flask (mg)	Mean wt. of each microtuber (mg)
75	556.81	105.13
90	577.83	116.95
105	808.67	121.47
LSD 0.05	71.78	14.83
LSD 0.01	96.66	19.97

The combined effect of CCC and duration of culture for the number of tubers per flask was significant (Table 3). The combination of CCC (500 mg/l) and duration of culture (105 days) produced the maximum of 7.00 microtubers per flask which was statistically similar to 300 mg/l CCC and 75 days of duration of culture (6.67), 300 mg/l CCC and 90 days of duration of culture (6.61), 500mg/l CCC and 75 or 105 days of duration of culture , each having 6.33 microtubers per flask against the minimum of 3.00 under 700 mg/l CCC and 90 days duration of culture. Whereas, the control treatment of CCC, irrespective of duration of culture gave higher number of tuber (Table 3).

4.1.1.3 Weight of microtuber per flask: The weight of microtuber per flask differed significantly due to addition of CCC in the culture media. The highest weight of microtuber per flask was 870.85 mg under 300 mg/l CCC, which was statistically similar to 500 mg/l CCC (865.26 mg). The minimum weight of microtuber per flask was 334.06 under 700 mg/l CCC against 401.44 mg/l under the control, while it was 433.91 mg under 100 mg/l CCC (Fig. 1). Bakul (2005) and Hossain (2006) recorded the maximum weight of

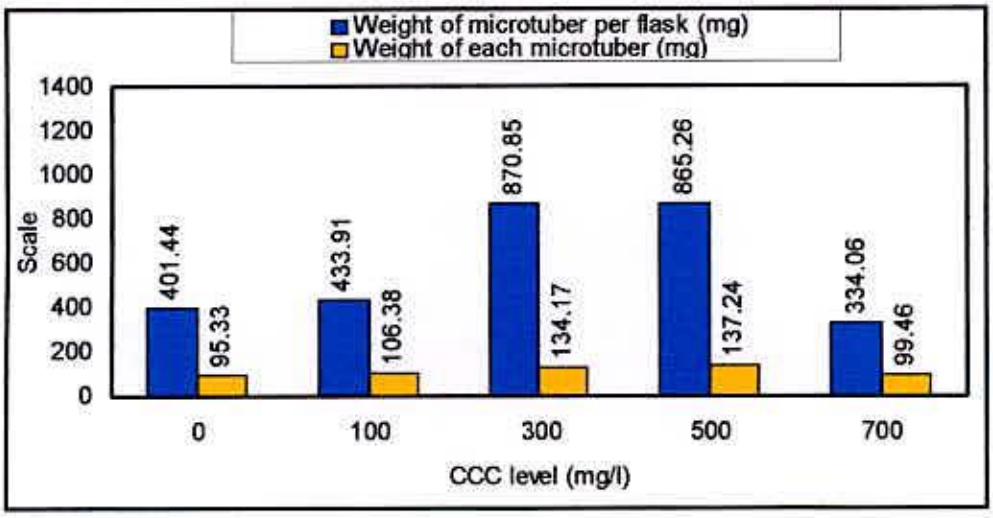


Fig. 1. Weight of microtuber per flask (mg) and mean weight of each microtuber (mg) as influenced by different levels of cycocel (CCC).

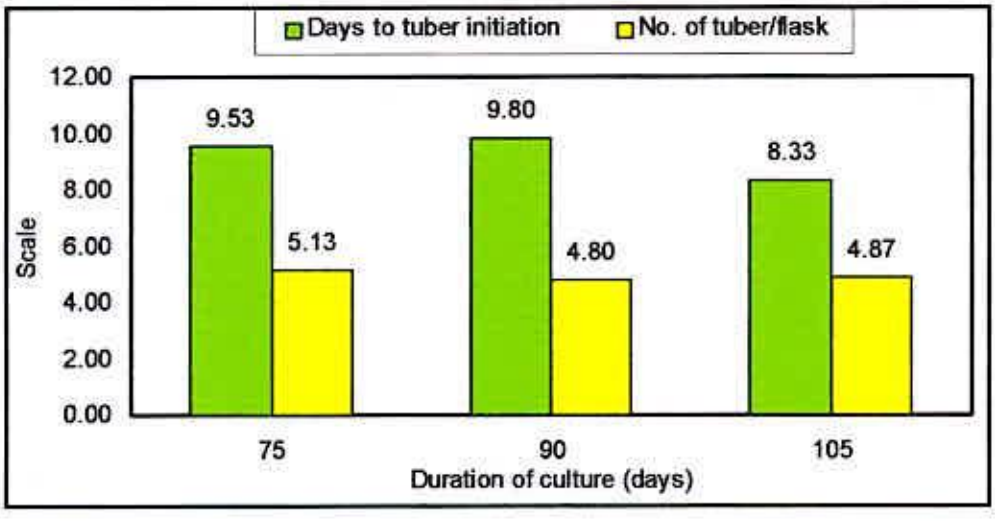


Fig. 2. Effect of duration of culture on days to tuber initiation and number of microtuber per culture jar.

Table 3. Combined effect of cycocel and duration of culture on the production of microtuber of the potato cv. Diamant

Treatment		Days to tuber initiation	No. of tuber per flask	Wt. of microtuber per flask (mg)	Wt. of each microtuber (mg)
Duration (days)	CCC level (mg/l)				
75	0	8.67	4.67	411.29	87.77
	100	11.00	4.33	383.77	90.47
	300	8.00	6.67	826.69	125.01
	500	7.33	6.33	828.15	132.80
	700	12.67	3.67	334.15	89.62
90	0	9.33	4.00	408.96	102.21
	100	11.33	4.33	503.05	117.61
	300	7.67	6.67	864.30	131.23
	500	8.33	6.00	852.67	146.27
	700	12.33	3.00	260.20	86.73
105	0	8.33	4.00	384.07	96.02
	100	10.00	3.67	414.90	111.06
	300	5.67	6.33	921.56	146.95
	500	6.33	7.00	914.90	131.99
	700	11.33	3.33	407.86	122.01
LSD 0.05		2.06	1.38	160.50	33.16
LSD 0.01		2.78	1.86	216.10	44.65

231.33 mg and 1040.00 mg microtuber from each tube and flask, respectively. In the present investigation, it was 870.85 mg per flask, which was quite good compared to the previous results.

The weight of microtuber per flask varied significantly in relation to the duration of culture period. The weight of microtuber per flask was 556.81 mg when cultured for 75 days, which increased gradually with increasing duration of culture period. However, it was 577.84 mg when cultured for 90 days, which was statistically similar to 75 days duration of culture period.

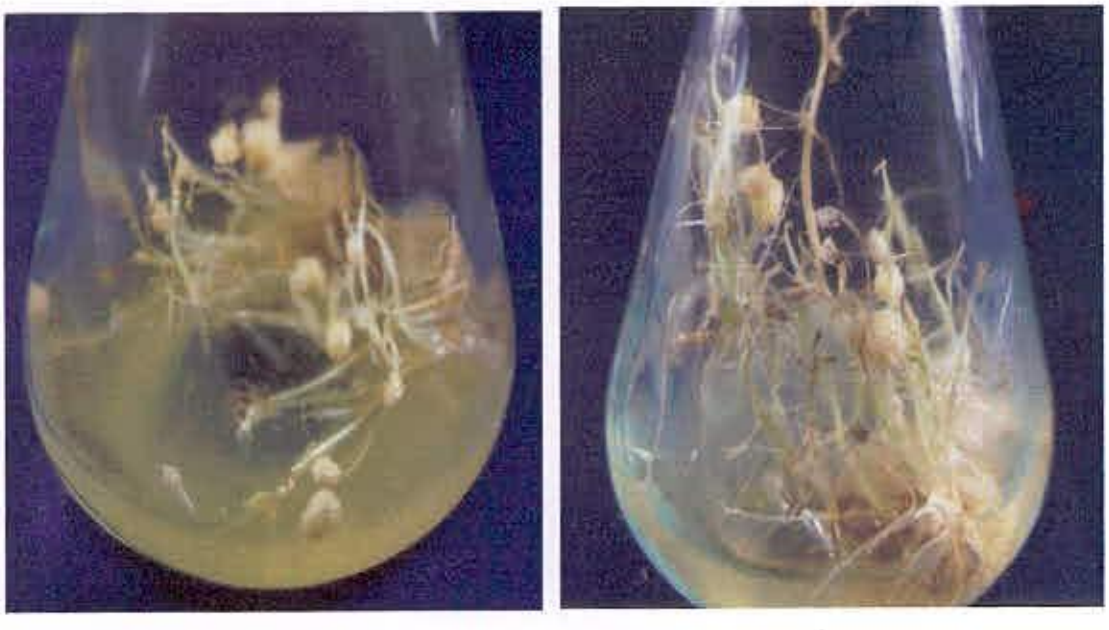
On the otherhand, the maximum weight of microtuber per flask was 808.67 mg after 105 days (Table 2). Hossain (2006) recorded 541.16 mg weight of microtuber per flask after 75 days and 637.76 mg after 105 days, which are in agreement with the results of the present investigation.

There was no interaction between the application CCC and duration of culture in relation to weight of microtuber per flask. But, their combined effect was significant. Irrespective of duration of culture period, 300 and 500 mg/l CCC gave the maximum weight of microtuber per flask (Table 3). However, the maximum weight of microtuber per flask was 921.56 mg under the combined treatment of 300 mg/l CCC and 105 days of duration of culture, which was statistically similar to a number of treatments. The minimum weight of microtuber per flask was 260.20 mg obtained from the treatment combination of 700 mg/l CCC and 90 days of duration of culture, while the control treatment of CCC, irrespective of duration of culture, had the tuber weight per flask in the range of 384.07 to 411.30 mg (Table 3). Hossain (2006) recorded the maximum weight of 747.21 mg microtuber per flask under 500 mg/l CCC and 105 days of culture duration. In the present investigation, it was 921.56 mg under 300 mg/l CCC and 105 days of culture duration, which was rather better as compared to the previous result.

4.1,1.4 Mean weight per microtuber: There existed significant variation among CCC levels on the mean weight per microtuber. The maximum mean tuber weight was 137.24mg obtained under 500 mg/l CCC which was statistically similar to 300mg/l CCC (134.17 mg). It was 106.37 mg for 100 mg/l. the mean weight per microtuber was 95.38 under 0 mg/l compared to 99.46 mg under 700 mg/l (Fig. 1). Hossain and Sultana (1998)

a b

Plate. 1	Maximum microtuber was formed when a) 300 and b) 500 mg/l CCC was used in the culture media.



a b

Plate 2	The number and size of microtuber was the minimum under a) 700 mg/l CCC and b) the control.

recorded the mean weight of each microtuber in the range of 91.0-115.6 mg, whereas, Zakaria (2003) harvested a little bit heavier microtuber, 145.7-190.1 mg and Bakul (2005) obtained mean weight in the range of 55.63-73.75 mg. In the present investigation, the range of mean weight of microtuber was 95.33-137.24 mg which was rather better as compared to the previous results.

The mean weight per microtuber due to addition of CCC in the culture media was statistically similar. However, the minimum mean weight of microtuber was 105.13 mg when cultured for 75 days. It was increased gradually with increasing duration of culture period. The maximum mean weight of each microtuber was 121.47 mg when cultured for 105 days as compared to 116.95 mg for 90 days duration (Table 2). Hossain (2006) recorded mean weight of each microtuber in the range of 89.35 mg after 75 days and 124.62 mg after 105 days which was at par with the previous results.

The interaction effect between CCC and duration of culture for mean weight of microtuber was not significant. However, their combined effect was found to be significant (Appendix-I). Irrespective of duration of culture, CCC@ 300 and 500 mg/l gave the mean weight of each microtuber. The maximum mean weight of each, microtuber was 146.95 mg obtained from the combination of 300 mg/l CCC and 105 days of duration of culture, which was closely followed by 500 mg/l CCC and 90 days. All the 300 and 500 mg/l CCC irrespective of duration of culture were statistically similar. Irrespective of duration of culture, the mean weight of each microtuber under 0 mg/l CCC ranged from 87.77 to 102.21 mg compared to 86.73 to

122.01 mg under 700 mg/l CCC (Table 3). Hossain (2006) recorded the maximum of 144.71 mg of mean weight of each microtuber under 300 mg/l CCC and 105 days of duration of culture. It was 146.95 mg under 300 mg/l CCC and 105 days of duration of culture, which are in agreement with the findings of the present investigation.

4. 1.1.5 Grading

4. 1.1.5.1 <100 mg: The percentage of microtuber of < 100 mg due to addition of CCC differed significantly (Appendix-II). The maximum percentage of number of microtuber of <100mg size was 48.70 obtained under 700 mg/l CCC against 46.85% under the 0 mg/l CCC. On the otherhand, 300 mg/l CCC produced the minimum percentage of number of microtuber of < 100 mg size which closely followed by 500 mg/l CCC (16.29%). A CCC level of 100 mg/l CCC also produced 46.29% of <100 mg size microtuber in number (Table 4). Yeasmin (2005) used different levels of N and K for the production of microtubers for 6 to 8 weeks in three potato cultivars, viz, Bintje, Binella and Baraka and graded microtubers in three sizes like, <50, 50-100 and 100-150 mg in number. The author recorded 12.50-75% microtuber of <50 mg, 0-87.50% of 50-100 mg and 0-31.25% of 100-150 mg sizes in number. In the present investigation, it was 13.30-48.70% of <100 mg which are contradictory to the previous results.

The size or grade of microtuber of <100 mg size in relation to the duration of culture varied significantly(Appendix-II).A culture period of 75 days duration produced the maximum percentage of number of microtuber <100

mg size (50.02), which decreased gradually with increasing duration of culture. The minimum was 19.11 % when the duration of culture was 105 days. It was 33.68% for 90 days culture period (Table 5). Yeasmin (2005) also reported that the percentage of large size microtuber increased with the increasing duration of culture.

The interaction effect of CCC and duration of culture on size or grading of microtuber was non significant(Appendix II). However, the combined treatment of 300 or 500 mg/l CCC and 105 days of duration of culture did not form tuber of < 100mg size in number, whereas, 700 mg /l CCC with 75 days of duration of culture produced the maximum of 71.11% tubers of <100 mg size in number and 100 mg/l CCC with 75 or 90 days of duration of culture produced 55% of <100 mg size microtuber. Irrespective of duration of culture, most of 300 or 500 mg/l produced the minimum percentage of microtuber compared to 0, 100 and 700 mg/l CCC, which produced 28.88 to 71.11 % microtubers of <100 mg size in number (Table 6).

4.1.1.5.2 100-200 mg: The maximum 50.06% of 100-200 mg size microtuber in number were produced due to the use of 500 mg/l CCC in the culture media, closely followed by 300 mg/l CCC (48.94%). The percentage of 100-200 mg size microtuber in number under 0 mg/l CCC was 39.81 against 41.11% under 700 mg/l CCC, while 100mg/l CCC produced 37.41% microtuber of 100-200 mg size microtuber in number (Table 4). Bakul (2005) divided microtubers in five grades viz, <50, 50-100, 100-150, 150-

200 and >200 mg and obtained only 3.61% microtuber of 150-200 mg size. In the present investigation, it was 37.41-50.07% of 100-200 mg size microtuber, which is rather much higher than the previous results.

Table 4. Main effect of cycocel (CCC) on the production of microtuber of different grades in number percentage of the potato cv. Diamant

CCC level (mg/l)	% microtuber in different size grades (by number)		
	<100 mg	100-200 mg	>200 mg
0	46.85 (2.00)	39.81 (1.67)	13.33 (0.56)
100	46.29 (1.89)	37.40 (1.56)	16.29 (0.67)
300	13.29 (0.89)	48.94 (3.22)	37.76 (2.44)
500	16.19 (1.00)	50.06 (3.22)	33.74 (2.22)
700	48.70 (1.67)	41.11 (1.33)	10.18 (0.33)
LSD 0.05	11.91 (0.59)	9.12 (0.53)	11.88 (0.46)
LSD 0.01	16.64 (0.79)	12.29 (0.72)	15.99 (0.62)

Figures in parenthesis are transformed values

Table 5. Main effect of duration of culture on the production of microtuber of different grades in number percentage of the potato cv. Diamant

Duration (days)	% microtuber in different size grades (by number)		
	<100 mg	100-200 mg	>200 mg
75	50.02 (2.40)	35.43 (1.87)	14.56 (0.86)
90	33.67 (1.40)	47.19 (2.26)	19.14 (1.13)
105	19.11 (0.67)	47.79 (2.46)	33.10 (1.73)
LSD 0.05	2.40 (0.46)	1.86 (0.42)	9.19 (0.48)
LSD 0.01	1.40 (0.62)	2.27 (0.56)	12.39 (0.65)

Figures in parenthesis are transformed values

The percentage microtuber of 100-200 mg size in number were almost similar for 90 and 105 days duration of culture, being 47.19 and 47.79

against the minimum of 35.43% for 75 days of duration of culture (Table 5). Although the interaction effect was non significant, the percentage of number of microtuber under 100-200 mg size varied significantly (Appendix-II) due to the combined effect of CCC and duration of culture.The maximum percentage of 62.10 tuber of 100-200 mg size was produced under the treatment combination of 500 mg/l CCC and 105 days of duration of culture, which was statistically similar to the treatment combinations 700mg/l and 90 days of duration of culture (55.55%) and 300 mg/l CCC and 105 days duration of culture (53.38%).Except 75 days of duration of culture, 300 or 500mg/l and 90 or 105 days of duration of culture produced >50% tuber of 100-200 mg size in number (Table 6).

4.1.1.5.3 >200 mg: The highest percentage of microtubers of >200 mg size was 37.76 when 300 mg/l CCC was added in the culture media. This value was statistically similar to that of 500 mg/l CCC (33.74%), whereas, the other treatments varied in the range of 10.185- 16.29%. The minimum percentage of microtuber of > 200 mg size in number was 10.18 under 700 mg/l CCC compared to 0 mg/l CCC (13.33%) (Table 4). Hossain (2006) obtained 4.80-8.39% of >200 mg size microtuber in number under 300-700 mg/l CCC while it was 16.30-36.76% under the same CCC levels, which were much higher than the previous results.

The percentage of >200mg microtuber in number varied significantly in relation to duration of culture period. The maximum was 33.10% when cultured for 105 days against 19.14% under 90 days of duration and 14.56% under 75 days of duration (Table 5).

There was no significant interaction between CCC and duration of culture production of microtubers of >200 mg size grade.However, percentage of microtuber >200 mg size grade combinedly varied significantly. Under the treatment combination of 300 mg/l CCC and 105 days of duration of culture produced the maximum percentage of >200 mg size microtuber in number (47.62%) while the same CCC level but 90 days of duration of culture gave 40.27% microtuber of >200mg size.

Table 6. Combined effect of duration of culture and cycocel (CCC) on the production of microtubers of different grades in number percentage of the potato cv. Diamant

Treatment		% microtuber in different size grades (by number)		
Duration (days)	CCC level (mg/l)	<100 mg	100-200 mg	>200 mg
75	0	63.33 (7.15)	28.33 (3.76)	8.33 (0.51)
	100	55.00 (7.02)	38.33 (4.16)	6.67 (0.46)
	300	30.16 (4.88)	44.44 (4.58)	25.39 (3.51)
	500	30.48 (4.92)	37.14 (4.17)	32.38 (3.95)
	700	71.11 (8.67)	28.89 (3.78)	0.00 (0.00)
90	0	52.22 (6.98)	41.11 (4.32)	6.67 (0.46)
	100	55.00 (7.02)	38.33 (4.16)	6.67 (0.46)
	300	9.72 (0.56)	50.00 (4.96)	40.27 (4.28)
	500	18.09 (2.67)	50.95 (4.98)	30.95 (3.91)
	700	33.33 (5.03)	55.56 (5.03)	11.11 (0.72)
105	0	25.00 (3.56)	50.00 (4.96)	25.00 (3.49)
	100	28.89 (3.69)	35.55 (4.02)	35.55 (4.02)
	300	0.00 (0.00)	52.38 (5.01)	47.62 (4.72)
	500	0.00 (0.00)	62.10 (5.09)	37.89 (4.21)
	700	41.67 (6.38)	39.89 (4.18)	19.44 (2.71)
LSD 0.05		20.63	15.80	20.57
LSD 0.01		27.78	21.78	27.70

Figures in parenthesis are transformed values

Combination of 0 mg/l CCC ,irrespective of duration of culture produced microtuber of >200mg size in the range of 6.67-25.00%, while 700 mg/l CCC and 75 days of duration of culture did not produce any tuber of >200mg size (Table 6)

4.2 Effect of sucrose on the production of microtuber of potato cv. Diamant

4.2.1 Days **to tuber initiation:** The number of days required for initiation of microtuber due to the addition of sucrose in the culture media varied significantly(Appendix-II). It occurred most early by 9.89 days when 9% sucrose was added in the culture media and most delayed by 16.33 days under 15% sucrose, which was statistically similar to 3% sucrose tratment (15.44 days). The effect of 6% and 12% sucrose was almost similar (11.78 and 13.89 days, respectively). Whereas, tuber formation did not occur when 0% sucrose was used (Fig. 3). Zakaria (2003) used eight levels of sucrose in microtuber production and recorded 15-16 days for low and high sucrose levels and Yeasmin (2005) got microtuber within approx. 9 days at 8 and 12% sucrose when used. In the present investigation, tuberisation was earlier at 6% (11.78 days) and 9% (9.89 days) sucrose treatment and those values were 15.44 and 16.83 days for 3 and 15% sucrose treatment, respectively, which are in agreement with the findings of the previous results.

The number of days required for initiation of microtuber in relation to the duration of culture did not vary significantly(Appendix-II). However, tuberisation occurred most early by 10.89 days for 105 days of duration of culture and most delayed by 11.44 days when cultured for 75 days (Table 7).

There was no interaction between sucrose treatment and duration of culture on days of tuber initiation. However their combined effect varied significantly(Appendix-III). The control treatment of sucrose (0%) did not form any microtuber. The minimum number of days required for initiation of microtuber was 9.0 at 9% sucrose and 105 days duration of culture, which was statistically similar to 9% sucrose treatment and 75 or 90 days of duration of culture (10.33 days). Most of the 3% and 15% sucrose use irrespective of duration of culture took the maximum days for initiation of microtuber, ranging from 14.67 to 16.67 days. On the other hand, the effect of 6% and 12% sucrose, irrespective of duration of culture, took almost similar number of days for initiation of microtuber, ranging from 11.67 to 12.00 days (Table 9).

4.2.2 Number of tuber per flask : The number of tuber per flask due to addition of sucrose in the culture media was highly significant (Appendix-III). The maximum number of tuber was 5.67 under 9% sucrose which was statistically similar to 12% (5.11) and 15% (5.11) sucrose levels (Fig. 3) (Plate 3). No tuber was formed under 0% sucrose but under 3% sucrose; it was 1.89 (Plate 4) and increased gradually with increasing sucrose level. Bakul (2005) and Yeasmin (2005) harvested approx. 5 and 2 microtubers from each flask and test tube, whereas, Zakaria (2003) harvested approx. 10 microtuber from each flask at 9-12%. There was a maximum (5.67) microtuber at 9% level of sucrose level in the present findings, which was a little bit lower than the results reported by Zakaria (2003).

The number of tubers per flask was independent of duration of culture. This parameter did not vary significantly. However, the maximum number of

microtuber per flask was 3.50 when cultured for 90 days; closely followed by 75 days (3.39) and 105 days (3.33) of duration of culture (Table 7).

There was no interaction between sucrose level and duration of culture for the number of tubers per flask. Their combined effect was significant. The maximum number of microtuber was produced under the combined treatment of 9% sucrose and 105 days of culture duration, which was statistically similar to the number of combined treatments. Most of the 9-15% sucrose treatments, irrespective of duration of culture, produced the maximum number of microtuber per flask. On the otherhand, the sucrose level of 6% and 12% produced statistically similar number of microtuber per flask, ranging from 2.67 to 5.00. There was no tuber formed under 0% sucrose, irrespective of duration of culture (Table 9).

Table 7. Main effect of sucrose level on the production of microtuber of the potato cv. Diamant

Sucrose level (%)	Wt. of microtuber per flask (mg)	Mean wt. of each microtuber (mg)
0	0	0
3	177.32	93.82
6	292.15	109.41
9	782.12	137.94
12	683.34	133.73
15	668.02	130.72
LSD 0.05	82.79	9.15
LSD 0.01	111.00	12.32

4.2.3 Weight of tuber per flask : The weight of microtuber per flask due to the addition of sucrose in the culture media varied significantly. It was found that weight of microtuber per flask increased gradually from 0% to 9% sucrose leveland then declined onwards. However, the highest weight of microtuber per flask was 782.12 mg under 9% sucrose level which was

statistically similar to 12% sucrose level (683.34 mg). Addition of 0% sucrose did not produce any tuber, though 3% sucrose gave 177.32 mg tuber per flask which increased to 292.15 mg at 6% sucrose used (Table 8). Hossain (2006) used 4, 6, 8, 10 and 12% sucrose for microtuber production and obtained the maximum of 802.05 mg microtuber from each flask at 10% sucrose, whereas it was 782.12 mg at 9% sucrose level in the present experiment, which is in agreement with the findings of the previous results.

Duration of culture influenced the weight of microtuber per flask significantly(Appendix-III). The minimum weight was 361.02 mg for 75 days of duration of culture, which gradually increased with the increasing duration and rose to 451.07 mg and 489.39 mg after 90 and 105 days respectively (Fig. 4). With increasing duration of culture the weight of microtuber per flask increased. Hossain (2006) harvested 575.44 mg after 90 days of duration of culture and 722.06 mg after 105 days while Yeasmin (2005) got 92.62 mg microtuber after 6 weeks and 104.45 mg after 8 weeks from each test tube or flask. In the present investigation, it was 361.02 mg after 90 days and 489.39 mg after 105 days which were rather lower than the results of Hossain (2006).

Table 8. Main effect of duration of culture on the production of microtuber of the potato cv. Diamant

Duration (days)	Days to tuber initiation	Number of microtuber per flask
75	11.44	3.39
90	11.33	3.50
105	10.89	3.33
LSD 0.05	ns	ns
LSD 0.01	-	-

ns, non-significant

a b

Plate . 3.	The maximum number of microtuber was produced when a) 9 and b) 12% sucrose were used in the culture media.

a b

Plate 4.	The minimum number of microtuber was produced under a) 3% and b) 6% levels of sucrose.

No interaction was found between effect of sucrose and duration of culture on weight of microtuber per flask. However, significant result was recorded when the treatments were combinedly applied(Appendix-III). The maximum weight of microtuber per flask was 938.8 mg obtained from the treatment combination of 9% sucrose and 105 days of duration of culture, which was statistically similar to 12% sucrose and 105 days (784.70 mg) or 9% sucrose and 90 days of duration of culture (749.00 mg). A sucrose level of 9-15% produced microtuber in the range of 544.1-658.6 mg at 75 days of culture,719.0-749.0 mg at 90 days and 740.4-938.8 mg at 105 days against 141.40 -246.40 mg, 218.0-330.2 mg and 172.6-299.80 mg under 3-6% sucrose and at 75, 90 and 105 days of duration of culture. Irrespective of duration of culture, 0% sucrose did not produce any microtuber (Table 9).

4.2.4 Mean weight of each microtuber : Different levels of sucrose influenced the mean weight of each microtuber .The maximum (137.94 mg) weight per microtubers was obtained when 9% sucrose was added in the culture media, which was statistically similar to 12% and 15% sucrose level, which was 133.73 and 130.72 mg respectively. Compared to these results, the mean weight of each microtuber under 3% and 6% sucrose were much lower, 93.82 and 109.41mg respectively. The control treatment of sucrose (0%) did not form any microtuber (Table 7). Hossain (2006) harvested maximum of 140.12 mg size microtuber at 10% sucrose while Zakaria (2003) harvested 271.20 mg size microtuber at 9% sucrose and Yeasmin (2005) 87.41 mg size microtuber at 8% sucrose after 8 weeks. In the present investigation, it was 137.94 mg at 9% sucrose which was at par with the

results reported by Hossain (2006) and rather lower than the results reported by Zakaria (2003).

Table 9. Combined effect of sucrose level and duration of culture on the production of microtuber of the potato cv. Diamant

Treatment		Days to tuber initiation	No. of tuber per flask	Wt. of microtuber per flask (mg)	Wt. of each microtuber (mg)
Duration (days)	Sucrose level (%)				
75	0	0.00	0.00	0.00	0.00
	3	15.67	1.67	141.40	82.00
	6	12.00	2.67	246.40	93.86
	9	10.33	5.67	658.60	116.00
	12	14.00	5.33	575.10	108.00
	15	16.67	5.00	544.60	109.00
90	0	0.00	0.00	0.00	0.00
	3	16.00	2.33	218.00	94.01
	6	11.67	3.00	330.20	110.40
	9	10.33	5.33	749.00	140.80
	12	14.00	5.00	690.20	138.00
	15	16.00	5.33	719.00	135.70
105	0	0.00	0.00	0.00	0.00
	3	14.67	1.67	172.60	103.40
	6	11.67	2.33	299.80	128.60
	9	9.00	6.00	938.80	156.50
	12	13.67	5.00	784.70	156.90
	15	16.33	5.00	740.40	147.90
LSD 0.05		1.95	1.10	143.40	15.91
LSD 0.01		2.62	1.48	192.30	21.33

There existed a highly significant variation on the mean weight of each microtuber due to different duration of culture. It was 106.49mg after 75 days culture, which increased gradually with increasing duration of culture. The maximum mean weight of each microtuber was 146.96mg, for 105 days of duration of culture. It was 128.81mg after 90 days of culture(Fig.4).

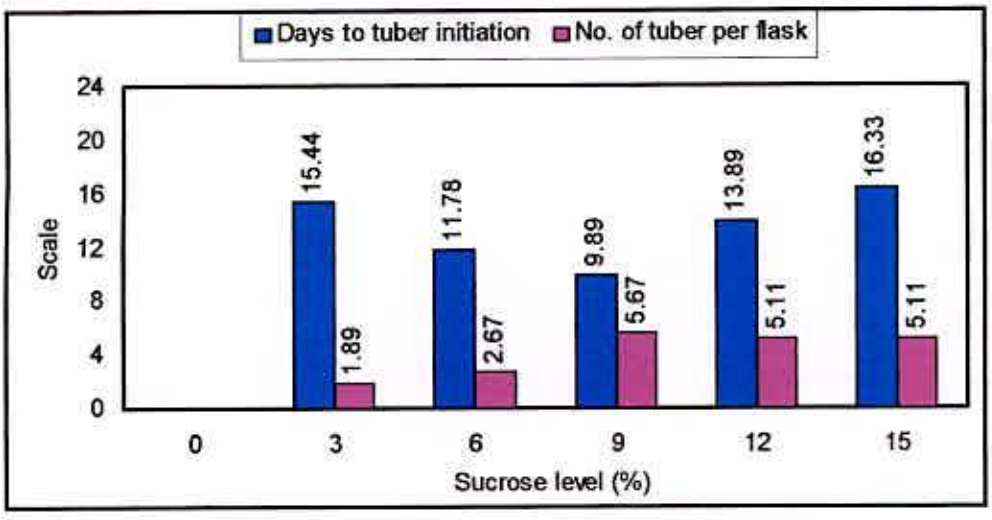


Fig. 3. Days to tuber initiation and the number of microtuber per culture jar as influenced by different levels of sucrose.

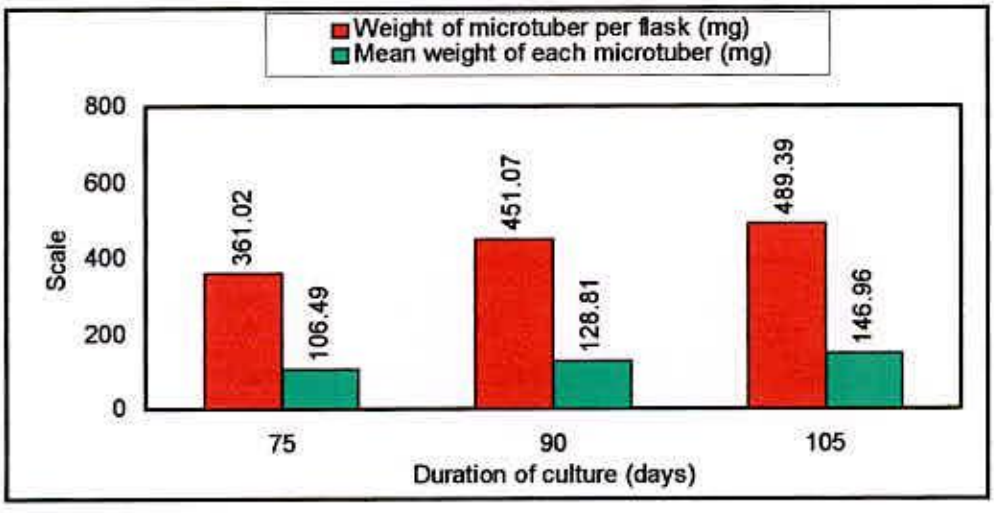


Fig. 4. The effect of duration of culture on weight of microtuber per culture jar (mg) and mean weight of each microtuber (mg)..

Sucrose level and duration of culture did not influence the mean weight of each microtuber. However, their combined effect showed significant result.The maximum mean weight of each microtuber was156.9 mg obtained

from 12% sucrose and 105 days of culture duration (156.5 mg). Moreover, irrespective of duration of culture, 9-15% sucrose level gave higher mean weight of each microtuber. In 9-15% sucrose level, the mean weight of each microtuber ranged from 108.0-116.0 mg after 75 days of culture, which were 138.0- 140.8 mg and 147.9-156.9 mg after 90 ad 105 days of culture, respectively. On the otherhand, these values for 3% and 6% sucrose were 82.0- 93.86 mg, 94.01-110.4 mg and 103.4-128.6 mg after 75, 90 and 105 days of culture. No tuber was found to form under 0% sucrose (Table 9).

4.2.5 Grading

4.2.5.1 < 100 mg: The percentage of microtuber of <100mg size grade varied significantly due to the addition of sucrose in the culture media(Appendix-IV). The maximum percentage of <100mg size microtuber was 57.41 under 3% sucrose level which was gradually reduced to 16.67% under12% sucrose level and rose to 21.48% under15% sucrose level (Table 10). Hossain (2006) and Zakaria (2003) reported almost similar results.

The maximum percentage of <100mg size microtuber was obtained after 75 days of culture (54.54) which was reduced to 31.39% after 90 days duration of culture. The minimum percentage of <100 mg size microtuber in number (18.33) obtained after 105 days of culture (Table 11). Hossain (2006) recorded the maximum 42% tuber of 100-200 mg size microtuber after 90 days and 28 % of >200 mg after 105 days.

Table 10. Main effect of sucrose level on the production of microtuber of different grades in number percentage of the potato cv. Diamant

Sucrose level (%)	% microtuber in different size grades (by number)		
	<100 mg	100-200 mg	>200 mg
0	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
3	57.41 (6.59)	37.04 (4.89)	5.56 (0.78)
6	33.33 (4.61)	55.56 (6.86)	11.11 (1.92)
9	19.63 (3.58)	45.55 (6.70)	34.81 (5.54)
12	16.67 (2.98)	50.37 (7.05)	32.96 (5.37)
15	21.48 (3.71)	46.30 (6.77)	32.22 (5.31)
LSD 0.05	16.81 (2.17)	16.50 (1.37)	11.95 (1.90)
LSD 0.01	22.54 (2.91)	22.12 (1.84)	16.03 (2.54)

Figures in parenthesis are transformed values

There was no interaction effect between sucrose level and duration of culture on the percentage of <100mg size microtuber in number. Their combined effect was significant(Appendix-IV). The treatment combination of 3% sucrose and 75 days culture duration produced cent percent tuber of <100 mg size , though this sucrose level at 90 and 105 days culture duration produced 38.89% and 33.33% of <100mg size tuber in number. On the otherhand, 9% sucrose level and 105 days of culture duration produced the minimum percentage of 5.56 of <100 mg size tuber in number. The treatment combination of 12% and 15% sucrose with 105 days of culture duration did not produce any tuber of this grade (Table 12).

4.2.5.2 100-200 mg : The maximum percentage of 100-200 mg size microtuber in number was obtained from 6% sucrose level(55. 56) which was statistically similar to 12% sucrose (45.55%) and15% (46.30%) sucrose level respectively while it was 45.55% for 9% sucrose level. On the otherhand, 3% sucrose level produced 37.04% microtuber of 100-200 mg

size microtuber in number (Table 10). Yeasmin (2005) used 4, 8, 12 and 16% sucrose for microtuber production for period of 6 and 8 weeks and did not find any tuber of >150 mg size in number with 4% sucrose, whereas, Hossain (2006) harvested at least 41% microtuber of 100-200 mg size in number with 6-9% sucrose used.

The percentage of microtubers of 100-200 mg size in number differed significantly in relation to duration of culture (Appendix-IV). The maximum percentage was 53.44 was obtained after 105 days of culture which was statistically similar to 90 days of duration of culture (43.26%), while this value for 75 days of duration of culture was 32.70% in number (Table 11).

Table 11. Main effect of sucrose level on the production of microtuber of different grades in number percentage of the potato cv. Diamant

Duration (days)	% microtuber in different size grades (by number)		
	<100 mg	100-200 mg	>200 mg
75	54.54 (7.05)	32.70 (4.15)	12.76 (1.98)
90	31.39 (5.54)	43.26 (6.14)	25.35 (3.71)
105	18.33 (3.33)	53.44 (6.35)	28.23 (3.94)
LSD 0.05	11.89	11.67	8.57
LSD 0.01	15.94	15.64	11.33

Figures in parenthesis are transformed values

Findings indicated that there was no interaction between sucrose level and duration of culture on the production of 100-200 mg size microtuber in number. However, their combined effect varied significantly.The maximum percentage of 100-200mg size microtuber in number was 66.67 for the treatment combination of 3 or 6% sucrose level and 105 days of duration of culture, which was statistically similar to number of treatments. The combined treatment of sucrose levels and 75 days of duration of culture gave minimum percentage of microtuber of 100-200 mg in size in number,

ranging from 38.89-46.67%, though no tuber in this grade was produced at 0 and 3 % sucrose used (Table 12).

Table 12. Combined effect of duration of culture and sucrose level on the production of microtuber percentage of different grades in of the potato cv. Diamant

Treatment		% microtuber in different size grades (by number)		
Duration (days)	Sucrose level (%)	<100 mg	100-200 mg	>200 mg
75	0	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	3	100.00 (10.00)	0.00 (0.00)	0.00 (0.00)
	6	61.11 (7.61)	38.89 (5.08)	0.00 (0.00)
	9	35.55 (5.95)	42.22 (6.43)	22.22 (3.85)
	12	36.67 (5.97)	44.44 (6.61)	18.89 (3.46)
	15	33.89 (5.61)	46.67 (6.82)	19.44 (3.59)
90	0	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	3	38.89 (5.07)	44.44 (6.63)	16.67 (2.36)
	6	27.78 (4.28)	61.11 (7.61)	11.11 (1.92)
	9	17.78 (3.41)	44.44 (6.61)	37.78 (6.13)
	12	13.33 (2.98)	46.67 (6.80)	40.00 (6.32)
	15	30.55 (5.51)	38.89 (6.20)	30.55 (5.51)
105	0	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	3	33.33 (4.71)	66.67 (8.05)	0.00 (0.00)
	6	11.11 (1.92)	66.67 (7.97)	22.22 (3.84)
	9	5.56 (1.36)	50.00 (7.07)	44.44 (6.63)
	12	0.00 (0.00)	60.00 (7.75)	40.00 (6.32)
	15	0.00 (0.00)	53.33 (7.29)	46.67 (6.82)
LSD 0.05		29.12 (3.77)	28.57 (2.38)	20.70 (3.29)
LSD 0.01		39.05 (5.05)	38.32 (3.19)	27.76 (4.41)

Figures in parenthesis are transformed values

4.2.5.3 >200 mg: Different sucrose levels differed significantly for the production of microtuber of >200mg size in number. The sucrose levels of 9-15% produced more than 30% of > 200mg size microtuber; it was 34.81 under 9% sucrose while it was 32.96% and 32.22% under 12% and 15% sucrose (Table 10). Zakaria (2003) did not find any tuber of 150-300 mg and >300 mg size in number when 3% sucrose was used in the culture media. The author got maximum of 56.9% of 150-300 mg and 33.1% of >300 mg size microtuber in number with 15% and 9% sucrose level respectively.

The percentage of microtuber of >200mg size in number in relation to duration of culture was significant (Appendix-IV). The maximum percentage of >200mg size microtuber was 28.23 for 105 days of duration of culture, which was statistically identical with 90 days duration of culture (25.35%), whereas, it was 12.76% for 75 days of duration of culture (Table 11).

No interaction was found between sucrose level and duration of culture on the production of percentage of microtuber sized >200mg while conducting the experiment. However, their combined effect showed significance(Appendix-IV). The sucrose levels of 0%,3% and 6% with 75 days of duration of culture, 0% sucrose with 90 days of duration of culture and 3% sucrose with 105 days of duration of culture did not form any tuber of > 200 mg size microtuber in number. Irrespective of duration of culture, the sucrose levels 9-15% produced the maximum of >200mg size microtuber in number. However, the maximum percentage was 46.67 at 15% sucrose level and 105 days duration of culture which was statistically similar to a number of treatments (Table 12).

4.3 Dormancy breaking of microtuber :Effects of GA_3 concentration and soaking time on dormancy breaking of peeled and suberised microtubers under controlled temperature.

4.3.1. Sprouting in microtubers: The levels of GA_3 (0.00, 0.1 and 0.5 & 1.0 mg/l) demonstrated statistically significant effect on sprouting of microtubers on 3rd, 5th and 7th days of germination (Appendix-V and Table 13). However, no sprouting of microtuber occurred with in 7 days when treated with only water, where as, on the same period, the maximum percentage of sprouted microtubers was 88.75 under 0.1 mg/l GA_3. It was 88.12% under 0.5 mg/l GA_3 & 85.62% under 1.0 mg/l GA_3 (Table-13). On 3rd day, maximum sprouted microtubers was 23.12% under 0.1 mg/l GA_3 compared to the minimum of 17.50% sprouted microtubers under 0.5 mg/lGA_3. With advancement of time the percentages of sprouted microtubers increased. On 7th day, the percentage of sprouted microtubers was maximum (88.75) under 0.1 mg/l GA_3 against 88.12% under 0.5 mg/l GA_3 (Table 13). The percentage of rot tubers varied from 11.25-14.37. Gibberellic acid is an important factor which regulates dormancy of potato tubers (Stallknecht and Farnsworth, 1982a). Suttle (1996a) and Wiltshire and Cobb (1996) observed a high gibberellin activity in peeled potato tuber, stimulating quick sprout. A very low concentration of GA_3 (0.1 to 1.0 mg/l) was found to be very effective when slightly peeled tubers were treated for a while on the otherhand peeled tuber without GA_3 treatment sprouted after 12-15 days (Hossain, 2006). Pruski *et al.* (2003) used randite and GA_3 for breaking dormancy and found randite much more effective than GA_3. While Couillerot (1993) observed an earlier and increased sprout elongation by

soaking the microtuber for 5 min in a 20000 ppm of ^{14}C-GA_3 solution. In the present investigation, the maximum sprouted microtuber was 94.16% after 7 days.

Table 13. Main effect of GA_3 on dormancy breaking of microtubers of potato cv. Diamant under control environment (22±2^{0}C)

GA_3 level (mg/l)	% Sprouted microtubers at different days (cumulative)			Rot or unsprouted tubers (%)
	3	5	7	
0.0	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	13.62 (3.09)
0.1	23.12 (4.48)	49.37 (6.90)	88.75 (9.40)	11.25 (2.68)
0.5	17.50 (3.67)	45.62 (6.63)	88.12 (9.37)	11.87 (2.79)
1.0	20.62 (4.09)	45.62 (6.67)	85.62 (9.23)	14.37 (3.31)
LSD 0.05	0.61	0.33	0.37	ns
LSD 0.01	0.88	0.58	0.63	ns

Note: Figures in parenthesis are transformed values, ns=Not significant

The percentages of sprouted microtubers were significantly higher under 5 min soaking time on 3rd day (23.75) and 5th day of germination (52.91) compared to 17.08% and 40.83%, respectively under 1 min soaking time, respectively. On 7th day, maximum sprouted microtubers were also with 5 min soaking time (90.00%) against 85.00% with 1 min soaking time (Table 14). The percentage of rot tubers was 15.00 under 1 min soaking time against 10.00 under 5 min soaking time (Table 14).

The interaction effect of GA_3 and soaking time was not significant. A significant combined effect of GA_3 and soaking time was recorded(Table 15). On 3rd day, the maximum percentage of sprouted microtubers was 27.50 under 0.1 mg/l GA_3 and 5 min soaking time, against 15.00% under 0.5 mg/l

GA_3 and 1 min soaking time (Table 15). With advancement of time, the percentage of sprouted microtubers increased. On 7th day, the maximum percentages of sprouted microtubers were 93.75 under 0.5 mg/l GA_3 and 5 min soaking time. The minimum was 82.50% under the same GA_3 levels and 1 min soaking time (Table 15). The percentage of rot tuber ranged from 6.25-17.50 across the combined treatments of GA_3 levels and soaking time (Table 15).

Suberisation of microtubers influenced the percentages of sprouted microtubers positively, giving 30% sprouting in microtubers under unsuberised condition against only 10.83% under suberised condition on 3rd day (Table 16). On 5th day, the percentages of sprouted microtubers under

Table 14. Main effect of soaking time on dormancy breaking of microtubers of potato cv. Diamant under control environment (22 ± 2^0C)

Soaking time (min)	% Sprouted microtubers at different days (cumulative)			Rot or unsprouted tubers (%)
	3	5	7	
1	17.08 (3.86)	40.83 (6.33)	85.00 (9.19)	15.00 (3.34)
5	23.75 (4.30)	52.91 (7.14)	90.00 (9.47)	10.00 (2.50)
LSD 0.01	(0.36)	(0.78)	ns	ns

Note: Figures in parenthesis are transformed values, ns=Not significant

unsuberised and suberised conditions were 57.08 and 36.66, respectively but on 7th day, the percentages of sprouted microtubers was significantly higher under suberised condition (92.50%) than unsuberised one (82.50) (Table 16). The unsuberised tubers suffered much from rot (17.50%) compared to suberised tubers (7.50%) (Table 16).

There was no significant interaction between GA_3 and tuber conditioning treatment. Their combined effect had a significant effect(Appendix-V).The treatment combination of 0.1 mg/l GA_3 and unsuberised condition of tuber had the maximum percentage of sprouted tubers (32.50) and the minimum was 7.50% under 0.5 mg/l GA_3 and suberised condition, on 3rd day. While the maximum sprouted tuber was 95% under 0.1 mg/l GA_3 and

Table 15. Combined effect of GA_3 and soaking time on dormancy breaking of microtubers of potato cv. Diamant under control environment (22±2°C)

Treatment		% Sprouted microtubers at different days (cumulative)			Rot or unsprouted tubers (%)
GA_3 level (mg/l)	Soaking time (min)	3	5	7	
0.0	1	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	5	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
0.1	1	18.75 (4.17)	40.00 (6.27)	6.25 (9.26)	13.75 (3.09)
	5	27.50 (4.78)	58.75 (7.52)	91.25 (9.53)	8.75 (2.26)
0.5	1	15.00 (3.27)	37.50 (6.05)	82.50 (9.06)	17.50 (3.83)
	5	20.00 (4.06)	53.75 (7.21)	93.75 (9.67)	6.25 (1.74)
1.0	1	17.50 (4.14)	45.00 (6.65)	86.25 (9.26)	13.75 (3.11)
	5	23.75 (4.04)	46.25 (6.70)	85.00 (9.20)	15.00 (3.50)
LSD 0.05		(1.32)	(0.82)	(0.54)	(1.85)
LSD 0.01		(1.78)	(1.10)	(0.72)	(2.48)

Note: Figures in parenthesis are transformed values

Table 16. Main effect of suberisation of peeled microtubers on dormancy breaking of potato cv. Diamant under control environment (22±2^{0}C)

Tuber condition	% Sprouted microtubers at different days (cumulative)			Rot or unsprouted tubers (%)
	3	5	7	
Unsuberised	30.00 (5.37)	57.08 (7.48)	82.50 (9.06)	17.50 (3.79)
Suberised	10.83 (2.79)	36.66 (5.98)	92.50 (9.61)	7.50 (2.06)
LSD 0.01	(0.72)	(0.45)	ns	(1.10)

Note: Figures in parenthesis are transformed values, ns=Not significant

suberised condition and the minimum was 80% under 1.0 mg/l GA_3 and unsuberised condition. The percentage of rot tuber varied from 5.00-20.00 across the combined treatments (Table 17).

Table 17. Combined effect of GA_3 and tuber condition on dormancy breaking of microtubers of potato cv. Diamant under control environment (22±2^{0}C)

Treatment		% Sprouted microtubers at different days (cumulative)			Rot or unsprouted tubers (%)
GA_3 level (mg/l)	Tuber condition	3	5	7	
0.0	Unsuberised	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	Suberised	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
0.1	Unsuberised	32.50 (5.57)	61.25 (7.75)	82.50 (9.05)	17.50 (3.77)
	Suberised	13.75 (3.38)	37.50 (6.05)	95.00 (9.74)	5.00 (1.58)
0.5	Unsuberised	27.50 (5.20)	57.50 (7.50)	85.00 (9.19)	15.00 (3.27)
	Suberised	7.50 (2.14)	33.75 (5.76)	91.25 (9.54)	8.75 (2.30)
1.0	Unsuberised	30.00 (5.32)	52.50 (7.20)	80.00 (8.92)	20.00 (4.32)
	Suberised	11.25 (2.86)	38.75 (6.15)	91.25 (9.54)	8.75 (2.30)
LSD 0.05		(1.32)	(0.82)	(0.54)	(1.85)
LSD 0.01		(1.78)	(1.10)	(0.72)	(2.48)

Note: Figures in parenthesis are transformed values

From Table 18, it can be observed that the combined treatment of 5 min soaking time and unsuberised condition of tuber had the maximum 37.50% sprouted tuber against the minimum of 10.00% under 5 min soaking time and suberised condition, on 3rd day. On 7th day, the maximum sprouted tuber was 94.16% under the combined treatment of 5 min soaking time and suberised condition. The percentage of rot tuber ranged from 5.83-20.83 across the treatments.

Table 18. Combined effect of soaking time and suberisation of peeled microtubers on dormancy breaking of potato cv. Diamant under control environment (22±2°C)

Treatment		% Sprouted microtubers at different days (cumulative)			Rot or unsprouted tubers (%)
Soaking time (min)	Tuber condition	3	5	7	
1	Unsuberised	22.50 (4.65)	45.83 (6.74)	79.16 (8.87)	20.83 (4.26)
	Suberised	11.66 (3.07)	35.83 (5.91)	90.83 (9.52)	9.16 (2.43)
5	Unsuberised	37.50 (6.08)	68.33 (8.23)	85.83 (9.24)	14.16 (3.32)
	Suberised	10.00 (2.51)	37.50 (6.06)	94.16 (9.69)	5.83 (1.69)
LSD 0.05		(1.08)	(0.67)	(0.44)	(1.51)
LSD 0.01		(1.45)	(0.89)	(1.59)	(2.02)

Note: Figures in parenthesis are transformed values

Plate 5.	Treatment of microtuber with 0.1-0.5 mg/l GA_3 for 1 min followed by a) suberisation showed much better sprouting than b) unsuberised tubers.

The results of combined effect of GA_3, soaking time and tuber condition are presented in Table 19. After 3rd day, the maximum percentage of sprouted microtuber was 42 under 1.0 mg/l GA_3, 5 min soaking time and unsuberised condition of microtuber, which was followed by 0.1 mg/l GA_3, 5 min soaking time and unsuberised condition (40%). The minimum percentage of sprouted microtuber was 5 under 0.5 or 1.0% GA_3, 1 or 5 minutes soaking time and suberised condition. The maximum sprouted micortuber recorded was 75% after 5th day under the combined treatments of 0.1 mg/l GA_3, 5 minutes soaking time and unsuberised condition and the minimum value was 30% under 0.5 mg/l GA_3, 1 minute soaking time and suberised condition. On 7th day, the maximum of 97.50% microtuber under the combined

treatment of 0.1 mg/l GA_3, 5 minutes soaking time and suberised condition were found to have sprouted (Table 19).

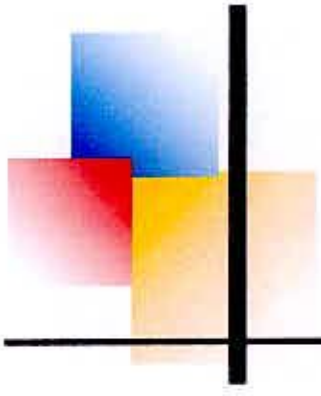
Table 19. Combined effect of GA_3, soaking time and suberisation on dormancy breaking of microtubers of potato cv. Diamant under control environment (22 ± 2^0C)

Treatment			% Sprouted microtubers at different days (cumulative)			Rot or unsprouted tubers (%)
GA_3 level (mg/l)	Soaking time (min)	Tuber condition	3	5	7	
0.0	1	Unsuberised	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
		Suberised	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	5	Unsuberised	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
		Suberised	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
0.1	1	Unsuberised	25.00 (4.85)	47.50 (6.86)	80.00 (8.90)	20.00 (3.81)
		Suberised	12.50 (3.49)	32.50 (5.68)	92.50 (9.61)	7.50 (2.37)
	5	Unsuberised	40.00 (6.29)	75.00 (8.63)	85.00 (9.20)	15.00 (3.74)
		Suberised	15.00 (3.27)	42.50 (6.42)	97.50 (9.87)	2.50 (0.79)
0.5	1	Unsuberised	25.00 (4.97)	45.00 (6.68)	77.50 (8.79)	22.50 (4.64)
		Suberised	5.00 (1.58)	30.00 (5.43)	87.50 (9.34)	12.50 (3.02)
	5	Unsuberised	30.00 (5.43)	70.00 (8.33)	92.50 (9.60)	7.50 (1.90)
		Suberised	10.00 (2.69)	37.50 (6.08)	95.00 (9.74)	5.00 (1.58)
1.0	1	Unsuberised	17.50 (4.14)	45.00 (6.69)	80.00 (8.92)	20.00 (4.32)
		Suberised	17.50 (4.14)	45.00 (6.61)	92.50 (9.60)	7.50 (1.90)
	5	Unsuberised	42.50 (6.51)	60.00 (7.71)	80.00 (8.92)	20.00 (4.32)
		Suberised	5.00 (1.58)	32.50 (5.68)	90.00 (9.47)	10.00 (2.69)
LSD 0.05			(1.87)	(1.16)	(0.76)	(2.62)
LSD 0.01			(2.15)	(1.55)	(1.03)	(3.51)

Note: Figures in parenthesis are transformed values

4.3.2 Rot or unsprouted tubers: GA_3 did not influence the percentage of rot or unsprouted microtubers significantly. However, it varied from 11.25% to 14.37% (Table 13). Effect of soaking time on the percentages of rot or unsprouted microtubers was also insignificant, having the range of 10.00-15.00 (Table 14). It varied from 6.25-17.50% under the treatment

combination of GA_3 and soaking time (Table 15). Suberisation of microtuber reduced the percentage of rot or unsprouted microtuber significantly. It was 17.50% for unsuberised and 7.50% for suberised tubers (Table 16). The percentages of rot or unsprouted microtubers varied significantly among the combined treatments of GA_3 tuber condition. The maximum was 20% under 1.0 mg/l GA_3 and unsuberised condition of tubers and the minimum was 5% under 0.1 mg/l GA_3 and suberised condition (Table 17). The rot or unsprouted microtuber under the treatment combination of soaking time and tuber condition varied significantly, the maximum was 20.83% and the minimum was 5.83% (Table 18). The percentages of rot or unsprouted microtuber among the combined treatments of GA_3, soaking time and tuber condition differed significantly, ranging from 2.50-20.00 (Table 19).



Chapter 5

Summary and Conclusion

CHAPTER 5

SUMMARY AND CONCLUSION

Experiments were conducted to study *in vitro* production of microtuber and breaking dormancy of produced microtuber of a standard potato cultivar Diamant at the Tissue Culture Laboratory, Horticulture Research Centre, Bangladesh Agricultural Research Institute, Joydebpur, Gazipur during the period from January to June, 2008.

The following experiments I and II were conducted for the production of microtuber of the potato cultivar Diamant and the experiment III for breaking dormancy of microtuber.

The experimens on effect of Chloro Choline Chloride (CCC) on the production of microtuber of potato cv. Diamant (Expt. I)

Effect of sucrose on the production of microtuber of potato cv. Diamant(Expt. II)

Effects of GA_3 concentration and soaking time on dormancy breaking of peeled and suberised microtubers under controlled temperature (Expt. III)

In expt. I. five levels of CCC (0, 100, 300, 500 and 700 mg/l) and in expt. II., six levels of sucrose (0, 3, 6, 9, 12 and 15%) were used in the culture media. In both the experiments, 5 mg/l BAP was used and tubers were harvested at three different dates (75, 90 and 105 days after addition of tuberisation media). In expt. III. three levels of GA_3 (0.1, 0.5 and 1.0) were

used to soak peeled microtubers for 1 and 5 minutes followed by suberisation of peeled microtubers at 85-90% RH and 15-18^{0}C or 22±2 °C for 48 hrs.

Experiment I., CCC @ 300 mg/l took the minimum days for tuber initiation (7.11 days) which was statistically similar to 500 mg/l CCC (7.33 days), while those two CCC levels also gave the maximum number of microtubers per flask (6.56 and 6.44, respectively). Similar trend for those two levels of CCC was observed for weight of microtuber per flask (870.85 and 865.26 mg, respectively) and mean weight of each microtuber (134.17 mg and 137.24 mg, respectively). CCC @ 300 and 500 mg/l produced the minimum and maximum percentage of <100 mg (13.30-16.19) and >200 mg size microtuber (33.74-37.76%) in number. While the percentage of 100-200 mg size microtuber ranged from 37.41-50.07 across the treatments.

The weight of microtuber per flask was gradually increased from 556.81 mg after 75 days of duration of culture to 808.67 mg after 105 days. Similar trend was also observed for mean weight of each microtuber. The percentage of small size microtuber (<100 mg) was the maximum under 75 days of duration of culture (50.16) and vice-versa for >200 mg size microtuber for 105 days of duration of culture (33.10%).

Tuberisation occurred with the minimum of 5.67 to 8.33 days for 300 and 500 mg/l CCC under most of the treatments of duration of culture (75, 90 and 105 days). Those two CCC levels gave the maximum number (7.00) and weight of microtuber per flask (914.96 mg) as well as mean weight of each micortuber (146.95). CCC @ 300 and 500 mg/l with any duration of culture

produced the minimum percentage of <100 mg size microtuber in number and vice-versa for >200 mg size microtubers.

Experiment II, The minimum days of 9.87 were required for tuber initiation when 9% sucrose was added to the culture media. The control treatment (0% sucrose) did not form any tuber. The maximum number and weight of tuber per flask and ; mean weight of each microtuber was the highest under 9% sucrose level, had being (5.67, 782.12 mg and 137.94 mg, respectively). The treatments of 9, 12 and 15% sucrose produced the minimum percentage of < 100 mg size microtuber in number (19.63, 16.67 and 21.48 respectively) and vice-versa for >200 mg size microtuber (34.81, 32.96 and 32.22 respectively).

The minimum weight of microtuber per flask and mean weight of each microtuber were 361.02 mg and 106.49 mg after 75 days of duration of culture respectively, which incresed to 489.39 and 146.96 mg after 105 days. After 75 days of duration of culture, the percentage of small size microtuber (<100 mg) in number was the maximum (54.54) and the large size tuber (>200 mg) the minimum (12.76) against those values after 105 days of culture (18.33 and 28.33).

The minimum days required for initiation of tuber was 9.00 for the treatment combination of 9% sucrose and 105 days culture duration. This combined treatment also gave the maximum number of tuber per flask (6.00), weight of microtuber per flask (938.80 mg) and mean weight of each microtuber (156.90 mg). The percentage of <100 mg size microtuber in number ranged

from 0-66.67% for 0-100,100-200 mg size and 0-46.67% for >200 mg size across the combined treatments.

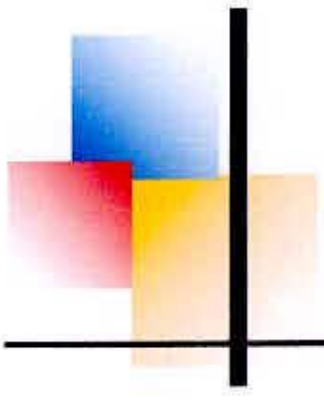
Experiment III., The microtubers under control treatment (treated with plain water) did not germinate at all. On 3rd day, the maximum sprouted microtuber was 23.12% under 0.1 mg/l GA_3 which incresed to 88.75% on 7^{th} day. Five minutes soaking time gave the maximum of 90% sprouting of microtuber on 7^{th} day against 85% for 1 minute soaking time. The treatment combination of 0.5 mg/l GA_3 and 5 minutes soaking time demonstrated the maximum percentage of sprouted microtubers (90.75). Suberisation of microtubers increased sprouting of microtubers positively, having 92.50% against 82.50% for unsuberised tubers on 7^{th} day. The treatment combination of 0.5 mg/l GA_3 and suberised microtubers sprouted the maximum of 95% on 7^{th} day and the minimum was 80% for unsuberised microtubers treated with 1.0 mg/l GA_3. On 7^{th} day, the maximum sprouted microtuber was 94.16% for the combined treatment of 5 minutes soaking time and suberised tuber, while the minimum was 79.16% for 1 minute soaking time and unsuberised microtuber. On the other hand, Microtuber treated with 0.1 mg/l GA_3 for 5 minutes followed by suberisation demonstrated the maximum 97.50% sprouting compared to the minimum of 77.50% under the treatment combination of 0.5 mg/l GA_3, 1 miniute soaking time and unsuberisation of tubers. In this experiment, the percentage of rot tubers varied from 2.5 to 22.50%.

Conclusion

From the results of the present study, it may be concluded that production of microtuber of the potato cultivar Diamant could be enhanced by using 300 or 500 mg/l CCC. Moreover, addition of 9% sucrose in the culture media was found to be very conducive to increased production of microtuber with higher proportion of >200 mg size microtuber. Treatment of peeled microtuber with 0.1 mg/l GA_3 for 5 minutes followed by suberisation was found to be very effective to break dormancy of 97.50% microtuber within 7 days.

Future Strategies

The results obtained in the present investigation may be very useful as a protocol for large-scale production of potato microtuber and subsequent breaking of dormancy of freshly harvested microtubers successfully. This protocol may be tried for microtuber production of other potato cultivars and also to break dormancy of microtuber. This will help a lot in the commercial tissue culture laboratory throughout the country for undertaking large-scale production of microtuber and to use the same after breaking dormancy. This would improve the tissue culture laboratory status as well as program related to potato.



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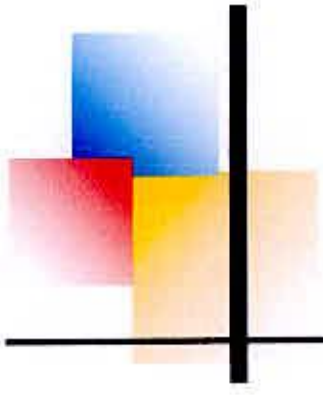

Appendices

Appendix I. Mean sum of square values of analysis of variance of the data on microtuber production as influenced by cycocel and duration of culture (Expt. 1).

Source of variation	Degrees of freedom	Mean sum of squares			
		Days to tuber initiation	No. of tubers per flask	Wt. of tubers per flask (mg)	Mean wt. per tuber (mg)
Treatment	(14)	10.555^{**}	4.390^{**}	165843.102^{**}	512.380^{ns}
CCC	4	42.722^{**}	19.478^{**}	629256.066^{**}	3518.806^{**}
Duration of culture (D)	2	9.156^{**}	0.467^{ns}	10205.203^{ns}	1066.848^{*}
CCC x D	8	0.572^{ns}	0.411^{ns}	7792.584^{ns}	353.444^{ns}
Error	30	1.533	0.689	9265.730	395.438

* and ** = Significant at 5% and 1% level of probability respectively, ns= Not significant

Appendix II. Mean sum of square values of analysis of variance of the data on size distribution of microtuber as influenced by , cycocel and duration of culture (Expt. 1).

Source of variation	Degrees of freedom	Mean sum of squares		
		% microtubers in different size grades (by number)		
		>200 mg	100-200 mg	<100 mg
Treatment	(14)	1128.769^{**}	94.39^{ns}	1.755^{ns}
CCC	4	3585.585^{**}	728.498^{**}	1400.237^{**}
Duration of culture (D)	2	2875.829^{**}	90.544^{*}	1424.941^{**}
CCC x D	8	215.152^{ns}	174.587^{ns}	113.278^{ns}
Error	30	153.098	89.810	152.170

* and ** = Significant at 5% and 1% level of probability respectively, ns= Not significant

Appendix III. Mean sum of square values of analysis of variance of the data on microtuber production as influenced by sucrose and duration of culture (Expt. 2).

Source of variation	Degrees of freedom	Mean sum of squares			
		Days to tuber initiation	No. of tubers per flask	Wt. of tubers per flask (mg)	Mean wt. per tuber (mg)
Treatment	(17)	79.034**	11.796**	243512.353**	108470.588**
Sucrose (S)	5	322.356**	45.674**	922470.669**	24691.220**
Duration of culture (D)	2	1.556ns	0.13ns	78164.482**	4300.980**
S x D	10	0.444ns	0.241ns	12135.220ns	241.592ns
Error	36	1.389	0.444ns	7498.45ns	92.30ns

* and ** = Significant at 5% and 1% level of probability respectively, ns= Not significant

Appendix IV. Mean sum of square values of analysis of variance of the data on size distribution of microtuber as influenced by sucrose and duration of culture (Expt. 2).

Source of variation	Degrees of freedom	Mean sum of squares		
		% microtubers in different size grades (by number)		
		>200 mg	100-200 mg	<100 mg
Treatment	(17)	2070.626**	1858.106**	998.875**
Sucrose (S)	5	6050.88**	1936.07**	1217.71**
Duration of culture (D)	2	3339.00**	3643.73**	2200.78**
S x D	10	552.18ns	517.23ns	165.65ns
Error	36	309.26	297.76	156.26

* and ** = Significant at 5% and 1% level of probability respectively, ns= Not significant

Appendix V . Mean sum of square values of analysis of variance of the data on sprouting of peeled and suberised microtubers treated with gibberellic acid for variable time and put on water soaked cotton pad contained in petriplates under controlled temperature (22±2°C) (Expt. 3).

Source of variation	Degrees of freedom	Mean squares			Rot or unsprouted tubers (%)
		% Sprouted microtubers at different days (cumulative)			
		3	5	7	
Treatment	(15)	426.31**	673.83**	214.8**	105.33ns
GA_3	3	231.03**	311.07**	255.78**	132.61ns
Soaking time (ST)	1	588.62**	11212.86**	314.28ns	365.11ns
GA_3 x ST	3	245.12**	332.82**	278.11**	266.18**
Tuber condition (TC)	1	3708.06**	5100.87**	305.62**	1403.18**
GA_3 x TC	3	255.16**	341.86**	305.12**	281.13**
ST x TC	1	613.86**	9106.32**	3068.16**	510.11**
GA_3 x ST x TC	3	304.86**	359.16**	360.92**	492.13**
Error	48	55.31	71.31	60.35	61.11

* and ** = Significant at 5% and 1% level of probability respectively, ns= Not significant