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IN VITRO MICROTUBERS AS AN ALTERNATIVE TECHNOLOGY FOR POTATO PRODUCTION

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SUMMARY

In vitro microtubers as an alternative technology for potato production

(G.A. Wattimena, L.W. Gunawan, Adri Ernawati, Agus Purwito, Brent H. McCown and Peter J. Joyce)

One of the main constrain of potato production in tropical countries such as Indonesia is the availability of elite potato propagule of well adapted cultivars. The elite propagule means the availability of potato propagules free from diseases especially the systemic diseases (potato vercoses and mycoplasm).

The objectives of the research was to produce potato microtubers as an elite potato micropropagules. The research was a joint research between staff of the Department of Bogor Agricultural University and the Staff of the Department of Horticulture, University of Wisconsin, Madison. The research was a three years research programs, consisted of :

1). *In vitro* shoot multiplication,
2). *In vitro* microtubers production, and
3). Field performance of micropropagules.

In vitro shoot multiplication and *in vitro* microtubers production were carried out in Bogor at the Horticultural Experimental Station Pasir Sarongge (_+ 1100 above sea level).

The starting *in vitro* explants for *in vitro* shoat and *in vitro* tuber experiments were a veins free explains (free from PVY, PVX, PLRV, PVA, PVS AND PVM). The elimination of ferns was a combination of meristem culture and chemical treatment with a virazol (Ribavirin)

There were ten *in vitro* shoat experiments consisted of screening shoat medium, evaluation the effect of cytokinins and cytokinins like activity, source of nitrogen, Ca-panthetonate and method of solidifying.

Twenty four *in vitro* tuberization experiments were carried out consisted of screening, optimalization and verification of level of concentration of growth retardants (cycocel, ancymidol, alar, and paclobutrazol), growth inhibitor (coumarin), cytokinin and cytokinin like activity (benzyl adenine, adenine, sulphate, kinetin, coconut water, and benomyl), sucrose, methods of tuberization and solidifying agent.

Nine of field performance experiments were evaluated of type of cutting and growth regulator on mini tuber production , direct and indirect planting of microtubers and comparison growth of potato derived from different potato propagules.

Technique of virus elimination with combined method of verazol and meristem culture were very

succesful. Thirty five potato cultivars were able to free from six potato virus (PVX, PVY, PLRV, PVS, PVA, PVM). Other cultivars carrying single virus were also kept as on *in vitro* collection of single virus such us : CIP 444 - PVY; Spunta - PVX; AVRDC 1282.16 - PVS; and AVRDC 77C45.88A - PLRV.

Applied cytokinin to induce *in vitro* multiple shoats from axillary nodes were unsuccessful but on the contrary inhibited the growth of *in vitro* shoat. C. panthotenate at least at 6 mg/L need to present in in vitro shoot multiplication media to prevent die back of *in vitro* shoat of potato.

Agar media was better than shallow layer liquid media for *in vitro* shoat production even though no significant different were found between the two types of media, single *in vitro* shoat usually produced one node in a three days. seedlings can be derived from single node cutting of *in vitro* shoat. This at least 9 single node cutting can be produced from one explant in 4 weeks. The economical *in vitro* shoat media will be liquid media of MS Complement with 6 mg/L calcium panthetonate and sucrose 3%.

In vitro tuberization had found optimal concentration level of sucrose, growth retardants, growth inhibitor, cytokinin, and optimal methots of *in vitro* tuberization . Optimum level for sucrose and coumarin were 9%, and 25 m/L consecutively. The optimum concentration level of BA, kinetin, adenine sulphate, benomyl, coconut water, cycocel, ancymidol, alar, uniconazole, paclabutrazol were: 5 mg/L, 10 mg/ l, 100 mg/L , 50 mg/L, 12 mg/L consecutively. Combination of retardants or inhibitor with cytokinin in inducing *in vitro* tuberization was better than retardants or cytokinin alone. Any combination of the optimum concentration level of retardants and cytokinin tested produced no significant different in the number of *in vitro* microtubers. From the 30 possible combination there were from combination highly recommended.

These four combination were :

1). MS media, sucrose 9%, BA 5 mg/L, cycocel 600 mg/L
2). MS media, sucrose 9%, BA 5 mg/L, coumarin 25 mg/L
3). MS media, sucrose 9%, coconut water 15 %, cycocel 600 mg/L
4). MS media, sucrose 9%, coconut water 15%, coumarin 25 mg/L

These combination were chosen based on the following criteria namely : early tuberization, bigger size tubers, greater number of microtubers, shorter microtuber dormancy and cheaper.

The field performance experiments showed no different in indirect and direct planting of microtubers as far as the microtubers had the sprout length of more than one centimetre. *In vitro* microtubers (100-150 mg/microtuber), and mini tubers (1.0-10 g/minituber) produced equal number and weight of tubers per plant and about 30-40% of tuber weight per plant of bigger size seed tubers (50- 100g). More experiments of potato micropropagules has to be carried out to be able to draw an acceptable conclusion.

The project contribute a lab, chemical and equipment that could accommodate of 10 student every

year. During the research period 20 student with special interest in bachelor graduate research in micropropagation of potato have been produced in 1989 two of these students (Miss Fatimah and Yasfianti) combining their research (producing of microtubers + field testing of microtuber) for taking part in "National Competition of Student Research" and have won the trophy for student research in agriculture. Since 1987 the potato tissue culture lab provide training and short courses to staff member of other Universities in total of 36 staff members from 18 state universities from different part of Indonesia.

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I. Introduction

1.1. Potato Production Area in Indonesia

The origin of potato is in South America. The tuberosum group (*Solanum tuberosum spp tuberosum*) is originated from Chili while the neotuberosum group (*Solanum tuberosum ssp andigena*) is originated from Peru and Bolivia.

No one known exactly when potato was introduced to Indonesia. According to Koens (1948) in 1794 potato was found to be grown around the district of Cimahi (West Java). In 1804 potato was grown in other areas in West Java in the area of Tengger in East Java. In 1812 the potato was spread to Magelang (Central Java) and Kerinci (Jambi). In 1905 the spread of the potato was extended to the other areas of Indonesia namely: Brastagi (North Sumatra), Takengon (Aceh), Curup and Kapahiang (Bengkulu), Pasemah (South Sumatra), Bukit Tinggi (West Sumatra), Tomohon (North Sulawesi), Goa (South Sulawesi), Tabanan (Bali), Flores, and Timor (East Nusa Tenggara), Manusela and Leksula (Maluku) and Arfak (Irian Jaya) (Koens, 1948).

At present the potato production areas are mainly on the Island of Java, Sumatra, and Sulawesi, namely: West Java (Pangalengan, Lembang, Ciwidey, Cikajang), Central Java (Wonosobo, Tawangmangu, Dieng), East Java (Malang, Pasuruan), North Sumatra (Brastagi, Dairi), West Sumatra (Solok, Bukit Tinggi,Tanah Datar) and South Sulawesi (Goa, Bontaing, Enrekang). The potential production areas and the current potato growing area with less then 1000 hectars including: Aceh, Riau, Jambi, South Sumatra, Bengkulu, Lampung, Central Sulawesi, North Sulawesi, Bali, Nusa Tenggara, Maluku, Irian Jaya, Timor and Timor Timur.

Indonesia grows annually about 25 000 hectares in these prime production areas (70% in Java) with average yield of 10 ton a hectares. Tuber yield of above 20 ton a hectares was obtained at the experimental field and by the best farmers of West Java and Central Java. In these prime production

areas potato is grown at the elevation of 900 to 2000 m above sea level. There are some potato cultivars (LT-1, LT-2, DTO 28, DTO 33, Red Pontiac and Desiree) that can produce a good crops at "mid elevation" (500-750 m above sea level). The available of the heat tolerant potato cultivars make it possible to extend the potato growing areas to the mid-elevation. The available areas in the mid-elevation is about 250 000 to 400 000 hectares (Kusumo, 1984). If potato production in Indonesia can be extended to these areas Indonesia will be the largest potato production countries in South East Asia and may be Asia. Now days the acreage of potato production in Indonesia is second to Nepal and bigger than Thailand and Philippines. However the average yield of Indonesia is higher than Nepal, Thailand and the Philippines.

1.2. Potato Growing in West Java.

West Java is the biggest potato production center of Indonesia. Annually about 33 percent of the potato production in Indonesia is produced in West Java (Pacet, Lembang, Pangalengan) (Rasjid, 1988). These centers are at elevations of 900 to 1800 m above sea level. There are nine or more consecutive wet months (month with more than 100 mm rainfall) around Pacet, Lembang and Pengalengan. The wet months are usually from October until July. The potato planting season is usually twice a year, however the planting season is not as obvious as in the temperate regions. The two planting season for potato, tomato and cabbage are October through November and March through April.

The Common practice is to follow the intensive fertilized crops (potato, tomato, Cabbage) with non fertilized crops (corn, sweet corn, carrot, peas, beans). Fertilized crops are treated with manufactured fertilizers of equal amount of N, P_2O_5, K_2O (100 - 150 kg/ha) and about 10 - 30 tons of farm manure (Chicken or Cow) a hectare. The seed piece used for planting is either local seed or imported seed. The local seed is usually small sized (less than 30 g) while imported seed is medium sized (30 - 50 g). All seed pieces are planted whole and pre sprout. For the production of ware potato the seeds are spaced 30 cm in rows and the distance between rows is 90 cm. For the production of seed potatoes the closer spacing is used namely 70 cm between rows and 25 cm in rows.

Hilling is done twice, first at planting and second at four weeks after emergence. The manures and fertilizers are applied by localized placement around the seed piece. The farm manures and phosphate fertilizer are applied once at planting time. The nitrogen and potassium fertilizers are split into two applications, half at planting time and the rest at second hilling.

The application of fungicides and insecticides are usually every three to six days during the growing season. During the rainy season crops the fungicides are used more frequently than the insecticides and the reverse is true for the dry season crops. Harvesting is done manually when all the vines have died. No chemical treatment is needed for the vines killing because the vines die off naturally.

1.3. Potential of potato production in Indonesia

There is a great potential to increase potato production in Indonesia by integration into established governmental programs

(1). The nutrition programs

Indonesia is self supporting in rice in 1984 and now government is emphasized on providing a well-balanced food for the Indonesian people. Some of the objectives can be met by balancing legume and grain foods. Legumes meet the sulphur-containing amino acid requirements. However, the potato is the best balanced food of any major crops (McCay and McCay, 1967, Sawyer, 1978). The balance between protein and calories is excellent. There are no limiting amino acid constituents such as in cereals and legume crops. The successful of transformed potato with a synthetic DNA coding for protein rich in essential amino acids (Dodds, 1987) will further increase it nutritive value. In addition potentially more food can be produced per unit of time and per unit of land than with the legumes and the grains.

(2). The low land peat reclaiming program

The low land peat area covers approximately 17 million hectares and is located manly along the Eastern Coast of Sumatra and in West, Central and South Kalimantan (Soepraptohardjo and Driessen, 1976). The program is called "Tidal Swamp Reclamation Project" (P4S). Rice, corn and soybean are the crop chosen for this area, however the results were not promising. Many horticultural crops with high market value will be tried. Potato can be included to this area when a heat tolerant potato cultivar becomes available.

(3). Increase non oil and gas export commodities

Potato can be considered as one of the non oil and gas export commodities. At present only small amount of potato is exported as fresh potato from North Sumatra (Brastagi) to Singapore, Hongkong and Malaysia. In other hand Indonesia is still imported potato as seed and as processed fries. Indonesia must be able to produce their own seed and fries. More research have to be done on fries quality due to the low dry matter content of potato produced in Indonesia. The early harvest due to the late blight attack and excessive used of manures and fertilizer were some of the culture practices that reduced the dry matter content of the potatoes in Indonesia.

1.4. Problems of seed tubers and alternatives

The potato crop is usually propagated by means of tubers. As a vegetative propagules, the tuber preserves not only the desirable genotype but viruses and other tissue born pathogens as well. Rather complex testing and certification programs are necessary to keep the incidence of such pathogen contamination of planting stock to a minimum.

Indonesia is always depended on the imported high quality seed tubers from Europe (The Netherlands, West Germany and Belgium). The depended on the imported seed tuber is the major problems of increasing potato production in Indonesia. Alternative propagation methods need to be

developed to encourage potato production in Indonesia. In a tropical country like Indonesia the disadvantages of using the seed tubers as propagules are as follows:

(1). High seed cost

The potato production cost in Indonesia is limited due to high seed cost. The imported seed tubers cost is 37 to 50 % of the total production cost (Wattimena, 1983, Santika and Adiyogo, 1985).

(2). Large quantity of seed tubers used per hectare

The amount of seed tubers required to plant a hectare of potato in Indonesia is 1 to 2 metric tons. This amount is about 10 to 20 % of the expected final yields.

(3). Limited availability of high quality seed tubers at the right planting time. The imported seed tubers is not of the highest grade because it will be too expensive. The shipments of the seed tubers are almost mismatch with regular planting time. Due to this mismatch planting time the seed tuber used is usually from the first reproduction seed (R1 seed) of the imported seed tubers.

(4). Lack of vector free production areas and seasons and a rapid virus accumulation. Indonesia is not able to produce its own certified seed tubers due to a lack of vector free areas or season. Virus contaminated plants increased at a rate of 60 to 100 % after 4 successive planting of virus free seed tubers (Sunarjo and Hadi, 1968).

(5). Late blight problems during planting and storage.

Late blight infection not only shortened the growing period of potato crop but also cause storage and marketing losses.

(6). Risk of being infected with exotic virulence potato pathogens. In 1988 the goverment of Indonesia has instructed to fumigate all the imported potato seed tuber after unloading and before being distributed to the farmers. This instructed was due to some imported seed tuber from the Netherlands was contaminated with golden cyst nematodes (*Glabodera rostochiensis*).

To overcome the seed tuber problems the Indonesian government has to look for other alternatives such as true potato seed (TPS), green house cutting, screen house tuber, minituber and microtubers. Research and development on TPS and greenhouse cutting are carried out at Lembang Horticultural

Research Institute (LEHRI). Due to non uniform field performance, non uniform tuber quality and late maturity of the crop originated from TPS, the TPS is not distributed to the farmers but there G1 tuber seed (first tuber generation from TPS). The rooted greenhouse cutting is also distributed to the farmers in Wonosobo and Dieng. These rooted greenhouse cutting is originated from *in vitro* virus free cutting. The draw back of greenhouse cutting is the high transportation cost and stagnation of field growth of some potato cultivar such as Nooksack. Nooksack propagated by greenhouse cutting is not performed well in the field, if the cutting was undergoing stress during the transportation handling.

The research and development of screenhouse tuber are carried out by P. T. Mustika Nusantara Abadi a private plant tissue culture company. The original propagules of the screen house tuber is also from *in vitro* virus free cutting. The *in vitro* shoot is propagated for producing the greenhouse plants. Cutting from the greenhouse plants are rooted and use as propagules for producing screen house tubers. In addition to those alternatives mentioned earlier, *in vitro* microtuber will be more advance. *In vitro* microtubers will be pathogens free if started with pathogen free explants. The *in vitro* microtubers as an alternative technology will be relevant to potato production not only for developing countries but also to the developed countries as well. In developing countries that not able to produce their own seed tubers this new technology will be replace the existing technology of producing seed tubers potatoes and as a complementary technology for the production of ware potatoes.

The advantages and disadvantages of *in vitro* seed tubers technology are as follow:

(1). *In vitro* seed tubers (microtubers) is disease free and trueness to type

(2). Less seed is needed per hectare. Seeding rate for *in vitro* microtubers is about 4 - 5 kg/ha, while for regular seed tubers is 1.0 to 2.0 metric ton. This amount can be redirected for food consumption.

(3). The available of *in vitro* microtubers as propagules will be at the right planting time and right physiological ages. The imported seed tubers after reach the developing countries most are not at the right planting time and the seed tubers are physiological to old (long multiple sprouts).

(4). The developing countries are able to utilize of proven will adapted local varieties and not depend on varieties supply by seed tubers producing countries

(5). Economic in storage and transportation

(6). The technique of producing *in vitro* microtubers can be possible mechanized.

(7). The management system in production of *in vitro* seed tubers will be less complicated than the regular seed tubers

(8). *In vitro* microtubers are about a pea size tuber so they can be possible planting with a seeder

(9). Easy to by pass the plant quarantine regulation and restriction

(10). Under favourable condition the growth of plant from small size *in vitro* seed tuber (60-100 mg) will growth as well as growth of plant from bigger size tuber (30 - 60 grams). However under stress condition *in vitro* seed tubers will be more vulnerable that the seed tubers, particularly at planting and emergence (disadvantages of *in vitro* microtubers).

(11). The production of *in vitro* tubers need a good laboratory, green house, equipments and their facilities.

1.5. Research assumptions, theories and Hypothesis

1.5.1. Research assumption

The underlying assumption for this research are:

(1). *In vitro* cutting can be induced to form *in vitro* tubers

(2). The inducers can be applied through the media under favourable light duration and favourable temperature

(3). The important inducer of *in vitro* tuberization are plant regulators, sucrose and nitrogen

(4). Tuber eyes in both *in vitro* tubers and seed tubers are potentials source of new plants. Both kind of tubers can be used as potato propagules, (5). Under proper cultivation technique the tuber yield of *in vitro* microtuber and seed tubers will be similar

1.5.2. Research theories

The theories supported the assumption can be divided into two part namely:
(1). the tuber formation and (2). the origin and growth of sprouts

(1). Process of tuber formation

Normal (non *in vitro*) tuberization of potato is induced by short days, high light intensity, low night temperature, low nitrogen and physiological old tubers. When the plant is in induced condition, tuberization is not restricted to underground stem (stolon) but every axil of the plant can produce tubers. The endogenous cytokinin, gibberellin like substance, and starch are different between the inducing and non inducing tissue. During the stolon formation (non induced) the endogenous gibberellin like substance are high but the endogenous cytokinin and starch are low. The reverse are true prior to tuberization and at tuberization.

These characters have been manipulated to produce *in vitro* tubers. The experimental evidence have shown that temperature, photoperiod, sugar, nitrogen, and plant growth regulators played and important role in *in vitro* tuberization (See Literature Review in chapter 3.0).

(2). The origin and growth of sprout

The potato tubers is morphological a stem with internode. The eye of the tubers consists of scale leaves and axillary buds. The axillary bud will produce sprout and when the sprout is rooted it produced a complete plant. The growth of this new plant with a well developed root system is not depend on tuber size (microtubers or seed tubers) but on the available of nutrient and water of the growing medium. With ample supply of water and nutrient the growth of the plant originated from *in vitro* microtubers will be similar to the growth of the plant originated from seed tubers. These characters will be manipulated to get an optimum cultivation method for *in vitro* microtubers and used them as potato propagules.

1.5 Research hypothesis.

The questions that are specifically addressed in this experiments are as follows:

(1). What rate of shoot multiplication and tuber multiplication can be obtained in *in vitro* systems

(2). How will *in vitro* microtubers potatoes perform in the field planting as compared to seed tubers propagated potatoes ?

(3). How will the spacing affect the growth and the tubers yield of potato plant originated from *in vitro* microtubers ?

The research hypothesis to answer these question are:

(1). Each of the factors tested will have an optimum level for *in vitro* tuberization

(2). There will be a combination of specific level of these factors tested that will give the best response for *in vitro* shoot multiplication and *in vitro* tuberization

(3). The growth and tubers yield of potatoes from *in vitro* microtubers and seed tubers will be equal.

(4). In row spacing will effect the growth and tuber yield of potatoes originated of *in vitro* microtubers.

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II. Shoot Multiplication

2.1 Introduction

In vitro shoot growth and multiplication is a central part of micropropagation technology. The manipulation of the growth regulator composition and balance is commonly the most successful method of regulating *in vitro* shoot multiplication (Lawrence, 1981). The growth regulator composition and balance is based on Skoog and Miller (1957) concept of auxin and cytokinin ratio of organogenesis. The auxin-cytokinin balance in controlling root and shoot initiation appears to be a general phenomena among plants (Murashige, 1974, 1977).

The total amount of nitrogen and the ratio between nitrate nitrogen and ammonium nitrogen is another controlling factors in shoot multiplication. No *in vitro* shoot can be grown on a single ammonium source of nitrogen and the growth of *in vitro* shoot is also very poor in single nitrate nitrogen (Winarso, 1986). The nitrate nitrogen and ammonium nitrogen have to be present in certain quantity and ratio to accelerate good shoot growth of *in vitro* culture (Gamborg and Shyluk, 1981; George and Sherington, 1984).

There are two source of *in vitro* shoots, namely the axillary shoots and adventitious shoots. Axillary shoots are derived from activation and proliferation of existing shoot meristems, while adventitious shoot arise through the induction and proliferation of adventitious meristems directly on parent tissue or via intermediate callus (Hussey and Stacey,1981; Lawrence, 1981; Minocha, 1980). Proliferation of the axillary bud is the most commonly used method for *in vitro* shoot multiplication. The advantages of this method are faster shoot development and genetic stability (Binns, 1981; Constantin, 1981; D Amato, 1975, 1978) eventhough it has a more limited level of multiplication as compared to the callus method of shoot proliferation (Lawrence, 1981; Murashige, 1974).

Enhanced axillary shoot production can now be accomplished with many different plant species. A limited number of plant species appear to require a low level of auxin along with cytokinin to induce the axillary branching (Hussey, 1980); most need only cytokinin (Murashige *et al*, 1977; Murashige, 1977). Theoretically, any plant species that produce axillary meristems and responds well to the available cytokinins can be clonally propagated through micropropagation. Representative examples are strawberry (Boxus and Druart, 1980), apple (Jones *et al*, 1977), gerbera spp (Murashige *et al*, 1977), sugar beet (Hussey, 1977), ferns (Lawrence, 1981) and birch (Mc Cown and Amos, 1977).

In vitro shoot production of potatoes can be based on regeneration from callus tissue. Nearly every part of the potato plant can be induced to produce callus and adventitious shoots. The organs commonly used are tubers (Jarret *et al*, 1980; Lam, 1975), shoots (Marani and Pisi, 1977), leaf mesophyll (Roest, 1977) and roots (Vasil and Vasil, 1980). However, potato plants derived from callus tissue may be variable (Roest, 1977) or genetically unstable (D'Amato. 1975). Thus, this method is unlikely to be useful for the propagation of commercially important cultivars or locally adapted cultivars. For micropropagation the axillary bud method of producing *in vitro* shoots is preferred.

In vitro shoot culture of potato based on axillary-bud growth has been used by Goodwin *et al.* (1980), Hussey (1980), Jarret *et al.* (1980), Lindsay (1987), Novak and Zadina (1987), Roca *et al.* and Wang (1977). Hussey (1980) used single node cuttings without growth regulators while others used single or multiply node cutting with growth regulators (Table 1.)

The following experiments were conducted to study the effect of some media components on shoot multiplication rates of potato. The medium components that were studied consisted of: (1) plant growth regulator, (2) nutrient carrier and (3) the amount and source of nitrogen.

Table 2.1. Plant growth regulator used in *in vitro* potato shoot culture

Culture medium	Plant growth regulator
1. Espinoza *et al.* (1986)	0.25 mg/l GA_3 0.5 mg/l BAP
2. Espinoza *et al.* (1986) (liquid)	0.4 mg/l GA_3 0.01 mg/l NAA 5 mg/l kinetin
3. Goodwin *et al* (1980)	0.01 mg/l GA_3 1.0 mg/l BAP
4. Jarret *et al* (1980)	0.3 mg/l GA_3 0.03 mg/l NAA 0.22 uM BAP
5. Lindsay (1987)	2.2 uM BAP 1.2 uM GA_3
6. Miller and Lipschutz (1984)	0.054 uM NAA 0.1 uM Kinetin
7. Novak and Zadina (1987)	1.0 uM GA_3 1.0 uM IAA

2.2. General methods

2.2.1. Medium preparation

The culture bottles (vessels), pipette, scalpel, forceps, sciccors and other utensils were first washing, drying and sterilized before using the culture bottles and utensils were steam sterilized in autoclave for 60 minutes at 17.5 psi and 121°C.

The mineral salt basal media containing the test level of growth regulator according to treatment was prepared (Murashige and Skoog, 1962). For the nitrogen manipulation experiments the major salts of nitrate and ammonium of Murashige and Skoog was adjusted according to treatments. The required plant growth regulator was added to the nutrient medium before autoclaving. The pH of the medium was adjusted to 5.6 and 20 ml of medium was dispersed into culture bottles. The bottles were capped with aluminium foil and steam sterilized in autoclave at 121°C and 17.5 psi for 30 minutes. The medium was stored at a room temperature for 10 to 15 days before subculturing of *in vitro* explants.

2.2.2. Subculture and harvest methodology

The explants for the experiments consisted of two to three nodes of *in vitro* shoot pieces. The shoot were taken from established cultures. The tip of the shoots was removed. The cultures were grown under continuous cool-white fluorescent light (1000-2000 lux/20-40 uE m^{-2} S^{-1}) and at room temperatures (25°-ã30°). The number of harvestable shoots was recorded. A harvestable shoot was defined as having four nodes. A shoot of eight nodes was counted as two harvestable shoots. The four node microshoot were suitable size for rooting in the greenhouse. The harvesting was based on non selective cuttings, because this type of harvest is more practical than the selective cutting. The first harvest was done 4 to 6 weeks after subculturing and the next successive harvest were conducted there after at 3 to 4 weeks interval.

2.3. Report on individuals *in vitro* shoot multiplication experiments

2.3.1. The effect of different shoot media on *in vitro* micro shoot production of PAS 3063

2.3.1.1. Introduction

The objective of this experiments was to evaluate the effectiveness of 4 shoot media on *in vitro* microshoot production of potato cultivar PAS 3063

2.3.1.2. Methods

The experiments consisted of 4 treatments carried out in a fully randomized design. The four treatments were as follows:

(a) Roca *et al* (1979): Control + GA_3 0.2 mg/l,(b) Goodwin *et al* (1980): Control + GA_3 0.3 mg/l + kinetin 5 mg/l, (c) Jarret *et al* (1980): Control + NAA 0.03 mg/l + BA 1.0 mg/l + GA_3 0.3 mg/l, (d) Hussey and Stacey (1981): Control + potato extract 60%.

The control consisted of MS salts and vitamins, Ca- pantothenic acid 2 mg/l, myoinositol 100 mg/l, sucrose 3% and agar 0.7%. The harvestable shoot were counted at 4 weeks and the shoot were harvested at 8 weeks.

2.3.1.3. Results.

The result of table 2.3.1. showed that:

(1). The media of Jarret *et al* (1980) produced the best number if shoot harvested at 8 weeks after subculture and significant different from three after media.

(2). The media of Hussey and Stacey (1980) and Roca *et al* (1978) produced the lowest number if shoot harvested.

(3). The media that contained cytokinin either 6-benzyl aminopurine (Jarret *et al*, 1980) or kinetin (Goodwin *et al*, 1980) produced higher number of shoot harvested than media without cytokinin (Hussey and Stacey, 1980; Roca *et al*, 1978) .

Table 2.3.1.1. The number of harvestable shoot and the number of shoot harvested per culture vials.

Treatments	Shoots Harvested	
	4 weeks	8 weeks
1. Hussey and Stacey (1980)	5.15 a	8.53 a
2. Goodwin *et al* (1980)	8.90 a	12.87 b
3. Roca *et al.* (1978)	5.90 a	8.73 a
4. Jarret *et al.* (1980)	8.30 b	18.73 c

The same letter in a column indicates no significant different at 5% level of its D test.

2.3.2. The effect of media on *in vitro* shoot production of three potato cultivars.

2.3.2.1. Introduction

The objective of the experiments was to evaluate the effectiveness of five shoot media on *in vitro* shoot production of three potato cultivars.

2.3.2.2. Methods.

The experiments consisted of two factors namely: (1) the kind of media and (2) potato cultivars. The media consisted of five level: MSO; Roca *et al.* (1978); Jarret *et al* (1980); Goodwin *et al* (1980) and Hussey and Stacey (1980). MSO medium was the medium of Murashige and Skoog (1962) without growth regulator. The other media were the same as in sections 2.3.2.1. The three potato cultivars were: DTO 28, Katahdin and Red Pontiac. The shoot were harvested and counted at 6 weeks after subculture.

2.3.2.3. Results

No microshoots could be harvested from the media of Hussey and Stacey (1980) and Jarret *et al* (1980). Both media produced branched and stunted microshoots. These microshoots did not fit the criteria for harvesting shoots (4 node shoots). The result for the other media were presented in table 2.3.2.1.

Table 2.3.2.1. The numbers of shoot harvested per vials 6 weeks after sub culture.

Treatment cultivars	Media	Number of shoot
DTO 28	MS O	5.33 ab
	Roca	6.87 bcd
	Goodwin	5.60 abc
Katahdin	MS O	7.00 bcd
	Roca	5.33 ab
	Goodwin	4.00 a
Red Pontiac	MS O	6.13 bcd
	Roca	7.53 d
	Goodwin	6.55 bcd

The same letter in a column indicates no significant different at 5% level of HSD test.

The conclusion that can be drawn from table 2.3.2.1. were as follows:

1. Different genotype response differently to different media
2. DTO 28 and Red Pontiac produced the most harvested microshoots with the medium of Roca *et al* (1978) eventhough it was no significant different from the other two media.
3. The best micro-shoots harvested for Katahdin was on MS O media at it was significant different from the media of Goodwin *et al* (1980).
4. The most harvested micro shoots was produced by Red Pontiac on media of Roca *et al* (1980) and the least by Katahdin on media of Goodwin *et al* (1980). The different was significant.

2.3.3. The effect of media on *in vitro* shoots production of five potato cultivars

2.3.3.1. Introduction

The objective of this experiments was to compare the effect of five different shoot medium on *in vitro* microshoot production of five potato cultivars.

2.3.3.2. Methods

This factorial experiments consisted of two factors each of five levels. The first factor was potato genotypes and the second factor was the medium. The potato genotypes were: Red Pontiac, Katahdin, DTO 28, Kennebec and Cipanas. The medium consisted of MS O, Roca *et al* (1978), Goodwin *et al* (1980), Jarret *et al* (1980), Hussey and Stacey (1980) the as in section 2.3.1.2. There were 25 treatments each treatment were replicated 15 times. The microshoots were harvested non selective at six, nine and twelve weeks after subculture.

2.3.3.3. Results

The number of shoots harvested at first, second, and third harvest were presented in table 2.3.3.1.

Table 2.3.3.1. The number of shoot harvested at first, second and third harvest

Medium	Cultivars				
	Red Pontiac	Katahdin	DTO 28	Kennebec	Cipanas
First harvest					
Control	12.9 b	12.9 b	13.2 c	13.2 c	10.5 b
Roca *et al*	16.3 c	16.3 c	12.2 bc	12.2 bc	11.9 b
Goodwin *et al*	14.6 bc	14.6 b	10.6 b	10.6 b	9.9 b
Jarret *et al*	7.1 a	7.1 a	7.5 a	7.5 a	4.3 a
Hussey and Stacey	11.2 b	11.2 b	8.6 b	8.6 b	6.3 a
Second harvest					
Control	5.5 b	5.5 b	6.0 c	6.0 c	5.9 c
Roca *et al*	5.5 b	5.5 b	6.0 c	6.0 c	7.6 d
Goodwin *et al*	4.9 ab	4.9 a	4.8 b	4.8 b	4.8 b
Jarret *et al*	4.1 a	4.1 a	2.8 a	2.8 a	2.4 a
Hussey and Stacey	4.1 a	4.1 a	5.1 bc	5.1 bc	3.7 b
Third harvest					
Control	3.0 b	3.0 b	3.0 b	3.0 b	3.3 b
Roca *et al*	2.9 b	2.9 b	2.9 b	2.9 b	3.2 b
Goodwin *et al*	3.1 b	3.1 b	2.3 a	2.3 a	2.1 a
Jarret *et al*	1.5 a	1.5 a	1.4 a	1.4 a	1.4 a
Hussey and Stacey	2.3 b	2.3 b	2.1 a	2.1 a	2.2 ab
Total of 1st, 2nd, 3rd					
Control	21.4	21.4	22.2	22.2	19.7
Roca *et al*	24.7	24.7	21.1	21.1	12.7
Goodwin *et al*	22.7	22.7	17.7	17.7	15.9
Jarret *et al*	12.7	12.7	11.7	11.7	8.1
Hussey and Stacey	17.6	17.6	15.8	15.8	12.2

Table 2.3.3.1. showed that:

(1). The media of MSO, Roca *et al* (1978), Goodwin *et al* (1980) produced the most *in vitro* shoots at very harvest and total harvest.

(2). Jarret *et al* (1980) medium produced the lowest number of shoot at every harvest and total harvest (3). For each treatment combination of cultivar and media, the number of shoots harvested continuous decrease from the first harvest, second harvest to the third harvest

2.3.4. The effect of benzyladenine and calcium pantothenate on potato microshoots

2.3.4.1. Introduction

The objective of the experiments was to evaluate the effectiveness of benzyladenine and calcium pantothenate on the production of potato microshoots of the cultivar PAS 3063.

2.3.4.2. Methods

The experiments is a factorial experiments of two factors. Each factors consisted of four levels. The levels of the first factor were 0, 0.5, 1.0 and 5.0 mg/l of benzyl adenine. The second factor was calcium pantothenate which the level of: 0, 2.0, 4.0 and 6.0 mg/l. The 16 treatments combination were carried out in a fully randomized design with 15 replicates. Each culture vial was considered as an experimental unit. The basic medium for this experiment was Murashige and Skoog medium (salt, organic and vitamins) plus 0.03 mg/l NAA and 0.3 mg/l GA_3. Benzyl adenine and calcium pantothenate were added to the basic medium according to the treatment combination. The shoots were harvested and counted at 6 weeks, 9 weeks and 12 weeks.

2.3.4.3. Results

The number of shoot harvested at 6 weeks (first harvest), node number and multiplication rate at 6 weeks and number of shoots harvested at a 9 weeks were presented at table 2.3.4.1. The total number of shoot harvested at first harvest (6weeks), second harvest (9 weeks), third harvest (12 weeks) and total shoot of the three harvest were presented at table 2.3.4.2.

Table 2.3.4.1. Node number at 6 weeks (A), node multiplication rate at 6 weeks (B), shoot harvested at 6 weeks(C) and shoot harvested at 9 weeks (D).

	Ca-P (ppm)	BAP (ppm)			
		0	0.5	1.0	1.5
A	0.0	14.50 abc	16.73 defg	16.60 defg	15.00 bcde
	2.0	15.87 bcdef	16.67 defg	17.97 g	14.70 abcd
	4.0	13.17 a	14.30 ab	15.23 bcdef	16.43 cdefg
	6.0	15.23 bcdef	17.27 fg	18.10 g	16.90 efg
B	0.0	7.273 ab	8.387 cde	8.333 cde	7.527 bc
	2.0	7.947 bcd	8.353 cde	9.007 e	7.367 ab
	4.0	6.607 a	7.193 ab	7.953 bcd	8.253 cde
	6.0	7.640 bc	8.653 de	9.073 e	8.480 cde
C	0.0	4.800 a	6.600 cde	7.267 efg	5.933 bc
	2.0	6.333 bcd	6.800 def	7.667 g	6.000 bc
	4.0	5.733 b	6.000 bc	7.000 defg	5.533 b
	6.0	6.333 bcd	7.467 fg	7.733 g	5.800 bc
D	0.0	2.667 a	4.000 cde	4.000 cde	3.733 bcd
	2.0	2.800 a	4.600 def	4.600 def	4.933 f
	4.0	2.733 a	3.800 bcd	3.800 bcd	3.600 bc
	6.0	3.000 ab	4.733 ef	4.733 ef	5.800 g

The same letters in row as well as in column for the same variables were not significant difference at 5 % student Newman Keul Test

Table 2.3.4.2. Harvested shoot at each harvest and total shoot harvested

Treatments BA (mg/l)	Ca-P (mg/l)	1 st Harvested	2 nd Harvested	3 rd Harvested	Total Harvested
0.0	0.0	4.8	2.7	3.5	11.0
	2.0	6.3	2.8	3.3	12.4
	4.0	5.7	2.7	3.3	11.7
	6.0	6.3	3.0	3.7	13.0
0.5	0.0	6.6	3.8	4.0	14.4
	2.0	6.8	3.9	4.0	14.7
	4.0	7.7	4.6	4.5	13.3
	6.0	7.5	4.5	4.8	16.8
1.0	0.0	7.3	4.0	4.5	15.8
	2.0	7.7	4.6	4.5	16.8
	4.0	7.0	3.8	4.5	15.3
	6.0	7.7	4.7	5.6	18.0
1.5	0.0	5.9	3.7	5.8	15.4
	2.0	6.0	4.9	4.0	14.9
	4.0	5.5	3.6	4.0	13.1
	6.0	5.8	5.8	6.0	17.6

The results in table 2.3.4-1 and table 2.3.4-2 showed that:

1. The combination of BA 1 mg/l with calcium pantothenate of 2,4,6 mg/l gave a good response for all variables measured. Without BA and calcium panthotenate the response was poor. This media was Murashige and Skoog medium plus 0.03 mg/l of NAA and 0.3 mg/l of GA_3
2. The higher number of each harvest and total harvest was achieved by calcium pantothenate of 6.0 mg/l in combination with 0.5, 1.0, or 1.5 mg/l of benzyl adenine.
3. Repeated harvest saved time, energy and cost of production. From three harvest of the repeated harvest during 12 weeks of incubation the combination of 6.0 mg/l of calcium pantothenate and 1.0 mg/l or 1.5 mg/l of benzyl adenine produced the most shoot numbers (18 shoots). It seems that for the repeated harvested the medium had to have a higher level of calcium pantothenate.

2.3.5. The effect of IBA, Kinetin and Benomyl on the production of *in vitro* potato shoots

2.3.5.1. Introduction

The objectives of the experiments was to evaluate the effectiveness of IBA, kinetin, and benomyl on the production of *in vitro* potato shoots of PAS 3063

2.3.5.2. Methods

The experiment was a factorial experiment consisted of 3 factors namely the level of IBA, kinetin and benomyl. The level of these factors were as follows: IBA 0, 0.05 mg/l, kinetin 0, 2.5, 5.0 mg/l and benomyl 0, 25, 50 mg/l. The 18 treatments combination were carried out in a fully randomized design with 15 replicates. Each cultures vessel was considered as an experimental unit. The basic medium for this experiment was Murashige and Skoog medium (salt, organic and vitamines), sucrose 3%, and agar 0.7%. The shoots were harvested at 6, 9 and 12 weeks after cultured of explants. The harvest was a non selective harvest.

2.3.5.3. Results

Table 2.3.5-1, and 2.3.5-2 presented the number of shoot harvested at first, second and third harvest. Table 2.3.5-3 showed the response curve of shoot harvested at six weeks.

These tables showed that:

1. The best treatment combination for node multiplication at 6 weeks, number of shoot harvested at 6 weeks and total number of shoots of three harvest were:
   a. IBA 0.05 mg/l, Benomyl 25 mg/l
   b. IBA 0.05 mg/l, Benomyl 50 mg/l
2. The worst treatment combination for node multiplication at 6 weeks number of shoot harvest at 6 weeks, and total number of shoot of threes harvest were a. kinetin 5 mg/l; b. kinetin 5 mg/l + benomyl 50 mg; and kinetin 5 mg/l + IBA 0.05 mg/l.
3. The optimal level of benomyl at IBA 0.05 mg/l was 42.7 mg/l. The calculated number of shoot harvest at 6 weeks at this optimum level of benomyl was 11.1 shoots.

Table 2.3.5.1. Number of nudes per explants (A), node multiplication rates (B) and number of shoot harvest at 6 weeks (C)

Variable	Benomyl (mg/l	IBA 0.0 mg/l			IBA 0.05 mg/l		
		Kinetin 0.0 mg/l	Kinetin 2.5 mg/l	Kinetin 5.0 mg/l	Kinetin 0.0 mg/l	Kinetin 2.5 mg/l	Kinetin 5.0 mg/l
A	0	25.20 fg	23.47 ef	15.20 a	24.60 fg	22.67 c	20.67 cd
	25	25.67 g	22.40 de	16.87 b	29.63 i	24.93 fg	21.60 cde
	50	28.00 h	21.67 cde	19.93 c	30.21 i	25.47 g	22.93 e
B	0	12.53 de	11.09 bcd	7.63 a	12.27 de	11.23 bcd	10.25 b
	25	12.85 e	11.16 bcd	8.31 a	14.76 f	12.37 de	10.75 bc
	50	3.96 f	10.75 bc	9.88 b	5.06 jk	11.96 cde	10.05 b
C	0	7.20 gh	6.53 defg	4.00 a	7.06 fgh	5.73 cd	4.73 b
	25	9.00 i	6.06 cde	4.66 b	10.50 jk	7.13 fgh	6.33 cdef
	50	9.33 j	5.66 c	5.93 cde	10.93 k	7.53 h	6.60 efg

The same letters in row as well as in column for the same variable were not significant different at 5% student Newman-Keuls Test

Table 2.3.5.2. Number of micro shoot harvested, second harvested (D), Third harvested (E), sum of first, second and third harvested (F)

Variable	Benomyl (mg/l	IBA 0.0 mg/l			IBA 0.05 mg/l		
		Kinetin 0.0 mg/l	Kinetin 2.5 mg/l	Kinetin 5.0 mg/l	Kinetin 0.0 mg/l	Kinetin 2.5 mg/l	Kinetin 5.0 mg/l
	0	4.6 bc	6.5 g	4.9 bcd	5.9 f	4.6 bc	4.5 bc
A	25	3.5 a	5.3 cde	3.7 a	5.7 ef	4.4 b	5.1 bcde
	50	4.5 bc	5.5 def	4.8 bcd	5.0 bcde	4.7 bc	5.2 bcde
	0	4.7 ab	7.1 fg	5.4 bc	5.6 c	5.9 cd	4.7 ab
B	25	5.7 cd	5.9 cd	5.7 bc	6.2 cde	4.4 a	6.8 efg
	50	5.9 cd	6.5 def	5.5 bc	6.1 cde	5.7 cd	7.4 g
	0	16.4 b	20.2 c	14.5 a	18.6 cd	16.3 b	13.9 a
C	25	18.2 cd	17.2 bc	13.9 a	22.3 f	15.9 b	18.3 cd
	50	20.3 e	17.7 cd	16.3 b	22.1 f	18.0 cd	19.1 a

The same letters in row as well as in column for the same variable were not significant different at 5% student Newman-Keuls Test

Table 2.3.5.3. Response of harvested micro shoot at 1st harvested at different Benomyl concentration at constant IBA and Kinetin

Concentration of		Response curve	Optimum Benomyl	Calculated shoot harvested
BA (mg)	Kinetin (mg)			
	0	Linier	-	-
	2.5	Linier	-	-
	5.0	Linier	-	-
	0	Quadratic	42.7	11.1
	2.5	Quadratic	47.5	7.5
	5.0	quadratic	41.2	6.6

2.3.6. *In vitro* shoot production of five potato genotypes on agar and static liquid medium

2.3.6.1. Introduction

The objective of the experiments was to evaluate the effect of agar medium and static liquid medium on *in vitro* shoot production of five potato cultivars.

2.3.6.2. Methods

The experiments was a two factors factorial experiments. The factors were medium and five potato genotypes. The medium consisted of agar medium (M_1) and static liquid (M_2) medium. The five potato genotypes were: Red Pontiac (V_1), DTO 28 (V_2), Kennebec (V_3), Katahdin (V_4) and Cipanas (V_5). The 10 treatments combination were run in a complete randomized design with 15 replicates. Each culture bottle was considered as an experiment unit. The medium for M_1 and M_2 were Murashige and Skoog medium (mineral, organic and vitamins), sugar 4%, ancymidol 0.1 mg/l, agar 0.7 % for M_1 and no agar for M_2. In each bottle there was 20 ml of medium. For static liquid medium these 20 ml of medium were distributed at four time of application. The time of application was before the culture of explants, and at each repeated harvest (three harvest). The repeated harvest was a non selective harvest. The *in vitro* shoots were harvested at 6, 9, 12 and 16 weeks.

2.3.6.3. Results

The number of shoot harvested at 6,9 and 12 weeks was presented on table 2.3.6-1. The result in table 2.3.6-1 showed that:

1. Different potato genotypes gave different response. There were three types of response namely:

a. No significant different of shoot production at each harvest and total number of shoot of four harvest between the agar medium and the static liquid medium (The Cipanas type).

b. There were significant different of shoot production at each harvest and total number of shoot of four harvest between the agar medium and static liquid medium (The Katahdin type). The agar medium was better than the liquid medium in the Katahdin type.

c. The response was between the Cipanas type and Katahdin type (the intermediate type).

2. The intermediate type of response showed that the agar medium produced more shoots of each harvest and total of four harvest than static liquid medium eventhough some of the different were not significant. These non significant different were found for: a. Kennebec (first harvest and total of three harvest), b. DTO 28 (first harvest), and c. Red Pontiac (first and third harvest).
3. The greatest number of shoot harvest were found in agar medium for Kennebec and Katahdin. The total number of shoot of four harvest were 60 shoots for both cultivars with an average more than 10 shoots at each harvest.

Table 2.3.6.1. Number of *in vitro* shoots, produced by five potato genotypes on agar medium and static-liquid medium.

Treatment	Harvest				
	First	Second	Third	Fourth	Total
Red Pontiac					
Agar	9.50 ab	13.55 bc	10.80 b	14.15 c	47.50 b
Liquid	8.80 a	8.40 a	10.10 ab	7.95 a	35.25 a
DTO 28					
Agar	11.30 bc	19.25 e	14.90 d	14.75 cd	60.20 c
Liquid	10.20 b	16.35 d	8.05 a	9.25 ab	43.85 b
Kennebec					
Agar	11.70 bc	16.60 d	12.20 c	13.55 c	54.05 b
Liquid	10.40 b	14.80 c	9.10 ab	7.80 a	42.10 b
Katahdin					
Agar	12.65 c	18.20 e	10.95 b	17.55 d	59.35 c
Liquid	9.20 ab	12.05 b	8.35 a	7.00 a	36.60 a
Cipanas					
Agar	8.10 a	9.05 a	8.65 a	7.25 a	33.05 a
Liquid	9.00 ab	11.60 ab	9.55 ab	9.85 ab	40.00 a

Letter in common in a column indicate no significant different at 5% LSD-Test

2.3.7. The effect of different level of NH_4NO_3 and KNO_3 on the growth and production of *in vitro* shoot of potato

2.3.7.1. Introduction

The objectives of the experiments was to evaluate the effect of different level of NH_4NO_3 and KNO_3 on the growth and production of *in vitro* shoot of potato.

2.3.7.2. Methods

There were two experiments. The first experiments. The first experiments was to evaluate the different level of NH_4NO_3 and KNO_3. These level were as follows:

1. NH_4NO_3: 5, 10, 15, 20, 25 mM and
2. KNO_3 10, 20, 30, 40 mM.

This factorial experiments with 20 treatments were arranged in a complete randomized design with 10 replicates.

The second experiments was the continuation of the first experiments. The three best medium from the first experiments was choosen for the repeated harvest. The second experiments consisted of three treatments and four replicates. The treatments of the second experiments was: 1. Medium 10-20 (10 mM NH_4NO_3 and 20 $_{mM\ KNO3}$, 2. Medium 15-20 (15 mM NH_4NO_3 and 20 mM KNO_3, 3. Medium 15-40 (15 mM NH_4 NO_3 and 40 mM KNO_3).

The basic medium for both experiments was Murashige and Skoog medium except for KNO_3 and NH_4NO_3. The amount of KNO_3 and NH_4NO_3 were used according to treatment combination. The explants for both experiments was *in vitro* cutting. The first used 2 explants per culture vials and 1 explant per culture vial for the second experiments. Each culture vial had 20 ml of culture medium.

The variables measured for the first experiments were number of shoots harvest and node multiplication rates. The shoot were harvested 6 weeks after culturing of explants. For the second experiments only the number of shoots harvested at first, second and third harvest.

2.3.7.3. Results

The result of the first experiments were presented in table 2.3.7.1, 2.3.7.2, 2.3.7.3 and for the second experiments in table 2.3.7.4. The data showed that:

1. There were 3 treatments combination of NH_4NO_3-KNO_3 that response well to number of shoot harvest and node multiplication rate. Those NH_4NO_3-KNO_3 combination were: 15-20; 10-20 and 15-40. The highest number of shoot produced (6 shoots/culture vials) was produced by treatment combination of 15-20 (15 mM NH_4NO_3 and 20 mM KNO_3).
2. To each level of NH_4NO_3 there was an optimum level of KNO_3. The level of NH_4NO_3 5, 10, 15, 20, 25 mM had the consecutive optimum level of KNO_3: 25, 15.3, 16.7, 25.0, 32.3 mM. (5-25; 10-15.3; 15-16.7; 20-25 and 25-32.3). These combination had a variable amount of N, NH_4^+, NO_3^- and K but a relative the same ratio of NH^+/NO_3^- (0.41 -0.47) except for the treatments 5-25 (NH_4^+/NO_3^- = 0.17).
3. The highest number of total shoot harvested of four repeated harvest was produced by the treatment combination 10-20 (18 shoots) and the lowest by 15-40 (10 shoots)
4. The treatment combination of 15-20 produced more shoots than 10-20 for the single harvest.

Table 2.3.7.1. Number of shoots harvested and nodes multiplication rate at 6 weeks after culturing of experiments

Variables	KNO3 (mM)	NH4NO3 (mM) 5.0	10.0	15.0	20.0	25.0
Shoot Harvested	10	2.088 abcdef	2.279 abcd	2.241 abcd	1.954 cdef	1.850 def
	20	2.111 abcdef	2.386 abc	2.502 a	2.035 bcdef	2.165 abcde
	30	2.173 def	1.701 f	2.260 abcd	2.047 bcdef	2.246 abcde
	40	1.840 def	14.30 ef	2.450 ab	1.790 ef	2.246 abcde
Node Multiplication Rate	10	3.115 cdefg	3.553 abcd	3.510 abcde	3.010 defg	2.885 efg
	20	3.268 bcdef	3.693 abc	3.970 a	3.236 bcdef	3.371 abcdef
	30	3.527 abcd	2.579 gh	3.475 abcdef	3.292 bcdef	3.432 abcdef
	40	3.008 defg	2.194 h	3.800 ab	2.868 fg	3.561 abcd

The same letters in rows as in column were no different at 5% Newman-Keuls Test. Transformed data (Vx + 0.5)

Table 2.3.7.2. Response of number of shoot harvested and node number of KNO_3 on constant of NH_4NO_3. Calculated value transformed data (Vx + 0.5)

Variables	NH4NO3 (mM)	Response Curve of KNO3	Optimum Conc. KNO3 (mM)	Calculated value
Numbers of shoot harvested	5	L, Q	25.0	2.022
	10	Q	15.3	2.289
	15	L, Q, C	16.7	2.271
	20	L	-	-
	25	L, Q	32.3	1.932
Node multiplication rate	5	Q	25.0	3.439
	10	L, C	15.3	3.905
	15	L	16.7	4.023
	20	Q	25.0	3.305
	25	L	-	-

L = Linear, Q = Quadratic, C = Cubic, L, Q, Qu = Significant

Table 2.3.7.3. Concentration of nitrogen, ammonium, nitrate, potassium, ratio of NH_4/NO_3 at best combination of NH_4NO_3-KNO_3 for number of shoot harvested and node multiplication rate.

Variables	NH4NO3-KNO3 (mM)	N (mM)	NH4 (mM)	NO3 (mM)	NH4/NO3 (mM)	K (mM)
Shoot harvested	5.0-25.0	35.0	5.0	30.0	0.17	25.0
	10.0-15.3	35.3	10.0	25.3	0.40	15.3
	15.0-16.7	46.7	15.0	31.7	0.47	16.7
	25.0-32.3	82.3	25.0	57.3	0.44	32.3
Node multiplication rate	5.0-25.0	35.0	5.0	30.0	0.17	25.0
	10.0-15.3	35.3	10.0	25.3	0.40	15.3
	15.0-16.7	46.7	15.0	31.7	0.47	16.7
	20.0-25.0	65.0	20.0	45.0	0.44	25.0

Table 2.3.7.4. Number of shoot at each harvest and total shoot harvest

Period of harvest	Combination of NH4NO3-KNO3 (mM)		
	(10-20)	(15-20)	(15-40)
I	5.25	5.75	4.75
II	4.25	3.50	3.25
III	5.00	4.00	2.25
IV	3.75	3.00	0.00
Total	18.25	16.25	10.25

2.3.8. The effect of nitrogen source and concentration on *in vitro* shoot production of potato

2.3.8.1. Introduction

The objective of this experiment was to evaluate the effect of level of nitrogen and nitrogen source (NO_3-N and NH_4) on *in vitro* shoot production of potato. This experiments was a continuation of experiments 2.3.7. The difference was that in this experiment the level of potassium was keeping at a constant level by substituting KNO_3 with $NaNO_3$ and K_2SO_4.

2.3.8.2. Methods

The experiments was a factorial experiment consisted of two factors, namely the sources of nitrogen (NO_3^-- or NH_4^+-N) and nitrogen concentration. There were 5 levels of ammonium and nitrate ratio (NH_4 : NO_3 = 1:3; 1:1; 1:2; 1:3; 0:1) and three level of nitrogen concentration (20,40,60 mM N). The symbol of treatment was indicated by ammonium and nitrate ratio and by the amount of nitrogen expressed in mM N. The treatment with the ratio of NH_4 : NO_3 was 1;0 and the amount of nitrogen was 20mM N was written a 1:0 - 20. There were in total 15 treatments combination as follows: 1:0-20; 1:1-20; 1:2-20; 1:3-20; 0:1-20; 1:0-40, 1:1-40; 1:2-40; 1:3-40; 0:1-40; 1:0-60; 1:1-60; 1:2-60; 1:3-60; 0:1-60. The experiments was in a fully randomized design with 20 replicates.

The medium was Murashige and Skoog medium with some modification. The KNO_3 of MS medium was substituted by $NaNO_3$ and K_2SO_4. The total nitrogen concentration and the ratio of NH_4:NO_3 was adjusted according to the treatment combination.

The shoots were harvested at 4, 7 and 10 weeks after culturing of explants. The pH of the medium was measured before autoclaving (pH = 5.6) and after the third harvest.

2.3.8.3. Results

The result were presented in table 2.3.8-1 (node shoot ratio), table 2.3.8-2 (rate of node number 1 shoot 1 week) and table 2.3.8-3 (the change of pH at the end of the experiments). These result showed that:

1. The growth of potato *in vitro* shoots were affected by the source of nitrogen, the concentration of the nitrogen d the interaction of source and concentration
2. The treatment with only ammonium gave the poorest growth and only the low nitrogen concentration that were able to produce weak shoots (20 mM). No growth was achieved at higher nitrogen concentration (60mM)
3. The treatment with only nitrate gave better growth than the ammonium. At higher nitrate-nitrogen concentration (40 mM, 60 mM) the shoot were not able to grow after the first harvest.

4. The best growth of *in vitro* shoots were achieved by mixed source of nitrogen (NH_4:NO_3 t 1:1; 1:2; 1:3). Even though there were no different in growth response, the NH_4 : NO_3 ratio of 1:3 gave more vigorous growth.
5. The rate of growth of plantlet grown on mixed nitrogen source were faster at second and third harvest than the first harvest. Maybe due to better rooting of plantlet after first harvest.
6. The pH of nitrogen source from nitrate increase the pH while from the ammonium source decrease the pH. The pH of the mixed source nitrogen (ammonium and nitrate) were more stable.

Table 2.8.3.1. The effect of nitrogen concentration and source of nitrogen on node-shoot ratio at first, second and third harvest

Treatments	Periods of Harvest		
	First	Second	Third
1:0-20	4.41 b	0.00 a	0.00 a
1:0-40	0.00 a	0.00 a	0.00 a
1:0-60	0.00 a	0.00 a	0.00 a
1:1-20	6.80 c	5.78 cd	5.36 b
1:1-40	6.57 c	5.52 cd	6.37 cde
1:1-60	6.70 c	6.13 d	6.36 cde
1:2-20	6.58 c	5.80 cd	5.73 bc
1:2-40	6.21 c	6.07 d	6.28 cde
1:2-60	6.84 c	5.78 cd	6.96 ef
1:3-20	6.63 c	5.16 c	6.02 cd
1:3-40	6.76 c	5.61 ed	6.56 def
1:3-60	7.04 c	6.12 d	7.20 f
0:1-20	6.34 c	4.41 b	0.00 a
0:1-40	7.01 c	0.00 a	0.00 a
0:1-60	6.55 c	0.00 a	0.00 a

Letters in common indicate no significant difference as determined by 5% Newman-Keuls Test

Table 2.8.3.2. The effect of nitrogen concentration and nitrogen source on shoot node number

Treatments	Node number/shoot/week		
	First	Second	Third
1:0-20	1.10	0.00	0.00
1:0-40	0.00	0.00	0.00
1:0-60	0.00	0.00	0.00
1:1-20	1.70	1.93	1.79
1:1-40	1.64	1.84	2.12
1:1-60	1.68	2.04	2.12
1:2-20	1.65	1.93	1.91
1:2-40	1.55	2.02	2.09
1:2-60	1.71	1.92	2.32
1:3-20	1.66	1.73	2.01
1:3-40	1.69	1.87	2.19
1:3-60	1.79	2.04	2.40
0:1-20	1.59	1.47	0.00
0:1-40	1.75	0.00	0.00
0:1-60	1.64	0.00	0.00

Table 2.8.3.3. The pH at the end of the experiment (10 weeks after culturing of explants). The starting pH was 5.6

NH4:NO3	Nitrogen concentration (mM)		
	20	40	60
1:0	4.2 ±0.1	4.3±0.1	4.4 ±0.1
1:1	4.9 ±0.1	5.2±0.1	5.4 ±0.1
1:2	5.4 ±0.1	5.2±0.1	5.3 ±0.1
1:3	5.7 ±0.1	5.8±0.1	5.4 ±0.1
0:1	7.0 ±0.1	6.9±0.1	7.0 ±0.1

2.4. Discussion

2.4.1. Plant Growth Regulator (Cytokinin)

The experiments were to verify the different shoot culture media (exp. 2.3.1.; 2.3.2.; 2.3.3.). The shoot culture media were different only in PGR.

Different potato genotypes response differently to different media. All genotypes response well to the media without cytokinin except for the genotype PAS 3063. This genotype produced good shoot only in media of Jarret *et al* (1980) and Goodwin *et al* (1980) that have cytokinin. The presence of cytokinin in media was inhibitory for shoot growth for the genotype Cipanas, DTO 28, and Katahdin. These cultivar produced good shoot in MS o media (no PGR) and medium of Roca *et al.* (1978) (GA_3 0.3 mg/l).

The presence and absent of cytokinins in shoot culture media did not affect the growth of shoot of Red Pontiac and Kennebec. These two cultivar produced shoot well in all four culture media namely MS O, Roca *et al.* 1978, Goodwin *et al.* 1980 and Jarret *et al.* 1980. Potato cultivar in these experiments can be differentiated due to their response of *In vitro* shoot production to cytokinin into three response type, namely: Cipanas response type (negative response), Red Pontiac response type (no response), and the PAS 3063 response type (positive response. The ineffectiveness of cytokinins (BA, Kinetin) to induce axillary shoot proliferation does not indicate that cytokinin in general is ineffective. Different cytokinin, different mixture of cytokinin, different concentration, and different method of application may give different result.

Cytokinins (BA, Zeatin, Kinetin) have been very effective in inducing shoot proliferation of potato callus derived from different explants namely: tuber (Lam 1975, Jarret *et al.* 1980), shoot (Marani and Pissi, 1977), leaf (Roest 1977), Cell (Cassel *et at.* 1987) and protoplast (Roest and Bokelmann 1983).

In general cytokinin overcome the inhibitory effect of terminal meristem on axillary bud growth (Sachs and Thiemann, 1964, 1967). The physiological basis as to why cytokinins are not effective in activating axillary shoot growth in potatoes is unknown, however several influences are apparent:

1. Potatoes (like tomatoes) have a very weak apical dominance, thus the necessity for stimulation fy exogenous control is less.
2.. Removal of the shoot tip of the explant eliminates the inhibiting effect of the apical meristem, thus further removing the need for exogenous stimulations.
3. The *in vitro* shoot used for explants may have high levels of endogenous cytokinin (non PAS 3063 response type). In such cases, addition of exogenous cytokinins can be expected to be inhibitory.

The toxic effect of an applied cytokinin (BA) on axillary shoot growth was demonstrated in *Solanum andigena* (Wang and Wareing 1979; Woolley and Wareing 1972). Woolley and Wareing (1972) treated single nodal cutting with BA. They found that only pretreated (exhausted from endogenous cytokinin) nodal cutting responded well to applied BA. Wang and Wareing (1979) treated non-pretreated nodal cutting with different concentration of BA. They found that applied BA inhibited shoot growth. The higher the concentration of BA, the less the shoot growth. BA at 10 mg/l was very inhibitory to shoot growth and 0.01 mg/l only slightly inhibitory. Wattimena (1983) also found that BA higher than 0.5 uM had inhibitory effect on *in vitro* shoot growth of the cultivar Red Pontiac and Norland.

2.4.2. Potato Extract

The potato extract of be 60 percent was inhibitory for *in vitro* shoot growth for all potato genotypes tested. The potato genotypes tested were Red Pontiac, Katahdin, Kennebec, Cipanas, DTO 8 and PAS 30633. Hussey and Stacey (1983) found that potto extract of 60 percent improved *in vitro* shoot growth for all the cultivar tested except for the cultivar Record. The cultivars tested in their experiment were: Arran Pilot, Majestic, King Edward, Ulter Sceptre, Red Graigs, Royal, Desiree and Record. Their cultivars that responded to potato extract developed more vigorous roots and greenish leaves.

It is not a fair comparison between our experiments with the experiments of Hussey and Stacey (1983) due to the cultivars difference. In addition it was hard to draw a conclusion from only one level of concentration of potato extract. Further experiments have to be done with different level of concentration of potato extract and from different cultivar.

2.4.3. Calcium Pantothenate

The optimum concentration level of Ca-pantothenate is this trial (exp. 2.3.4.) was 6 mg/l. This optimum level in combination with 0.5-1.0 mg/l benzyladenine produced the highest *in vitro* shoot. The potato cultivar used in this trial was the cytokinin response type (PAS 3063).

Ca-pantothenate 6 mg/l was the highest concentration level in this trial so the true optimum level of level concentration may lay beyond this concentration (6 mg/l). Sha *et al* (1985)found that to overcome the shoot tip necrotic of potato in culture need Ca-pantothenate at the concentration of 4 mg/l to 30 mg/l. In some tissue culture media Ca-paanthothenate was used as one of the vitamins at the concentration of 0.5 to 2.5 mg/l (pierik, 1987). Ca-pantothenate was found to stimulate tissue proliferation in Willow and Blackbane but it was not effective for carrot, vine, and Virginia creeper (George and Sherringon, 1984).

2.4.4. Static shallow layer liquid-liquid medium

In this rial (exp. 2.3.6) the response of potato genotype o static liquid medium can be classified in two type of response, namely the Cipanas response type and Katahdin response type. The Cipanas response type, the static liquid medium produced more shoot in each harvest and total of four harvest than agar medium even though the difference was not statistical significant. The reverse was true for the Katahdin response type. To this type of response the shoot produced at each harvest and total of four harvest were always greater in agar medium than in static liquid medium. Some of these difference were statistical significant. The shoot growth of all potato genotype in static liquid medium look normal and healthy with prolific roots. This static liquid medium was shallow,with the thickness of the liquid medium of less than one centimetres. This shallow layer of static liquid medium have also been practised for micropropagation of carnation, potato and *Lilium speciosum* (George and

Sherrington 1984). This trial showed that the static shallow layer liquid (S 2L2) medium look promising for *in vitro* nodal culture of potatoes. The S2L2 medium has been used now in our laboratory as standard method for potato micropropagation either for production of microshoots or microtubers. The advantage of S2L2 medium that is no need for agar and agitated apparatus (shaker).

2.4.5. Nitrogen: Source and Amount

The experiment 2.3.5.7. was to evaluate the best combination of NH_4NO_3 of Murashige and Skoog (1962) medium on *in vitro* shoot production in potato. To each level of NH_4NO_3 the optimum level of KNO_3 was calculated from the response curve. These calculated optimum combination of NH_4NO_3 - KNO_3 (in mM) were as follows: 5-25; 10-15.3; 15:16.7; 20-25 and 25-32.3. These combination had a variable amount of N, NH_4^+, NO_3^- and K^+, but a relative the same ratio of ammonium and nitrate. The ammonium nitrate ratio (NH_4^+ : NO_3^-) for all the combination was between 0.41-0.47 except for the treatment combination of 5-25 where the ammonium nitrate ratio was 0.17.

Under single source of nitrogen (experiment 2.2.8.) either ammonium or nitrate, the culture were able to survive only under the low nitrogen level (20 mM). The culture produced healthy and vigorous shoot only under the mixed source of nitrogen (ammonium and nitrate). The ammonium nitrate ratio of 1:3 produced more vigorous shoot than the ammonium nitrate ratio of 1;1 and 1:2. There was trend for each ammonium nitrate ratio (1:1; 1:2; 1:3) that the increased of the amount of nitrogen also increased the growth of *in vitro* shoot (table 2.8.3.-1.)

The pH of the medium changed due to the source of nitrogen (table 2.8.3.3). The pH changed drastically when the nitrogen consisted of single source; ammonium-N (NH_4-N) or nitrate-N (NO_3-). When the nitrogen source was NO_3-N the initial pH drop from $\pm$ 5.6 to $\pm$4.3 at the end of the experiments. The reverse was true when the nitrogen source was NH_4-N, the pH rose from $\pm$5.6 to $\pm$7.0 The pH of the culture medium was more stable when the nitrogen source consisted of NH_4-N

and NO_3-N. The stability of pH was increased with the decreased of NH_4 N: NO_3-N (1:1; 1:2; 1:3). The NH_4 N:NO_3 -N of 1:3 produced the most stable pH (table 2.8.3.3).

These data showed that the effect of nitrogen in the growth of potato culture was an indirect effect. The direct effect of nitrogen source was o the stability of the pH of the medium. The change of the pH of the medium determined the uptake and toxicity of nitrogen and other ions. Uptake of NO_3^- and NH_4^+ is pH sensitive. NH_4^+-N took place best neutral pH and it was depressed as the pH dropped. The reverse was true for NO_3^-absorption. NO_3^- was absorbed rapidly at low and neutral pH values and it was depressed as the pH increased (Rao and Rains, 1976). Both for of nitrogen; NH_4-N and NO_3-N, were absorbed at equal rate at pH of 6,8 (Michael *et al.*, 1965). The presence of both nitrogen source increased the rate of nitrogen uptake (Biondel and Blave, 1973, Cox and Reiseauer, 1973).

The presence of low NH_4-N and high carbohydrate status of the plant stimulated the reductio of nitrate (NO_3-NO_2-NH_3) (Kirkby ad Hughes, 1970). The presence of high nitrate (NO_3) and nitrite (NO_2) in tissue is toxic to plant (Megel and Kirkby, 1982). At higher pH or at very acid pH, the presence of NH_4-N as nitrogen source in the medium could be toxic to plant due to the presence of ammonium in aqueous solution (NH_3 (aq.)) (Bennett, 1974, Maynard and Barker, 1969).

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III. *In vitro* tuberization

3.1. Introduction

3.1.1. Non *in vitro* tuberization

The potato tuber is morphologically a modified stem with a shortened and broadened axis with rather poorly developed scale leaves (Slater, 1963). Tuber initiation and growth are the result of cell division and cell enlargement (Plaisted, 1957; Reeve *et al*, 1973). The first indication of tuber initiation is a thickening of the first internode behind the apical bud of the stolon (Plaisted, 1957). The continued growth of the tuber depends primarily on the expansion of internodes already present in the apical buds (Cutter, 1978). The expansion of internodes proceeds acropetally from proximal end to the distal end. The size of the tuber depends on the number of internodes that make up the tuberous portion of the apical axis (Cutter, 1978).

Tuberization in potato can be induced by short days, high light intensity, low night temperature, low nitrogen level, physiological old seed tubers, and any combination of these factors (Boadlander, 1963; Gregory, 1956, 1965; Slater, 1963, 1968; Steward *et al*, 1981; Sattelmachner, 1978a, b, c; Werner, 1935). The combinations of light and temperature that are naturally occurring in the potato growing areas in Indonesia are: short days, low temperature, and low light intensity during the rainy season and short days, low temperature and hight light intensity during the dry season.

The movements of the tuberization stimulus have been followed by grafting techniques. The root stock of non-induced plants can be tuberized by grafting a scion from induced plants (Chapman, 1958, Gregory, 1956, 1965; Kumar and Wareing, 1973). The other technique have been performed by reciprocal grafting of tolerant and non tolerant potato cultivars to high temperature and grown them at the high temperature environments (Adisarwanto, 1989). Such experiments have shown the existence of a hormone like tuberization stimulus and its translocation across the graft union.

The participation of hormones in tuberization have been investigated by analyzing endogenous hormones from induced and non induced plants (Forsline and Langille, 1976, 1976; Hammes and Nel, 1975; Mauk and Langille, 1976; Sattelmachner, 1978 a, b, c) and by application of exogenous growth regulators (Abdullah and Achmad, 1980; Adisarwanto, 1989; El-Antalbly *et al*, 1967; Gifford and Moorby, 1967; Hammes, 1971; Kumar and Wareing, 1974; Menzel, 1980). Cytokinins, gibberellin-like substances, and abscisic acid (ABA) are endogenous hormones that have been extracted from induced and non induced plants. Mauk and Langille (1978) identified the cytokinins in potato as zeatin ribosides and the level of cytokinins was higher in induced tissue than in non-induced tissue (Forsline and Langille, 1976a; Mauk and Langille, 1978; Sattelmachner, 1978a, b, c). The reverse was true for the gibberellin-like substances (Catchpole and Hillman, 1969; Chapman, 1955; Krauss and Marschner, 1982; Kumar and Wareing, 1974). No different was found in the level of abscisic acid (Krauss and Marschner, 1982; Kumar and Wareing, 1974).

Most of the exogenous applications of growth regulators have been applied in *in vitro* systems rather than to whole plants. Application to whole plants is complicated by many problems including absorption at the site of applications and translocation to the site of action. Gibberellic acid (GA), ABA, CCC are the most used for non *in vitro* system. In general application of ABA and CCC promote tuberization (Abdullah and Achmad, 1980; Adisarwanto, 1989; El-Antably *et al*, 1967; Gifford and Moorby, 1967; Hammes, 1971; Hammes and Nel, 1975; Kumar and Wareing, 1974), while GA inhibited tuberization (Abdullah and Achmad, 1980; Adisarwanto, 1989; Kumar and Wareing, 1974; Lovell and Booth, 197 4). However, Smith and Rappaport (1969) reported the ineffectiveness of ABA in promotion of tuberization. They used low concentrations of growth substance, while other workers generally used high concentrations (El-Antably *et al*, 1967; Hammes, 1971; Hammes and Nel, 1975). Recently, ethylene has been shown to induced tuberization but starch was very low or absent in the little tubers so induced (Catchpole and Hillman, 1969). The changes in endogenous hormones and carbohydrates during tuber initiation and development have also been studied. Cytokinin levels are high in tiny tubers and decline when tuber reach maturity (Okazawa, 1970). Gibberellin activity is high at the growing stolon tip and decreases rapidly at the stage just before tuberization (Smith and Rappaport, 1969).

3.1.2. *In Vitro* tuberization

Potato tuberization is not restricted to the underground stem (stolon); every axil of the plant can produce tubers. This character has been manipulated to produce *in vitro* tubers. Morphologically tubers found *in vitro* are the same as other tubers. *In vitro* tubers can be sessile on the nodes of explant stem (Catchpole and Hillman, 1969; Mes and Menge, 1954) or can be axillary or terminally formed on new growing shoots (Barker, 1953; Hussey and Stacey, 1981; Palmer and Smith, 1972; Wang and Hu, 1982; Wattimena, 1983). The size of *in vitro* produced tubers are usually one centimetre or less (Stallknecht and Farnsworth, 1982; Wattimena, 1983). For this reason Wattimena (1983) have termed *in vitro* produced tubers as 'microtuber'.

Wattimena (1983) has used *in vitro* method of tuberization for the production of microtubers while most of the *in vitro* method of tuberization has been used mainly for tuber initiation studies. The explant tissue has varied widely and has included induced and non induced nodal cutting (Catchpole and Hillman, 1969, Chapman, 1958; Forsline and Langille, 1976; Harmey *et al*, 1966), nodal cutting of young sprout (Barker, 1953; Chapman, 1955; Garcia-Tarres and Gomez-Campo, 1973; Tizio and Biain, 1973), excised stolons (Lawrence and and Barker, 1963; Smithand Rappaport, 1969). *in vitro* apical stolon (Mauk and Langille, 1978;Mingo-castel *et al*, 1974; Obata-Sasamoto and Suzuki, 1979; Palmer and Bakker, 1973; Palmer and Smith, 1969a, b, 1970; Stallknecht and Farnworth, 1974, 1980a, b) and *in vitro* stem cutting (Anonymous, 1977; Hussey and Stacey, 1981; Wang and Hu, 1982; Wattimena, 1983).

These explant tissues were used to evaluate factors involved in the tuberization process such as the inducing stimulus (Catchpole and Hillman, 1967; Forsline and Langille, 19776b; Lawrence and Barker, 1963), Carbohydrate level (Catchpole and Hilman, 1967; Harmey *et al*, 1966; Palmer and Smith, 1970), nitrogen level (Stallknecht, 1972; Stallnecht and Farnsworth, 1979; Wattimena, 1983), light and temperature (Chapman, 1955, 1958; Lawrence and Barker, 1963), carbon dioxide (Mingo-Castel *et al*, 1974; Obata-Sasamoto and Suzuki, 1979b) exogenous growth regulators (Forsline and Langille, 1976b; Mauk and Langille, 1978; Mingo-Castel *et al*, 1974, 1976; Palmer and

Smith, 1969a, B; Parrot, 1975; Smith and Rappaport, 1969; Tizio and Biain, 1973; Wattimena, 1983). starch and starch relating enzymes (Mingo-Castel *et al.* 1974; Obata-Sasamoto and Suzuki, 1979b; Palmer and Bakker, 1973; Palmer and Smith, 1969b).

The type of tissue used depends on the suitability of that tissue to a particular study. *In vitro* apical stolons were suitable for the study of the exogenous growth regulator requirements, carbohydrate accumulations and their related enzymes (Forsline and Langille, 1976b, Harmey *et al*, 1966, Mauk and Langille, 1976b; Mingo-Castel *et al*, 1976; Parrot, 1975; Smith and Rappaport, 1969; Tizio and Biain, 1973), while inducing and neon inducing nodal stem cuttings were suitable for evaluating the tuberization stimulus (Catchpole and Hillman, 1969; Forsline and Langille, 1976b; Lawrence and Bakker, 1973).

A variety of basal media (Chapman, 1955; Forsline and Langille, 1976b; Mes and Menge, 1954; Stalknecht, 1972; Wang and Hu, 1982; Wattimena, 1983) have been used for *in vitro* tuberization studies such as described by White (1973), Nitsch (1951), Katsura *et al* (1970) and Murashige and Skoog (1962). White's medium is a low salt medium while Murahige and Skoog's medium is a high salt medium. White used only nitrate as a nitrogen source, while Murashige and Skoog used both nitrate and ammonium. The amount of nitrogen in Murashige and Skoog (60 mM N/l) is 24 times nitrogen in White's medium (2.5 mM N/l).

Carbohydrate concentration in medium appears to be the most important organic nutrient variable effecting rate and percentage of tuberization. The concentration used for testing optimum doses ranged from no sucrose to 20% sucrose (Catchpole and Hillman, 1969; Lawrence and Barker, 1963; Okazawa, 1967; Palmer and Smith, 1970, Stallknecht and Farnsworth, 1979; Wang and Hu, 1982; Wattimena, 1983). Response to these concentrations depended on the stage of induction. Highly induced tissue can be tuberized at lower sucrose concentrations while non-induced tissue can not ever be tuberized at the highest sucrose concentrations tested (Palmer and Bakker, 1970). Increased sucrose concentration increased the percentage and earliness of tuberization of induced tissue. The optimum sucrose concentration range was between 6% to 8% (Catchpole and Hillman, 1969; Law-

rence and Barker, 1963; Palmer and Bakker, 1970; Stallknecht and Farnsworth, 1979, 1982a, Wang and Hu, 1982; Wattimena, 1983). Levels higher than 18% are strongly inhibitory (Stallknecht and Farnsworth, 1979) and at 20%, tuberization was completely inhibited (Catchpole and Hillman, 1969). As a source of carbohydrate, sucrose can be substituted by other sugars such as fructose, glucose and maltose (Mes and Menge, 1954; Okazawa, 1967).

Effect of nitrogen on *in vitro* tuberization has been studied by Okazawa (1967), Stallknecht and Farnsworth (1979) and Wattimena (1983). Okazawa compared tuberization of induced basal sprout tissue in medium with no nitrogen and with 250 ppm nitrogen. With no nitrogen tuberization was rapid in both 2% and 8% sucrose. At 250 ppm nitrogen, tuberization was limited in 2% sucrose but rapid in 8% sucrose. Stallknecht and Farnsworth (1979) and Wattimena (1983) found that low nitrogen (2.5 and 4.0 mM N/l) in both the explant medium and tuberization medium was best for coumarine-induced tuberization. Farnsworth (1979) found that no tubers were formed if high nitrogen (60 mM N/l) was present in both media, while Wattimena (1983) found that high nitrogen (60 mM N/l) influence tuberization only if present in tuberization medium. Farnsworth (1979) also found inter action of nitrogen and sucrose at intermediate levels of nitrogen (15 mM N/l). Inhibition of coumarin-induced tuberization at intermediate levels of nitrogen can be overcome by 12% sucrose but not by 6% sucrose. Kinetin and BA induced tuberization was not inhibited with high levels of nitrogen as in coumarin-induced tuberization (Palmer and Smith. 1969b, 1970; Wang and Hu, 1982; Wattimena, 1983).

The optimum temperature range for *in vitro* tuberization appears quite large. The experiments were run on a constant day and night temperature or alternating high day and low night temperature (Hussey and Stacey, 1981; Lawrence and Barker, 1963; Mes and Menge, 1954; Okazawa, 1967; Palmer and Smith, 1970; Stallknecht and Farnsworth, 1982b; Wang and Hu, 1982;Wattimena, 1983). The constant optimum temperature was better than alternating high day and low night temperature (Wang and Hu, 1982). The optimum range from 15 °C to 26 °C (Hussey and Stacey, 1981; Lawrence and Barker, 1963; Okazawa, 1967; Palmer and smith, 1970; Wang and Hu, 1982; Wattimena, 1983). At temperature lower than 12 °C or higher than 28 °C tuberization was strongly inhibited (Okazawa,

1967; Palmer and Smith, 1970; Wang and Hu, 1982; Wattimena, 1983). No tubers were formed at either 30 °C or 35 °C (Palmer and Smith, 1970; Stallknecht and Farnsworth, 1982a). Most *in vitro* tuberization experiments using *in vitro* apical stolons as the explant tissue were at a constant temperature of 25 °C (Hussey and Stacey, 1981; Mauk and Langille, 1978; Mingo- Castel *et al*, 1974, 1976; Palmer and Smith, 1969a, b, 1970).

Different light requirements for *in vitro* tuberization have been reported. These requirements vary from continuous dark (Barker, 1953; Forsline and Langille, 1976b; Smith and Rappaport, 1969; Wattimena, 1983), alternating continuous light and dark (Chapman, 1955; Tizio and Biani, 1973) to continuous light (Hussey and Stacey, 1981; Wattimena, 1983). Chapman (1955) reported that tuberization was better if the culture tissue was incubated for four weeks in continuous light at 21 °C to 26 °C and then transferred to continuous dark at 16 °C for several days. Others have found that prior incubation of the cultured tissue for several days in diffuse light (400 lux) followed by transfer to continuous dark for the rest of tuberization was best (Chapman, 1955; Tizio and Biani, 1973). Lawrence and Barker (1963) found that their culture could be tuberized only under continuous dark interrupted with low light. No tubers were produced under 8, 16, and 24 hour photoperiods. However Wang and Hu (1982) found that 8 hour photoperiods were better than 16 hour photoperiods and low light intensity (100 lux) was better than hight light intensity (400 lux). Hussey and Stacey (1981) observed from their *in vitro* shoot culture that after four month incubation period, only cultures under 16 and 24 hour photoperiods were forming tubers. Wattimena (1983) found than *in vitro* tuberization was faster under continuous dark than under continuous light.

Carbon dioxide (CO_2) has been reported to play an important role in *in vitro* tuberization. Mingo-Castel *et al* (1974, 1976) found that exposing cultures for at least five days to CO_2 at 8% to 10% can induce tuberization *in vitro* cultured stolons. They also found that the total absence of CO_2 is detrimental for *in vitro* tuberization and that a certain level of CO_2 is required for *in vitro* tuberization.

The effect of exogenous growth regulators on *in vitro*, tuberization was quite variable due to different types of tissue used as mentioned previously. Type of explant tissue used determined the amount of endogenous hormones and thus the response to exogenously applied growth regulators can be grouped into auxin, gibberellic acid, cytokinin, growth retardants, inhibitors and ethylene.

The auxin applied *in vitro* tuberization experiments are indole acetic acid (IAA), 2-naphthalene acetic acid (NAA) and 2,4-dichlorophenoxy acetic acid (2,4-D). The response to IAA, 2,4-D and NAA on *in vitro* tuberization depended on concentration. At lower concentration (1 ppm or less) induction is observed (Harmey *et al*, 1966; Okazawa, 1967; Stallknecht and Farnsworth, 1982a; Tizio and Biain, 1963). At higher concentration, NAA and IAA were less effective in inducing *in vitro* tuberization, Stallknecht and Farnsworth (1982a) also found that IAA at 25 ppm and NAA at 1 ppm completely inhibited coumarin induced tuberization.

Different from auxin regulators. GA's do not have any stimulatory concentration for *in vitro* tuberization. GA has been found to have an inhibiting effect on *in vitro* tuberization even at low concentration (Garcia-Tarres and Gomez-Campo, 1973; Palmer and Smith, 1970; Stallknecht and Farnsworth, 1982b). GA at 2.5 ppm completely eliminated the *in vitro* tuberization induced by coumarin (Stallknecht and Farnsworth, 1982b).

Cytokinin has the reverse effect of gibberellic acid. All applied exogenous cytokinin induced *in vitro* tuberization (forsline and Langille, 1976b, Mauk and Langille, 1978; Mingo-Castel *et al*, 1974, 1976; Palmer and Bakker, 1973; Palmer and Smith, 1969a, b; Stallknecht and Farnsworth, 1982a; Tizio and Biain, 1973; Wang and Hu, 1982; Wattimena, 1983). The induced concentration depended on the kind of cytokinin and the explant tissue. The optimum doses of less potent cytokinins such as kinetin for *in vitro* tuberization is about twice as much as the more potent compound such as zeatin riboside (ZR) and 6-benzyladenin (BA). The optimum dosage for kinetin using non *in vitro* shoot explant is about 5 ppm (Mingo-Castel *et al*, 1974, 1976; Palmer and Bakker, 1973; Palmer and Smith, 1969b, 1970; Tizio and Biain, 1973) while for *in vitro* shoot explant is about 10 ppm (Wattimena, 1983). The optimum dosage for ZR and BA using non *in vitro* shoot explant is about 3 ppm (Muak and

Langille, 1978; Palmer and Smith, 1969b), The optimum dosage of BA using apical stolons and in *in vitro* cutting as explant tissue was 2.5 ppm and 10 ppm, respectively (Mauk and Langille, 1978; Wang and Hu, 1982; Wattimena, 1983). Kinetin has also been reported to increase the coumarin-induced *in vitro* tuberization (Stallknetch and Farnsworth, 1982a). Many kinds of growth retardants and growth inhibitors have use for *in vitro* tuberization studies. These compounds included: ABA, CCC, Maleic hydrazide (MH), Alar, Amo-1618, and coumarin. ABA has been inhibitory in *in vitro* tuberization studies (Palmer and Smith, 1969a; Smith and Rappaport, 1969)_. Even kinetin-induced tuberization was completely inhibited by ABA (Palmer and Smith, 1969a). CCC and MH were found to induce *in vitro* tuberization (Parrot, 1975; Tizio and Biain, 1973). On the other hand, Palmer and Smith (1969a) reported that CCC and Amo 1618 have the same reversing effect on kinetin-induced tuberization as ABA. Stallknecht and Farnsworth (1982a) found that CCC and Alar do not effect coumarin-induced tuberization. Coumarin tuberization was faster than kinetin tuberization (Stallknecht, 1972; Stallknecht and Farnsworth, 1979, 1982a; Wattimena, 1983). Coumarin-induced tuberization required both low nitrogen in the explant medium and in the tuberization medium (Stallknecht and Farnsworth, 1979(. Inhibitors of protein and nucleic acid significantly inhibited the coumarin-induced tuberization (Stallknecht and Farnsworth, 1982b), while no comparable inhibiting effect on kinetin-induced tuberization was observed (Palmer and Smith, 1969a). Stallknecht and Farnsworth (1982b) suggested that the mode of coumarin induction is distinctly different from that of kinetin.

The importance of ethylene in *in vitro* tuberization has also been studied. Ethylene was not only ineffective in inducing *in vitro* tuberization (Palmer and Bakker, 1973) but also inhibitory to the inducing effect of kinetin and CO_2 (Mingo-Castel *et al*, 1974, 1976). Ethylene has been applied as either ethylene gas (Mingo-Castel, *et al.* 1974, 1976) or as water soluble ethylene-releasing compound such as 2-chloroethyl phosphoric acid (CEPA, Ethrel)(Palmer and Bakker, 1973).

Starch accumulation and the related enzymes has been studied for *in vitro* stolons treated with kinetin (Mingo-Castel *et al*, 1976; Obata-Sasamoto and Suzuki, 1979b; Palmer and Bakker, 1972, 1973; Palmer and Smith, 1969b). Kinetin-treated stolons accumulated starch at stolon tip prior to

tuberization but not in control or CEPA-treated stolons. Reducing sugars decreased in kinetin-treated stolon but not in control (Palmer and Bakker, 1973). The decrease in reducing sugars coincided with an increase of starch synthesis (Palmer and Bakker, 1973).

The importance of factors inducing *in vitro* tuberization andthe mechanism of *in vitro* tuberization have been reviewed. This has shown that the following factors are particularly important: temperature, photoperiod, sugar, nitrogen, cytokinin, growth retardants and inhibitors, and the synergistic effect or additive effect of growth regulators. Thus, many factors appear to influence tuberization. The application of these factors on maximization of *in vitro* microtuber production was studied.

3.2. General methode of *in vitro* tuberization

3.2.1 Source of explant

Auxillary shoot of *in vitro* virus free stock derived from cultivars ; Red pontiac, Katahdin, Cipanas, DTO 28 and Kennebec are used all *in vitro* tuberization experiments. Initiial cultures were abtained firm University of Wisconsin in 1986 and propagated continously in tissue culture laboratory of Bogor Agryculture University (IPB).

In vitro shoots were cut into 2 node segment and culture in tuberization media for the direct method of tuberization or cultured in shoot multiplication media for the indirect method of tuberization method *in vitro* shoot derived from these 2 node segment were tuated later with various tuberization media (indirect method).

3.2.2 Culture media

There were 2 types of culture media in the experiments namely shoot multiplication and tuberization media. Basal composition of Murashige and Sloog (1962) supplemented with tiamin - HCl,

pyridoxine-HCl, nicotinic acid, Ca - panthotenate and glycerine was used both in shoot multiplication as well as tuberization media. For tuberization media other component were added according to their treatment combination. The media components tasted as factors in *in vitro* tuberization experiments level of sucrose, type and level of cytokinin, type and level of retardants, level of coconut water, level of coumarin, and level of nitrogen on addition the pH and tuberization methods were also tested. The media preparation was the same as in section 2.2.1 *in vitro* shoot experiments.

3.2.3. *in vitro* tuberization method

There were 5 *in vitro* tuberization method tested in these series of experiments.

1. Direct method: One - step tuberization in solid media, explants derived from *in vitro* axillary shoot were planted in solid tuberization media.
2. Direct method: On - steptuberization on liquid media explants derived from *in vitro* axillary shoot were culture in static shallow liquid tuberization media.
3. Indirect method: Two - step tuberization method using double layer technique.
   Explants derived from *in vitro* axillary shoot were grown in solid shoot multiplication media for 4. weeks. After 4 weeks, liquid tuberization medium was added to original culture bottle resulting in double layer system.
4. Indirect method: Two - step tuberization method liquid - liquid by substitution
   Explant were planting in liquid multiplication medium. After 4 weeks original shoot multiplication media was added to the cultures .
5. Indirect method: Two - step tuberization in shallow layer liquid -liquid media .
   Explant were grown their layer of liquid multiplication media. After 4 weeks of the time culture used up all multiplication medium and a new liquid media were added to the culture to form a new shallow layer.

3.2.4. Incubation environment and observed variables.

In vitro shoot cultures were incubated for 4 weeks in light and at the temperature of 22 - 26° C. Lighting was provided by inflorescent lamp at the intensity of 1000 - 2000 lux. Illumination was given with the cycle of 16 hours light and 8 hours dark. *in vitro* shoots cultures were incubated for 8 weeks in dark and at the room temperature of 18 - 20 ° C.

In shoot multiplication studies shoot were observed weekly for 4 weeks . In *in vitro* tuberization studies weekly or fortnightly reservation were carried out for earliness of tuberization: number of tubers and percentage of tuberization . The micotuber were harvested at 8 weeks and the measured variables consisted of: numbers of tubers, tuber size, tubers fresh weight, tuber dry weight and percentage of dry weight.

3.2.5. The design and analysis of the experiment.

The factors affect *in vitro* tuberization and their approximate level have been known or can be approximate determined. The experimental design for these experiments must be able to find the optimum level and combined all these factors at their optimal level at the verification experiments.

To meet these objectives the experimental approach is a two factor factorial design step wise two by two at a time until all the factor have been tasted. When the optimum level of all the factor have been determined the next series of the experiments are the evaluation and verification of all these factors at their optimum level.

Each culture vessel / bottle is considered as an experimental unit. Due to the problem of contamination each treatment consist of 20 to 25 bottles . The data are collected only from the uncontaminated culture vessel. The count data and the percentage data are transformed before analysing statistically while the measured data are analysing without transformation. The optimum level of each factors tested are determined by orthogonal polynomial methods

3.3.1. Effect of Coumarin, Cytokinin, and Retardant on *in vitro* tuberization

3.3.1.1. Introduction

The objective of the experiment was to evaluate the effect of coumarin, cytokinin, and retardant on *in vitro* tuberization on solid system of tuberization.

3.3.1.2. Methods

The experiment was a factorial experiment consisted of two factors, the cytokinins and the growth retardants. The cytokinins consisted of 3 levels namely: kinetin 10 mg/l, BA 5 mg/l, benomyl 50 mg/l. There were 4 levels of retardants as follows: Alar (B-9) 5 mg/l, ancymidol 5 mg/l, cycocel 500 mg/l and paclobutrazol 5 mg/l. The 12 treatment combination were arranged in Completely Randomized Design with 11 replication. All the treatments combination were combined with coumarin 25 mg/l.

The treatment combination were as follows:

KJ1= C0C0R1	KJ5= C0C2R1	KJ9 = C0C3R1
KJ2= C0C1R2	KJ6= C0C2R2	KJ10= C0C3R2
KJ3= C0C1R3	KJ7= C0C2R3	KJ11= C0C3R3
KJ4= C0C1R4	KJ8= C0C2R4	KJ12= C0C3R4

C0 = Coumarin 25 mg/l
C1 = Kinetin 10 mg/l
C2 = Benzyl adenin 5 mg/l
C3 = Benomyl 50 mg/l
R1 = Alar (B-9) 5 mg/l
R2 = Ancymidol (A-Rerst) 5 mg/l
R3 = Cycocel (CCC) 500 mg/l
R4 = Paclobutrazol 5 mg/l

The shoot propagation medium consisted of MS mineral salts,MS vitamins, sucrose 3% and agar 0.8%. The tuberization medium consisted of MS mineral salts, MS vitamins, sucrose 6%, agar 0.8% and growth regulators according to the treatment combination. The shoot cultures were incubated

for 4 weeks at the temperature of 20 - 25 °C, light intensity of 200 lux and a photo period of 16 hours. The tuberization cultures were incubated at the same room but in the dark.

3.3.1.3. Result

The result of experiment were presented in table 3.3.1.1 3.3.1.2 and 3.3.1.3. The table in table 3.3.1.1 to table 3.3.1.3

Showed that:

1. Not all treatments combination could induced a hundred percent of tuberization at 5 weeks of incubation period only 4 treatments were able to induce a 100 percent of tuberization. These treatments combination were: (a) coumarin 25 mg/l + kinetin 10 mg/l + cycocel 500 mg/l; (b) coumarin 25 mg/l + BA 5 mg/l + ancymidol 50 mg/l: (c) coumarin 25 mg/l + BA 5 mg/l + cycocel 500 mg/l; coumarin 25 mg/l + BA 5 mg/l + paclobutrazol 5 mg/l.

2. The treatment combination of:
   (a) coumarin 25 mg/l + kinetin 10 mg/l + cycocel 500 mg/l produced the largest numbers of tuber per culture vial (4 tubers/vial)
   (b) coumarin 25 mg/l + kinetin 10 mg/l + paclobutrazol 5 mg/l produced the lowest numbers of tubes per culture vials.

3. Coumarin 25 mg/l + BA 5 mg/l + ancymidol 5 mg/l produced the:
   (a) largest size micro tubers (length of 6.5 mm, diameter of 4.9 mm).
   (b) Biggest tuber fresh weight (112.0 mg/microtuber)
   (c) Biggest tuber dry weight (1.9 mg/micro tuber)

4. The precentage of tuber dry matter was low for all treatmentc combination range 9.3% to 12.9%.

Table 3.3.1.1. The effect of Coumarine, Cytokininsand growth retardants on percentage of *in vitro* tuberization

Treatments	Weeks of Incubation (WOI)					
	3 WOI	5WOI	7 WOI	9 WOI	11 WOI	13 WOI
KJ1	20.0	66.7	93.3	92.9	85.7	92.3
KJ2	13.6	100.0	100.0	100.0	94.4	93.3
KJ3	78.6	100.0	100.0	100.0	100.0	100.0
KJ4	30.8	53.9	69.2	61.5	46.2	46.2
KJ5	53.3	80.0	100.0	100.0	100.0	100.0
KJ6	66.7	100.0	100.0	100.0	100.0	100.0
KJ7	93.3	100.0	100.0	100.0	100.0	100.0
KJ8	56.3	75.0	100.0	100.0	100.0	100.0
KJ9	60.0	60.0	86.7	80.0	93.3	93.3
KJ10	0.0	40.0	93.3	86.7	86.7	86.7
KJ11	33.3	73.3	86.7	93.3	93.3	93.3
KJ12	53.3	80.0	86.7	86.7	86.7	86.7

Table 3.3.1.2. The effect of Coumarine, Cytokinins and retardantson the numbers of tubers per culture vials. (V x + 0.5)

Trear-ments	Weeks of Incubation (WOI)					
	3 WOI	5 WOI	7 WOI	9 WOI	11 WOI	13 WOI
KJ1	0.848a	1.331ab	2.086b	2.120c	1.709bc	1.836cb
KJ2	1.559c	1.788bc	2.256b	2.222c	2.008c	1.737cb
KJ3	1.532c	2.015c	2.272b	2.312c	2.133c	1.937cb
KJ4	0.913a	1.081a	1.422a	1.209a	1.150a	1.013a
KJ5	0.993ab	1.272ab	1.915b	1.805cb	1.644bc	1.654cb
KJ6	1.069ab	1.478ab	2.200b	2.042cb	1.720bc	1.667cb
KJ7	1.413bc	1.726bc	2.220b	2.203c	1.931bc	1.906cb
KJ8	1.040ab	1.441ab	2.021b	1.913cb	1.704bc	1.495b
KJ9	1.119ab	1.257ab	1.998b	1.904cb	1.918bc	2.012c
KJ10	0.707a	1.009a	1.737b	1.635b	1.430ab	1.624cb
KJ11	0.954a	1.394ab	2.156b	2.226b	2.047c	1.943cb
KJ12	1.113ab	1.535abc	2.230b	2.222c	1.919bc	1.671cb

The same letter at the same columns were not significant different at 5% HSD Test

Table 3.3.1.3. The effect of Coumarin, Cytokinins and retardants on tuber length, tuber diameter, tuber fresh weight, tuber dry weight and percentage of tuber dry weight. Harvest at 13 weeks at incubation period

Treat-ments	Length (mm)	Diameter (mm)	Fresh weight (mg)	Dry weight (mg)	% of dry matter
KJ1	4.425a	3.294a	37.06a	0.755ab	9.6
KJ2	5.017a	3.586a	48.66a	1.107ab	10.2
KJ3	4.622a	3.500a	38.37a	1.269ab	11.8
KJ4	4.400a	3.300a	26.22a	0.408a	9.5
KJ5	5.800a	3.800a	48.74a	0.742ab	9.3
KJ6	6.519a	4.923b	112.00b	1.900b	11.2
KJ7	5.819a	4.194ab	69.10a	1.474ab	12.9
KJ8	4.500a	3.225a	31.11a	0.659ab	11.9
KJ9	4.679a	3.513a	49.04a	1.035ab	9.6
KJ10	5.058a	3.942a	54.87a	0.897ab	12.7
KJ11	5.135a	4.297ab	62.33a	0.896ab	10.3
KJ12	6.077a	4.077ab	63.56a	1.237ab	10.0

The same letter at the same columns were not significant different at 5 % HSD Test

3.3.2. Effect of benzyladenine and sucrose on micro tuber production of potato

3.3.2.1. Introduction

The objection of the experiments was to evaluate the effect of B.A. and sucrose on *in vitro* micro tuber production of potato of the cultivar Cipanas.

3.3.2.2. Methods

The experiment was a factorial experiment of two factors. The factors and their level were as follows: (a) B.A.: 1.25, 2.5, 3.75. 5.0 mg/ml and (b) sucrose: 70, 80, 90 g/l. The 12 treatments combination were arranged in a Completely Randomized Design with 9 replication. The method of tuberization was a liquid-liquid system of tuberization.

The shoot medium consisted of MS mineral salts, MS vitamines and organic, Ca-panthotenate 2 mg/l, sucrose 3% and ancymidol 0.1 mg/l. The tuberization medium consisted of MS salts, MS vitamins and organic, Ca-panthotenate 2 mg/l, cycocel 600 mg/l, sucrose and benziladenin were added according to treatments combination. The incubation periods and environments for both shoot and tuberization cultures were the same as described in general method in 3.2.-

3.3.2.3. Results

The results of the experiment were presented in table 3.3.2.1

The data in table 3.3.2.1 showed that:

1. Numbers of microtubers per culture bottle

   The sucrose concentration 80 g/l gave the best response of numbers of microtubers produced per bottle at B.A. concentration of 1.25; 2.25, and 5.0 mg/l. At B.A. concentration of 3.75 mg/l the best sucrose concentration was 70 g/l.

2. Microtubers diameter (size)

   At B.A. concentration at 3.75 mg/l, the sucrose concentration (70, 80, 90 g/l) did not effect the microtuber size. The best sucrose concentration that produced the bigger size microtubers for BA 1.25; 2.5; 5.0 mg/l was 90, 80, and 90 g/l respectively.

3. The biggest fresh weight of microtubers produced by BA of 1.25, 2.50, 3.75 and 5.0 mg/l was at the sucrose concentration of 80, 80, 70 and 90 g/l respectively.

4. There were a tendency that for all the BA level of concentration the higher the concentration the higher the percentage of dry weight of microtubers.

Table 3.3.2.1. Effect of benzyladenine and sucrose on numbers, size, fresh weight and percentage of dry weight of microtubers of potato the cultivar Cipanas.

Treatments B.A. mg/l Sucrose g/l	Microtubers Number	Diameter (mm)	Fresh Weight (g)	Percentage dry weight (%)
1.25				
70	8.00 b	3.68 a	0.062 c	14.37
80	9.67 c	4.67 ab	0.065 c	14.55
90	8.56 b	6.33 c	0.100 e	15.83
2.50				
70	8.67 bc	4.00 a	0.060 bc	14.64
80	10.22 d	5.00 b	0.079 d	15.00
90	6.33 a	4.22 ab	0.058 b	15.09
3.75				
70	9.67 c	4.44 ab	0.078 d	15.09
80	7.89 ab	4.33 ab	0.043 ab	15.25
90	8.11 b	4.22 ab	0.062 c	15.55
5.00				
70	9.67 c	4.89 b	0.064 c	14.09
80	9.33 c	4.00 a	0.037 a	15.22
90	6.33 a	5.22 bc	0.077 d	15.42

The same letter at the same column indicate no significant differensce as determinated by HSD 5%.

3.3.3. The effect of benzyl adenine (B.A) and sucrose on the production of potato microtubers of PAS 3063.

3.3.3.1. Introduction

The objective of this experiments was to find the best combination of benzyl adenine and sucrose on *in vitro* tuberization of potato of PAS 3063.

3.3.3.2. Methods

The experiments consisted of two factors. The first factor was BA with 3 level of concentration namely 1,2,3 ppm. The second factors was sucrose with 2 level of concentration namely 40 and 80

mg/liter. The experiment was run in a completely Randomyzed Designs with 10 replicates. The tuberization methods was direct method in a agar solidified medium. The micrc tubers were harvested at 8 weeks after treatment.

3.3.3.3. Results.

The result were presented in table 3.3.3.1. Table 3.3.3.1. showed that:

1. There was no interaction betwen BA and sucrose.
2. There were no difference in the nur ıber of microtuber with different level of BA concentration.
3. The sucrose concentration of 40 gr/liter produced significantly more tubers than 80 gr / liter.

Table 3.3.3.1. The effect of BA concentration on number and fresh weight of microtubers.

Treatment	Average number of microtuber per vial	Average tuber fresh weight (g)
Benzyl adenine (mg / liter)		
1	3.65 a	0.142 a
2	2.95 a	0.132 a
3	3.25 a	0.127 a.
Sucrose (gr / liter)		
40	3.61 b	0.10 a
80	2.97 a	0.17 a.

The same letter in a column for each factors indicates no significant different at 5 % level of HSD test.

3.3.4. Effect of ancymidol, B-9 (alar), and cycocel on *in vitro* microtuber production

3.3.4.1. Introduction

The objection of this experiments was to evaluate the appropriate concentration of ancymidol, alar and cycocel to induce *in vitroo* tuberization.

3.3.4.2. Methods

Each growth retardants was run as a single experiment, so there were 3 seperate experiments. The experiments and their treatments (level of concentration) were as follows:

- experiment 1 : Ancymidol (0, 0.5, 1.0, 2.0, 4.0, 8.0, 16.0 and 32.0 mg/l)
- experiment 2 : Alar (0.0, 0.25, 0.50, 1.0, 2.0 and 4.0 mg/l)
- experiment 3 : Cycocel (CCC) (0, 100, 200, 400, and 600 mg/l)

Each of these experiments was arrange.. in a Completely Randomized Designs with 15 replication.

The tuberization method used in these experiments was a direct solid system of tuberization. The shoot propagation medium consisted of MS salts, MS organic, Ca-panthotenate 2 mg/l, GA_3 0.3 mg/l, NAA 0.01 mg/l, sucrose 3% and agar 0.8%. The tuberization medium consisted of MS salts, MS organic, Ca-panthotenate 2 mg/l, sucrose 6%, agar 0.8% and the concentration of growth retardants was applied according to treatment combination.

The shoot propagation cultures were incubated at a temperature of 22-25 °C, light intensity of 1500-2000 lux and a photoperiods of 16 hours. The tuberization cultures were incubated at same room, without light. The shoot propagation cultures were incubated for 6 weeks and a tuberization cultures for 12 weeks.

3.3.4.3. Results

The result of these experiments were presented at table 3.3. 4.1 and table 3.3.4.2.

Table 3.3.4.1. The numbers of microtubersharvested per bottle

Retardants	Cultivars					
(mg/l)	RP	RB	KE	KA	K	NP
Ancymidol						
0.132	3.0 a	3.2 b	4.4 b	2.4 b	2.4 b	3.0 b
0.264	4.6b	3.8 b	5.2 bc	2.4b	2.4 b	4.8 c
2.64	5.2 c	4.8 c	5.4 c	2.6 b	2.8 b	2.5 b
26.4	2.0 a	1.2 a	1.8 a	1.2 a	1.4 a	1.4 a
Alar						
0.5	5.0 b	4.8 ab	5.6 a	4.2 a	5.8 b	5.8 b
1.0	6.2 b	5.2 bc	6.2 bc	5.6 b	5.8 b	5.6 b
10.0	6.4 b	6.8 c	7.8 c	6.6 c	5.2 b	6.0 b
100.0	3.6 a	4.2 a	4.8 a	3.6 a	3.2 a	3.4 a
Cycocel						
200	5.0 a	5.0 a	4.4 a	4.4 a	4.0 a	4.4 a
400	5.6 a	5.8 a	5.8 b	5.2 a	5.4 b	5.2 a
600	7.8 b	7.0 b	7.0 c	6.2 b	6.4 c	6.8 b

The same letters at the same column of each growt retardants denotes no significant different at 5 % of HSD Test. RP = Red Pontiac; RB = Russet Burbank; KE = Kennebec;KA = Katahdin; NK = Nooksack; NP = Norchip

Table 3.3.4.2. Average micro tuber fresh weights (grams)

Retardants (mg/l)	Cultivars RP	RB	KE	KA	NK	NP
Ancymidol						
0.132	0.0770 b	0.0752 b	0.0720 b	0.0870 c	0.1648 b	0.0754 a
0.264	0.1024 c	0.1084 c	0.1802 c	0.0949 c	0.1428 b	0.1002 c
2.64	0.0828 b	0.0590 b	0.0572 a	0.0548 b	0.0594 a	0.0498 a
26.4	0.0344 a	0.0316 a	0.0720 b	0.0284 a	0.0350 a	0.0320 a
Alar						
0.5	0.0494 a	0.0522 a	0.0800 b	0.0356 a	0.0562 a	0.0650 a
1.0	0.0632 a	0.0654 a	0.0630 a	0.0598 a	0.0614 a	0.0858 b
10.0	0.1016 b	0.1116 b	0.0970 b	0.0994 b	0. 216 b	0.1244 c
100.0	0.0492 a	0.0732 a	0.0640 a	0.0592 a	0.0580 a	0.0804 b
Cycocel						
200	0.0514 a	0.0534 a	0.0618 a	0.0558 a	0.0638 a	0.0614 a
400	0.1022 b	0.1042 b	0.1128 b	0.1124 b	0.1088 b	0.0948 b
600	0.0996 b	0.0938 b	0.0980 b	0.0948 b	0.0806 b	0.0770 b

The same letters at the same column of each growth retardants denotes no significant different at 5 % of HSD Test. RP = Red Pontiac; RB = Russet Burbank; KE = Kennebec; KA = Katahdin; NK = Nooksack; NP = Norchip

The data from the table showed that:

1. Earliness of tuberization

   Ancymidol showed an early tuberization than cycocel and Alar. Each retardants at their best concentration, ancymidol (8 mg/l) showed a 100 % of tuberization at 3 weeks of incubation, while cycocel (400 mg/l) and B-9 (2 mg/l) only 25.0 % and 21.4% respectively.

2. Number of microtubers

   The appropriate level of concentration that produced the greater number of micro tuber for each retardants was as follows: ancymidol 4 mg/l with 2.7 microtuber/culture, b-9 2 mg/l with 1.0 microtuber/culture and cycocel 400 mg/l with 1.3 microtuber/culture

3. Different level of ancymidol, B-9 and cycocel did not have significant effect on micro tuber size, micro tuber fresh weight, micro tuber dry weight and percentage of microtuber dry matter.

3.3.5. The effect of coumarin and kinetin on *in vitro* microtuber production of 5 potato cultivars

3.3.5.1. Introduction

The objective of these experiment was to evaluate the effect of coumarin and kinetin on microtuber production of 5 potato cultivars

3.3.5.2. Methods

The experiment was a two factors factorial experiments consisted of 6 tuberization medium and 5 potato cultivars. The 30 treatments combination were arranged in a Completely Randomized Designs with 15 replication. The 5 potato cultivars were Red Pontiac, Kennebec, Cipanas, and DTO 28. The tuberization medium were as follows:

(1). Coumarine liquid tuberization medium (CLT).
Modified Murashige and Skoog, coumarin 170 uM, and sucrose 6%, Nitrogen of MS was modified from 60 mM N to 4 mMn N

(2). Coumarine Solid Tuberization medium (CST) was CLT + agar 0.7%.

(3). Kinetin Liquid Tuberization medium (KLT)
Murashige and Skoof Medium , kinetin 45 uM and sucrose 6%.

(4). Kinetin Solid Tuberization medium (KST)
KLT + agar 0.7 %

(5). Coumarin-Kinetin liquid tuberization medium (CKLT).
Murashige and Skoog Medium, coumarin 170 uM, kinetin 45 uM and sucrose 6%.

(6). Coumarin-kinetin-solid tuberization medium (CKST)
CKLT + agar 0.7%

In each culture vials were planted 2 two nodes explants. The culture were incubated in dark at the temperature of 18-22 °C. The microtubers were harvested at 8 weeks after treatment

3.3.5.4. Results

The results were presented in table 3.3.5.1, 3.3.5.2, 3.3.5.4 and 3.3.5.5. The data in these table showed that:

(1). The coumarin tuber induction (liquid or solid) gave high percentage of tuberization than kinetin tuber induction medium (liquid or solid) (table 3.3.5.1)

(2). At 8 weeks after treatment all coumarin tuber induction medium had a 100 percent of tuberization while kinetin tuber induction medium only 70-90 percent of tuberization (table 3.3.-1).

(3). The cultivar DTO 28 response differently from Red Pontiac, Katahdin, Kennebec and Cipanas. With coumarin tuber induction medium or coumarin-kinetin tuber induction medium these 4 cultivars had a 100 percents of tuberization while DTO 28 had only 45 to 55 percent of tuberization (table 3.3.5.1).

(4). Numbers of microtubers

The coumarin-kinetin tuber induction medium (liquid or solid) produced the greatest numbers of tubers per bottle for all 5 cultivars (table 3.3.5.2).

(5). Fresh weight of microtubers

The coumarin-kinetin tuber induction medium (liquid or solid) produced the heaviest tuber frest weight for all 5 cultivars (table 3.3.5.3).

(6). Microtuber size

The coumarin-kinetin tuber induction medium (liquid or solid) produced the bigger tuber size for all 5 cultivars but only in solid medium. For the liquid medium the bigger tuber size induced by coumarin-kinetin tuber induction medium only for Red Pontiac, Katahdin, Cipanas and Kennebec. For Cipanas, kinetin tuber induction medium (liquid) produced the bigger size (table 3.3.5.4).

(7). Percentage dry weight of microtubers

The coumarin tuber induction medium had a high percentage of dry matter compared to type of response was for both type of medium (solid and liquid) as well as for 5 cultivars (Table 3.3.5.5)

Table 3.3.5.1. The percentage of tuberization at 4 and 8 weeks

	4 weeks					8 weeksMedium				
	RP	KA	DT	KE	CI	RP	KA	DT	KE	CI
Solidmedium										
CST	80	75	45	87.5	75	100	100	45	100	100
KST	44	68	25.5	75	63	70	80	30.5	87	85
CKST	88.5	92.5	40.0	80	87	100	100	55	100	100
Liquid medium										
CLT	85	80	37.5	75	100	100	100	40	100	100
KLT	65	70	20	70	80	80	82.5	37.5	100	90
CKLT	88.5	82.5	20	81	95	100	100	45	100	100

RP = Red Pontiac; KA = Kathadin; DT = DTO 28;
KE = Kennebec; CI = Cipanas

Table 3.3.5.2. The number of tubers produced per bottle (8 weeks)

Medium	Cultivars				
	Red Pontiac	Katahdin	DTO 28	Kennebec	Cipanas
Solid medium					
CST	1.93 a	2.07 a	2.93 b	3.36 a	3.10 b
KST	1.43 a	1.93 a	1.93 a	3.29 a	2.40 a
CKST	2.43 b	2.86 b	3.10 a	3.50 a	3.70 c
Liquid medium					
CLT	2.43 a	2.21 a	1.64 a	2.29 b	2.55 b
KLT	2.43 a	2.00 a	1.54 a	1.93 a	1.76 a
CKLT	3.07 b	3.14 b	2.14 b	3.35 c	2.77 b

Table 3.3.5.3. Fresh weight of microtubers (grams)

Medium	Cultivars				
	Red Pontiac	Katahdin	DTO 28	Kennebec	Cipanas
Solid medium					
CST	0.0321 a	0.0515 b	0.0270 a	0.0471 b	0.03 1 b
KST	0.0310 a	0.0234 a	0.0166 a	0.0241 a	0.6172 a
CKST	0.2351 b	0.1650 c	0.0609 b	0.0608 c	0.0837 c
Liquid medium					
CLT	0.0350 a	0.1109 b	0.0325 a	0.0580 a	0.0421 a
KLT	0.0734 b	0.0579 a	0.0531 b	0.0430 a	0.0372 a
CKLT	0.1780 c	0.1374 c	0.0531 b	0.1194 b	0.1231 b

Table 3.3.5.4. Microtuber diameter (size in mm)

Medium	Cultivars				
	Red Pontiac	Katahdin	DTO 28	Kennebec	Cipanas
Solid medium					
CST	2.93 a	3.79 b	2.93 b	3.57 b	3.77 b
KST	3.00 a	2.64 a	1.43 a	2.79 a	2.15 a
CKST	6.57 b	5.86 c	3.43 b	4.00 c	3.98 b
Liquid medium					
CLT	3.57 a	6.29 c	3.14 a	3.86 a	3.19 a
KLT	5.10 b	4.14 a	5.43 b	4.43 b	3.71 a
CKLT	6.14 c	5.40 b	4.43 a	5.50 c	4.32 b

Table 3.3.5.5. Percentage of dry matter (%) of microtuber

Medium	Cultivars				
	Red Pontiac	Katahdin	DTO 28	Kennebec	Cipanas
Solid medium					
CST	18.15	14.07	16.06	13.65	15.70
KST	14.14	14.95	15.48	16.81	14.53
CKST	14.14	12.91	13.06	11.47	13.73
Liquid medium					
CLT	17.70	13.26	15.28	19.25	16.37
KLT	14.80	16.89	12.45	14.60	15.91
CKLT	15.90	13.56	13.26	16.57	15.73

3.3.6. *In vitro* potato micro tuber production of PAS 3063 induced by kinetin and benzyladenine.

3.3.6.1. Introduction

The objective of the experiment was to evaluate the effectiveness of kinetin and benzyladenine in inducing *in vitro* micro tuber production of the cultivar PAS 3063.

3.3.6.2. Methods

The experiment was a factorial experiments with two factors, kinetin and benzyladenine. Each factors consisted of 4 level of concentration. The consecutive level of kinetin was: 0.5,0, 10.0, 15,0 mg/liter while the consecutive level of benzylodenine was: 0.2,5, 5.0 and 7.5 mg/liter.The 16 treatment combination were arranged in a complette Randomized Designs with 14 replication. The shoot propagation medium consisted of MS mineral salts, MS vitamins, Ca - panthotenate 2 mg/liter, NAA 0.01 mg/liter, GA3 0.3 mg / liter, sucrose 3 % and agar 0.8 %.

The tuberization medium consisted of hyponex (20-20-20) 4 gr/liter, sucrose 8 %, agar 0.8% and growth regulator according the treatment combination. The shoot cultures were incubated by light intensity of 2000 lux, 16 hours photoperiod at the temperature 20 - 25 °C. The tuberization cultures were incubated in the dark at the same room (temperature). The tuber were harvested at 8 weeks after culturing in solid tuberization medium.

3.3.6.3. Results

The results presented in table 3.3.6.1., 3.3.6.2., 3.3.6.3, 3.3.6.4., 3.3.6.5., 3.3.6.6., and 3.3.6.7. Were as follow:

1. The effect of kinetin and benziladenine on percentage and earliness of tuberization (Table 3.3.6.1).
2. The effect of kinetin and benzilodenine average number of tuber per culture vial at 4.6 and and 8 weeks (table 3.3.6.2)
3. The effect of kinetin and benziladenine on percentage of tuberization, number of tubers, tuber size, tuber fresh weight and percentage of dry matter. Harvest at 8 weeks after culturing in tuberization medium (table 3.3.6.3)
4. Response curve of kinetin an benzil adenine on:
   a. Number of tuber (table 3.3.6.4)
   b. Tuber size (table 3.3.6.5)
   c. Tuber fresh weight (table 3.3.6.6)
   d. Tuber dry weight (table 3.3.6.7).

The data from these tables showed that

1. At 4 weeks after culture in tuberization medium (WAT) only 2 treatments have a 100 % of tuberization . The two treatments were:Kinetin 10 mg/liter and BA 2.5 mg/liter(table 3.3.6.1)

2. At harvest (8 weeks) only 7 treatments had achieved a 100 % of tuberization. These treatments were : kinetin 5 mg / liter, kinetin 10 mg/liter, kinetin 15 mg/liter, BA 2.5 mg/liter + kinetin 15 mg / liter and BA 5 mg / liter (table 3.3.6.1)
3. At harvest a control treatment produced the greatest number of tubers per culture vials, eventhough it was not satistical different from the treatment of: Kinetin 5 mg/liter, kinetin 10 mg / liter , kinetin 15 mg / liter , BA 2.5 mg /liter , BA 2.5 mg/liter + kinetin 5 mg/liter, BA 2.5 mg/liter + kinetin 15 mg / liter and BA 5 mg / liter(table 3.3.6.2).
4. No additional tubers were formed after 6 weeks for the treatments: BA 2.5 mg / liter and kinetin 10 mg/liter (table 3.3.6.3)
5. The response of number of micro production to cytokinin treatment was better on single source of cytokinin (B.A 5 mg/ liter , B.A 7.5 mg / liter, kinetin 10 mg / liter, kinetin 15 mg / liter) than sources of cytokinin except for treatment combination of B.A 2.5 mg / liter + kinetin 5 mg /liter (table 3.3.6.4)
6. The greatest tuber size, tuber fresh weight, tuber dry weight were produced by the control treatment and kinetin 10 mg / liter (table 3.3,6.3)
7. At every level of kinetin, increased the level of concentration of B.A significantly decreased the number of micro tuber (table 3.3.6.4.4), tuber size (table 3.3.6.5) tuber fresh weight (table 3.3.6.7), tuber dry weight (table 3.3.6.7).

3.3.7. The effect of growth regulator (kinetin and coumarin) and method of *in vitro* tuberization on potato microtuber production of PAS 3063

3.3.7.1. Introduction

The objective of this experiment was to find the effect of kinetin, coumarin and *in vitro* tuberization method on potato microtuber production of PAS 3063.

3.3.7.2. Methods

The 12 treatment combination consisted of 3 level of tuberization media and 4 level of tuberization methods. These 12 treatment were arranged in a Completely Ramdomized Design with 10 replicates. The tuberization methods consisted of: direct solid tuberization method, direct liquid tuberization method, solid-liquid method, and liquid-liquid tuberization method.

The basic media components for the tuberization media were the same, the different was due to the growth regulators. The basic medium components were MS salt, MS vitamins, sucrose 6%, and agar 0.7 % for solid tuberization medium. For coumarin tuberization medium, nitrogen was decreased from 60 mM N to 4 mM N but not for coumarin plus kinetin. The growth regulator for tuberization media were as follows: (1) coumarin 25 mg/l, (2) kinetin 10 mg/l and (3) coumarin 25 mg/l + kinetin 10 mg/l. The incubation environment for shoot multiplication as well as for *in vitro* tuberization were the same in 3.3.6 The microtubers were harvested at 8 weeks after incubating in tuberization medium.

3.3.7.3. Result

The result were present ted in table 3.3.7.1 The results in table 3.3.7.1 showedd that:

(1) the solid-liquid orliquid-liquid method of tuberization produced bigger size tubers than the solid method of tuberization.

(2) The liquid method of tuberization produced no tubers until 8 weeks of incubation periods.

Table 3.3.7.1. The effect of kinetin, coumarin and method of tuberization on number of microtubers fresh weight and percentage of tuberization

Treatment	Number of micro tubers	Micro tuber fresh weight (g)	Percentage of tuberization	
	..... 8 weeks		4 wks	8 wks
Solid				
Coumarin	3.25	0.047	100	100
Kinetin	2.63	0.075	100	100
Coumarin + Kinetin	3.25	0.012	100	100
Liquid				
Coumarin	-	-	0	0
Kinetin	-	-	0	0
Coumarin + Kinetin	-	-	0	0
Solid-liquid				
Coumarin	2.63	0.259	100	100
Kinetin	4.50	0.225	100	100
Coumarin + Kinetin	4.37	0.276	100	100
Liquid-liquid				
Coumarin	3.12	0.116	100	100
Kinetin	2.88	0.115	100	100
Coumarin + Kinetin	4.12	0.143	100	100

3.3.8. Single effect of Cycocel, Benzyladenin, Adenin sulfat and sucrose on *in vitro* microtuber production of potato in liquid-liquid system of tuberization.

3.3.8.1. Introduction

The objective of these experiment were to evaluate the effect of single factor of cycocel, benzyladenin, adenin sulfate and sucrose on *in vitro* microtuber production of potato in liquid-liquid system of tuberization.

3.3.8.2. Methods

There were 4 simple experiment as follows:

(1). The level of cycocel (CCC): 400, 500, 600 mg/l the level of benzyladenin and sucrose were 5 mg/l and 90 g/l respectively.

(2). The level of benzyladenin: 2.50, 3.75, 5.0 mg/l the level of cycocel and sucrose were 600 mg/l and 90 g/l respectively.

(3) The level of adenin sulfate: 100, 200, 300, 400 mg/l. The level of cycocel and benzyladenine were 600 mg/l and 5 mg/l respectively.

(4). The level of sucroese: 70, 80, 90 g/l. The level of cycocel and benzyladenin were 600 mg/l and 5 mg/l respecti vely.

Each experiments were analyzed in a completly Randomized design with 12 replication. The tuberization method was a liquid-liquid system of tuberization. The *in vitro* shoot liquid medium and the incubation environments was same as described in general method of *in vitro* tuberization (3.2.). After 4 weeks incubation in shoot liquid medium, the liquid tuberization medium were added and incubated for 8 weeks at the temperature of 150 ° to 18 °C without light.

3.3.8.3. Results.

The result were presented in table 3.3.8.1. The data in table 3.3.8.1 showed the effect of each factor as follows:

(1). Level of cycocel.

At constant sucrose concentration of 90 g/l and benzyladenin 5 mg/l:

(a). Cycocel 600 mg/l produced bigger size microtubers than cycocel 400 mg/l and 500 mg/l.

(b). Cycocel 500 mg/l produced greater microtuber fresh weight than cycocel 400 mg/l and 600 mg/l.

(2). Level of adenine sulfate.

At constant sucrose concentration of 90 g/l and cycocel of 600 mg/l:

(a). adenin sulfate at 100 mg/l and 400 mg/l produced more microtubers, bigger size of microtubers, and greater microtubers fresh weight than adenine sulfate of 200and 300 mg/l.

(b). The reverse was true for the precentage dry weight of microtubers.

(3). Level of benzyl adenine

At constant concentration of sucrose 90 g/l and cycocel 600 mg/l:

(a). The number of microtubers was not effected by the level of benzyladenine

(b). Microtuber size and fresh weight were greater at benzyl adenine 2.5 mg/l and 3.75 mg/l than at Benzyl adenin 1.25 mg/l and 5.0 mg/l.

(4). Level of sucrose

At constant concentration of Benzyl adenin 5 mg/l and cycocel 600 mg/l:

(a). the level of sucrose (70, 80, 90 g/l) did not effetthe: number, size, fresh weight and percentage dry weight of microtubers.

(b). There was a tendency that sucrose at 90 g/l gave a better response to the number, siiziei and percentage dry weight of microtubers.

Table 3.3.8.1. Single effect of cycocel, adenine sulfate, benzyl adenine and sucrose on number, size, fresh weight and percentage dry weight of microtubers.

Concentration mg/l		Micro tubers			
		Number	Diameter (mm)	Fresh Weight (g)	Percentage dry weight (%)
CCC	400	5.4 a	4.0 a	0.098 ab	15.29
	500	6.8 b	4.0 a	0.116 b	15.29
	600	7.8 c	4.2 a	0.075 a	15.67
A.S.	100	8.2 q	5.4 pq	0.123 p	15.61
	200	7.4 p	4.6 p	0.199 q	14.89
	300	7.6 p	6.6 q	0.188 q	14.90
	400	8.2 q	5.6 pq	0.104 p	15.55
B.A.	1.25	7.0 y	3.4 x	0.120 x	15.44
	2.50	6.0 xy	4.4 y	0.186 y	15.33
	3.75	5.4 x	4.6 y	0.181 y	14.66
	5.00	6.6 xy	3.6 x	0.122 x	15.55
Sucrose	70	5.6 m	3.8 m	0.122 m	14.78
	80	4.6 m	4.8 m	0.167 m	15.10
	90	5.8 m	4.9 m	0.111 m	15.87

The same latter at the same column for the same faktor indicate no significant difference as determined by lsd 0.05

3.3.9. Effect of Hyponex, Ancymidol and benzyl adenine on in vitro micro tubers production

3.3.9.1. Introduction

In addition to plant growth regulator, mineral salt and vitamines are very important for *in vitro* microtuber production and quality. Murashige and Skoog medium (1962) is usually used for *in vitro* shoot production as well as *in vitro* tuber production. Murashige dan Skoog mineral salt can be substituted by compound fertilizer available at the market. On of these fertilizer is hyponex, contain not only macro nutrient but micro nutrient as well. It also contain nitrogen in both ammonium nitrogen as well as nitrate nitrogen. Substitution of MS medium by hyponex or any compound fertilizer for their mineral salts will be more economical and practical. The objection of this experiments was to evaluate the effect of hyponex, benzyl adenin and ancymidol on *in vitro* micro tuber production.

3.3.9.2. Methods

The experiment was a factorial experiment of three factors. The factors and their level were as follows: (a) Hyponex 4 g/l, 8 g/l; (b) benzyl adenine : 0 mg/l, 5 mg/l and ancymidol 0, 0.66, 1.32, 2.64 mg/l. The 16 treatments combination were arranged in Completely Randamozed Design with 25 replication. The method of tuberization used in experiment was solid-liquid system of tuberization. The shoot medium consisted of MS salt, MS vitamines and organic, sucrose 3% and agar 0.7%. The tuberization consisted of sucrose 6%, plus hyponex, ancymidol, and benzyl adenine according to the treatment combination.

Each culture vessels was planted with 4 two node explants. The shoot cultures were incubated at 1000 - 2000 lux of light intensity, 16 hours photoperiod and at a temperatures of 23 - 25 ° C for 4 weeks. After 4 weeks the tuberization medium were added to the shoot culture and then incubated in in same room, in the dark for anather 8 weeks. Micro tubers were harvested at eight after incubation in the tuberization medium, observed and analyzed.

3.3.9.3. Results

The results were presented in table 3.3.9.1, 3.3.9.2, 3.3.9.3 and 3.3.9..4. The data from those table showed that:

(1). The treatments combination of benzyladenine 5 mg/l, hyponex 2 g/l, with any level of ancymidol (0.66, 1,32, and 2.64 mg/l) tuberized eraly and produced a hundret percent of tuberization.

(2). More axillary micro tubers were produced than the terminal microtubers for all the treatments combination.

(3). Bigger size tuber (4 18 mm diameter) was produced by the treatment combination of hyponex 2 g/l, and ancymidol 2.64 mg/l

(4). The treatmenmt combination of hyponex 4 g/l and ancymidol 2.64 mg/l produced the heaviest tuber fresh weight (117.7 mg/tuber)

(5). The number of micro tubers produced was depent on benzyladenine and ancymidol. Benzyl adenine of 5 mg/l and ancymidol of 0.66 mg/l produced the greater number of microtubers

3.3.10. The effect of different level of growth retardants on *in vitro* microtubers production of different ptotato cultivars

3.3.10.1. Introduction

The objectives of this experiment was to evaluate different level of different growth retardants on *in vitro* microtuber production of different potato cultivars in solid-liquid system of tuberization.

3.3.10.2. Methods

The experiment consisted of different level of concentration of growth retardants and different potato cultivars. The growth retardants and their concentration were as follows:

(1). Arest (ancymidol):0.5, 1.0, 10.0, 100.0 mg/l

(2). Cycocel (CCC) . 200, 400, 600 mg/l

(3). Alar (SADH) : 0.5, 1.0, 10.0, 100.0 mg/l

The potato cultivars consisted of: Red Pontiac, Katahdin, Russet Burbank, Kennebect and Norchip. Each retardant in combination with potato cultivars re[resent an experiment, so there were in totaol 3 experiments arranged were in Completely Randamized Designs with 10 replication.

The solid-liquid system of *in vitro* tuberization was used in these experiments. The shoot growth medium consisted of MS salts, MS vitamines, sucrose 3%, Ca-panthotenate 2 mg/l, and agar 0.7%. The shoot culture were incubated for six weeks at a light intensity of 1000 - 2000 lux, 16 hours photoperiod and a temperature of 25 °C for 6 weeks.

After 6 weeks tuber induction medium were addcd to the shoot culture. Thr induction medium consisted MS mineral salts, MS vitamines, sucrose 6% and retardants according to treatemts. The culture were incubated for 8 weeks in dark at a temperature of 18-22 °C. The microtubers were harvested at 8 weeks, observed and analyzed.

3.3.10.3. Results

The effect of different levels of growth retardants on number of microtuber produced and average microtuber fresh weight of 6 cultivars were presented in table 3.3.10.1, and 3.3.10.2.

The data in table 3.3.10.1 and 3.3.10.2 showed that:

(1). Number of micro tuber produced (table 3.3.10.1)

(a). The best concentration of ancymidol was 2.64 mg/l for all cultivars, except for Norchip was 0.264 mg/l

(b). The best concentration for lar was 10 mg/l for all potato cultivars

(c). The best concentraion of cycocel was 600 mg/l for all cultivars

(d). The toxic levels for ancymidol was 26.4 mg/l and for Alar 100 mg/l.

(2). Microtuber fresh weight (Table 3.3.10.2)

(a). The best concentration of ancymidol that produced the heaviest microtuber fresht weight was 0.264 mg/l, different from the best concentration of Alar and Cycocel were similar to their concentration that produced the highest number of microtubers.

(b). The best concentration of Alar was 10 mg/l and cycocel 600 mg/l that produced the heaviest microtuber fresh weight for all cultivars. These concentration of Alar and cycocel were similar to their concentration that produced the highest number of microtuber.

3.3.11. Effect of Sucrose and Benzyladenine on Potato microtubers

3.3.11.1. Introduction

The objective of the experiment was to evaluate the effect of different level of concentration of sucrose and benzyladenine on potato micro tuber production

3.3.11.2. Methods

The experiment was a factorial experiment consisted of 2 factors, namely sucrose and benzyladenine. There were 4 level of concentration of sucrose (3, 6, 9, 12 %) and 3 level of concentration of benzyladenine (0, 2, 4 mg/l). The 12 treatments combination were arranged in a Completely Ramdomized Design with 12 replication. The liqyuid shoot propagation medium consisted of MS mineral salt, MS vitamines, Ca-panthothenate 2 mg/l, ancymidol 0.5 mg/l and sucrose 3 %. The liquid tuberization medium consisted of MS mineral salt, MS vitamines, cycocel 400 ppm, sucrose and benzyladenine were added according to the treatemnt combination.

Each culture vials was planted with 3 to 4 nodes explants. The cultures vials were incubated at a light intensity of 100 lux 16 hours photoperiod and at room temperature of 20 - 23 °C for 4 weeks. After 4 weeks the tuberization medium added to the shoot culture. Each culture vials was added 20 ml of tuberization medium. The culture were incubated in darek room with the average temperature of about 20 °C. The micro tubers were harvested at 8 weeks of incubation period.

3.3.11.3. Result

The result of the experiments were presented in table 3.3.11.1. to table 3.3.11.7 under the following titles.

(1). Effect of sucrose and benzyladenine (BA) on percentage of tuberization (table 3.3.11.1).
(2). Effect of sucrose and benzyladenin on percentage of axillary and terminallly microtubers (table 3.3.11.2)
(3). Effect of sucrose oni the number of microtubers (3.3.11.3)
(4). Effect of sucrose on the size of microtubers (table 3.3.11.4)
(5). Effect of sucrose and benzyladenine on microtuber fresh weight (table 3.3.11.5)
(6). Effect of sucrose and benzyladenine on the percentage of micro tuber dry weight (table 3.3.11.6)
(7). Effect of sucrose and benzyladenine on microtubers dry weight (table 3.3.11.7)

Data from those tables showed that:

(1). Earliness of tuberization was induced at the higher sucrose concentration and was deleyed by benzyladenine (table 3 3.11.1). A 100 percent of tuberization was achieved at 1 weeks after incubation at sucrose concentration of 6, 9, 12% with- out benzyladenine. At the same sucrose concentration (6, 9, 12%) in combination with B. A. of 2 mg/l and 4 mg/l a 100 percent of tuberization was achieved 2 weeks after incubation

(2). No difference on earliness of tuberization between the axillary type of tuberization and terminal type of tuberization. At 1 week oof incubation period, both type of tuber have been initiated. The percentage of axillary tubers was greater than the terminal tubers (table 3.3.11.2)

(3). Sucrose concentration had a significant effect on number of tuber per culture vials. The optimum sucrose concentration was 9% with 7 microtuber per culture vials (tabel 3.3.11.3)

(4). Sucrose concentration of 9% produced the largest tuber size with an average diameter of 5.82 mm (table 3.3.11.4)

(5). The treatmen combination of sucrose 9% and benzyladenine 2 mg/l produced the highest average tuber fresh weight of 225.79 mg/micro tuber (table 3.3.11.5)

(6). At each level of benzyl adenine concentration (0, 2, 4) mg/l) the higher the concentration of sucrose the higher the percentage of tuber dry weight (table 3.3.11.6)

(7). The treatment combination of sucrose 9% and benzyladenin of 2 mg/l produced the highest average tuber dry weight of 41.57 mg/micro tuber (table 3.3.11.7).

Table 3.3.11.1. The effect of sucrose and benzyl adenine (BA) on the percentage of tuberization

Sucrose	BA	Weeks of Incubation (WOI)				
		1	2	4	6	8
ppm						
3	0	25.00	41.67	50.00	50.00	50.00
	2	41.67	66.67	83.33	91.67	91.67
	4	50.00	75.00	75.00	75.00	75.00
6	0	100.00	100.00	100.00	100.00	100.00
	2	91.67	100.00	100.00	100.00	100.00
	4	91.67	100.00	100.00	100.00	100.00
9	0	100.00	100.00	100.00	100.00	100.00
	2	91.67	100.00	100.00	100.00	100.00
	4	75.00	100.00	100.00	100.00	100.00
12	0	100.00	100.00	100.00	100.00	100.00
	2	83.33	100.00	100.00	100.00	100.00
	4	75.00	100.00	100.00	100.00	100.00

Table 3.3.11.2. The effect of sucrose and benzyl adenine (BA) on the percentage of axillary tubers and terminal tubers

Sucrose	BA	Weeks of Incubation (WOI)					
		1 WOI		4 WOI		8 WOI	
		AX	TER	AX	TER	AX	TER
	ppm						
3	0	60.00	40.00	50.00	50.00	58.33	41.76
	2	66.67	33.33	53.33	46.67	60.00	40.00
	4	50.00	50.00	81.25	18.75	81.82	18.18
6	0	82.35	17.65	79.55	20.45	78.43	21.57
	2	96.15	3.85	83.64	16.36	79.41	20.59
	4	79.31	20.69	79.92	23.08	70.44	29.51
9	0	96.36	3.64	93.90	6.10	90.80	9.20
	2	81.63	18.37	77.63	22.37	74.71	25.29
	4	85.71	14.29	86.76	13.24	85.53	14.47
12	0	79.49	20.51	82.19	17.81	78.72	21.28
	2	84.62	15.38	92.98	7.02	91.67	8.33
	4	50.00	50.00	76.92	23.08	78.08	21.92

Table 3.3.11.3. The effect of sucrose on the number of tuber (V x + 0.5)

Sucrose	Weeks of Incubation (WOI)			
	2	4	6	8
3	1.139a	1.211a	1.318a	1.334a
6	1.955b	2.137b	2.221b	2.319b
9	2.394c	2.556c	2.606c	2.689c
12	2.151b	2.364c	2.426bc	2.522bc

The same letters at each columns were not significant different at 5% HSD Test

Table 3.3.11.4. The effect of sucrose on tuber size harvest at 8 weeks of incubation (WOI)

Sucrose (%)	Tuber diameters (mm)
3	5.19
6	5.65
9	5.82
12	5.31
Linear	**
Quadratic	**
Cubic	NS

* highly significant
NS = not significant

Table 3.3.11.5. The effect of sucrose and benzyl adenine(BA)on tuber fresh weight

Sucrose	Benzyl Adenine		
	0 ppm	2 ppm	4 ppm
%	..... mg		
3	120.33 Xa	103.65 Xa	139.65 Xa
6	147.25 Xa	140.54 Xab	149.81 Xa
9	167.38 Xa	164.22 Xbc	166.23 Xa
12	115.60 Xa	225.79 Xc	127.44 Xa

The same letters in rows (a,b,c) and the same letters in columns were not significant different at 5 % HSD Test

Table 3.3.11.6. The effect of sucrose and benzyl adenine (BA) on the percentage of tuber dry weight

Benzyl Adenin	Sucrose		
	0 ppm	2 ppm	4ppm
%	..... mg		.....
3	5.86	7.63	8.29
6	11.40	10.90	12.08
9	17.05	16.60	15.55
12	18.27	18.41	17.83

Table 3.3.11.7.The effect of sucrose and Benzyl adenine(BA)on tuber dry weights

Sucrose	Benzyl Adenine (BA)		
	0 ppm	2 ppm	4 ppm
%	..... mg		.....
3	7.05 Xa	7.91 Xa	11.58 Xa
6	16.79 Xab	15.32 Xa	18.10 Xab
9	30.05 Xc	27.26 Xb	25.85 Xb
12	21.12 Xbc	41.57 Xc	22.72 Xb

The same letters in row (a,b,c) and in columns (x,y) were not significant different at 5% HSD Test

3.3.12. Effect of nitrogen and pH on *in vitro* microtuber production of potato induced by cycocel and benzyl adenine.

3.3.12.1. Introduction

The objective of the experiment was to evaluate the effect of nitogen and pH on *in vitro* microtuber production of potato induced by cycocel and benzyl adenine.

3.3.12.2. Methods

The experiments was a two factors factorial experiment. The factor in their level were as follows:

a. Nitrogen 7.5; 15.0; 30.0; 60.0 mM and
b. pH 4.7; 5.7; 6.7; 7.7.

The 16 treatments combination were arranged in a Completely Randomized Designs with 11 replications. The system of tuberization was a liquid. Liquid system of tuberization. The shoot multiplication medium consisted of MS mineral salts and MS vitamins , ancymidol0.1 ppm and sucrose 3 %. The cultures were incubated for 4 weeks at room temperature of 22 - 25 °C, 16 hours photoperiod and a light intensity of 1000-2000 lux. The tuberization medium consisted of MS mineral salts were nitrogen was ajusted according to the treatment combination. MS organic, Ca - panthotenate 2 mg/ liter, sucrose 9 %, CCC 600 mg / liter and B.A 5 mg / liter. The pH of the medium was ajusted conespondingly to treatment combination. After the explant have been incubated for 4 weeks in shoot culture medium, tuberization medium was added. The tuberization cultures were incubated in dark at a temperature of 15 °C to 18 °C. After 8 weeks of incubation the microtubers were harvested, observed and analysed.

3.3.12.3. Results

The results of the treatment were presented in table 3.3.12.1

The data on table 3.3.12.1 showed that:

1. Effect of pH on number of microtuber per culture vials, size of microtubers and microtuber fresh weight depent on level of nitrogen.
2. Number of microtubers

   The highest numbers of micro tubers produced per culture vials of the pH of 7.7, 6.7, 5.7 and 4.7 was acheived at the nitrogen concentration of 30 mM respectively

3. Microtuber size (diameter)

   The microtuber size was effected by nitrogen concentration only at the pH 5.7, 6.6 and 7.7. At pH 4.7 the nitrogen concentrations did not effec the microtuber size. At the pH 7.7 the lowest diameter was produced by the lowest nitrogen concentration . At the pH 5.7 and 6.7 the biggest size microtubers was produced by 30 mM N.

4. Microtuber fresh wight.

   At all pH the highest nitrogen concentration (60 mM N) produced the largest microtuber fresh weight.

3.3.13. Effect of pH and Nitrogen on *in vitro* microtuber production of potato as induced by coumarin and kinetin

3.3.13.1. Introduction

The objective of these experiments was to evaluate the effect of pH and nitrogen on *in vitro* microtuber production of potato (cv Red Pontiac) as induced by coumarin and kinetin.

3.3.13.2. Methods

All the methods were the same as in 3.3.12. Except the growth regulator used for microtuber induction. In this experiment the growth regulators used in tuberization medium were: coumarin 25 mg/l and kinetin 10 mg/l.

3.3.13.2. Results

The results were presented in table 3.3.13.1. Data intable 3.3.13.1 showed that:

(1). The level nitrogen concentration determined the number of microtubers, the microtuber size and microtuber fresh weight.

(2). Theappropriate nitrogen concentration that gave the best response for the number of microtubers, microtuber size, microtuber fresh weight and percentage of dry weight of microtubers was 15 mM for all level of pH (4.7, 5.7, 6.7 and 7.7).

Table 3.3.13.1. Effect of pH and nitrogen on number of microtuber size, microtuber fresh weight and percentage dry weight of microtubers as induced by coumarin by Coumarin and kinetin

Treatment pH mM N	Numer of micro tuber	Micro tuber diameter (mm)	Micro tuber fresh weight (g)	Percentage dry weight at microtuber (%)
7.7				
60	8.33 a	5.32 a	0.115 a	16.23
30	8.75 a	6.27 ab	0.142 b	16.21
15	9.85 ab	6.61 b	0.122 ab	16.47
7.5	9.31 a	5.35 a	0.143 c	15.22
6.7				
60	9.10 a	6.31 b	0.121 ab	16.35
30	9.33 a	6.27 ab	0.125 b	16.34
15	10.33 b	6.41 b	0.143 c	16.73
7.5	9.95 ab	6.41 b	0.124 b	14.32
5.7				
60	10.22 ab	6.51 b	0.123 ab	16.55
30	10.24 ab	6.31 b	0.147 c	16.55
15	11.33 c	7.25 c	0.143 c	17.31
7.5	10.05 ab	6.33 b	0.112 a	14.22
4.7				
60	8.95 a	5.11 a	0.115 a	15.71
30	9.22 a	6.23 ab	0.121 ab	15.77
15	10.43 b	6.27 ab	0.143 c	16.85
7.5	10.21 ab	5.33 a	0.142 c	15.71

The same letters at the same column indicate no significant difference as ditermined by HSD 5%

3.3.14. Effect of height of liquid tuberization medium on *in vitro* microtubers production in liquid-liquid system of tuberization

3.3.14.1. Introduction

Liquid-liquid system of *in vitro* tuberization wa usually incubated on shaker or rolling bottles. In case of no shaker or rolling bottles available, the tuberization culture had to be induced in static condition. In static condition the height of the medium or volume of the liquid tuberization medium would determine the number of *in vitro* microtubers production. In Indonesia height of the liquid culture

medium will be more appropriate than the volume since the culture bottles available are from different size collected from different sources.

The objective of the experiment was to determine the appropriate height of the liquid medium of the tuberization medium on *in vitro* tuber production in liquid-liquid system of tuberization of the Red Pontiac.

3.3.14.2. Methods

In vitro shoots were culture in liquid shoots medium in a 100 ml erlen meyer flask. The volume of the liquid shoots culture medium was 25 ml (1 cm height). In each flask were added two nodes explants of the cultivar Red Pontiac. The culture were incubated at the light intensity of 1000 - 2000 lux, 16 hours photoperiod and a room temperature of 25 °C. The liquid shoot medium used in this xperiment consisted of MS mineral salt, MS organic, Ca-panthothenate 2 mg/l and sucrose 4%.

Four weeks after incubation the liquid medium almost used up and dry up and the average size of micro shoots was 4 cm height. At this time liquid tuberizaation medium were added to the shoots medium. The amount of the liquid medium added was according to the treatments. There were 4 treatments namely: 1 cm (25 ml), 2 cm (50 ml), 3 cm (70 cm), and 4 cm (90 ml). Each treatment was replicated ten times and arranged in completely Randomized designs.

The liquid tuberization medium consisted of MS mineral salt, MS organic, cycocel 400 mg/l, BA 5 mg/l and sucrose 9%. The cultures were incubated in dark at room temperature of 15-18 °C. Eight weeksd after incubation period the microtubers were harvested observed and analyzed.

3.3.1.4.3. Results

The result of the experiment were presented in table 3.3.14.1 Table 3.3.14.1 showed that:

(1). The best height of liquid medium tuberization medium were 1cm and 2 cm for the number of microtuber production

(2). At the height of 4 cm the number of micro tubers as well as micro tuber fresh weight was low.

(3). From the economic stand point the appropriate height of liquid tuberization medium would be 1 cm (25 ml).

Tabel 3.3.14.1. Effect of height of liquid tuberization medium on the average number of microtubers, microtubers fresh weight, microtubers dry weight, and percentage dry weight

Height and volume of medium cm (ml)	Number of tubers	Fresh Weight (gram)	Dry Weight (gram)	Percentage dry weight (%)
1 (25)	19.83 c	0.112 b	0.0177 a	15.80 a
2 (50)	18.17 c	0.158 b	0.0274 b	17.34 a
3 (70)	16.14 b	0.127 b	0.0268 b	21.10 b
4 (90)	3.40 a	0.084 a	0.0142 a	16.90 a

The number with the same letter at the same column was not significant different at 5% HSD test.

3.3.15. Effect of plant growth regulator and method of tuberization as *in vitro* micro tuber productions of PAS 3063

3.3.15.1. Introduction

In vitro microtuber production not only depend on growth regulator but on tuberization method as well. Tuberization methods were the methods of adding liquid tuberization media to liquid or solid shoot propagation mathods. This could be biphasic media (solid liquid) or monoplastic media (liquid - liquid or liquid subtituted liquid). Plant growth regulator used in this experiment were combination of plant growth retardants (CCC,paclobutrazol)and cytokinins (B.A, kinetin). Each at their effective level of concentration.

The objective of the experiments was to find the best combination of plant growth regulator and *in vitro* micro tuber production.

3.3.15.2. Methods

The liquid shoot propagation media unsisted of MS mineral salt vitamins, sukrose 3 % and Ca-penthotenate 2 mg / Liter.The solid shoot propagation was the same as liquid propagation media but with the addition of agar 0,7 %. The treatment combination consisted of incomplete combination of tuberization methods. The tuberization media were as follows:

1. MS mineral salt and vitamins, CCC 500 mg / liter , BA 5mg / liter and sucrose 6 %.
2. MS muneral salt and vitamins, paclobutrazol 100 mg / liter and sukrose 6 %

3. CCC 500 mg / liter, B.A. 5 mg / liter and sukrose 8 %.
4. Paclobutrazol 100 mg / liter, kinetin 10 mg / liter and sukrose 8 %.

Tuberization method consisted of

1. Solid shoot media + liquid tuberization media (SS + LT).
2. Liquid shoot media + liquid tuberization media (LS + LT).
3. Liquid shoot media subtituted by liquid tuberization media (OLS + ST)

The treatments combinations were as follows:

1. SS + LT 1	5. LS + LT 2
2. LS + LT 1	6. OLS + LT 2
3. OLS + LT	7. SS + LT 3
4. LS + LT 2	8. SS + LT 4

The number of LT (LT 1, LT 2, LT 3, LT 4) denote the number of liquid tuberization media. These 8 treatments combinations were arranged in Complettely Randomized design with 10 replicates. The micro tubers were harvested at 8 weeks after incubation in tuberization media. The incubations environment were the same as

3.3.15. 3. Result

Theresultsof the experiments werepresentedintable 3.3.15.1

Tabel 3.3.15.1. The effect of plant growth retardants and tuberization methods on the average number of tuber, tuber fresh weight, and percentage of tuberization

Treatments percenta	No of micro tuber (V X + 0,5)	microtuber freshweight (gr)	percentage of tuberi-zation	
	..... 8 weeks.....		4 wks	8 wks
1. SS+LT 1	2.34 bc	0.829ab	100	100
2. LS+LT 1	2.08 bc	0.944c	100	100
3. OLS+LT 1	2.66 c	0.846b	100	100
4. SS+LT 2	2.01 bc	0.761a	100	100
5. LS+LT 2	1.07 a	0.742a	0	25
6. OLS+LT 2	1.17 a	0.728a	0	50
7. OLS+LT 3	2.13 bc	0.768a	100	100
8. OLS+LT 4	1.59 ab	0.745a	100	100

The same letter in a column indicates no significant different at 5 % level of HSD test

The conclusion that can be drawn from table 3.3 were:

1. There were no significant different among the methods of tuberization on number of micro tubers production for tuberization media 1. However, the micro tuber fresh weight was influenced by the method of tuberization, the liquid - liquid (LS + LT) tuberization method produced the harvers micro tuber frsh weight for liquid tuberization media 1.
2. For solid - liquid tuberization method (SS + LT) there were no significant different on number of micro tubers production among the different tuberization media (media 1,2,3 and 4)
3. The low number of micro tuber production in treatment LS +LT2 were due to low percentage of tuberization.
4. For biphase tuberization methods (solid - liquid method), the tuberization media could consist only of sucrose and growth regulators (Media 3 and 4) without inorganic salts and vitamins.

3.3.16. The effect of growth retardants on *in vitro* potato microtuber production of PAS 3063 in a solid - liquid system of tuberization.

3.3.16.1. Introduction

The objective of this experiments was to test the effect of different level of concentration of different growth retardants on *in vitro* potato microtuber production of PAS 3063 in a solid - liquid system of tubrization.

3.3.16.2. Methods

The same as 3.3.15, except that the shoot propagation medium consisted of MS mineral salts and vitamins, Ca - panthotenate 2 mg / liter, sucrose 3 % and agar 0.7 %

3.3.16.3. Results

The number of microtubers average microtuber fresh weight and the percentage of tuberization as a response to different level of concentration of retardants were presented in table 3.3.16.1

The data in table 3.3.16.1 showed that:

1. The concentration of 100 mg / liter was already toxic for paclobutrazol and ancymidol but was not yet for B.9.
2. The effective level of concentration to induce *in vitro* tuberization for all three growth retardants range from 0.5 to 10 mg / liter.
3. *in vitro* tuberization was very fast with all three growth retardants at the inducing level of concentration. A hundred percent tuberization was acheived in 4 weeks period at this inducing level of concentration.

4. The concentration level of 0.5 to 10 mg / liter for all three growth retardants produced the harvest microtuber fresh weight.

Table 3.3.16.1. Number of microtubers average fresh weight of microtubers, percentage of tuberization of different growth retardants.

Growth retardants. (gr / l)	No of micro tubers.	fresh weight of microtubers.	Percentage of tuberization	
	.... 8 wks		4 wks	8 wks
B.9 (alar)				
0.5	11.6 c	0.130 b	100	100
1.0	9.2 b	0.129 b	100	100
10.0	8.8 ab	0.073 ab	100	100
100.0	8.2 a	0.082 a	100	100
Paclobutrazol				
0.5	4.4 a	0.180 b	100	100
1.0	6.8 a	0.220 c	100	100
10.0	9.4 c	0.085 a	100	100
100.0	2.0 c	0.031	0	20
Ancymidol				
0.5	9.6 b	0.122 b	100	100
1.0	7.6 b	0.129 b	100	100
10.0	11.4 c	0.069 a	100	100
100.0	0	0	0	20

The same letter in a column indicates no significant different at 5 % levels of HSD for each growth retardants.

3.3.17. The effect of growth retardants on *in vitro* microtuber production of PAS 3063 in liquid-liquid system of tuberization.

3.3.17.1. Introduction

The objective of this experiment was to test the effective- ness of different growth retardant on *in vitro* microtuber production of PAS 3063.

3.3.17.2. Methods

Three growth retardant chemicals namely: B 9 (alar), ancymidol (Arest) and paclobutrazol each was tested at 0.5, 1.0, 10 and 100 ppm. Each growth retardants was tested seperately in a completes randomized designs each with 15 replicates.

The explants were first grown in shoot multiplication media for 4 weeks. The liquid multiplication media (media - 11) consisted of MS mineral salt and vitamins, Ca penthetonate 2 mg / liter, BA 0.5 mg / liter, GA3 0.5 mg /liter and sucrose 3 %. The culture were incubated without shoking at light intensity of 1000 - 2000 lux and at an average temperature of 18 - 22 ° C.

After a month in multiplication media, the liquid tuberization media (media - 2) were added to the plantlet in media - 1. The liquid tuberization media consisted of MS mineral salt and vitamins, sucrose 9 % and retardants according to treatments. The cultures were incubated for 8 weeks in dark at in average temperature 18 - 22 °C. The microtubers were harversted at 8 weeks after incubation in tuberization media.

3.3.17.3. Results.

The number of tubers, tuber fresh weight and percentage of tuberization were presented in table 3.3.17.1.

Table 3.3.17.1. The average number of microtubers, microtuber fresh weight and percentage of tubrization.

Growth retardant (mg / l)	no. of tuber	tuber fresh weight (g)	% of tuberization.	
	.....8 wks.....		4 wks	8 wks
B9 (alar)				
0.5	7.8 c	0.120 a	100	100
1.0	4.2 ab	0.230 d	100	100
10.0	3.8 ab	0.148 b	100	100
100.0	3.0 a	0.210 c	100	100
Paclobutrazol				
0.5	5.2 b	0.170 b	100	100
1.0	7.2 c	0.115 a	100	100
10.0	3.6 a	0.220 c	100	100
100.0	1.0	0.180	0	20
Ancymidol				
0.5	4.4 a	0.184 b	100	100
1.0	8.4 c	0.133 a	100	100
10.0	7.4 b	0.178 b	100	100
100.0	0	0	0	0

The same letter in columer for each growth retardants indicates no significant different at 5 % level of HSD test

The data in table 3.3.17.1 showed that:

1. The concentration of 100 ppm for both paclobutrazol and ancymidol was already toxic, while for B - 9 it was not yet toxic
2. For all three growth retardant the effective. Concentration level range from 0.5 to 10 ppm. The best concentration for B-9, paclobutrazol and ancymidol were 0.5, 7.2 and 8.4 c ppm consecutively
3. The tuberization was faster at the effective concentration level. Ahundred percent level was achievedat4 weeks period.

3.3.18. The Effect of coconut water on *in vitro* microtuber production.

3.3.18.1 Introduction

In Indonesia expensive cytokinins can be substituted by coconut water. Coconut water can be received free at the market. Coconut water contain both zeatin and diphenylurea. Zeatin is an cytokinin phytohormones and diphenylurea have cytokinins activiting. The objective of the experiment was to evaluate the effect of coconut water on *in vitro* microtuber production.

3.3.18.2 Methods

Liquid system of *in vitro* tuberization was used in this experiments. The liquid shoot medium consisted of MS medium, 30 g/l sucrose, 0.01 mg/l NAA, and 0.1 mg/l ancymidol. The liquid tuberization medium were MS medium, 90 g/l sucrose, 600 mg/l CCC and coconut waater according to treatments. The shoot were growth for 6 weeks in shoot medium before adding tuberization medium. After 8 weeks in tuberization medium to microtuber were haarvested and evaluated. The coconut water treatment consisted of 5 levels namely: 0, 5.0, 10.0, 15.0 and 20.0%.

3.3.18.3 Results

The result were presented in tabel 3.3.18.1. Data in table showed that:

1. The level of coconut water did not affect the microtuber size, fresh weight and percentage dry weight
2. Coconut water at 15% produced significant higher microtuber numbers.
3. Coconut water at 15% produced bigger tuber size, fresh weight and percentage dry weight, eventhough no statistical different with the other level.

Table 3.3.18.1. The effect of level of coconut water on numbers, size, fresh weigh of dry weight of microtuber

Coconut water %	Microtuber			
	Number	size (mm)	Fresh weight (mg)	% of dry weight
0.0	9.12 ab	5.72	153	15.75
5.0	8.79 a	5.47	147	17.41
10.0	10.55 b	6.37	145	16.79
15.0	10.71 c	5.87	171	17.31
20.0	10.67 bc	6.79	178	16.91

The same letter at same colum indicate no statistical difference as determined by HSD 5%.

3.3.19. The effect of kind of cytokinins and retardants on *in vitro* tuberization of potato

3.3.19.1. Introduction

This experiment was a verification experiment based on the result of the previous experiments. Some of the previous experiments were an optimazing experiments of growth retardants and cytokinins on *in vitro* tuberization of potato. The previous experimental results showed that the optimum level of cytokinins for *in vitro* tuberization were: BA 5 mg/l, kinetin 10 mg/l, benomyl 50 mg/l and adenine sulphate 100 mg/l. The optimum level to induce *in vitro* tuberization for retardant were: coumarin 25 mg/l, CCC 600 mg/l, paaclobutrazol 10 mg/l and uniconazole 3 mg/l.

Theoritically if there were no interaction between retardants and cytokinins any combination of optimum level of any kind of cytokinins and optimum level of any kind retardants would have the same effect of *in vitro* tuberization. The objective of this experiment was to evaluate the combination of kind of cytokinins and retardants at their optimum level on *in vitro* tuberization of potaato.

3.3.19.2. Material and method

The shallow liquid system of *in vitro* tuberization was used in this experiments. The liquid shoot medium consisted of MS medium, 3% sucrose and 4 mg/l of Ca. pentothenate. The liquid tuberization consisted of MS medium 9% sucrose, combination of cytokinins and retardants according to treatments.

In each bottle was planted with 4 two nodes *in vitro* cutting of cv. Red Pontiac. After 4 weeks in hoot medium 20 ml of tuberization medium was added and incubated for 8 weeks in dark at 17^0C. *In vitro* tuber were harvest after 8 weeks in tuberization and evaluated.

The experiment consisted of combination 4 kinds of cytokinins (BA 5 mg/l; kinetin 10 mg/l, benamyl 50 mg/l, adenine sulphate 100 mg/l) and 4 kinds of retardant (coumarin 25 mg/l; CCC 600 mg/l; paclobutrazol 10 mg/l; uniconazole 3 mg/l).

These 16 treatments combination were aarranged in a complete randomized design with 10 replication. Each bottle represented a treatment unit.

3.3.19.3. Result

The result in table 3.3.19.3 showed that:

(1) There were no significant different in number of microtuber perbottle microtuber fresh weight perbottle and diameter of microtuber.
(2) The diameter of microtuber and microtuber fresh weight were lower than usually.
(3) The microtuber used as propagules should have a diameter of 0.5 - 1.0 mm, microtuber fresh weight of more than 100 mg and percentage dry weight of more than 14%.
(4) Considered the number of microtubers, microtu ber dry matter, and percentage dry weight of microtuber the combination of kinetin 10 mg/l or benomyl 50 mg/l with coumarin 25 mg/l or CCC 600 mg/l seems to be better.

(5) Any cytokinins combination with uniconazole 3 mg/l produced bigger microtuber size but lower number of microtubers and percentage dry weight of microtuber.

Table 3.3.19.1 The effect cytokinins and growth retardants on number, diameter, fresh weight and percentage dry weight of potato microtubers

Treatment	Number per bottle V(x+0.5)	Diameter (mm)	Fresh weight mg/microtuber	Percentage dry weight (%)
BA				
Coumarin	2.49 ab (5.9)	3.39	36.9	19.92
CCC	2.17 a (4.4)	3.42	41.6	21.96
Paclobu-trazol	2.67 ab (6.8)	3.52	33.8	22.26
Unicona-zole	2.44 ab (5.7)	3.87	51.0	19.61
Kinetin				
Coumarin	2.69 ab (7.0)	3.37	38.4	20.45
CCC	2.72 ab (7.0)	3.44	36.5	18.34
Paclobu-trazol	2.28 ab (4.9)	3.56	41.8	21.49
Unicona-zole	2.25 ab (4.7)	4.43	60.4	17.08
Benomyl				
Coumarin	2.93 b (9.0)	3.41	41.1	18.22
CCC	2.50 ab (6.0)	3.96	56.7	18.98
Paclobu-trazol	2.67 ab (6.8)	4.05	50.0	19.68
Unicona-zole	2.28 ab (4.8)	4.15	53.9	16.77
Adenine suphate				
Coumarin	2.45 ab (5.6)	3.45	35.9	18.56
CCC	2.63 ab (6.6)	3.44	37.1	20.15
Paclobu-trazol	2.35 ab (5.1)	3.74	44.3	14.90
Unicona-zole	2.16 a (4.2)	4.04	53.4	14.35

The same letter at same colum denoted no significant different at 5% HSD test. a(b) —> a = transformed V(x+0.5), (b) detransformed data. Percentage dry weight was not tested statiscally.

3.4. Discussion

3.4.1. Method of *in vitro* tuberization

Several methods of direct or indirect *in vitro* tuberization had been evaluated. These methods were: direct solid medium (exp 3.3.1, 3.3.3, 3.3.6, 3.3.7), direct liquid medium (exp 3.3.5, 3.3.9, 3.3.10, 3.3.15, 3.3.16), liquid-liquid medium (exp 3.3.2, 3.3.8, 3.3.9, 3.3.11, 3.3.15) and liquid subtituted by liquid medium. These methods have been explained *in vitro* tuberization methods (point 3.2.3). Based on the number of microtuber produced, fresh weight of microtuber, tuber size and percentage dry matter of microtuber these methods can be grouped into two groups namely:

(1) Tuberization methods produced low number of microtuber and smaller size tuber or lower microtubers fresh weight (TM - LS).

(2) Tuberization methods produced large number of microtuber and bigger size tuber or large mi crotuber fresh weight (TM - HL).

(1) TM - LS (tuberization methods to produce low number and small size microtubers.

Direct methods of solid medium or direct method of liquid medium produced lower number of microtuber, small size *in vitro* tuber (< 50 mg). *In vitro* tubers produced by these methods were usually sessile on the axil of the node of the explants.

Tuber produced axillary were usually small and the number of tuber depend on the response of the node of explants to the induced tuberization experiments showed the direct method produced less number (less than 5 microtuber/bottle) of microtuber and smller size microtuber. The number of microtubers and microtubers size produced in these experiments by the direct methods were similar to that of mank and Langgile (1978), palmer and Smith (1969a, 1969b) and Wattimena (1983).

(2) TM - HL 9Tuberization methods to produce high number and larger size microtubers, solid-liquid, liquid-liquid and liquid substituted liquid of tuberization method in general produced

higher number of microtuber (more than 5 microtubers/bottle) and bigger size microtubers. Experiments 3.3.15. all these methods produced unusually bigger size microtubrs range from 728 mg to 944 mg per microtubers. The average normal size tuber produced by these methods range from 100 to 250 mg/ mcrotubers such as in experiments. These normal size microtuber also produced by CIP (TOvar et al 1985) with liquid-liquid method of tuberization using sucrose90 g/l, BA 5 mg/l and cycocel 500 mg/l to induced *in vitro* tuberization. Size of microtubers depend on cultivars and growth regulation used for induced *in vitro* tuberization. Tuberization using BA 5 mg/l and cycocel 500 - 600 mg/l usually produced bigger size tubers than using paclobutrazol, ancymidol as growth retardants (exp 3.3.15, Wattimena et al 1985). Wattimena and Purwito (1989) modified the liquid system and named it shallow liquid-liquid system of tuberization (SL2S). The SL2S consisted of growing *in vitro* shoot a very thin layer of liquid shoot medium (5 ml in a bottle of 4.5 cm diameter) for three weeks and then added 20 ml of liquid tuberization medium aand incubated in dark for 8 weeks. The SL2S now became a standard method for *in vitro* tuberization in Bogor (Wattimena et al 1991, veronica 1992).

3.4.2. Nitrogen and pH

Nitrogen not only directly effect *in vitro* tuberization of potato but indirectly effect the pH of the media.the source of nitrogen determined the change of the pH of the media. NH_4^+ nitrogen alone will increase the pH of media and NO_3-nitrogen alone will decrease the pH of medium. *In vitro* culture media always combine this two sources of nitrogen. In Murashige and Skoog (MS) medium 40 mM of nitrogen is in NO_3-nitrogen and 20 mM of nitrogen is in NH_4^+-nitrogen.

Experiments 3.3.13 showed that the best nitrogen level all from level of pH (4.7,5.7; 6.7; 7.7) was 15 mM. The nitrogen at 15 mM et all four level of pH produced higher number of microtubers, microtuber fresh weight, percentage dry weight of microtubers than lower level of nitrogen (7.5 mM) or higher level of nitrogen (30; 60 mM). This experiments used 90 g/l sucrose, 5 mg/l kinetin and 25 mg/l coumarine to induce *in vitro* tuberization.

Stallknecht and Farnsworth (1979) found that low level of nitrogen (2.5 mM) in both the explant medium and the tuberization was best for coumarine induced tuberization no tuber were formed if high nitrogen (60 mM) were present in both mediaa. They also found interaction of nitrogen and sucrose at intermediate level of nitrogen (15 mM).

Inhibition of coumarin induced tuberization medium at intermediate level of nitrogen can be overcome by 12% of sucrose but not by 6% of sucrose. Kinetin and BA induced tuberrization medium was not inhibited with high nitrogen level as in coumarine induced tuberization (Palmer and Smith, 1970, Wang anf Hu 1982). Wattimena (1983) found that high nitrogen level (60 mM) in both shoot medium and coumarine induced tuberization medium decrease the number of microtubers. There were no different in number of tuber produced either at low level of nitrogen (4 mM) in shoot medium or at low level of nitrogen (4 mM) in coumarine induced tuberization medium compared to both level of nitrogen (4 mM, 4 mM) in shoot medium and coumarine induced medium.

3.4.3 Sucrose

sucrose concentration in the medium to be most important organic nutrient variable affecting rate and percentage of *in vitro* tuberization of potato. Sucrose of 9% in tuberization medium (exp 3.3.8, 3.3.11) produced higher number of microtubers, microtuber fresh weight and percentage dry weight of microtubers. Thus from the level of sucrose 9% showed the optimum level. Sucrose 3% showed only 50% of the experiment unit produced microtuber and at sucrose 12% all the experimental unit produced microtubers. The nimber of microtubers and microtuber fresh weight produced by sucrose 12% was lower than sucrose 6% and 9% but the reverse was true for percentage of microtuber dry weight.

In vitro tuberization experiments by Palmer and Baker (1970) and other with *in vitro* apical stolon as explants showed similar result. They found the optimum sucrose concentration range between 6% and 8% (Catchpale and Hillman 1969, Palmer and Baker 1970, Stallknecht and Farnsworth 1979) and at 20% tuberization was completely inhibited (Catchpole and Hillman 1969).

3.4.4. Cytokinins

Cytokinins have been known to be able to induce *in vitro* tuberization of potato (Mingo-Castel 1974, 1976, wattimena 1983). In these *in vitro* tuberization experiments of potato we used cheaper cytokinins (BA, kinetin) or cytokinins like activity (adenine sulphate/AS), coconut water (cw) and benomyl to induce *in vitro* tuberization. Media for testing optimal concentration level of cytokinins or cytokinins like activiting were MS media, sucrose 9%, cycocel (CCC) 600 or 800 mg/l and different level of cytokinins concentration. The results of the experiment showed the optimum level for different cytokinins such as: kinetin (exp 3.3.6), BA (exp 3.3.6, 3.3.8), AS (exp 3.3.8), coconut water (exp 3.3.8) and benomyl (exp 3.3.19) were: 10 mg/l, 5 mg/l, 100 mg/l, 15% aand 50 mg/l respectively.

The optimum concentration level of kinetin and BA of these experiments were similar as found by wattimena (1983), wattimena and Purwito (1989). The optimum concentration level of coconut water was similar as found by wattimena and Purwito (1989). No experiment else where with coconut water to subtitute cytokinins to induce *in vitro*tuberization of potato, so no data to compere with our optimum concentration level.

3.4.5. Growth retardants and coumarine

The growth retardants have been evaluated for optimum concentration level to induce *in vitro* tuberization of potato were: cycocel (exp 3.3.8, 3.3.10), ancymidol (exp 3.3.10, 3.3.16, 3.3.17), alar (exp 3.3.10, 3.3.16) and paclobutrazol (3.3.16, 3.3.17). The optimum level for cycocel was 600 mg, while for ancymidol, alar, aand paclobutrazol were in the range of 1.0 mg/l to 10.0 mg/l. The media used for tested optimum concentration level of retardants were MS media, sucrose 9%, kinetin 10 mg/l or BA 5 mg/l and growth retardants according to treatments. Cycocel (CCC) at the concentration of 600 mg/l haave been used at CIP Peru for producing *in vitro* microtubers potato (Trovar et al 1985). No data available for the other retardants (ancymidol, alar, paclobutrazol).

The optimum concentration level of retardants was not evaluated in these experiments, but used the optimum coccentration level of Stallknecht and Farnsworth (1979) that was veryfied by wattimena (1983) (25 mg/l) coumarine at 25 mg/l was evaluated in combination with sucrose 9% and BA 5 mg/l (exp 3.3.1) or kinetin 10 mg/l (exp 3.3.5, 3.3.7, 3.3.13) consistently produced high number of micro-tubers, microtubers fresh weight, and earlier tuberization. The late tuberization of kinetin aand BA has been overcome by coumarine.

3.4.6. Synergistic effect of cytokinins and growth retardants

Gibberellic acid has an inhibiting effect on *in vitro* tuberization of potato even at low concentration (Garcia-Tarres and Gomez- Campo 1973, Palmer and Smith 1970). GA at 2.5 mg/l completely inhibi-ted the *in vitro* tuberization of poato induced by coumarine (Stallknecht and Farnsworth 1982b). Cytokinins has the reverse effect of gibberellic acid (Stallknecht and Farnsworth 1982a, Wang and Hu 1982). Retardants inhibited biosynthesis of gibberellic acid, thus inderectly induced *in vitro* tuberization of potato.

Based on these literatures the experiments have been design to find out if:

(1) Combination of cytokinins and retardants at their optimum concentration level will induce better *in vitro* tuberization than cytokinins or retardants/inhibitor alone.
(2) Any combination of cytokinins aand retardants at their optimum concentration level will have similar effect on *in vitro* inducing tuberization of potato.

The result of experiments 3.3.5 and 3.3.7 showed that coumarine 25 mg/l and kinetin 10 mg/l were better in inducing *in vitro* tuberizationof potato than coumarine 25 mg/l or kinetin 10 mg/l. These have been tested for different form of *in vitro* tuberization media, namely: solid media (exp 3.3.1), liquid media (exp 3.3.5), solid-liquid media (exp 3.3.7) and liquid-liquid media (exp 3.3.7).

The experiment 3.3.19 showed thaat aany combination of cytokinins (BA 5 mg/l, kinetin 10 mg/l, AS 100 mg/l, benomyl 50 mg/l) and retardants (coumarine 25 mg/l, CCC 600 mg/l, paclobutrazol 10

mg/l, uniconazole 3 mg/l) have the same effect on *in vitro* tuberization of potatoes. From the 16 treatments combinations there were no significant different in number of microtuber fresh weight and percentage dry weight of microtubers.

These experimental data second our two assumptions mentimed before. Thus any combination aand retardants at their optimum concentrations level can be used to induced *in vitro* tuberization of potato.

From point 3.4.4 and 3.4.5 have been listed the optimum concentration level of cytokinins and retardant/inhibitor as followed:

(1) Cytokinins or Cytokinins like activity	Optimum concentration
BA (benzil adenine)	5 mg/l
Kinetin	10 mg/l
AS (adenine sulphate)	100 mg/l
Benomyl	50 mg/l
CW (coconut water)	15%

(2) Retardants/Inhibitor	Optimum concentration
Cycocel	600 mg/l
Ancymidol	10 mg/l
Alar	10 mg/l
Uniconazole	3 mg/l
Paclobutrazol	10 mg/l
Coumarine	25 mg/l

There is a possibility of 30 combination that can be used to produce *in vitro* microtuber of potatoes. We recommended one of these four combination namely:

(1) MS media, sucrose 9%, BA 5 mg/l, cycocel 600 mg/l

(2) MS media, sucrose 9%, BA 5 mg/l, coumarine 25 mg/l

(3) MS media, sucrose 9%, coconut water 15%, cycocel 600 mg/l

(4) MS media, sucrose 9%, coconut water 15%, coumarine 25 mg/l

The form of media will be shallow liquid-liquid system (SL2S). These recommendation based in earlier of tuberization, bigger size tuber, greater number of tuber, easy to get the chemicals and cheaper.

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IV. The development of microtubers as a feasible method for potato production

Section I

4.1. INTRODUCTION

A. Role of microtubers

The potato is conventionally propagated asexually by the tuber. This method not only preserves the desired genotype, but also the tissue borne viruses and bacteria that lead to degeneration of yields (Burton, 1966). Certified seed potatoes need to be propagated from a pathogen free plant, under disease free conditions (Johansen, *et al.*, 1984). Through techniques of heat treatment and meristem isolation, plant pathogens can be eliminated from *in vitro* potatoes. Micropropagation provides a controlled disease free environment in which to rapidly multiply the desired potato genotype (Figure IA1).

Potato micropropagation is used extensively all over the world, from developed countries in North America and Europe, to lesser developed countries such as Vietnam and Indonesia (Jones, 1987; Van Uyen and Vander Zaag, 1984; Wattimena, 1983). The advantages of potato micropropagation over the traditional stem cuttings are the higher yield per plant, less labor, a faster rate of multiplication, reduced testing and a increased flexibilility of scheduling with less dependence on mother tuber physiology. Also yield increases of 10-38% have been reported, from micropropagated stocks, as well as an increased ability to withstand and recover from environmental stress (Jones, 1987). In the North America potato micropropagation is used extensively in all seed potato producing states. *in vitro* plantlet production in North America totals 579,700 while stem cutting production is 44,300 (Jones, 1987).

Microtubers can play an important role as an alternative to microcuttings in that they, if started from a properly prepared mother plant, are true to type and pathogen free. In addition microtubers may be produced less expensively than microcuttings by minimizing the need for a high maintenance population stock. Microtubers are easier to handle and can be transported long distances without decreasing in vigor. Microtubers are adaptable to planting by "seed technology" and are not as delicate as microcuttings. Microtubers are more flexible in their planting time than microcuttings.

Microtubers are more flexible in their planting time than microcuttings, which allows for adjustment for the weather with little additional cost. In addition, microtubers have been reported to have a greater vigor than microcuttings when transplanted to the field (McCown and Wattimena, 1987). A disadvantage of microtubers is the additional developed from microcuttings (Wattimena, *et al.*, 1983). Microtubers may be especially useful to growers in tropical countries where problems encountered with conventional potato propagation is so severe that alternative methods of propagation is so severe that alternative methods of propagation are a necessity.

B. Terminology

There has been considerable confusion in the literature as to the meaning of the words minituber and microtubers. Wattimena *et al.* (1983) first used the term microtubers referring *in vitro* potatoes. "Micro" is a commonly used prefix in tissue culture, in words such as micropropagation and microcutting, and stems from the general term for *in vitro* culture, microculture. "Micro" is a descriptive prefix, but by definition indicates *in vitro* culture (Harman and Kester, 1983). According to Wattimena (1983), microtubers are *in vitro* produced tubers, generally less than one centimeter in diameter (Figure IB1). Hussey and Stacey (1984) used the term minituber to describe *in vitro* tubers. Wiersma *et al.* (1987) refers to microtubers as very small ex vitro tubers. Ortiz-Montiel and Lozoya-Saldana (1987) use both minituber and microtuber interchangeably to describe tubers produced *in vitro*. Other researchers referred to them as *in vitro* tubers (Palmer and Smith, 1970; Stallknecht and Farnsworth, 1982a; Mauk and Langille, 1978; Estrada *et al.*, 1986). Wang and Hu (1982) called *in vitro* tubers "miniature tubers." In this paper we will refer to *in vitro* produced tubers

as microtubers, and small ex vitro tubers produced in a nonsterile environment from *in vitro* stock as minitubers. It is important that there be an international general agreement among researchers as to the meaning of the terms microtuber and minituber.

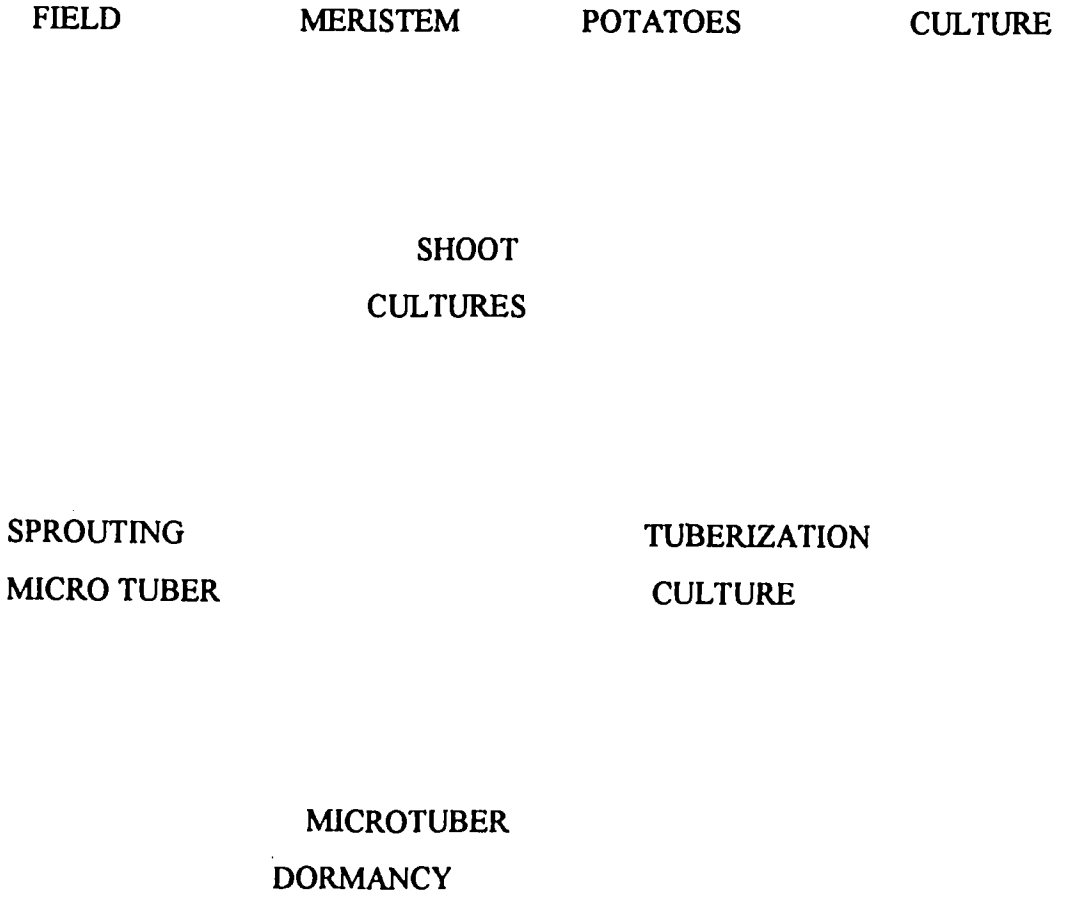

Figure A1. A generalized flow chart showing overall scheme for rapid multiplication of potatoes through micropropagation and microtubers.

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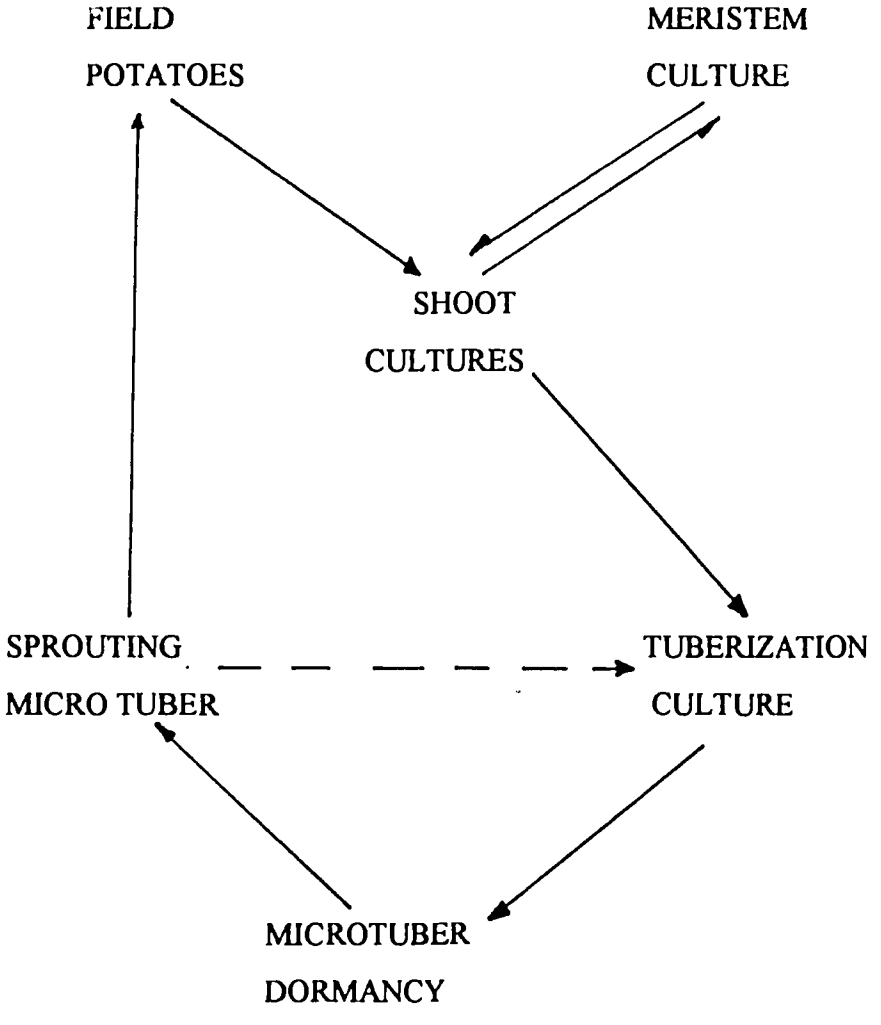


Figure A1. A generalized flow chart showing overall scheme for rapid multiplication of potatoes through micropropagation and microtubers.

Section II

4.2. LITERATURE REVIEW

A. Difference in laboratory technique

Differences in laboratory technique can drastically affect results, sometimes making between-lab comparisons difficult. Well established, stabilized *in vitro* plants have a number of anatomical and physiological differences with ex vitro plants. Cell differentiation and photosynthetic capacity are also altered by the *in vitro* environment (Smith, *et al.*,1986). Because of these physiological and anatomical differences between ex vitro and stabilized *in vitro* plants, they should not be expected to respond in an identical fashion to various stimuli. Often "*in vitro*" experiments are conducted after sterilization of an ex vitro plant placed in pathogen free conditions without time for the tissue to adapt to its new environment. This type of tissue has not gone through the stabilization phase of tissue culture (McCown, 1987). For potatoes, it may take several generations of long photoperiods and constant temperatures *in vitro* in order for the tissue to take on a uniform, juvenile growth habit. Thus the response of a sterilized ex vitro potato grown *in vitro* can vary from a well established, stabilized *in vitro* culture.

In addition to unstabilized culture, differences in vessel types, vessel closure, and gelling agents will affect results in each laboratory. Vessel type, size and motion will influence rate and type of growth. The vessel closure can have a dramatic effect on plant injury due to calcium shoot tip necrosis (Sha *et al.*, 1985). Using Magenta B caps as the vessel closure increases air exchange and transpiration, and decreases calcium deficiency injury (Sha *et al.*, 1985). Vessel type also influences potato anatomy and physiology. Potato shoot cultures grown in test tubes generally have larger more differentiated leaves and thicker stems than shoot cultures grown in cultures jars. There also can be striking differences in agar quality and concentration which affects availability of cytokinin and mineral elements, as well as conductivity and solidity of the gel (Debergh, 1983ü. Subtle differences in laboratory iquipment and procedures can influence a tissues response in different experiments. Therefore comparisons between laboratories have to take into account these differences.

B. *in vitro* tuberization

Various factors can affect the tuberization of *in vitro* potatoes. Temperature, light, photoperiod, sucroce concentration, syntetic growth regulators, and gas composition all affect potato *in vitro* tuberization. These factors were reviewed by my predecessor in this lab, Gustaaf A. Wattimena (1983). This review, and updates it with recently published literature.

Temperature

The optimal temperature range for *in vitro* tuberization appears to vary from system to system. Wang and Hu (1982) found that 28°C and a 12 hour alternating high/low temperatures of 27/22°C, 27/17°C, and 27/12°C were not as productive as a constant 20°C. They also reported that temperatures over 28°C were inhibitory to tuber formation. For a solid media system, Hussey and Stacey (1984) found no difference in tuber formation between 10 and 25°C, and selected 25°C as their optimal temperature. Wattimena (1983) found that 25°C was inhibitory and that 10 to 15°C was optimal for both kinetin and no growth regulator based tuberization with axillary nodes. Bourque *et al.*, (1987) using axillary buds as explants found 19°C to be better than 25°C. Palmer and Smith (1970) reported that 15°C was suboptimal for a kinetin based tuberization. but their explants were sterilized ex vitro stolons. Several researchers used 20-25°C as their optimal temperature, but did not use *in vitro* axillary buds as their explants (Stallknecht and Farnsworth, 1982; Palmer and Smith, 1970; Mauk and Langille, 1978; Mingo-Castel *et al.*, 1974). Other researchers that used 20-25°C have given no significant data on constant temperatures below 20°C showing inhibition of tuber formation (Ortiz-Montiel and Lozola-Saldana, 1987; Wang and Hu, 1982; Rossell, *et al.*, Estrada, *et al.*,1986). In summary the researchers who have used *in vitro* explants and compared temperatures above and below 20°C have found temperatuures below 20°C and above 5°C to be better for tuberization to temperatures above 20°C (Wattimena, 1983; Bourque, *et al.*, 1987; Hussey and Stacey, 1984).

Sucrose

Using a liquid shaker system, Wang and Hu (1982) reported that potatoes grown on media amended with 8% (wt/wt) sucrose produced more tubers than potatoes on media with 6% and 9% sucrose.

Wattimena (1983) using a solid medium reported that potatoes grown on media amended with 6% sucrose produced more microtubers than 4% and 8% sucrose, but the difference was not significant. Other researchers found the optimal sucrose level to be between 6 and 8% (Estrada, *et al.*, 19887; Hussey and Stacey, 1984).

Photoperiod

Photoperiod requerements for tuberizing potatoes vaary between laboratories. Hussey and Stacey (1984) found taht shoot cultures in solid media under 16 and 24 hour photoperiod tuberized, but cultures under 8 hour photoperiod did not. Wang and Hu (1982), using shaken liquid media, reported that an 8 hour photoperiod gave more microtubers than a 16 hour photoperiod, but neither laboratory tested tuberization in the dark. Wattimena (1983) tested photoperiods at 0, 8 and 24 hours for tuberization and reported that the longer the photoperiod, the better the tuberization. Ortiz-Montiel and Lozoya-Saldana (1987) found that the cultivar Atzimba tuberized with higher fresh weight in an 8 hour photoperiod better tha diffuse light and darkness. Other workers tuberize cultures in the dark (Wattimena, 1982, Estrada, *et al.*, 19886).

Gas

Experiments by Mingo-Castel, *et al.* (1974, 1976a) showed that 8-10% CO_2 for 3-5 days promoted tuberization in sterilized stolons cultured *in vitro*. In addition, the total absence of CO_2 is detrimental to tuberization. Ethylene gave a slight promotion of tuberization for some researchers (Stallknecht and Farnsworth, 1982; Garcia-Torrez and Gomez-Campo,1973), but was inhibitory to tuber induction for others (Mingo-Castel, *et al.*, 1974, 1976a). For *in vitro* cultures, Wang and Hu (1985) found that $KMnO_4$ soaked filter paper completely alleviated ethylene-induced inhibition.

Synthetic growth regulators

Gibberellic acid (GA): For *in vitro* potatoes there is strong evidence that GA inhibits tuberization. Garcia-Torrez and Gomez-Campo (1973) reported that even low concentrations of GA inhibits *in vitro* tuberization. Stallknecht and Farnsworth (1982) reported no inhibition of *in vitro* tuberization at 0.01 ppm GA and comlete inhibition of tuberization at 2,5 ppm GA.

Auxin: Most *in vitro* tuberization experiments with exogenous auxins have used indole acetic acid (IAA), 2-naphthalene acetic acid (NAA) 2,4-dichlorophenoxy acetic acid (2,4-D). The activity of the auxin is dependant on its concentracion. Stallknecht and Farnsworth (1982) reported that 25ppm IAA and 1.0 ppm totally inhibited coumarin based tuberization, but only slightly inhibited non-coumarin based tuberization.

Cytokinins: Exogenous citokinins have the effect of promoting *in vitro* tuberization (Koda and Okazawa, 1983; Hussey and Stacey, 1984; Mingo-Castel *et al.* 1976; Mauk and Langille, 1978; Palmer and Smith, 1970; Ortiz-Montiel and Lozaya-Saldana, 1987). An optimal dosage for liquid shaker culture was 44.4 μM benzyl adenine (BA) (Wang and Hu, 1982) and the for solid media was 44.4 μM kinetin (Wattimena, 1983). Citokinins promote cell devision, cell enlargement and inhibit cell elongation, which is needed for tuberization (Palmer and Smith, 1970).

Coumarin: Coumarin also induces potatoes to tuberization for *in vitro* cultures (Stallknecht, 1972; Stallknecht and Farnsworth, 1979, 1982a). The optimal coumarin concentration is 170 μM (Stallknecht and Farnsworth, 1983a). Coumarin tuberization appears to be different than cytokinin based tuberization. Coumarin induced tuberization is inhibited by protein and nucleic acid biosynthesis inhibitors, indicating that the tuberization required de novo protein synthesis (Stallknecht and Farnsworth, 1982b). No comparable effect was seen for kinetin induced tuberization (Palmer and Smith, 1970). In addition kinetin was metabolized in the potato while coumarin's structure remained intact (Stallknect and Farnsworth, 1982) and coumarin based tuberization was inhibited by media high nitrogen levels while kinetin based tuberization was not (Stallknect and Farnsworth 1979; Palmer and Smith, 1970). Thus the two modes of inducing tuberization may be distinct (Stallknecht and Farnsworth, 1982a).

Other: Abscisic acid (ABA) has been found to be inhibitory to *in vitro* tuberization (Palmer and Smith, 1969). Chlorochlorine (CCC) was found to be stimulatory for *in vitro* tuberization, presumably by inhibiting GA biosynthesis (Tizio and Biain, 1973; Estrda, *et al.*, 1986). The resulting microtubers seem to exhibit a uniform dormancy (Dodds, 1987).

There are several different ways of inducing microtubers. CCC, cytokinins and coumarin have successfully induced 100% tuberization. None of the inducing growth regulators, CCC, cytokinins and coumarin appear to be stritly necessary for tuberization without the others. Tuberization probably is a general response of the potato to a range of stimuli and stress.

C. Research goals

Although the are several viable methods for microtuber induction, no one method seems to be commercially viable. The work of McCown and Wattimena (1987) indicated that the lenght of dormancy between microtubers was not consistent. Some microtubers would sprout within two weeks of harvers, while others would not sprout for 3 months. A commercially feasible production system requires that microtubers sprout uniformly. Since then our lab has changed several procedures in order to get more uniform production. We now use 280 ml. culture jars with Magenta B caps, and add 3μM calcium gluconate to media. These two innovations improved uniformity in tuberization. My work at making microtubers took two approaches in order to achieve uniform microtuber sprouting: 1) to optimize tuber induction and size testing the effects of growth regulators (Section IIIA), explant leaft (Section IIIB), shoot culture propagule (Section IIIC), axillary node position (Section IIID), and gelling agent (Section IIIE); 2) to break dormancy in microtubers using GA, cytikinins or rindite (Section IIIF).

A second set of problems that needs to be addressed is the lack of multiplication, and the high labor input in microtuber production. Meristems (nodes) multiply in shoot culture at arate of 6-10 meristems/month. Microtuber production gives a multiplication rate of 0.25-0.5 microtubers/month or 24 times less than shoot culture multiplication (Wattimena, 1983; Estrada *et al.*, 1986). Production oriented systems need to raise multiplication rates in order to make microtuber production more feasible commercially.

In order to improve multiplication rates, reduce culture time amd transfer time my work took two approaches: 1) to stimulate the growth of axillary buds through the use of a synthetic growth regulator Thidiazuron (Section IVA); 2) to test *in vitro* culture systems desigened for lower labor input (Section IVB), and higher multiplication rates (SectionIVC).

Section IIIA

4.3. REPORT INDIVIDUAL IN VITRO EXPERIMENTS

MICROTUBERS INDUCTION, GROWTH AND DORMANCY

Introduction: Microtuber production is influenced by physical, hormonal, and nutritional parameters. Previous research by Wattimena (1983) showed that 15°C, dark and 6% sucrose gave optimal microtuber production on a solid medium. Optimal concentrations for cytokinin and coumarin were found to be 45 and 170μM respectively. CCC was not tried based on a fear of acarryover effect on subsequent growth. The semisolid medium microtuber production system, because of its uniformity and reproducibility, was used to investigate microtuber culture, growth, dormancy, storage and calcium levels.

Materials and Methods: Red Pontiac shoot cultures, virus-indexed and stabilized, were used as source material for tuberization. Shoot cultures were maintained at 22°C, with 40 μ Einsteins of light, in 280 ml jars covered with Magenta B caps for sterility and air exchange. The madium was composed of MS mineral salts (Murashige and Skoog, 1962), amended with 3.0 mM calcium gluconate (Zeldin and McCown, 1986), 87 μM sucrose (30 g/L), and solidified with 3.0 g/L of agar and 0.75 g/L of gel rite. Shoot tips were transferred on 30 mls of media without synthetic hormones. For tuberization ten axillary buds were transferred into 120 ml glass jars, embedded in one of the four treatment media at 175 mM sucrose (6%), and placed into a dark growth chamber at 15°C. There were ten replicates each with ten explants, for a total of one hundred explants per treatment, arranged in a Randomized Complete Block. Each treatment had the following medium growth regulator combinations: 1) 45μM BA only 2) 170 μM coumarin only; 3) no growth regulators; 4) both 45 μM and 170 μM coumarin. Data on the number of tuberized explants was

recored once a week for eight weeks. After eight weeks of growth the microtubers were harvested and the data were taken recording the fresh weight, dry weight, percent dry matter and percent calcium. The calcium content of the microtubers was determined by atomic absorbtion analysis. All microtubers within a treatment were pooled in order to run the calcium analysis.

In a separate experiment, microtubers were induced according to the four different growth regulator treatments, as described previously. The diameters and fresh weights of the microtubers were measured once a week for 11 weeks. Five jars with ten microtubers each for a total of fifty microtubers were sampled eachweek. Since sampling was destructive, 45 jars for a total of 450 microtubers were sampled. Each microtuber was individually measured for diameter. Diameter was measured in the plane perpendicular to longitudinal axis. Microtuber fresh weight was measured in groups of ten.

Different groups of Red Pontiac microtubers induced by 45 μM BA and 170 μM coumarin were held in storage at 5°C. Over a period of a year the treatments were planted in seeding trays in the greenhouse under a mist of 2 seconds per minute. Treatments of 7, 12, 16, 20, and 33 weeks had 10 microtubers while the 24 week treatment had 45 microtuber. Observations on the percentage of seedlings for a given storage time were taken once a week for four weeks.

Results and discussion: Coumarin induces microtuberization quicker than BA. Coumarin amended medium induces 100% tuberization after only 3 weeks, while 100% tuberization took four to five weeks For BA only amended media (Fig IIIA1). Potatoes in media that have no growth regulators added did not have as high of a tuberization percentage as those potatoes in media with either BA or coumarin. The combinaation of high sucrose, dark, and 15°C did induce tuberization in Red Pontiac, but the tuberization percentage was improved with the addition of both BA and coumarin. The combinations of hormones stimulated tuberization in 100% of the eight different cultivars tested. The only significants genotife-syntetic hormone interaction was DTO-28 which did not tuberize with the coumarin-only treatment.

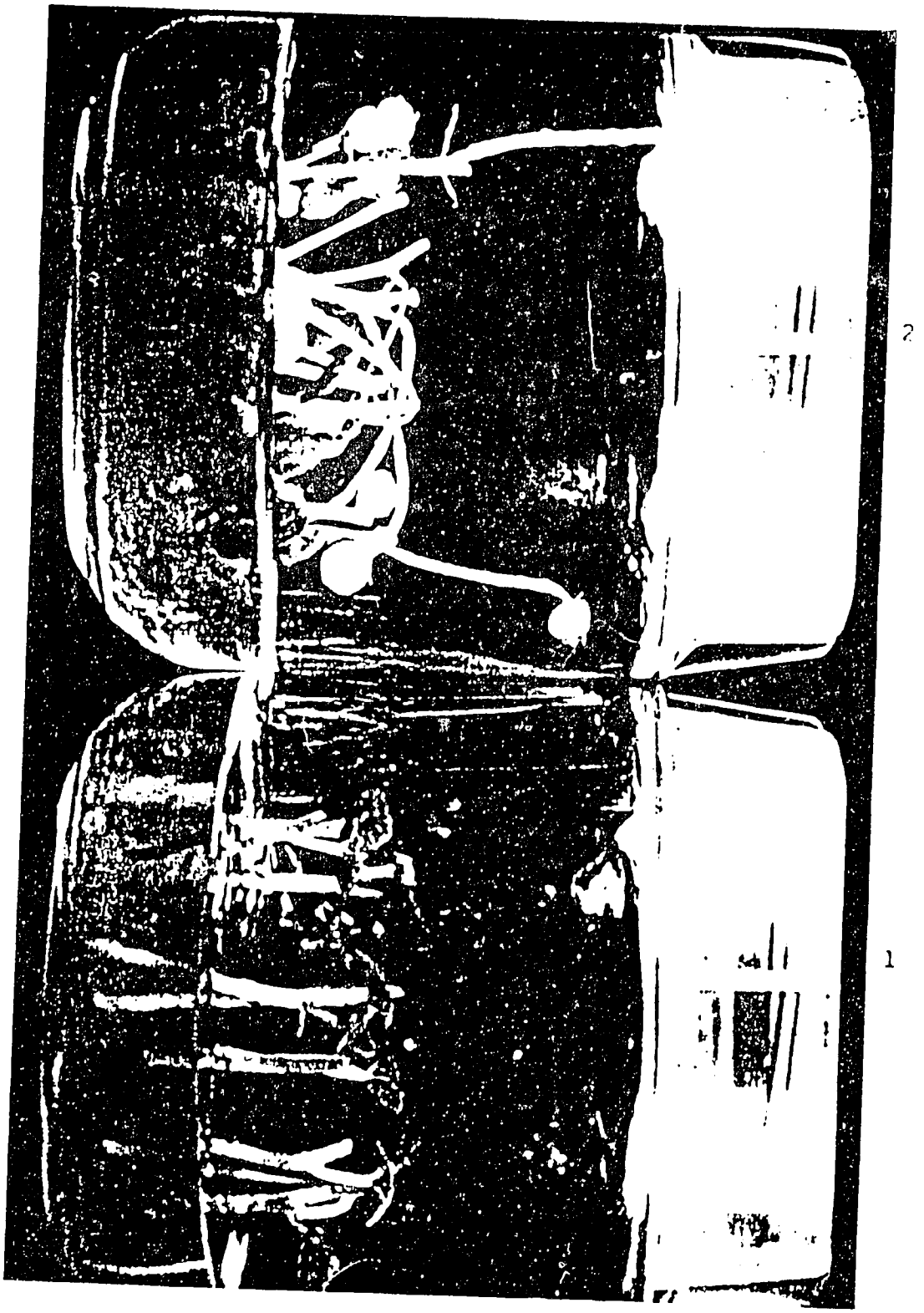


Figure IB1. 1) Explants on the day of transfer; 2) Microtubers eight weeks after transfers.

In addition, BA and coumarin amended media induce larger sized microtubers than a no growth regulator media (Fig. IIIA2). The microtuber diameter and fresh weights are largest for Red Pontiac grown on a media amended with both BA and coumarin (Table IIIA; Fig. IIIA3).

Red Pontiac microtubers grown on media amended with BA and BA + coumarin have a higher percentage dry matter than the microtubers grown with no growth regulators or with coumarin only (Table IIIA2; Fig. IIIA4). In addition the same two treatments have a lower percentage of calcium (Fig. IIIA5; Table IIIA2). A hypothesis that expalins the low calcium in BA induced microtubers, is that BA at the 45 μM level totally inhibits rooting of the explants gives a lower water uptake. The water uptake is responsible for the uptake of calcium, since calcium is only mobile in plant's xylem tissue. Therefore the microtubers on treatments with BA have no roots, little water uptake, and little calcium uptake. The treatments that have no BA in the media, have roots, and have a stronger transpirational stream. Water droplets were observed on the microtuber grown on coumarin only treatment media, indicating guttation.

Potatoes grown under field conditions tyfically will range in calcium levels in medullary tissue from .02 to .06 percent without any visual symptoms of a calcium deficiency (Kratzke, 1987). Microtubers grown on a BA amended media have marginally low calcium levels. When such microtubers are planted, care should be taken to insure they get enough calcium.

The fresh weight of the Red Pontiac microtubers increased steadily over and showed no sign of decreasing after being in tuberization culture for 11 weeks (Fig. IIIA6). The increase in fresh weight was linear, with an average increase of approximately 7 mg. per week. This indicates that it would take a prohibitively long length of time to get microtubers to be the size of microtubers. For example it would take a microtuber approximately 2000 weeks growing at 7 mg. per week to reach the 1/2 ounce (14 grams) size. A different *in vitro* system would be needed to produce a significantly larger microtuber.

In contrast the rate of increase in diameter seems to decrease after 6 to 8 weeks (Fig IIIA7). Assuming the microtuber is a sphere and that it has a constant density, the diameter would increase as the cubed root af the fresh weight. Thus the increase in diameter is much slower than the increase in fresh weight.

Throughout all trials of tuberization with 45 µM BA and 170 µM coumarin, the sprouting of microtubers when stored at 5°C has been uniform . Red Pontiac microtubers gave 100% emergence between 24 and 33 weeks when stored at 5°C (Fig IIIA8). The microtubers generally did not emerge unless they had a sprout of approximately 2 mm. in length (Wattimena, 1983). These data have not definitely defined the period of emergence of Red Pontiac microtubers. Rather this data shows the biological for microtubers to emerge uniformly if induced and stored as described earlier.

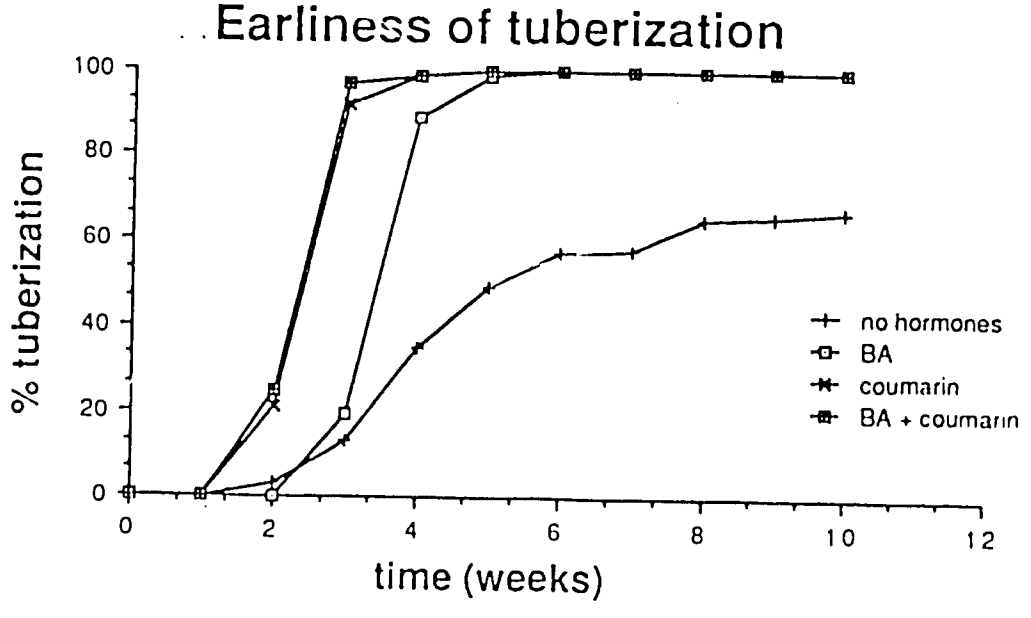


Figure IIIA1. The percentage of Red Pontiac microtuberization vs. time under four different tuberization regimes. Data was collected from five vessels containing 10 explants each.

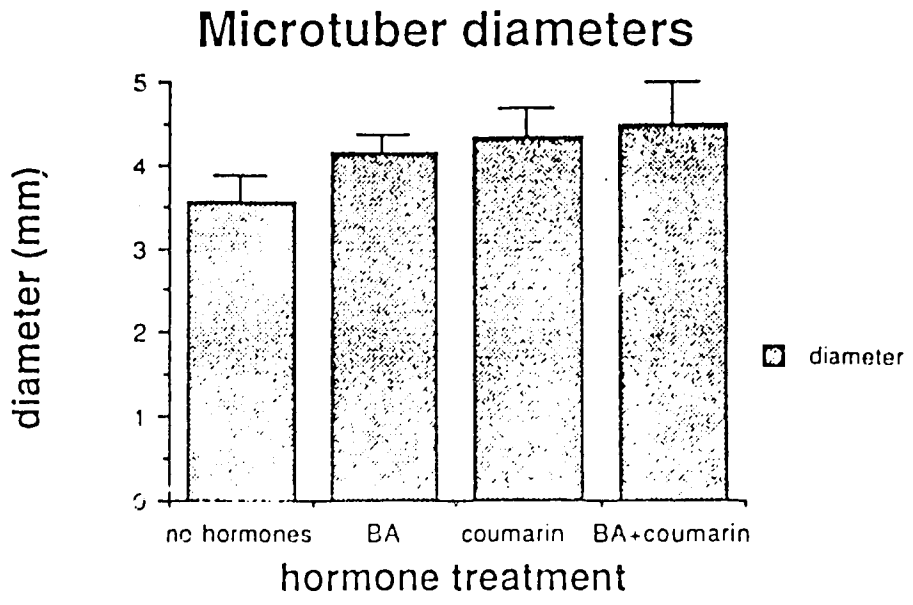


Figure IIIA2. The mean diameter of Red Pontiac microtubers for four different tuberization regimes. Data was collected from four vessels containing 10 explants each. The bars indicate standard deviation.

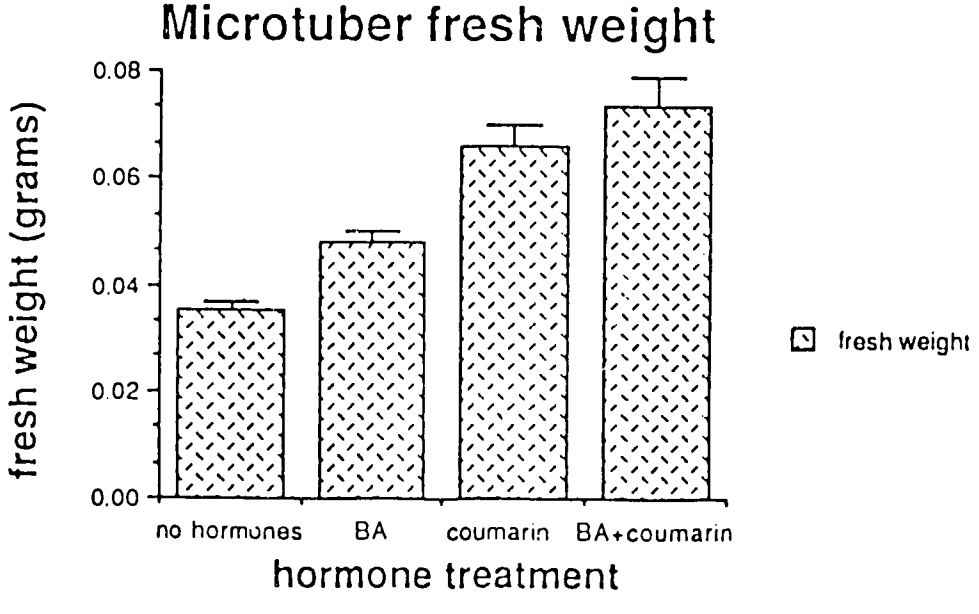


Figure IIIA3. The mean fresh weight of Red Pontiac microtubers for four different growth regulator combinations. Data was collected from ten vessels containing 10 explants each.

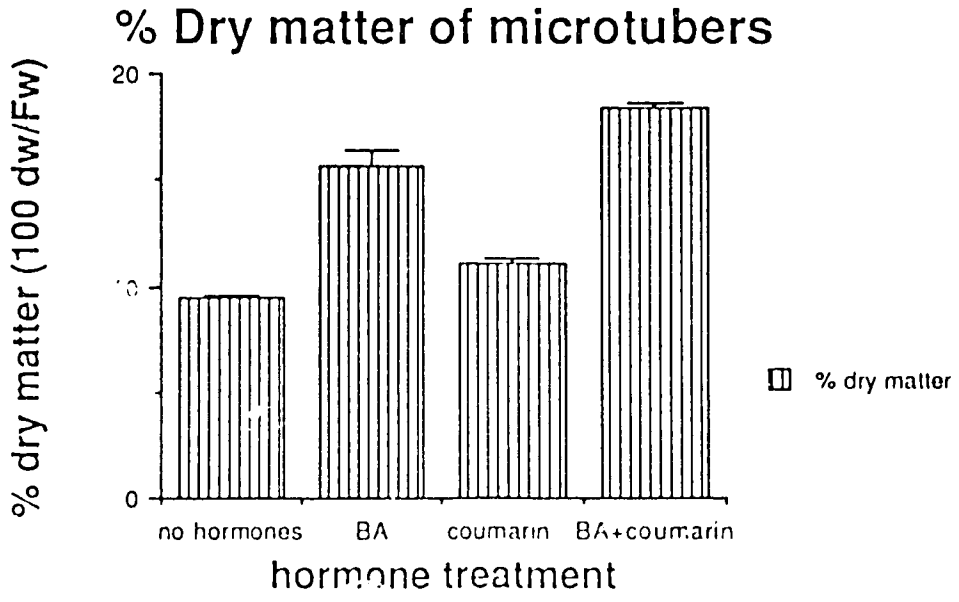


Figure IIIA4. The percent dry matter of Red Pontiac microtubers for four different growth regulatir combinations. Data was collected from ten vessels containing 10 explants each.

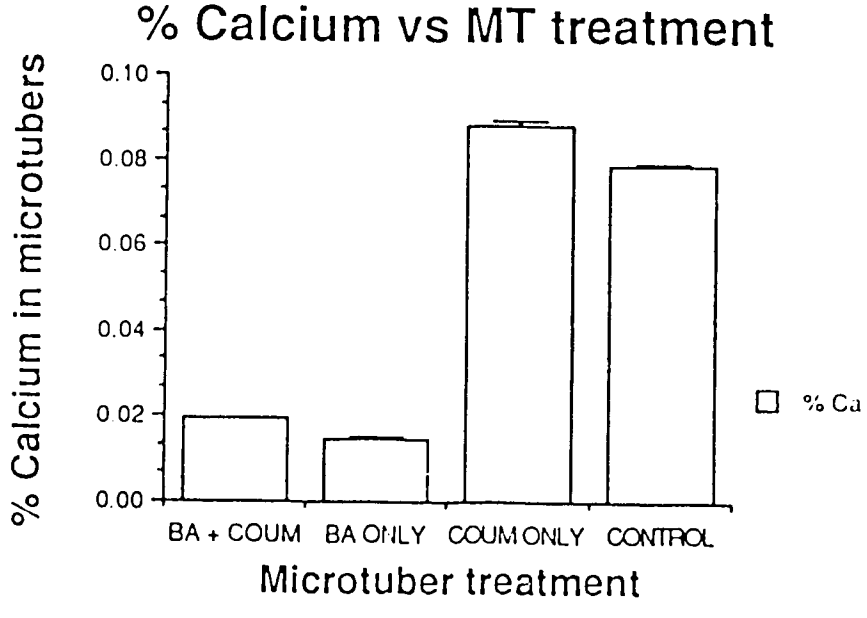


Figure IIIA5. The percent calcium of Red Pontiac microtubers for four different growth regulator combinations. Data was collected from 100 microtubers in each treatment pooled together and analyzed three times.

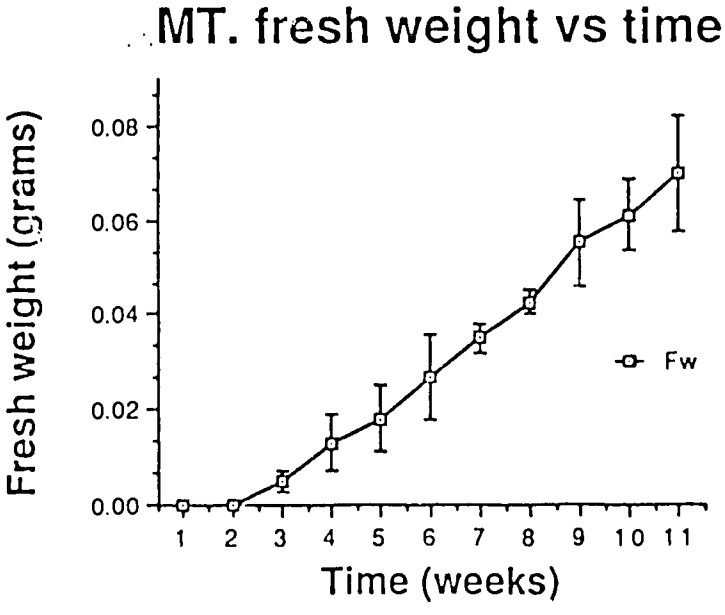


Figure IIIA6. The increase in fresh weight over time of Red Pontiac microtubers grown on 45 μM BA and 170 μM coumarin amended media. Data was collected from five vessels containing 10 explants each. Bars indicate standard devitions.

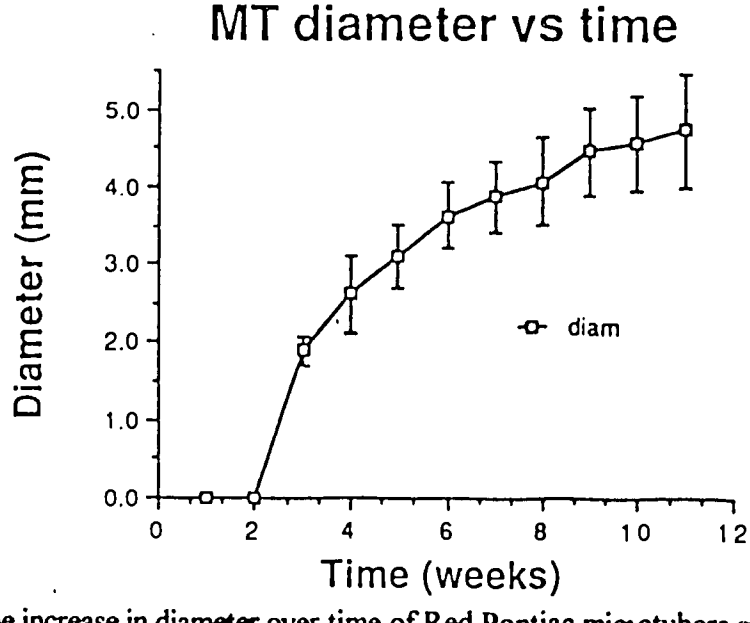


Figure IIIA7. The increase in diameter over time of Red Pontiac microtubers grown on 45 μM BA and 170 μM coumarin amended media. Data was collected from five vessels containing 10 explants each. Bars indicate standard devition.

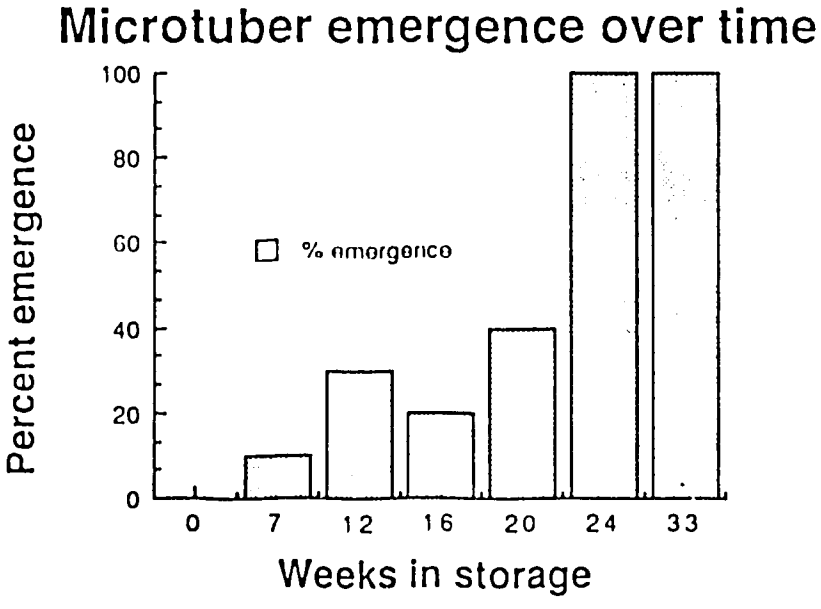


Figure IIIA8. The percent sprouting of Red Pontiac grown on 45 μM BA and 170 μM coumarin amended media for weeks and stored at 5°C.

Table IIIA1. Fresh weights and diameters of Red Pontiak microtubers grown on 45 μM BA and 170 μM coumarin amended media for 8 weeks.

diameter	fresh weight	
Treatment	(mm)	(grams)
no hormones	3.56 (.64)	.0355 (.0012)
BA only	4.14 (.46)	.0484 (.0019)
coumarin only	4.35 (.70)	.0659 (.0042)
BA and coumarin	4.49 (.50)	.0735 (.0054)

The standard deviations are in parentheses.

Table IIIA2. % Dry matter and % calcium in Red Pontiac microtubers grown on 45 μM BA and 170 μM coumarin amended media for 8 weeks.

Treatment	% dry matter	% calcium
no hormones	9.4 (0.2)	.079
BA only	15.7 (0.7)	.014
coumarin only	11.1 (0.2)	.088
BA+coumarin	18.4 (0.2)	.019

The standard deviations are in parentheses.

Section IIIB

EFFECTS OF THE EXPLANT LEAF

Introduction: There are many factors in a semisolid medium system that potentially can affect the uniformity and quality of *in vitro* tuberization. This section will look at a parameter that may have and influence on either the percent of tuberization or on the fresh weight of the microtuber.

Experiments with cuttings have indicated that when environmental conditions are equal, cuttings with a large leaf area are more likely to develop tubers than cuttings with a small leaf area (Ewing, 1985). However when environmental conditions for tuberization are favorable the difference in leaf area plays a smaller role. Although *in vitro* cuttings generally have small, undifferenteated leaves, they may play a role in the tuberization stimulus. In this experiment the tuberization of microcuttings with and without leaves were compared in order to lean if the leaves on microcuttings have a significant influence on tuberization of potatoes.

Materials and Methods: Single node axillary buds from DTO-28 shoot cultures were randomized in sterile distilled water. Upon removal from the water, buds were cut on the petiole to remove the leaf. Ten axillary buds were transferred to each jar, five buds with leaves and five without. The jars were arranged in a completely randomized block. Tuberization procedurs were conducted as described in section IIIA. There were two tuberuzation media, one with 45 μM BA and the other with 45 μM BA and 170 μM coumarin. After transfers, the cuttings were placed in the dark at 15°C. After 70 days in the dark, the microtubers were harvested, counted and weighed.

Results and Discussion: There were no significant differences in either the percent tuberization or the fresh weight per microtuber between micricuttings with or without leaves (Table IIIB1). In an *in vitro* system tuberization is stimulated by the physical, nutritional and hormonal factors. There were no significant differences in the fresh weight and percent tuberization between explants with and without leaves for two different types of tuberization (Table IIIB1). Apparently the *in vitro* physical,

chemical and hormonal environment overwhelms any difference in induction stimulus that may be present in the leaves.

It must also be considered that DTO-28 tuberized well *in vitro*. Russett Burbank shoot cultures grown in test tubes have larger, more differentiated leaves. Test tube grown Russet Burbank potatoes may be a more appropriate cultivar to examine the more subtle leaf influences than DTO-28).

Table IIIB1. The mean fresh weight per microtuber and percent tuberization of DTO-28 explants, with and without leaves. Data was collected from vessels containing five explants with leaves and five explants without leaves.

BA (μM)	coumarin (μM)	Fw (grams) cuttings w leaves	Fw (grams) cuttings no leaves
45	170	0.0678	0.0690
45	0	0.0472	0.0484

BA (μM)	coumarin (μM)	Percent tuberization cuttings w leaves	cuttings no leaves
45	170	96	80
45	0	92	88

Means are not significantly different by the LSD test at the 5% significance level.

Section IIIC

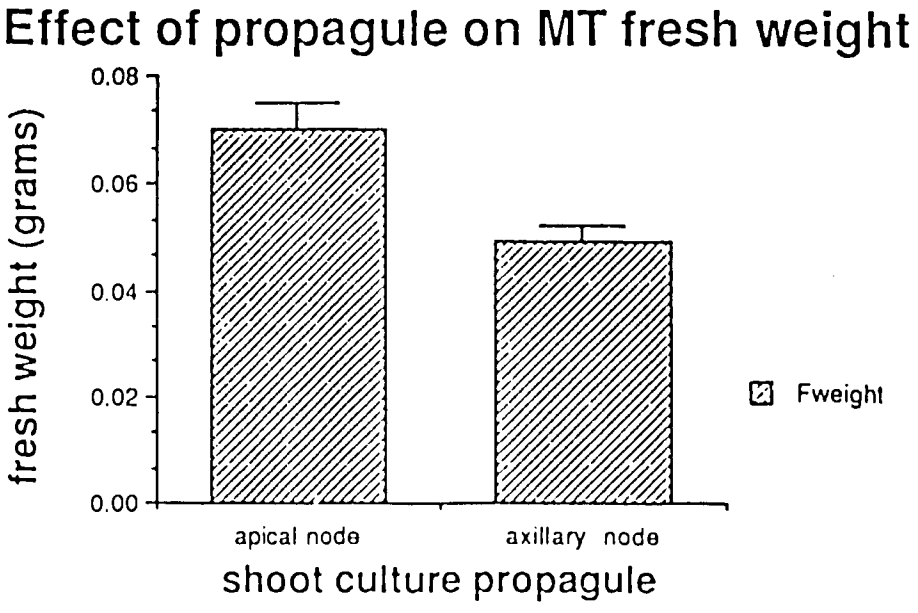
EFFECTS OF SHOOT CULTURE PROPAGULE

Introduction: High quality shoot cultures are essential for optimal microtuber production. Shoot cultures can be propagated by either axillary or apical meristems. Preliminary observations indicated that apical buds produced shoots with thick stems, large leaves, and evenly spaced nodes. Shoots derived from axillary buds tend to have univenly spaced nodes, small leaves, and thin stems. This experiment was conducted in order to measure the influence of shoot culture propagule on the production of microtubers.

Materials and Methods: All axillary and apical buds of Red Pontiac shoot cultures were separated and randomized in sterile distilled water. Buds from each group were randomly selected and transferred to separete 280 ml media jars, with 10 explants per jar. The jars were arranged in a completely randomized block at 22°C under constant light. After three weeks five axillary buds per jar from both types of shoot cultures were transferred to media with 45 μM BA and 170 μM coumarin and stored at 15°C in the dark for eight weeks. There were 20 jars or a total of 100 explants per shoot culture source. Each jar was harvested individually, weighing the fresh weight per microtuber for each shoot culture source.

Results and Discussion: Microtubers derived from shoot cultures propagated by apical buds a significantly larger fresh weight than microtubers derived from shoot cultures propagated by axillary buds (Fig. IIIC1).

In a production system it works well to use the apical bud to propagate shoot cultures and the axillary buds to propagate microtubers (Fig. IIIC2). In this fashion, all meristems are used effeciently and the meristems produce the highest quality shootculturesanmicrotubers.



The means are different significantly as determined by the LSD test at 5% significance level.

Figure IIIC1. Microtuber fresh weights for explants derived from shoot cultures propagated by axillary buds and by apical buds. aaData was collected from twenty vessels containing ten microtubers each.

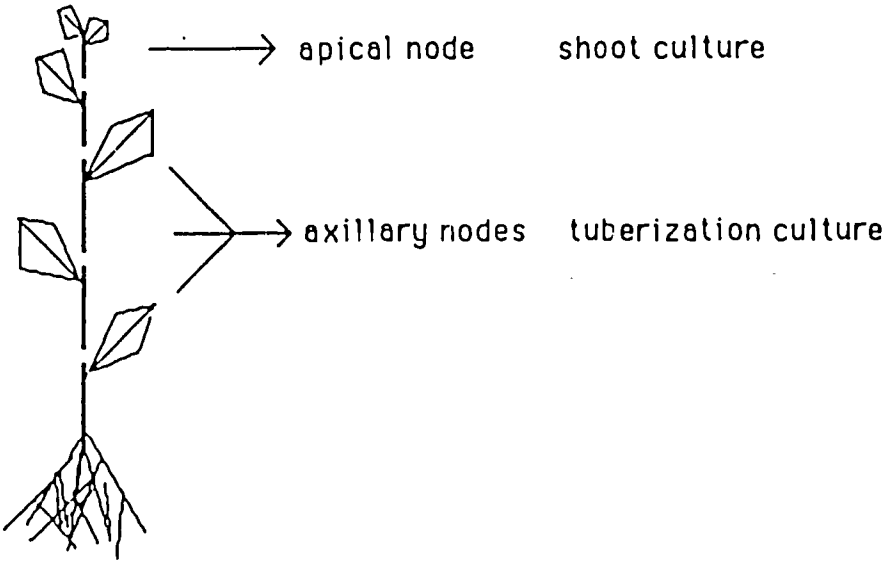


Figure IIIC2. A schematic diagram of a potato microshoot showing that the apical bud is used for shoot cultures and the axillary buds are used for tuberization culture.

Section IIID

EFFECT OF NODE PLACEMENT ON TUBERIZATION

Introduction: In a microtuberization media, very high levels of growth regulators may be used in order to get uniform tuberization. Preliminary observations indicated that some axillary meristems in 45 µM BA and 170 µM coumarin became necrotic. The object of microtuberization, is to get a high percentage of tuberization. The object of this study was to learn if the position of node relative to the medium had an effect on the fresh weight and percent tuberization of the explant.

Materials and Methods: Axillary buds, from Red Pontiac shoots cultures maintained as described in section IIIA, were selected for long internodes and randomized in sterille destiled water. The axillary buds were than transferred to 45 µM BA and 170 µM coumarin tuberization media as described in section IIIA, at varying height levels (Fig. IIID1). Below indicated that the node was beloow the surface of the media; even meant that the node was level with the media; low indicated that the node was less than 1 cm above the media; and high meant that the node was more than 1 cm above the media. Each jar had 8 explants, two explants per position. There were 13 replicates. After 8 weeks the microtubers were harvested, and measured for fresh weight per microtuber and percent of tuberization.

Results and Discussion: Ba is generally not translocated in shoot culture. Coumarin is translocated, but its translocation is inhibited by high nitrogen cotent (62.5 mM) of the media (Stallknect and Farnsworth, 1982a). Considering the limited hormone translocation, there may be hormone gradients along the axis of the explant of both coumarin and BA. Therefore nodes at different heights above the medium probably are subject to different hormone concentrations. A meristem's reactions to the hormones might be a wounding response, or tuberization. There may be significant correlation between relative distance of the node to the media and the type of tuberization.

A significantly lower percentage of nodes tuberized when they were placed below the medium (Table IIID1). Although the fresh weight per microtuber did not vary significantly between treatments (Fig. IIID2), the standard deviation of the fresh weight per microtuber did increase as the node got closer to the medium (Fig. IIID3). If a node tuberized below the media, it was either very big or very small. There was little difference in fresh weight and percent tuberization for the Red Pontiac explants at the high and low tuberization positions. The level position had and intermediate effect in percent tuberization, standard deviation, and fresh weight (Fig. IIID2,3, Table IIID1).

Genarally the node needs to be about 1 cm above the media in order to get uniform tuberization and fresh weight using the 45 μM BA and 170 μM coumarin media.

Table IIID1. The number of tuberized Red Pontiac nodes placed at different relative distances in relation to the media surface. Data was collected from jars containing 10 explants.

node position	Percent tuberize
high	0.96a
low	0.93a
even	0.81ab
below	0.50b

Means with a smilar letter indicates no significant difference by the LSD test at the .05 level of significance.

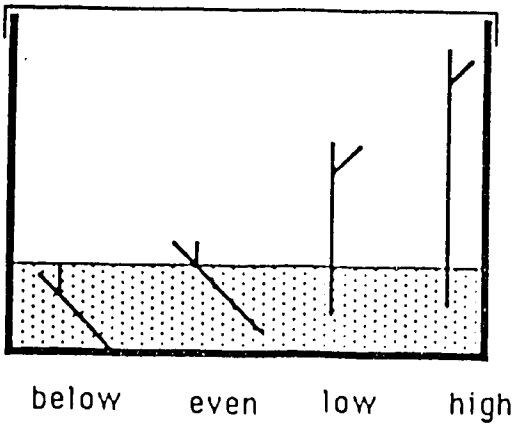


Figure IIID1. The positions of potato axillary nodes relative to the medium.

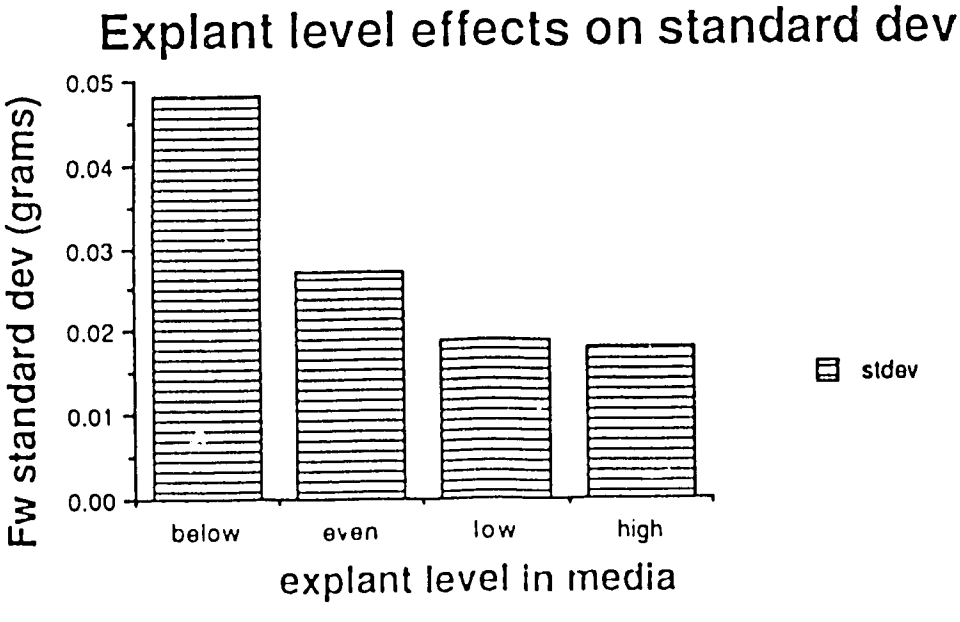


Figure IIID3. The standard deviation of the fresh weight of Red Pontiac microtubers of explants placed at varying positions relative to the medium.

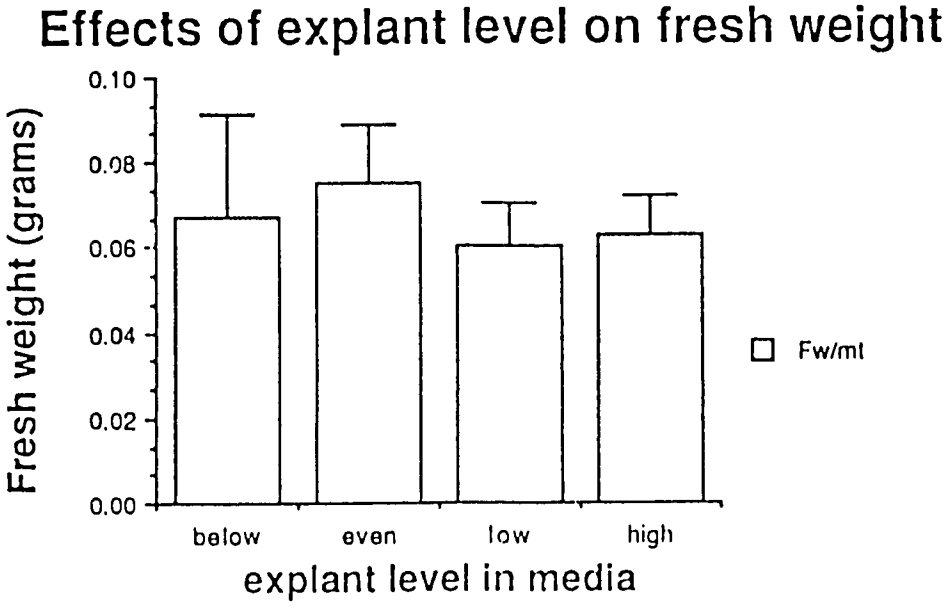


Figure IIID2. The fresh weight of Red Pontiac microtubers of explants placed in varying positions relative to the medium.

Section IIIE:

A COMPARISON OF AGAR AND GELRITE FOR POTATO SHOOT CULTURES AND MICROTUBERS

Introduction: Gelrite is a polisaccaride derived from bacterial exudate. It is chemically composed of uronic acid, rhamnose, and glucose (Kelco, 1982). Gelrite can be used as a gelling agent, like agar, with some different physical and biological properties. Gelrite is clearer than agar, allowing for better detection of contamination. Gelrite has been essential for the growth of ginseng, Panax quinquepolium, in culture, (Thompson, 1985). When used in combination with cytokinins, gelrite has been associated with vitreous growth in Populus and Malus (Pasqualetto, *et al*, 1986). Gelrite used in combination with agar has been found to be effective in reducing the vitreousness of shoot cultures (McCown, 1987; Pasqualetto, *et al* 1986). Most research labs working on the micropropagation of potatoes have used agar (Rosell, *et al*, 1987; Roca, *et al* 1978; Thorton and Knutson, 1986; Hussey and Stacey, 1984; Koda and Okazawa, 1983; Stallknecht and Farnsworth, 1982; Uyen and Vander Zaag, 1983; Wattimena *et al.*, 1983; Wang, 1987), but some use gelrite (Slack, 1986; Bourque, *et al*, 1987). Borque, *et al* (1987) found that gelrite gave more reproducible and larger microtubers (*in vitro* tubers) with their system. Commercial and research potato micropropagation demand both optimal production and high reproducibility. This experiment was undertaken to compare the growth of potato shoot cultures and microtuber media with agar, gelrite, and agar/gelrite compositions to see if there is a significant difference in response the potato.

Materials and Methods: Stabilized and virus indexed Red Pontiac and Russet Burbank shoot cultures were grown on medium as described in section IIIA. To evaluate shoot cultures, ten shoot tips of Russet Burbank were transferred to each culture jar in Randomized Complete Block design. The treatments consisted of the following media gelling agents: 1) 1.5 g/L gel rite; 2) 6.0 g/L agar; 3) 0.75 g/L gelrite-3.0 g/L agar. Each treatment had eight replicates. Each shoot culture was measured for nomber of nodes, shoot height, shoot and root fresh weight, dry weight and oercent dry matter. Roots were first separated from the gel with water and then were blotted dry before being weighed,

and dried at 60°C for three days, and weighed again. Procedures were repeated for Red Pontiac shoot cultures.

Microtubers were made by the semisolid medium method as described earlier (Section IIIA). There were five explants of Russet Burbank transferred per jar, which were arranged in a Completely Randomized Block design. There were eight replicates of the agar treatment and seven replicates of the gelrite treatment. The shoot cultures were measured for fresh weight, dry weight and percent dry matter.

Bacterial contamination observations were made on Norland shoots grown on agar for several generations. Explants were randomly distributed to three jars each of both the gelrite and agar treatments.

Results: The production of nodes of Russet Burbank was not significantly different on media gelled with agar and on media gelled with gelrite (Table IIIE1). However there were significant differences in the height of the explants.

Red Pontiac shoots grown on medium with gelrite were found to have a significantly larger fresh weight and dry weight than shoots grown on either agar or agar/gelrite (Table IIIE2). Roots of Red Pontiac were significantly heavier in term of fresh weight when grown on gelrite media, but dry weights differences in the means were not significant. Russet Burbank showed similar differences in means for fresh weights and dry weights of shoots and roots.

The percent dry matter significsntly increased for both Red Pontiac and Russet Burbank shoots when grown on gelrite, but significantly decreased for roots grown on gelrite (Table IIIE2). Russet Burbank microtubers grown on gelrite had a significantly higher fresh weight, dry weight and percent dry matter than microtubers grown on agar (Table IIIE3). In addition for Red Pontiac, the fresh weight and diameters were greater for microtubers grown on gelrite, than for microtubers grown on agar (Table IIIE4).

Discussion: One important goal of shoot production is to multiply the number of nodes as quickly as possible. For this criteria the use of gelrite does not make a significant difference (Table IIIE1). Visually the shoots grown on gelrite do appear bigger, and they are significantly taller. The increase in height is due to an increase in internode space as well as number of nodes.

There are consistent significant differences in both the shoot and microtuber dry matter accumulation. Gelrite appears to be less inhibitory to biomass accumulation, than does agar. A yellow bacteria was seen growing at the base of Norland explants transferred to gelrite media, but the bacteria did not grow on any Norland explants grown on agar, even though the explants were randomized (Fig. IIIE1). Gelrite did not supress the growth of an endogenous bacteria on Norland, as did agar. The presence of the endogenous bacteria in the potato shoots was confirmed by streaking on a plate of bacterial growth medium. It is advantagenous that all opportunistic bacteria are expressed so that they can be regued out of culture.

Essentially more growth is better, as long as it does not lead to vitreousness. In our lab were grown potatoes *in vitro* on gelrite for several generations without any observations of viteous growth.

Table IIIE1. Shoot height and number of nodes for Russet Burbank grown *in vitro* on gelrite, agar/gelrite, and agar gelled media. Data was collected from vessels containing 8 explants.

	shoot height(cm)	# of nodes
Gelrite	59.5a	49.0a
Ag/GR	53.3b	47.0a
uAgar	52.9b	45.4a

Identical letters with a colum indicate no significant difference at the .05 significance level using the LSD test.

Table IIIE2. Red Pontiac fresh weights, dry weights and percentage dry matter for shoots and roots grown on agar, agar/gelrite and gelrite. athere were eight vessels per treatment.

Red Pontiac (10 explants per vessel)

	SHOOT			ROOT		
	.Fw	.Dw	.%dm	.Fw	.Dw	.%dm
Gelrite	1.118a	.064a	.057a	1.440a	.046a	.029b
A/gr	.840b	.048b	.057a	.990b	.038a	.039a
Agar	.769b	.042b	.051a	1.046b	.039a	.038a

Russet Burbank (8 explants per vessel)

	SHOOT			ROOT		
	.Fw	.Dw	.%dm	.Fw	.Dw	.%dm
Gelrite	0.80a	0.051a	6.6a	0.57a	0.023a	.039b
A/GR	0.70a	0.042b	5.9a	0.43b	0.019b	.045a
Agar	0.67a	0.043b	6.5a	0.39b	0.019b	.047a

Identical letters within a column indicate no significant diference at the .05 significanca level using the LSD test.

Table IIIE3. Russet Burbank microtuber fresh weights, dry weights, and percent dry matter grown *in vitro* on agar and gelrite gelled media. Data was collected from vessels containing 5 explants.

	.Fw(g)	.Dw(g)	.%dm
gelrite	.037a	.0056a	.151a
agar	.027b	.0032b	.124b

Identical letters within a colum indicate no significant difference at the .05 significance level using the LSD test.

Table IIIE4. Red Pontiac microtuber fresh weights and diametters grown on agar and gel rite gelled media. Data was collected from vessels containing 10 explants.

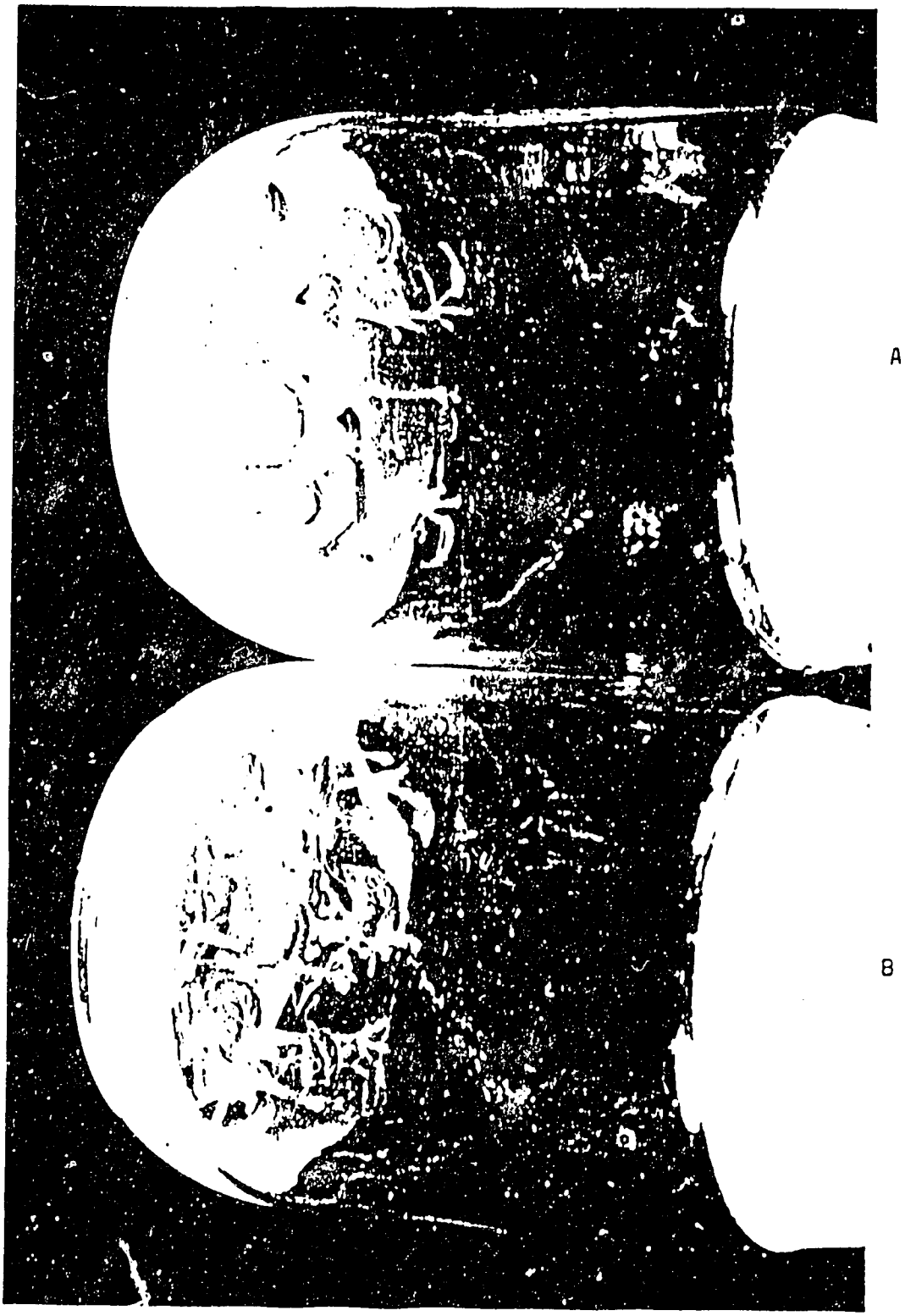
	.Fw(g)	.Diameter (mm)
gel rite	0.070a	4.9a
agar	0.044b	4.lb

Identical letters within a colum in indicate no significant difference at the .05 significance level using the LSD test.

Section IIIF

ARTIFICAL SPROUT INDUCTION

Introduction: It is convenient that all microtubers from a single planting emerge at the same time. Therefore, in order for any microtuber research and production regime to function, microtubers must have a uniform and reproducible dormancy. One method to insure uniform emergence of microtubers is to stimulate sprouting artificially throughthe use of synthetic growth regulators. An ideal solution would be induce 90-100% of the microtubers of all physiological ages to sprout simultaneously. Artifical sprout stimulation would also allow microtubers of varying physiological ages to be bulked together for planting. Microtuber production could then be spread over a longer period of time, thereby saving on laboratory capital costs. Dormancy manipulations can increase the flexibility of microtuber production. Endogenous cytokinins have also been implicated in regulating dormancy in potatoes. Zeatin riboside concentrations in potato tuber buds increased during and after emergence (Turnbull and Hanke, 1985b). Koda (1982) also found that butanol soluble cytokinin levels increased prior to the end of dormancy. Treatments of exogenouscytikinin were found to break the rest period of the cultivar Majestic whichis known for its long dormancy period (Hemberg, 1970; Turnbull and Hahke, 1985a). The effect of cytokinins varied with the age ofthe tuber. Tubers that werr harvested prematurely and tubers 11-12 weeks old sprouted in response to exogenous cytokinins. Tubers of other ages were not responsive to exogenous cytokinins.



Figgure IIIEl. Two Norland shoot cultures contaminated with a yellow bacteria. Culture A contains agar as a gelling agent and the yellow bacteria is not visible. Culture B contains gelrite as the gelling agent and the yellow bacteria is visible. Both cultures came from the same source of tissue.

Several workers have used gibberellic acid (GA) to manipulate the dormancy of ex vitro tubers. Bruinsma and Swart (1970) found that exogenous GA interrupted dormancy and promoted sprout growth in several potato cultivars. Endogenouslevels of GA were found to change during and after emergence from rest in the cultivar Red Pontiac (Smith and Rappaport, 1960). Others workers found that GA only promoted sprout growth, but did not break dormancy (Doorenbos, 1958; Mioduszewska and Bielenska-Czarnecka, 1984). *in vitro* tubers did not sprout any better when dipped into 5 mg/L of GA (Ewing, *et al.* 1987).

Rindite is commonly used to break dormancy (Denny,1945). When Wattimena (1983) used rindite to break dormancy, it was effective for 40% of the microtubers treated. Currently, low temperatures (5°C) are used to prevent microtuber sprouts from growing excessively in stroage (Estrada *et al.*, 1986). Cutting microtubers is also an effective way for inducing sprouting, but still needs to break dormancy in a higher percentege of the microtubers in order to be a viable method (Ewing,*et al*, 1987).

CYTOKININS

Materials and Methods: Red pontiac, Katahdin and DTO-28 shoot cultures, virus indexed and stabilized were used as the source material for tuberization. The three cultivars were tuberized with 45 uM BA, 170 uM coumarin, 6 % sucrose, at 15°C in the dark.

The microtubers were stored at 5 °C for 8 to 36 days in sterile one once vials sealed with parafilm. After storage the microtubers were transferred to sterile 45 ml vials along with 4.5 mls of the treatment solution. There were 3 microtubers per vial and 5 vials per treatment. The treatment solutions had TDC and BA added, and the pH adjusted to 5.6 before autoclaving for 20 minutes. The vials were sealed with a cap, and rolled horizantally at one rpm at 27 °C under 20 u Einstein of light. Observations on the number of microtubers sprouted per vial were recorded after four to six days.

Results and Discussion: There are several factors which can influence the percentage of sprouting of dormant microtubers. Light was necessary to get any sprouting of microtubers when combined with cytokinins (Table IIIF 2). This observation was confirmed in other non-replicated experiments. The light may have activated a phytochrome mediated response necessary for sproting. It is also possible that the photosynthates produced by light were necessary for sprouting. Tubers always turned green before they sprouted. In addition rooting did not seem inhibited by light.

Different synthetic cytokinins at different concentrations had no significant effect on the percentage of microtubers sprouted. Percent sprouted microtubers for some treatments, especially for 0.04 uM BA, were higher than the no growth regulator treatment. but not significantly so (Table IIIF 3). DTO-28 is a short dormancy cultivar with a substantial part of its lineage from Solanum phureja. 0.04 uM BA induced a higher percentage sprouting for DTO-28 than it did for either Katahdin or Red Pontiac (Table IIIF 3).

We are looking for a stimulus that will sprout 90-100% of the microtubers at all physiological ages. Syntetic cytokinins did nor induce a high percentage of sprouting for microtubers 0 to 1 month after harvest.

GIBBERELIC ACID

Materials and Methods: Red Pontiac and DTO-28 microtubers were tuberized with 45 uM BA, 170 uM coumarin, in 15°C, in the dark. The DTO-28 microtubers were harvested and completely randomized before being placed in the treatment solution. the five treetments consisted of 1) air; 2) distilled water; 3) 200 uM GA; 4) distilled water shaken at 120 rpm; 5) 200 uM GA shaken at 120 rpm. Then DTO-28 microtubers were placed in the five treatment solutions for 10 minutes 1 day, 3 days and 7 days. After the treatments ended the microtubers were rinsed twice with distilled water and planted in a shallow Jiffy mix seedling tray. Seedlings were maintained the greenhouse under a 2 seconds of mist per minute. Observations on the numbers of microtubers sprouted was made once a week for 6 weeks. The second experiment involved different microtubers of both DTO-28 and

Red Pontiac, and varied dosage levels of GA. The dosage levels ranged from 20 uM min to 2 x 106uM min. The GA levels were from 2, 20, 200 uM and the time of soaking of the microtubers per treatment. After soaking in the treatment solution, microtubers were planted in jiffy mix seedling flat s under a 2 seconds of mist per minute in the greenhouse. Observations on the numbers of microtubers sprouted were made once a week for 6 weeks.

Results and Discussion: In these preliminary experiments GA did not show the potential of being able to break dormancy uniformly, especially for Red Pontiac. Sprouts were effectively stimulated to grow for DTI-28 microtubers with 2000 uM minutes of GA in the first trial (Table IIIF 4) and with 2 x 105 uM minutes of GA in the second trial (Table IIIF 5) Agitation on a shaker (120 rpm)was tried to see if that would help the GA penetrate the microtuber. Shaking may have stimulated GA activity slightly after 10 minute, but it physically destroyed the microtuber after 3 days. The destructive effects of agitation rule against its use for general application of GA on microtubers.

GA used on Red Pontiac at varying stages of chronological age was not effective at stimulating sprouting. Red Pontiac microtubers at 0, 6, and 12 weeks of age were not responsive to GA at levels ranging from 20 to 2 x 105 uM minutes. GA may be effective in stimulating sprout growth on short dormancy cultivars like DTO-28, but was not effective for longer dormancy cultivars such as Red Pontiac.

RINDITE

Materials and Methods: Ninety Red Pontiac microtubers were tuberized with 45 uM BA, 170 coumarin, 15°C, in the dark. After harvest the microtubers were stored one week in the dark at 22°C. Sixty microtubers were placed in a sealed ceramic bowl with Rindite (7 parts ethylene chlorohydrin; 3 parts ethylene dichloride; 1 part carbon tetrachloride) released at rate of 4 mls/ft3/day for three days. Afterwards the microtubers were stored at 22°C for one week. Thirty of the sixty microtubers went through the same Rindite treatment for the second week. The microtubers were stored at 22°C for another five weeks before photographs of microtubers were taken.

Results and Discussion: Red Pontiac microtubers treated with Rindite showed extreme dessication with no sprout stimulation(Fig.IIIF1). The dose of rindite was probably too stressful for the microtubers. A dosage curve of of rindite is recommended for any futher attempsts to use rindite stimulate sprouting.

TableIIIF2. The percentage sprouting of Katahdin microtubers after six days of bathing in water and Murashige and Skoog (MS) medium, with 0.001 uM TDZ and 0.4 uM BA, in the light and dark. Data was collected from five vessels containing 3 microtubers each.

light/ dark	percent sprouted
light	13.3%
dark	00.0%

The differences between treatments were significantly different as determined by the LSD test at the 5% level of significance.

Table IIIF3. The percentage of Red Pontiac and DTO-28 microtu- bers after six of bathing in water, and MS medium salt with 0.04 uM BA and 4.0 uM TDZ. Data was colected from vassels containing 3 microtubers; there were 3 replicates per treatment.

TDZ Conc (uM)	BA Conc (uM)	DTO-28 Percent Sprouted
0.0	0.0	56 a
4.0	0.0	22 b
0.0	0.04	67 a
4.0	0.04	0.0 b

TDZ Conc (uM)	BA Conc (uM)	Red Pontiac Percent Sprouted
0.0	0.0	11y
4.0	0.0	0.0z
0.0	0.0	433x
4.0	0.0	411y

Treatment that shared a common letter are not significanty different as determined by Duncans Multiple Range Test at the 0.5 significance level.

Table IIIF4. The number of DTO-28 microtubers emerged 3 weeks after GA treatment. Data was collected from 10 microtubers per treatment

	Length of time of treatment			
Treatment	10 min	1 day	3 days	7 days
Air	1	1	0	2
water	1	3	7	1
GA	8	3	2	0
shaken water	1	1	0	0
shaken GA	10	2	0	0

Table IIIF5. The number of emerged microtubers four weeks after treatment with GA. Data is determined from 10 microtubers per treatment

	Microtuber chronological age after harvest			
Dosage (uMxmin)	0 weeks DTO-28 Sprouted	0 weeks Red Pon. Sprouted	6 weeks Red Pont. Sprouted	12 weeks Red Pont. Sprouted
0 x 1000	0	0	1	1
0 x 10	1	4	0	2
20 x 10	3	0	2	3
200 x 10	0	0	1	3
2000 x10	2	1	1	3
2000 x 1000	0	2	4	0
20,000 x 1000	2	5	2	1
200,000 x 1000	9	3	2	1

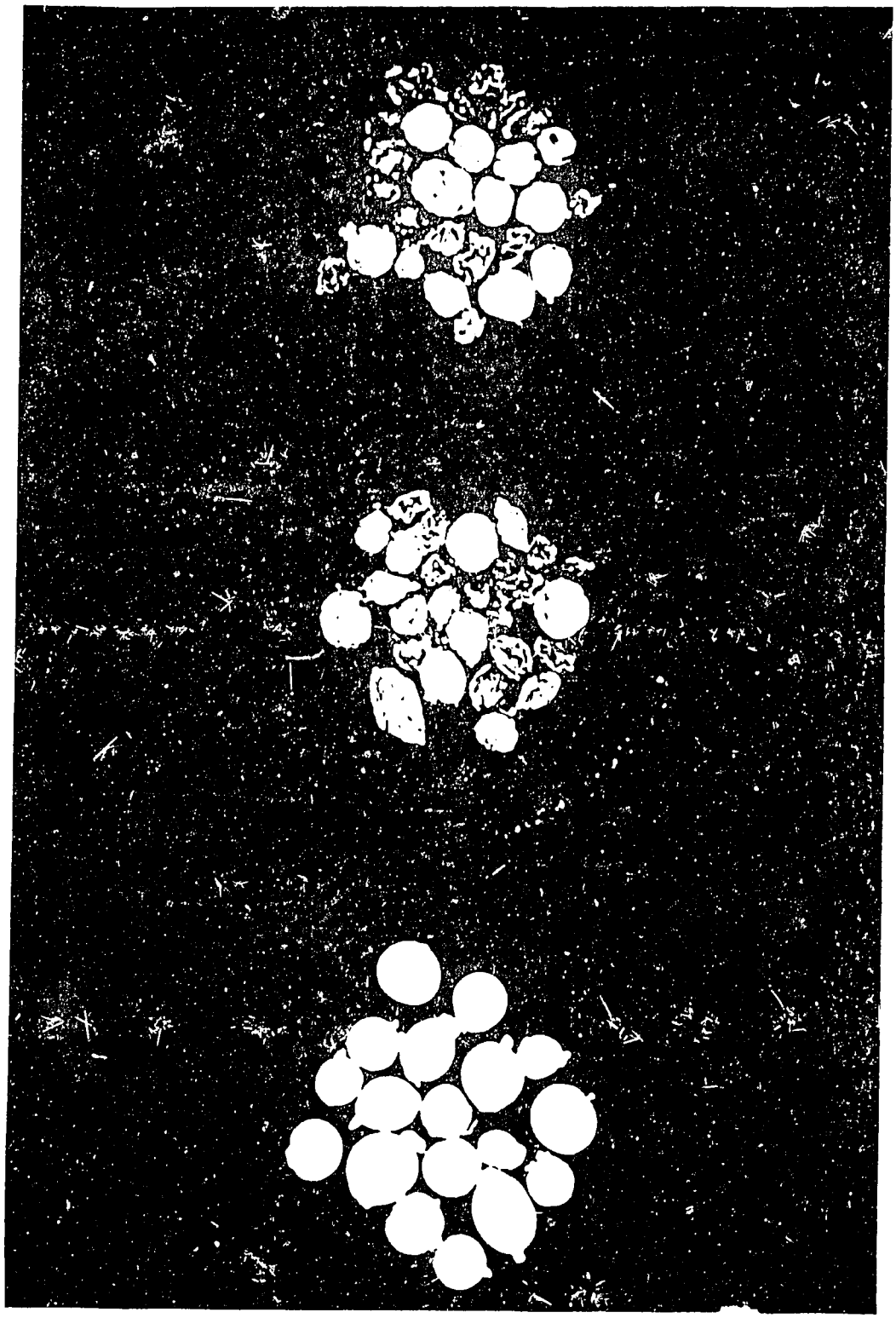


Figure IIIF1. Red Pontiac microtubers eight weeks after harvest a) microtubers treated with one 3 day dose of rindite, b) microtubers treated with two 3 day doses of rindite; c) microtu- bers stored at 22°C without rindite.

Section IVA

EFFECT OF THIDIAZURON ON SHOOT CULTURE MULTIPLICATION

Introduction: Tissue culture is rapidly becoming an essential part of high quality potato seed cerification program (jones, 1987). Tissue culture provides an ideal disease free environment for potato certification system would be to multiply as many generation as possible *in vitro* instead of in the field. How ever the use of potato tissue culture is limited by the low multiplication rates in culture resulting in a very labor intensive, expensive process.

In other plant species high rates of multiplication are achieved by breaking apical dominace and stimulating axillary but growth. This is usually accomplished with synthtic cytokinins such as benzyladenin (BA). Potatoes in culture characteristically have a strong apical dominace and are not highly responsive to BA, thus multiplication rates in shoot culture are limited.

Thidiazuron (TDZ) is a higly active phenyl urea derivative showing cytokinin-like activity *in vitro* (Capelle, *et al*, 1983). It has been used with plant tissues of maple (Kerns and Meyer, 1986). Poplar (Russell and McCown, 1986), and soybeans (Thomas and Katterman, 1986). In maple and poplar, TDZ was able to stimulate a cytokinin-like response in the plant tissue when BA could not. For potato, TDZ was found to not be as effective as BA for inducing *in vitro* tubers (Slimmont and Souza-Machado, 1987). This experiment will compare TDZ, BA and combination of the two, for axillary bud growth in the potato.

Materials and Methods: The experiments were conducted with stabilized virus-indexed shoot cultures of Katahdin. Shoot cultures were maintained in 280 ml culture jars covered by magenta B caps at 22 °C under constant light at as intensity of 20 u Einstain. The medium was composed of MS mineral salt (Murashige and Skoog, 1962) amended with 3 mM calcium gluconate and solidified with 3.0 g/l of agar and 0.75 g/l of gelrite. Shoot tips were propagated on medium without growth regulators to maintain the cultures.

Three-node explants with leaves removed were laid horizon- tally on the test media which consisted of different concentra- tion and combination of BA and TDZ. Each treatment had eight replicates and each replicate had three, three-node explant. The treatments were arranged in a randomized complate block on the culture shelves. Observations were recorded weekly. Each experiment ended when the firt shoots touched the top of culture jar.

Result: BA was ineffective at stimulating axillary bud growth at low concentration and inhibitory to shoot growth at higher concentration (Table IV A1). The response of Katahdin as measured by the number of nodes sprouted and number of nodes per jar did not increase when BA was added.

Preliminary experiments indicated that an optimal concentration of TDZ to stimulate the sprouting of axillary buds of potato existed between 0.1 uM abd 1.0 uM (Table IV A2). More detailed experimentation showed that TDZ increased the number of sprouts per jar and number of nodes per jar with minimal inhibi- tory effects to shoot length. There were no significant differences in number of nodes of Katahdih sprouted between TDZ levels ranging from 0.1 uM to 2.0 uM (Table IV A3).

Using concentration of TDZ of 0.10 uM and BA of 0.4 uM, the response of Katahdin to TDZ and BA was compared (Table IV A4). As Proviously shown, the response of Katahdin to only BA was not significantly different from that of the no growth regulator me- dium, except that significantly inhibited increases in shoot length. TDZ at 0.1 uM significantly stimulated axillary bud growth as measured by the number of sprouts, without any significant inhibition of shoot length. BA and TDZ combined significantly increased the number of sprouts and total number of nodes, but also decreased shoot length. Theshoot multiplication response of Katahdin to both TDZ and BA was not significantly different than the response to TDZ alone.

Discussion: For potatoes, TDZ is significantly more effective synthetic cytokinin for shoot multiplication based on axillary bud stimulation than is BA. TDZ induced the synthesis and/or accumulation of endogenous cytokinins in soybean callus (Thomas and Katterman, 1986). However, we have not found any evidence of a synergistic interaction of TDZ with BA. The fact that Katahdin

responded favorably to TDZ at a level as as high as 2.0 uM, a level toxic to many other plants, shows that potatoes are insensitive to exogenous cytokinins.

The rate of multiplication achieved with optimal levels of TDZ although significantly greater tahn controls, have not yet achieved a level high enough to significantly alter the economic viability of potato micropropagation. TDZ, and other phenyl urea derivatives, may prove to be useful as a more effective synthetic cytokinin for *in vitro* propagation systems designed to achieve higher rates of multiplication.

Table IV A1. Effect of BA on *in vitro* shoot growth of Katahdin. Data collected from eight vessels containing three, three-node explants.

BA conc.(uM)	Total on.of Sprouts/jar	Total no. of nodes Per jar	shoot length/jar(cm)
0	3.2(0.4)	15.4 (1.6)	14.2 (3.8)
0.4	3.2(0.4)	12.8 (1.6)	10.4 (1.0)
1.0	3.2(0.4)	13.2 (1.2)	11.7 (1.3)
4.0	3.6(0.5)	14.4 (1.6)	10.7 (1.8)
10.0	3.4(0.5)	14.2 (2.9)	8.4 (2.2)
40.0	0.6(0.8)	2.6 (4.3)	1.2 (1.8)

Numbers is in the parentheses are standard deviations

Tabel IV A2. Effect of different concentration of TDZ on the number of *in vitro* shoots of Katahdin. Data was collected from eight vessels containing four three-node explants

TDZ conc. (uM)	total no.of sprouts/jar
0.0	3.9 c
0.001	4.6 bc
0.01	4.0 c
0.1	5.8 ab
1.0	7.3 a
10.0	3.5 c

Any two means with a common letter are not sognificantly different at a .05 significance level the LSD test.

Table IV A3. Effects of different TDZ concentrations on the number of sprouts per jar. Data was collected from eight vessels containing three, three-node expiants

TDZ conc. (uM)	total no.of sprouts/jar
0.00	3.8 (0.7) a
0.10	5.1 (0.8) a
0.30	5.4 (1.2) a
0.70	4.6 (1.1) ab
1.00	5.3 (1.6) a
2.00	5.1 (1.1) a

Numbers in the perentheses are standard deviations. Any two means with a common letter are not significantly different at a 0.05 significance level by the LSD test.

Table IV A4. Effects of BA and TDZ on Katahdin *in vitro* shoot growth. Data collected from eight vessels containing three, three-node explants.

BA-TDZ conc. (uM)	total no.of Sprouts/jar	total no.	of nodes per jar	shoot length/jar (cm)
0.0-0.0	3.9 b		19.3 no	19.2 y
0.4-0.0	3.5 b	15.5 o	13.3 z	
0.0-0.1	5.6 a	21.8 mn	18.[illegible]	
0.4-0.1	6.1 a	23.4 m	16.[illegible]	

Idential letters indicate no significant differences between means at a 0.05 level by LSD test.

Section IV B

STATIC LIQUID CULTURE MICROTUBER PRODUCTION

Introduction: Liquid cultures are easier to prepare, easier to refresh, and less expensive than semi solid media. Microtubers from liquid cultures are significantly heavier and larger, but are not different in their percent dry matter and percent tuberization from microtubers grown on semi solid cultures (Wattimena, 1983). A potential disadvantage of liquid microtubers is their lenticels.

The major advantage of liquid media microtubers is that the transfer time required is less than the time required for transfer on solid media. A liquid media saves a significant amount of transfer time by eliminating the need the embed the explant. Labor required for transferring explants is a major expense of amicrotuber production operation. Reducing the time spent transferring cultures would significantly reduce operating costs.

This experiment examines the growth of microtubers in different quantities of liquid media and different quantities of growth regulators. Because there always is vessels-growth regulator interaction. We tested varying concentrations of BA and coumarin in order to find the optimal growth regulator balance.

Materials and Methods: Potato shoot cultures of the cultivar Superior, virus indexed and stabilized, were used as source material for tuberization. Shoot cultures were maintained as described in section IIIA. Five axillary buds were transferred to jars containing the treatment media. The media consisted of amended MS mineral salt (MS= MS salt with consisted low nitrogen, 2.06 uM $NH4^+$ and 22.10 uM $NO3^-$) and sucrose 8% sucrose. There were four experiments each lasting approximately four weeks. Experiment one was designed to determine the quantity of liquid media optimal for tuberization. Treatments consisted of jars with 1, 5, 10 and 20 mls. per jar, with four replicates per treatment. The liquid media was dispensed before autocalving. Experiment two was designed to determined what the optimal level of coumarine was. Using 4 mls. of liquid media per vessel, treatments consisted of MS media with 0, 42.5, 85, 170, and 340uM coumarin with five replicates per treatment. Experiment three tested BA levels of 0.0, 1.0, 10.0,.and 22.5 uM with 5 mls. of liquid per vessel and five replicates per treatment. Experiment four was designed to detrmine what level of BA would be optimalwith 85 uM coumarin, and 6 mls. of media per jar. BA treatment levels of 0, 1.0,

10.0 and 22.5 uM were tested combined with 85 UM coumarin with five replicates per treatment. All experiments were transferred and randomized complete block.

Results and Discussion: One promblem with a liquid culture is that tissues can suffer from the lack of aeration. Low aeration can be over come with a gentle agitation from a shaker or a roller apparatus. A different approach to getting plant tissues more air is to expose the tissues to the air by lowering the amount of liquid in a stationary vessel. Five mls. of liquid media produced a significantly higher number and fresh weight of microtubers per jar than 1, 10, and 20 mls. of media (Table IVB1). Twenty mls. of media, although producing a significantly higher fresh weight per microtuber, produces a prohibitively low number of microtubers. Microtubers in one ml. of media tuberized well, but did not accumulate enough fresh weight to withstand dessication. Vessels that initially had one ml. of media were dry after 28 days.

Tuberization induced with 42.5, 85, and 170 uM coumarin produced significanly higher microtuber fresh weight per culture jar than did microtubers induced with no coumarin and with 340 uM coumarin (Table IVB2). 85 uM coumarin was selected as being optimal although there was no significant difference in the num- ber and fresh weight of microtubers between 42.5, 85 and 170 uM coumarin.

BA was significantly inhibitory to tuberization at 10 and 22 uM levels (Table IVB3). One hypothesis to explain this phenomenon is that BA is phytotoxic at those levels. This hypothesis would also agree with the resuls in section IIID. Wattimena (1983) tu- berized potooes in liquid media using 32 uM kinetin, which has similar cytokinin-like activity as BA. Differences in Techniqoe between experiments include vessel closure, quantity of media, vessel size, vessel shape, sucrose concentration and growth regu- lators. Nonetheless the results of this experiment and Wattime- na's experiment were elevated above the media, which is different than this experiment, which immersed the nodes in the media. When BA was combined with 85 uM coumarin, there were no significant differences in microtuber fresh weight between 0 and 1.0 uM BA (Table IVB4). 85uM coumarin overcome the 'inhibitory' effects of 10 uM BA.

Table IV B1. Microtuber number, fresh weight per jar and fresh weight per microtubers for Superior potatoes in a stationary liquid system with MS media amended with 85 uM coumarin. Treatments varied in a quality of liquid dispensed in each jar. Data was collected from four vessels containing five explants 32 days after transfers.

media quality mls)	no. of microtubers Per jar	fresh weight per jar (grams)	fresh weight per microtuber (grams)
1	3.0 b	0.049 c	0.015 c
5	4.3 a	0.255 a	0.060 b
10	1.3 c	0.105 b	0.090 a
20	0.0 d	0.000 c	0.000 d

Numbers with different letters within a column are significantly different from each other as determined by the LSD test at the 0.05 level of significance.

Table IV B2. Microtuber number, fresh weight per jar and fresh weight per microtuber for Superior potatoes in a stationary liquid system with MS media. Treatment varied in the amount of coumarin added to the liquid media. Data was collected from five vessels containing five explants.

coumarin conc. (uM)	no. of microtubers per jar	fresh weight per jar (grams)	fresh weight per microtuber (grams)
0.0	3.2 a	0.292 q	0.098 xy
42.5	3.0 a	0.324 pq	0.114 x
85.0	3.8 a	0.412 p	0.111 x
170	3.6 a	0.390 pq	0.109 x
340	2.8 a	0.186 r	0.065 y

Numbers with different letters within a column are significantly different from each other as determined by the LSD test at 0.05 level of significance.

Table IV B3. Microtuber number, fresh weight per jar and fresh weight per microtuber for Superior potatoes in a stationary liquid system with MS media. Treatment varied in the amount of BA added to the liquid media. Data was collected from five vessels containing five explants.

BA conc. (uM)	no. of microtubers per jar	fresh weight per jar (grams)	fresh weight per microtuber (grams)
0.0	4.0 a	0.200 a	0.060 a
1.0	3.4 a	0.188 a	0.054 a
10.0	2.2 b	0.098 b	0.046 a
22.5	0.6 c	0.026 c	0.018 b

Numbers with different letters with a column are significantly different from each other as determined by the LSD test at the 0.05 level of significance.

Table IV B4. Microtuber number, fresh weight per jar and fresh weight per microtuber for Superior potatoes in a stationary liquid system with MS media amended with 0.85 coumarin. Treatment varied in the amount of BA added to the liquid media. Data was collected from five vessels containing five explants.

BA conc. (uM)	no. of microtubers per jar	fresh weight per jar (grams)	fresh weight per microtuber (grams)
0.0	3.4 b	0.231 a	0.058 a
1.0	4.4 a	0.193 a	0.054 a
10.0	0.9 c	0.041 b	0.047 a
22.5	0.3 c	0.012 b	0.047 a

Numbers with different letterswithinacolumnare significantly different from from each other as determined by the LSD test at the 0.05 level of significance.

Section IVC

ROLLER BOTTLE LIQUID CULTURE MICROTUBER PRODUCTION

Introduction: A major goal of potatoes micropropagation is to economically produce microtubers on a large scale (McCown and Wattimena, 1987). Thus far this goal has been limited by low multiplication rates in culture. Typically, only one microtuber forms per explant although there may be many more meristems. One limitation to this problem is the strong source-sink relationship that exists, with the microtuber dominating over all the other meristems on the explant.

We wish to explore the potential of roller bottle system for inducing more than one microtuber per explant. A roller bottle system has several differences from liquid stationary and shaker cultures. First roller bottle cultures are gravity neutral. The potato is not oriented to grow in vertical fashion in the roller bottle. Second, each node is ini direct contact with all the growth regulators and nutrients in media. Terminal nodes are in contact with the same concentrations of exogenous growth regulators as basal nodes. A roller bottle system gives all nodes equal exposure to exogenous growth regulators. Thirdly, the transpirational stream is dirupted. Since the leaves is the same as the water potential at the roots.

Hypothetically, the uniformity of the environment should allow meristems to overcome apical dominace and become indepen- dant. A roller bottle system gives all nodes equal exposure to exogenous growth regulators, to induce tubers at every node.

Material and Methods: Shoot culture of the cultivar Superior were maintained as described in section III A. Axillary buds of these shoot cultures were transferred to 250 ml. Corning media bottles. The bottles were sealed with plastic scew caps, and modified with teflon seals to ensure sterility and air exchange (Figure IV C1). Inside the bottle was a 6 by 17 cm piece plastic mesh commonly used for 25 lb sacks of citrus from a local grocery store. A 9 cm piece of half cm diameter silicon tubing was inserted in order to pin the mesh againt the bottle 25 ml of MS media were added to each bottle. 10 axillary buds were transferred to each bottle. The bottle were rotated constantly at 1/2 rpm, in the dark at 22 °C. After 2 weeks of rotation one ml. of a sterilized concentrated solution of sucrose, BA and coumarin were added to bring the final concentration to 8% sucrose, 85 uM coumarin, and 22.5 uM BA. The bottles were then rolled in the dark at 15 °C for four weeks. After four weeksd data was taken on the number of microtubers and fresh weight per bottle.

Results and Discussion: Air excange was essential for tuberiza- tion. Potato cultures that were sealed with nonmodified caps did not form microtubers, while potato cultures with caps that were modified did form microtubers (Fig. IVC2). Microtubers were consistently induced on 90-100 % of the explants. However growth regulators were not able to induce tuberization on more then one node per stem (Fig. III C3). The cultures maintained their apical dominace inspite of the manipulations. Each shoot maintained its apical dominance over the inactive axillary buds. In orde for more than one microtuber to form on each stem, meristems have to have the capability to compete with each other, rather than dominate.

Further research should center on systems to active more meristems in culture. TDZ, or other phenyl urea derivatives, may be able to activate more meristems in a roller bottle system. Calcium shoot tip mecrosis may also be a way to break apical do- minace and stimulate axillary buds in roller bottle culture.

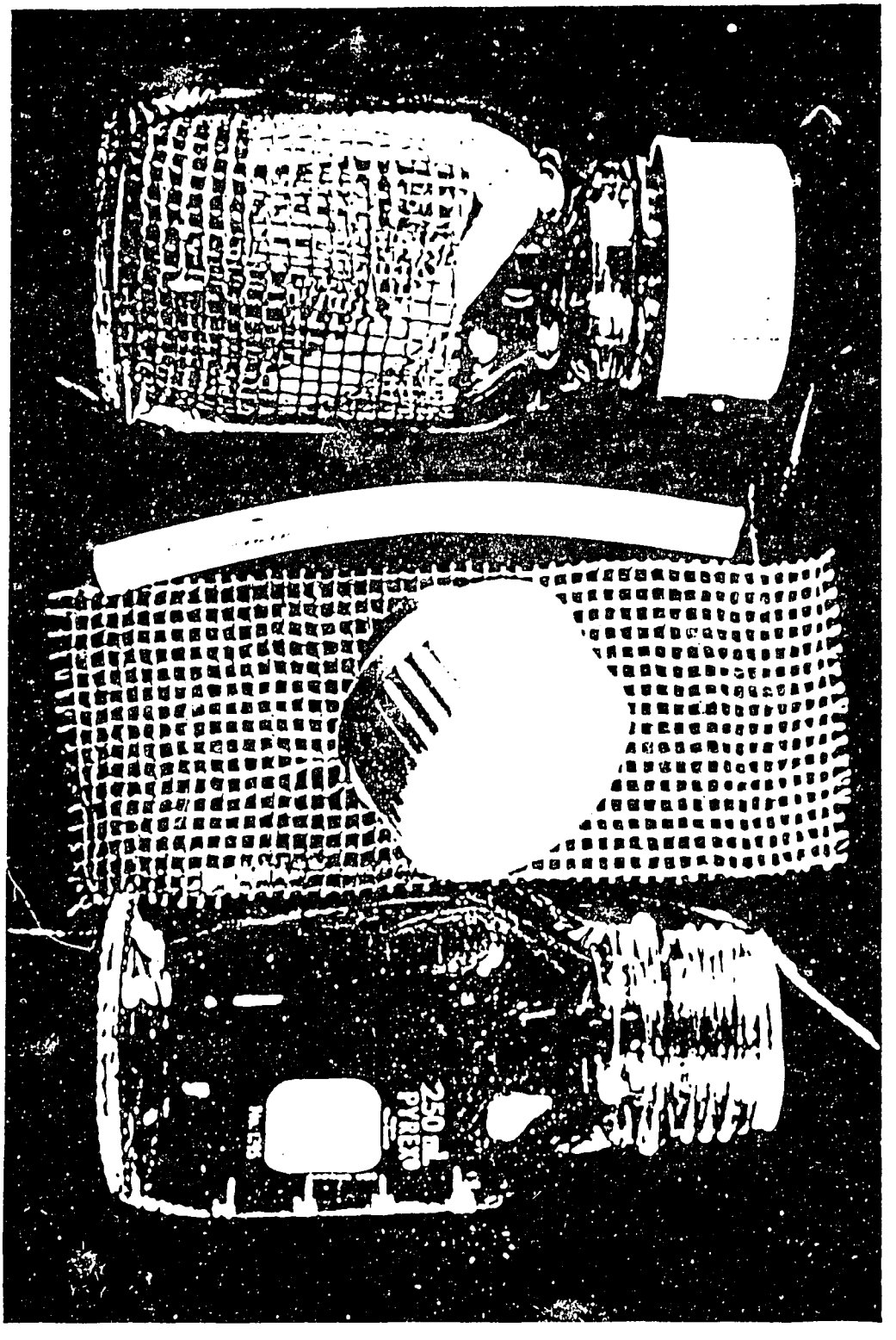
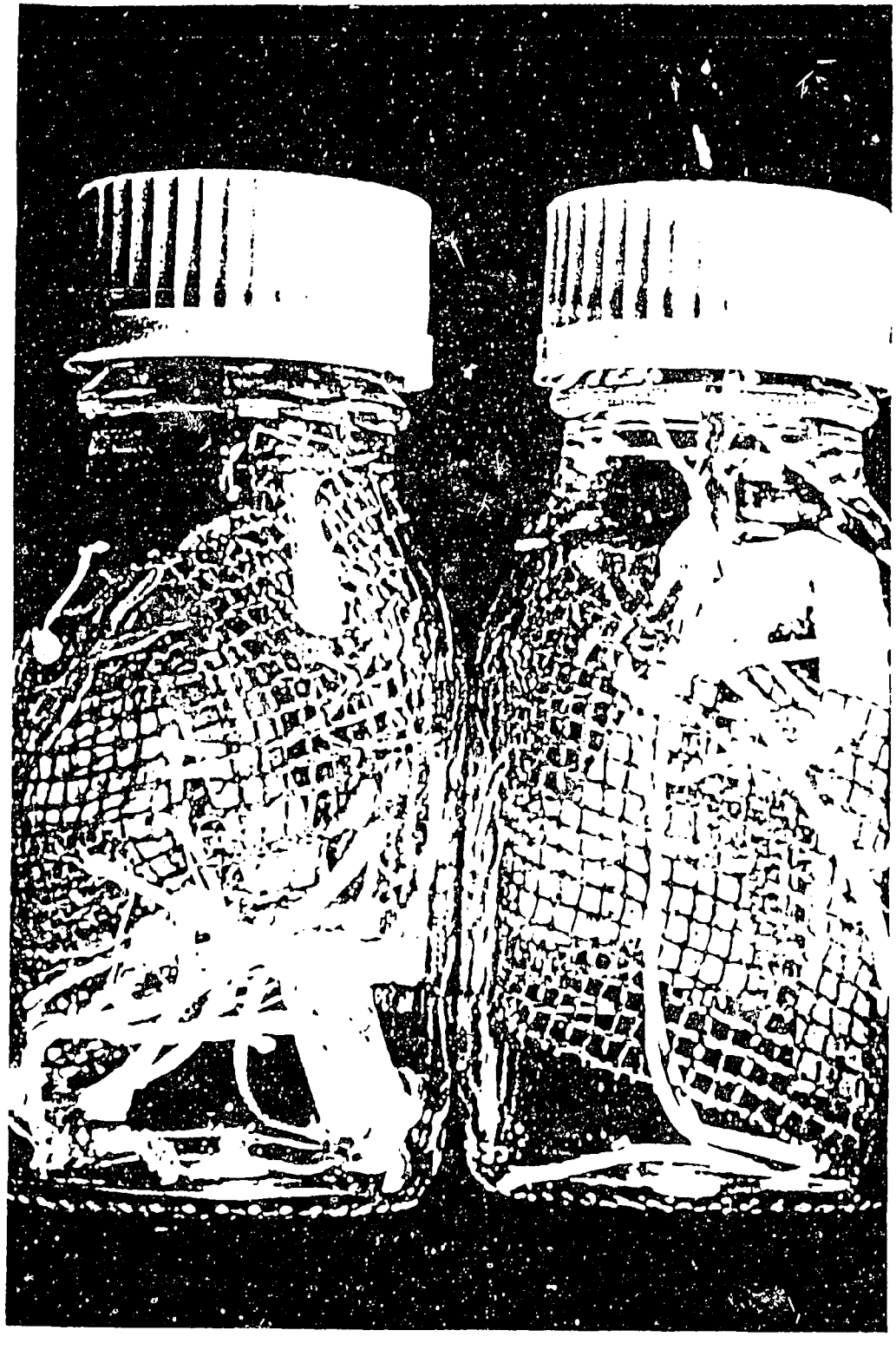


Figure IVC1. Individual and assembled roller bottle equipment for potato cultures. Equipment includes a 1) 250 ml Corning bottle, 2) a 9 cm oiece of silicon tubing, andd 30 a 6 by 17 cm strip of plastic mesh



1 2

Figure IVC2 Superior tuberization cultures with 1) the modified screw cap The media was composed of MS salts amended with 8% sucrose, 45 uM BA, and 170 uM coumarin and was rooled at 15 °C in the dark for 30 days Vessels contained ten explants each

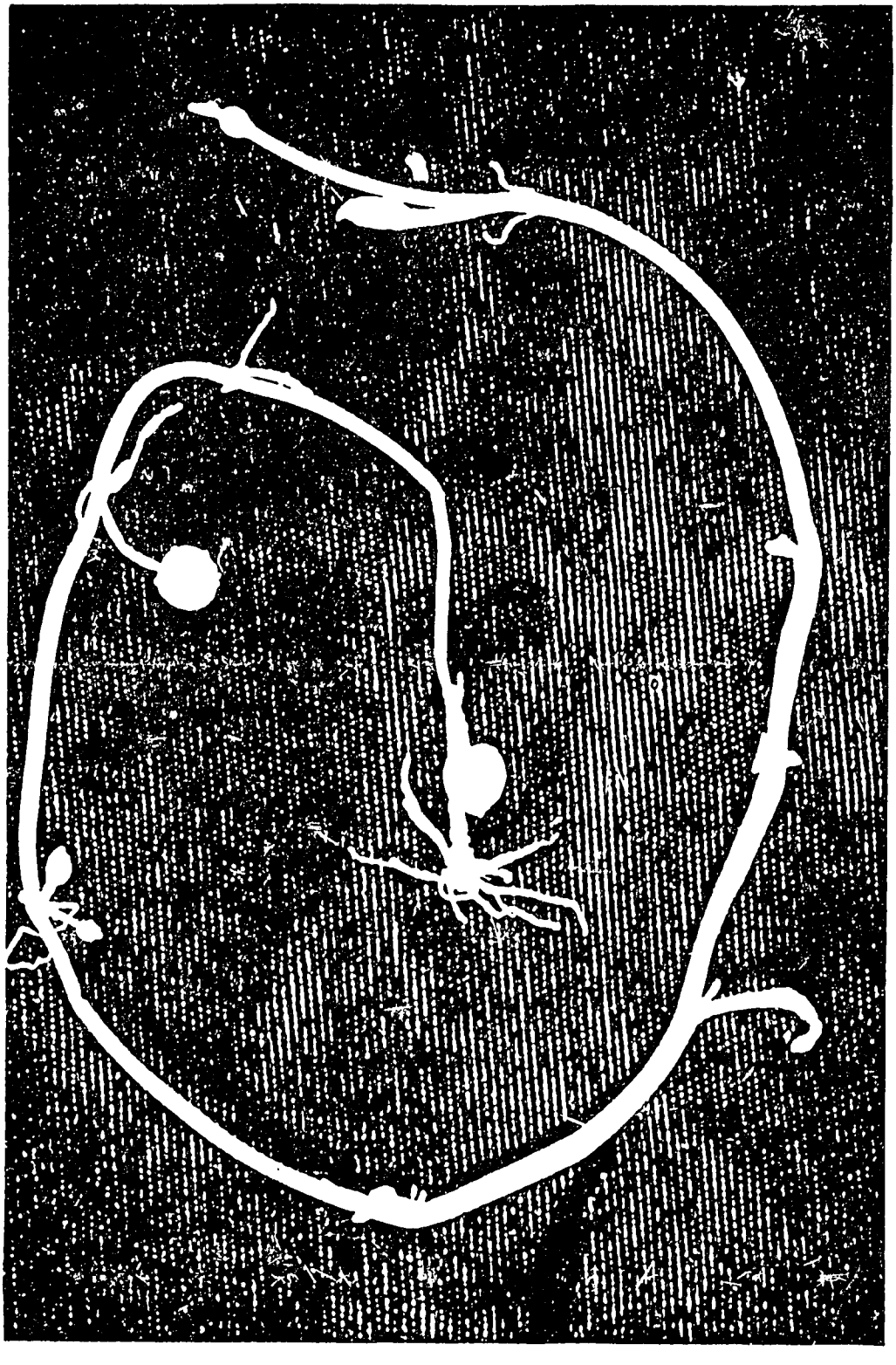


Figure IVC3. A tuberized Superior etiolated stem with a single large microtuber out of total of eight nodes. The tissue was removed from a roller bottle with mendia composed of MS salts amended with 8% sucrose, 22 uM BA, and 85 uM coumarin and was rolled at 15 °C in the dark for 30 days

Section V

4.4. CONCLUSIONS AND PROJECTION

The first goal of microtuber production, uniform dormancy is readily achieved through uniform microtuber induction and proper storage at 5 °C. Rindite, GA3 and synthetic cytokinins were not effective at inducing microtuber sprouting at the dosages discussed in section III F. Other findings to optimize microtu- ber production include:

1) Use of gelrite as a gelling agent
2) Use of apical buds as the shoot culture propagule
3) Nodes for tuberization should be kept above the media
4) Coumarin and BA are effective for inducing microtubers
5) The explant leaf is not important for *in vitro* tuberi-
   zation

The second goal of microtuber production, an economical, large scale ability to produce microtubers, has not yet been achieved. However the following conclution can be made:

1). TDZ does significantly stimulate axillary bud sprouting, but not at level hight enough to affect the commercial viability of micropropagation.
2). Static liquid culture, a system which requires less transfer time than semi solid media, is amenable to producing microtubers. An appropriate quantity of liquid media for the vessel is important for plant survival. Optimal tuberization was achieved with 85 uM coumarin, 1.0 uM BA with five mls. of liquid media per culture jar.
3). The roller bottle liquid system does not overcome the potatoes source-sink relationshhip. Further manipulations are necessary to increase multiplication rates.

TDZ and other phenyl urea derivatives may prove to be more effective in increasing multiplication rates in the roller bottle. Calcium shoot tip necrosis may also be effective to break apical dominance in roller bottle cultures.

Another important aspect of the commercialization of potato micropropagation is to manipulate cultures in the dark. Lights re major operating and capical expense for laboratories. In addition lights make temperature more difficult to control. Tissiu culture potatoes derive very few carbohydrates from photosynthesis, getting most of their energy from the sucrose in medium. Tuberization culture

in this lab is always conducted in the dark. It would be advantegeous to initiate shoot culture system that also do not need light. Roller bottle shoot culture system may not need light.

It may be possible to get of shoot cultures altogether. Microtuberization of microtubers should be investigated. With only a multiplication factors of two, microtuber populations could be cycled every year with half going toward field production and half going to storage (Fig. IA1). Shoot cultures would than only be used to multiply stocks and not to maintain them, thus decreasing energy costs significantly.

Whatever system is used for potato microtuberization, research efforts should concentrate on increasing the multiplication rates while maintaining uniform dormancy

Section VI

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V. FIELD PERFORMANCE OF MICRO PROPAGULES

5.1. INTRODUCTION

The main ion straint in creasing potato production in Indonesia is the availability of virus free seedtubers. It is hard to produce virus free seedtubers in Indonesia dueto the present of virus transmitter at every place and every season. The conventional method of producing virus free seedtubers is almost imposible in Indonesia (Watimena et.al, 1983).

To overcome the virus free seedtubers problems has to be with inconventional methods. Nearly all part or organ of plant can be used for propagation. Organ or part of potato plants used for propagation are called propagules.

According to Wattimena (1986) potato propagules consisted of : seedtubers, stem cutting, *in vitro* shoat cutting, mini tubers, *in vitro* micro tubers and botanical seed. Mini tubers are 1 to 10 gram size tubers produced in insect screen houses. The propagules for producing minitubers derived from microtubers, micro shoot or botanical seed. Microshoots and microtebers are propagules produced *in vitro* and are general called micro propagules (Mc Cown ans Wattimena, 1987).

Micropropagules and botanical seeds (True potato seed = TPS) are potential virus free propagules or propagules for production elite potato seedtubers. Eventhough both are a potential propagules but each of them have their own weakness. TPS can be nifected by PSTV Potato Spridle Tubervid virus), in additim TPS are late maturing and has great variability in maturing, vegetative growth, and tuber size and firms. Micropropagules are now succesful produced in laboratory scales, the production in a commercial scales at a reasmable price is the problem to be solved in an intergrated system.

The field planting of microprapagules is another probem too that will be different than conventional seedtubers. There are very little field experiment of potato mikro propagules so far have been done.

most of the field experiments were arrived from the temperate countries. Goodwin and Brown (1980) from Australia have found that no different in growth of potato plant derived from mikro cutting and seedtubers. The number of plant per hill, the number of tuber and tuber fiesh weight were no difference of potatoplant derived from microshoot and seedtubers. two years experiment in wisconsin. USA showed no difference in tuber yield per hill or per plot between mikropropagules and seedtubers (Wattimena, 1983). The difference of the two types of propagules were in tuber size, number of them per hill and number of tuber per hill. The similarities and the difference between the two types of propagules were found in all four cultivar tested, consisted of Norland, Red Pontiac, Ruset Burbank, and Katahdin (Wattimena, 1983,Me Cown and Wattimena, 1987).

None of the potato micropropagules have been field tested in Indonesia so far. These series of experiments will be performed to evaluated the field performance of the micropropagules.

These series of experiments consisted of:

1. Greenhouse studies of *in vitro* shoot propagules of potato. The effect of Kinetin and cultivars.
2. Field studies of *in vitro* shoat propagules of potato. The effect of 2,4 D for mulation and cultivars.
3. Field studies of *in vitro* microtuber propagules. The effect of of seeding method and cultivars.
4. Field studies of potato prpagules of three potato cultivars.

5.2. REPORT ON INDIVIDUAL FIELD PERFORMANCE OF MICROPROPAGULES EXPERIMENTS

5.2.1. Greenhouse studies of *in vitro* shoot propagules of potato. The effect of and cultivars

5.2.1.1 Introduction

Many experiments showed that *in vitro* tuberization was induced by BAP, kinetin and zeatin wich the concentration range firm 5 - 10 ppm (Wang and Hu 1982, Wattimena 1983) Field experiments of

Blunden and Wildgoose (1977) showed that field sprangs of kinetin of 2.8 g/ ha increase tuber field and uniform tubersize. The objective of this experiments evas to evaluate the effect of kinetin on growth and tuberfield of greenhouse growing potato plant of the cultivar Red Pontiac, DTO 28 and granola.

5.2.1.2 Material and Methods

The experiments was carried out at the IPB. Experiment Station at Pasir Sarongge (± 1100 m above sea level). The source propagules was *in vitro* growing shoot of the cultivar Red Pontiac, DTO 28, and Granola.

The experiment was a two factors expeiments in a split plot design with six replicates. The main plot was potato cultivar of : Red Pontiac, DTO 28 and Granola and the concentartion of kinetin (0, 5, 10 mg/l) as subplot.

Three nodes *in vitro* shoot cutting were aclimatisized in a steril soil medium in s small plastic posfor sweeks. For the first two weeks it was growth under full shade and afterwqeds the shade were removed little by little until complete shadeless at 5 weeks. Where the seedlings were transplanted in a bigger size plastic pot (diameter of 35 cm and 45 cm high). At transplanting each plastic bag was fertilized with 4 g urea, 5.5 g TSP and 4.2 g KCl and another 4 g urea was applied at 4 weeks after transplanting. Kinetin of 0, 5 and 10 mg/l were applied as foliage spray at 4 weeks after transplanting. For controlling pest and disease the plants were spray at there days interval with Baryrusil (2 cc'l), Difolatan 0.2 % and Agrimycin 0.6 g/l.

The variables measures were : Plant height, number of nodes, length of internode, number of bianches, number of tuber per pot, fresh weight of tuber per pot, dry weight of tuber per pot, and percentage tuberdry weight.

The vegetative variables were evaluated every weeks until the last measured at 11 weeks after transplanting. The tuber variables were evaluatd at harvest at 12 weeks after transplanting.

5.2.1.3 Results

The result were showed in table 4.1.1 and 4.1.2. The table showed that :

1. DTO 28 was higher than Red Pontiac and Granola. The higher manistem was due to the more number of nodes as well as a longer length of the internodes.
2. DTO 28 has more branches than Granola and Red Pontiac. Red Pontiac had the smallest number of branches.
3. The kinetin treatment decreased plant hight, tuber fresh weight tuber dry weight of Red Pontiac and DTO 28 but the reversed was true for Granola.
4. There was a trend that kinetin secreased the number of tuber per plant ofcultivar DTO 28 and increase in cultivar Granola.
5. Kinetin did not increased percentage tuber dry weight. DTO 28 and granola has a higher persentage dry weight than Red Pontiac.

Table 5.2.1.1. The effect of Kinetin on height of meristem, number of tubers, tuber fresh weight tuber dry weight of three potato cultivars

Cultivar/Kinetin	Height Meristem (cm)	No. Tuber/plant	Tuber Fwt/Plant (g)	Tuber Dwt/plant (g)
Red Pontiac				
0.0 ppm	39.97 bc	8.25	100.00 cd	14.62 c
5.0 ppm	34.37 cde	8.75	57.24 d	8.25 c
10.0 ppm	32.12 de	8.75	79.04 d	11.65 c
DTO 28				
0.0 ppm	52.32 de	14.00	232.10 a	42.27 a
5.0 ppm	48.73 ab	13.75	170.93 b	29.81 b
10.0 ppm	46.20 ab	12.75	147.29 b	25.89 b
Granola				
0.0 ppm	24.92 e	9.50	55.48 d	9.01 c
5.0 ppm	33.10 de	14.25	96.29 c	15.04 c
10.0 ppm	37.37 bcd	15.00	136.84 bc	25.01 b

The same letter at each column indicated not significant different at 5% HSD Test

Table 5.2.1.2. Number of node of meristem, length of internode, number of branches, pecentage tuber dry weight of three potato cultivars and three level of kinetin.

Treatments (Main Factors)	No. of Node (Vx)	Internode Length (cm)	No. of Branches (Vx)	% Tuber Dwt
Cultivars				
Red Pontiac	4.968 xy	1.39 y	1.927 z	14.61
DTO 28	5.131 x	1.93 x	3.543 x	17.80
Granola	4.718 y	1.48 y	2.198 y	17.00
Kinetin				
0 ppm	4.967	1.62	2.622	17.00
5 ppm	5.871	1.62	2.379	16.36
10 ppm	4.984	1.59	2.651	17.22

The same letter at each columns for cultivars or kinetin indicated not significant different at 5% HSD Test.

5.2.2. Field studies of potato *in vitro* shoats propagules. Effect of 2,4-d formulation and cultivars.

5.2.2.1. Introduction

in vitro shoats propagules have to be roated before field planting. Goodwin and Brown(1980) found that 6 weeks after roating was the best time to transplanted the seedling to the field. Later than 6 weeks the growth and fields were decreased. Field studies with potato shoat propagules in Wisconsin Showed no difference in growth and field of different ages of seedlings (Wattimena 1983). The treatment during roating and acclunatization that determined the growth and field in the field.

Hydrasil a 2,4-D for mulated with addition of macro nutrients (N,P,K,Mg,S) and micro nutrients (Mn,Zn,Cu) was recommended by goverment in Indonesian for horticultural craps nicluding potato. The price of hydrasil (2,4-D 400 mg/L) compared to 2,4-D as herbicida or standard 2,4-D plus metalik. Metalik is micro nutrient fertilizer consisted of Mn 5%, Fe 1.7%, Zn 0.86%, B 2%, Mo 0.24%, Mg 0.2%, protein 5% and organic acid 4.5%.

Auxin 2,4-D have found to have some benificial effect on growth of potato plants. Applied of 2,4-D have to be mixed with some micro nutrients (Fe, B, Mn, Zn, and Cn) to eliminated the toxic effect of 2,4-D (Wort 1965). Application of 2,4-D as faliage spray at tuber mitiation phase in creased tuber field (Ellis 1949, Nelson and Nylund 1963), intensifield the red skin tuber (Ellis 1949, Fults and Payne 1955), and a more Uniform tubersize (Nelson and Nylund 1963). The objective of the experiments was to evaluated the effect of 2,4-D formulation growth and tuberfield of three potato cultivars namely : Red Pontiac, Katahdin, Kennebec, DTO 28, and Cipanas.

5.2.2.2. Materials and Methods

The experiments was carried out at the IPB Experiment Station at Pasir Sarongge (± 1100 m above sea level) and the trilis a volcanic Andosal. The saurce explants of Red Pontiac, Katahdin, Kennebec, DTO 28 and Cipanas was a 5 weeks old *in vitro* shoats. The acclinatization procedures the same as experiments 4.1.

The 5 weeks old seedling coke transplanted to field with the distance of 30 cm x 70 cm. The fertilizer was applied per hill with the doses of 12 gr Urea, 9 gr TSP, and 4 gr KCL. The fertilizer was applied at planting except Urea half at planting and the other half at 4 weeks after planting. Hilling was done twice namely at 2 weeks and 4 weeks after planting.

Insecticides and fungicides was applied regularly. Furadan 3 G applied at planting the fungicides (Dithane M-45.2g/l, Antracol 2g/l) every 3 days and insecticides (Baryrusil 1.5 ml/l, Supracide 1 ml/l) every weeks.

The 2,4-D formulation was sprayed as foliage sprayed at 2 weeks and 4 weeks after planting with the 2,4-D concentrations of 0,6 mg/l.

The experiments was a two factors experiments. First factors consisted of 5 potato cultivars namely: Red Pontiac, Katahdin, Kennebec, DTO 28 and Cipanas. The second factors was formulation of 2,4-D namely : 0, 2,4-D, hidrasil, 2,4-D + metalic. The experiments was arranged in a vandomized block design with four replication. Each experimental unit consisted of 20 plants/hills.

The measure variables were : manistem height, number of nodes of manistem, length of intermode, number of plants per hill, number of tuber per hill, tuber fiesh weight per hill, tuber dry weight per hill and percentage tuber dry weights.

5.2.2.3. Results.

1. Formulation of 2,4 D did not effect the manistem height, mumber of nodes of manistem, length of manistem internodes and number of manistem per hill (tabel 4.2.1)
2. DTO 28 has the heighest manistem and length of manistem internode, Red Pontiac and Cipanas the intermediate; Katahdin and Kennebec the lowest (tabel 4.2.1)
3. Cipanas and DTO 28 produced higher stem number per hill compared to Red Pontiac, Katahdin and Kennebec (tabel 4.2.1)

4. DTO 28 produced the highest tuber fresh weight and dry weight per hill; Red Pontiac and Cipanas the intermediate ; Katahdin and Kennebec the lowest (tabel 4.2.2)
5. Cipanas produced the highest number of tuber per hill; Red Pontiac and Cipanas the intermediate; Katahdin and Kennebec the lowest (tabel 4.2.2)
6. The effect of 2,4-D formulation on tuber variables were cultivars dependent. Hydrasil tend to increase number of tuber per hill, fresh and dry tuber weight per hill for cultivars Cipanas and Red Pontiac. DTO 28 treated with 2,4-D + metalic tend to increase tuber fresh weight and dry weight per hill
7. Red Pontiac treated with hydrasil or metalic + 2,4-D tend to increase number of tuber per hill, tuber fresh weight per hill and tuber dry weight per hill. In this respect Kennebec and DTO 28 response better to 2,4-D than hydrasil or metalic + 2,4-D

Table 5.2..2.1. The effect of 2,4-D formulation on height of manistem, number of nodes number of manistem per hill, length of internodes of five potato cultivars. (10 WAP = Weeks After Planting)

Cultivars	2,4-D Formulation				Average
	Control	Hydrasil	2,4-D	2,4-D +Metalik	
Meristem Height (cm)					
Red Pontiac	59.4	58.8	51.6	53.8	55.9 b
Katahdin	48.9	40.8	45.1	53.7	47.1 a
Kennebec	50.1	47.5	48.5	46.3	48.1 a
DTO 28	72.2	65.1	69.5	72.0	69.7 c
Cipanas	55.0	54.5	56.2	55.8	56.8 b
Number of Nodes					
Red Pontiac	28.9	29.3	26.6	29.5	28.58 ab
Katahdin	27.9	26.2	28.1	28.6	27.71 ab
Kennebec	27.6	27.2	28.4	26.6	27.44 a
DTO 28	30.1	28.8	28.9	29.8	29.37 ab
Cipanas	29.4	29.4	33.0	28.1	29.97 ab
Number of Meristem/Hill					
Red Pontiac	3.0	3.2	2.4	2.6	2.81 a
Katahdin	2.1	1.7	2.6	1.5	1.96 a
Kennebec	3.2	5.6	4.4	3.4	3.39 a
DTO 28	7.4	7.5	8.1	7.9	7.74 b
Cipanas	7.7	7.6	8.9	9.5	8.64 b
Length of Internode (cm)					
Red Pontiac	2.1	2.0	2.0	1.9	2.0 c
Katahdin	1.8	1.6	1.7	1.9	1.7 a
Kennebec	1.9	1.8	1.8	1.8	1.8 ab
DTO 28	2.5	2.3	1.9	2.5	2.3 d
Cipanas	1.9	2.1	1.8	2.1	1.9 bc

The same letter at the same column for each variables denote no significant different at 5% HSD test

Table 5.2.2.2. The effect of 2,4-D formulation on number of tuber, tuber fresh weight, tuber dry weight, percentage tuber dry weight of five potato cultivars. (10 WAP = Weeks After Planting).

Cultivars	2,4-D Formulation				Average
	Control	Hydrasil	2,4-D	2,4-D +Metalik	
Number of tuber per hill					
Red Pontiac	13.2 abc	15.2 cd	13.3 abc	14.6 abc	14.06
Katahdin	7.4 abc	8.1 abc	7.1 abc	7.9 abc	7.66
Kennebec	5.7 ab	6.7 abc	9.9 abc	5.7 a	7.01
DTO 28	12.2 abc	16.1 cd	14.4 bc	13.6 abc	14.12
Cipanas	45.4 ef	48.3 f	29.2 de	30.9 ef	38.48
Tuber fresh weight per hill (g)					
Red Pontiac	202.2 abcde	248.62 abcdefg	192.5 abcd	235.5 abcde	219.73
Katahdin	104.5 ab	76.95 a	88.0 a	129.1 abc	99.63
Kennebec	84.4 a	61.10 a	182.2 abcd	69.2 a	99.26
DTO 28	367.0 def	315.60 cdef	442.5 f	394.2 ef	379.84
Cipanas	256.4 abcdef	299.00 bcdef	129.9 abc	171.0 abcd	214.11
Tuber dry weight per hill					
Red Pontiac	29.6 abcde	40.8 bcdefg	29.7 abcd	35.0 abcdef	33.78
Katahdin	17.8 abcd	11.6 ab	13.9 abc	20.9 abcd	16.05
Kennebec	13.5 abc	8.1 ab	32.9 abcdef	9.5 a	16.02
DTO 28	58.0 efg	44.2 cdefg	69.1 g	61.2 fg	58.16
Cipanas	42.5 cdefg	48.3 defg	20.9 abcd	28.9 abcde	35.18
Percentage tuber dry weight (%)					
Red Pontiac	15.1 abc	16.4 abc	15.5 abc	14.8 abc	15.46
Katahdin	17.0 bc	15.0 abc	15.6 abc	16.2 abc	15.97
Kennebec	15.7 abc	12.9 ab	17.8 c	12.7 a	14.79
DTO 28	15.9 abc	14.3 abc	15.7 abc	15.5 abc	15.36
Cipanas	16.6 abc	16.3 abc	15.5 abc	16.7 abc	16.27

The same letter at the same column for each variables denote no significant different at 5% HSD test

5.2.3. Field studies of *in vitro* microtuber propagules. The effect of sedding methods andc cultivars.

5.2..3.1. Introduction

Microtuber is a small tuber of 5 mm to 10 mm. Size tuber fresh weight or about 50 to 150 mg tuber produced *in vitro*. the microtubers have to break the dormancy before used as micropropagules. The dormancy period depends on cultivars and growth retardants used for induced *in vitro* tuberization. Paclabutiazal gave the longest domancy while coumarine or cycocel (CCC) produced the shortest dormancy.

In Wisconsin the sprouted microtubers had to be rooted first and then transplanted as seedings. Direct seeding of sported microtuber did not germinated due to low soil temperature at planting time in the spring. In Indonesia with the high temperature all the year round, the planting of sproted microtuber is possible. Prelemininany tasting showed that the direct seeding of sprouted microtuber is possible if the sprout is between 1 to 2 cm long. Sprouted less than 1 cm produced uneven staud in the field.

The objective of the experiment was to evaluated the direct seeding and indirect seeding of sprouted microtubers on the growth and yild of the cultivar Cipanas, DTO 28 and Red Pontiac.

5.2.3.2. Materials and Methods

The experiment was carried out at the IPB experiment station at Pasir Sarongge (_+ 1100 m above sea level) and the soil is a valanic Andosol.

The source sprouted microtubers were dervied from *in vitro* tuber, induced by 5 mg/L Kinetin, 600 mg/L cycocel and 90 g/L sucrose with a shollow liquid. Liquid sistem of tuberization. Half of the sprouted (1cm - 2 cm sprout) microtubers were planted directly in a tray and planted two weeks later. The application of fertilizer, insectisides and fungicides were the same as experiments 4.2.

The treatment combination consisted of 3 potato cultivars (Cipanas, DTO 28, Red Pontiac) nad 2 planting methods(direct and transplanting microtubers). The experiments were arranged in a randomized block design with 4 replication.

The variable measured consisted of plant height, number of nodes, tuber fresh weight, number of tuber and tuber size. The potato plants were harvested 12 weeks afer planting for direct planting and 10 weeks for transplanting.

5.2.3.3. Results

The results in table 4.3.1 showed that:

1. Direct planting plants (DP. plants) and trasplanting plants(TP. plants) of each cultivars response differently to the Variables measured.
2. Marin stem height. TP plants of DTO 28 had siguificant higher stem than DP. plants but the reverse was true for Red Pontiac. TP plants and DP plants of Cipanas showed no different in heighest of the manistem.
3. Number of nodes of manistem.

   T.P plant and D.P plants of the cultivar Red Pontiac showed no different in number of nodes of manistem. T.P plants of DTO 28 produced less number was true for the cultivar Cipanas.
4. Tuber fresh weight per hills.

   T.P plants of cultivar Red Pontiac and Cipanas had higher tuber fresh weight per hills than D.P plants but the reversed was true for DTO 28. T.P plants as well as D.P plants of Red Pontiac produced significant higher tuberfresh weight per hill than T.P plants and D.P plants of Cipanas and DTO 28.
5. Number of tubers per hill.

   T.P plants of DTO 28 produced higher number of tube. than D.P plants but there were no different betwen T.P plants and D.P plants for the cultivar Red Pontiac and Cipanas. T.P plants of Cipanas produced higher tuber numbers per hill than T.P plants of Red Pontiac and DTO 28 and the same hold true for D.P plants.

6. Tuber diameter (size).

No different in tuber diameter between D.P plants and T.P plants of all three cultivars (Cipanas, DTO 28 and Red Pontiac). T.P plants of Red Pontiac had higher tuber diameter of T.P plants of DTO 28 and Cipanas and the same hold true for D.P plants.

Table 5.2.3.1. The effect of method of planting sprouted microtubers on plant height, number of nodes, number of tubers, tuber fresh weight, and tuber size of three potato cultivars.

Treatments	Plant Height (cm)	No. of nodes	Tuber fresh weight/hill (g)	No. of tuber/Hill	Tuber diameter (cm)
Cipanas					
- Direct	107.2 bc	32.0 c	228.7 a	21.1 c	2.43 a
- Transplanting	95.2 b	28.2 b	262.2 b	20.0 bc	2.39 a
DTO 28					
- Direct	109.7 c	29.2 bc	363.5 d	17.2 a	2.54 a
- Transplanting	117.0 d	28.5 b	280.5 bc	19.2 bc	2.27 a
Red Pontiac					
- Direct	95.7 b	27.2 a	309.7 c	18.0 a	3.56 bc
- Transplanting	78.7 a	27.2 a	380.2 e	17.2 a	3.70 b

The same letter at the same column denoted not significant different at 5% HSD test

5.2.4. Studies of potato propagules of three potato cultivars

5.2.4..1. Introduction

A potato plant is actually a sprout with a well developed root. The different between the micropropagules and seed propagules are the number of plant per hill. The micropropagules produced single plant per hill while the seed propagules produced multiple plants per hill. Eventhough micropropagules produced single plant per hill, they produced the same number of emergent astems as the seed propagules. Emergent stems of a micropopagules plants are subsurface branches while the emergent stems of seed propagules plants are true potato plants.

To do an acurate comparison between the micropropagules plants and the seed tubers plants both have to be compared at a single plant per hill.

The objective of the experiment was to evaluate the growth and yield of a microtuber plants with a seed tubers plants of a single and multiple plants per hill of the cultivar Cipanas, DTO 28 and Red Pontiac.

5.2.4.2. Material and Methods

The experiments was carried out at IPB Experiments Station at Pasir Sarongge (1100 m above sea level, volcanic andosol). The source propagules consisted of sprouted microtubers (Go) and first generation of microtubers (G1) of the cultivar Cipanas, DTO 28 and Red Pontiac.

These experiments consisted of three single factors experiment. The single factor treatments were:

1. Microtubers (Go)
2. G1 50 g - single stem (G1 big-SS)
3. G1 20-50 g single stem (G1 medium-SS)
4. G1 20 g - single stem (G1 small-SS)

5. G1 50 g - multiple stems (G1 big-MS)
6. G1 20-50 - multiple stems (G1 medium-MS)
7. G1 20 g - multiple stems (G1 small-MS)

The treatments were arranged in a randomized block design with 5 replicates each for Cipanas, DTO 28 and Red Pontiac.

For all three experiments individual plant was an experimental unit. Each observed variables was an average of 10 plants. The variables measured consisted of: plant height, number of nodes of plants, emergent stem, number of tuber per hill, tuber fresh weight per hill and percentage dry matter.

The planted propagules were all spronted. The microtubers had only 1 sprent with a minimum length of 0,5 cm. G1propagules consisted of single spront and multiple spronts according to treatmant. The propagules were planted at a distance of 30 cm by 70 cm. Chicken manure of 1 kg per hill were applied 1 week before planting. At planting each hill was applied with Furadan 3 G 2 kg, Urea 4 kg, TSP 12 kg, and KCL 9 kg. The secontd application of Urea 4 g/hill was at 4 week after planting.

The plants were treated every 3 days with Dithane M-45 (1 g/L), Ridomil (1 g/L) and Antracol 75 WP (2 g/L) to control the fungi. The control the bacteria and insects the plants were treated every week with Agrimycin 15 WP (1,5 g/L) and Bayrusil 250 EC (1.5 cc/L). The plant were haversted at 90 days after planting.

5.2.4.3. Results

The result as tabulated in table 4.4.1 and 4.4.2 ahowed :

1). Height and node numbers

a. G1 microtuber propagules plants were sigmificantly shorter than plants of G1. Big and G1.Medium for all three potato cultivars (Cipanas, DTO 28, Red Pontiac)

b. G1 small (SS+MS) plants of DTO 28 were significantles shorter than G1. Medium and Big but wereno different with G0- microtuber plants.

c. The shorter plants of G0-microtuber propagules of Red Pontiac were due to smaller number of nodes while for Cipanas and DTO 28 were due to shorter internodes length (no different in the number of nodes).

2). Number of emergent stems

G0 microtubers and G1. small MS produced lower number of emergent stems compared to G1.Medium (SS+MS) and G1. Big (SS+MS) eventhough the significantly different were only found in Red Pontiac and DTO 2.

3). Tuber fresh weight per hill

a. There were no sinificant different of tuber fresh weight per hill among the small tuber propagules (G0. microtuber, G1. small SS, G1. small MS) for cultivar Cipanas and DTO 28. In Red Pontiac no significant different were only found between G0. microtubers and G1. small MS propagules .

b. G0. microtubers propagules produced significant lower tuber fresh weight per hill than G1.Medium (SS+MS) and G1. Big (SS+MS) propagules for all three cultivars (Cipanas, DTO 28, Red pontiac). Small tubersize propagules (G0. microtubers, G1. small SS, G1. small MS) of DTO 28 produced significantly lower tuberfresh weight per hill than G1. Medium (SS+MS) and G1. Big (SS+MS) propagules.

c. Propagules of G1. Big MS and SS in Red Pontiac produced significantly higher tuberfresh weight per hill than propagules of G1. Medium MS and SS.

4). Number of tubers per hill

a. Three was no effect of different kind of propagules on the numbers of tubers per hill of the cultivar Cipanas.

b. The numbers of tubers per hill of small propagues (G0. microtubers, G1. small. SS, G1. small MS) were not significant different in cultivar DTO 28 and Red Pontiac.

c. G1. Big (SS+MS) propagules of Red Pontiac produced higher number of tuber propagules while no different was found between the medium size tuber propagules and small size tuber propagules.

There were no different of number of tubers per hill between the G1. Big (SS+MS) propagules and G1. Medium (SS+MS) propagules of DTO 28 Pontiac but both were different with small size propagules (G0. microtubers, G1. small SS, G1. Small MS).

Table 5.2.4.1. The effect of propagules on plant height, number of nodes and number of emergent stem of the cultivar Cipanas, DTO 28 and Red Pontiac.

Propagules	Plant Height (cm)	Number of nodes	Number of stems
Cipanas			
Go-microtubers	61.3 a	17.9 a	2.55
G1-Big-SS	90.0 bc	20.4 ab	3.39
G1 Medium-SS	84.3 bc	20.9 b	3.70
G1 Small-SS	75.5 b	20.3 ab	2.28
G1-Big-MS	92.1 c	21.6 b	3.62
G1 Medium-MS	88.1 bc	21.1 b	4.10
G1 Small-MS	82.8 bc	21.6 b	2.80
DTO 20			
Go-microtubers	83.8 j	21.2	1.51 a
G1-Big-SS	103.4 m	22.0	6.37 d
G1 Medium-SS	95.2 klm	21.6	5.60 cd
G1 Small-SS	91.0 jkl	20.7	3.87 bc
G1-Big-MS	102.0 lm	22.4	7.63 d
G1 Medium-MS	98.4 lm	21.2	5.65 d
G1 Small-MS	86.7 jl	20.5	1.68 b
Red Pontiac			
Go-microtubers	59.9 r	20.1	1.30 r
G1-Big-SS	93.3 tu	22.4	4.44 tu
G1 Medium-SS	91.5 tu	22.4	4.03 tu
G1 Small-SS	82.1 st	21.1	2.76 st
G1-Big-MS	67.9 s	22.4	5.28 u
G1 Medium-MS	92.2 tu	22.3	3.67 tu
G1 Small-MS	73.9 s	21.2	1.99 rs

Numbers followed by the same letter at the same cultivar and column are not significantly different at 5% HSD Test.

Table 5.2..4.2. The effect of propagules on tuber fresh weight, number of tuber per hill, and percentage dry matter of the cultivar cultivar Cipanas, DTO 28 and Red Pontiac.

Propagules	Tuber fresh weight per hill (g)	Number of tuber per hill	Number of stems
Cipanas			
Go-microtubers	212.6 a	17.9 ab	13.9
G1-Big-SS	539.6 d	16.2 ab	13.6
G1 Medium-SS	465.7 bcd	14.8 ab	13.8
G1 Small-SS	354.2 abc	12.5 a	14.8
G1-Big-MS	528.8 d	14.6 ab	13.9
G1 Medium-MS	537.4 d	20.9 ab	14.0
G1 Small-MS	330.7 ab	15.2 ab	15.1
DTO 20			
Go-microtubers	210.6 j	5.3 j	12.8
G1-Big-SS	885.2 l	7.9 klm	13.6
G1 Medium-SS	861.7 l	10.3 lm	13.7
G1 Small-SS	451.7 jk	5.9 jkl	13.6
G1-Big-MS	987.7 l	10.1 lm	13.7
G1 Medium-MS	754.7 l	9.2 m	13.3
G1 Small-MS	325.8 jk	5.8 jk	12.5
Red Pontiac			
Go-microtubers	141.2 r	6.1 r	12.8
G1-Big-SS	908.2 u	9.7 tu	13.0
G1 Medium-SS	652.9 st	8.9 rst	13.6
G1 Small-SS	538.2 s	6.2 rs	14.0
G1-Big-MS	978.6 u	12.7 u	13.9
G1 Medium-MS	675.4 st	8.0 rst	13.4
G1 Small-MS	326.8 r	6.3 rs	13.4

Numbers followed by the same letter at the same cultivar and column are not significantly different at 5% HSD Test.

5.2.5. The Effect of Kinetin on the Growth and Development of 3 Potato Cultivars Derived from *in vitro* Cuttings

5.2.5.1. Introduction

in vitro tuberization can be induced by application of cytokinins, anti giberellin or both. The application of cytokinins for inducing tuberization for field growing potato almost no experiments have been done due to the expensive of cytokinins. The objective of this research was to evaluate the effect of kinetin in the growth, develop and the production of tuber of three potato cultivars derived from *in vitro* shoot.

5.2.5.2. Methods

The experiments was conducted at Pasir Sarongge Horticulture Experiment Station of IPB at an elevation of 1030 m above sea level. The average daily temperature of 22-23°C. The experiments was started on November 1988 till March 1989.

The experiments consisted of two factors, each of three levels. The cultivars as the first factor consisted of Red Pontiac, DTO 28 and Granola. The second factor kinetin consisted three levels 0, 5 Ind 10 mg/l. The experiments were run in a Randomized Block Design with 6 replicates.

The micro shoots were planted for 5 weeks in the plastic house before transplanting to a plastic bag. The medium for rooting of micro rooting as well as for growing the seedling consisted of 3 parts of soil and 1 part of stable manure. Each polybag contained only 1 plant/polybag. The kinetin were applied only once at 4 weeks after planting.

The variable measured were as follows:

(1). The height of the plants; the number of nodes per plant; the number of branches per plants; and the average lenght of internode.

(2). The number of tuber per plant; the tuber fresh weight per plants; the tuber dry weight per plants; the average tuber weight and the harvest index.

5.2.5.3. Results

The results of the experiments were presented on Table 2.1.1, 2.1.2, 2.1.3, 2.1.4 and 2.1.5.

(1). The main effect of cultivars and level of kinetin were presented on Table 2.1.4 (cultivars) and Table 2.1.5 (level of kinetin)

(2). The interaction effect of cultivars and kinetin were presented on Table 2.1.1 and 2.1.3.

(3). Table 2.1.2. The significant test for all variables measured at harvest for the main effect and the interaction.

From the table presented the following conclusion can be drown:

1. Different varieties gave significant effect on all variable measure. Except for the numbers of tubers and harvest index (Table 2.1.4).
2. From all the variables measured DTO 28 was significant different from Red Pontiac and Granola, while there were no significant different between Red Pontiac and Granola except for tuber size (Table 2.1.4). 3. DTO 28 had a various vegetatif growth bigger tuber yield, bigger tuber size, than Granola and Red Pontiac (Table 2.1.4).
4. The level of kinetin did not have significant effect on the variables measured. Kinetin decreased the tuber size (Table 2.1.5).
5. Haulm fresh and dry weight, tuber fresh and dry weight, average tuber weight increased with increased the level of kinetin, while tha reversed was true for Granola. Red Pontiac reacted more similar to DTO 28 (Table 2.1.3) for tuber variables but similar to Granola for the haulm variables (Table 2.1.1).

Table 5.2.5.1. The effect of cultivars and kinetin on haulm fresh weight, haulm dry weight, percentage of dry weight and harvest index

Treatments	Haulm fresh weight (g)	Haulm dry weight (g)	Percentage dry weight (g)	Harvest Index Based on	
				Fresh weight	Dry weight
Red Pontiac					
0 mg/l kinetin	14.37y	3.97y	27.63	0.88xy	0.79x
5 mg/l kinetin	6.95y	2.16y	31.10	0.91xy	0.77x
10 mg/l kinetin	17.43xy	6.04xy	34.62	0.85y	0.73x
DTO 28					
0 mg/l kinetin	39.10x	11.93x	30.52	0.86y	0.79x
5 mg/l kinetin	35.23x	10.09x	28.63	0.83y	0.75x
10 mg/l kinetin	29.67x	8.07x	27.20	0.84y	0.76x
Granola					
0 mg/l kinetin	3.79y	1.58y	41.87	0.93x	0.85x
5 mg/l kinetin	7.69y	2.99y	39.09	0.92x	0.84x
10 mg/l kinetin	9.86y	3.55y	36.05	0.93x	0.88x

The same letter at the same column are not significant different at 5% HSD Test.

Table 5.2.5.2. The anova for all variables measures at harvest (12 weeks)

Variables	F-test		
	Varieties	Kinetin	Interaction
- Number of tuber per plant	ns	ns	ns
- Tuber frsh weight per plant	**	ns	**
- Tuber dry weight per plant	**	ns	**
- Average tuber sizer	**	**	**
- Percentage of tubers	**	**	**
greater than 20 g - Percentage of tuber smaller than 20 g	**	**	**
- Haulm fresh weight	**	ns	ns
- Haulm dry weight	*	ns	ns
- Harvest index (fresh weight)	**	ns	ns
- Harvest index (dry weight)	ns	ns	ns

**, *, and ns denotes significancy at 0.01, 0.05 and no significant of F test.

Table 5.2.5.3. The interaction effect of cultivars and kinetin on tuber fresh weught, tuber dry weight, average tuber weight, percentage of tuber above and below 20 g

Treatments cultivars/ level of kinetin (mg/l)	Tuber weight			Size of tuber	
	Fresh (g)	Dry (g)	Average (g)	Above 20 g (%)	Below 20 g (%)
Red Pontiac					
0	100.08cd	14.62c	12.19b	24.65ab	75.35bc
5	57.24d	8.25c	7.70cd	0.00db	100.00a
10	79.04d	11.65c	8.48cd	7.50c	92.50ab
DTO 28					
0	232.10a	42.27a	15.92a	35.37a	64.62c
5	170.93b	29.81b	12.51b	18.32b	81.67bc
10	147.29b	25.89b	11.60b	14.25b	85.70b
Granola					
0	55.48d	9.01c	6.28c	0.00d	100.00a
5	96.29c	15.04c	6.75de	1.58cd	98.43ab
10	136.84bc	25.01b	9.04c	5.15c	94.05ab

The same letter at the same column are not significant different at 5% HSD Test.

Table 5.2.5.4. The main effect of cultivars on the 15 variables measured

	Variables (per plant)	Red Pontiac	DTO 28	Granola
1.	Plant height (cm)	35.48y	49.08x	31.79y
2.	Number of nodes	24.83xy	26.44x	22.37y
3.	Internode length	1.39y	2.02x	1.50y
4.	Number of levels	37.78y	89.28x	51.98y
5.	Number of branches	4.50y	13.50x	12.92x
6.	Number of tubers	8.58y	13.50x	12.92x
7.	Tuber fresh weight	78.79y	183.44x	96.21y
8.	Tuber dry weight	11.51y	32.66x	16.36y
9.	Average tuber weight	9.25y	13.35x	7.36z
10.	Percentage of tuber above 20 g	10.72y	22.65x	2.24z
11.	Percentage of tuber below 20 g	89.28y	77.32z	97.76x
12.	Haulm fresh weight	12.92y	34.67x	7.09y
13.	Haulm dry weight	4.06y	10.03x	2.71y
14.	Harvest index (fresh)	0.88xy	0.84y	0.93x
15.	Harvest index (dry)	0.76x	0.77x	0.85x

The same letter at the same column are not significant different at 5% HSD Test

Table 5.2.5.5. The main effect of kinetin on the 15 variables measured

	Variables (per plant)	Kinetin (mg/l)		
		0	5	10
1.	Plant height (cm)	39.07a	38.73a	38.56a
2.	Number of nodes	24.83a	23.83a	24.94a
3.	Internode length	1.66a	1.65a	1.60a
4.	Number of levels	49.39a	50.50a	56.06a
5.	Number of branches	8.28a	8.00a	8.83a
6.	Number of tubers	10.58a	12.25a	12.17a
7.	Tuber fresh weight	129.22a	108.16a	121.06a
8.	Tuber dry weight	21.97a	17.70a	20.85a
9.	Average tuber weight	11.46a	8.76b	9.71b
10.	Percentage of tuber above 20 g	20.01a	6.63b	8.97b
11.	Percentage of tuber below 20 g	79.99b	93.37a	91.02a
12.	Haulm fresh weight	19.08a	16.61a	18.95a
13.	Haulm dry weight	5.83a	5.08a	5.89a
14.	Harvest index (fresh)	0.89a	0.89a	0.87a
15.	Harvest index (dry)	0.81a	0.79a	0.79a

The same letter at the same column are not significant different at 5% HSD Test.

5.2.6. The Effect of Paclobutrazol on the Growth and Develop of Three Potato Cultivars Derived from *in vitro* Cuttings

5.2.6.1. Introduction

In vitro tuberization is induced by cytokinins and growth retardants. In nature tuberization is retarded by Giberellin. Application of growth retardant for field growing potato can increased tuber production by earliness of tuberization and realocation of carbohidrat from haulm to tubers. The effect of retardants to increase tuber production is depend on the: (1) kind of growth retardants, (2) the concentartion, (3) time of application, (4) cultivars and (5) plant vigors.

The objectives of the experiments is to evaluate the effect of paclobutrazol on the haulm growth and tuber production of three potato cultivars derived from micro shoots.

5.2.6.2. Methods

The experiments consisted of three levels of paclobutrazol (0, 500, 1000 mg/l) and three potato cultivars (Red Pontiac, Katahdin and Cipanas). The experiments were arranged in a split plot design, with cultivars as main plot and levels of paclobutrazol as subplot with six replication.

The micro shoot of 3 or more nodes were rooted in sterile medium. After 5 weeks the seedling were transplanting in 10 kg black plastic bag.

Four week after transplanting the paclobutrazol were sprayed to the haulm. The variable measure consisted of:

(1). Haulm variables: plant height, number of nodes, length of internodes, number of branches and haulm dry weight.

(2). Tuber variables: number of tuber, tuber fresh weight, tuber dry weight, percentage of dry matter, percentage of tuber above and below 20 g.

(3). Harvest index.

5.2.6.3. Results

The results of the experiment were presented in Table 2.2.1, 2.2.2 and 2.2.3. From those tables the following conclusion can be drawn:

1. Application of paclobutrazol 500 mg/l to Red Pontiac, Katahdin and Cipanas decresed plant height by 33.65%, number of nodes by 7.14% and internode by 21.05%. These figures for paclobutrazol 1000 mg/l were: 33.33%, 9.28% and 17.54% (Table 2.2.1).
2. The decreased of haulm dry weight for all three cultivars by the application of paclobutrazol was due to decrease of plant heights, number of nodes and internode length. For the cultivar Cipanas the decrease of haulm dry weight was not only due to those 3 variables but also due to the decrease of the number of branches (Table 2.2.1)
3. The effect of interaction was significant for the number of branches. The application of paclobutrazol 500 mg/l and 100 mg/l to the cultivar Cipanas decreased the number of

branches by 47.85% and 54.37%. The application of paclobutrazol did not effect the number of branches of Red Pontiac and Katahdin (Table 2.2.1).

4. Katahdin was higher and longer internode than Red Pontiac and Cipanas. There were no significant different for these variables between Red Pontiac and Cipanas due to the longer internodes of Katahdin, because there were no different in the number of nodes among these three cultivars. The heght of the stem is determined by the internode length and the number of nodes.

5. The application of paclobutrazol decreased tuber fresh weight and tuber dry weight, for all three cultivars. The application of paclobutrazol 500 mg/l and 1000 mg/l decreased the tuber fresh weight by 42.01% and 53.65%. These figures for tuber dry weight were 38.33% and 49.44% (Table 2.2.3).

6. The effect of interaction was significant for the number of tubers per plant. The application of paclobutrazol 500 mg/l decreased the number of tubers of Katahdin by 47.73% and Cipanas by 37.84%. These figures for paclobutrazol 1000 mg/l were 52.27% and 51.35%. The application of paclobutrazol did not effect the number of tubers of Red Pontiac (Table 2.2.2).

7. The application of paclobutrazol did not effect the percentage of tuber dry matter, tuber size, different class of tubers, tuber haulm rat o and harvest index for Red Pontiac, Cipanas and Katahdin (Table 2.2.2 and Table 2.2.3).

Table 5.2.6.1. The interaction effect of paclobutrazol and cultivars on plant heights, number of nodes, length of internodes, number of branches and haulm dry weights

Varieties	Level of Paclobutrazol (mg/l)			Average
	0	500	1000	
		Plant height (cm)		
Red Pontiac	20.70	18.07	16.57	18.44a
Katahdin	29.93	22.03	23.55	25.17b
Cipanas	28.23	16.22	16.47	20.31a
Average	26.29b	18.72a	18.86a	
		Number of nodes		
Red Pontiac	23.33	22.33	21.17	22.28
Katahdin	22.17	21.50	20.83	21.50
Cipanas	24.67	21.33	21.67	22.56
Average	23.39b	21.72a	21.22a	
		Length of internodes		
Red Pontiac	0.91	0.84	0.81	0.86a
Katahdin	1.33	1.07	1.18	1.19b
Cipanas	1.19	0.80	0.80	0.93a
Average	1.14b	0.90a	0.93a	
		Number of branches		
Red Pontiac	1.50	1.33	1.67	1.50a
Katahdin	1.17	1.17	1.33	1.28a
Cipanas	7.67b	4.00a	3.50a	5.00b
Average	3.50b	2.11a	2.17a	
		Haulm dry weght (g)		
Red Pontiac	2.04	0.92	0.90	1.29a
Katahdin	3.62	1.94	2.14	2.57b
Cipanas	4.58	1.93	1.55	2.69b
Average	3.41b	1.60a	1.53a	

The same letter by column or rows denotes no significant different different at 5% HSD Test.

Table 5.2.6.1. The effect of cultivars and paclobutrazol on number of tubers, average tuber weights, the percentage of tuber above and below 20 grams

Varieties	Level of Paclobutrazol (mg/l)			Average
	0	500	1000	
	Number of tuber/plant			
Red Pontiac	6.00	4.50	5.50	5.33a
Katahdin	11.00b	5.75a	5.25a	7.33a
Cipanas	18.00b	11.50a	9.00a	13.00b
Average	11.83b	7.25a	6.58a	
	Average tuber size (g)			
Red Pontiac	12.95	14.06	6.38	11.13b
Katahdin	8.61	11.10	8.58	9.43ab
Cipanas	6.04	5.17	6.26	5.82a
Average	9.20	10.11	10.41	
	Percentage of tuber less than 20 g			
Red Pontiac	89.42	75.00	100.00	85.14
Katahdin	85.32	86.75	91.67	87.90
Cipanas	92.37	97.50	100.00	96.62
Average	86.04	86.42	97.22	
	Percentage of tuber greatly than 20 g			
Red Pontiac	19.58	25.00	0.00	14.88
Katahdin	14.68	13.40	8.33	12.14
Cipanas	7.55	0.00	0.00	2.52
Average	13.93	12.80	2.78	

The same letter by column or rows denotes no significant different different at 5% HSD Test.

Table 5.2.6..3. The effect of cultivars and paclobutrazol on tuber fresh weight, tuber dry weight, percentage of dry matters, and harvest index

Varieties	Level of Paclobutrazol (mg/l)			Average
	0	500	1000	
	Tuber fresh weight/plant (g)			
Red Pontiac	68.75	36.82	34.08	46.55a
Katahdin	92.25	60.64	38.29	63.73b
Cipanas	108.84	59.03	52.71	73.53b
Average	89.95b	52.16a	41.69a	
	Tuber dry weight/plant (g)			
Red Pontiac	10.20	5.45	4.52	6.72a
Katahdin	14.20	10.35	6.85	10.47b
Cipanas	15.60	8.85	8.85	11.10b
Average	13.33b	8.22a	6.74a	
	Tuber dry matter (%)			
Red Pontiac	14.83	14.81	13.26	14.30
Katahdin	15.39	17.08	17.88	16.78
Cipanas	14.33	14.99	16.78	15.37
Average	14.85	15.62	15.97	
	Tuber haulm ratio			
Red Pontiac	5.81	7.03	5.34	6.06
Katahdin	3.92	5.33	3.87	4.37
Cipanas	3.89	4.64	5.68	4.74
Average	4.54	5.67	4.68	
	Harvest index			
Red Pontiac	0.84	0.86	0.84	0.85
Katahdin	0.80	0.84	0.78	0.81
Cipanas	0.78	0.82	0.85	0.82
Average	0.81	0.84	0.82	

The same letter by column or rows denotes no significant different different at 5% HSD Test.

5.2.7. The Ability to Produce Greenhouse Cuttings and Minitubers of Four Potato Cultivars Derived from Two Types of *In Vitro* Cutting

5.2.7.1. Introduction

The green house cuttings and mini tubers can be used as propagules for potato production either as seed potato production as well as ware potato production. The source of plant materials are virus cuttings of *in vitro* plantlet. These micro cuttings are usually microshoots. The micro cuttings can be increased if the micro cuttings are not a microshoots but a single node cuttings.

The advantages of microshoots as cutting is the greater ability to root and higher survival rate. The disadvantage is that it produce less cutting due to a microshoot consisted of 3 to 4 nodes. The reverse is true from a single node cutting. The rooting ability and the survival rate is low but a greater number of cuttings derived from the same plantlet.. The resultant of the disadvantages and advantages of both type of micro cutting may yield the same amount of propagules produced from both micro cutting or single node cutting may produce more propagules than the microshoot cutting.

The objective of this experiments was to evaluate the ability of microshoot cutting and a single node cutting to produce greenhouse cutting and mini tubers of four potato cultivars.

5.2.7.2. Methods

The experiment consisted of two types of cuttings (microshoot and single node) and four cultivars namely: PAS 3061 (V1), Majestic (V2), Norship (V3) and Nooksack (V4).

The experiment consisted of three steps as follows:

Step 1. Transplanting of cutting from *in vitro* medium to soil medium. Each variety has 14 trays of 20 plants. Seven trays for microshoots cutting and seven trays for single node cuttings. Variables measured in the first were: The survival rate, the height of the seedling, the number of nodes and the number of root per cuttings.

Step 2. The production of greenhouse cuttings. From the experiments from the first step 3 trays for each variety of each cutting were evaluated for the ability to produce greenhouse cuttings. The cuttings were taken every two for five cutting period. The variable measured were the number of cuttings produced per cutting period and total cutting period.

Step 3. The production of mini tubers. Greenhouse cuttings produced in step 2 were rooted. After three weeks in the rooting medium the rooted cuttings were transfer to polybag. Each polybag was planted with one rooted cutting. For each treatment there were 8 replicated. Each polybag was considered as an experimental unit. So there were in total 64 experimental units (64 polybag). The plant were harvested at 10 weeks after transplanted. The variables measured consisted of: plant height, the number of branches, the number of nodes of the mainstem, the internode length, the number of tuber per plant, tuber fresh weight per plant, tuber dry weight per plant and the grade of tubers. Tuber smaller than 30 grams were considered as mini tubers. In this experiments the production of mini tubers was indirect. The mini tubers were produced from greenhouse cutting derived from microshoot cutting and single node cutting.

5.2.7.3. Results

The results of the experiments were presented in Table 5.2.7.1 to Table 5.2.7.6. In those table the following abreviation were used: C1 = microshoot cutting; C2 = single node cutting, V1 = PAS 3063; V2 = Majestic; V3 = Norchip; and V4 = Nooksack.

Table 5.2.7.1 - Table 5.2.7.2	: Result of Step 1.0
Table 5.2.7.4	: Result of Step 2.0
Table 5.2.7.5 - Table 5.2.7.6	: Result of Step 3.0

From those tables following conclusion can be drawn:

Step 1.0

1. The survival rate of microshoots cutting was significant greater than the single node cutting (Table 5.2.7.1).

2. There were no significant different of the survival rate among PAS 3063, Majestic and Norchip. Nooksack had the lower survival rate and was significant different from those three cultivars (Table 5.2.7.1).
3. Plant of Nooksack was significant shorter than plant from PAS 3063 and Norchip (Table 5.2.7.1).
4. There were no significant different in height between plant derived from microshoot cutting and single node cutting only for Majestic and Norchip. In PAS 3063 and Nooksack plant derived from microshoot cutting was significant taller than plant derived from single node cutting (Table 5.2.7.1).
5. There were significant different in number of nodes of plant derived from microshoot cutting and single node cutting for all four cultivars. Plant from microshoot cutting produced significant more node than plant from single node cutting (Table 5.2.7.2).
6. The number of node of the four cultivars were significant different. The cultivar thet produced the greatest node number to the lowest node number was in the following order: Norchip> PAS 3063 > Majestic > Nooksack (Table 5.2.7.2).
7. Plant derived from microshoot cutting produced better root system than plant derived from single node cutting for all four cultivars (Table 5.2.7.3).

Step 2

8. The number of harvested cutting was significant greater from plant derived from microshoot cutting than plant from single node cutting only for Majestic and Nooksack. In PAS 3063 and Norchip there were no different in the number of harvested cutting between plant derived from the two kind of cutting (Table 5.2.7.4).
9. There were significant different on the number of cutting produced among the four cultivars. The cultivars that produced the greatest number of cutting to the lowest number of cutting was in the following order: Norchip > Majestic > PAS 3063 > Nooksack (Table 5.2.7.4).

   In step 3.0 the term plant derived from microshoot or single node cutting is different from Step 1.0 and Step 2.0. In Step 3.0 the meaning of microshoot/single node cutting must be the first generation of microshoot cutting and single node cutting. We will abbreviate as follows:

G1-microshoot = First generation from microshoot cutting

G2-single node = First generation from single node cutting

10. There were no significant different in plant height, node number, and internodelength between G1-microshoot plant and G1-single node plant (Table 5.2.7.5)
11. The different of plant-height, node number and internode were due to varietal difference. Majestic was significant taller tha PAS 3063, Norchip and Nooksack. No significant different in height were found among these three cultivars. The different in height can be due to: node number, the length of internode or noth node number and internode as found in this experiment as follows:

(1). Node number

Majestic was taller than PAS 3063 due to the Majestic produced more number of node than PAS 3063. There were no difference in internode length between the two cultivars (Table 5.2.7.5).

(2). The length of internode

Majestic was taller than Norchip due to the different in internode length. Majestic had longer internode than Norchip. There were no different in node number between the two cultivars (Table 5.2.7.5).

(3) Both node number and length of internode Majestic was taller than Nooksack due to both node number and length of internode. Majestic produced more number of node as well as longer internode than Nooksack (Table 5.2.7.5).

12. G1-plant from Majestic and Norchip produced significant more branches than PAS 3063 and Nooksack. There were no significant difference in branches produced between Majestic and Norchip as well as between PAS 3063 and Nooksack (Table 5.2.7.5).
13. There were no significant different in the number of tuber per plant, tuber fresh weight, tuber dry weight between G1-microshoot plant and G1-single node plant (Table 5.2.7.6; 5.2.7.7).
14. The different in the number of tuber, tuber fresh weight were due to varietal difference. Norchip had significant greatest number of tuber than the three other cultivars. Majestic and Nooksack had more number of tuber than PAS 3063. There were no difference in tuber number between Majestic and Nooksack. Nooksack had the highest tuber fresh weight and dry weight

followed by Majestic and Norchip. PAS 3063 had the lowest tuber fresh and dry weight. There were no significant difference between Majestic and Norchip in tuber fresh and dry weight. The two cultivars were significant with PAS 3063 in tuber fresh weight and tuber dry weight (Table 5.2.7.6, Table 5.2.7.7).

Table 5.2.7.1. The height of plant and survival rate derived from two types of cutting and four cultivars

Treatment	Survival rate (%)	Weeks after transplanting 3 weeks 1	2	3
		..... cm		
Cutting				
C1	92.92a	1.60a	3.22a	5.42a
C2	85.85b	0.86b	2.14b	4.16a
Cultivars				
V1	93.33a	1.41a	3.21a	5.86a
V2	95.83a	1.29ab	2.00bc	4.58bc
V3	95.00a	1.21b	2.64b	5.13ab
V4	73.33a	1.18b	2.27c	3.59c
Interaction				
C1V1	95.00	1.58a	3.80a	6.65a
C1V2	98.33	1.47b	2.81c	4.73b
C1V3	100.00	1.51b	2.79c	5.02b
C1V4	78.33	1.90a	3.47b	5.29ab
C2V1	91.67	0.96c	2.61c	5.06b
C2V2	93.33	1.11c	2.39c	4.44b
C2V3	90.00	0.90c	2.49c	5.24ab
C2V4	68.33	0.45a	1.06d	1.89c

The same letters at the same column for cutting, cultivar, or interaction were not significant different at 5% DMR Test.

Table 5.2.7.2. The number of nodes of mainstem derived from two types of cutting and four cultivars

Treatment	Weeks after transplanting		
	1	2	3
Cutting			
C1	4.60a	6.40a	7.92a
C2	2.92b	4.15b	6.10b
Cultivars			
V1	4.20a	5.47b	7.63b
V2	4.20a	5.77b	7.13c
V3	4.27a	6.30a	8.10a
V4	2.37b	3.57c	5.17d
Interaction			
C1Vi	4.87a	5.93b	8.13b
C1V2	4.87a	7.13a	8.13b
C1V3	4.93a	7.73a	9.13a
C1V4	3.73b	4.80c	6.27db
C2V1	3.53b	5.00c	7.13c
C2V2	3.53b	4.40c	6.13d
C2V3	3.60b	4.87c	7.07c
C2V4	1.00c	2.33d	4.07d

The same letters at the same column for cutting, cultivar, or interaction were not significant different at 5% DMR Test.

Table 5.2.7.3. The number of roots of cutting derived from two types of cutting and four cultivars

Treatments Interaction	Root Scoring					Number of cutting
	1	2	3	4	5	
1. Microshoot Cuttings (C1)						
PAS3063 (V1)	0	2	5	9	2	18
Majestic (V2)	0	0	0	5	13	18
Norchip (V3)	0	1	3	4	10	18
Nooksack (V4)	1	2	7	6	2	18
2. Single node Cuttings (C2)						
PAS3063 (V1)	0	0	8	5	4	17
Majestic (V2)	0	7	10	0	0	17
Norchip (V3)	1	10	4	2	0	17
Nooksack (V4)	3	8	3	2	1	17

Scoring: 0 = no roots, 1 = 1-3 short roots, 2 = root more than three but short, 3 = 3-4 long roots 4 = more than 4 long roots

Table 5.2.7.4. The number of cutting harvested per plant per harvest derived from two types of cutting and four cultivars

Treatments Combination	Number of cutting harvested at each harvest					Total cutting harvested
	I	II	III	IV	V	
Cuttings						
1. Microshoot (C1)	0.99a	1.58a	1.38a	1.96a	1.71a	7.61a
2. Single node (C2)	0.72b	1.13b	1.13b	1.77a	1.22b	5.97b
Cultivars						
1. PAS3063 (V1)	1.00a	1.40b	0.98b	0.99c	1.39a	5.75c
2. Majestic (V2)	0.80b	1.33b	1.58a	2.11b	1.29a	7.11b
3. Norchip (V3)	0.96a	1.76a	1.70a	3.20a	1.52a	9.15a
4. Nooksack (V4)	0.56c	0.73c	0.65c	1.09c	1.46a	4.50d
Interaction						
C1V1	1.00a	1.56b	0.92c	1.00a	1.64a	6.11b
C1V2	1.00a	1.97a	2.17a	2.36a	1.67a	9.17a
C1V3	1.00a	2.03a	1.39b	3.03a	1.72a	9.17a
C1V4	0.97a	0.77c	1.03c	1.44a	1.79a	6.00b
C2V1	1.00a	1.29b	1.02c	0.98a	1.22a	5.51b
C2V2	0.67b	0.90c	1.20bc	1.94a	1.04a	5.75b
C2V3	0.94a	1.59b	1.90a	3.31a	1.39a	9.14a
C2V4	0.29c	0.71c	0.41d	0.86a	1.23a	3.50c

The same letters at the same column for cutting, cultivar, or interaction were not significant different at 5% DMR Test.

Table 5.2.7.5. Plant height, number of nodes, length of internodes and number of branches of the mainstem derived from two types of cutting and four cultivars (10 weeks after transplanting)

Treatments Combination	Height (cm)	Number of node	Length of internode (cm)	Number of branches above the soil	Number of branches below the soil surface
Cuttings					
1. Microshoot (C1)	30.29a	23.92a	1.28a	6.92a	0.54a
2. Single node (C2)	31.93a	24.54a	1.36a	6.62a	0.83a
Cultivars					
1. PAS3063 (V1)	29.17b	19.92c	1.43a	3.83b	0.17b
2. Majestic (V2)	40.34a	27.58a	1.52a	9.58a	1.00a
3. Norchip (V3)	27.39b	25.92a	1.10b	8.58a	0.50ab
4. Nooksack (V4)	27.93b	23.50b	1.23b	5.08b	1.08a
Interaction					
C1V1	28.78	19.50	1.55	4.17	0.17
C1V2	38.95	26.83	1.57	9.33	0.83
C1V3	26.30	25.83	1.57	9.33	0.33
C1V4	27.93	23.50	1.23	4.83	0.83
C2V1	29.55	20.33	1.53	3.50	0.17
C2V2	41.75	28.33c	1.53	9.83	0.17
C2V3	28.48	26.00c	1.14	7.83	0.67
C2V4	27.93	23.50	1.23	5.33	1.33

The same letters at the same column for cutting or cultivar were not significant different at 5% DMR Test.

Table 5.2.7 6. The number of tubers, tuber fresh weight, tuber dry weight and percentage dry matter of potato derived from two types of cutting and four cultivars (10 weeks after transplanting)

Treatments Combination	Number and weight per plant			Percentage of dry matter (%)
	Number of tuber	Tuber fresh weight (g)	Tuber dry weight (g)	
Cuttings				
1. Microshoot (C1)	11.62	64.45	10.25	15.78
2. Single node (C2)	10.46	62.34	9.35	14.65
Cultivars				
1. PAS3063 (V1)	5.42c	41.05c	5.98c	14.29
2. Majestic (V2)	11.42b	61.47b	8.91b	14.48
3. Norchip (V3)	16.67a	69.91ab	10.59b	15.15
4. Nooksack (V4)	10.67b	81.15a	13.71a	16.94
Interaction				
C1V1	5.67	47.70	7.63	16.00
C1V2	10.33	63.63	9.02	14.18
C1V3	20.17	68.85	10.07	14.63
C1V4	12.00	77.62	14.27	18.38
C2V1	5.17	34.40	4.32	12.56
C2V2	12.50	59.30	8.80	14.84
C2V3	14.83	70.97	11.12	15.15
C2V4	9.33	84.68	13.15	15.50

The same letters at the same column for cutting or cultivar were not significant different at 5% DMR Test.

Table 2.3.7. The number of tuber produced in each class of potato derived from two types of cutting and four cultivars (10 weeks after transplanting)

Treatments Combination	Number of tuber per plant			
	Smaller than 2.5 g	2.5-20.0 g	20.1-30.0	Greater than 30 g
Cuttings				
1. Microshoot (C1)	5.79	5.54	0.54	0.17
2. Single node (C2)	4.29	5.50	0.58	0.00
Cultivars				
1. PAS3063 (V1)	1.33b	3.75b	0.33	0.00
2. Majestic (V2)	5.00a	5.50ab	0.67	0.08
3. Norchip (V3)	7.08a	7.00a	0.67	0.00
4. Nooksack (V4)	6.75a	5.83a	0.58	0.25
Interaction				
C1V1	1.00	4.17	0.50	0.00
C1V2	4.83	4.50	0.83	0.17
C1V3	11.00	9.00	0.17	0.00
C1V4	6.33	4.50	0.67	0.50
C2V1	1.67	3.33	0.17	0.00
C2V2	5.17	6.50	0.50	0.00
C2V3	3.17	5.00	1.17	0.00
C2V4	7.17	7.17	0.50	0.00

The same letters in each column for cultivars were no significant different at 5% DMR Test.

5.2.8. The Effect of Plant Density On Mini Tuber Production of two Potato Cultivars Derived from *In Vitro* Cuttings

5.2.8.1. Introduction

There are two kind of small tuber, used as propagules in potato production. Production of seed potatoes as well as ware potatoes. The small tuber produce by *in vitro* methods is called micro tuber and small tuber produced in the greenhouse is called mini tubers. Mini tubers are usually bigger than micro-tubers. The size and the number of mini tubers produced in a unit area determined by plant density and cultivars.

The objectives of this experiments were to evaluate the effect of plant density on mini tuber production of two potato cultivars.

5.2.8.2. Methods

The experiment was done in a insectproff greenhouse of Mustika Nusantara Abadi at Cisarua, Bogor at an alevation of 1000 m above sea level. The factorial experiments consisted of four levels (30, 60, 90, 120 plants/m^2) of plant density and two cultivars (Norchip and majestic). The experiments were arranged in a randomized block design with four replicates. *In vitro* micro cutting of the two cultivars were planted 2.5 cm by 2.5 cm in a plastic trays. The soil medium consisted compost and sand (1 : 1 by volume). The cuttings were placed in room with controlled temperature and humidity. After three weeks the cutting were put in a regular insect proff greenhouse. After 1 weeks stem cuttings of 3 to 4 nodes were taken from these cutting trays and plant in a new trays with the density according to treatments. The media consisted of soil and manure with the volume ratio of 3 : 1. Four weeks after transplanting new sterilize medium were added to the cutting trays. The plant were harvested at eight weeks after transplanting.

The variables measured consisted of:

(1) The number of nodes per mainstem
(2) The height of the mainstem
(3) The number of branches per plant
(4) Haulm dry weights
(5) Number of tuber per plant and per m^2
(6) Number of tuber according to size
(7) Tuber fresh weight and tuber dry weight
(8) The percentage tuber dry matter.

5.2.8.3. Results

The result were presented in Table 5.2.8.1, 5.2.8.2, 5.2.8.3 and 5.2.8.4. From those tables following conclusion can be drawn:

1. Majestic had taller mainstem than Norchip due to more number of nodes produced (Table 5.2.8.1).
2. The higher density the lower the number of node and the number of branches (Table 5.2.8.1 and Table 5.2.8.4).
3. The higher the density the lower the haulm dry weight per plant due to lower number of branches produced (Table 5.2.8.1 and Table 5.2.8..4).
4. The higher the plant density the lower tuber fresh weight and tuber dry weight per plant. The reverse was true for tuber fresh weight and tuber dry weight per m^2 (Table 5.2.8.1, Table 5.2.8.2 and table 5.2.8.4).
5. The higher the plant density the lower the number of tuber per plant but reverse for the number of tuber per m^2. This relationship hold true not only for total number of tubers but for the number of tuber smaller than 2.5 g; 2.5 g to 20 g and greater than 20 g as well.
6. Majestic and Norchip response similar to plant density for all variables measured (Table 5.2.8.1, 5.2.8.2, 5.2.8.3 and 5.2.8.4).
8. For both varieties the plant density had not reached the optimum density for all the variables measured (Table 5.2.8.1, 5.2.8.2, 5.2.8.3 and 5.2.8.4).

Table 5.2.8.1. The effect of plant density and cultivars on plant height, number of nodes, number of branches, haulm dry weight and tuber fresh weight (8 weeks)

Cultivars	Plant density (plants/m²)				Average
	30	60	90	120	
		Plant height (cm)			
Norchip	22.0	25.4	26.8	25.8	25.0
Majestic	39.0	35.4	36.1	34.8	36.3
Average	30.4	30.4	31.5	30.3	30.7
		Number of nodes			
Norchip	20.8	20.2	19.7	19.2	20.0
Majestic	23.7	23.5	21.4	20.8	22.4
Average	22.3	21.9	20.6	20.0	21.2
		Number of branches			
Norchip	5.6	5.6	4.8	2.9	4.7
Majestic	6.3	5.0	4.7	4.1	5.0
Average	6.0	5.3	4.8	3.5	4.9
		Haulm dry weights (g)			
Norchip	3.84	3.32	2.80	2.22	3.05
Majestic	4.83	3.14	2.91	2.25	3.28
Average	4.34	3.23	2.86	2.24	3.17
		Tuber fresh weight (g/plant)			
Norchip	56.81	41.40	39.42	28.55	41.57
Majestic	47.73	29.22	33.28	22.40	33.16
Average	52.27	35.36	36.35	25.48	37.37
		Tuber fresh weight (g/m²)			
Norchip	1704.24	2489.24	3547.71	3426.27	2791.88
Majestic	1431.99	1753.22	2995.11	2688.36	2217.17
Average	1568.14	2121.23	3271.41	3057.32	2504.53

Table 5.2.8.2. The effect of plant density and cultivars on tuber dry weight, percentage of dry matter, the number of tubers per plant, average fresh tuber weight, number of tuber per plant and number of tuber per m²

Cultivars	Plant density (plants/m²)				Average
	30	60	90	120	
	Tuber dry weight (g/plant)				
Norchip	10.30	7.62	7.34	5.48	7.69
Majestic	8.80	5.27	6.13	3.92	6.03
Average	9.55	6.45	6.74	4.70	6.86
	Percentage of dry matter (%)				
Norchip	18.30	18.23	18.56	19.14	18.56
Majestic	18.99	18.08	18.48	17.82	18.34
Average	18.65	18.16	18.52	18.48	18.45
	The number of tubers per plant				
Norchip	5.7	3.9	3.8	2.9	4.1
Majestic	7.2	5.4	5.6	4.4	5.7
Average	6.5	4.7	4.7	3.7	4.9
	Average fres tuber weight (g/tubers)				
Norchip	12.73	12.18	11.54	12.09	12.14
Majestic	6.91	6.31	8.09	5.20	6.63
Average	9.82	9.25	9.82	8.65	9.39
	Number of tuber per plants				
Norchip	5.6	3.0	2.9	2.8	4.1
Majestic	7.2	5.7	5.6	4.4	5.8
Average	6.4	4.8	4.8	3.7	4.9
	Number of tuber per m²				
Norchip					273.4
Majestic					389.8
Average	192.2	279.0	423.1	432.2	

Table 5.2.8.3.The effect of population density and cultivars on number of tuber per m² of each class

Treatments	Total	Number of tubers (%) in each class		
		Less 2.5 g	2.5-20.0 g	Greater 20.0 g
Cultivar				
Norchip	273.4	89.7(30.3)	156.6(51.8)	52.9(18.2)
Majestic	398.8	145.8(37.1)	227.6(58.6)	34.8(4.4)
Population density (number/m²)				
30	192.2	72.9(33.6)	137.3(57.0)	29.6(9.4)
60	279.0	101.5(33.4)	154.7(53.7)	26.8(12.9)
90	423.1	159.5(37.4)	220.4(51.4)	43.5(11.8)
120	432.2	137.1(30.4)	255.9(58.6)	39.2(11.0)

a(b) - a = number of tuber; (b) = percentage

Table 5.2.8.4. Variables and their linear response curves (density)

Variables	Linear Response Curve	R^2 value
1.	Number of nodes per main stem (Node)	
	YNode 5 = 4.50865 - 0.00219 X	0.970
	YNode 6 = 4.64475 - 0.00258 X	0.992
	YNode 7 = 4.72345 - 0.00280 X	0.999
2.	Number of branches per main stem (BR)	
	YBR4 = 2.252755 - 0.00452 X	0.909
	YBR5 = 2.477865 - 0.00588 X	0.954
	YBR6 = 2.509810 - 0.00590 X	0.953
	YBR7 = 2.531845 - 0.00603 X	0.935
	YBR8 = 2.647395 - 0.00613 X	0.945
3.	Haulm dry weights per mainstem (Hdw)	
	YHdw = 4.83100 - 0.02330 X	0.955
4.	Tuber fresh weight per plant (Tfp)	
	YTfp = 57.20918 - 0.26461 X	0.855
5.	Tuber fresh weight per m² (Tfm)	
	YTfm = 1162.588 + 17.47576 X	0.767
6.	Tuber dry weight per plant (Tdp)	
	YTdp = 10.41875 - 0.04748 X	0.840
7.	Number of tuber per plant (Tp)	
	YTp = 2.625332 - 0.00616 X	0.867
8.	Number of tuber smaller than 2.5 g per plant (Tsp)	
	YTsp = 2.706875 - 0.01261 X	0.858
9.	Number of tuber per m² (Tm)	
	YTm = 83.16375 + 1.433712 X	0.948
10.	Number of mini tubers (2.5-20 g/tuber) per m² (mm)	
	Ymm = 55.00875 + 0.836212 X	0.716
11.	Number of tubers smaller than 2.5 g per m² (Tsm)	
	YTsm = 11.65662 + 0.081598 X	0.911

- 4,5,6,7,8 = weeks after transplanting

5.3. DISCUSSION

1. Field performance

Field performance of the experiments 4.1 on black plastic containers considered to be about 70 percent of the field growing plants. Field performence of the experiments 4.3 considered to be a good craps. The experiment was carried out on the dry season with supplement inigation. During the dry season the light duration was 12 hours, high light intensity, low temperatures.

Field performance of the experiment4.2 and 4.4 considered to be an average of the mediocre craps. The experiments were carried out during the rainy season with heavy rain, short days, low temperature and low light intensity. The sail was to wet and a heavy application of pesticides to control pest and diseases.

These field performance have to be considered to evaluate correctly the experimental result. These experimental results can be compared with the similar experiment in Wisconsin USA. The same source of *in vitro* propagules of the cultivar Red Pontiac was used for the potato propagules experiments both in Wisconsin and in Indonesia. Comparison among the similar propagules of Red Pontiac between these two climatic region (tropics or temperate) will enriched the experimental results.

2. Microshoats propagules

The height of stem was influenced both by the environtment and genotypes. In both experiments DTO 28 was higher than the other cultivar due to more number of nodes and longer internodes. The field planting plants of both Red Pontiac and DTO 28 had higher plant than pot planting plants. Higher plants were due to longer internodes and higher number of nodes.

The yield growing plants of Red Pontiac in Wisconsin were higher than the field growing plants of Red Pontiac in Indonesia, while the reversed was due to longer internodes. Lower number of nodes Red Pontiac in Wisconsin may be due to slow growth or the manistem stap growing several weeks before harverst (harvest at Wisconsin 125 days, harvest at Pasir Sarongge 90 days). The fresh tuber weight of Red Pontiac of Pasir Sarongge was about 14 % (200g/hill),of Wisconsin Red Pontiac (1400g/hill) while no different in number of tuber per hill (13/hill).

The effect of Kinetin on stem height and tuber fresh weight depend on cultivars. Increased in Granola but decreased in Red Pontiac and DTO 28. Increased in tuber field and number of tubers has been reported by Blunden and Wildgoose (1977). This increasing in field and number of tuber due to kinetin teratment were also cultivars dependent.

The effect of 2,4-D on all cultivars in creased number of tuber and fresh weight. Most cultivar response well to 2,4-D metalic or hydrasil (2,4-D + micronutrient + makronutrient) than 2,4-D alone. These 2,4-D plus may be not only the effect of 2,4-D but also the effect of micro nutrients. Applied of 2,4-D to broad leave plants have to be applied with micro nutrient to eliminate the toxie effect (Wort 1965). Increased of tuber fresh weight and number of tubers have also been reported (Nelson and Bristal 1975, Ellis 1949)

3. Microtuber Propagules

Application of microtubers as potato propagules at least two condition have to be fulfil namely:

1). The length of the spront must be longer than one centimeter.

2). The soil condition have to be favorable to in duce roat initation and proliferation. Favorable soil structure, soil moisture, soil temperature and avoilale nutrient.

In Wisconsin the sponted microtuber have to be roated and than roated microtuber seedlings was transplanted to the field. Direct transplanting microtubers to the field were emergence one month after planting. The irabilaty of the icrotubers to roat was due to the low soil temperature at planting in the spring (May).

Stress condition at planting and early growth is detri mental to micropropagules in general. Experiment 4.3 with a very favorable soil condition and weather induce very good growth of micropropagules, while the unfavorable soil condition reduced the growth in experiments 4.4 (table 4.4.3; 4.3.1; 4.5.1; 4.5.2). The higher plants, higher node numbers higher tuber fresh weight and higher tuber numbers of experiments 4.4 indicated that full potensial growth of potato his not yet been reached. The field growing potato palants will never reached this full potensial.

Eventhough the experiments 4.3 produced a very good vine growth (height and node numbers) the tuber yeild will still lower than the similar microtuber plants growing in Wisconsin (table 4.4.3). The different chinatic condition and duration of the growing season were the main cause of the different of tuber yield.

4. Micropropagules and seed propagules

The similarities and difference of micro propagules and seed propagules in Bogor and Wisconsin as fallows:

Wisconsin:	Bogor:
Tuber multiple stem	Big Tuber multiple shoat
Tuber single stem	Medium Tuber multiple shoat
Microshoat	Small Tuber multiple shoat
Microtubers	Big Tuber single shoat
	Medium Tuber single shoat
	Small Tuber single shoat
	Micro Tubers

Big Tuber multiple stem and single stem of Bogor equal to tuber multiple stem and single stem of Wisconsin. Both tuber propagules wer of Wisconsin. Both tuber propagules were of equal size (a 50 grams whole tubers). The culrivar used in cammon in these two location was red pontiac.

The microtuber propagules and the smaller seed propagules either multiple stem and single stem produced all produced shorter plants, less tuber fresh weight and less number of tuber compared to big and medium sized tubers. Indicated smaller tuber size either from *in vitro* tubers performed similar growth and tuber yield. More nunber of nodes and number of tuber of micropropagated plants did not show in experiments 4.4. Except from experiments 4.3. More node numberand more tuber number of micropropagated plants did not show up (table 4.5.2). Comprehensive experiments on these line have to be set up where the growth regulator (cytokinins, retardants, auxin) to produce *in vitro* shoat and *in vitro* tuber have to be considered as an important factors.

5. Potatoes of temperature climate and tropical climate

General false conclusion that the low potato yield in the tropics such as Indonesia due to the leas intensive culture than the temperature zone. The truth is that the reserse is true.

The problems of low potato yield in Indonesia are:

1). Low quality seed tubers

2). Short photo periods

3). Shorter vine growth

4). Heavy investation of pest and diseases

From table 4.5.2 comparison can be made of Red Pontiac seed tuber single stem from the two location. Poin (4) can be eliminated due the seed tubers were derived from *in vitro* cutting growth in insect proof screen. The spray of insectisides and fungicides were very intensive.

Red Pontiac is a cultivar of Solunum tuberosum spptuberosum sennitive to short days. Red Pontiac in Indonesia The vines killed natural at 90 days after planting. In Wisconsin the vine killing was by chemical treatments with paraquat. Let take TS from both location Bogor and Wisconsin of cultivar Red Pontiac :

1). Tuber yield in Bogor was 908.2g/hill

2). Tuber yield in Wisconsin was 1575.0g/hill

3). Bulking rate in Bogor is : 90 day -45 days=908.2g/45days=20.18g/day/plant

4). Bulking rate in Wisconsinis: 126 days -45 days=1575.0g/81day/plant

Red Pontiac started first flowering in both location at about 1,5 month after planting and this time tuberization started. Based on that calculation the tuber yield of the two location must be the same if harvested at the sama age.

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The other problems was the number of nodes. In both location the number of nodes perplant were the same. Thereare two possibilities:

1). The time need to produce 1 node is foster in Bogor than in Wisconsin.

2). The time need to produce 1 node is the same in Bogor and Wisconsin. The vine stop growing when rapid tuber development ("bulking") occur (Milthorhope 1963, Moorby 1978)

Table 5.3.1. Effect of micropropagules and chimatic region on the height, number of nodes, number of tuber, and tuberfresh weight.

Location and Experiments	Height (cm)	No. of nodes	Tuber fresh weight (g)	No. of tuber
Micro shoot Propagules				
DTO 28				
- Exp. 4.1	52.3	26.3	232.1	14.0
- Exp. 4.2	72.3	30.1	367.0	12.0
Red Pontiac				
- Exp. 4.1.	39.9	24.7	100.0	8.2
- Exp. 4.2	59.4	29.3	202.0	13.2
- Wisc. 1.0	69.3	21.6	1476.0	13.2
- Wisc. 2.0	68.7	18.8	1400.0	13.0
Microtuber Propagules				
DTO 28				
- Exp. 4.3	117.0	28.5	280.5	19.2
- Exp. 4.4	83.8	21.2	210.0	5.3
Cipanas				
- Exp. 4.3	107.0	32.0	228.0	21.2
- Exp. 4.4	61.3	17.9	212.0	17.9
Red Pontiac				
- Exp. 4.3	78.7	27.2	380.2	17.2
- Exp. 4.4	59.9	20.1	141.2	6.1
- Wisc. 1.0	74.1	23.1	1570	12.0
- Wisc. 2.0	-	-	1656	24.6

Exp. 4.1 = Pot Exp. Bogor, Exp 4.2, 4.3, 4.4 = Field Exp. Bogor, Wisc. 1,2,3 = Field experiments (Wisconsin)

Table 5.3.2. Comparison of similar experiments carried out at Bogor and Wisconsin at different yield of the cultivar Red Pontiac.

Location and Propagules	Height (cm)	No. of nodes	Tuber fresh weight (g)	No. of tuber
Bogor				
- TM	67.9	22.4	978.6	12.7
- TS	93.3	21.1	908.2	9.7
- MT	59.9	20.1	141.2	6.1
- MT1*	95.9	27.2	309.7	18.0
- MS*	59.4	29.3	202.0	13.2
Wisconsin				
- TM	73.5	19.1	1975.0	10.9
- TS	68.7	18.8	1575.0	8.4
- MT	74.1	23.1	1570.0	12.0
- MS	70.8	22.7	1410.0	12.9

TM= tuber multiple stem, TS= tuber single stem, MT= microtuber, MS= microshoot, *= other seperate experiments

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VI. PRODUCTION COST OF MICROTUBERS

6.1. Component of Inputs

Inputs in potato microtuber production include : chemicals, electricity and labor. Cost for electricity is the highest, followed by chemicals and labor. Of all cost spent, 80% of the cost is allocated for electricity to run the equipment, air condition, and lighting. Lighting can be minimized since tuberization is best in dark condition. However, energy for maintenance of low room temperature and operation of equipment cannot be reduced. Efforts are then focused on reducing chemicals used.

Following the studies on physiology of initiation and development of microtuber, studies to develop effective, reliable, and low cost microtuber production method (s) were conducted. From all tuberization methods developed, the 2 step tuberization procedure using 2 different liquid media is the best from the standpoint of number and quality of tuber obtained.

In liquid medium, the cost of agar can be omitted. In Indonesia, agar is the most expensive component, five times higher than other components. Usually in liquid medium, continuous shaking is essential. But in our method, the liquid medium is provided in thin layer, so the *in vitro* cuttings planted are not submerged entirely. Thus shaking of cultures is not necessary. The flow charts show cost calculations of potato microtuber production with liquid-liquid system.

6.2. Cost Calculation

Calculation on cost of microtuber production were made on :

1. Cost for growth medium, which is Murashige and Skoog basal composition plus vitamines and various growth regulators (Table 5.1 - Table 5.2)
2. Electricity for autoclave, laminar air flow cabinet, lighting, air condition, and other equipments (Table 5.7)
3. Labor cost for media preparation, transplanting/subculture and harvest of microtubers.

4. Cost recapitulation for labor, electricity and chemicals.
5. The microtubers production was calculated based on : (1) the capacity of the culture of 10 000 vials, (2) five laminar air flow cabinet, (3) a person was able to subculture 40 vials per hour. For multiplication of explants need 2 000 vials (20%) and 8000 vials for shoot induction followed by *in vitro* tuberization. Average contamination was about 10%. In one year period there will be five harvest.
6. The incubation for explants and shoot culture was in light culture room each of 4 weeks and 16 hours/day. The incubation for *in vitro* tuberization was a dark incubation for 8 weeks.

6.3. Discussion

In a year can be harvested 360 000 microtubers with total cost of Rp 13 544 093,00. Thus a microtuber cost Rp 37,60 or Rp 1 692 000,00 for a hectare (1 Ha = 45 000 microtubers). The electric cost contributed 82.43% of the total production and air condition alone was 46.38%. If the laboratory is at high elevation (more than 1 000 m above sea level), the air condition cost can be eliminated and the price of microtuber would be reduced from Rp 37,60/microtuber to Rp 17,30/microtuber or from Rp 1 692 000,00/hectare to Rp 778 000,00/hectare.

Table 6.1. Cost of 1 liter stock solution for MS medium

Stock Solution	Concen-tration (g/l)	Price (Rp)	Volume Used per liter Medium (ml)
I. Inorganic salt			
A. NH_4NO_3	82.50	2 858.63	20
B. KNO_3	95.00	7 847.00	20
C. H_3BO_3	1.24	43.40	
KH_2PO_4	34.00	2 380.00	
KI	0.166	25.73	5
Na_2MoO_4	0.05	22.75	
$CoCl_2.6H_2O$	0.005	4.88	
D. $CaCl_2$	88.00	3 185.60	5
E. $MgSO_4.7H_2O$	74.00	3 515.00	
$MnSO_4.4H_2O$	4.46	842.94	5
$ZnSO_4$.			
$CuSO_4.5H_2O$	1.72	287.59	
F. $FeSO_4.7H_2O$	0.005	0.47	5
Na_2EDTA	5.57	540.29	
II. Vitamines			
G. Thiamin-HCl	0.02	27.64	
Nic. Acid	0.10	35.60	
Pyridoxin-HCl	0.10	820.00	5
Glycine	0.40	94.00	
Myo-inositol	20.00	25 600.00	
Sucrose	-	90.00	30 g/l
T O T A L		48 221.52	

Table 6.2. Cost of 1 liter medium for *in vitro* shoot propagation of potato (liquid medium)

Stock Solution	Concen-tration (g/l)	Price (Rp)	Volume Used per liter Medium (ml)	Price per liter Medium (Rp)
I. Inorganic salt				
A. NH_4NO_3	82.50	2 858.63	20	57.17
B. KNO_3	95.00	7 847.00	20	156.94
C. H_3BO_3	1.24	43.40		0.22
KH_2PO_4	34.00	2 380.00		11.90
KI	0.166	25.73	5	0.13
Na_2MoO_4	0.05	22.75		0.11
$CoCl_2.6H_2O$	0.005	4.88		0.03
D. $CaCl_2$	88.00	3 185.60	5	15.93
E. $MgSO_4.7H_2O$	74.00	3 515.00		17.58
$MnSO_4.4H_2O$	4.46	842.94	5	4.22
$ZnSO_4$.				
$CuSO_4.5H_2O$	1.72	287.59		1.44
F. $FeSO_4.7H_2O$	0.005	0.47	5	0.002
Na_2EDTA	5.57	540.29		2.70
II. Vitamines and growth regulators				
G. Thiamin-HCl	0.02	27.64		0.14
Nic. Acid	0.10	35.60		0.19
Pyridoxin-HCl	0.10	820.00	5	4.10
Glycine	0.40	94.00		0.47
Myo-inositol	20.00	25 600.00		128.00
A-rest	-	100.00	0.5	100.00
NAA	0.05	20.00	2	0.04
Sucrose	-	30.00	30 g/l	30.00
Aluminium Foil				400.00
Aquadest				150.00
T O T A L		48 221.52		1 111.312

Table 6.3. Cost of 1 liter medium for *in vitro* tuberization induced by cycocel and benzyl-adenine with liquid-liquid system

Stock Solution	Concentration (g/l)	Price (Rp)	Volume Used per liter Medium (ml)	Price per liter Medium (Rp)
I. Inorganic salt				
A. NH_4NO_3	82.50	2 858.63	20	57.17
B. KNO_3	95.00	7 847.00	20	156.94
C: H_3BO_3	1.24	43.40		0.22
KH_2PO_4	34.00	2 380.00		11.90
KI	0.166	25.73	5	0.13
Na_2MoO_4	0.05	22.75		0.11
$CoCl_2.6H_2O$	0.005	4.88		0.03
D. $CaCl_2$	88.00	3 185.60	5	15.93
E. $MgSO_4.7H_2O$	74.00	3 515.00		17.58
$MnSO_4.4H_2O$	4.46	842.94	5	4.22
$ZnSO_4.$ $CuSO_4.5H_2O$	1.72	287.59		1.44
F. $FeSO_4.7H_2O$	0.005	0.47	5	0.002
Na_2EDTA	5.57	540.29		2.70
II. Vitamines and growth regulators				
G. Thiamin-HCl	0.02	27.64		0.14
Nic. Acid	0.10	35.60		0.19
Pyridoxin-HCl	0.10	820.00	5	4.10
Glycine	0.40	94.00		0.47
Myo-inositol	20.00	25 600.00		128.00
Cycocel	30.00	31 800.00	20	636.00
Benzyl-adenine	1.00	38 000.00	5	190.00
Sucrose	-	90.00	90 g/l	90.00
Aquadest				150.00
T O T A L		118 021.52		1 467.272

Table 6.4. Cost of 1 liter of *in vitro* tuberization medium induced by benzyl-adenine and paclobutrazol with liquid-liquid system

Stock Solution	Concentration (g/l)	Price (Rp)	Volume Used per liter Media (ml)	Price per liter Media (Rp)
I. Inorganic salt				
A. NH_4NO_3	82.50	2 858.63	20	57.17
B. KNO_3	95.00	7 847.00	20	156.94
C. H_3BO_3	1.24	43.40		0.22
KH_2PO_4	34.00	2 380.00		11.90
KI	0.166	25.73	5	0.13
Na_2MoO_4	0.05	22.75		0.11
$CoCl_2.6H_2O$	0.005	4.88		0.03
D. $CaCl_2$	88.00	3 185.60	5	15.93
E. $MgSO_4.7H_2O$	74.00	3 515.00		17.58
$MnSO_4.4H_2O$	4.46	842.94	5	4.22
$ZnSO_4.$ $CuSO_4.5H_2O$	1.72	287.59		1.44
F. $FeSO_4.7H_2O$	0.005	0.47	5	0.002
Na_2EDTA	5.57	540.29		2.70
II. Vitamines and growth regulators				
G. Thiamin-HCl	0.02	27.64		0.14
Nic. Acid	0.10	35.60		0.19
Pyridoxin-HCl	0.10	820.00	5	4.10
Glycine	0.40	94.00		0.47
Myo-inositol	20.00	25 600.00		128.00
Benzyl-adenine	1.00	38 000.00	5	190.00
Paclobutrazol	2.00	1.60	5	0.008
Sucrose	-	90.00	90 g/l	90.00
Aquadest				150.00
T O T A L		118 021.52		1 467.272

Table 6.5. Cost of 1 liter of *in vitro* tuberization medium induced by benzyl-adenine and coumarin with liquid-liquid system

Stock Solution	Concentration (g/l)	Price (Rp)	Volume Used per liter Media (ml)	Price per liter Media (Rp)
I. Inorganic salt				
A. NH_4NO_3	82.50	2 858.63	20	57.17
B. KNO_3	95.00	7 847.00	20	156.94
C. H_3BO_3	1.24	43.40		0.22
KH_2PO_4	34.00	2 380.00		11.90
KI	0.166	25.73	5	0.13
Na_2MoO_4	0.05	22.75		0.11
$CoCl_2.6H_2O$	0.005	4.88		0.03
D. $CaCl_2$	88.00	3 185.60	5	15.93
E. $MgSO_4.7H_2O$	74.00	3 515.00		17.58
$MnSO_4.4H_2O$	4.46	842.94	5	4.22
$ZnSO_4.$ $CuSO_4.5H_2O$	1.72	287.59		1.44
F. $FeSO_4.7H_2O$	0.005	0.47	5	0.002
Na_2EDTA	5.57	540.29		2.70
II. Vitamines and growth regulators				
G. Thiamin-HCl	0.02	27.64		0.14
Nic. Acid	0.10	35.60		0.19
Pyridoxin-HCl	0.10	820.00	5	4.10
Glycine	0.40	94.00		0.47
Myo-inositol	20.00	25 600.00		128.00
Benzyl-adenine	1.00	38 000.00	5	190.00
Coumarin	5.00	901.00	5	4.51
Sucrose	-	90.00	90 g/l	90.00
Aquadest				150.00
T O T A L		118 021.52		1 467.272

Table 6.6. Cost of media through different phases of *in vitro* tuberization for 1 year (360 000 microtubers)

Medium	Price per liter medium (Rp)	Total price (Rp)
I. Multiplication of explants	1361,312	81 678,72(60 ltrs)
II. *In vitro* shoots	1 111,312	44 452,80 (40 ltrs)
III. *In vitro* tuberization		
a. Cycocel	1 467,272	234 763,52 (160 ltrs)
b. Paclobutrazol	740,280	118 444,80 (160 ltrs)
c. Coumarin	835,782	139 645,12 (160 ltrs)

Table 6.7. Electric cost for producing *in vitro* microtubers in a year (360 000 microtubers in 100 000 culture vials)

Instruments	Watt	Hours	Total KWH	Price (Rp)
I. Preparation of explants (6 000 vials)				
1. Kitchen				
a. autoclave (5)	5 000	24	120	10 320,00
b. oven (2)	3 000	30	90	7 740,00
c. dishwasher (1)	500	240	120	10 320,00
2. Culture Room				
a. air condition (1)	1 500	182	273	23 478,00
b. laminar air flow (5)	2 500	182	455	39 130,00
II. *In vitro* shoot and *in vitro* tubers (36 000 vials)				
1. Kitchen				
a. autoclave (5)	5 000	144	720	61 920,00
b. oven (2)	3 000	180	540	46 440,00
c. dishwasher (1)	500	1 440	70	6 192,00
2. Culture Room				
In vitro shoot				
a. air condition (1)	1 500	1 215,55	1 823,3	156 803,80
b. laminar air flow (5)	2 500	1 215,55	3 038,9	261 345,40
In vitro tuber				
a. air condition (1)	1 500	1 215,55	1 823,3	156 803,80
b. laminar air flow (5)	2 500	1 215,55	3 038,9	261 345,40
III. Incubation Room (Light)				
Lamp (230)	9 200	11 months 16 hrs/day	48 576	4 177 536,00
Air condition (4)	6 000	11 months	47 520	4 086 720,00
IV. Incubation Room (Dark)				
Air condition (2)	3 000	10 months	21 600	1 857 600,00
T O T A L	30 700		129 810,40	11 163 694,40

Table 6.8. Ammount of working hours and cost to produce 360 000 microtubers

Activities	Total Hours	Cost (Rp)
I. Explants multiplication		
1. Cleaning and washing	240	60 000,00
2. Drying (oven)	30	7 500,00
3. Medium preparation	300	75 000,00
4. Autoclave	24	6 000,00
5. Subculture	182	45 000,00
II. *In vitro* shoot and *in vitro* tubers		
1. Cleaning and washing	1 440	360 000,00
2. Drying (oven)	180	45 000,00
3. Medium preparation (shoot)	1 800	450 000,00
4. Medium preparation (microtuber)	1 000	250 000,00
5. Autoclave	144	36 000,00
6. Subculture	1 039,50	259 875,00
7. Medium filling	1 039,50	259 875,00
III. Harvest	1 039,50	259 875,00
T O T A L	8 458,50	2 114 125,00

Table 6.9. Recapitulation of cost of production of 360 000 microtubers in one year

Activities	Cost (Rp)	Percentage of Total Cost (%)
I. LABOR		
a. Preparation	1 289 500,00	9.52
b. Subculture	565 250,00	4.17
c. Others	259 875,00	1.92
SUBTOTAL I	2 114 625,00	15.61
II. ELECTRICITY		
a. Air condition	6 281 409,00	46.38
b. Lamp	4 177 536,00	30.84
c. Laminar air flow	561 816,00	4.15
d. Autoclave	72 240,00	0.53
e. Others	70 693,00	0.52
SUBTOTAL II	11 165 694,00	82.43
III. CHEMICALS		
a. Inorganic salt	69 776,00	0.51
b. Plant growth regulator	75 678,00	0.56
c. Others	120 320,00	0.89
SUBTOTAL III	265 774,00	1.96
T O T A L	13 544 093,00	100.00

VII. IMPACT OF THE PROJECT ON RESEARCH EDUCATION AND DEVELOPMENT OF BIOTECHNOLOGY IN INDONESIA

The principal constrain to research and higher education in Indonesia is lack of research resources. In the past, much of research needs and student interest could not be attended. However, since 1987, through the PSTC research grant, the quality and quantity of research improved significantly. Purchase of additional essential equipment, chemicals, and hiring of higher quality technician are made possible through the project. Different tempeature culture room s has olso been set up. The out put of the project could be summarized as follow (Fig 7.1.)

The project contributed a lab that could accomodate an average of 10 student every year. During the research period, 20 students with specials interest bacheler guided research in micro propagation of potato have been produced. The chemicals and lab facilities also shared with other students doing research in tissue culture. A total of 50 students have finished their studies, with their bachelor guided research in tissue culture.

A part from the qraduate, our lab has already develop rapid and low cost microtuber production technique and produced virus free planting material from 6 commercially important cultivars. The micro propagation techniques and the graduates are absorbed by private agribussiness companies to initiate large scale production of planting material. As for an axemple: four of the 50 student that have taken thier guided research in potato micropropagation have adapted some of the technique (in vitro shoot propagation) and have developed their own private potato micropropagation laboratory to produced high quality seed tubers for the farmers. Their micro propagation company is named 'Mustika Nusantara Abadi' located at Tugu about 40 km South of Bogor. The ultimate goal is to help the farmers in obtaining higher production and higher income as well.
Beside the direct output, our mission is that helping other universities outside Java to establish their lab and of improving their quality of researchs. The lab fasilities and activities became a model for other universities. Since 1987 we provide training and short course to staff member of other universities. The number of staf and university of origin are summerized in table 1.

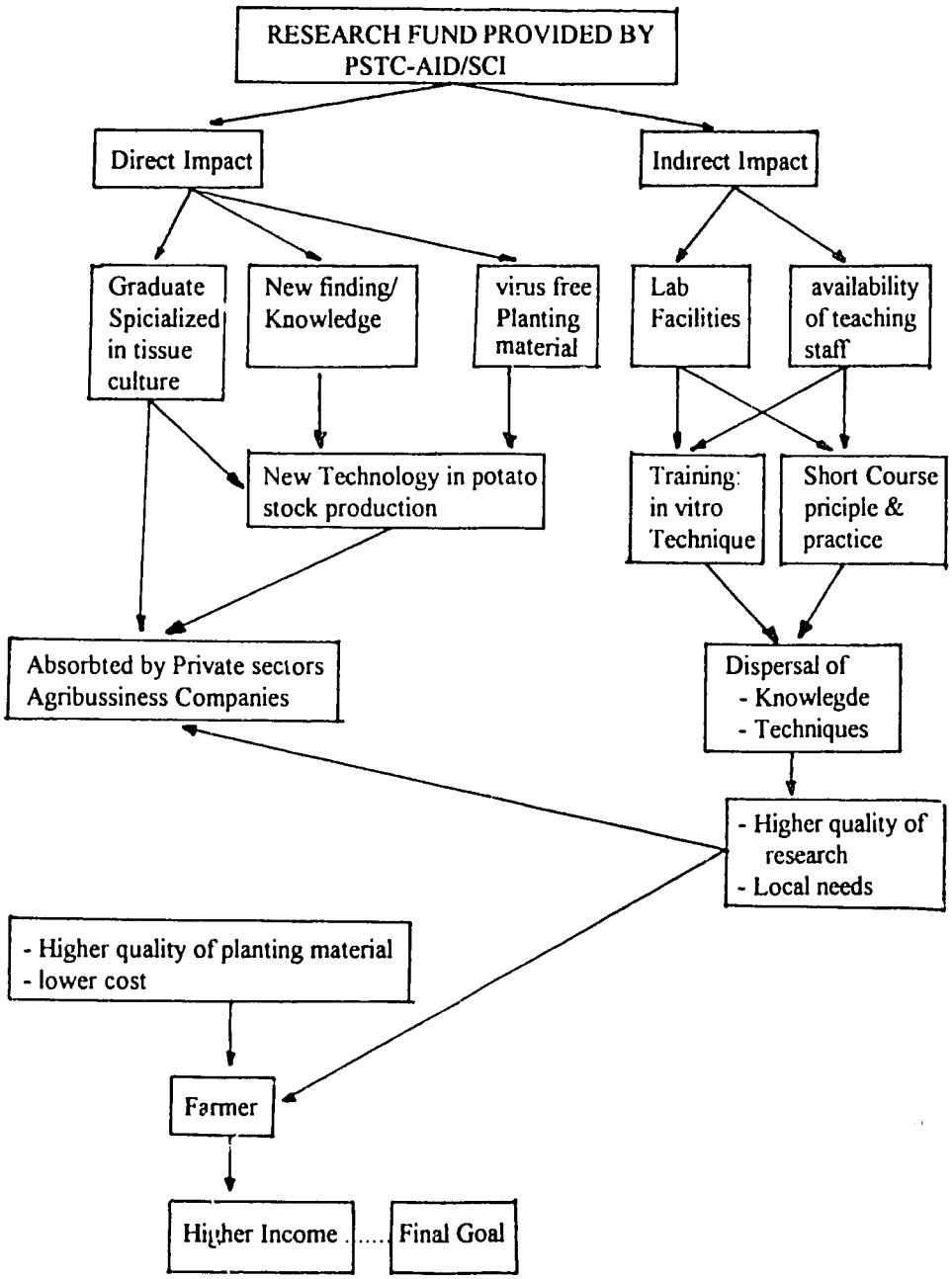

Fig 7.1. The impact of PSTC-AID/SCI research and project on plant biotechnology development in Indonesia

Table 7.1. Training and Short Course Activities during 1987 - 1989

Year	Type of Training	During	Number of Staff	University
-1987	Short Course in Tissue Culture	3 months	2	Universitas Pattimura
Feb. 1988	Short Course (intensive) Plant Biotechnology	2 months	12	Universitas Hassanuddin (2) Universitas Haluoleo (1) Universitas Mulawarman (1) Universitas Tadulako (2) Universitas Palangkaraya (1) Universitas Andalas (2) IPB (2)
June 1988	Short Course (intensive) Cooperated with WUAE-Project University of Kentucky Topics: Tissue Culture Technique	1 month	15	Universitas Lampung (2) Universitas Sriwijaya (2) Universitas Andalas (2) Universitas Jambi (2) Universitas Sumatra Utara (2) Universitas Tanjung Pura (2) Universitas Syah Kuala (2)
January 1989	Training in Lab Procedure: in vitro technique	3 months	3	IPB (1) IKIP Manado (1) IKIP Manado (1)
April 1989	Training in Lab Procedure in vitro Technique	3 months	2	Universitas Pattimura (2)
September 1989	Training in lab procedure in vitro technique	3 months	2	Universitas Sebelas Maret (1) IKIP Manado (1)

By providing and intensive short course in Biotechnology to teaching staffs of various University in Indonesia, we hope to disperse the the knowlegde to other part of Indonesia. In turn they can utilize the techniques to solve their local problems.

This lab have been visited by many people. People from private company, high school student, high school teacher, university student, scientist from Indonesia as well as from abroad. The foreign scientist that have visited the lab is listed by countries in table.

In september 1989 two of the student namely Fatimah and Yasfianti have taken part in "National Competation of Student Research" by combining their quided research (producing of microtubers and field testing of microtubers) and have won the trophy for student research in agriculture.

VIII. CONCLUSIONS

1. *In vitro* Shoot

The good and low cost medium of *in vitro* shoot will be shallow layer liquid medium of MS, sucrose 3%, and Ca-pantothenate 6 mg/l. It is faster and economical to use single node cutting of *in vitro* shoot as potato micro-propagule than *in vitro* shoot induced by cytokinins.

2. *In vitro* Tuberization

The best method of *in vitro* tuberization is liquid-liquid system of tuberization. Four weeks in shallow layer liquid medium of shoot culture followed by adding shallow layer ofliquid *in vitro* tuberization medium, and kept in dark for eight weeks. The recommended *in vitro* tuberization medium are : MS medium, sucrose 9%, cytokinins (BA 5.0 mg/l or coconut water 15%) and retardants (cycocel 600 mg/l or coumarin 25 mg/l).

With this recommended method, the price of a microtuber was 0.02 cents (US $). The percentage distribution of the production for labor, chemicals and electricity was 15.6%, 2.0% and 82.4% consecutively. The distribution of 82.4% of the electricity cost was 42.4% for air conditioning and 36.0% for running autoclaves, ovens, transfer boxes and lighting in the culture room.

3. Field performance of microtubers

Sprouted microtubers of one centimeters or longer can be field planted direct as sprouted microtubers or indirect as seedlings. Sprouted microtubers can be used as potato propagules for producing seed tubers but not for producing ware potatoes. The tuber yield of microtuber plants were still lower (30 - 40%) than the tuber yield of seed potato tuber plants.

4. Laboratories Facilities

The research grant contributed a tissue culture laboratory, important equipments and chemicals that can accomodate the researchs of students and staffs, training and short courses in plant tissue culture for students and staffs.

5. Research of Students

A total of 20 students specialized in potato micropropagation for their bachelors thesis have been graduated during the research project. Two of these students (Miss Fatimah and Miss Yasfianti) combining their research (producing micro-tubers and field tested microtubers) for taking part in "National Competition of Students Research" and have won the trophy for students research in Agriculture.

6. Training Staff of Other State Universities

Since 1987 to 1989, this potato tissue culture laboratory have trained 36 staff members from 18 state universities from different parts of Indonesia.