In vitro potato microtuber production

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Award date:
1993

Awarding institution:
University of Bath

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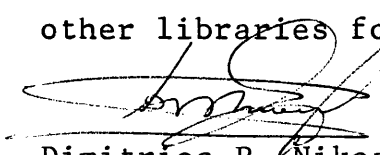
submitted by Dimitrios P. Nikopoulos

for the degree of Ph. D

of the University of Bath

1993



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ABSTRACT

This study was directed at finding a single and reliable *in vitro* tuberization procedure for use in the seed potato industry and at the same time at the theoretical approaching of the microtuberization by the investigation of factors as cytokinin, sucrose, light intensity, photoperiod, pH of the medium fluidity of medium e.t.c. affecting it and other factors concerning the use of microtubers for elite seed potato production.

Two first early, one second early and seven maincrop cultivars were used for tuberization. Single nodes excised from *in vitro* microplants were used as inoculum, and for all the tested cultivars there was found at least one liquid or semi-solid MS - based medium which gave a productivity of approximately one microtuber per one explant.

Sucrose at concentrations between 4 and 10% (w/v) were used in combination with BAP at concentrations between 0 and 4 $mg \cdot l^{-1}$ with semi-solid media and 0 and 0.5 $mg \cdot l^{-1}$ with liquid media.

For microplant production the cultures were incubated at 22 ± 1°C with a 16h photoperiod and 140 $\mu M \cdot m^{-2} \cdot sec^{-1}$ PAR irradiance and for tuberization the cultures were incubated at 22 ± 1°C with an 8h photoperiod and 11.5 $\mu M \cdot m^{-2} \cdot sec^{-1}$ PAR irradiance.

Investigating the effect of BAP, sucrose and level of irradiance on microtuberization, a triple interaction among them was found with regard to microtuber productivity , mean fresh weight and percentage of elongated microtubers. At both tested levels of irradiance (11.5 and 140 $\mu M \cdot m^{-2} \cdot sec^{-1}$ PAR), at least one medium was identified which gave 100% productivity, the mean fresh weight with this medium for cv

Kennebec did not differ significantly between these two levels of irradiance but for cv Spunta the microtubers were significantly smaller at the higher level of irradiance; the proportion of abnormally elongated microtubers increased as the light level and the sucrose concentration increased and where the BAP concentration was higher than a particular level which depended on the cultivar.

Kinetin and zeatin tested for microtuberization on MS semi-solid media showed similar promotive effects to BAP but zeatin riboside found to be less effective.

Mannitol tested as sucrose substitute for microtuberization, inhibited both explant growth and tuberization even in the media which were supplemented with sucrose at concentrations suitable for plantlet growth (3% w/v) or tuberization (6% w/v).

Photoperiods of 8 or 16h and initial medium pH between 5 and 8 did not produce any significant differences concerning either productivity or size of microtubers.

The position of the node on the mother plantlet did not affect microtuber productivity and differences concerning size and quality of microtubers although significant, were not so large as to cause problems concerning microtuber quality.

Nodes excised from plantlets grown on MS semi-solid media with 3% (w/v) sucrose for periods ranging between 15 and 50 days, did not show any significant difference concerning microtuber productivity and size of microtubers; the plantlets of 30 and 50 days age, however, gave the best results regarding the proportion of abnormal microtubers.

Nodes with and without their leaves, showed the same performance regarding microtuberization on semi-solid media suggesting that mechanical chopping of the plantlets could be employed.

Densities of explants in the tuberization culture ranging between o.6 and 1.8 per ml of semi-solid medium did not show significant differences concerning microtuber productivity and the proportion of abnormal microtubers, although the mean fresh weights decreased gradually as the planting density increased.

Liquid media and semi-solid media of high fluidity (o.3% w/v agar) compared with semi-solid media (0.7 w/v agar) when used for shoot culture, gave significantly more nodes per explant and the nodes excised from plantlets grown on liquid medium when used for microtuberization on semi-solid media, improved significantly the productivity, size and quality of microtubers.

The use of two successive MS liquid media for plantlet growth and for microtuberization, without the addition of exogenous cytokinin, gave a microtuber productivity per node of 100%, the microtubers were much more heavier than with semi-solid media and without abnormalities. This indicated that liquid media are preferable for <u>in vitro</u> tuberization as with shoot culture.

Plantgar polymer used instead of agar for shoot culture on MS medium, inhibited root formation and delayed plantlet growth.

Nodes randomly distributed on the surface of semi-solid media of high fluidity (o.3% w/v agar) for microtuberization, gave the same high productivity and the microtubers had

statistically equal or higher fresh weight depending on the cultivar, in comparison with nodes cultured on semi-solid medium (0.7% w/v agar) by the conventional method of individually inserting the end of each node in the medium, suggesting that this procedure would be preferable for routine microtuberization.

Rice-seed husks were unacceptable as an alternative support material but preliminary studies with vermiculite gave promising results.

The majority of microtubers were found to be dormant after harvest for a period of 95-110 days depending on the cultivar but dormancy could be broken by applying GA_3 solution in the tuberization medium two days before harvest, or by wounding the dormant microtubers at the stolon site before treatment with GA_3 solution. Microtubers treated with GA_3 for two days before harvest could be stored at low temperature (2 ± 1°C) for immediate use at any time after harvest. It was found that dormant microtubers could be stored at 4 ± 1°C for 20 months and at 2 ± 1°C for at least 25 months.

Comparing four alternative propagation systems (microtubers, microplants, normal tubers and "small tubers"), for elite seed-potato production, the microtubers gave a higher number of seed-grade tubers than either microplants or "small tubers" and an equal or higher number than the normal tubers.

The proportions of seed grade tubers produced by microtubers planted in compost at densities of 25,50 and 100 per m^2 were not significantly different. There was a minimum size of microtubers above of which depending on the cultivar, the

productivity of plants grown from them did not differ significantly concerning the number of seed grade tubers. The majority of microtubers when sprouted, gave one apical sprout which was found to be dominant on the emergence of the other sprouts. On excision of this apical sprout, more than one sprout was formed on each microtuber and when such treated microtubers were planted, the productivity of the plants from them was increased significantly, indicating that this treatment is necessary for optimization of the productivity when microtubers are used for seed potato production.

ACKNOWLEDGEMENTS

I gratefully acknowledge my supervisor Professor G.G. Henshaw for his guidance encouragement and helpful criticisms throughout the course of this study.

Special thanks are extended to Dr. Jane O'Hara, Dr. A. Kanakis and Dr J. Stamp for their interest and support during the laboratory experiments, and to P.K. Nandi, statistician of the School of Mathematics, for his assistance in the statistic analysis of the data. I also wish to express my deep appreciation to Chris Davey, Experimental Officer, and Peter Clark, Senior Technician of the School of Biological Sciences of the University of Bath for their assistance and cooperation during this research work and I am also pleased to recall the friendship of the rest staff and the postgraduate students of the School of Biological Sciences of the University of Bath.

D.P. NIKOPOULOS

LIST OF ABBREVIATIONS

BAP 6 - Benzyl aminopurine

CCC 2 - Chloroethyl trimethyl ammonium chloride

CIP International Potato Centre

cm Centimetre

Cu Copper

cv Cultivar

EDTA Ethylene - dinitrilo - tetra - acetic - acid

F Ferrum

GA3 Gibberellic acid

h Hour(s)

K Potassium

Kg Kilogram

l litre

m metre

Mg Magnesium

mg Milligram(s)

$mg.l^{-1}$ Milligram per litre

ml Millilitre(s)

MOS Miliosmols

Mo Molybdenium

NAA 1 - Naphthalene acetic acid

N Nitrogen

PAR Photosynthetically active radiation

P Phosphorus

sec Second

sp Species

subsp Subspecies

v/v Volume/volume

w/v Weight/volume

Zn Zinc

µM Micromolar

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Plates III and IV Microtuber production for first early cv Maris Bard, on MS semi-solid media supplemented with factorial combinations of the following concentrations of BAP and Sucrose:

BAP 0, 0.5, 2, 4 ($mg \cdot l^{-1}$)

Sucrose: 4, 6, 9 (% w/v)

A: media without BAP (0 - 4, 0 - 6, 0 - 9)

B: media supplemented with 0.5 $mg \cdot l^{-1}$ BAP (0.5-4, 0.5-6, 0.5 - 9)

C: media supplemented with 2 $mg \cdot l^{-1}$ BAP (2 - 4, 2 - 6, 2 - 9)

D: media supplemented with 4 $mg \cdot l^{-1}$ BAP (4 - 4, 4 - 6, 4 - 9)

Culture conditions: 22±1°C, 8h photoperiod, 11.5 $\mu M \cdot m^{-2} \cdot sec^{-1}$ PAR irradiance.

Plate V Microtubers of cv Spunta produced on MS semi-solid media supplemented with Kinetin and sucrose in factorial combinations of the following concentrations

Kinetin: 0.5, 2, 4 ($mg \cdot l^{-1}$)

Sucrose: 4, 6, 9 (%w/v)

A: media with 0.5 $mg \cdot l^{-1}$ Kinetin (0.5-4, 0.5-6, 0.5-9)

B: media with 2 $mg \cdot l^{-1}$ Kinetin (2 - 4, 2 - 6, 2 - 9)

C: media with 4 $mg \cdot l^{-1}$ Kinetin
(4 - 4, 4 - 6, 4 - 9)
Culture conditions: 22±1°C, 8h photoperiod, 11.5 $\mu M \cdot m^{-2} \cdot sec^{-1}$ PAR irradiance.

Plate VI

Microtubers of cvs Spunta and Kennebec produced on MS semi-solid media supplemented with zeatin and sucrose in factorial combinations of the following concentrations:
zeatin: 0.05, 0.5, 2, 4 $mg \cdot l^{-1}$
sucrose: 6, 9% (w/v) for cv Spunta and 4, 6, 9% (w/v) for cv Kennebec
Z: zeatin ($mg \cdot l^{-1}$)
S: sucrose (% w/v)
Culture conditions: 22 ± 1°C, 8 h photoperiod, 11.5 $\mu M \cdot m^{-2} \cdot sec^{-1}$ PAR irradiance.

Plate VII

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Culture conditions: 22 ± 1°C, 8 h photoperiod, 11.5 $\mu M \cdot m^{-2} \cdot sec^{-1}$ PAR irradiance.

B: Microtubers of cv Majestic produced on MS semi-solid medium supplemented with 4 $mg \cdot l^{-1}$ BAP + 9% (w/v) sucrose from (1) shoot-tips and (2) nodes.
Culture conditions: 22 ± 1°C, 8h photoperiod, 11.5 $\mu M \cdot m^{-2} \cdot sec^{-1}$ PAR irradiance.

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cvs: D.Y: Duke of York
S: Spunta
D: Desirree
B: Bintje
J: Jaerla
K: Kennebec

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B colour: sprouted microtuber with only one apical sprout before planting (cv Spunta).

A B/W : yield of plants grown on compost in the greenhouse from microtubers of cv Spunta and Kennebec treated as above

B B/W : yield of plants grown on compost in the greenhouse from microtubers of cv Spunta and Kennebec untreated.

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GENERAL INTRODUCTION

1.1 BIOLOGY AND BIOSYSTEMATICS, ECOLOGY USES AND BREEDING OF POTATO

1.1.1 Biology and Biosystematics of Potato

The cultivated potato is a grown as an annual but it is actually a perennial plant which produces underground tubers formed from stolons. Some of the cultivated varieties, form flowers, others not, and some of the flowering varieties produce berries. The failure to set fruit, depends on reasons ranging from pollen sterility to competition for assimilate among fruits and other organs, especially tubers (White and Sadik 1983). Botanically the potato belongs to the family SOLANACEAE-genus Solanum and has the following species:

A. Diploids 2n=24

(i) S. ajanhuiri (ii) S. goniocalyx

(iii) S. phureja (iv) S. stenotomum (Juz et Buk)

B. Triploids 2n=36

(i) S. x chaucha (Juz et Buk)

(ii) S. x jureperukii (Buk)

C. Tetraploids 2n=48

(i) S. tuberosum L. with two subspecies

ia S. tuberosum subsp tuberosum

ib S. tuberosum subsp andigena (Juz et Buk)

D. Pentaploids 2n=60

(i) S. x curtilobum (Juz et Buk)

In addition to above species, C. Ochoa (1984), described a new potato species Solanum hygrothermicum, which is cultivated by native inhabitants of the warm humid lowlands of Peru. This species belongs to the series tuberosa (2n=48 chromosomes), and it is the only potato cultivated under the climatic regime of the warm rainforest in the Peruvian Amazon basin where it is native.

1.1.2. Ecology

The wild species are found in a very wide range of habitats and over a wider latitude range than the cultivated species, since they are known to occur from the United States (Colorado, Utah etc), southwards through Mexico and Central America, as far south as latitude 45°S. In both sub-continents, they are briefly plants of medium to high altitudes, although in South America, they are found in the coastal areas of Peru and Chile, as well as in the plains of Argentina, Paraguay, Uruguay and Southern Brazil.

All historical and archeological evidence, points to the fact that cultivated potatoes were once entirely confined to the high Andes of South America and the coastal strip of central to southern Chile.

The common cultivated potato, is raised now in most parts of the world, although in the tropics it needs to be grown in the winter season and even so, cannot be considered to be well adapted to these conditions. In fact, cultivated potatoes are generally best adapted to the cool temperate zones of the high altitudes in the Andes (2.000 - 3.500m), at sea levels in temperate regions of North America, Europe, southern Chile and Argentina, and at appropriate altitudes in intermediate latitudes

1.1.3. Uses of Potato

The potato is cultivated for its tubers which are used for :

(i) human consumption (approximately one half)

(ii) stock feed (one third)

(iii) starch industry etc

Among the major crops worldwide, the potato ranks fourth after wheat, rice and maize in terms of total production (see figure No 1), and among the major crops of the Europe, it ranks second after wheat (see figure No2).

Figure 1

World production for the four most important crops 1991
(FAO Bulletin of statistics V5 - 1992)

1.	WHEAT	:	550.993.000	M.T.
2.	RICE PADY	:	519.869.000	M.T.
3.	MAIZE	:	478.775.000	M.T.
4.	POTATO	:	261.162.000	M.T.

Figure 2

Production for the three most important crops in Europe - 1991
(FAO Bulletin of Statistics V5 - 1992)

1.	WHEAT	:	132.407.000	M.T.
2.	POTATO	:	85.054.000	M.T.
3.	BARLEY	:	72.280.000	M.T.

1.1.4. Breeding of Potato

In potato there are two alternative methods of reproduction :

(i) the sexual method which is particularly common in wild species and less so in the cultivated ones;

(ii) the asexual method which is by tubers.

The majority of wild and cultivated diploid potato species can cross without difficulty and the resulting F1's are fertile and vigorous. With almost all the diploid species, outbreeding is obligatory, through the functioning of genetically controlled self-incompability mechanism.

In most tetraploids, and all known hexaploids, self-pollination is possible but insect pollinators seem to be needed to transfer pollen (White 1983). The "gene pool" of potatoes is undoubtedly large, which is of considerable advantage for those who wish to transfer characters or blocks of genes from one species to another, but from another point of view the potato is a difficult crop for breeders because of the poor flowering and low fertility of many cultivars and because of the high degree of heterozygosity and the low heritability of many important characters (Broertjes and Van Harten 1978). Somatic mutations occur in vegetatively propagated potatoes with a frequency of about one plant per $2\text{-}5\times10^{5}$ (Heiken 1958) and the plants are frequently chimerical in structure (Howard 1970). Studies on the variation between plants derived from adventitious shoots produced by tissue culture techniques from various tissues such as leaf, rachis, petiole, stem pieces and protoplast cultures have been reported (Behnke 1979, Shepard et al 1980, Van Harten et al 1981, Thomas et al 1982, Austin and Cassells 1983, Webb et al 1983, Karp et

al 1984, Wheeler et al 1985). From these studies we can conclude that there is a possibility that plants derived from tissue culture procedures might be used as a source of variants ("Somaclonal variants") for commercial purposes.

Currently, however, the production of new potato varieties nearly always, involves sexual reproduction. Breeding objectives include: the number of tubers and tuber size, disease resistance, suitability of the tubers for a particular manufacturing process, appearance and edibility of the cooked tubers, tuber shape and depth of eyes, flesh colour, earlyness of the harvest etc (Howard 1978).

1.2. PROPAGATION OF POTATO

1.2.1. Tubers as propagation material

The cultivated potato is generally propagated asexually with the tubers being used as propagation material. Potato tubers have the properties of the whole plant and they can be transferred, stored and planted quite easily. After the harvest, the tubers are dormant for 3-5 months depending on the variety. In many countries, especially in the Third World, farmers use a part of their crop as propagation material for the next crop over a number of successive years, but they usually encounter severe problems of declining yield and poor quality, because of

the accumulation of tuber-borne pathogens, particularly viruses, during these successive generations. In the developed countries this problem is overcome by the creation of a seed potato industry, which provides certified "seed potato tubers" for the farmers. In this industry a stock of virus-tested material is maintained on a long-term basis for the production of the "certified seed potato tubers". A severe disadvantage of propagation with tubers, however, is the high cost of this certified propagation material; to plant one hectare of field, approximately 2.5 tones of seed tubers of optimum size are required and the seed tubers can be stored usually only from one growing season to the next and not longer even under optimum storage conditions (Burton 1982).

1.2.2. Propagation by cuttings

The potato plant can also be propagated by stem cuttings or leaf bud cuttings. These cuttings can be taken from plants grown in pots under specific conditions, which protect them from disease infections, or from sprouts produced by tubers. These cuttings can produce either whole plants or small tubers according to the conditions. Propagation by cuttings is used in the seed potato industry at the stage of multiplication of the pathogen tested stock material. This method can also be used by

farmers for their ware potato production and as an efficient method for the rapid increase of selected stocks during the early stages of developing a new cultivar (Lauer and Florian 1977, Bryan 1982, Rowberry and Coffin 1983).

1.2.3. True seed as propagation material

In developing countries, the lack of high quality, inexpensive planting material (traditionally seed tubers) is the main factor limiting potato production. Use of locally-produced seed tubers, infected by tuber-born diseases, results in significant yield reduction. Importation of seed tubers from zones relatively free of diseases, can provide a solution to the problem but the increase in production costs is very high. The International Potato Centre has, therefore, attempted to find a technique for the developing countries which could provide propagation material of good quality as an alternative to the tuber (White and Sadik 1983).

It is suggested that the true seed shows promise either for direct ware potato production or as a source of disease-free material for seed-tuber production; the potential problem of increased genetic variability can probably be overcome by selection of suitable parental material. True seed has the advantage for propagation of low cost, the suitability for long-term storage (Barker

and Johnston 1980) and reduced pathogen transmission (Jones 1982), but there are still some problems including the increased genetic variability, the failure of the cultivars to flower or set fruit, the poorer vigour, the difficulties in the establishment in the field and the slower growth of the seedlings and the lower yield compared with plantlets from tubers (Accatino 1980), Sadik 1983, Burton 1989). Martin (1983) suggested that, despite these problems, for tropical and less developed countries, the production of potatoes from a handful (25g per ha) of true seed which is inexpensive, disease-free, easy to produce, transport, store and plant, is certainly to be preferred to the use of two to three tonnes of tuber-seed which are expensive, a carrier of serious diseases, and difficult or impossible to produce, transport, store and plant.

1.3 TUBERIZATION OF POTATO

In potato plants tubers develop from the sub-apical region of stolons which are lateral shoots with spirally arranged scale leaves, elongated internodes, and a hooked tip. Tuber formation is regarded as the sum of two separate processes: stolon formation and tuberization of the stolon tip.

1.3.1. Stolon formation

Even in S. tuberosum subsp. andigena, which forms tubers only under short-day conditions, stolons do not have a comparable requirement and they will develop under long day conditions (Booth 1959). Other environmental factors, however, do have some importance; for example, moist, dark conditions favour stolon development (Clark 1921) but only in the presence of a dominant apex (Kumar and Wareing 1972, Woolley and Wareing 1972b). Generally a lateral bud can develop, depending on the conditions, either as a stolon or as a leafy shoot and further, a developing stolon can be converted into a leafy shoot (Kumar and Wareing 1972). Application of GA to the stems of intact plants, resulted in the development of aerial stolons in the apical regions (Kumar and Wareing 1972b). According to Woolley and Wareing (1972b), in the case of lateral buds which are not subjected to apical dominance, a high ratio of cytokinin to gibberellin favours the development of leafy shoots, and a low ratio, favours stolon development. Also according to Woolley and Wareing (1972c), plants of Solanum tuberosum subsp. andigena, grown under low light conditions, formed aerial stolons and these could be induced in high light conditions by application of GA.

The induction of aerial stolons in decapitated shoots of Solanum tuberosum subsp. andigena by application of IAA

and GA, occurs to a greater extent when plants are grown under short day conditions: in long day plants, a high percentage of induced aerial stolons, became upright when the IAA-GA mixture was replaced by plain lanolin, but no stolons became converted to leafy shoots in plants grown under short-day conditions. Levels of endogenous cytokinins were lower in short-day plants (Woolley and Wareing 1972c) and according to Cutter (1978), the observations concerning endogenous hormones are compatible with the view that the control of lateral shoot development depends on the balance between cytokinins and gibberellins.

1.3.2. Tuberization

Tubers are usually formed from the basal stolons of a plant but under special conditions (for example very high carbohydrate status of the plant) they may develop from nearly every vegetative bud irrespective of its location on the plant (Werner 1954). As Artschwager (1924) pointed out, the potato tuber is a modified stem with a shortened (and broadened) axis and eyes with rather poorly developed leaves. Tuber formation is affected by various conditions; for example, tuber formation occurs earlier at low temperatures (Mes and Menge 1954, Slater 1963-68, Ivins and Bremmer 1964) and it is delayed at high temperatures (Slater 1963). Gregory (1956) using cv

Kennebec, has found that tuber yield was good if plants were grown in short days with low night temperature, but no tubers were formed in short-days with high night temperature. Low temperature treatment may be effective in inducing tuber formation and, for example, even in young rapidly growing plants, tubers can be induced to develop by maintaining the plants for 7 days at 7°C or less (Burt 1964). Independently of temperature, there is a quantitative effect of photoperiod on tuber formation and plants grown in short days form tubers earlier than those kept in long days (Madec and Parennec 1959, Slater 1963, Ivins and Bremmer 1964). In S. tuberosum subsp. andigena short photoperiods are an absolute requirement for tuber formation (Booth 1959). Kummar and Wareing (1973) showed that a stimulus to tuber formation is formed under short-day conditions and, according to Slater (1968) and Hammes and Nel (1975), tuber formation may be regulated by the relative activities of several substances rather than the absolute concentration of a single substance.

Palmer and Smith (1969a and b) showed that excised stolons of S. tuberosum subsp. tuberosum grown in vitro, formed tubers if supplied with a cytokinin and kinetin was the most effective of those tested, inducing 80-100% tuberization.

A certain period of continuous treatment was required and kinetin induced tuber formation could be completely

inhibited by a temperature of 35°C (Palmer and Smith 1969b, 1970). Experiments with excised nodal segments of induced and non induced potato plants, cultured under aseptic conditions, showed that the presence of kinetin in the culture medium could substitute for the stimulatory effect of induction on tuber formation in the axillary buds (Forsline and Langille 1976). The results of these experiments suggest that a natural cytokinin could be the tuber forming stimulus.

There is some evidence that ethylene also, may play a role in tuber formation; when sprouts of cv Arran Pilot bearing several stolons were treated with gaseous ethylene, extension growth was inhibited and swelling of all rapidly growing regions occurred. Sub-apical swellings on stolon were similar morphologically to normal tubers, but contained no starch (Catchpole and Hillman 1969). Ethrel (a substance releasing ethylene) led to an increase in tuber formation when applied in the soil (Garcia-Torres and Gomez-Campo 1972). In cultured stem segments, also, ethrel enhanced tuber formation and counteracted the effects of applied GA (Garcia-Torres and Gomez-Campo 1973). Thus ethylene may play some role in the process of stolon and tuber formation.

A number of workers have concluded that sucrose is required for tuber formation (Gregory 1956, Madec and Parennec 1959, Madec 1963). In cultured stem segments in media, tubers were formed in the presence of 8% but not

2% sucrose (Harmey et al 1966). In other experiments with cultured stolons, however, tubers did not develop in the presence of sucrose unless kinetin was also supplied and for the induction of tuber formation by kinetin, 6, 8 or 10% sucrose was required, 2 or 4% being inadequate (Palmer and Smith 1970). Tuber formation seems to be associated with a high concentration of soluble sugars in the stolon tip and with conditions which lead to this (Slater 1963, 1968 and Burt 1964). In cultured stolons treated with kinetin, the level of reducing sugars decreased, as compared with controls; this coincided with the rapid synthesis of starch in cultures with kinetin (Palmer and Barker 1973). Palmer and Smith (1969a) considered that cytokinins may act by mobilizing metabolites to the site of the tuber formation and in 1970 they suggested that kinetin may exert its stimulatory effects on tuber formation by promoting the activity of starch synthetase and suppressing that of starch hydrolase, and that gibberellic acid may have the converse effect on these enzymes, thus inhibiting tuber formation.

Mares et al (1985) investigating the sugar metabolism in developing tubers have found that during tuberization, Sucrose is translocated from the leaves to the developing stolon tips and then this is converted to starch once the tubers are established.

Morell and Rees (1986) also investigating the sugar metabolism in developing tubers have found that sucrose was the dominant sugar among the three (sucrose-glucose-fructose), that the sugar concentration in potato tubers unlike that of starch, does not increase throughout the period of development, and that all three sugars per g fresh weight declined as the tubers grew larger.Batutis and Ewing (1981) supported the hypothesis that phytochrome is involved in tuberization of Solanum tuberosum subsp. andigena. Ewing (1981) considered that the tuberization stimulus favours both tuber initiation and tuber enlargement and that it is possible, if a balance or ratio of compounds controls tuberization, then short photoperiods might increase the concentration of one group of compounds, whereas cool temperatures might produce similar morphological effects through decreases in a counterbalancing group of compounds. Menzel (1981) considered that buds are major sites of gibberellin synthesis in the potato and that high temperatures, stimulate the synthesis of gibberellin, and his conclusion that tuberization is controlled by gibberellin levels, is strongly supported by the responses of the potato plant to exogenous GA. Menzel (1985), working on the interaction between temperature and irradiance, reported that potato cv Sebago responded similarly to high temperatures and low irradiance by diverting dry matter to shoots rather than to the tubers, and changes

were noted in a range of morphological characteristics. He proposed that the effect of both high temperature and low irradiance is brought about by the increased production of a growth substance, possibly gibberellin, which inhibits tuber formation, and that tuber yield is determined by the balance between temperature and irradiance and also that long photoperiod has the same influence.

Sattelmacher and Marschner (1979) working with water cultures of potato plants with continuous nitrogen supply via the roots, found that interruption of the nitrogen supply induces tuberization.

Studies have shown also that the growth retardant 2 chloro-ethyltrimethyl ammonium chloride (CCC) which inhibits the synthesis of gibberellins in plants (Kumar and Wareing 1974), can promote tuberization in potato grown under non-inductive conditions of photoperiod (Hammes 1971, Hammes and Nel 1975), temperature (Menzel 1981) and nitrogen supply (Krauss and Marschner 1976). It has been proposed (Hammes and Nel 1975, Krauss 1978) that these factors exert their control over tuberization, through their effect on the levels of endogenous phytohormones, especially gibberellins and inhibitors. According to Menzel (1983), the tuber production may be controlled by at least three factors : (a) a promoter produced by the buds at cool temperatures, (b) an inhibitor derived from the buds but dependent on warm

temperatures for its formation, and (c) a second inhibitor derived from the mature leaves and produced in response to warm temperatures. Pereira and Valio (1984) have noticed in their experiments that GA3 applied to stem cuttings induced by 30 short days, inhibited the photoperiodic effect and orthotropic shoots without tubers were formed. Also, the GA3, caused a reduction in the starch content of induced buds, lowering it to the same level as that found in long-day treated plants, but α-amylase activity of buds of induced plants was not affected by the GA3, suggesting that GA3 does not inhibit tuberization by promotion of starch hydrolysis.

It has been suggested that cytokinin glycosides are bound or storage forms of the active compounds (Parker and Letham 1973) or that they could be a form in which cytokinins are translocated through the phloem of plants (Van Staden and Drimalla 1977). Van Staden and Drimalla (1977) working with small tubers formed on tubers stored for long period at low temperature in the dark, have found that zeatin riboside is the major cytokinin throughout the whole tuber system during the tuberization of such stored tubers.

The zeatin reboside was present in the highest levels at the stolon tips and in the smallest induced tubers; that is, in those regions where a high rate of cell division and metabolism could be expected to occur. The presence of this cytokinin at such high levels where tuberization

takes place, could mean that it was actively involved in the tuberization process. According to Van Staden and Drimalla (1977), if zeatin glucoside represents a storage form, then a decrease in its level would be expected to result in an increase in the free zeatin pool but zeatin levels in the stolon tips and induced potatoes remained fairly constant; the overall distribution pattern might therefore be explained by the hypothesis that zeatin glycoside is hydrolised followed by a rapid conversion of the zeatin released to zeatin riboside which was found to be the dominant cytokinin detected in stolon tips where the tuberization process begins.

Koda(1982) working on the nature of the water-soluble cytokinin in potato tubers and the changes in the levels of both butanol-soluble and water-soluble cytokinins during their life cycle, found also that the level of butanol-soluble cytokinin in elongating stolon tips was low while that of water-soluble cytokinin was extremely high; the main cytokinin detected in the water-soluble fraction had the same chromatographic behaviour and susceptibility to enzymes as zeatin riboside, and upon swelling of the stolon tips, the butanol-soluble cytokinins increased greatly as the water-soluble decreased. Koda, therefore, concluded that the increased butanol-soluble cytokinins, play an important role as cell division promoting factors, which are responsible

for the subsequent vigorous thickening growth of the stolons to form tubers and that the water-soluble cytokinin is a temporary storage cytokinin.

Maux and Langille (1978), using high pressure liquid chromatography, the cucumber cotyledon bioassay and mass spectometry, isolated from potato plant tissues (cv Katadhin) a cytokinin which has been identified as cis-zeatin riboside; its levels in induced plants, were significantly higher than in non-induced plants and its highest level was noted in below-ground tissue after four days exposure to inducing conditions with tuber initiation observed after eight days. A companion study, conducted to determine the effect of zeatin riboside on in vitro tuberization of non-induced nodes revealed that, after one month in culture, controls exhibited 0% tuberization while zeatin riboside treatments of 0.3 and 3.0 $mg \cdot l^{-1}$ showed 39 and 75% tuberization respectively.

Jameson et al (1985) working on changes in cytokinins during initiation and development of potato tubers, found that the soluble sugars did increase as stolons were induced and during the late stage of tuberization, the concentration of soluble sugar dropped markedly and the starch concentration rose. Also they found that non-induced stolon tips and tubers up to 7.5mm in diameter, contained a low level of compounds which respectively had elution volumes similar to those for zeatin, zeatin-riboside, isopentenyladenine and isopentenyladenosine.

Increases in cytokinin like activity, occurred subsequent to tuber formation which indicated that the tuberization stimulus is unlikely to be cytokinin-like in nature.

1.4 IN VITRO TUBERIZATION OF POTATO

The conventional potato propagation techniques are convenient but the seed-tubers that are used must be free of disease and so it is necessary for them to be produced under conditions which assure good quality. In addition to this problem, the conventional way of propagation requires a long term production program lasting 5-8 years, to allow "certified" stocks tuber to be produced from the breeder's material. The possibility of producing high quality certified seed-tubers at an acceptable cost, depends mainly on the following factors:

(i) The maintenance of a disease free "nuclear stock" protected from contaminations for long term use.

(ii) The use of techniques which assure a very rapid multiplication rate of this disease-free material for the production of the required amount of "basic" and "certified" seed tubers at the appropriate times.

In vitro techniques have given useful alternative solutions to the above requirements for a well organized modern seed potato industry, based on the following general scheme:

(i) disease elimination through thermotherapy and shoot meristern culture

(ii) establishment of a facility for the long term maintenance of the necessary disease-free

nuclear stock under *in vitro* conditions

(iii) rapid micropropagation of the material according to the requirements of the production program

(iv) hardening of the micropropagated plantlets and transplantation to disease-controlled conditions for "elite" seed-tuber production

The most demanding stage in the above procedure, however, occcurs after the rapid multiplication of the disease-free material, when a large number of plantlets must be transplanted to the field or to an insect-proof glasshouse at a particular time. The establishment of these plants is successful only under favourable conditions including high relative humidity, favourable temperature and light, and moreover there is a high labour demand. It is obvious therefore, that it would be particularly valuable to the seed potato industry if an alternative type of disease-free planting material could be produced independently of the planting season and then stored, transported and planted more easily than the *in vitro* plantlets. Such an alternative material could be the *in vitro* microtubers, which have all the characteristics of variety and the properties of a normal tuber; in addition they have the advantages of material produced under *in vitro* conditions and as they are not bulky, they are easy to store and transport. According to Bajaj (1987) this is likely to revolutionise, the seed potato industry.

Since 1970, interesting studies of in vitro tuberization have been carried out by a number of workers. Palmer and Smith (1970) and Palmer and Barker (1973) studied the tuberization process working with stolons maintained under in vitro conditions.

Wang and Hu (1982) in their work on in vitro mass tuberization and virus-free seed potato production in Taiwan examined the following factors for their effect on in vitro tuberization:

BAP and sucrose concentrations in the culture medium, temperature, daylength and light intensity during incubation. Instead of the single-node cuttings technique used by other workers, they used an in vitro "layering" procedure, which was carried out repeatedly at twenty-day intervals, to produce large quantities of shoots which were then transferred to MS liquid medium. Large Erlenmeyer flasks (500 ml) were used for in vitro mass tuberization under low light conditions during the incubation period (100 Lux). The above technique was used for the large scale production of microtubers which after three successive plantings, gave 1800 kg of seed-potato tubers, for use by farmers. It was the first reported use of microtubers for commercial purposes.

Koda and Okazawa (1983) examined the influence of environmental, hormonal and nutritional factors on in vitro tuberization and concluded that a reduction of the GA level in the tissue is a prerequisite for tuberization

and that cytokinin and ABA are not involved in tuber initiation, although they may stimulate tuber growth. Hussey and Stacey (1981, 1984) used as starting material nodal cuttings taken from cultures grown on MS medium for tuberization, and the MS medium was supplemented with myo-inositol, vitamins and a mixture of aminocompounds based on analysis of potato tubers. The following factors were examined for their effect on tuberization: daylength, cytokinins, sucrose concentration, gibberellin, CCC, ABA, ethylene. They arrived at the following conclusions:

(i) Short days have some promotive effect on tuberization.

(ii) The most efficient treatment promoting stolons formation, followed by tuberization, was the addition of a cytokinin to the medium particularly under short-day conditions.

(iii) The addition of GA3 consistently inhibited tuberization, while CCC enhanced the process and reinforced the promoting effect of cytokinin.

(iv) Abscisic acid under certain conditions could stimulate tuberization but generally it inhibited both growth and tuberization.

(v) Restriction of gaseous-exchange between the culture vessel and the external atmosphere, inhibited tuberization an effect that appeared to be due mainly to ethylene accumulation.

(vi) Higher sucrose levels, favoured tuberization in

short-days but not in continuous light and the promotive effect of BAP was enhanced in both day-lengths by higher sucrose levels.

(vii) Stolon development and tuber formation were both encouraged by a high ratio of cytokinin to gibberellin which is opposite to the results from the experiments of Wareing and his co-workers with Solanum tuberosum subsp. andigena when with lateral buds not subject to apical dominance (including single isolated nodes), the development of leafy shoots was favoured by a high ratio of cytokinin to gibberellin, while stolon development was encouraged by a low ratio (Woolley and Wareing 1972).

The micro tubers produced by Hussey and Stacey had a mean size of 60-120mg and their efforts to obtain microtubers from cultures in liquid media, gave larger tubers of up to 200mg but they had distorted shapes and tended to develop leafy shoots precociously.

Wattimena (1984) in his investigations on micropropagation as an alternative technology for potato production in Indonesia, reported in vitro tuberization in 4-week cultures in continuous dark, using growth regulators (kinetin, ancymidol, coumarin) and 6% (W/V) sucrose.

Tovar et al (1985) using MS medium supplemented with CCC (500ppm), BAP (5ppm) and 8% Sucrose on 2-3 weeks old

microplantlets in a three-stage procedure involving tuberization in the dark, were successful with ten cultivars but the dormancy period of the microtubers was very long (210 days) instead of 60 days when the microtubers were formed under 8h day.

Estrada et al (1986) subsequently used this procedure succesfully with a range of 50 genotypes but the problem of the prolonged dormancy of the microtubers still existed.

These authors have induced in vitro tuberization on MS liquid medium containing CCC in combination with an increased concentration of sucrose and BAP. Hammes and Nel (1975) have concluded that the ability of CCC to stimulate tuber formation is due to its anti-gibberellin action.

Recently Harvey et al (1991) testing the effect of the growth retardants CCC, ancymidol, daminozide and paclobutazol on the tuberization process in vitro, found that ancymidol and paclobutrazol stimulated tuber initiation at concentrations which did not inhibit subsequent microtuber growth in the tested cultivars; daminozide strongly reduced microtuber fresh weight and the CCC included in media to stimulate tuber initiation by recalcitrant genotypes could have deleterious effects on microtuber growth.

The influence of temperature on in vitro tuberization was examined by Kwiatwowski et al (1988). They found that

microcultures of three clonal potato cultivars and two wild species kept at low temperatures (4° C and 10° C) produced microtubers after 21 months in storage without subculture or supplementation of the media.

Garner and Blake (1989) have reported microtuber production by shoot cultures of two potato cultivars grown on MS medium without the addition of growth regulators.

They found that 8% (w/v) sucrose in comparison with 4 or 12% advanced the initiation of tuberization and gave more and large microtubers ; an incubation period of one month with 16h days, followed by transfer to 8h days, gave most rapid microtuber development and a reduction in the total nitrogen supply or an increase in the ratio of ammonium to nitrate reduced the size and number of microtubers.

Slimmon et al (1989) also reported that short days (8h) are beneficial for tuberization in vitro.

In a recent work Koda et al (1991) investigating the tuber inducing properties of jasmonic acid, a naturally occurring substance in potato leaves, found that this compound and its methyl ester (methyl-jasmonate) showed strong tuber inducing activity.

Paet and Zamora (1991) report an improved in vitro tuberization protocol using MS medium with normal or half the normal concentration of salts supplemented with BAP, coumarin, coconut water and high sucrose concentration.

All authors who have investigated microtuber production,

have reported that the microtubers, when planted in soil under favourable growth conditions after the dormancy period gave normal potato plants.

Wang et al (1991) have reported that in China during the period 1988-1991 six million microtubers produced in a factory have been sold to farmers as prebasic seed.

Wattimena et al (1983) examined the growth and the yield of potato plants of two cultivars, produced from three different propagation sources: tubers, shoot cultures and in vitro microtubers. They found that although there were no differences in total yield among plants produced by the three propagation methods, the number of tubers per plant for the micropropagated plants (from both microcuttings and microtubers) was greater than for the tuber-produced plants.

Tovar et al (1985) testing the seed tuber yield from in vitro tubers planted in beds at a density of 100 microtubers per m2 have found that the yield for the four tested cultivars ranged between 195 and 648 tubers per m2 bed. Also, testing the influence of the size of microtubers on the yield by planting microtubers of three sizes (3, 3-7, 7mm in diameter) at the same density, they found good yield for all sizes but smaller microtubers gave poorer yields.

Wiersema et al (1987) working with microtubers of three sizes (0.63, 1.25, 2.50g) and in vitro plantlets planted in nursery beds at densities of 24 and 48 plants per m2

have found that the tuber number and tuber weight were higher at 48 plants per m2, in vitro plantlets produced significantly more but smaller tubers than did plants grown from microtubers and that plants grown from the larger microtubers produced a similar number of tubers but larger ones than did plants grown from small microtubers.

Alsaton et al (1988) reported also that the weight of seed microtubers show a positive correlation with the yield obtained from them.

Although microtubers provide a useful alternative to in vitro produced plantlets, as a basic material for propagation, the higher cost of their production compared with the cost of plantlet production is a disadvantage associated with potato microtubers (Dodds, 1988).

1.5 AIM OF THE RESEARCH

The main objectives of this research were to test the followings:

(i) The reliability of the *in vitro* tuberization method by testing it on a number of potato cultivars with varying degrees of earliness with various combination of BAP and sucrose concentrations in the medium and various light conditions, and the establishment of general procedures for application with any cultivar.

(ii) The influence on the *in vitro* tuberization process of cytokinins other than the more commonly used BAP including kinetin, zeatin and zeatin riboside.

(iii) The role of sucrose and the photoperiod in the *in vitro* tuberization procedure.

(iv) The influence of other possible factors which might be beneficial in the development of a large scale *in vitro* tuberization system for commercial purposes; such factors could concern any part of the procedure from the plantlet production to tuberization and they are concerned with lower cost materials, the better quality microtubers, improved yields of microtubers and reduced duration of the culture period. The mechanization of the procedure and especially the mechanical chopping of

the plantlets and distribution of the material in medium will be of great practical importance in the future so it was necessary to investigate problems concerning the performance of the explants when they were just dropped onto the media and the behaviour of the non-homogenous products of the mechanical chopping which include nodes with a leaf, nodes without a leaf, internodes, petioles, leaves, roots.

(v) The measurement of the dormancy of the microtubers and the investigation of possible reliable methods of the breaking of the dormancy.

(vi) The investigation of the optimum conditions for long-term storage of the microtubers.

(vii) The main factors affecting the use of the micro-tubers in the seed potato industry for better yield of Elite seed tubers and a comparison of this yield with those of other alternative materials such as conventional tubers, microplants or small tubers produced on common tubers in storage.

The Laboratory experiments were carried out at Bath University - England during the years 1984, 1985, except for the experiments concerning the storage of microtubers , the pH of the tuberization media and the use of the rice husks as support material which were carried out during 1986 - 87 at the Institute of Olive, Fruit and Vegetables of Kalamata, Greece. The field trials carried out in the greenhouses and the fields of the latter Institute and at the seed potato region of Taygetos mountain - Kalamata, Greece during the years 1986 - 1991.

2. MATERIALS AND METHODS

2.1. Plant material for *in vitro* tuberization

The following ten cultivars of *Solanum tuberosum* subsp. *tuberosum* were examined :

(i) Maincrop cultivars : Majestic, Record, Pentland, Ivory, Pentland Squire, Desiree, Kennebec, Bintje.

(ii) Second early cultivar : Spunta

(iii) First early cultivars : Maris Bard, Duke of York.

In vitro microplants were available in the laboratory for five of the maincrop cultivars (Majestic, Record, Pentland Ivory, Pentland Squire, Desiree). Cultures of these cultivars were maintained at 18-20° C temperature, 100-140 $\mu M.m^{-2}$ sec^{-1} PAR and 16h photoperiod in semi-solid MS medium (0.7% w/v agar).

For the remaining cultivars, virus-tested tubers were provided by the Department of Agriculture and Fisheries for Scotland Edinburgh.

2.2 Establishment and maintenance of stock cultures *in vitro*

The following procedure was used :

(i) The virus-tested tubers, after storage at room temperature (15-18°C) for their dormancy period, were washed well with distilled water and kept

for sprouting in darkness (in paper bags) at room temperature

(ii) When their sprouts had reached the length of 6-7 cm (3-4 nodes), a number of sprouts were taken for surface sterilization by 6-10% Domestos solution (0.5-0.8% w/v available chlorine) for 10-15 minutes; the exact concentrations of chlorine and the duration of Domestos application, were varied according to the cultivar and the vigour of the sprouts. Each tuber provided at least 50 nodes in 3-5 successive harvests over a period of six months.

(iii) The sprouts were washed 4-5 times with sterile distilled water.

(iv) In a laminar -flow cabinet, single nodes were excised and transferred to MS3 or MS4 semi-solid (0.7% agar) or liquid medium under aseptic conditions.

(v) These cultures were maintained at 22±1°C temperature, 16h daylength and 100-140$\mu M.m^{-2}.sec^{-1}$ irradiance, and after 20-25 days, the explants had produced new well-rooted plantlets with 4-6 nodes, ready for subculture.

(vi) Successive subcultures at 20-30 day intervals by the "single node" method, produced the necessary material for the experiments.

In some cases, when the sprouts of the tubers had

formed small roots which is common with sprouts more than 40 days old, the growth of the plantlets from the first passage after sterilization, was very rapid, giving 3-4 well-developed nodes in 10 days.

(vii) The maintenance of the material *in vitro* was succeeded by cultures on MS3 or MS4 semi-solid medium in vented disposal 9cm petri dishes at $22 \pm 1^{o}C$ temperature for short-term use and on MS semi-solid medium (0.7% w/v agar) with 9%(w/v) sucrose in 1l Twyford autoclavable containers at $16^{o}C$ for long-term storage (5-6 months)

Each 9cm plate contained 20ml of the medium for ten explants, and each Twyford container contained 140-150 ml medium for 50 plantlets.

2.3 Media for *in vitro* tuberization

MS medium supplemented with sucrose and growth regulators as required, was used for all the experimental work. Mannitol was tested as a sucrose substitute in a specific experiment.

For the preparation of the media, salt mixture of MS basal medium (Flow Loboratories Ltd) without sucrose or growth regulators was used (see Appendix 1).

Distilled water was used in all cases

The pH of the media was adjusted to 5.7 before

autoclaving at 1.4 Bar and 121° C for 15 minutes.

Whenever semi-solid medium was required, 0.7% (w/v) Oxoid Number 3 agar was added to the medium before autoclaving and whenever semi-solid medium of high fluidity was required, 0.3% (w/v) agar was added. As agar substitutes, rice by-products, vermiculite or plantgar polymer in specific experiments were tested.

Whenever liquid medium was required, 3-4 drops of the surfactant Tween-80 (Polyoxethylene) was added in 1l of medium before autoclaving for better spreading of the medium into the narrow plastic containers. No negative influence of the Tween-80 on the cultures had been observed.

For the determination of the amount of mannitol which could substitute for part or all of the sucrose without changing the osmolarity of the medium, (section 5.2.1) calibration curves were made for the osmolarities of sucrose solution between 1 and 15% (w/v) and also for the osmolarities of the mannitol solutions of between 1 and 8% (w/v) from measurements with a CAMBLAB automatic osmometer and from these curves the equivalent amounts of mannitol for substituting part or all of the sucrose, as the experiment demanded, was found. The osmolarity of the MS tuberization medium was measured before the addition of the agar and all measurements concerning sucrose or mannitol solutions or MS medium with sucrose and/or mannitol, were replicated at least three times.

2.4 Containers

The following containers were used:

(i) 9cm diameter vented disposal petri dishes (Sterilin Ltd) sealed with Parafilm (American Can Co)

(ii) 14cm diameter vented disposal petri dishes (Sterilin Ltd) sealed also with Parafilm

(iii) 11 (14x10x7cm) Twyford's autoclavable containers (Twyford's Ltd)

(iv) 100ml disposable jars sealed with metallic screw-caps (Sterilin Ltd)

(v) 150ml disposable jars sealed with metallic screw-caps (Sterilin Ltd)

when disposable jars were used, the screw caps were fitted loosely to allow gaseous exchange.

2.5 Aseptic technique

All transfers were carried out under sterile conditions in a laminar-flow cabinet (Microflow, Pathfinder Ltd., Hampshire). All tools were flame-sterilised after dipping into 90% (v/v) ethanol and all work surfaces were swabbed with 70%(v/v) ethanol.
Where necessary, the plant material was surface sterilised with 6-10% Domestos (0.5-0.8%(w/v) available chlorine) and washed with sterile distilled water.

2.6 Incubation conditions

The experiments were carried out under various combinations of the following values of temperature, photoperiod, light intensity, in A. Gallenkamp and Co Ltd or Controlla Rodas Ltd, Thessaloniki growth cabinets.

Temperature : 22 ± 1° C or 16 ± 1° C

Photoperiod : 8h or 16h day

Irradiance : 1.3 - 11.5 - 20 - 140 $\mu M \cdot m^{-2} \cdot sec^{-1}$ PAR

2.7 Plant Material for the trials in the greenhouse and the field

The field trials and the trials in the greenhouse were carried out with the following plant materials of the cultivars Desirée , Spunta and Kennebec :

(i) microtubers produced in vitro
(ii) microplantlets produced in vitro
(iii) small tubers produced by normal seed tubers during long-term storage
(iv) normal seed tubers

All these materials were virus-tested.

2.8 Media for the trials in the greenhouse and the field

Whenever the trials were carried out in the field, the plant material was planted in light, well-cultivated soil which was fertilized before planting with 2.000kg.ha^{-1} of 16-20-0 NPK compound. No herbicides were used before planting or during growth.
Water demands were covered by sprinkler irrigation.
The plants were fully protected against nematodes, fungi and insects by chemical treatments and during the growing season weeds were removed by hand.
Whenever the trials were carried out in the greenhouse, in pots or on the nursery bench, a mixture of Hy-Pot compost 75% (v/v) and Perlite 25% (v/v) or Triohum-

Potground P compost, or BAS VAN BOUREN B.V (pH 5.5-6.0), supplemented with 300 $mg.l^{-1}$ N, 200 $mg.l^{-1}$ P_2O_5, 300 $mg.l^{-1}$ K_2O and 0.6$gr.l^{-1}$ Fertilon Combi micronutrients compound containing 4%(w/v) MgO, 1.5%(w/v) Mn, 1.5%(w/v) Fe, 0.5%(w/v) Cu, 0.5%(w/v) Zn, 0.3%(w/v) B and traces of Mo were used.
As in the field trials the plants in the greenhouse were fully protected against fungi and insects by chemical treatments and irrigated by sprinklers. During the growing period nitrogen fertilizer was applied to the plants 2 or 3 times.

2.9 Culture procedure during the trials in the greenhouse and in the field

With microtubers, tubers or small tubers, it was necessary that they should be out of dormancy before planting. Before planting in soil, microtubers, small tubers and microplantlets were first grown for a period in compost, for better acclimatization and synchronization of growth with the growth of the seed tubers that were also used in the trials.
In the field the plant material was planted in rows on the side of narrow furrows for better drainage, at a density of 100,000 plants. ha^{-1} (planting spacing : 20 x 50cm). When the plants had reached a height of approximately 20cm the bases of the stems were covered by soil from the bottom of the furrows (hilling) for better

stolon formation.

Growth lasted for three and a half to four months depending on the cultivar and plants were harvested, in the field or in the greenhouse, when they were fully senesced. Immediately after harvest the tubers were cleaned and measurements were made.

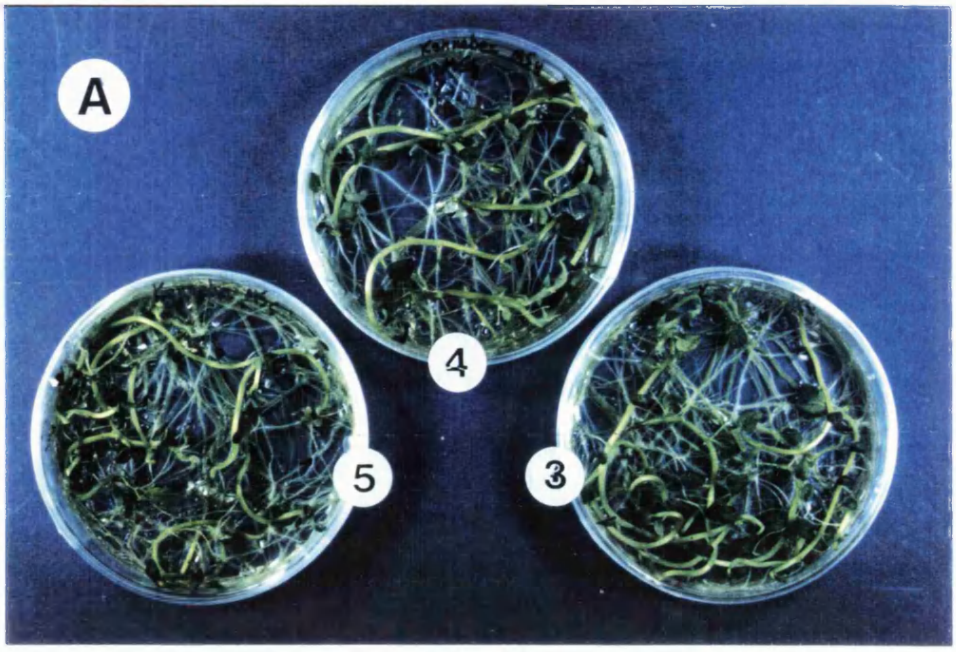
2.10 Statistical analysis of the data

The experiments with more than two treatments were analysed by the F value; when the F value showed significant difference, the evaluation of the data was made by the Lower significant difference test (Lsd) at p=.05. When the experimental data concerned two treatments, the statistical analysis was made by t value test at p=.05.

Plate I. Plantlet and Microtuber production on MS semi-solid media (cv Kennebec)

A: Plantlets cultured on MS_3 (3), MS_4 (4), or MS_5 (5) semi-solid medium for node production at 22 ± 1° C, with a 16h photoperiod and 140 $\mu M \cdot m^{-2} \cdot sec^{-1}$ PAR irradiance.

B: Microtuber production on MS semi-solid medium supplemented with 0.5 $mg \cdot l^{-1}$ BAP + 6% (w/v) sucrose at 22 ± 1° C with an 8h photoperiod and 11.5 $\mu M \cdot m^{-2} \cdot sec^{-1}$ PAR irradiance.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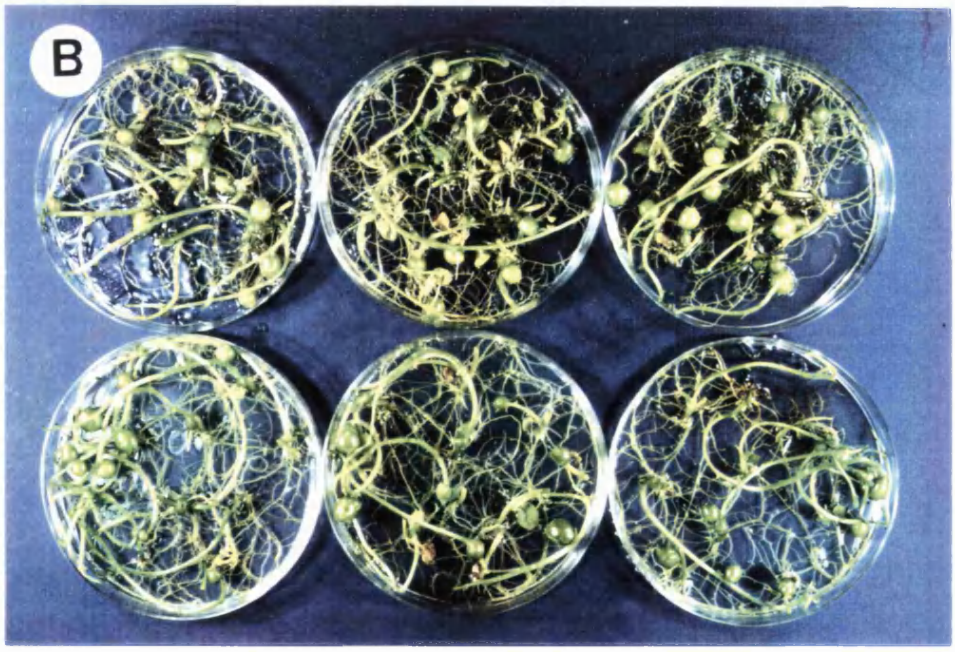

B

3. THE EFFECTS OF BAP, SUCROSE CONCENTRATION AND LIGHT ON THE IN VITRO TUBERIZATION OF POTATO

3.1 Introduction

Essentially, the work of Wang and Hu (1982) concerning mass in vitro tuberization of two potato cultivars (see Section 1.4) showed that the process is promoted by 10 $mg.l^{-1}$ BAP, 8%(w/v) sucrose and 100 Lux irradiance. Hussey and Stacey (1984) working with cultured nodes of two potato cultivars (see section 1.4) reported that $2mg.l^{-1}$ BAP, 6%(w/v) sucrose and 6.000 Lux irradiance promoted in vitro tuberization. It was not clear, however, whether the above procedures which had only been applied to a limited number of cultivars, would be applicable to a wider range. The experiments described here were carried out using nodes of nine potato cultivars (two first early, one second early, 6 maincrop) cultured in MS semi-solid medium supplemented with sucrose and BAP at various concentrations. Different levels of irradiance were also tested.

3.2 Results

3.2.1 The effect of BAP and Sucrose on *in vitro* tuberization with four maincrop cultivars, using semi-solid media and low irradiance

Four maincrop cultivars, Majestic, Pentland Ivory, Pentland Squire and Record were used in this experiment. Nodes taken from shoot cultures grown on MS_3 semi-solid medium were cultured in nine different MS semi-solid media supplemented with BAP and sucrose in factorial combinations of the following concentrations :

(i) BAP : 0, 2.0 and 4.0mg.l^{-1}

(ii) Sucrose : 6, 9 and 12%(w/v).

Forty explants for each treatment were divided evenly between four 9cm petri dishes each containing 20 ml medium.

The cultures were incubated for 90 days at 22 ± 1^{o} C temperature with an 8h photoperiod and 1.3μM.m^{-2}.sec^{-1} PAR irradiance. This irradiance is approximately equivalent to the light level (100 Lux) which had been used by Wang and Hu (1982) but they had started the experiment with whole plantlets instead of nodes.

At harvest the following measurements were made :

(i) number of microtubers produced

(ii) weight of microtubers

(iii) number of precociously sprouted microtubers

(iv) number of abnormally elongated microtubers (only

for cv Record)

(v) number of microtubers formed on adventitious shoots.

Concerning the abnormally elongated microtubers, in the media supplemented with 2 or 4 $mg.l^{-1}$ BAP and 6 or 9% (w/v) sucrose, a proportion of the microtubers (less than 6.3%) were abnormally elongated. Adventitious microtubers were not found in any of the tested cultures or media.

Figure 3a. Percentage Productivity (number of microtubers per 100 explants)

	MEDIA								
Cultivars	BAP ($mg.l^{-1}$)								
	0			2.0			4.0		
	Sucrose (% w/v)			Sucrose (% w/v)			Sucrose (% w/v)		
	6	9	12	6	9	12	6	9	12
Majestic	0	0	0	90ab	83b	63c	50d	98a	80b
Record	10	0	0	80	98	10	45	83	30
P.Ivory	0	0	0	68	78	33	60	93	38
P.Squire	0	0	0	60	98	60	68	90	80

Lsd at p=.05 for cv Majestic = 11.2

For cvs Record, Pentland Ivory, Pentland Squire no interaction was found between BAP and sucrose.

For interaction diagrams see Fig 3b.

Figures followed by the same letter are not significantly different at p=.05 (Appendix II-ia)

Culture conditions :

Basal medium : MS semi-solid

Temperature : 22 ± 1°C

Irradiance : 1.3 $\mu M.m^{-2}$ sec^{-1}

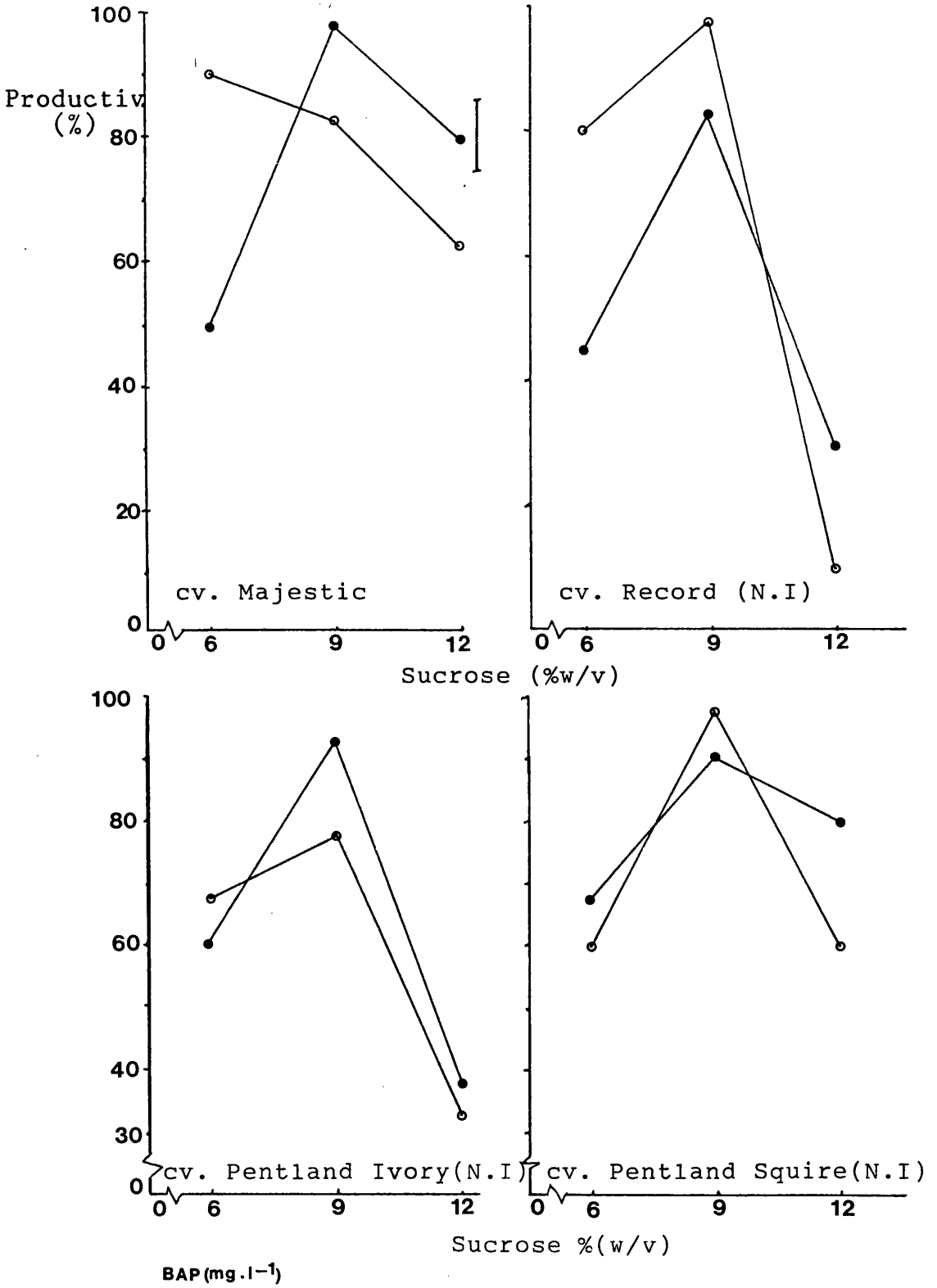
Photoperiod : 8 h

Number of replicates : 4 x 10 nodes

Volume of Medium : 20 ml per each petri dish

Statistical analysis in the appendix II-ia

Figure 3b. Interaction diagrams showing the effect on percentage microtuber productivity of node cultures, of the interactions between the BAP and sucrose supplemented to MS semi-solid medium.



Statistical analysis : appendix II-ia
Experimental details as in fig. 3a.

N.I. = No Interaction

Figure 4a. Mean fresh weight of microtubers in mg

MEDIA

Cultivars	BAP ($mg.l^{-1}$)								
	0			2.0			4.0		
	Sucrose %(w/v)			Sucrose %(w/v)			Sucrose %(w/v)		
	6	9	12	6	9	12	6	9	12
Majestic	0	0	0	19.0c	17.5cd	15.0e	15.8de	31.0a	28.5b
Record	22	0	0	23.5b	28.3a	20.3bc	21.0bc	19.5c	19.5c
P.Ivory	0	0	0	36.5b	27.5d	19.3e	32.3 c	43.0a	28.5cd
P.Squire	0	0	0	21.8d	23.0d	25.0c	28.0b	37.6a	27.5b

Experimental details as in fig 3a.

Statistical analysis in the appendix II-ib

For interaction diagrams see fig 4b.

LSD at p=.05 : (i) Majestic : 2.12

(ii) Record : 3.40

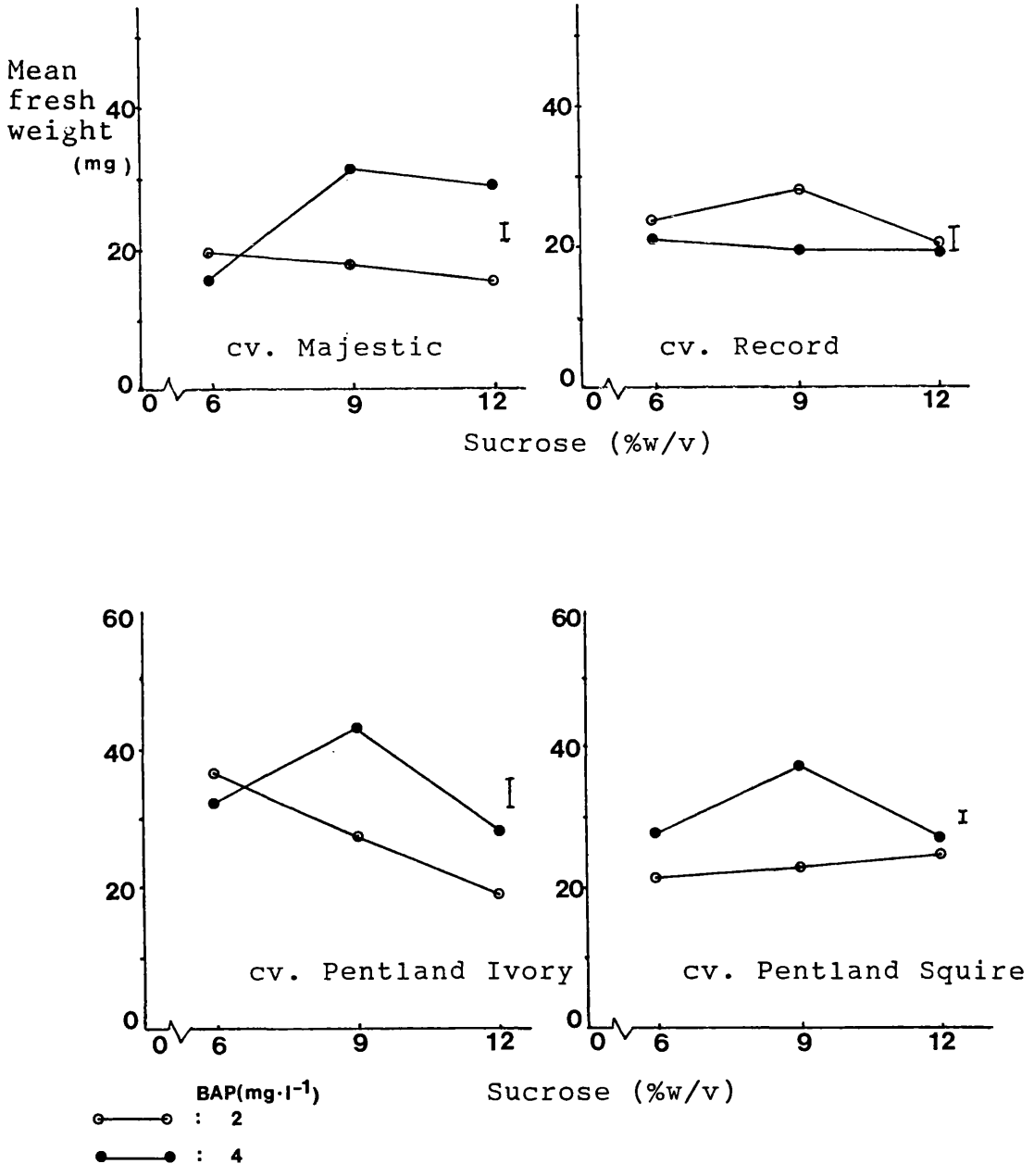
(iii) Pent. Ivory : 3.90

(iv) Pent. Squire : 1.87

Figures of the same row followed by the same letter are not significantly different at p = .05

Experimental details as for Figure 3a.

Figure 4b. Interaction diagrams showing the effect on mean fresh weight of microtubers of the interactions between the BAP and sucrose supplemented to MS semi-solid medium.



Statistical analysis : appendix II-ib
Experimental details as in fig. 3a.

Figure 5. Sprouted microtubers at harvest (%)

Cultivars	MEDIA								
	BAP ($mg.l^{-1}$)								
	0			2			4		
	Sucrose (% w/v)			Sucrose (% w/v)			Sucrose (% w/v)		
	6	9	12	6	9	12	6	9	12
Majestic	0	0	0	22	0	0	55	0	0
Record	0	0	0	6	5	0	6	9	0
P.Ivory	0	0	0	7	16	0	33	0	0
P.Squire	0	0	0	42	0	0	0	0	0

Experimental details as for figure 3a.

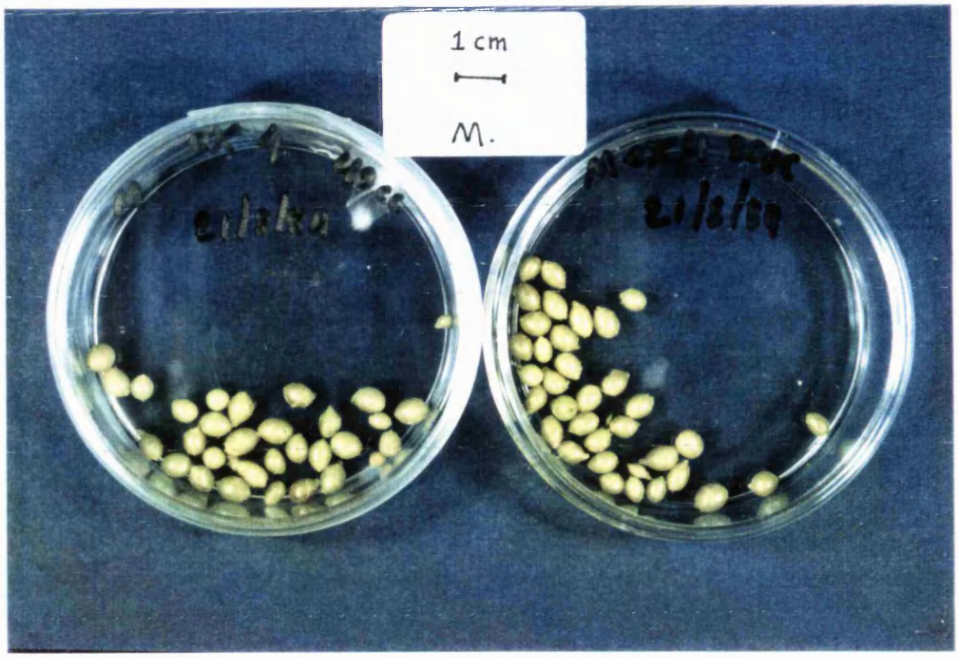
Plate II

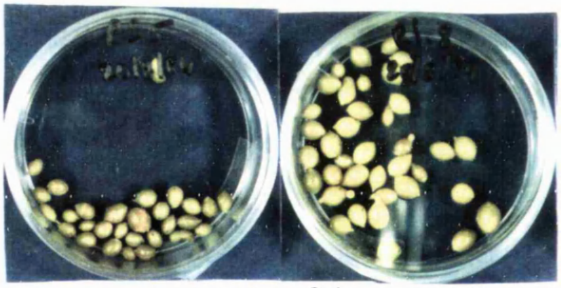
Microtubers of maincrop cvs Majestic, Pentland Squire and Pentland Ivory produced on MS semi-solid medium supplemented with 4mg·l^{-1} BAP+ 9% (w/v) sucrose at 22±1°C, with an 8h photoperiod and 1.3 µM·m^{-2}·sec^{-1} PAR irradiance.

M. : cv Majestic

P.S.: cv Pentland Squire

P.I. : cv Pentland Ivory





P.S. P.I.

1 cm

From the statistical analysis of the data and the interaction diagrams (figures 3a, 4a, 3b, 4b) and the data of the fig. 5 it can be concluded that :

(i) there is interaction between BAP and sucrose concentrations concerning microtuber productivity for cv Majestic with higher productivity in the media with 2mg.l^{-1} BAP + 6%(w/v) sucrose and 4mg.l^{-1} BAP + 9%(w/v) sucrose but there is no interaction for the remaining cvs Record, Pentland Ivory and Pentland Squire.

(ii) No significant difference was found between the effects of BAP at 2 and 4mg.l^{-1} concerning microtuber productivity for the cvs Majestic, Pentland Ivory and Pentland Squire, but for cv Record significantly higher productivity was obtained with 2mg.l^{-1} BAP than with 4mg.l^{-1}.

(iii) None of the tested cvs produced microtubers in the absence of cytokinin except for a few microtubers on medium with 6%(w/v) sucrose for the cv Record (10%).

(iv) Interaction was found between BAP and sucrose levels concerning the mean fresh weight of all tested cultivars with significantly heavier microtubers formed on the medium supplemented with 4mg.l^{-1} BAP + 9%(w/v) sucrose for the cvs Majestic, Pentland Ivory and Pentland Squire,

and on the medium supplemented with 2mg.l^{-1} BAP + 9%(w/v) sucrose for the cv Record.

(v) In terms of both productivity and mean fresh weight, the medium with 4mg.l BAP + 9%(w/v) sucrose was the best for the cvs Majestic, Pentland Ivory and Pentland Squire and the medium with 2mg.l BAP + 9%(w/v) sucrose for the cv Record.

The productivity for these specific media ranged between 93 and 98%.

(vi) Precociously sprouted microtubers were not found in the media with 12%(w/v) sucrose but this incidence was severe in the media containing lower concentrations of sucrose, particularly 6%(w/v) for cvs Majestic and Pentland Squire and 6 and 9%(w/v) for cvs Record and Pentland Ivory.

(vii) With cv Record, the proportion of the abnormally elongated microtubers never exceeded 6.3%. Data were not collected for the other cultivars.

(viii) Adventitious microtubers were not found in any of the tested cultivars or media.

3.2.2 Effect of BAP, Sucrose and Light on *in vitro* tuberization

In the previous experiment (3.2.1), in all tested cultivars, a high productivity was obtained (practically one microtuber per one node) in at least one medium, using very low irradiance, as with Wang and Hu (1982).
In this experiment two other cultivars (the second early cv Spunta and the maincrop cv Kennebec), were tested in a range of media and at the same time the effects of two levels of irradiance on microtuberization were examined.
Because of the very poor development of the plantlets of all tested cultivars in the previous experiment when 1.3 $\mu M.m^{-1}$ sec^{-2} PAR irradiance was used, the levels of 11.5 and 140 $\mu M.m^{-2}$ sec^{-1} PAR were tested in this experiment. Nodes excised from shoot cultures grown on MS3 semi-solid medium were cultured on MS semi-solid media supplemented with BAP and sucrose in factorial combinations of the following concentrations :

(i) BAP : 0, 0.5, 2.0, 4.0, 6.0 $mg.l^{-1}$

(ii) Sucrose : 6, 9, 12%(w/v)

40 replicate nodes for each medium-cultivar combination were distributed evenly between four 9cm petri dishes, each containing 20ml medium.
The cultures were incubated at 22 ± 1° C with an 8h photoperiod and the two above described levels of irradiance.

After 90 days the same measurements as in experiment 3.2.2 were made.

No adventitious microtubers were found in any of the tested media and cultivars.

The data were analysed statistically in two ways:

(i) as a factorial of 3 factors separately for each cultivar (BAP, sucrose, irradiance)

(ii) for each level of irradiance and for each cultivar separately, by Lsd at p=.05 among all tested media in order to determine which had given high productivity and high mean fresh weight.

Figure 6a. The effect of BAP, sucrose and light on microtuberization with cv Spunta

Media		Microtubers productivity(%)		Mean fresh weight(mg)		Elongated microtubers(%)	
BAP	Sucrose						
$mg.l^{-1}$	%(w/v)	Irradiance ($\mu M.m^{-2}sec^{-1}$)		Irradiance ($\mu M.m^{-2}sec^{-1}$)		Irradiance ($\mu M.m^{-2}sec^{-1}$)	
		11.5	140	11.5	140	11.5	140
0	6	50.0c	67.5e	79.0ab	94.3a	0	3.5
	9	87.5ab	80.0cd	68.5abc	70.8b	0	0
	12	7.5e	10.0h	25.0f	24.8f	0	0
0.5	6	77.5b	90.0abc	51.0cde	35.0ef	11.5	11.0
	9	32.5d	50.0fg	24.8f	36.0ef	5.0	50.0
	12	10.0e	10.0h	27.0f	30.0f	0	0
2	6	100.0a	100.0a	90.5a	54.3cd	7.5	12.5
	9	100.0a	87.5bc	53.0cd	28.5f	5.0	23.3
	12	50.0c	40.0g	25.8f	29.0f	48.8	100.0
4	6	97.5a	95.0ab	61.5bc	62.8bc	13.0	66.5
	9	100.0a	97.5a	58.0bcd	47.5de	2.5	47.5
	12	80.0b	70.0de	32.0ef	29.0f	63.0	100.0
6	6	100.0a	97.5ab	85.5a	99.8a	2.8	62.5
	9	100.0a	100.0a	63.3bc	57.5c	5.0	60.0
	12	77.5b	52.5f	39.3def	32.5f	64.0	100.0

Lsd at p=.05 for

(i) Microtuber productivity among media of low light : 13.2

(ii) Microtuber productivity among media of high light : 10.2

(iii) Mean fresh weight among media of low light : 22

(iv) Mean fresh weight among media of high light : 12.88

Figures of the same column followed by the same letter do not differ significantly. For statistical analysis details see appendix : II-ii

Cultures conditions :

Basal medium : MS semi-solid

Temperature : 22±1°C

Photoperiod : 8h

Replicates : 4 petri dishes x 10 nodes

Volume of medium : 20ml per petri dish

6b. The effect of BAP, sucrose and light on micro-tuberization with cv Kennebec

Media BAP	Sucrose	Microtuber productivity(%)		Mean fresh weight(mg)		Elongated microtuber(%)	
$mg.l^{-1}$	%(w/v)	Irradiance ($\mu M.m^{-2}sec^{-1}$) 11.5	140	Irradiance ($\mu M.m^{-2}sec^{-1}$) 11.5	140	Irradiance ($\mu M.m^{-2}sec^{-1}$) 11.5	140
	6	90.0ab	87.5ab	69.8abc	64.5b	0	0
0	9	85.0bcd	85.0b	51.3de	52.5cdef	0	0
	12	75.0de	40.0d	48.5de	57.8bce	0	0
	6	100.0a	100.0a	79.3a	80.0a	0	0
0.5	9	15.0g	5.0f	53.3de	52.0cdefg	0	0
	12	7.5g	5.0g	70.0ab	54.0bcd	0	0
	6	72.5de	67.5c	45.8def	48.3cdefg	65.5	70.0
2	9	35.5f	35.0de	33.0f	41.5fgh	18.8	70.0
	12	10.0g	5.0f	46.0def	45.0defgh	50.0	100.0
	6	97.5ab	100.0a	54.3de	49.5cdefg	12.5	35.0
4	9	85.0bcd	30.0de	39.5ef	40.0gh	3.5	41.8
	12	32.5f	2.5f	49.8de	43.0efgh	0	100.0
	6	82.5cd	60.0c	56.3cd	59.3bc	53.0	41.5
6	9	63.5e	27.5e	59.8bcd	34.3h	32.8	100.0
	12	90.0abc	67.5c	50.5abc	56.0bcd	100.0	100.0

Lsd at p=.05 for :

(i) Microtuber productivity among media of low light : 14.5

(ii) Mean fresh weight among media of low light : 15.2

(iii) Microtuber productivity among media of high light : 11.8

(iv) Mean fresh weight among media of high light : 12.11

Figures of the same column followed by the same letter do not differ significantly. For statistical analysis details see appendix II-ii

Cultures conditions as in fig. 6a.

From the above data in can be concluded that :

(i) BAP, sucrose and irradiance are three factors which affect microtuber productivity, mean fresh weight of microtubers and percentage of elongated microtubers and a triple interaction among them was found from the statistical analysis of the data concerning these three values for both tested cultivars.

(ii) Comparing separately the 15 media at low irradiance and the 15 media at high irradiance by Lsd at p=.05 it can be suggested that although the results were quite variable, there are some similar trends concerning productivity between low and high

irradiance for each cultivar. Over the range of concentrations tested, BAP did not have a pronounced effect on productivity with cv Kennebec whereas the higher concentrations (2, 4, 6mg.l^{-1}) were apparently beneficial for cv Spunta ; in these media the productivity was higher not only at the levels of 6 and 9%(w/v) sucrose but also at 12%(w/v) sucrose, at which level 0, 0.5mg.1 BAP gave very low productivity. This comparison also showed that productivity for cv Kennebec was favoured by the lower concentration of sucrose (6% w/v) for both irradiances and all levels of BAP, except at BAP 6mg.l^{-1} when 12%(w/v) sucrose also gave high productivity; for cv Spunta it was favoured by 6 or 9%(w/v).

(iii) Concerning mean fresh weight of microtubers, the general trends were similar for both levels of irradiance.

(iv) The proportion of abnormal elongated microtubers increased severely as the sucrose concentration increased and as the irradiance level increased under conditions where the BAP concentration was higher than a particular level which depended on the cultivar and on the level of irradiance; for cv Spunta this level was 2mg.l^{-1} BAP for low irradiance and 0.5mg.l^{-1} BAP for high irradiance and for cv Kennebec 6mg.l^{-1} BAP at low

irradiance and 2mg.l^{-1} at high irradiance.

(v) Comparing separately the media at low irradiance and at high irradiance, the following media gave at the same time high productivity (approximately one microtuber per one node), the highest mean fresh weight and low percentage of abnormal elongated microtubers:
a) for cv Spunta : at low irradiance 0-9, 2-6, 6-6 and at high irradiance 2-6 (mg.l^{-1} BAP and % w/v sucrose respectively) and
b) for cv Kennebec : 0 or 0.5 mg.l^{-1} BAP and 6%(w/v) sucrose. These media can be recommended for the conditions of the experiments as the best for <u>in vitro</u> tuberization of the tested cultivars.

3.2.3 *In vitro* tuberization of two first-early cultivars using semi-solid media

Considering the results of the experiment 3.2.2 carried out with Spunta and Kennebec cultivars we can conclude that an irradiance of 11,5$\mu M.m^{-2}\,sec^{-1}$ and 8h photoperiod are favourable for *in vitro* tuberization and that the acceptable higher level for sucrose is 9% (w/v) and for BAP is $4mg.l^{-1}$. For comparison in this experiment two first-early cultivars (Maris Bard and Duke of York) were tested for tuberization using a procedure where the above levels of irradiance, BAP and sucrose were applied, together with a range of other conditions.

Nodes excised from shoot cultures grown on MS3 semi-solid medium were cultured on MS semi-solid media supplemented with sucrose and BAP in factorial combinations of the following concentrations :

(i) BAP : 0, 0.5, 2, $4mg.l^{-1}$

(ii) sucrose : 4, 6, 9% (w/v)

Ten nodes were cultured in each 9cm petri dish containing 20ml medium and, as replicates, 5 petri dishes were used for cv Duke of York and 4 for cv Maris Bard. The cultures were incubated at 22±1° C temperature with an 8h photoperiod and $11.5\mu M.m^{-2}\,sec^{-1}$ PAR irradiance.

The cultures were maintained for 90 days for cv Maris Bard and 60 days for cv Duke of York (because of

contaminations).

Percentage productivity was measured for both cultivars, but mean fresh weight and abnormally elongated microtubers only for the Maris Bard because of early harvest of the Duke of York.

Figure 7a. Microtuber productivity (%) in cultures of two first-early cultivars (Maris Bard and Duke of York)

Media		Cultivars	
BAP($mg.l^{-1}$)	Sucrose(%w/v)	Maris Bard	Duke of York
0	4	100.0a	0d
	6	72.5c	12d
	9	92.5ab	68c
0.5	4	97.5ab	0d
	6	97.5ab	86ab
	9	67.5c	74bc
2.0	4	95.0ab	0d
	6	100.0a	94a
	9	87.5b	86ab
4.0	4	97.5ab	0d
	6	100a	96a
	9	97.5ab	100a

Lsd at P=.05 for :

(i) Maris Bard : 12.9

(ii) Duke of York : 16.8 (Appendix II-iiia)
The figures followed by the same letter do not differ significantly).

For interaction diagrams see Figure 7b.

Culture conditions :

Basal medium : MS semi-solid

Temperature : $20 \pm 1^{\circ}$ C

Irradiance : 11.5 $\mu M.m^{-2}sec^{-1}$ PAR

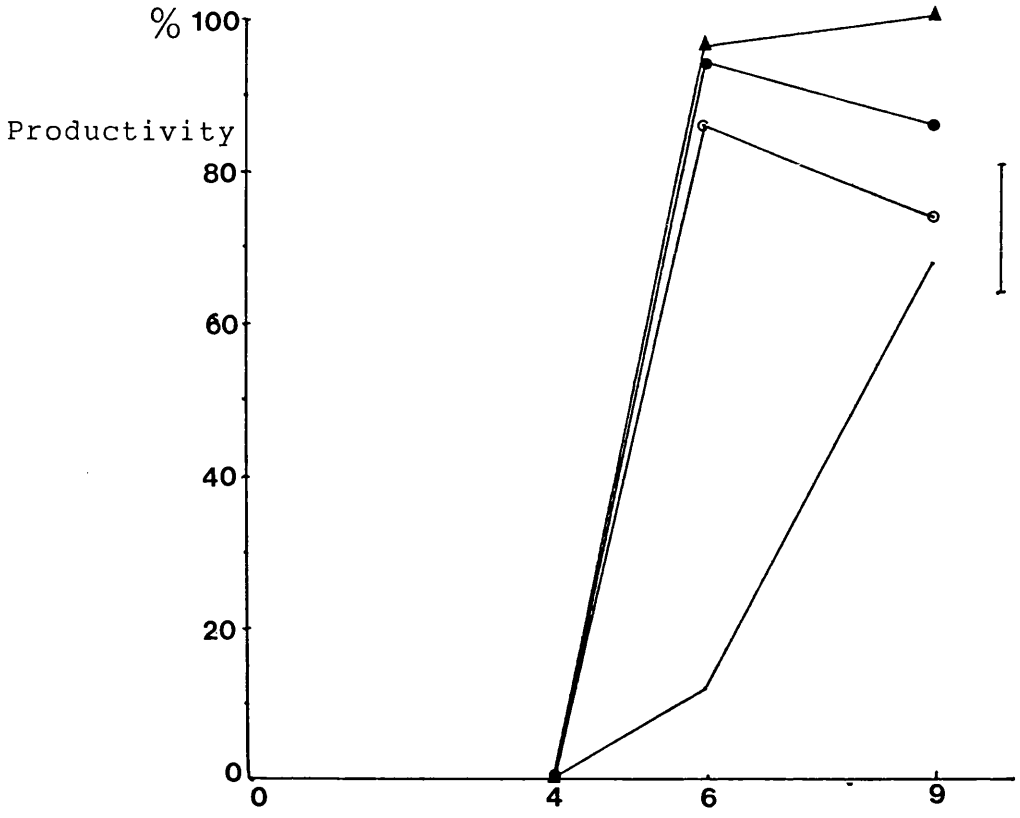
Photoperiod : 8h

Replicates : 5 petri dishes x 10 nodes for Duke of York

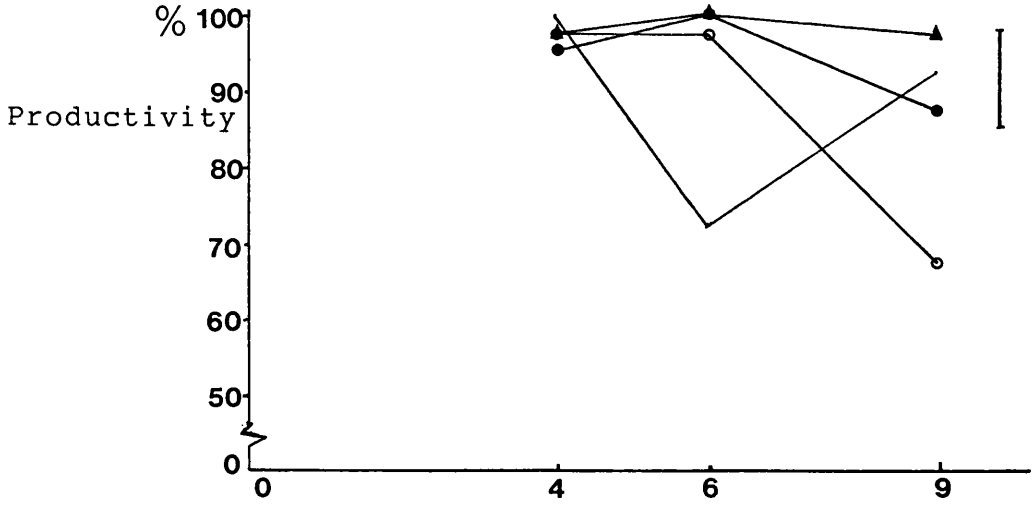
4 petri dishes x 10 nodes for Maris Bard

Volume of Medium : 20ml per each petri dish

Figure 7b. Interaction diagrams showing the effect on microtuber productivity of node cultures of two first early-cultivars, of the interactions between the BAP and sucrose supplemented to MS semi-solid medium.



cv. Duke of York Sucrose %(w/v)



cv. Maris Bard Sucrose %(w/v)

BAP(mg.l^{-1})
0
0·5
2·0
4·0

Statistical analysis : appendix II-iiia
Experimental details as in fig. 7a

Figure 8a. Mean fresh weight of microtubers (mg) and proportion of abnormal elongated microtubers (%) in cultures of cv Maris Bard

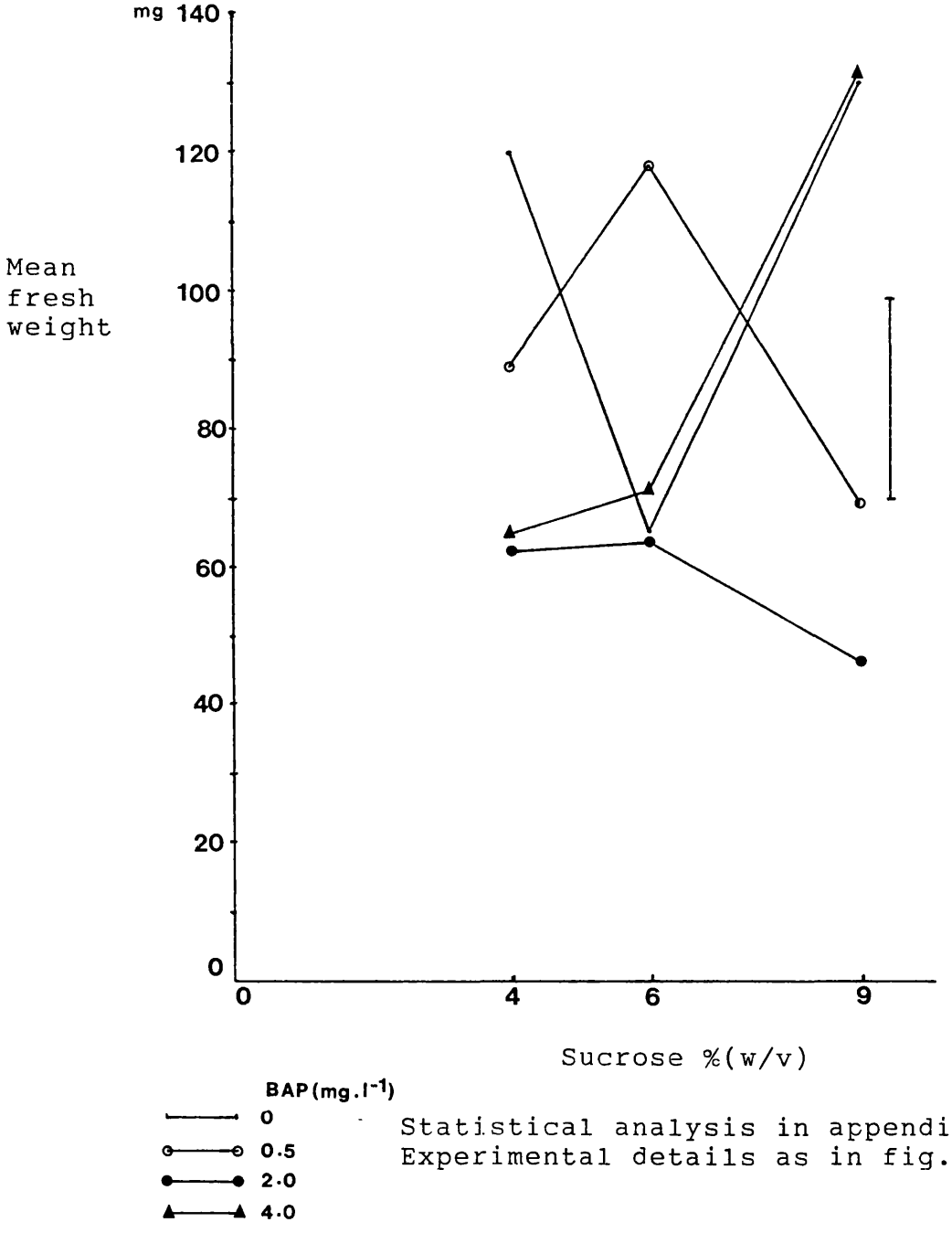
Media		Mean Fresh	Elongated
BAP ($mg.l^{-1}$)	Sucrose (%w/v)	weight (mg)	microtubers (%)
	4	119.75a	0
0	6	65.75 cd	0
	9	130.00a	0
	4	89.25bc	0
0.5	6	118.00ab	2.6
	9	69.25 cd	11.1
	4	62.50 cd	5.3
2.0	6	64.00 cd	3.3
	9	46.75d	0
	4	65.00 cd	0
4.0	6	71.25 cd	0
	9	131.25a	0

Lsd at p=.05 for mean fresh weight : 29.11

(Appendix II-iiib)

Culture conditions : as indicated for Figure 7a

Figure 8b. Interaction diagram showing the effect on mean fresh weight of microtubers of cv Maris Bard, of the interactions between the BAP and sucrose supplemented to MS semi-solid medium.



Statistical analysis in appendix II-iiib
Experimental details as in fig. 7a

The figures followed by the same letter do not differ significantly.

The precocious sprouting of microtubers before harvest, did not occur on any medium. For interaction diagram see Figure 8b.

From the above data it can be concluded that:

(i) There is interaction between BAP and sucrose, concerning microtuber productivity for both tested cultivars and on mean fresh weight of the tested cv Maris Bard.

(ii) Over the range of concentrations tested, BAP did not have a pronounced effect on productivity and fresh weight with cv Maris Bard, whereas it was beneficial with cv Duke of York with regard to productivity.

(iii) All the tested concentrations of sucrose (4,6, 9%w/v) were beneficial for high productivity for cv Maris Bard but only 6 and 9%(w/v) for cv Duke of York, which did not tuberize at 4%(w/v) sucrose, independently of the concentration of BAP.

(iv) The proportion of abnormal elongated microtubers in cv Maris Bard ranged between 0 and 11,1% but this was not a problem with any of the media.

(v) No sprouted microtubers were found in any medium.

Plates III and IV Microtuber production for first early cv Maris Bard, on MS semi-solid media supplemented with factorial combinations of the following concentrations of BAP and Sucrose:

BAP 0, 0.5, 2, 4 ($mg \cdot l^{-1}$)

Sucrose: 4, 6, 9 (% w/v)

A: media without BAP (0 - 4, 0 - 6, 0 - 9)

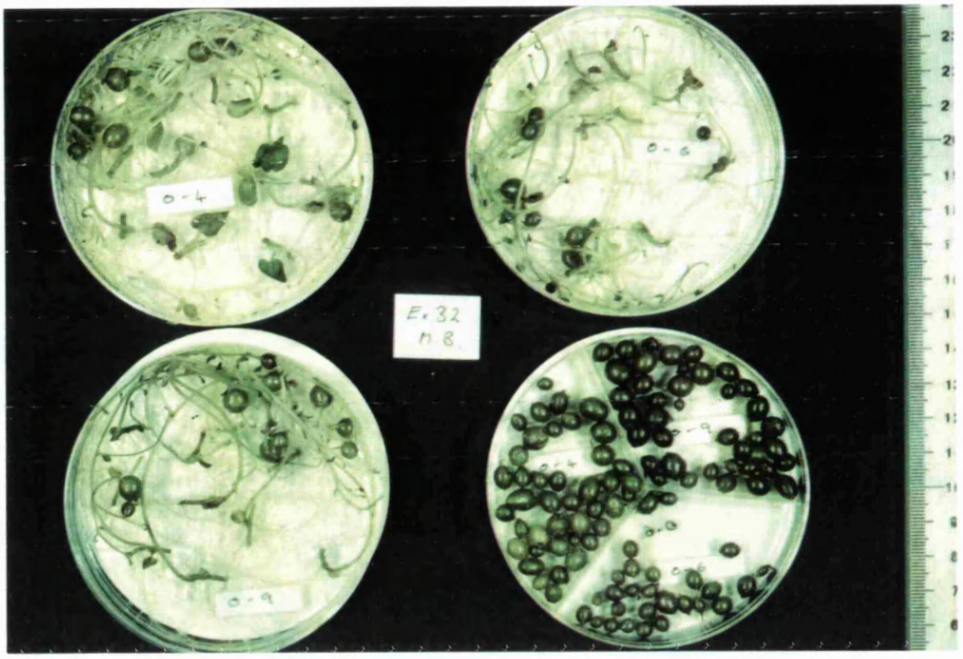
B: media supplemented with 0.5 $mg \cdot l^{-1}$ BAP (0.5-4, 0.5-6, 0.5 - 9)

C: media supplemented with 2 $mg \cdot l^{-1}$ BAP (2 - 4, 2 - 6, 2 - 9)

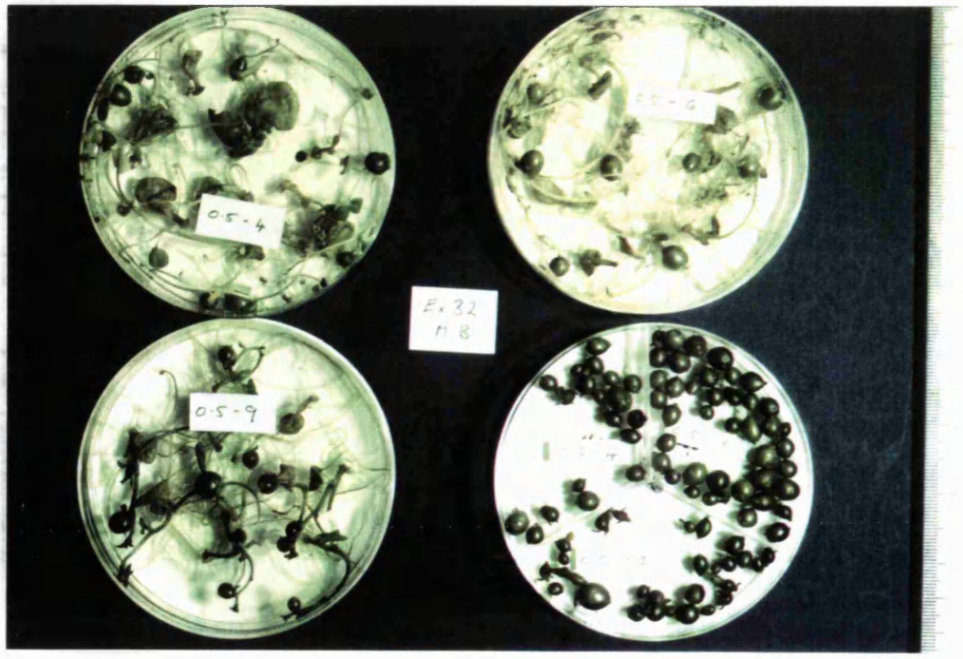
D: media supplemented with 4 $mg \cdot l^{-1}$ BAP (4 - 4, 4 - 6, 4 - 9)

Culture conditions: 22±1°C, 8h photoperiod, 11.5 $\mu M \cdot m^{-2} \cdot sec^{-1}$ PAR irradiance.

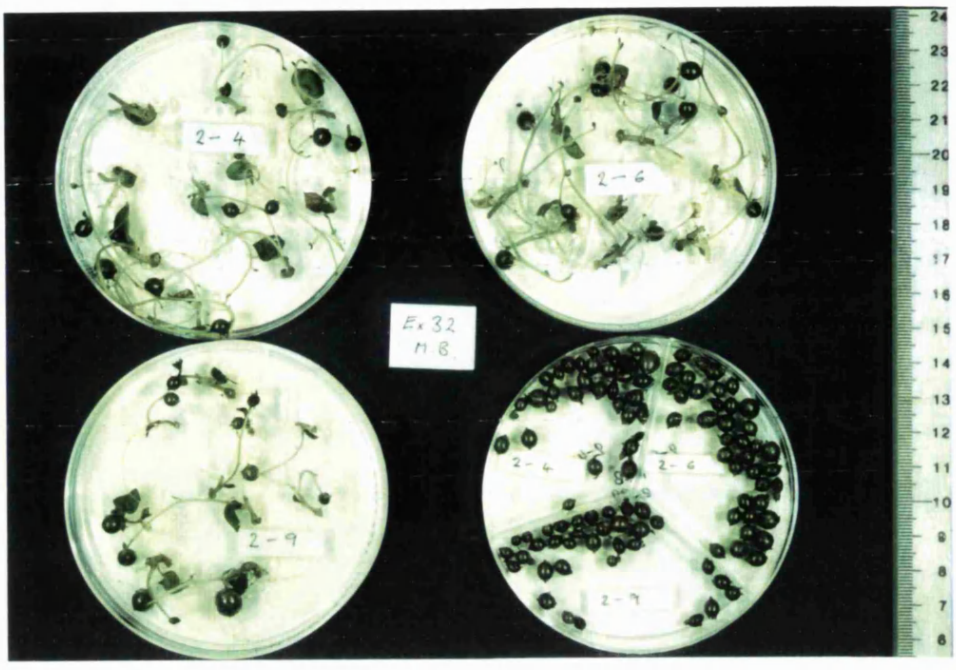
A



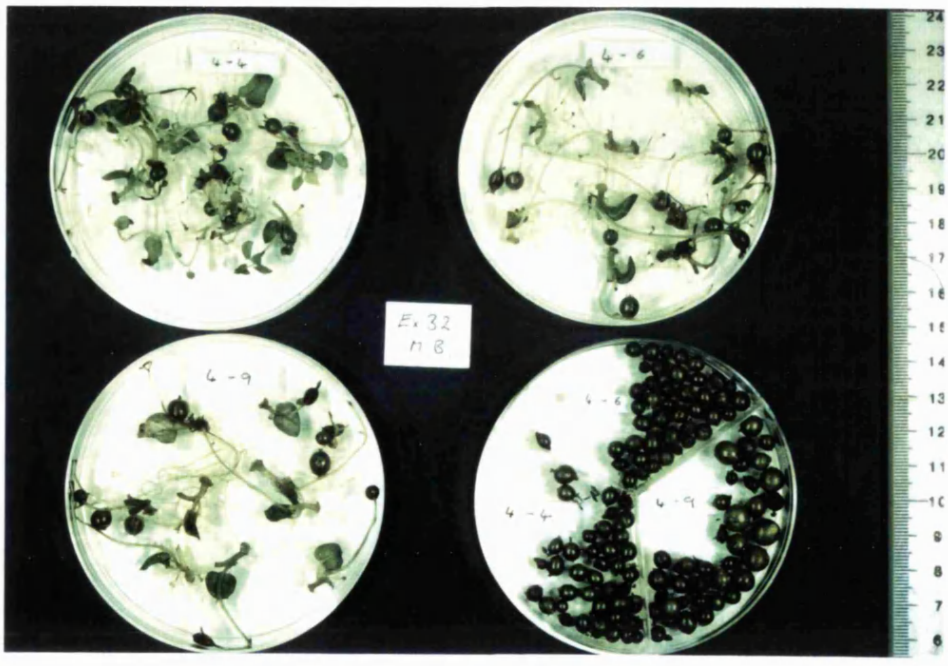
B



C



D



3.2.4 *In vitro* tuberization of cv Bintje

The most important maincrop cultivar in Europe, cv Bintje, was used in a last test of the reliability of the method of *in vitro* tuberization as determined by the experiments 3.2.2. and 3.2.3. These experiments involve four levels of BAP (0, 0.5, 2, 4mg.l^{-1}) and two levels of sucrose (6 and 9%(w/v)) in factorial combinations and an irradiance of 11,5µM.m^{-2}. sec^{-1} PAR and had given evidence of a reliable procedure for *in vitro* tuberization at 22 ± 1° C temperature and with an 8h photoperiod.

Nodes of cv Bintje, excised from shoot cultures grown on MS3 semi-solid medium, were cultured in MS semi-solid media supplemented with BAP and sucrose in factorial combinations of the above described concentrations.

Ten nodes were cultured in each 9cm petri-dish containing 20ml medium, and as replicates 5 petri dishes were used.

The cultures were incubated at 22± 1°C temperature with an 8h photoperiod and 11.5 µM.m^{-2} sec^{-1} PAR irradiance for 90 days.

Microtuber productivity, mean fresh weight of microtubers, proportion of sprouted and proportion of elongated microtubers were measured.

3.2.4 *In vitro* tuberization of cv Bintje

The most important maincrop cultivar in Europe, cv Bintje, was used in a last test of the reliability of the method of *in vitro* tuberization as determined by the experiments 3.2.2. and 3.2.3. These experiments involve four levels of BAP (0, 0.5, 2, 4mg.l^{-1}) and two levels of sucrose (6 and 9%(w/v)) in factorial combinations and an irradiance of 11,5μM.m^{-2}. sec^{-1} PAR and had given evidence of a reliable procedure for *in vitro* tuberization at 22 $\pm$ 1° C temperature and with an 8h photoperiod.

Nodes of cv Bintje, excised from shoot cultures grown on MS3 semi-solid medium, were cultured in MS semi-solid media supplemented with BAP and sucrose in factorial combinations of the above described concentrations.

Ten nodes were cultured in each 9cm petri-dish containing 20ml medium, and as replicates 5 petri dishes were used.

The cultures were incubated at 22$\pm$ 1°C temperature with an 8h photoperiod and 11.5 μM.m^{-2} sec^{-1} PAR irradiance for 90 days.

Microtuber productivity, mean fresh weight of microtubers, proportion of sprouted and proportion of elongated microtubers were measured.

Figure 9a. In vitro tuberization of cv Bintje

Media		Productivity	Mean fresh weight(mg)	Sprouted microt.(%)	Elongated microt.(%)
BAP ($mg.l^{-1}$)	Sucrose %(w/v)	(%)			
0	6	100abc	88.8a	0	0
	9	54d	48.6bc	0	0
0.5	6	98abc	73.2a	0	0
	9	88c	43.8cd	0	0
2	6	108 a	61.6 b	0	0
	9	102ab	49.4bc	0	0
4	6	90bc	29.8d	0	0
	9	102ab	72.8a	0	0

Lsd at p=.05 (i) Productivity % : 12.3

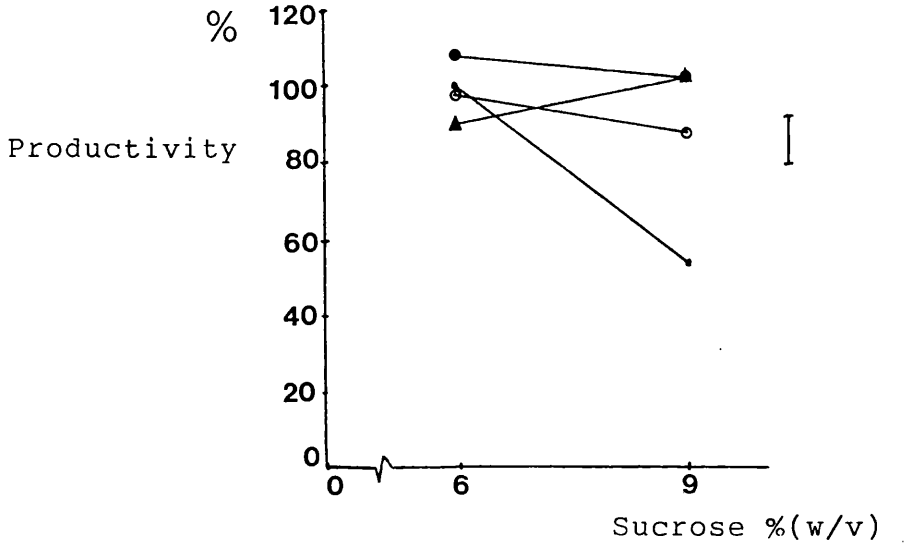
(ii) Mean fresh weight : 16.02

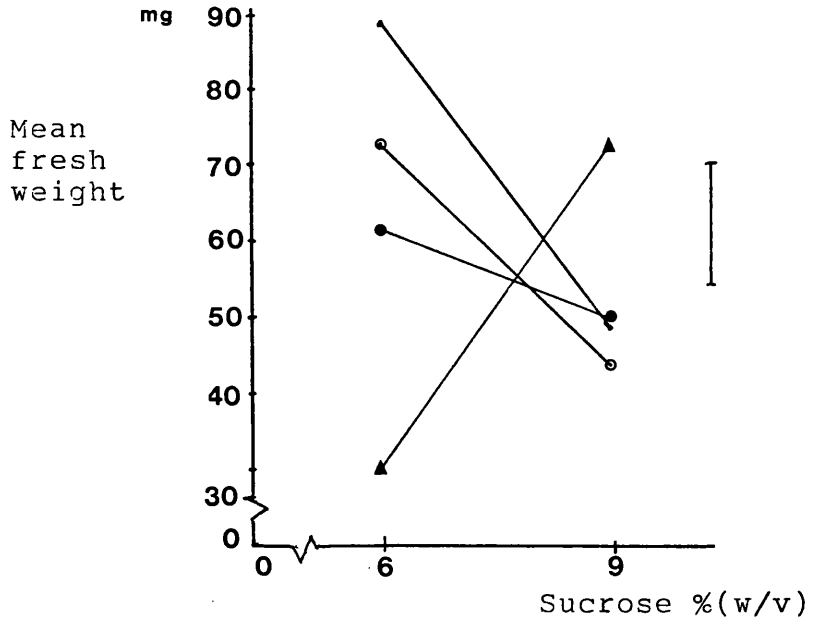
(Appendix II-iv)

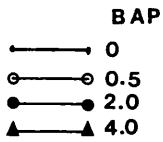
Figures followed by the same letter do not differ significantly.

For interaction diagram see figure 9b.

Figure 9b. Interaction diagrams showing the effect on microtuber productivity and mean fresh weight of microtubers of cv Bintje, of the interactions between the BAP and sucrose supplemented to MS semi-solid medium.







Statistical analysis : appendix II-iv
Experimental details as in fig. 9a

Culture conditions :

Basal medium : MS semi-solid

Temperature : 22 ± 1°C

Irradiance : 11,5μM.m^{-2} sec^{-1} PAR

Photoperiod : 8h

Number of Replicates : 5 x 10 nodes

Volume of medium : 20 ml per each petri-dish

From the above data (fig. 9a, 9b) it can be concluded that :

(i) For the best yield (100% productivity and the maximum size of microtubers) no cytokinin was required as in the case of cv Maris Bard (section 3.2.3)

(ii) There is interaction between BAP and sucrose concerning both microtuber productivity and mean fresh weight with maximum productivity for the media 6%(w/v) sucrose + 0 or 0.5 or 2 mg.l^{-1} BAP and 9%(w/v) sucrose + 2 or 4 mg.l^{-1} BAP and maximum fresh weight for the media 6% (w/v) sucrose +0 or 0.5mg.l^{-1} BAP and 9% (w/v) sucrose + 4mg.l^{-1} BAP

(iii) None sprouted microtuber or elongated microtuber was formed on any medium

3.3 Conclusions

From the results of the experiments of this chapter it can be concluded the following :

(i) Sucrose, BAP and irradiance are among the determining factors in microtuber formation

(ii) The optimum level of concentration of sucrose depends on the cultivar and the concentration of BAP and with the tested cultivars it ranged from 4 to 9%(w/v)

(iii) For conditions consisting of 22 ± 1^{o} C temperature, 8h day photoperiod, 11.5 $\mu M.m^{-2}.sec^{-1}$ PAR irradiance, there are cultivars which need cytokinin in the medium for a good yield (as cv Duke of York), but there are others which can give excellent yields without cytokinin (cv Maris Bard, cv Bintje)

(iv) Very low irradiance (1.3$\mu M.m^{-2}.sec^{-1}$ PAR) promotes the sprouting of microtubers before harvest but at levels of 11,5 or 140$\mu M.m^{-2}.sec^{-1}$ PAR, this phenomenon disappears

(v) The very high irradiance (140$\mu M.m^{-2}.sec^{-1}$ PAR) promotes the abnormal elongation of the microtubers and this becomes more severe with the higher levels of BAP and/or sucrose

4. USE OF OTHER THAN BAP CYTOKININS FOR IN VITRO TUBERIZATION

4.1 Introduction

Palmer and Smith (1969 a,b) showed that excised stolons of Solanum tubercosum subsp tubercosum grown in vitro, formed tubers if supplied with kinetin (see section 1.3.2), Mingo-Castel et al (1976) working with etiolated potato sprout sections cultured in vitro found that kinetin promoted tuberization when sufficient sucrose was present in the medium and Hussey and Stacey (1984) produced microtubers in vitro on MS media supplemented with kinetin in continuous light.

Wattimena (1984) have also reported tuberization in vitro in darkness with media supplemented with kinetin, ancymidol, cumarin and sucrose (see section 1.4).

Van Staden and Dimalla (1977) investigating tuberization of potato stolons during long-term storage of tubers in dark at low temperatures, had found that zeatin riboside was the dominant cytokinin detected in stolon tips where the tuberization process begins, Koda (1982) had found that the increased butanol-soluble cytokinin is responsible for the subsequent vigorous thickening growth of the stolons to form tubers and that the water-soluble cytokinin as the zeatin riboside is a temporary storage form, and according to Koda and Okazawa (1983) zeatin

riboside is less effective from other cytokinins in tuberization.

By the following experiments the effects of the cytokinins: kinetin, zeatin, zeatin riboside on the *in vitro* tuberization of potato were investigated.

4.2 Results

4.2.1 The effect of kinetin on the *in vitro* tuberization

Nodes of cv Spunta excised from shoot cultures grown on MS3 semi-solid medium were cultured on MS semi-solid medium containing kinetin and sucrose in factorial combinations of the following concentrations:

kinetin $mg.l^{-1}$: 0.5, 2, 4

sucrose % (w/v) : 4, 6, 9

Ten nodes were cultured in each 9cm petri dish containing 20ml medium and, as replicates four petri dishes were used.

The cultures were incubated at 22± 1° C temperature with an 8h photoperiod and 11.5 $\mu M.m^{-2}.sec^{-1}$ PAR irradiance and were grown for 90 days.

Figure 10a. The effect of kinetin on *in vitro* tuberization

Media		Productivity(%)	Mean fresh weight of microt.(mg)	Elongated microt. (%)	Adventitious microt. (%)
Kinetin ($mg.l^{-1}$)	Sucrose (% w/v)				
	4	82.5	145.00d	34.3	0
0.5	6	95.0	106.25cd	5.0	0
	9	87.5	138.75bc	0	0
	4	102.5	182.75a	61.0	0
2.0	6	97.5	91.75de	0	0
	9	100.0	141.75b	3.3	0
	4	80.0	64.00ef	6.7	0
4.0	6	92.5	79.75def	13.5	0
	9	72.5	50.50f	3.4	0

Lsd at p=.05 for Mean fresh weight : 33.12

For productivity no significant interaction was found.

Details of statistical analysis in appendix III-i

Culture conditions :

Basal medium : MS semi-solid

Temperature : $22 \pm 1^{\circ}$ C

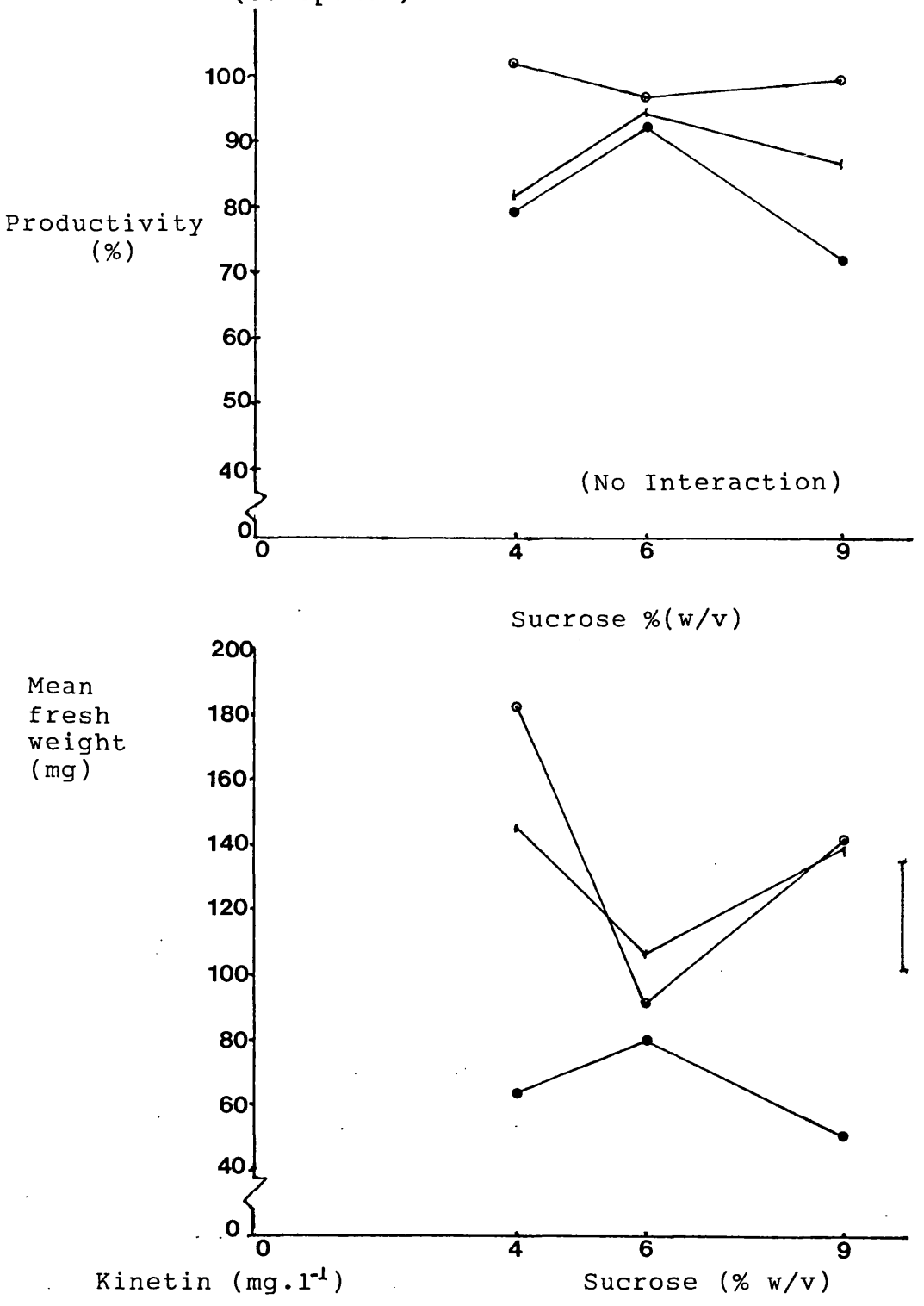
Irradiance : 11.5 $\mu M.m^{-2}.sec^{-1}$ PAR

Photoperiod : 8h

Replicates : 4 petri dishes x 10 nodes

Volume of medium : 20ml per petri dish

Figure 10b. Interaction diagrams showing the effect on microtuber productivity and mean fresh weight of microtubers, of the interaction between the kinetin and sucrose supplemented to MS semi-solid medium (cv Spunta)



Statistical analysis : appendix III-i
Experimental details as in fig. 10a

From the above data the following can be concluded :

(i) No interaction between sucrose and kinetin concerning microtuber productivity was found

(ii) No significant difference among the three levels of sucrose concerning the microtubers productivity was found

(iii) Kinetin of 2mg.l^{-1} gave significantly higher productivity than 0.5 and 4mg.l^{-1}

(iv) There was interaction between kinetin and sucrose concerning mean fresh weight of microtubers with maximum mean fresh weight in the medium with 2mg.l^{-1} kinetin and 4% (w/v) sucrose and minimum in the medium with 4mg.l^{-1} kinetin and 9% (w/v) sucrose

(v) 4% (w/v) sucrose promoted strongly the abnormal elongation of microtubers at levels of 0.5 and 2mg.l^{-1} kinetin (34.3 and 61%) but not at 4mg.l^{-1} kinetin (only 6.7%)

(vi) No adventitious microtubers were found in any medium

Plate V Microtubers of cv Spunta produced on MS semi-solid media supplemented with Kinetin and sucrose in factorial combinations of the following concentrations

Kinetin: 0.5, 2, 4 ($mg \cdot l^{-1}$)

Sucrose: 4, 6, 9 (%w/v)

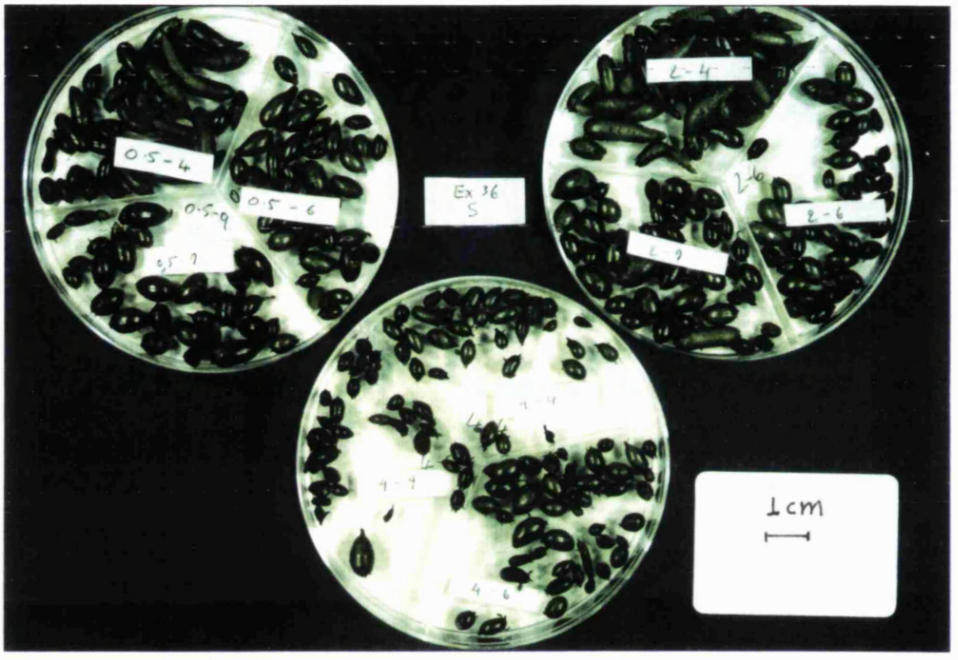
A: media with 0.5 $mg \cdot l^{-1}$ Kinetin (0.5-4, 0.5-6, 0.5-9)

B: media with 2 $mg \cdot l^{-1}$ Kinetin (2 - 4, 2 - 6, 2 - 9)

C: media with 4 $mg \cdot l^{-1}$ Kinetin (4 - 4, 4 - 6, 4 - 9)

A

B



C

4.2.2 The effect of zeatin on *in vitro* tuberization

Nodes of the cultivars Spunta and Kennebec excised from shoot cultures grown on MS3 semi-solid medium were cultured on MS semi-solid medium supplemented with factorial combinations of the following concentrations of zeatin and sucrose :

zeatin : 0.05, 0.5, 2, 4 $mg.l^{-1}$

sucrose : 4, 6, 9% (w/v)

Because of contamination data from the cultures of cv Spunta with 4% (w/v) sucrose are not included.

Ten nodes were cultured in each 9cm petri dish containing 20ml medium and, as replicates five petri dishes were used.

The cultures were incubated at 22^{+} 1° C temperature with an 8h photoperiod and 11.5 $\mu M.m^{-2}.sec^{-1}$ PAR irradiance and were grown for 90 days.

Figure 11a. The effect of zeatin on *in vitro* tuberization of potato

Cultivar	Media		Productivity(%)	Mean fresh weight (mg)	Elongated microt. (%)	Adventitious microt. (%)
	Zeatin ($mg.l^{-1}$)	Sucrose (%w/v)				
Spunta	0.05	6	94a	108.8c	25.5	0
		9	98a	126.0b	14.3	0
	0.50	6	100a	122.8b	7.5	0
		9	98a	110.4c	37.9	0
	2.00	6	96a	138.6a	12.5	0
		9	98a	142.0a	2.6	0
	4.00	6	100a	108.4c	10.0	0
		9	96a	108.6c	0	0
Kennebec	0.05	4	92ab	57.0	0	0
		6	104a	120.4	15.4	0
		9	94ab	85.6	0	0
	0.50	4	74bc	30.0	0	0
		6	78bc	67.4	38.5	0
		9	74bc	55.6	29.7	0
	2.00	4	64c	70.6	0	0
		6	102a	103.2	0	0
		9	96ab	126.0	10.4	0
	4.00	4	22d	32.2	0	0
		6	102a	93.8	5.0	0
		9	94ab	80.8	0	0

F value for : productivity with Spunta : 1.04 (No significant difference)

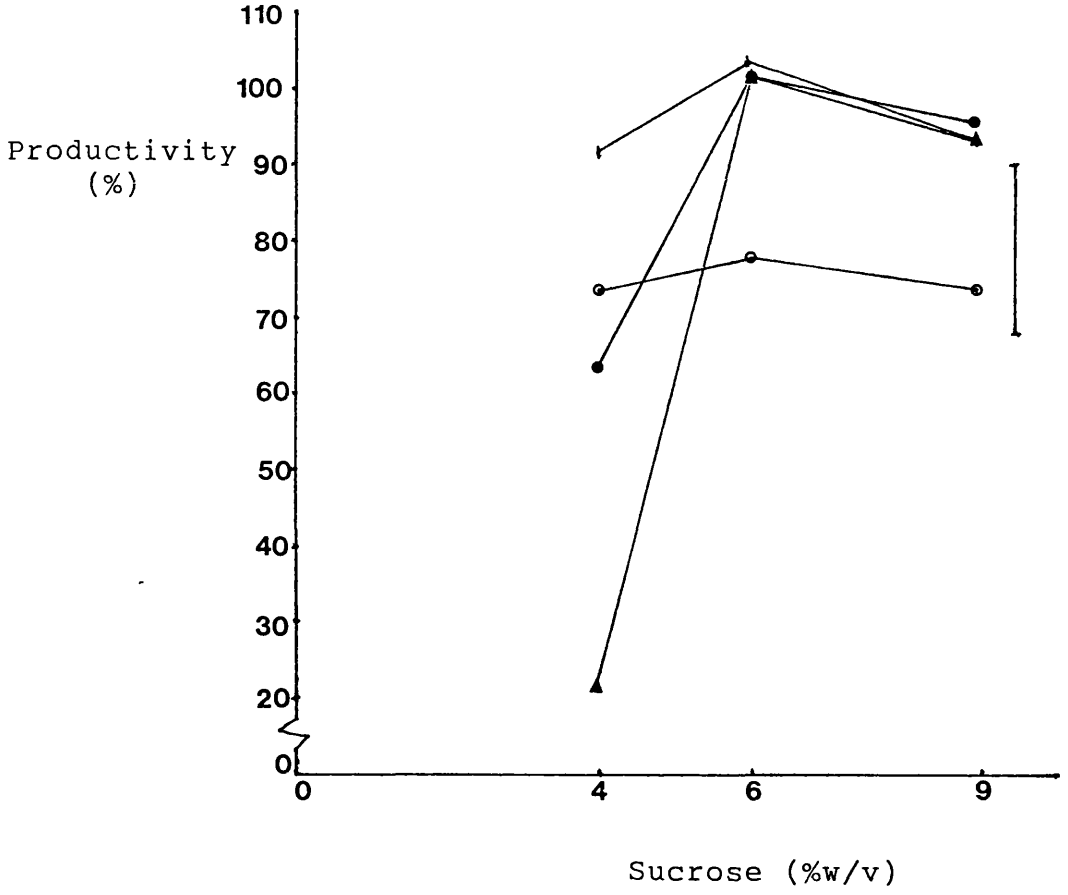
LSD at p=.05 for : (i) productivity with Kennebec : 22.2

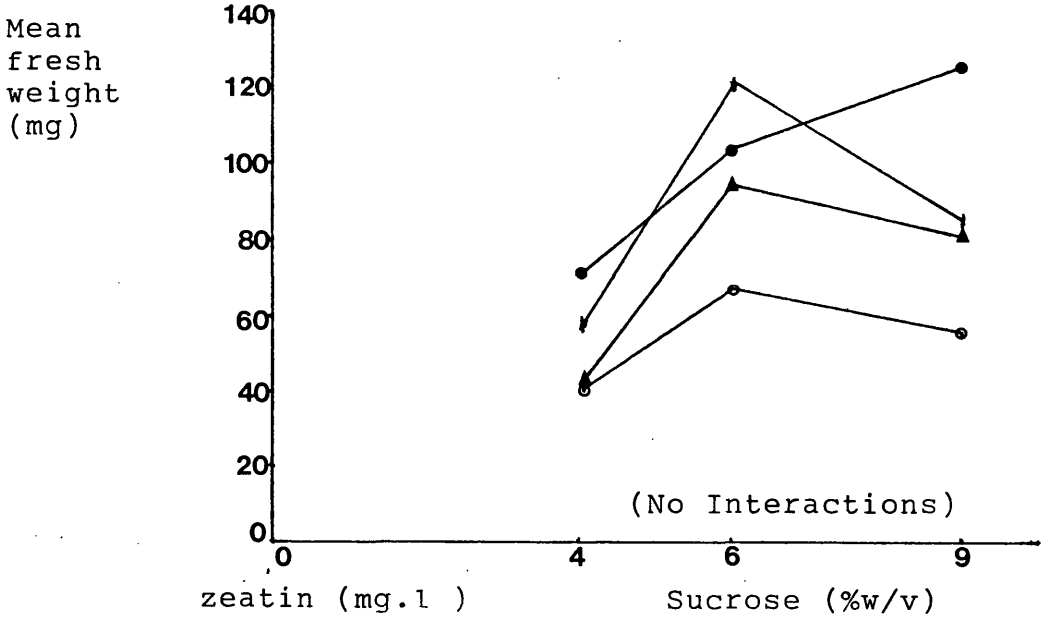
(ii) mean fresh weight with Spunta : 11.33

For mean fresh weight with Kennebec no significant interaction was found

For each cultivar figures followed by the same letter do not differ significantly.

Figure 11b. Interaction diagrams showing the effect on microtuber productivity and mean fresh weight of microtubers, of the interactions between the zeatin and sucrose supplemented to MS semi-solid medium (cv Kennebec)

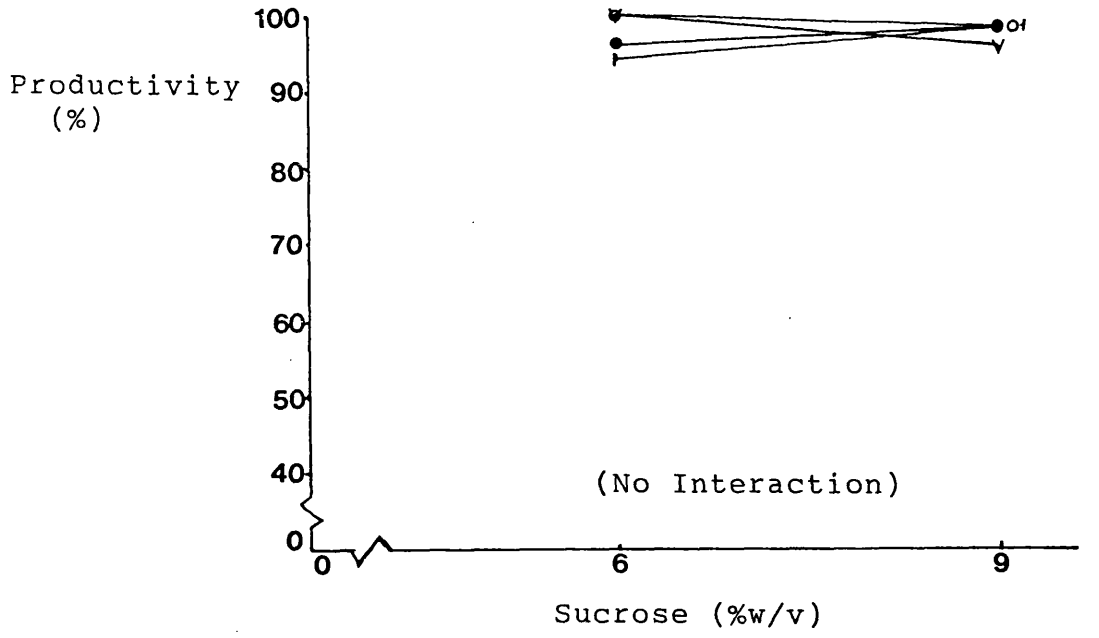


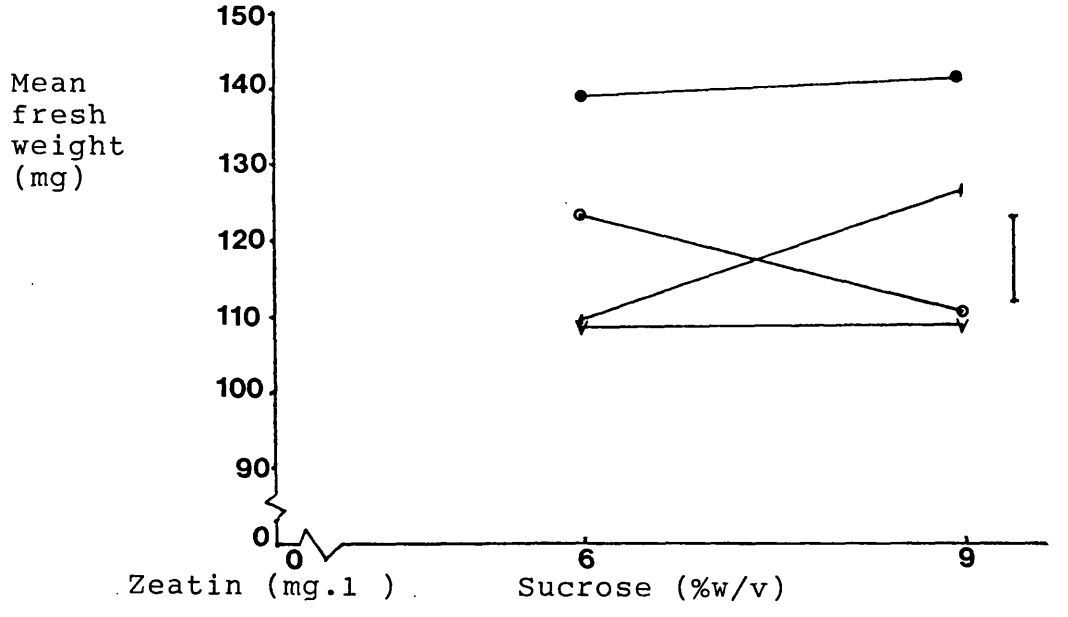


:0.05
:0.5
:2.0
:4.0

Statistical analysis : appendix III-ii
Experimental details as in fig. 11a

Figure 11c. Interaction diagrams showing the effect on microtuber productivity and mean fresh weight of microtubers, of the interactions between the zeatin and sucrose supplemented to MS semi-solid medium (cv Spunta)





:0.05
:0.50
:2.00
:4.00

Statistical analysis appendix III-ii
Experimental details as in fig.11a

For interaction diagrams between zeatin and sucrose for cv Kennebec see Figure 11b, and for cv Spunta Figure 11c.

Details of statistical analysis in appendix III-ii

Culture Conditions : Basal medium : MS semi-solid

Temperature : 22±1°C

Irradiance : 11.5 $\mu M.m^{-2}.sec^{-1}$ PAR

Photoperiod : 8h

Replicates : 5 petri dishes x 10 nodes

Volume of medium : 20 ml each petri dish

From the data of this experiment it can be concluded that :

(i) Interaction was found between zeatin and sucrose concerning mean fresh weight but no interaction concerning microtuber productivity for cv Spunta ; all media gave practically one microtuber per each node for cv Spunta. Heavier microtubers gave all media supplemented with $2mg.l^{-1}$ zeatin

(ii) For cv Kennebec interaction was found between zeatin and sucrose concerning microtuber productivity with maximum yield in all media containing $0.05mg.l^{-1}$ zeatin and the media of 2 or $4mg.l^{-1}$ zeatin and 6 or 9% (w/v) sucrose;

all the media containing 0.5mg.l^{-1} zeatin gave low productivity

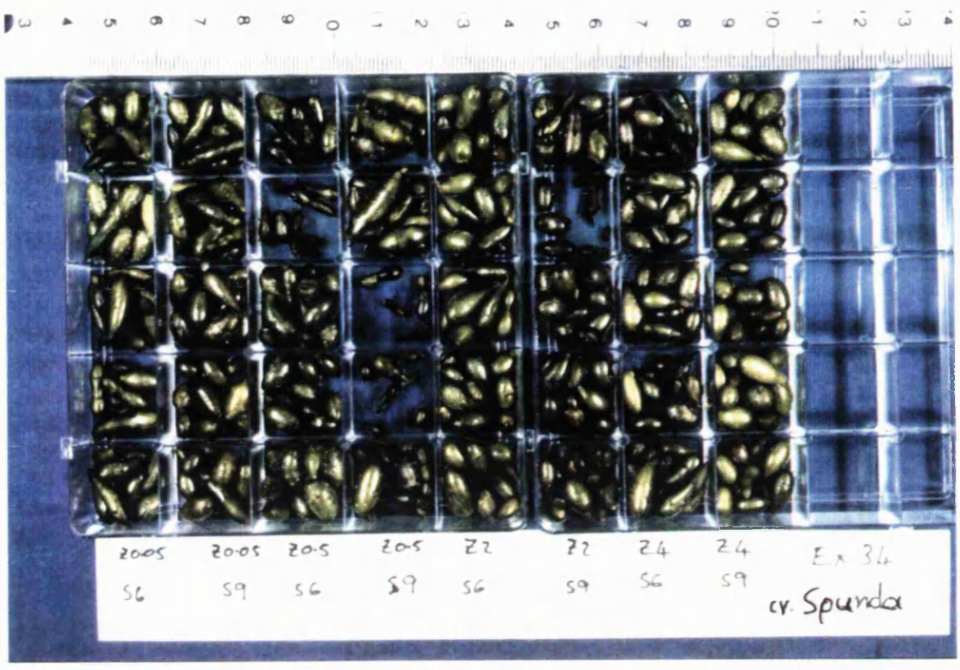
(iii) No interaction was found between zeatin and sucrose concerning the mean fresh weight of microtubers of cv Kennebec but significant difference among the levels of zeatin and among the levels of sucrose was found; higher mean fresh weight gave zeatin of 0.05 and 2mg.l^{-1} and sucrose of 6 and 9% (w/v)

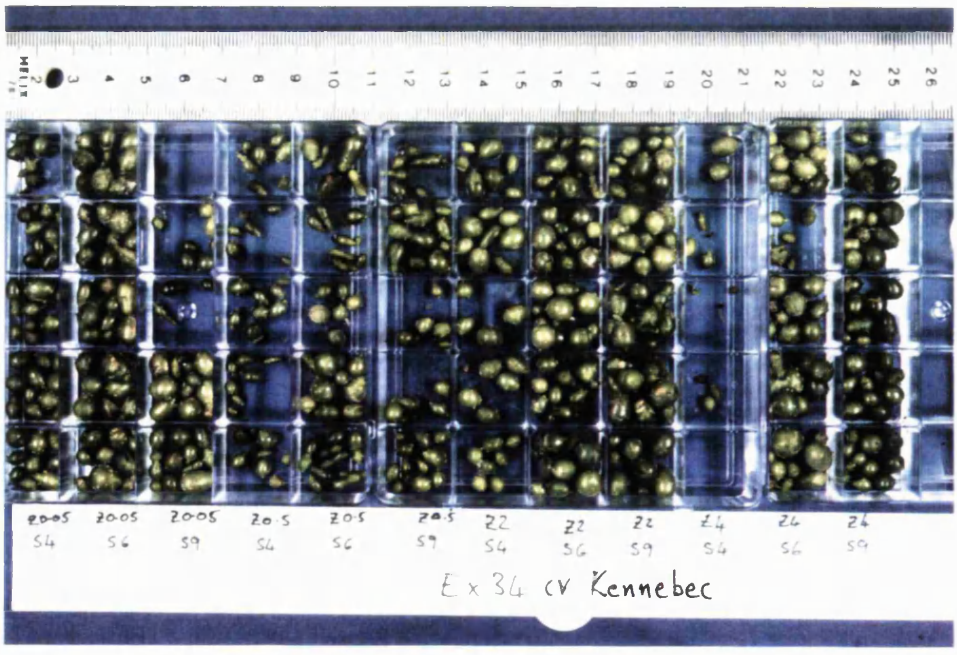
(iv) Abnormal elongation of microtubers was strongly promoted at low concentrations of zeatin (0.5, 0.05mg.l^{-1}) for both cultivars; at 4%(w/v) sucrose no elongated microtubers were found for the tested cv Kennebec

(v) No adventitious microtubers were found in any medium

Plate VI

Microtubers of cvs Spunta and Kennebec produced on MS semi-solid media supplemented with zeatin and sucrose in factorial combinations of the following concentrations:
zeatin: 0.05, 0.5, 2, 4 $mg \cdot l^{-1}$
sucrose: 6, 9% (w/v) for cv Spunta and 4, 6, 9% (w/v) for cv Kennebec
Z: zeatin ($mg \cdot l^{-1}$)
S: sucrose (% w/v)
Culture conditions: 22 ± 1°C, 8 h photoperiod, 11.5 $\mu M \cdot m^{-2} \cdot sec^{-1}$ PAR irradiance.


20·5 S6
20·5 S9
20·5 S6
20·5 S9
22 S6
22 S9
24 S6
24 S9
Ex 34
cv. Spunda


20·5 S4
20·5 S6
20·5 S9
20·5 S4
20·5 S6
20·5 S9
22 S4
22 S6
22 S9
24 S4
24 S6
24 S9
Ex 34 cv Kennebec

4.2.3 The effect of zeatin riboside on the *in vitro* tuberization of potato

The zeatin riboside as factor affecting the *in vitro* tuberization was examined in this experiment.

Nodes of cv. Majestic excised from shoot culture grown on MS3 semi-solid medium were cultured in MS semi-solid medium supplemented with zeatin riboside and sucrose in factorial combinations of the following concentrations:

zeatin riboside : 0, 0.5, 2.0, 4.0, 6.0, 9.0 $mg.l^{-1}$

sucrose : 6, 9, 12%(w/v)

The following experimental conditions were applied :

Incubation conditions : $22 \pm 1^{o}C$ temperature, 8h photoperiod, $1.3\mu M.m^{-2}.sec^{-1}$ PAR irradiance

Replicates : four 9 cm petri-dishes containing 20ml medium with 10 nodes cultured in each petri dish

The cultures were grown for 110 days, 20 days more than in previous experiments, in order to investigate the possibility of microtubers being formed on adventitious shoots, which were produced rapidly in some of these media in contrast to media containing BAP, kinetin or zeatin.

For comparison of the results of this experiment with those of experiment 3.2.1., where the same cultivar was cultured in media supplemented with BAP, the same incubation conditions were applied.

Figure 12. Effect of zeatin riboside on *in vitro* tuberization of potato

Media		Productivity	Mean Fresh	Elongated	Sprouted
Zeatin riboside ($mg.l^{-1}$)	Sucrose %(w/v)	(%)	weight(mg)	microt.(%)	microt.(%)
0	6	0	0	0	0
	9	0	0	0	0
	12	0	0	0	0
0.5	6	10.0	24.0	0	0
	9	50.0	29.5	0	0
	12	20.0	29.0	0	0
2.0	6	37.5	37.5	0	0
	9	97.5	72.5	0	5.1
	12	42.5	31.0	0	0
4.0	6	50.0	41.5	0	0
	9	15.0	24.0	0	0
	12	12.5	32.0	0	0
6.0	6	22.5	29.0	0	0
	9	65.0	47.0	0	0
	12	52.5	70.0	0	0
9.0	6	2.5	8.0	0	0
	9	60.0	64.0	0	0
	12	7.5	33.0	0	0

Culture Conditions :

Basal medium : MS semi-solid

Temperature : $22 \pm 1^{0}C$

Photoperiod : 8h

Irradiance : 1.3 $\mu M.m^{-2}\ sec^{-1}$ PAR

Replicates : 4 petri dishes x 10 nodes each

Volume of medium : 20 ml each petri dish

Adventitious shoot formation was observed especially after the 26th day of culture. In some media many adventitious shoots were formed on callus on the basipetal end of the node and, as they grew rapidly, special care was taken to detect any associated microtuber formation. In the medium supplemented with 2.0mg.l^{-1} zeatin riboside and 6% (w/v) sucrose, 55% of the explants gave at least one adventitious shoot which at the 60th day of the culture, had reached the length of 2-3 cm and in the medium with 4.0mg.l^{-1} zeatin riboside and 6% (w/v) sucrose, 60% of the explants gave at least one adventitious shoot which at the 60th day had reached the length of 2-10 cm. However, even after delaying the harvest for 20 days, no microtubers were found to be formed on any of these adventitious shoots.

From the data of this experiment it can be concluded that :

(i) Zeatin riboside promoted microtuberization.

(ii) In contrast to the other cytokinins tested (BAP, kinetin, zeatin), only one of the tested media ($2mg.l^{-1}$ zeatin riboside + 9% (w/v) sucrose) showed productivity of approximately one microtuber per each node; the other media gave very low productivities (maximum 65%).

(iii) Zeatin riboside did not promote the abnormal elongation of the microtubers.

(iv) With the medium supplemented with $2mg.l^{-1}$ zeatin riboside and 9% sucrose, 5% of the microtubers were sprouted at the harvest but this did not occur with any other medium.

Overall, it can be concluded that zeatin riboside is less effective than BAP in promoting <u>in vitro</u> tuberization, at least under the conditions of these experiments.

4.3 Conclusions

From the results of the experiments of this chapter can be concluded that all three tested cytokinins (kinetin, zeatin, zeatin riboside) promote the *in vitro* tuberization of potato, the highest productivity for each of them was practically one microtuber per each node, as in the media supplemented with BAP (see section 3), and the optimal concentrations of sucrose in association with each of these cytokinins were 6 and 9% (w/v), as in the case of BAP; there was some evidence that the microtubers produced in media with each of these cytokinins were larger than those produced in media with BAP, but more work would be necessary to confirm this.

5.0 THE EFFECT OF MANNITOL, PHOTOPERIOD AND PH ON THE *IN VITRO* TUBERIZATION OF POTATO

5.1 Introduction

The importance of sucrose as a factor affecting the *in vitro* tuberization of potato had been suggested by many authors (Wang and Hu 1982, Koda and Okozawa 1983, Hussey and Stacey 1984). This has been found also from the experiments in the previous chapters (3,4). There seems to be a general agreement that low levels of sucrose (lower than 4% w/v) independently of the use of growth regulators do not promote tuberization, but levels of 6-9% (w/v) strongly promote this process. The use of sugar other than sucrose in tuberization media had not been reported by any authors at the time of these experiments. In order to investigate whether the effect of higher concentrations of sucrose on *in vitro* tuberization is a result of their osmotic or their nutritional qualities, an experiment was carried out in which mannitol was substituted for sucrose in various combinations. Using as control MS semi-solid media supplemented with $2mg.l^{-1}$ BAP and two levels of sucrose, 6 and 9% (w/v), the whole or part of the sucrose was replaced by mannitol so that the osmolarity of the media remained unchanged and the performance of the nodal cultures in these modified media was investigated.

Photoperiod is another factor which seems to play an

important role in the in vitro tuberization of potato. Wang and Hu (1982) reported that the optimum photoperiod for mass tuberization was 8h, and Hussey and Stacey (1984) also found that short days (8h) have some promotive effect on tuberization, but shoots raised in long days and then subjected to periods of short days were not affected as far as tuberization is concerned. In the experiment described here, was investigated the effect of short (8h) and long (16h) days on in vitro tuberization with three potato cultivars growing on media optimized for cytokinin and sucrose concentrations.

In most reports concerning in vitro tuberization, the pH of the medium was adjusted to 5.6-5.7 before autoclaving but no author referred to the reasons for the use of that pH value. In order therefore, to investigate the effect of pH on in vitro tuberization, in a specific experiment the pH was adjusted to levels in the range from 4 to 10 and the performance of two cultivars at these pH values was examined.

5.2 Results

5.2.1 The effect of Mannitol as a sucrose substitute on *in vitro* tuberization

Nodes of cv Majestic excised from microcultures grown on MS3 semi-solid medium were cultured for 100 days on seven different MS semi-solid media supplemented with $2mg.l^{-1}$ 6BAP and the following combinations of the concentrations of sucrose and mannitol :

Media	Sucrose %(w/v)	Mannitol %(w/v)	Osmolarity(MOS)
1	6	0	
2	3	1.680	282
3	0	3.333	
4	9	0	
5	6	1.740	385
6	3	3.380	
7	0	4.920	

Preliminary studies were carried out to determine the equivalent amounts of mannitol which were necessary to replace the indicated proportions of sucrose without changing the osmolarity of the medium.

Incubation conditions :

Temperature : 22±1° C

Photoperiod : 8h

Irradiance : 1.3 $\mu M.m^{-2}.sec^{-1}$ PAR

Containers : 100 ml plastic jars containing 20ml medium

Replicates : 4 jars with 10 nodes each

A low irradiance level (1.3 $\mu M.m^{-2}.sec^{-1}$ PAR) was selected in order to reduce the possibility of an influence from sugars formed by photosynthesis in the cultures.

Figure 13. The effect of mannitol as a sucrose substitute on in vitro tuberization of potato (cv Majestic)

Media No	Sucrose (%w/v)	Mannitol (% w/v)	Osmolarity MOS	Microtuber Productivity	Explant growth
1	6	0		80	normal growth
2	3	1.680	282	0	30% of explants did not grow
3	0	3.333		0	by 10 days the explants died
4	9	0		75	normal growth
5	6	1.740	385	50	fairly normal growth
6	3	3.380		0	30% of explants did not grow
7	0	4.920		0	by 10 days the explants died

Culture conditions :

Basal medium : MS semi-solid

Temperature : $22 \pm 1^{\circ}C$

Photoperiod : 8h

Irradiance : $1.3\ \mu M.m^{-2}\ sec^{-1}$ PAR

Replicates : 4 jars x 10 nodes

Volume of medium in each container : 20ml

From the above data it can be concluded that :

(i) Mannitol inhibited in vitro tuberization at both levels of osmolarity even in the media containing 6% (w/v) sucrose which on its own (see medium No 1) promoted tuberization.

(ii) Mannitol inhibited the growth of the explants even in the media with 3% (w/v) sucrose which on its own is adequate for a good explant growth.

(iii) With both levels of osmolarity and with no sucrose at all, the explants died by the 10th day of culture.

(iv) Mannitol was apparently less inhibiting in the presence of 6% (w/v) sucrose than with 3% (w/v) sucrose.

(v) There was no evidence in comparison with either the 6% or the 9% (w/v) levels of sucrose, that mannitol could substitute in part for the effect of sucrose on tuberization. This would suggest either that the effect of the higher sucrose

concentrations is nutritional rather than osmotic or that the mannitol is toxic.

5.2.2 The effect of photoperiod on the *in vitro* tuberization of potato

Nodes on the cvs Spunta, Kennebec and Majestic excised from microcultures grown on MS4 liquid medium for Spunta and Kennebec and MS4 semi-solid for Majestic, were cultured on MS semi-solid media as follows:

(i) Spunta : MS supplemented with 2mg.l^{-1} BAP and 6% (w/v) sucrose

(ii) Kennebec : MS supplemented with 6% (w/v) sucrose

(iii) Majestic : MS supplemented with 4mg.l^{-1} BAP and 9% (w/v) sucrose

These media were selected as being suitable for each cultivar (sections 6.2.9, 3.2.1, 3.2.2) for *in vitro* tuberization with an 8h photoperiod, 22±1°C temperature, 11.5µM.m^{-2}.sec^{-1} PAR irradiance.

Two photoperiods (8h and 16h) were investigated.

Five 9cm petri dishes each containing 20ml medium and 10 nodes were used for cv Spunta and cv Kennebec and four petri dishes of 14cm containing 50ml medium and 25 nodes for cv Majestic. The cultures were grown for 90 days.

Figure 14. Effect of photoperiod on *in vitro* tuberization of potato

Cultivars	Photoperiod (h)	Microt. productivity	Mean Fresh weight (mg)	Elongated microt.(%)
Spunta	16	92.0a	112.6a	4.3
	8	100.0a	99.5a	6.3
Kennebec	16	98.0a	154.5a	0
	8	96.0a	165.3a	0
Majestic	16	101.0a	57.5a	0
	8	97.5a	54.9a	0

Culture conditions :

Basal medium : MS semi-solid

Temperature : 22 ± 1 ° C

Irradiance : 11.5 $\mu M.m^{-2}.sec^{-1}$ PAR

Replicates: 4 for Majestic, 5 for Spunta, Kennebec

Volume of medium : 20ml for each 9cm petri dish and 50ml for each 14cm petri dish.

The statistical analysis of the data in Fig. 14 (see appendix IV-i) shows that, for all the tested cultivars under the conditions of this experiment, there were no significant differences between the effects of the 8h and 16h photoperiods on either microtuber productivity or the mean fresh weight of the microtubers.

5.2.3. The effect of the pH of the medium on the *in vitro* tuberization of potato

Nodes of the cultivars Spunta and Kennebec excised from microcultures grown on MS4 media of high fluidity (0.3% (w/v) agar) were cultured in MS medium of high fluidity supplemented with 8% (w/v) sucrose, without cytokinin, for tuberization. The pH of the medium was adjusted before autoclaving by solutions of NaOH and HCI at the following seven different levels : 4,5,6,7,8,9,10.
Ten 9cm petri-dishes each containing 20ml medium and 10 nodes were used for each cultivar at each pH value.
The cultures were incubated at 20 ± 1°C temperature with an 8h photoperiod and 20 $\mu M.m^{-2}.sec^{-1}$ PAR irradiance.
The cultures were grown for 80 days and the pH of the medium was measured again at the time of harvest.

Figure 15. The effect of pH on *in vitro* tuberization of potato

pH before autoclaving	Microtuber productivity(%)		Mean Fresh weight(gr)		pH at harvest	
	cv S	cv K	cv S	cv K	cv S	cv K
4	0	86c	0	170ab	5.90	5.00
5	92a	100ab	182a	176ab	5.09	5.98
6	96a	100ab	186a	182ab	5.06	5.60
7	95a	99ab	178a	186a	5.04	5.56
8	85a	106a	173a	168ab	4.88	5.00
9	85a	107a	172a	162b	4.86	4.87
10	88a	94bc	112b	98c	4.89	4.83

S = Spunta K = Kennebec

Values of the same column followed by the same letter do not differ significantly on the basis of P=.05 (see appendix IV-ii)

F for Productivity for Spunta = 2.03 (No significant difference)

Lsd at p=.05 for

(i) Productivity for Kennebec = 9.59

(ii) Mean fr. weight for Spunta = 30.33

(iv) Mean fr. weight for Kennebec = 20.6

Culture conditions :

Basal medium : MS semi-solid with 0,3% (w/v) agar

Temperature : 22 ± 1^{c} C

Irradiance : 20 $\mu M.m^{-2}.sec^{-1}$ PAR

Photoperiod : 8h

Replicates : 10 petri dishes x 10 nodes for each

Volume of medium : 20ml

From the above data it can be concluded that :

(i) Initial pH values between 5 and 10 for Spunta and between 5 and 9 for Kennebec did not produce significant differences concerning productivity.

(ii) Initial pH values between 5 and 9 for Spunta and between 4 and 8 for Kennebec did not show significant differences concerning the mean fresh weight of microtubers.

(iii) The pH values at harvest were very different from the values before autoclaving, ranging between approximately 4.8 and 6 for both cultivars.

5.3 Conclusions

From the results of the experiments in this chapter it can be concluded that mannitol cannot substitute in part for the effect of sucrose or tuberization, which would suggest either that the effect of the higher concentrations of sucrose on tuberization is nutritional rather than osmotic or that the mannitol is toxic. Further experiments, possibly using other osmotic agents, would be required to distinguish between these two possibilities.

Under the conditions of these experiments and with the cultivars tested, there seemed to be no significant effect of photoperiod on the microtuberization process since similar results were obtained both under short-days (8h) and long-days (16h).

Concerning the pH, the microtuberization process appeared to be indifferent to the initial pH of the medium, in the range of pH 5 to 9 for cv Spunta and 5 to 8 for cv Kennebec, lower pH value (pH 4) were inhibitory but higher pH values (10 for cv Spunta and 9, 10 for cv Kennebec) have not showed severe negative effect on tuberization.

6. FACTORS AFFECTING PRODUCTIVITY, QUALITY AND COST OF MICROTUBERS IN A COMMERCIAL SCALE PRODUCTION

6.1 Introduction

The productivity of the *in vitro* tuberization procedure together with the quality and cost of production are all of great importance for commercial microtubers production.

The experiments in this section which were concerned with these three factors, examined the mechanization of chopping, the distribution of this material on the media, the better exploitation of the medium, the possibility substituting of inexpensive ingredients and the use of the lowest possible amounts of ingredients.

6.2. Results

6.2.1 The effect of the position of the nodes on the mother plantlet on *in vitro* tuberization

In the *in vitro* tuberization procedure described in the previous experiments, single nodes were used as plant material. In a system involving the mechanical chopping of the plantlets, all of the nodes of the plantlets would be used for tuberization. This experiment was concerned with differences in the performance of the nodes from various positions on the mother plantlet when used for *in vitro* tuberization.

Nodes of cv Majestic were excised from plantlets with six nodes grown on MS3 semi-solid medium.

The performance of the second and third nodes from the base was compared with the fifth and sixth nodes, after cutting, on MS semi-solid medium supplemented with $4mg.l^{-1}$ BAP and 9%(w/v) Sucrose.

Ten nodes were placed in 9cm petri dishes containing 20ml medium and four petri dishes were used for each set of nodes. The cultures were incubated at $22 \pm 1^{\circ}$ C temperature with an 8h photoperiod and $1.3\mu M.m^{-2}.sec^{-1}$ PAR irradiance for 90 days.

Figure 16. The effect of the position of the nodes on the mother plantlet on *in vitro* tuberization

Treatments	Productivity (%)	Mean Fresh weight (mg)	Sprouted microt.(%)	Elongated microt.(%)
2nd+3rd nodes	100	39.1a	7.5	12.5
5th+6th nodes	100	29.0b	5.0	5.0

t at p=.05 for mean fresh weight = 12.25 (Appendix V-i)

Culture Conditions :

Medium : MS semi-solid with 4mg.l^{-1} BAP + 9%(w/v) sucrose

Temperature : 22 ± 1°C

Photoperiod : 8h

Irradiance : 1.3µM.m^{-2}.sec^{-1} PAR

Replicates : 4 petri dishes containing 20ml medium x 10 nodes

Cultivar : Majestic

From the data of this experiment it can be concluded that :

(i) The two sets of nodes gave the same productivity of one microtuber per each node.

(ii) The mean fresh weight of the produced microtubers was influenced by the position of

nodes on the mother plantlet with the basal nodes giving significantly heavier microtubers but the difference was not sufficiently large to cause problems of quality, if both types of nodes were to be used together for tuberization.

(iii) Concerning abnormalities such as sprouting before harvest and elongation of the microtubers, the basal nodes produced higher proportions but not sufficiently high to cause problems.

(iv) It can be suggested that all nodes of the plantlet can be used in commercial scale in vitro tuberization.

6.2.2 Comparison of apical shoot tips and nodes as material for *in vitro* tuberization

In previous experiments the shoot tips were used together with nodes for tuberization. In this experiment the performance of shoot tips in the tuberization media was compared with nodes.

This material was excised from microplants of cv Majestic grown on MS3 semi-solid medium and it was cultured for tuberization on MS semi-solid medium supplemented with 4mg.l^{-1} BAP and 9% (w/v) sucrose. 25 explants were placed in each 14cm petri dish containing 50ml medium and four petri dishes were used for each type of explant. The cultures were incubated for 84 days at 22 $\pm$ 1°C with an 8h photoperiod and 11.5μM.m^{-2}.sec^{-1} PAR irradiance.

Figure 17. Comparison of apical shoot tips and nodes as material for *in vitro* tuberization

Treatments	Productivity (%)	Mean Fresh weight(mg)	Sprouted microt.(%)	Elongated microt.(%)
Shoot tips	93a	43.5a	5.5	10
Nodes	96a	40.0a	14.0	13

t at ρ=.05 for Productivity = 1.029

t at p=.05 for Mean fresh weight = 1.35 (Appendix V-ii)

Culture Conditions :

Medium : MS semi-solid with 4mg.l^{-1} BAP and 9%(w/v) sucrose

Temperature : 22 ± 1° C

Photoperiod : 8h

Irradiance : 11.5μM.m^{-2}.sec^{-1} PAR

Replicates : 4 petri dishes containing 50ml medium x 25 nodes each

Cultivar : Majestic

From the above data it can be concluded that :

(i) Shoot tips were equivalent to the nodes of the plantlet concerning the productivity and the mean fresh weight of the microtubers that were produced

(ii) The proportions of microtubers sprouting before harvest and showing abnormal elongations was lower than those produced from nodes

6.2.3 The effect of the age of the mother plantlet on *in vitro* tuberization

For commercial *in vitro* tuberization the age of the mother plantlet could have a significant effect on productivity and therefore on the economics of the process. Nodes of cv Majestic were therefore cultured on MS3 semi-solid medium for the following times before nodes were excised and transferred to the *in vitro* tuberization medium : 15,20, 25, 30, 35 and 50 days. 25 nodes were placed in each 14cm petri dish supplied by 50ml medium and there were four petri dishes for each treatment.

For tuberization the cultures were incubated for 90 days at $22 \pm 1^{\circ}C$ temperature, with an 9h photoperiod and 11.5 $\mu M.m^{-2}_{s}\ sec^{-1}$ PAR irradiance in MS semi-solid medium supplemented with 4mg.l BAP and 9%(w.v) sucrose.

The following measurements were made :

(i) productivity in plant material for each treatment (number of plantlets used for obtaining 100 nodes)

(ii) % productivity of tuberization cultures

(iii) mean fresh weight of microtubers

(iv) % microtubers sprouted before harvest

(v) % abnormal elongated microtubers

Figure 18. Effect of age of mother plantlet on *in vitro* tuberization of potato

Age in days	Number of plantlets for 10 nodes	Productivity(%)	Mean fresh weight(mg)	Sprouted microt.(%)	Elongated microt.(%)
15	48	99a	39.2a	11bc	10 c
20	34	97a	39.8a	17b	17a
25	27	100a	39.7a	6c	11bc
30	20	99a	47.3a	5c	6c
35	18	97a	39.1a	26a	16ab
50	11	98a	42.8a	5c	0d

Figures followed by the same letter do not vary significantly.

F : (i) for Productivity = 0.88

(ii) for mean fresh weight = 1.77

Lsd at p=.05 (i) for sprouted microtubers % = 8.5

(ii) for elongated microtubers % = 5.6(Appendix V-iii)

Culture Conditions :

Medium : MS semi-solid with 4mg.l^{-1} BAP and 9%(w/v) sucrose

Temperature : 22 ± 1°C

Photoperiod : 8h

Irradiance : 11.5μM.m^{-2}.sec^{-1} PAR

Replicates : 4 petri dishes x 25 nodes each

Cultivar : Majestic

Volume of medium : 50 ml each petri dish

From the data of this experiment it can be concluded that

(i) On increasing the duration of plantlet culture from 15 days to 50 days, the growth continued, thus increasing the number of available for subculture nodes; 48 plantlets were necessary for obtaining 100 nodes when 15 day old plantlets were used in comparison with 11 plantlets when 50 day old plantlets were used.

(ii) The productivity and the mean fresh weights of microtubers produced by these explants of various ages did not show any significant difference which means that any tested culture period would be suitable for providing nodes for in vitro tuberization.

(iii) Concerning the proportion of sprouted microtubers, the cultures derived from 20 and 35 day old plantlets gave significantly higher proportions. Nodes from 25, 30 and 50 day old plantlets gave very low proportions.

(iv) A rather similar situation occurred for abnormally elongated microtubers as for sprouted microtubers; the nodes from the 20 and 35 day

old plantlets gave the highest proportion of these abnormal microtubers and those from 30 and 50 day old plantlets gave the lowest.

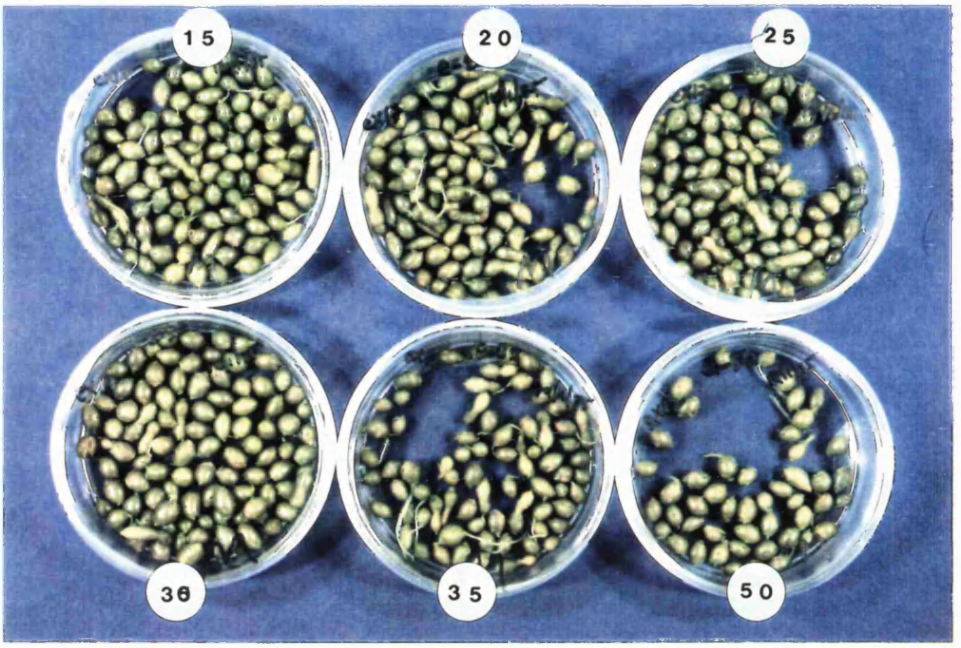
(v) The general conclusion from the above observations is that the most suitable material with regard to productivity, mean fresh weight and quality of microtubers would be the nodes excised from 30 and 50 day old plantlets.

Plate VII

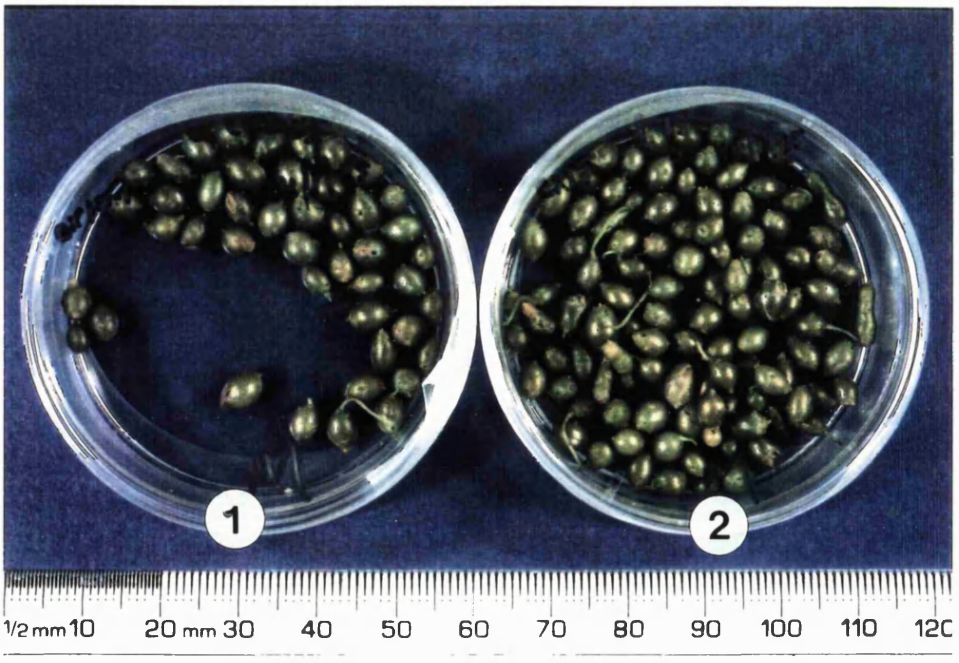
A: Microtubers of cv Majestic produced on MS semi-solid medium supplemented with 4 $mg \cdot l^{-1}$ BAP+9% (w/v) sucrose from nodes excised from plantlets of various ages. Age of mother plantlets as indicated on the plate.
Culture conditions: 22 ± 1°C, 8 h photoperiod, 11.5 $\mu M \cdot m^{-2} \cdot sec^{-1}$ PAR irradiance.

B: Microtubers of cv Majestic produced on MS semi-solid medium supplemented with 4 $mg \cdot l^{-1}$ BAP + 9% (w/v) sucrose from (1) shoot-tips and (2) nodes.
Culture conditions: 22 ± 1°C, 8h photoperiod, 11.5 $\mu M \cdot m^{-2} \cdot sec^{-1}$ PAR irradiance.

A



B



6.2.4 The effect of the presence of the leaf on the nodes on their tuberization ability

If groups of plantlets were to be chopped mechanically before their use for tuberization, varying proportions of nodes would lose their leaves. In this experiment the ability of these non leaf-bearing nodes to produce microtubers was tested.

Nodes of cv Majestic, with and without leaves excised from plantlets grown on MS3 semi-solid medium were used as inoculum. 25 nodes were placed in 14cm petri dishes containing 50ml MS semi-solid medium supplemented with 4mg.l BAP + 9% (w/v) sucrose and four petri dishes were used for each type of explant. The cultures were incubated for 90 days at 22 ± 1° C temperature, 8h photoperiod, 11µM.m^{-2}.sec^{-1} PAR irradiance.

Figure 19. Effect of the presence of the leaf on the nodes of their tuberization ability

Inoculum	Productivity (%)	Mean Fresh weight(mg)	Elongated microt.(%)
Nodes with leaf	97a	48.7a	16.0
Nodes without leaf	98a	48.3a	16.2

No sprouted microtubers were found at the harvest.

Culture conditions :

Medium : MS semi-solid with 4mg.l^{-1} BAP and 9%(w/v) sucrose

Temperature : 22±1° C

Photoperiod : 8h

Irradiance : 11µM.m^{-2} sec^{-1} PAR

Replicates : 4 petri dishes containing 50ml medium x 25 nodes

Cultivar : Majestic

For Statistical analysis : appendix V-iv

From the data in this experiment it was clear that, for the conditions of the experiment, the leaf did not play any role in the tuberization process that affected productivity, fresh weight or number of abnormal microtubers (sprouted or elongated).

6.2.5. The effect of the density of explants in the cultures on microtuber production

Of economic importance regarding commercial microtuber production is the determination of the optimal planting densities for the nodes on the tuberization medium, in terms of the effect on productivity, mean fresh weight and quality of microtubers and the most efficient exploitation of the medium.

For this investigation, nodes of cv Majestic excised from plantlets grown on MS3 semi-solid medium were cultured for tuberization on MS semi-solid medium supplemented with 4mg.l^{-1} BAP and 9% (w/v) sucrose.

The following densities of nodes per 20ml medium contained in each 9cm petri dish were used :

12, 16, 20, 24, 28, 36

Four petri dishes were used for each density.

These cultures were incubated at 22 $\pm$ 1° C with an 8h photoperiod and 1.3 $\mu M.m^{-2}.sec^{-1}$ PAR irradiance for 90 days.

The exploitation of the medium was calculated by :

(i) the weight of microtubers per ml of medium

(ii) the number of microtubers per ml of medium

The low irradiance was selected for the lowest possible interference of photosynthesis in the growth and productivity of the explants, especially at the higher densities.

Figure 20. Effect of the planting density of the nodes on *in vitro* tuberization

Number of nodes : per 20ml medium	Product-ivity (%)	Mean fresh weight (mg)	Sprouted microt. (%)	Elonga-ted microt. (%)	Weight of microt. per ml medium(mg)	Number of microt. per ml medium(mg)
12	100a	32.2a	0	8.3	19.3	0.60
16	100a	28.5b	0	1.6	22.8	0.80
20	98.8a	25.7c	1.3	1.3	25.4	0.99
24	96.9a	23.8d	0	2.2	27.7	1.16
28	95.5a	23.5d	0.9	0.9	31.4	1.34
36	95.8a	22.7e	0	0.7	39.1	1.72

Figures followed by the same letter do not vary significantly

Lsd for mean fresh weight at p=.05 : 0.88 (Appendix V-v)

Culture Conditions :

Medium : MS semi-solid with 4mg.l^{-1}BAP and 9%(w.v) sucrose

Temperature : 22± 1° C

Photoperiod : 8h

Irradiance : 1.3 µM.m^{-2}.sec^{-1} PAR

Replicates : 4 petri dishes containing 20ml medium

Cultivar : Majestic

From the above data it can be concluded that :

(i) On increasing the density of the nodes three times (from 12 to 36 nodes per 20ml medium), the productivity of the cultures remained unchanged at the level of approximately one microtuber per node.

(ii) The mean fresh weights of microtubers decreased gradually as the density increased, but at the highest density the mean fresh weight had only decreased by 30% .

(iii) The quality of microtubers, expressed by the proportion that were sprouted or elongated was not affected by the density of explants.

(iv) As far as the exploitation of the medium was concerned, it followed the increase in planting density ; on increasing the density three times, the weight of microtubers per ml of the medium increased twice and the number of microtubers increased approximately three times.

Overall, it can be concluded that for the conditions of the experiment the density of the explants in the tuberization medium can range between 12 and 36 nodes per 20 ml medium without any negative influence concerning productivity and quality of microtubers; the only factors that would have to be taken into account for the selection of the planting density would be the gradual reduction in the size of the microtubers as the

density increased and the efficiency of exploitation of the medium.

6.2.6 The effect of the orientation of explant growth on the production of nodes

From the preceding experiments it is evident that a productivity of practically one microtuber per one node can be achieved with the *in vitro* tuberization procedure.

It is therefore very important for large scale microtuber production that the most efficient method for the initial production of the nodes should be used. The type of container used for plant material culture and its influence on the orientation of growth and productivity of the shoot cultures and on the exploitation of incubator space, was therefore investigated in this experiment.

Nodes and apical shoot tips excised from plantlets of cv Spunta and cv Kennebec grown on MS3 semi-solid medium, were cultured in the two different containers containing MS3 semi-solid medium :

(i) Inverted "Twyford" container (14x10x7cm high)

(ii) 14cm petri dishes (1.9cm high).

Both types of containers were supplied with 50ml medium and inoculated with 25 explants. Four of each type of container were incubated at 22 ± 1° C with a 16h photoperiod and 140 $\mu M.m^{-2}$ sec^{-1} PAR irradiance and the cultures were grown for 30 days. The number of nodes produced by the 25 plantlets were measured.

The apical shoot tips were cultured separately from the nodes in order to detect any alteration in performance in comparison with the meristems of the nodes.

The two types of containers tested here, have approximately the same basal surface (140 and 153cm^2) but their height is very different, providing vertical and horizontal growth of the explants respectively for the inverted "Twyford" container, and the petri-dish.

Figure 21. Effect of the orientation of plantlet growth on node production

Cultivar	Type of explant	Container	Mean number of nodes per 100 plantlets
Spunta	Nodes	Inverted "Twyford"	328.5a
		Petri dish	623.0b
	Shoot tips	Inverted "Twyford"	383.0a
		Petri dish	639.0b
Kennebec	Nodes	Inverted "Twyford"	389.0a
		Petri dish	607.0b
	Shoot tips	Inverted "Twyford"	468.0a
		Petri dish	641.5b

Cultures conditions :

Medium : MS3 semi-solid

Temperature : 22 ± 1°C

Photoperiod : 16h

Irradiance : 140$\mu M.m^{-2}.sec^{-1}$ PAR

Replicates : 4 containers x 25 nodes each

Volume of Medium per container : 50ml

For statistical analysis : appendix V-vi

From the above data it can be concluded that :

(i) For both tested cultivars the cultures showing vertical growth (in inverted "Twyford" containers) gave significantly lower numbers of nodes than the cultures showing horizontal growth (in petri-dishes).

(ii) The promoting effect of the horizontal growth was found with both the nodes and the shoot tips

(iii) The differences in the productivity between vertical and horizontal growth ranging from 77% for cv Spunta and 46% for cv Kennebec strongly suggests the use of narrow flat containers for shoot cutting production. Using such containers significantly better exploitation of the incubation space is achieved; approximately 3.5 times as many plantlets can therefore be grown in a certain volume of incubator, producing 6.2 times as many nodes for cv Spunta and 5.2 for cv Kennebec.

6.2.7 Shoot culture production in liquid medium

Investigating the factors which could affect the production of shoot cultures for use in commercial scale *in vitro* tuberization, the use of liquid media was examined with the aim of improving the productivity of the cultures, reducing the cost of the media used and shortening the culture period. Agar is one of the ingredients of the medium which does not significantly provide nutrients necessary for plantlet growth, and its cost is high.

In this experiment the culture of plantlets in liquid media was investigated.

Nodes of the cvs Kennebec and Spunta excised from plantlets grown on MS3 semi-solid medium were cultured on two types of media :

(i) MS4 liquid medium

(ii) MS4 semi-solid medium

MS4 medium was used after preliminary studies (data not included) comparing MS3, MS4 and MS5 semi-solid media for shoot culture growth, which had shown that MS4 gave slightly more vigorous plantlets than either MS3 or MS5. 14cm petri dishes were used as containers with 50ml medium and 25 nodes each.

In the liquid medium 3 drops $.1^{-1}$ of Tween -80 was added before autoclaving for better spreading of the medium on the bottom of the dishes. No negative influence of

Tween-80 was noticed in preliminary studies (data not included) comparing the effect of medium with and without Tween-80. 4 petri dishes were used for each type of medium and the cultures were incubated for 18 days at 22± 1°C, with a 16h photoperiod and 140μM.m^{-2} sec^{-1} PAR irradiance.

Figure 22. Liquid media used for material production

Cultivar	Medium	Number of Nodes per 100 explants on 18th day	Mean length of nodes on on 18th day (mm)
Kennebec	liquid	606a	19.5a
	semi-solid	364b	13.0b
Spunta	liquid	583a	18.0a
	semi-solid	320b	12.5b

The thickness of the internodes was at least 50% greater in the liquid media.

Culture Conditions :

Medium : MS4

Temperature : 22± 1° C

Photoperiod : 16h

Irradiance : 140μM.m^{-2} sec^{-1} PAR

Replicates : 4 petri dishes x 25 nodes

Volume of medium per petri dish : 50 ml

For statistical analysis : appendix V-vii

From the above data it can be concluded that :

(i) The presence of the agar was not only unnecessary for the plantlet growth but it also inhibited it significantly.

(ii) The growth of the explants was accelerated in the liquid media giving approximately 66% more nodes for cv Kennebec and 82% more for cv Spunta; for this yield, 30 days of culture would normally be needed in semi-solid media.

(iii) The promoting effect of the liquid media was observed not only on the number of nodes but also on their length and on their vigour.

Applying this procedure for the cvs Bintje, Duke of York, Maris Bard, Desirree and Majestic, (data not included), similar results were obtained as for cvs Spunta and Kennebec.

6.2.8 Use of nodes from shoot cultures grown on liquid medium for *in vitro* tuberization on semi-solid media

After the promising results of the previous experiment (6.2.7) concerning the duration of culture, the vigour of the explants and the cost of the plant material production on liquid medium, this material was tested for *in vitro* tuberization.

Nodes of cvs Kennebec and Spunta excised from plantlets grown on MS3 liquid medium were cultured in MS semi-solid media for tuberization.

These media and incubation conditions were selected from those used in experiment 3.2.2 as follows (also see figures 6a, 6b):

(i) media which had given high productivity for cv kennebec (0 or 0.5 $mg.l^{-1}$ BAP + 6%(w/v) sucrose at 11 $\mu M.m^{-2} sec^{-1}$ PAR irradiance)

(ii) media which had given intermediate productivity for cv Kennebec (9%w/v sucrose without BAP at both irradiances 11.5 and 140$\mu M.m^{-2} sec^{-1}$ PAR)

(iii) media which had given low productivity for cvs Spunta and Kennebec (12%w/v sucrose without BAP at 11.5 and 140$\mu M.m^{-2} sec^{-1}$ PAR irradiance for cv Spunta and for cv Kennebec 12%w/v sucrose without BAP at 140$\mu M.m^{-2} sec^{-1}$ PAR irradiance and 2$mg.l^{-1}$ BAP + 6%w/v sucrose at 140$\mu M.m^{-2} sec^{-1}$ PAR

irradiance.

10 nodes were placed in each 9cm petri dish containing 20ml medium and four petri dishes were used as replicates. The cultures were incubated for 90 days at 22 ± 1°C with an 8h photoperiod and irradiance as above is described.

For comparison purposes the results of this experiment are given in figure 23 together with the results of the experiment 3.2.2 (figures 6a, 6b) in which the same cultivars, media and irradiance were used with nodes excised from cultures grown on semi-solid medium.

Figure 23. Use of nodes produced on liquid media for *in vitro* tuberization

cv	Medium BAP ($mg.l^{-1}$)	Sucrose (%w/v)	Irradiance ($\mu M.m^{-2}sec^{-1}$)	Productivity (%) I	Productivity (%) II	Mean Fresh weight (mg) I	Mean Fresh weight (mg) II	Abnormal elong.(%) I	Abnormal elong.(%) II
	0	6	11.5	105a	90.0a	125.8	69.8	0	0
	0.5	6		100	100.0	149.8	79.3	0	0
K	0	9 *		98a	85.0a	134.1	51.3	6	0
	0	9 *	140	85	85.0	201.2	52.5	0	0
	0	12 **		85	40.0	235.3	57.8	0	0
	2	6 **		100	67.5	129.5	48.3	3	70
Sp	0	12 **	11.5	100	7.5	180.5	25	0	0
	0	12 **	140	95	10.0	245.6	24.8	0	0

Code : I : Shoot cultures grown on liquid medium

II : Shoot cultures grown on semi-solid medium (experiment 3.2.2)

cv : cultivar

K : Kennebec

Sp : Spunta

Culture Conditions :

Basal medium : semi solid MS

Temperature : $22 \pm 1^{o}C$

Photoperiod : 8h

Replicates : 4 petri dishes containing 20ml medium x 10 nodes

* media on which intermediate productivity was obtained in experiment 3.2.2

** media on which poor productivity was obtained in experiment 3.2.2

For statistical analysis : appendix V-viii

Although care has to be taken in comparing the results from two separate experiments the following tentative conclusions can be drawn :

(i) The productivity of the nodes from plantlets grown on liquid medium increased considerably for both cvs and for all tested media which had given low productivity with nodes of plantlets grown on MS3 semi-solid medium.

(ii) The fresh weight of the microtubers for both tested cultivars and for all tested media also increased when nodes of plantlets grown in liquid medium instead of semi-solid medium were cultured for in vitro tuberization.

(iii) There was a low percentage of abnormally elongated microtubers in two of the tested media and in the remaining six media none were formed when nodes from liquid were used ; in the medium containing $2mg.l^{-1}$ BAP and 6%(w/v) sucrose at $140\mu M.m^{-2}.sec^{-1}$ PAR irradiance the proportion of elongated microtubers decreased considerably

when nodes from liquid medium instead of nodes from semi-solid medium were used.

Overall, it can be concluded that the quality of the plant material used as inoculum has an important effect on *in vitro* tuberization and the use of the liquid media for production of plant material can be recommended.

6.2.9 Use of semi-solid media of high fluidity for shoot culture production

The use of liquid media for shoot culture production showed very promising results concerning productivity, quality and cost and for subsequent *in vitro* tuberization. The use of liquid media, however is not always convenient and the depth of the medium in the containers has to be low in order to avoid the drowning of the small nodes during the early stages of the culture period.

To overcome this disadvantage, the use of semi-solid media of high fluidity was tested. This medium had to permit good contact with the explants when the nodes are mechanically distributed and yet it had to minimise the inhibiting effect of the agar, to avoid the drowning of the small nodes, and to permit easy transport of the containers.

In preliminary studies MS4 media supplemented with the following concentrations of agar oxoid No 3 were tested : 1, 2, 3, 4, 5 and 6 $g.l^{-1}$. After autoclaving, 20ml aliquots of the media were distributed in 9cm petri dishes and kept for ten hours at 20° C; after that time the medium with $1g.l^{-1}$ agar was rejected as being too liquid and the media with 4,5 and 6 $g.l^{-1}$ agar were rejected as being too solid.

The media containing 2 and 3 $g.l^{-1}$ agar were further

tested by distributing 20ml aliguots in two types of containers:

(i) 9cm petri dishes (height of medium-approx.0.3cm)

(ii) 100ml jars (height of medium-approx.1.6cm).

Nodes of cv Kennebec excised from MS3 semi-solid medium were scattered on these media and then were incubated at 22 ± 1° C temperature, 16h photoperiod, 140μM.$m^{-2}sec^{-1}$ PAR for 12 days. On the 12th day the numbers of grown plantlets with at least two nodes was measured. Five containers with 10 nodes were used as replicates.

Figure 24. Growth of plantlets on MS4 media of different fluidities

Agar ($g.l^{-1}$)	Container	Mean % of grown plantlets per container
2.0	Jars	92.5
	Petri-dishes	87.5
3.0	Jars	97.5
	Petri-dishes	100.0

Culture conditions :

Temperature : 22± 1° C

Photoperiod : 16h

Irradiance : 140μM.m^{-2}.sec^{-1} PAR

Replicates : 5 x 10 nodes

Volume of medium per container = 20ml

cv : Kennebec

After this test, nodes of cv Kennebec were cultured in MS4 medium supplemented with 3 or 7g.l^{-1} agar for 20 days under the same conditions as above described. Five 9cm petri dishes with 10 nodes each were used as containers; with the medium containing 3g.l^{-1} agar the nodes were just scattered on the medium but with the medium containing 7g.l^{-1} agar each node was placed with the basipetal end inserted 2-3mm into the medium.

Figure 25. Growth of plantlets on MS4 semi-solid media with 3 or 7g.l^{-1} agar

Agar (g.l^{-1})	Number of nodes from 100 explants on 20th day
3.0	592
7.0	380

Culture Conditions as in figure 24.

The plantlets on MS4 medium with 3g.l^{-1} agar were more vigorous than those in 7g.l^{-1} agar. From the data of these experiments it can be concluded that :

(i) MS4 medium of high fluidity ($3g.l^{-1}$ agar) is more convenient than MS4 with $7g.l^{-1}$ agar providing cultures of higher productivity and better quality.

(ii) Comparing the data of the figure 25 with the data of the figure 22 it can be suggested that MS liquid medium of high fluidity ($3g.l^{-1}$ agar) gave similar results concerning the production of nodes

Overall, it can be concluded that by using $3g.l^{-1}$ agar in comparison with $7g.l^{-1}$ agar in the MS medium for shoot culture production, mechanical distribution of the explants on the medium can be applied, resulting in a significantly higher yield and more vigorous explants for microtuber production. Further, the transportation of petri-dishes containing media with $3g.l^{-1}$ agar is much easier than with liquid media and there is no problem caused by the drowning of small nodes.

6.2.10 Use of plantgar polymer as an agar substitute for shoot culture production

After the promising results of experiment 6.2.9 where MS semi-solid medium of high fluidity was used for shoot culture production, a substitute of agar, the plantgar polymer was tested for use in MS4 medium of high fluidity.

In preliminary studies the concentrations of 2, 4, 6, 8, 10 $g.l^{-1}$ plantgar polymer gave media which failed to prevent the drowning of the small explants and concentrations of more that 16$g.l^{-1}$ plantgar polymer gave media which were too solid. Thereafter, the concentrations in the range of 12-16 $g.l^{-1}$ of plantgar polymer were compared with a concentration of 3 $g.l^{-1}$ agar.

Nodes of cv Spunta and Bintje excised from plantlets grown on MS4 semi-solid medium of high fluidity (3 $g.l^{-1}$ agar) were cultured on MS4 medium supplemented with 3 $g.l^{-1}$ agar or plantgar polymer at the following concentrations : 12, 14, 16 $g.l^{-1}$. Ten nodes were placed in 9 petri dishes containing 20ml medium and five petri dishes were used as replicates. The cultures were incubated at $20 \pm 1^{o}C$ with a 16h photoperiod and 20$\mu M.m^{-2}.sec^{-1}$ PAR irradiance for 25 days.

The number of grown plantlets having at least 3 nodes and the fresh weights of the explants (without their

roots) at the 26th day were measured. Also observations concerning the growth of the root system were made.

Figure 26. Use of plantgar polymer as agar substitute for shoot culture production

Cultivar	Number of grown plantlets (%)				Fresh weight of 100 plantlets (g)			
	plantgar polymer ($g.l^{-1}$)			Agar ($g.l^{-1}$)	plantgar polymer ($g.l^{-1}$)			Agar ($g.l^{-1}$)
	12	14	16	3	12	14	16	3
Spunta	66	62	82	96	18.3	24.5	26.7	40.6
Bintje	66	82	84	100	27.3	35.0	48.3	80.2

Culture Conditions :

Basal medium : MS4

Temperature : $20 \pm 1^{o}C$

Photoperiod : 16h

Irradiance : $20\mu M.m^{-2}sec^{-1}PAR$

Replicates : 5 x 10 nodes

Volume of medium per container : 20ml

The growth of the root system in the media with plantgar polymer was strongly inhibited; roots were rare and when they were formed they were shorter than the roots of

plantlets grown on media with agar.

Approximately 3-4 times more roots which were 8-10 times longer were formed on plantlets grown on medium with agar than on media with plantgar polymer.

From the above data it can be concluded that plantgar polymer cannot be recommended for use with the MS media for potato shoot culture production.

6.3 Conclusions

From the results of the experiments of this chapter the following can be concluded :

(i) All nodes including the apical shoot tip, have the ability when cultured for *in vitro* tuberization to produce one microtuber.

(ii) The lower nodes produced larger microtubers than the nodes near to the apical shoot tip.

(iii) The age of the mother plantlets in the range of 15-50 days did not affect productivity and size of microtubers produced from the nodes but it affected the proportion of elongated microtubers and sprouted microtubers giving evidence for a recommendation of ages of 30 and 50 days as being most suitable.

(iv) The leaf of the node did not influence the productivity, the size of the microtubers nor the proportion of elongated and sprouted microtubers.

(v) Density of explants in the tuberization media ranging from 12 to 36 nodes per 20ml of medium, did not affect the productivity of the nodes, but the size of microtubers was significantly affected and also the exploitation of the medium.

(vi) Horizontal growth of explants in narrow flat

containers (petri-dishes) resulted in a significantly higher number of nodes than vertical growth of explants in taller containers

(vii) Plantlets grown on liquid MS media gave significantly more nodes of better quality in comparison with plantlets grown on semi-solid media giving suggestion that agar inhibited their growth

(viii) Nodes of large size excised from plantlets grown on liquid medium gave significantly higher yield and large microtubers than smaller nodes excised from plantlets grown on semi-solid media.

(ix) Plantlets grown on semi-solid medium of high fluidity ($3g.l^{-1}$ agar) produced significantly higher number of nodes of better quality than those produced by plantlets grown on semi-solid medium ($7g.l^{-1}$ agar).

(x) Plantlets grown on semi-solid medium of high fluidity ($3g.l^{-1}$ agar) produced approximately the same number of nodes of the same quality as those produced by plantlets grown on liquid medium.

(xi) Plantgar polymer inhibited both the formation and growth of roots and the growth of plantlets when used as an agar substitute.

7. LIQUID MEDIA AND SEMI-SOLID MEDIA OF HIGH FLUIDITY FOR IN VITRO TUBERIZATION OF POTATO

7.1 Introduction

Wang and Hu (1982) using the layering method for potato tuberization in liquid media (details in Introduction 1.4) succeeded in the production of small size microtubers. Hussey and Stacey (1984) culturing nodes in liquid media (see section 1.4) produced large microtubers (up to 200mg) but they had distorted shapes and they precociously developed leafy shoots.

From the results of the experiments concerning shoot culture production in liquid media or in media of high fluidity (6.2.7, 6.2.8, 6.2.9) came strong evidence of the positive influence of these media on plantlet growth, and on microtuber production when nodes from those plantlets were cultured for in vitro tuberization.

In the experiments of this chapter, the use of liquid media or media of high fluidity ($3g.l^{-1}$ agar) for in vitro tuberization of potato nodes was investigated. Also the use of rice industry by-products instead of agar as support material in the tuberization media was examined.

7.2 Results

7.2.1 The use of two successive liquid media for *in vitro* tuberization

In order to avoid the abnormalities of microtubers produced from nodes on liquid media supplemented with BAP, as described by Hussey and Stacey (1984), the following two stage procedure was applied for *in vitro* tuberization on liquid media.

Nodes of the cvs Bintje, Kennebec and Desirée excised from plantlets grown on MS4 liquid media, were cultured for 12 days in MS4 liquid medium at 22±1° C temperature, with a 16h photoperiod and 140μM.m^{-2}.sec^{-1} PAR irradiance (first stage) and then the remaining MS4 medium was rejected and MS liquid medium supplemented with 4mg.l^{-1} BAP + 6%(w/v) sucrose was added to the containers. The cultures with the new medium were incubated at 22 ± 1° C temperature with an 8h photoperiod and 11.5μM.m^{-2}.sec^{-1} PAR till the senescence of the plantlets (68 days). As containers, 150ml jars were used containing 20ml of tuberization medium and 10 nodes each. The card-board liners were removed from the caps, which were loosely fastened to allow adequate gas exchange. During the first stage, 8ml medium was used in the containers so that the depth of the medium was kept low (4cm) to avoid the submergence of the nodes.

Figure 27. Use of two successive liquid media for *in vitro* tuberization of three potato cultivars

Cultivar	Microtuber productivity(%)	Mean fresh weight(mg)	Sprouted microt.(%)	Elongated microt.(%)
Bintje	100	192.7	0	0
Kennebec	100	161.5	0	0
Desirree	100	256.1	0	0

No adventitious microtubers were formed.

All microtubers were normal without any distortion

Culture conditions :

1st stage : Medium : MS4 liquid

Temperature : 22±1° C

Photoperiod : 16h

Irradiance : 140μM.m^{-2}.sec^{-1} PAR

Containers : 150ml jars

Replicates : 4 jars x 10 nodes

Volume of medium : 8ml per jar

Duration of culture : 12 days

2nd stage : Medium : MS liquid with 4mg.l^{-1}BAP + 6%(w/v)sucrose

Temperature : 22±1° C

Photoperiod : 8h

Irradiance : 11.5μM.m^{-2}.sec^{-1} PAR

Containers : 150ml jars

Replicates : 4 jars x 10 nodes

Volume of medium : 20ml per jar

Duration of culture : 68 days

From the above data it is clear that this procedure involving two-stage liquid media was satisfactory for the *in vitro* tuberization of the three tested cvs with regard to productivity, size and quality of microtubers; the productivity for all cvs was one microtuber per each node, their mean fresh weight ranged between 161.5 and 256.1mg depending on the cultivar and no abnormal microtubers were formed.

7.2.2. Use of various containers in the two-stage liquid media procedure for *in vitro* tuberization

After the results of the previous experiment and the results of experiment 6.2.6 concerning plantlets growth in various containers, the two stage tuberization procedure as described in the previous experiment was applied using nodes of cv Kennebec excised from plantlets grown on MS4 liquid medium and two types of container : (i) 14 cm petri dishes
(ii) 150 ml disposable jars
Four containers of each type containing 25 nodes and 20ml MS4 liquid medium for the first stage and 50ml liquid medium with 6%(w/v) sucrose + 4mg.l^{-1} BAP for the second stage, were grown for 12 days (1st stage) at 22±1° C with a 16h photoperiod and 140 $\mu M.m^{-2}.sec^{-1}$ PAR irradiance and then (2nd stage) at 22±1° C with an 8h photoperiod and 11.5$\mu M.m^{-2}.sec^{-1}$ PAR irradiance till the senescence of the plantlets. The card-board liners were removed from the caps of the jars, which were loosely fastened to allow adequate gas exchange and the nodes in the jars were placed vertically to avoid their submergence.
The plantlets in the petri dishes were senescent by 66 days from the establishment of the culture and the plantlets in the jars by 80 days, and at this time the

microtubers were harvested. Microtuber productivity, fresh weight of microtubers, number of sprouted and number of abnormal elongated microtubers were measured.

Figure 28. Use of various containers in the two-stage liquid media procedure for *in vitro* tuberization

Container	Microtuber productivity(%)	Mean fresh weight(mg)	Sprouted microt.(%)	Elongated microt.(%)
Jars	100a	160	0	0
Petri dishes	108a	320	0	0

No adventitious microtubers were formed.

Statistical analysis for microtuber productivity : appendix VI-i

Culture conditions :

1st stage : Medium : MS4 liquid

Temperature : 22±1° C

Photoperiod : 16h

Irradiance : 140µM.m^{-2}.sec^{-1} PAR

Replicates : 4 (x 10 nodes)

Volume of medium : 20ml per container

Duration of culture : 12 days

2nd stage : Medium : MS liquid + 4mg.l^{-1}BAP +
6%(w/v) sucrose
Temperature : 22±1° C
Photoperiod : 8h
Irradiance : 11.5μM.m^{-2} sec^{-1} PAR
Volume of medium : 50ml per container
Duration of culture : 54 days for petri dishes and 68 days for jars

From the above data it can be concluded that:

(i) Significantly larger microtubers were produced when petri dishes instead of jars were used.

(ii) The duration of the tuberization culture was shorter in the petri dishes than in jars because the plantlets were senescent respectively earlier in the petri dishes than in jars.

(iii) The microtuber productivity was not affected significantly by the type of containers.

7.2.3 One stage culture for tuberization on liquid medium

In order to simplify the procedure of tuberization on liquid media and supposing that distortions of the microtubers produced by Hussey and Stacey (1984) when they used liquid media were caused by BAP in the media employed, nodes of cvs Kennebec and Spunta excised from plantlets grown on MS4 liquid media were cultured for in vitro tuberization directly after their excision on MS liquid media supplemented with factorial combinations of the following BAP and sucrose concentrations :

(i) BAP ($mg.l^{-1}$) : 0, 0.05, 0.5

(ii) Sucrose(%w/v) : 4, 6, 8, 10 for cv Spunta and 4, 6, 8 for cv Kennebec

The cultures were grown in 14cm petri dishes, each containing 30ml medium and 15 nodes. 4 dishes were used for each medium/cultivar combination. The following incubation conditions were used :

Temperature : 22 ± 1^{o} C

Photoperiod : 8h

Irradiance : $20\mu M.m^{-2}\ sec^{-1}$ PAR

The incubation lasted for 90 days in order to obtain the maximum yield, but by 70 days the microtubers could have been harvested.

The concentrations of BAP were selected on the basis of

preliminary studies which showed that higher concentrations (1.0 and 2.0 $mg.l^{-1}$) caused a mass of callus formation on the explants.

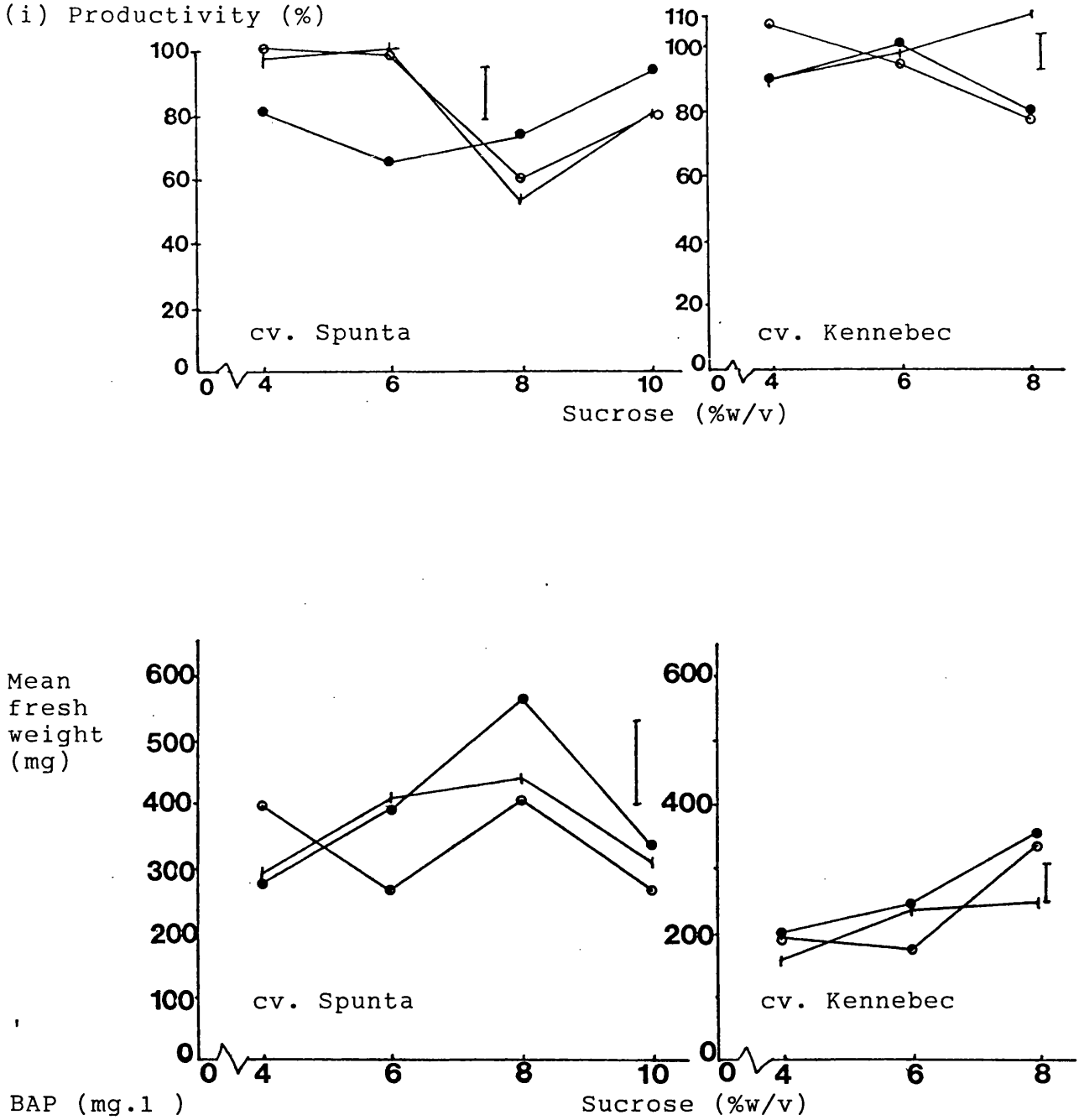
The numbers and fresh weights of microtubers, together with the numbers of elongated microtubers and percentage survival after 30 days storage at 4° C were determined.

Figure 29a. Tuberization of nodes on MS liquid medium supplemented with various concentrations of sucrose and BAP

Cultivar	Medium BAP ($mg.l^{-1}$)	Sucrose (%w/v)	Microtuber productivity (%)	Mean fresh weight (mg)	Elongated microt. (%)	Loss of microt. (%) by 30 days storage
Spunta	0	4	97a	293.3cd	0	3
		6	100a	405.8bc	3	7
		8	53e	439.0ab	0	42
		10	80bc	308.5cd	0	63
	0.05	4	100a	397.5bc	27	3
		6	98a	266.5d	18	16
		8	60de	404.5bc	19	61
		10	80bc	266.5d	8	38
	0.5	4	80bc	278.0cd	21	8
		6	65cde	389.0cd	14	31
		8	73cd	526.5a	18	36
		10	93ab	335.5cd	18	7
Kennebec	0	4	90cd	154.3e	0	0
		6	98bc	230.0bcd	2	0
		8	110a	244.8b	6	0
	0.05	4	107ab	187.8bcde	0	0
		6	95c	172.8de	0	0
		8	78e	335.0a	14	0
	0.5	4	90c	192.0bcde	0	0
		6	100abc	244.0bc	0	0
		8	80d	351.3a	0	0

Lsd at p=.05 for: a) productivity(%)for cv Spunta:16.3
b) mean fresh weight for cv Spunta:130.1
c) productivity(%)for cv Kennebec:11.33
d) mean fresh weight for cv Kennebec:61.35

Figure 29b. Interaction diagrams showing the effect on productivity and mean fresh weight of microtubers, of the interaction between Sucrose and BAP supplemented to MS liquid medium



Culture conditions :

Basal medium : MS liquid

Temperature : 22±1°C

Photoperiod : 8h

Irradiance : 20μM.m^{-2}.sec^{-1} PAR

Replicates : 4 petri dishes x 15 nodes

Volume of medium : 30 ml per each petri dish

Statistical analysis : appendix VI-ii

From the above data it can be concluded that :

(i) For cv Spunta a proportion of microtubers by 30 days storage were died because of drying and this problem did not appear in the microtubers of cv Kennebec. This drying of the microtubers was not severe at the low level of sucrose (4%w/v) but severe at the levels of 6, 8, 10% except with the medium containing 10%(w/v) sucrose and 0.5mg.l^{-1} BAP.

(ii) With regards to the microtuber productivity for both cultivars interactions between BAP and sucrose concentrations was found and higher productivities for cv Spunta gave the media with the factorial combinations of 0 and 0.05 mg.l^{-1} BAP with 4 and 6% (w/v) sucrose and the medium of 0.5mg.l^{-1} BAP x 10%(w/v) sucrose, and for cv Kennebec the media of 0 x 8, 0.05 x 4 and 0.5 x 6, concentrations of BAP (mg.l^{-1}) x sucrose %(w/v) respectively

(iii) Regarding the mean fresh weight of microtubers for both cultivars interaction was found between BAP and sucrose and a higher mean fresh weight for cv Spunta gave the media containing 8%(w/v) sucrose and 0 or 0.5mg.l^{-1}BAP and for cv Kennebec the media containing 8%(w/v) sucrose and 0.05 or 0.5mg.l^{-1} BAP.

(iv) Compared with the experiments with semi-solid media the microtubers, for the same volume of medium per node (2ml), were significantly larger; for cv Spunta their weight ranged between 266.5 and 526.5mg and for cv Kennebec between 154.3 and 351.3 mg, depending on the medium.

(v) The presence of BAP in the medium was not necessary for obtaining 100% productivity and large microtubers with either cultivar.

(vi) The proportion of elongated microtubers was high (8-27%) in the media containing BAP but not in the media without BAP (0-3%) for cv Spunta and for cv Kennebec it was high (14%) only in the medium containing 0.05mg.l^{-1} BAP + 8%(w/v) sucrose.

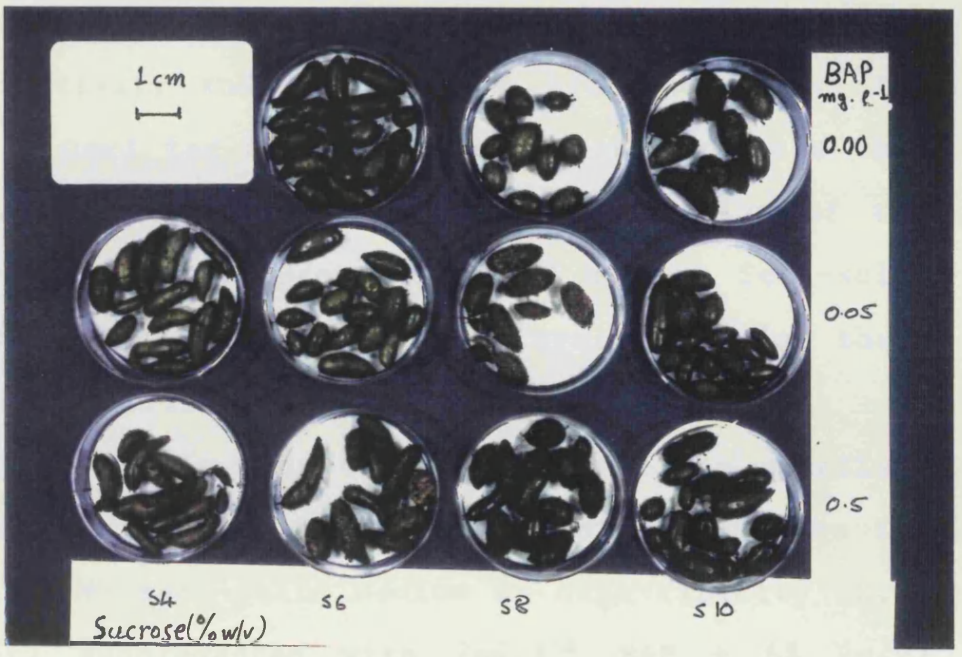
Plate VIII Microtubers produced on MS liquid media (one stage culture)

A: Microtubers of cv Spunta from media of various concentrations of BAP and sucrose

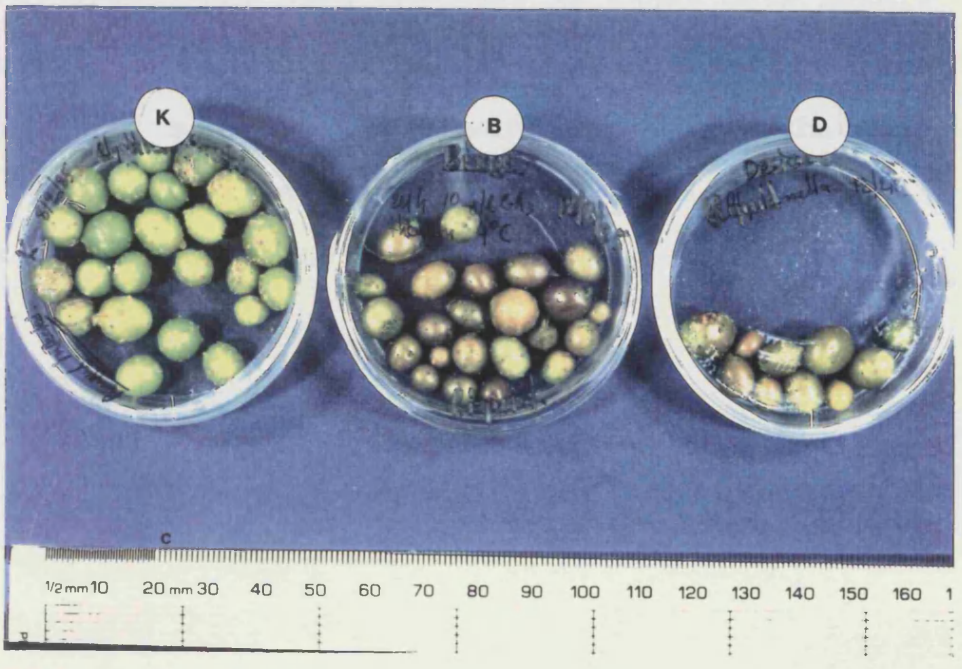
B: Microtubers of cvs: K: Kennebec, B: Bintje, D: Desirree.

Culture conditions: 22 ± 1°C, 8 h photoperiod, 11.5 $\mu M \cdot m^{-2} \cdot sec^{-1}$ PAR irradiance.

A


1 cm
BAP
mg. l-1
0.00
0.05
0.5
S4
S6
S8
S 10
Sucrose(% w/v)

B


K
B
D
1/2 mm 10
20 mm 30
40
50
60
70
80
90
100
110
120
130
140
150
160

7.2.4 Semi-solid media of high fluidity for *in vitro* tuberization

The liquid media had given promising results regarding the productivity and the size of microtubers and the lack of a need for BAP, but with certain vessels they can cause difficulties for the transportation of the cultures and the submergence of small nodes. Semi-solid media of high fluidity were therefore tested for their effects on tuberization.

Nodes of cv Spunta and Kennebec excised from plantlets grown on MS3 semi-solid medium were distributed on the surface of MS semi-solid medium of high fluidity (0.3% w/v agar) supplemented with $2mg.l^{-1}$ BAP + 6% (w/v) sucrose for cv Spunta and 8% (w/v) sucrose for cv Kennebec and as controls the same media were used with 0.7% (w/v) agar. For each cultivar 50 nodes were evenly distributed between five 9cm petri dishes each containing 20ml medium. They were incubated then for 90 days at 22 ± 1^{o} C temperature, with an $11.5\mu M.m^{-2}.sec^{-1}$ PAR irradiance and 8h photoperiod.

Plate IX (A) Node production and (B) microtuber production on MS media of high fluidity (0.3% agar Oxoid 3).

A: Plantlets grown on MS_4 medium of high fluidity at 22 ± 1°C, with a 16h photoperiod and 140 $\mu M \cdot m^{-2} \cdot sec^{-1}$ PAR irradiance. (cv Spunta)

B: Microtuber produced on MS medium of high fluidity supplemented with 8% sucrose at 22 ± 1°C, with an 8h photoperiod, 11.5 $\mu M \cdot m^{-2} \cdot sec^{-1}$ PAR irradiance(cv Kennebec).

A

B

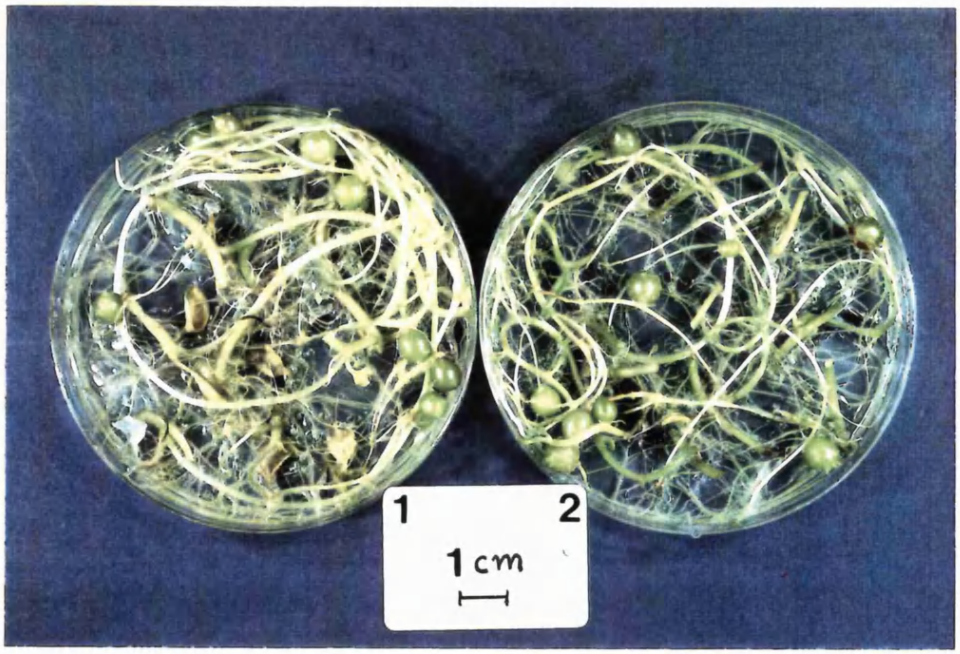


Figure 30. Effect of the fluidity of the MS semi-solid medium on *in vitro* tuberization

Cultivar	Agar (%w/v)	Microtuber productivity(%)	Mean fresh weight(mg)
Spunta	0.7	98	70.0b
	0.3	98	125.8a
Kennebec	0.7	90a	121.8a
	0.3	96a	136.6a

t value for :

(i) mean fresh weight of microtubers for cv Spunta : 3.97
(Significant difference)

(ii) productivity with cv Kennebec = 2.19
(No significant difference)

(iii) mean fresh weight with cv Kennebec = 1.07
(No significant difference)

Statistical analysis : appendix VI-iii

Culture conditions :

Basal medium : MS

Temperature : 22±1° C

Photoperiod : 8h

Irradiance : 11.5μM.m^{-2}.sec^{-1} PAR

Replicates : 5 petri dishes containing 20ml medium and 10 nodes

From the above data it can be concluded that for both cultivars the productivity was high (approximately one microtuber per node) for both fluidities, the mean fresh weight of microtubers for cv Spunta was significantly higher on the medium with 0.3%(w/v) agar, and for cv Kennebec no significant difference was found between the mean fresh weights of microtubers on the two media.

7.2.5 The use of a rice-industry by-product as a support material with liquid tuberization media

In an attempt to solve the problem of providing support for the small nodes, thus avoiding their submergence when cultured on liquid media, a rice-industry by-product was used as support material. This material which is produced during the threshing of paddy-rice, is very cheap and it keeps its shape after autoclaving. This material contains the husks of the paddy rice seed. Nodes of cvs Kennebec and Spunta, excised from plantlets grown on MS4 semi-solid medium of high fluidity (0.3%(w/v) agar), were cultured on two MS liquid media supplemented with :

(i) 8% (w/v) sucrose

(ii) 6% (w/v) sucrose and 0.5mg.l^{-1} BAP

As containers, 250ml Erlenmeyer flasks sealed with cotton bungs were used, each containing 20ml medium and 10 nodes. The rice-husks were added to the medium before autoclaving until they reached the surface. An agar solidified medium was used for comparison, with the concentration of agar (0.3% w/v) being selected to provide good contact with the explants when scattered on the surface.

Five flasks were used for each treatment and the cultures were incubated for 90 days at 20± 1° C

temperature with an 8h photoperiod and 20μM.m^{-2}.sec^{-1} PAR irradiance.

Figure 31. Rice-industry by-product (seed husk) as support material in tuberization media

Cultivar	Media BAP mg.l^{-1}	Media Sucrose (%w/v)	Support material	Microtuber productivity (%)	Mean fresh weight (mg)
Spunta	0	8	Rice-husks	57.5	20
			Agar	105	185
	0.5	6	Rice-husks	66	26
			Agar	95	292
Kennebec	0	8	Rice-husks	40	61
			Agar	102.5	150
	0.5	6	Rice-husks	34	32
			Agar	128	107

Culture conditions :

Basal medium : MS

Temperature : 20±1° C

Photoperiod : 8h

Irradiance : $20\mu M.m^{-2}.sec^{-1}$ PAR

Replicates : 5 flasks x 10 nodes

Volume of medium : 20ml each flask

From the above data it can be concluded that :

The productivity of the cultures on the media with rice-seed husks as support material was significantly lower for both tested cultivars and both tested media than on the media with agar and the mean fresh weight of the microtubers was significantly lower (2.5-11.25 times less) on the media with rice-seed-husks as support material for both tested cultivars and both tested media than on the media with agar.

Preliminary studies with vermiculite instead of rice-seed-husks (data not included) gave evidence that this material could be used as support material for <u>in vitro</u> tuberization but more work has to be done in this topic.

7.3 Conclusions

From the results of the experiments in this section it can be concluded that liquid media used for *in vitro* tuberization of single potato nodes, either in two-stage or in one-stage procedures, gave productivity of 100% (one microtuber from each node) and the microtubers were significantly heavier than the microtubers produced on semi-solid media.

The microtubers were larger when 14cm petri dishes instead of 150ml jars were used with the two-stage liquid media.

Cultures of nodes distributed on the surface of semi-solid medium of high fluidity (0.3%w/v agar) for tuberization in comparison with nodes cultures on semi-solid medium with 0.7%w/v agar, gave similar high productivity of approximately one microtuber per node for both tested cultivars, similar size of microtubers for the cv Kennebec but for cv Spunta on the medium with 0.3%w/v agar the microtubers were significantly heavier.

Rice-seed husks were significantly inferior as support material in the tuberization medium, in comparison with agar concerning productivity and size of microtubers.

8. DORMANCY OF MICROTUBERS

8.1 Introduction

Wang and Hu (1982) reported that the microtubers produced by the layering method (details in section 1.4) were dormant and Hussey and Stacey (1984) also reported that most microtubers produced on node cultures (details in section 1.4) remained dormant for approximately five months after harvest, irrespective of the storage temperature. The duration of the dormancy period and the method of breaking this dormancy were both examined in the experiments in this chapter, since they are both very important factors affecting the storage of the microtubers and their use.

8.2 Results

8.2.1 Measurement of the period of dormancy of microtubers

In this experiment the duration of the dormancy of microtubers was investigated. 35 microtubers each of cvs Majestic, Pentland Ivory and Pentland Squire produced on MS semi-solid medium supplemented with 4mg·l^{-1} BAP and 9% (w/v) sucrose from nodes incubated for 90 days at 22+1°C temperature, 8h photoperiod, 1.3 µM·m^{-2}·sec^{-1} PAR irradiance, were sown immediately after harvest in Levington compost mixed with 50% (v/v) sand and incubated at 18±1°C temperature, 16h photoperiod, 20µM·m^{-2}·sec^{-1} PAR irradiance.

After 20 and 60 days, the numbers of sprouted microtubers were measured and then all the microtubers were maintained under these conditions until all had sprouted. The time from the harvest to the sprouting of all the microtubers was measured for all the tested cultivars.

Figure 32 Dormancy period of microtubers

Cultivar	Percentage of sprouted microtubers 20 days after harvest	Percentage of sprouted microtubers two months after harvest	Time taken for all microtubers to sprout (days)
Majestic	0	34	110
Pentl.Ivory	40	45	95
Pentl.Squire	0	14	110

20 microtubers of the same cultivars maintained at the above incubation conditions in 9cm petri-dishes, sprouted at the same time as the microtubers sown in the Levington compost/sand mixture.

From the above data it can be concluded that:

(i) The microtubers in proportions ranging according to the cultivar from 0 to 40% were non-dormant immediately after harvest.

(ii) The total dormancy period of the microtubers varied, depending on the cultivar, from 95 to 110 days.

(iii) Dormancy was not broken simultaneously with any of the cultivars and the proportion of microtuber sprouting increased gradually over the total period of 95 to 110 days, depending on the cultivar.

(iv) For the conditions of the experiment, the micro tuber dormancy period was the same whether the microtubers were sown on damp compost/sand mixture, or kept into petri dishes without compost.

8.2.2 Application of GA_3 for breaking of dormancy of the microtubers 15 days after their harvest.

The storage capacity of normal potato tubers is considerably improved if they are cured after harvest for approximately 15 days at a temperature of 16 ± 2° C since during this period all cuts or wounds are healed. Similarly keeping the microtubers for 15 days at 16 ± 2° C for 15 days allowed their wounds to be healed.

30 dormant microtuber sample of cv Majestic, after their 15 days curing period were treated by dipping into different concentrations of GA_3 for varying periods of time as follows:

(i) GA_3 ($mg \cdot l^{-1}$) : 0.1, 1, 10

(ii) Dipping time : 10 minutes, 3 hours, 20 hours.

After the treatment the microtubers were transferred into wet vermiculite and incubated at 20±1° temperature, with a 16h photoperiod and 20$\mu M \cdot m^{-2} \cdot sec^{-1}$ PAR irradiance for 20 days. The microtubers were observed after 10 and 20 days and it was obvious that dormancy had not been broken by any of the treatments.

8.2.3 Application of GA_3 immediately after harvest for the breaking of microtuber dormancy

Since the ineffectiveness of the GA_3 treatment on microtuber dormancy, when applied after the curing period, might have been due to the impermeability of the epidermis of the microtuber, in this experiment the GA_3 treatment was applied immediately after harvest. Dormant microtubers of each of the cultivars Majestic, Bintje, Kennebec and Spunta, were dipped for 2 or 20 days in GA_3 solutions of concentrations 1 and 10 $mg \cdot l^{-1}$ for Majestic and 10 $mg \cdot l^{-1}$ for the remaining three cultivars.

After the treatment, the microtubers were transferred onto damp vermiculite and incubated at 20±1°C temperature with a 16h photoperiod and 20$\mu M \cdot m^{-2} \cdot sec^{-1}$ PAR irradiance.

At the 4th day, the number of sprouted microtubers, the proportions of the microtubers with one sprouted eye and the proportions of the microtubers with more than one sprouted eye were measured. 20 microtubers were used for each treatment.

Figure 33. Breaking of dormancy of microtubers by GA_3 treatment immediately after harvest

Cultivar	GA_3 concentration ($mg \cdot l^{-1}$)	Treatment time (h)	Percentage of sprouted microtubers	Percentage of microtubers with more than one sprouted eye
Majestic	1	2	80	25
	1	20	80	25
	10	2	100	20
	10	20	100	60
Bintje	10	20	80	25
Kennebec	10	2	40	0
	10	20	30	0
Spunta	10	2	30	0
	10	20	20	0

From the data in the figure 33 it can be concluded that:

(i) The GA_3 treatment immediately after harvest succeeded breaking the dormancy of the microtubers in proportions ranging from 20 to 100% depending on the cultivar.

(ii) The concentration of the GA_3 for the cultivar Majestic affected the proportion of the sprouted microtubers which ranged from 80 to 100%.

(iii) The proportion of microtubers producing more than one sprouted eye after the GA_3 treatment, varied from 0 to 60% depending on the cultivar; for

the cv Majestic the maximum proportion of such microtubers was found at the higher level of GA_3 ($10\ mg \cdot l^{-1}$) and longer-time treatment (20 hours).

8.2.4 Wounding of microtubers and GA_3 application for breaking their dormancy

After the results of the experiment 8.2.3 in would seem that the effectiveness of GA_3 in the breaking of the dormancy of the freshly harvested microtubers depends on the absorbtive capacity of the microtubers. The results of experiment 8.2.2 indicated that similar treatments were not effective with dormant microtubers in storage. In this experiment the breaking of dormancy of such dormant microtubers was investigated and in an attempt to solve the possible problem of impermeability of the epidermis of the microtubers to GA_3 the following procedure was applied:

30 dormant microtubers of each of the cvs Kennebec, Bintje, Spunta for 15 days after harvest were maintained at 18°C (curing period) and then, to test their germinability, they were incubated for 4 days at 20 ± 1° C with a 16h photoperiod and 20$\mu M \cdot m^{-2} \cdot sec^{-1}$ PAR irradiance on damp vermiculite.

None of these microtubers sprouted during that period and 20 of them were then wounded by slicing off approximately 2mm^2 tissue from the stolon-pole before being dipped into a GA_3 solution of 10$mg \cdot l^{-1}$ GA_3 concentration for 20 hours.

After this treatment their germinability was measured by measuring the number of sprouted microtubers when they were again incubated for 4 days on damp vermiculite as described above. Ten unwounded microtubers of each cultivar were treated by GA_3 as controls.

Figure 34. The effect of a combination of wounding and GA_3 treatment on the dormancy of microtubers

Cultivar	Sprouted microtubers (%)	
	Wounded	Control
Kennebec	95	0
Spunta	90	0
Bintje	85	0

All sprouts emerged from the apical eye giving one sprout each.

The above data indicate that GA_3 can be used very effectively to break the dormancy of microtubers that have undergone a curing period, as long as the epidermis is damaged to allow the penetration of the GA_3 solution.

8.2.5 Breaking of dormancy of microtubers by GA_3 application in the culture medium before harvest.

After the results of the previous experiment indicating that GA_3 would break the dormancy of the microtubers when absorbed though a wounded surface at the stolon-pole, an alternative method for the breaking of dormancy, involving GA_3 application in the tuberization medium before harvest, was examined.

Tuberizing cultures of cv Record, on MS semi-solid medium supplemented with 4 $mg \cdot l^{-1}$ BAP + 9% (w/v) sucrose, and of cv Kennebec, in the second stage of a procedure involving liquid media (see details in section 7.2.1), were treated with GA_3 four days before harvest. 2ml of 10 $mg \cdot l^{-1}$ GA_3 solution per microtuber were added to the tuberization medium in the case of cv Record and in the case of cv Kennebec the whole liquid medium was replaced by GA_3 solution of the same concentration 10 $mg \cdot l^{-1}$) in the amount of 2ml solution per microtuber. At harvest the number of sprouted microtubers was measured. Microtubers harvested from untreated cultures were used as control.

Figure 35. Breaking of dormancy of microtubers by GA_3 application into the tuberization culture before harvest.

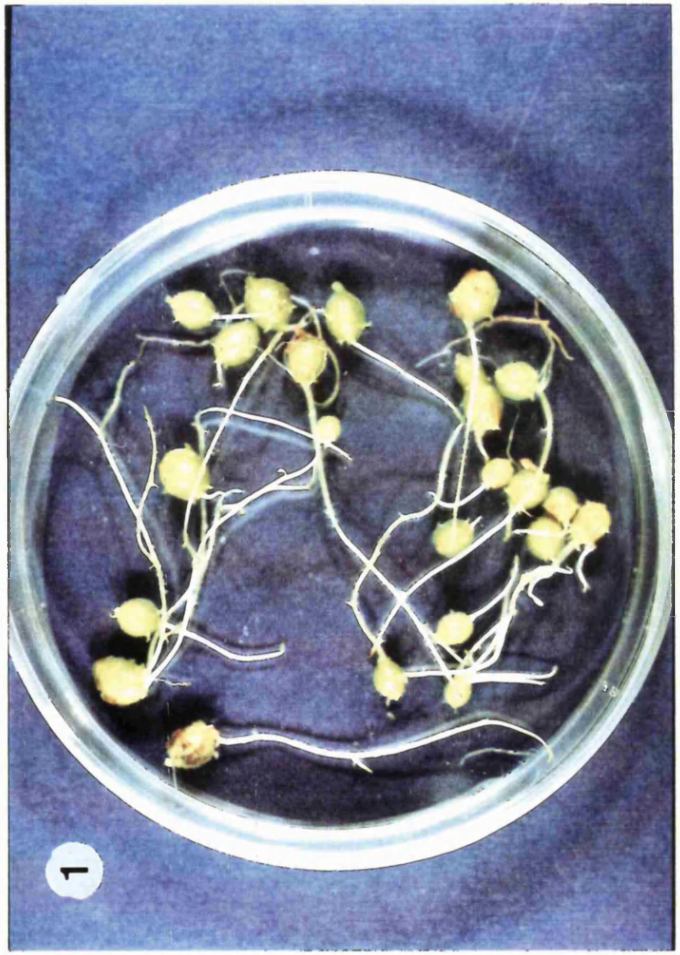
Cultivar	Number of treated micro-tubers	Number of untreated micro-tubers	Proportion of sprouted microtubers at harvest (%)	
			Treated microtubers	Untreated microtubers
Record	24	20	100	0
Kennebec	28	22	93	0

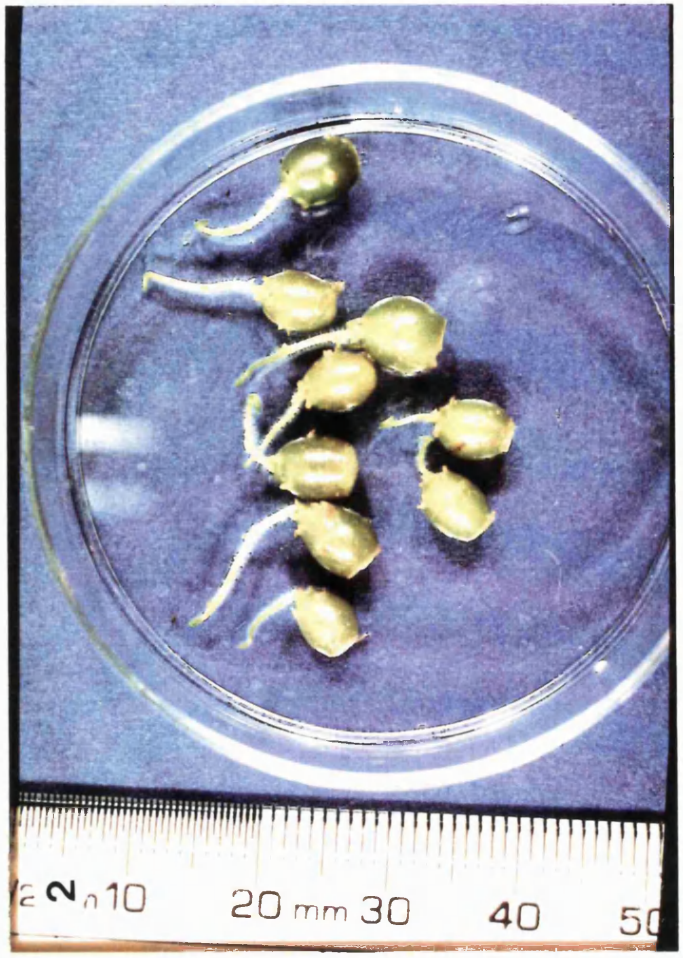
From the above data it can be concluded that for both tested cultivars the addition of GA_3 to the culture medium before harvest very effectively prevented the development of a state of dormancy in the microtubers.

Plate X Sprouted microtubers of cv Kennebec after GA_3 treatment

1: GA_3 treatment in the tuberization medium before harvest

2: GA_3 treatment on dormant microtubers which were sliced at the stolon-pole.


1


2
10
20 mm 30
40

8.2.6 GA_3 application into the tuberization medium before harvest and the production of unsprouted non-dormant microtubers for planting at any time.

After the promising results of the previous experiment, the further possibility was examined of producing non- dormant unsprouted microtubers for planting at any time. These microtubers would be stored at low temperature and when they were needed for planting they would just have to be transferred to favourable conditions.

Tuberizing cultures of cvs Kennebec and Bintje grown on the two successive liquid media procedure (see section 7.2.1. for details) were treated two days before harvest, replacing the medium by 10 $mg \cdot l^{-1}$ GA_3 solution in the ratio of 2ml medium per microtuber. At harvest the number of sprouted microtubers was measured and the microtubers were transferred to damp vermiculite for the sprouting test as in section 8.2.5.

At the 4th day the number of sprouted microtubers was measured; the sprouts were then excised and the microtubers were maintained under the same conditions for a further three days before the numbers of microtubers which had given new sprouts were measured. As control, 20 untreated microtubers fore each cultivar were used.

20 treated by GA_3 microtubers of the cv Bintje, directly after harvest, instead of planting in damp vermiculite were stored at 4°C for 20 days; then they were transferred to damp vermiculite and their germinability was tested at the 4th day as described in section 8.2.5.

Figure 36. Production of unsprouted non dormant microtubers by GA_3 treatment 2 days before harvest

Cultivar	Number of treated microtubers	Proportion of sprouted microtubers (%)			
		at harvest		4th day after harvest	
		Treated microtubers	Control	Treated microtubers	Control
Kennebec	30	0	0	100	0
Bintje	20	0	0	100	0

All microtubers which were stored at 4°C for 20 days, sprouted when transferred to damp vermiculite.

All microtubers at the germination test, sprouted only from the apical eye and microtubers from which the first sprout was excised, three days later gave sprouts from more than one eye.

From the above results it can be concluded that:

(i) GA_3 applied into the culture medium for two days before harvest prevented the initiation of dormancy with all treated microtubers for both tested cultivars.

(ii) The fact that the GA_3 treated microtubers, following their storage at 4°C for 20 days after harvest, sprouted under fovorable conditions, gave evidence that they had not become dormant.

(iii) When the apical sprout was excised, sprouts from more than one eye emerged indicating that more than one eye was not dormant.

8.3 Conclusions

From the results of the experiments of this chapter it can be concluded that the majority of the microtubers are dormant after harvest for a period varying for each cultivar; GA_3 can break the dormancy of the microtubers when it can be absorbed through a small wound at the stolon-pole. Alternatively, for some cvs (f.i.cv Majestic), dormancy can be broken or prevented by application of GA_3 immediately after harvesting, and for all tested cvs by application of GA_3 two days before harvest. Microtubers obtained in the latter manner and stored at 4°C for at least 20 days were still capable of sprouting soon planted under suitable conditions, indicating that it is possible to store them in a non-dormant state.

9. STORAGE OF MICROTUBERS

9.1 Introduction

The majority of microtubers remain dormant for a period of 3-5 months according to the results of experiment 8.2.1 and the results of Hussey and Stacey (1984). During the dormancy period the microtubers can be stored without difficulties but beyond this period the mictotubers would sprout and further investigation was required to indentify conditions suitable for longer periods of storage. The sprouting of microtubers means energy consumption and since for such small structures this loss would be damaging, the storage conditions must keep the non dormant microtubers alive, preferably in an unsprouted state or at least with very slow sprouting. In this section therefore, the survival of microtubers stored at various storage temperatures was investigated.

9.2 Results

9.2.1 Long-term storage of microtubers

The storage ability of not treated by GA_3 microtubers was examined by keeping them at various temperatures immediately after harvesting and testing their germinability or viability by sowing them on damp sand or vermiculite under favourable conditions (20±1°C temperature, 16h photoperiod, 20μM·m^{-2}·sec^{-1} PAR irradiance) after their dormancy period. The following four temperature regimes were used for the microtuber storage:

(i) 20 ± 1°C for 6 months (cvs Spunta, Kennebec, Majestic)

(ii) 6 ± 1°C for 6 months (cvs Spunta, Kennebec, Majestic)

(iii) 4 ± 2°C for 20 months (cvs Duke of York, Spunta, Bintje, Desirree, Jaerla, Kennebec)

(iv) 2 ± 1°C for at least 25 months (ten new potato clones).

9 cm petri dishes sealed with parafilm were used as storage containers to avoid excessive loss of water during the storage period. 40 microtubers of minimum weight 50 mg were used as replicates. During storage at 20±1°C the microtubers sprouted after their normal dormancy period and by the sixth month their sprouts had a length of more than 8cm. All of the microtubers planted at the end of the sixth month, produced plants which arose from the sprout which formed roots after planting.

With storage at 6 ± 1°C the growth of sprouts on the microtubers during the storage was slower than at 20 ± 1°C reaching a maximum length of 8cm at the end of the 6 month period.

All microtubers planted at the end of the 6 month period, produced plants.

Following storage at 4 ± 2°C for 20 months there was more than 90% survival of the microtubers for the cvs Duke of York, Spunta, Desirée, Bintje and Kennebec but there was only 65% survival for cv Jaerla (see plate XIB); this gives evidence that the optimum temperature for long term storage of microtubers depends on the cultivar.

Storage at 2 ± 1°C has succeeded in keeping alive for at least 25 months microtubers of 10 new potato clones at the Institute of Olive Fruit and Vegetables of Kalamata-Greece.

Form the above observations it can be concluded that:

(i) A temperature of 6 ± 1°C or 20 ± 1°C are suitable for storage of microtubers for periods at least as long as 6 months.

(ii) With a temperature of 4 ± 2°C it is possible to store microtubers for at least 20 months with survival rates of between 65% and more than 90%

(iii) At a temperature of 2 ± 1°C it has been possible to keep alive microtubers of 10 tested clones for at least 25 months.

Plate XI Storage of microtubers;

A: Dormant microtubers 15 days after harrest (cv Bintje).

B: Sprouted microtubers after 20 months storage at 4±2° C

cvs: D.Y: Duke of York

S: Spunta

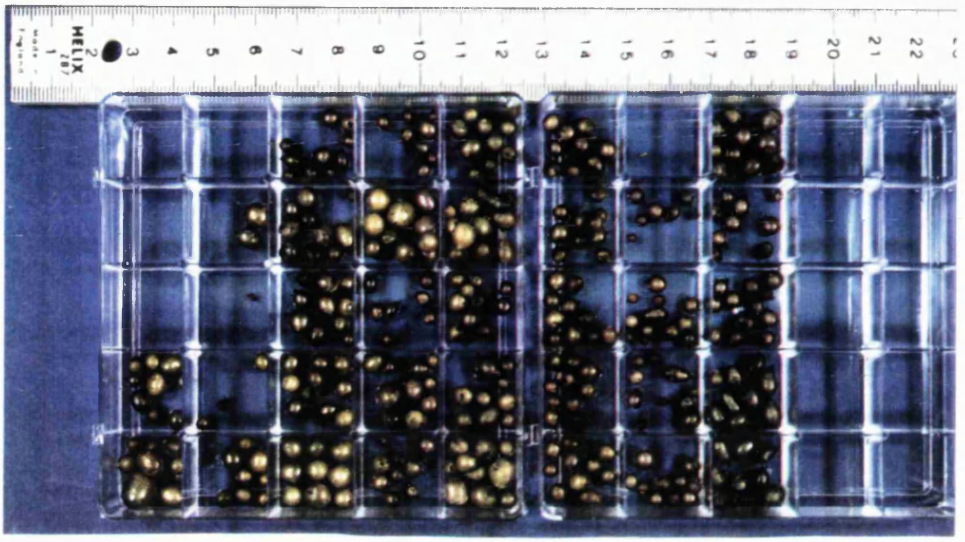
D: Desirree

B: Bintje

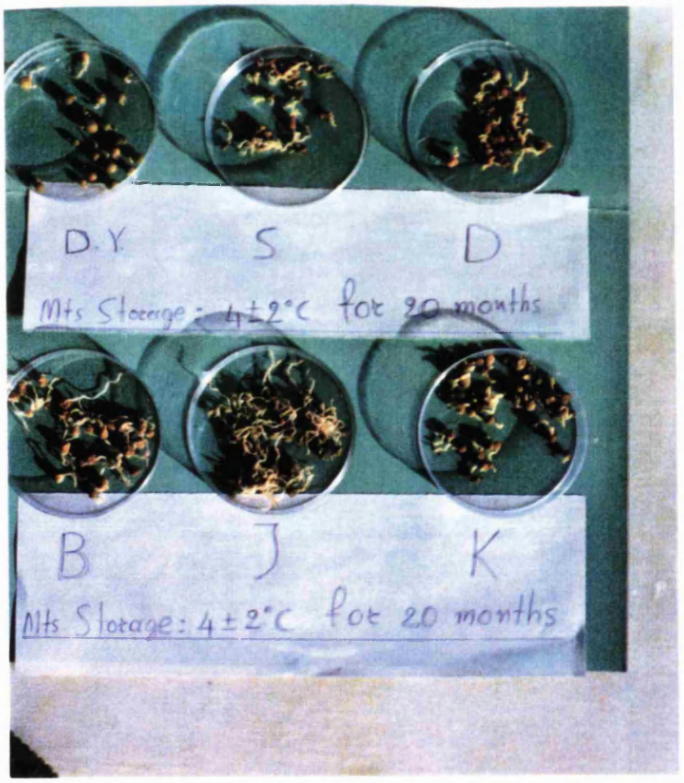
J: Jaerla

K: Kennebec

A



B


D.Y S D
Mts Storage: 4±2°C for 20 months
B J K
Mts Storage: 4±2°C for 20 months

9.3 Conclusions

From the experiment in this section it can be concluded that storing microtubers of minimum size 50mg at 2 ± 1°C temperature the storage capacity of the majority of these microtubers can be extended to 25 months for the tested cultivars.

10. USE OF MICROTUBERS IN SEED POTATO INDUSTRY

10.1 Introduction

Wang and Hu (1982) reported that from 36.000 microtubers after three successive plantings in soil, 1.800 Kg of virus-free seed- potatoes were obtained. It was the first time that microtubers have been used for large scale seed potato production. Wattimena et al (1983) investigating the yield of potato plants of two cultivars grown from three different propagation sources (tubers, shoot-culture microplantlets, microtubers) had found that although there were no differences in total yield among these materials, the number of tubers per plant for the micropropagated plants, from both microplantlets and microtubers, was greater than for the tuber-produced plants and the tubers respectively smaller.

The microtubers produced from virus-free material could substitute for the microplantlets as being easier for storage, transport and planting, and for the normal tubers as being less bulky and more reliable concerning freedom from virus infection. The small tubers produced on seed tubers during long-term storage at low temperatures are quite similar to microtubers in their properties.

For evaluation of the microtubers as plant material for use in the seed-potato industry these four alternative materials were tested by a field comparative trial.

Of great importance for the use of microtubers by the seed

potato industry would be the investigation of factors which could affect the productivity of plants grown from microtubers and this was the second topic which was investigated.

10.2 Results

10.2.1 Comparative trial with microplantlets, microtubers, small tubers and tubers for elite seed potato production

This trial was aimed at the evaluation of four alternative sources of planting material for use in the seed-potato industry. These planting materials were:

(i) rooted microplantlets grown on MS4 medium of high fluidity (0.4% w/v agar) at 20± 1° C temperature, with 16h photoperiod and 20μM · m^{-2}·sec^{-1} for 8 days

(ii) microtubers of 100 mg minimum fresh weight produced on MS_8 semi-solid medium of high fluidity (0.3% Agar) and allowed to produce 2-5 cm sprouts

(iii) "small tubers" of approximately the same size as the above microtubers produced on seed tubers during long-time storage at 4° C in the dark and allowed to produce 2-5 cm sprouts.

(iv) seed tubers (100-150 gr) out of dormancy and out of the dominance of the apical eye.

Two cultivars, Spunta and Kennebec, were tested for all materials except for "small tubers" which were only available for the cv Spunta.

As replicates for each category, 10 plants were grown in 15 l pots containing 15 Triohum-Potgrond P compost supplemented with nutrients as described in section 2.8.

Microtubers, microplantlets and "small tubers" (but not seed tubers), before planting in the pots, were planted in 280 ml Jiffy pots containing compost (without extra fertilizers)

and were kept in a growth chamber for 20 days at 16± 1° C temperature with a 12h photoperiod and 190 µM · m^{-2}·sec^{-1} PAR irradiance. The plantlets were then transfered from the jiffy pots to the pots and at the same time seed tubers were planted in the pots. This later planting of the seed tubers was aimed at keeping a balance in the growth of the different categories of plants since plants produced form seed tubers are faster in growth than those from the other three planting materials.

All above planting materials were virus tested and special care was taken to keep the plants free from diseases and well maintained (see for details sections 2.8, 2.9) The minimum temperature during the culture was 10° C and the maximum temperature 28° C. The experimental lay-out involved the completely randomised design of equal samples.

The following measurements were made after harvest:

(i) the number of tubers produced by each plant

(ii) the weight of tubers for each plant

(iii) the number and weight of tubers of minimum length 3 cm

(iv) the number and weight of tubers of minimum length 2cm (only for cv Kennebec).

The data were analysed statisticaly as in appendix VII

For cv Kennebec, tubers of minimum length 2cm can be used as elite seed tubers because they are round (but not for cv Spunta which has long tubers).

Figure 35. The yield of potato plants grown in compost from four different planting materials (average of 10 plants)

Cultivar	Number of tubers				Total weight of tubers (g)			
	Planting material				Planting material			
	a	b	c	d	a	b	c	d
Spunta	15.2c	26.8b	14.6c	32.6a	665.2b	630.6bc	620.4c	754.4a
Kennebec	9.8 b	17.8a	-	15.8a	597.0a	653.4a	-	671.4a

code: a: microplantlets

b: microtubers

c: "small tubers"

d: seed tubers

Figures on the same row followed by the same letter do not vary significantly (Appendix VII ia).

Lsd at p= 0.5 for: (i) number of tubers for cv Spunta : 3.54

(ii) number of tubers for cv Kenebec : 2.03

(iii) total weight of tubers for cv Spunta: 44.39

Culture conditions: replicates: 10 plants

compost :15 l Triohum-Potgrond P compost per pot. Microplantlets, microtubers, "small tubers" were cultured for 20 days in 280 ml jiffy pots with compost at 16± 1°C then in 15 l pots, but seed tubers directly in 15 l pots.

Culture temperatures: minimum : 10°C

maximum : 28°C

Figure 36. Number and weight of tubers of minimum length 3cm produced by potato plants grown in compost from four different planting materials

Cultivar	Number of tubers				Weight of tubers (g)			
	Material				Material			
	a	b	c	d	a	b	c	d
Spunta	13.8b	24.2a	14.2b	24.2a	662.0b	622.6b	619.5b	728.0a
Kennebec	7.6b	12.8a	-	10.6ab	585.0a	621.9a	-	644.3a

code: a: microplantlets, b: microtubers

c: "small tubers", d: seed tubers

Figures on the same row followed by the same letter do not vary significantly (Appendix VII ib).

Lsd at p= o.5 for: (i) number of tubers for cv Spunta : 2.75

(ii) number of tubers for cv Kennebec : 3.11

(iii) total weight of tubers for cv Spunta: 46.3

Experimental details as in fig.35

Figure 37. Number and weight of tubers of minimum length 2cm produced from potato plants of cv Kennebec grown on compost from three different planting materials.

	Plant materials		
	a	b	d
Number of tubers	9.0c	16.8a	14.0b
Weight of tubers (g)	595.2a	650.9a	667.3a

code: a: microplantlets, b: microtubers, d: seed tubers

Figures on the same row followed by the same letter do not vary significantly (Appendix VII ic)

Lsd at p = .05 for number of tubers: 2.19

Experimental details as in fig. 35

From the above it can be concluded that:

(i) Plants form seed tubers gave higher total numbers of tubers and higher yield than those from the remaining three planting materials for cv Spunta. For cv Kennebec no significant difference was found between plants from microtubers and seed tubers regarding the total numbers of tubers but there were significantly lower numbers of tubers for plants from microplantlets and no significant difference between the plants form the three planting materials regarding total yield.

(ii) Concerning the tubers of minimum length 3cm, plants from microtubers and seed tubers gave similar numbers (exactly equal for cv Spunta) which were significantly higher than the numbers produced by plants from microplantlets and "small tubers" and there were no significant defferences between the weights of tubers from the different types of planting materials for cv Kennebec but for cv Spunta the plants form seed tubers gave significantly higher weight of tubers.

(iii) Concerning the tubers of minimum length 2cm for cv Kennebec, plants form microtubers gave significantly higher numbers of tubers than plants from seed tubers which gave significantly higher numbers than plants from microplantlets. No significant differences were found between the yields form the different types of plants.

10.2.2 Tubers produced from plants grown from microtubers for elite II seed-potato production

In the previous experiment no significant difference was found between plants form microtubers and seed tubers concerning the numbers of tubers of minimum length 3cm for both tested cultivars and significantly higher number of tubers of minimum length 2cm gave the plants from microtubers than plants from seed tubers for the cv Kennebec. These tubers could be used for elite II seed-potato production in the field.

This experiment was aimed at determing any differences in the yield when tubers produced from plants grown from seed-tubers and microtubers were planted for elite II seed-potato production. Tubers of cvs Spunta and Kennebec of length between 3 and 6 cm, produces by plants from microtubers and seed-tubers, out of dormancy and out of the dominance of the apical eye, were planted in the field in the seed potato region of Taygetos mountain of Kalamata-Greece in a comparative trial of randomized complete-block design

The tubers were planted at a density of $10^5 \cdot$ hectare^{-1} with planting spacing 0.20 X 0.50 m .

Replicates: 4 X 20 plants

Fertilizers were applied as described in section 2.8.

Tubers of cv Bintje were planted at the same time as quard rows.

During the culture, the minimum temperature was 6°C and the maximum 24° C.

The treatments of the plants were as described in sections 2.8 and 2.9

After harvest the number of tubers and the yield in Kg were measured.

Figure 38. Elite II seed potato production from tubers of Elite I plants grown from microtubers and seed tubers.

	cvs			
	Spunta		Kennebec	
	Planting Material		Planting Material	
	From micro-tubers	From seed-tubers	From micro-tubers	From seed-tubers
Number of tubers from 20 plants	126a	132.5a	165.5b	171.8b
Yield of 20 plants (Kg)	9.4a	9.9a	8.8a	10.2a

Figures on the same row followed by the same letter do not vary significantly.

For statistical analysis: Appendix: VII ii

From the above data it can be concluded that no significant difference was found between the tested planting materials concerning both the number and yield of tubers for both tested cultivars.

Plate XII Elite I seed tubers produced from microtubers planted on compost in the greenhouse.
K: cv Kennebec, S: cv Spunta.
Density of planting: 100 microtubers·m^{-2}


ELITE I
K S
KALAMATA 1990

10.2.3 The effect of the density of the microtubers planted in nursery bench on their productivity.

From the previous experiments, 10.2.1. and 10.2.2. it was found that microtubers can substitute for seed-tubers for elite I seed-potato production. In this experiment the effect of the planting density of the microtubers on their productivity was investigated.

Microtubers of minimum size 100 mg of cv Kennebec, kept in storage at 2 ± 1° C for 12 months, were planted in an insect-proof greenhouse on benches of 25 cm height. At planting, the benches were covered to a depth of 15 cm by a mixture of 75% Hy-pot compost and 25% perlite and fertilizers were added as described in section 2.8.

When the plants had grown to approximately 20cm in height, mixture of compost was added to the benches up to their upper edge.

The following three densities of planting were tested:

(i) 25 microtubers · m^{-2} with 20 cm X 20cm spacing

(ii) 50 microtubers · m^{-2} with 20 cm X 10cm spacing

(iii) 100 microtubers · m^{-2} with 20 cm X 5cm spacing

Four replicates of 20 microtubers each were used.

during growth the plants were treated against diseases and they were irrigated as described in section 2.8.

the completely randomized equal samples design was applied and, as a quard-row, microtubers of cv Bintje were used.

The numbers of tubers and their weights per replicate (20 plants) were measured.

Figure 39. Effects of density of microtubers planted on compost for elite seed-potato production

Measurements	Densities (numbers of micro-tubers · m^{-2})		
	25	50	100
Number of tubers (20 plants)	181.25a	134.0b	80.5c
Weight of tubers (20 plants) in g	1567.5a	1100.0b	577.5c
Number of tubers · m^{-2}	226.6c	335.0b	402.5a
Weight of tubers · m^{-2} in g	1959.4c	2750.0a	2887.5a
Proportion of tubers longer than 2cm (%)	48.5a	50.0a	52.5a
Proportion of tubers longer than 3cm (%)	31.4a	31.2a	25.5a

The figures on the same row followed by the same letter do not vary significantly.

Lsd at p= .05 for: (i) number of tubers per 20 plants:24.93

(ii) weight of tubers per 20 plants: 24.93

(iii) number of tubers · m^{-2} : 40.77

(iv) weight of tubers · m^{-2} : 676.2

For statistical analysis details, see appendix VII iii.

From the above data it can be concluded that:

(i) The numbers of tubers and the weights of tubers per plant and the number of tubers per m^2 of the bench were significantly different for the three tested planting densities, with maximum productivity of the plants at the lower density and maximum number of tubers per m^2 at the higher density.

(ii) The weights of tubers per m^2 do not vary significantly for the densities of 50 and 100 microtubers · m^{-2} but there was significantly lower weight of tubers at the density of 25 microtubers per m^2.

(iii) The proportions of the tubers of minimum length 2 cm or 3 cm were not significantly different for the three tested planting densities.

10.2.4 The effect of the size of microtubers on the productivity of derived plants

It is apparent from the experiments concerning microtuber production, that many factors affect their size including the composition of medium, the density of the culture, the nature of the plant material etc.

In this experiment the effect of the size of microtubers on the productivity of derived plants was investigated.

Microtubers of the cvs Desirée and Spunta kept in storage at 2 ± 1°C for 9 months, of the following mean fresh weight at the harvest were tested:

(i) 20-40 mg

(ii) 80-110 mg

(iii) 150-180 mg

(iv) minimum 220 mg

Ten microtubers of each of the above sizes, were planted in 280 ml Jiffy pots with Triohum-Potgrond P compost and were grown in a growth chamber at 20 ± 1 C° with a 16h photoperiod and 60μM· m^{-2}· sec^{-1} PAR irradiance for 20 days. The plantlets were then transferred to 15 l pots with compost supplemented with fertilizers, as described in section 2.8, in an insect-proof greenhouse for further growth. Irrigation and other treatments were as described in sections 2.8 and 2.9.

At harvest the following measurements were made:

(i) number of tubers per plant

(ii) weight of tubers per plant

(iii) number of tubers of minimum length 2 cm per plant

(iv) number of tubers of minimum length 3 cm per plant

(v) weight of tubers of minimum length 2cm per plant

(vi) weight of tubers of minimum length 3cm per plant

The completely randomized-equal samples design was applied.

Figure 40. Effect of size of microtubers on the productivity of derived plants.

Measurements (per plant)	Cultivars							
	Spunta				Desirée			
	Size of microtubers				Size of microtubers			
	a	b	c	d	a	b	c	d
Number of tubers	6.6a	6.8a	9.2a	9.1a	4.1a	4.6a	5.6a	5.4a
Weight of tubers (g)	96.6b	123.4ab	177.0a	174.1a	76.7b	101.8ab	110.8a	118.0a
Number of tubers of minimum length 2 cm	6.6a	6.8a	9.2a	9.1a	3.9a	4.6a	5.2a	5.2a
Number of tubers of minimum length 3 cm	6.1b	6.4b	9.0a	9.1a	3.6a	4.4a	4.3a	4.3a
Weight of tubers of minimum length 2cm(g)	96.6b	123.4ab	177.0a	174.1a	75.1a	101.0a	108.4a	117.2a
Weight of tubers of minimum length 3cm(g)	90.2b	115.5b	171.9a	173.1a	73.5a	100.3a	104.5a	113.2a

Code for size of microtubers : a: 20- 40 mg

b: 80-110 mg

c: 150-180 mg

d : minimum 220 mg

Figures on the same row for each cultivar followed by the same letter do not differ significantly

Lsd at p = 0.5 for: (i) weight of tubers for cv Spunta :55.65

(ii) weight of tubers for cv Desirée:30.12

(iii) number of tubers of minimum length 3cm for cv Spunta : 2.46

(iv) weight of tubers of minimum length 2cm for cv Spunta : 55.65

(v) weight of tubers of minimum length 3cm for cv Spunta : 55.10

For statistical analysis details, see appendix VIIiv.

From the above data it can be concluded that:

(i) The numbers of tubers did not differ significantly among the four tested sizes of microtubers for both cultivars.

(ii) Microtubers of minimum fresh weight 80mg for both cultivars did not differ significantly concerning tuber weight per plant but smaller microtubers gave significantly lower tuber weights.

(iii) The numbers of tubers of minimum length 2cm did not differ significantly among the four tested sizes for both cultivars

(iv) The numbers of tubers of minimum length 3cm did not differ significantly among the four tested sizes for cv Desirée but for the cv Spunta the two higher sizes gave significantly higher numbers of tubers of minimum size 3cm than the two lower sizes.

(v) The weights of tubers of minimum size 2 cm did not differ significantly among the four tested sizes for cv Desirée and for the three higher sizes for cv Spunta but the microtubers of 20-40 mg for cv Spunta gave significantly lower weights of tubers of minimum size 2cm.

(vi) The weights of tubers of minimum size 3cm did not differ significantly among the four tested sizes for cv Desirée and for the two higher sizes for cv Spunta; the two lower sizes of microtubers for cv Spunta did not show significant difference, but they differed significantly than the weights of tubers of the two higher sizes of microtubers.

10.2.5 The effect of the dominance of the apical sprout of the microtubers on the productivity of derived plants.

During the experiment concerning the storage of microtubers. (9.2.1), the majority of microtubers after their dormancy period, sprouted giving only one sprout emerging from the apical eye.

In preliminary studies keeping 200 microtubers of the cvs Spunta and Kennebec for 10 months at 3 ± 1° C and measuring after that period the number of microtubers having one or more than one sprout, it was found that:

(i) for cv Spunta, 162 microtubers had one sprout and 38 microtubers more than one sprout.

(ii) for cv Kennebec, 187 microtubers had one sprout and 13 microtubers more than one sprout.

After this test, from 200 microtubers of the cv Kennebec stored at 3 ± 1° C for 6 months and which had formed one apical sprout, this sprout was excised and after ten days at the same temperature, the number of the microtubers having 1,2,3,4,5,6 or more sprouts was measured. From the 200 tested microtubers:

(i) 26 microtubers had only one sprout (sum of sprouts :26)

(ii) 48 microtubers had two sprouts (sum of sprouts :96)

(iii) 62 microtubers had three sprouts (sum of sprouts :186)

(iv) 30 microtubers had four sprouts (sum of sprouts :120)

(v) 20 microtubers had five sprouts (sum of sprouts :100)

(vi) 14 microtubers had at least six sprouts (sum of sprouts 84)

giving a total number of 612 sprouts.

After these tests the productivities of microtubers having only their apical sprout and microtubers for which this apical sprout was excised before planting, were investigated in the following comparative trial.

Sprouted microtubers of minimum fresh weight 100mg at harvest of the cvs Spunta and Kennebec stored at 3 ± 1° C for six months having either one apical sprout or having more than one sprout after the excision of the apical sprout, were planted in Jiffy pots with compost and grown for 20 days in a growth chamber at 18±1°C temperature with a 12h photoperiod and 190$\mu M \cdot m^{-2} \cdot sec^{-1}$ PAR irradiance and then transplanted in to 15l pots containing compost (BAS VAN BOUREN B.V. - pH 5.5-6.0) and transferred in an insect-proof greenhouse. Irrigation, fertilization and other treatments were as described in sections 2.8, 2.9. As replicates 10 microtubers were used.

At the harvest the following measurements were made per plant:

(i) number of stems

(ii) number of tubers

(iii) weight of tubers (g)

(iv) number of tubers of minimum size 2 and 3 cm

(v) weight of tubers of minimum size 2 and 3 cm

Figure 41. Effect of excision of the apical sprout of microtubers on the number of stems on derived plants and on the productivity of these plants (mean of 10 microtubers)

a. number of stems.

Cultivar	Untreated Microtubers	Treated Microtubers
Spunta	1	5.6
Kennebec	1.3	3.7

b. productivity of plants

Measurements	Cultivar			
	Spunta		Kennebec	
	Microtubers		Microtubers	
	Untreated	Treated	Untreated	Treated
Number of tubers	8.8b	18.7a	9.4b	23.1a
Weight of tubers (g)	139.1a	156.0a	161.3b	230.1a
Number of tubers of min. length 2 cm	6.3b	9.7a	6.5b	14.1a
Weight of tubers of min. length 2 cm (g)	136.2a	149.2a	155.7b	213.8a
Number of tubers of min. length 3 cm	4.3b	6.2a	5.0b	8.6a
Weight of tubers of min. length 3 cm (g)	127.9a	137.6a	143.2a	174.7a

Figures of the same row for each cultivar followed by the same letter do not differ significantly.

Details of the statistical analysis are in appendix VIIv

From the above data it can be concluded that:

(i) Alter the excision of the apical sprout, the microtubers formed significantly higher numbers of sprouts than the untreated microtubers.

(ii) The total number of tubers and the number of tubers of minimum length 2 or 3cm was significantly higher in both cultivars than for the non treated microtubers.

(iii) The weight of all produced tubers or tubers of minimum length 2 or 3cm did not differ significantly between treated and untreated microtubers for the cv Spunta but for cv Kennebec the treated microtubers gave significantly higher total weight of tubers and weight of tubers of minimum size 2cm than the untreated microtubers, and concerning the weights of tubers of minimum length 3 cm no significant difference was found between treated and untreated microtubers.

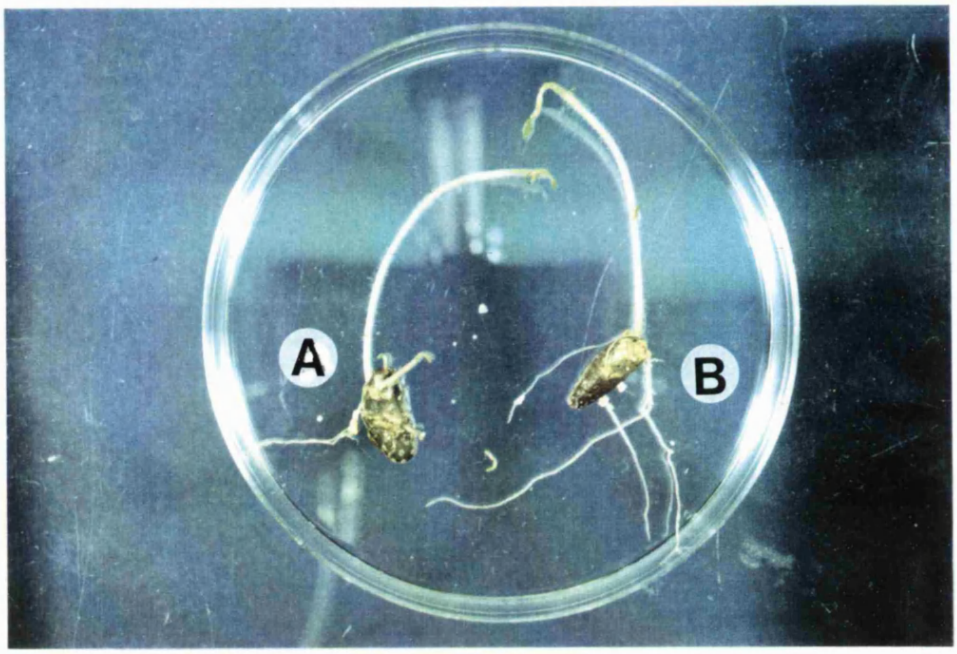
Plate XIII The effect of the dominance of the apical sprout of the microtubers on the productivity of the plants grown from them.

A colour: sprouted microtuber with four lateral sprouts after the excision of the apical sprout before planting (cv Spunta).

B colour: sprouted microtuber with only one apical sprout before planting (cv Spunta).

A B/W : yield of plants grown on compost in the greenhouse from microtubers of cv Spunta and Kennebec treated as above

B B/W : yield of plants grown on compost in the greenhouse from microtubers of cv Spunta and Kennebec untreated.



YIELD OF MTS WITH MORE THAN ONE SPROUTS

10.3 Conclusions

From the experiments in this section, it can be concluded that the microtubers produced plants compared with seed tubers, microplantlets and "small tubers" produced plants, gave significantly higher or equal number of tubers of size which can be accepted for elite II seed-potato production in the field (section 10.2.1).

No significant difference was found in the yield when tubers produced from seed-tubers and microtubers were planted for elite II seed potato production (section 10.2.2.). There were significant differences among the three tested planting densities for microtubers concerning the number of tubers and the weight of tubers produced per plant and the number of produced tubers per square metre of space but the proportion of tubers of minimum length 2 or 3 cm did not differ significantly (section 10.2.3). However the effect of the size of microtubers on the number of tubers of minimum length 2 or 3 cm produced per plant which is the main interest for the seed-potato industry, depends on the cultivar and the minimum weight of microtubers (section 10.2.4). The dominance of the apical sprout of the microtuber affects significantly the total number of tubers and the number of tubers of minimum length 2 or 3 cm produced from plants grown from these microtubers, so that the numbers increase significantly when this apical sprout is excised before planting (section 10.2.5).

11. DISCUSSION, CONCLUSION AND SUGGESTIONS FOR FUTURE WORK

11.1 Discussion

11.1.1 The effect of BAP, sucrose concentration and light on *in vitro* tuberization of potato

Main purposes of this work were to devise an effective practical way of producing microtubers for commercial use and at the same time to gain a better understanding of the mechanism of *in vitro* tuberization.

For commercial application an *in vitro* tuberization technique can be characterised as reliable if:

(i) it is applicable in practically any potato cultivar (first early, second early or maincrop).

(ii) the microtubers produced by this technique are non-adventitious in origin, thus ensuring the genetic stability of the propagation material.

(iii) The optimum conditions for each cultivar concerning productivity, size of microtubers, quality of microtubers and cost have been established.

Testing nine potato cultivars (6 maincrop, 1 second-early, 2 first-early) in nodal cultures grown on MS solid media supplemented with various concentrations of sucrose and BAP at 22 ± 1° C, 8h photoperiod and various irradiance levels as demanded by the experiments for 90 days, all tested cultivars

gave, in at least one of the tested conditions, a productivity of approximately one microtuber per node; no adventitious microtubers were formed with any cultivar or any medium; under very low irradiance (1.3 $\mu M \cdot m^{-2} \cdot sec^{-1}$) the presence of BAP was necessary for tuberization with all tested cultivars. It was concluded that each cultivar has its own optimum tuberization medium particularly with regard to sucrose and BAP concentrations and this affects on yield, productivity, mean fresh weight and quality of microtubers (low levels of elongated microtubers).

These results concerning sucrose concentration were broadly in agreement with those of Wang and Hu (1982, Tovar et al (1985), Estrada et al 1986). Rosell et al (1987), Garner and Blake (1989) suggesting 8% (w/v) as the optimum concentration for in vitro tuberization of potato and with those of Hussey and Stacey (1984), Abbot and Belcher (1986) suggesting 6% (w/v) sucrose. It was shown that there is a strong influence of genotype on the necessity of BAP for the induction of in vitro tuberization so at under otherwise similar conditions the cv Duke of York needed at least 0.5 $mg \cdot l^{-1}$ BAP to produce a high yield whereas cv Maris Bard did not require any cytokinin. Other factors which may also have significant effect on the requirement for cytokinin for tuberization include the incubation conditions (photoperiod, irradiance level, temperature and the kind of the plant material).

It is likely therefore that the requirement for the addition of exogenous cytokinin for tuberization is affected by the balance of endogenous growth regulators thus explaining the contradictions between reports suggesting either that

exogenous cytokinin is necessary for optimum tuberization (Wang and Hu 1982, Hussey and Stacey 1984, Tovar et al 1985, Estrada et al 1986, Abbot and Belcher 1986, Rosell et al 1987) or that it is not required (Garner and Blake 1989).

Concerning the effect of irradiance on tuberization, at both tested levels (11.5 and 140 $\mu M \cdot m^{-2} \cdot sec^{-1}$PAR) at least one medium was identified which gave 100% productivity; the mean fresh weight with this medium for cv Kennebec did not differ significantly between these two levels of irradiance but for cv Spunta the microtubers were significantly smaller at the higher level of irradiance. Li and Wang (1990) investigating the influence of light intensity on in vitro tuberization have also found that tuber number was not significantly affected by light intensity but that higher light intensity significantly enhanced tuber yield; however no further details were given concerning the tested levels of irradiance, the cultivars or the media. At lowest level of irradiance tested (1.3 $\mu M \cdot m^{-2} \cdot sec^{-1}$ PAR) a percentage of the microtubers had sprouted before harvest and this response was found to be inhibited by the use of the higher concentration of sucrose (12% w/v) but not by the concentration of BAP for all (four) tested cultivars. Precociously sprouted microtubers were not found in any of the cultivars when higher levels of irradiance (11.5 or 140 $\mu M \cdot m^{-2} \cdot sec^{-1}$) were used. As the sprouted microtubers are not dormant, they are unsuitable for any form of long term storage. Wang and Hu (1982) working with a light intensity of 100 Lux which is approximately equivalent to 1.3 $\mu M \cdot m^{-2} \cdot sec^{-1}$ did not make any comment about this type of response.

The microtubers, just as the normal tubers from the field, have a characteristic shape depending on the cultivar, but with some *in vitro* tuberization procedures abnormally elongated microtubers were formed. The proportion of these elongated microtubers has been found to be affected by the irradiance level and the concentration of sucrose and BAP and it increased as the sucrose concentration increased (from 6 to 12% w/v) and /or the irradiance level increased from 11.5 to 140 $\mu M \cdot m^{-2} \cdot sec^{-1}$ PAR) assuming that the BAP concentration was higher than a particular critical level, depending on the cultivar; for cv Spunta the critical level was 2 $mg \cdot l^{-1}$ BAP at both tested irradiance levels and for cv Kennebec it was 6 $mg \cdot l^{-1}$ BAP for 11.5 $\mu M \cdot m^{-2} \cdot sec^{-1}$ AR irradiance and 2 $mg \cdot l^{-1}$ BAP for 140 $\mu M \cdot m^{-2} \cdot sec^{-1}$ PAR irradiance.

These results are in agreement with those of Abbot and Belcher (1986) who found that elongated microtubers occurred mainly in long day high light (16h, 160 $\mu M \cdot m^{-2} \cdot sec^{-1}$) conditions; when the amount of light was halved in duration (8h) or in photon flux density (80 $\mu M \cdot m^{-2} \cdot sec^{-1}$) the incidence of long tubers was reduced to less than 20% and in dim light (3 $\mu M \cdot m^{-2} \cdot sec^{-1}$) or in the dark, long tubers were completely eliminated. No investigations concerning the effect of BAP concentration on this phenomenon were reported by these authors. Marinus (1190) working with *in vitro* tuberization has also reported the formation of abnormal tuber shapes, including long thin tubers, thickened branches instead of tubers or thickened apices of the stems in some media, but no more details were given.

11.1.2 Use of other cytokinins for *in vitro* tuberization of potato

Using kinetin or zeatin or zeatin riboside instead of BAP in the MS semi-solid media for *in vitro* tuberization of potato it was found that all of these cytokinins promoted tuberization, giving at least on one of the tested media maximum productivity of one microtuber per one node as in the media supplemented with BAP and the optimum concentrations of sucrose were 6 and 9% (w/v) als as on media supplemented with BAP. Zeatin riboside was found to be less effective than the other tested cytokinins and this agrees with the results of Koda and Okazawa (1983) and supports the hypothesis of Koda (1982) that butanol-soluble cytokinin is responsible for the subsequent vigorous thickening growth of the stolons to form tubers and that the water-soluble cytokinin (zeatin riboside) is a temporary storage form.

Concerning the promoting effect of kinetin, Palmer and Smith (1969) with excised stolons grown *in vitro* and Forsline and Langille (1976) with excised nodal segments of potato plants cultured *in vitro* obtained similar results, and Mingo-Castel *et al* (1986) working with etiolated sprout sections cultured *in vitro* found that kinetin promoted tuberization when sufficient sucrose was present in the medium. Hussey and Stacey (1984) working with node cultures also reported tuberization on media supplemented with kinetin, ancymidol, coumarin and sucrose at concentrations 45, o.1, 170 $\mu M \cdot l^{-1}$ and 6% (w/v) respectively. In the current investigation a high percentage of elongated microtubers (34-61%) was found in low kinetin concentrations in companion

with a low sucrose concentration (4% w/v) but this percentage was considerably reduced (6.7%) at 4.0 mg · l^{-1}) kinetin and with 9% (w/v) sucrose (0-3.4%) independently of the level of kinetin. Similarly with low levels of zeatin (0.05 and 0.5 mg · l^{-1}) a higher proportion of microtubers was elongated in comparison with higher zeatin levels (2.0,4.0 mg · l^{-1}), but these effects were complicated by a triple interaction of cultivar, sucrose concentration and cytokinin concentration.

11.1.3 The effect of mannitol, photoperiod and pH on the *in vitro* tuberization of potato.

Sucrose and mannitol were used in various combinations in the *in vitro* tuberization medium in order to investigate whether the sucrose at high concentrations (6 and 9% w/v) promotes tuberization by acting solely as a carbon source or whether its osmolarity is also important. It was found, however, that mannitol inhibited the growth of the explants even in the medium - supplemented also with 3% (w/v) sucrose which on its own is adequate for good plantlet growth and it inhibited tuberization at both tested levels of osmolarity (282,385 MOS), even in the media containing 6% (w/v) sucrose. There was no evidence in comparison with either 6% or 9% (w/v) levels of sucrose that mannitol could substitute in part for the effect of the sucrose on tuberization which would suggest either that the effect of the higher sucrose concentration is nutritional rather than osmotic or that the mannitol is toxic.

The results of this work are in complete agreement with the results of a similar investigation by Garner (1987) who, using mannitol to raise the osmolarity of MS + 4% (w/v) sucrose medium to that of MS + 8% (w/v) sucrose, found that the microtuberization was delayed by four weeks on media containing mannitol and by 14 weeks only a few microtubers had been formed compared to around twice as many on 4% (w/v) sucrose on its own and four times as many on 8% (w/v) sucrose. Similarly, Chandra *et all* (1988) found that 8% (w/v) sucrose was optimal while smaller microtubers were obtained on media with glucose of fructose and none with mannose or mannitol.

Even after this recent work, however, the role of sucrose on tuberization still needs clarification and possibly the use of an osmotic agent other than mannitol in combination with sucrose would provide useful information.

Regarding the effect of photoperiod on *in vitro* tuberization, it was found with three cultivars, on media optimized for an 8h photoperiod, that there was no significant difference between the effects of 8h and 16 h photoperiods on microtuber productivity, mean fresh weight of microtubers and percentage of abnormal elongated microtubers. In contrast, Hussey and Stacey (1984) had found that in long days (24h) the tuberization rate was lower than in short days (8h); Garner and Blake (1989), however, suggested that photoperiod does not have a major effect on number or weight of microtubers and although there were requirements for photoperiod in the tested two cultivars, one of which formed significantly more microtubers with 16h days than with 8h days, they concluded that the major effect of the 8h photoperiod was advance microtuber formation particularly in combination with 8% (w/v) sucrose. Abbot and Belcher (1986) suggested that photoperiod is among the determining factors in tuber formation and more BAP was needed in long (16h) days to achieve maximum tuberization in the tested early cultivars but not in cv Desirée. Tovar *et al* (1985) and Estrada *et al* (1986) recommend for tuberization absolute darkness for the last stage of a two stage process involving liquid media supplemented with BAP and CCC. On the other hand Rosell *et al* (1987) suggest continuous light of 5.000 Lux for semi-solid media supplemented with BAP, NAA, and/or CCC. Slimmon *et al*

(1989) comparing an 8h photoperiod with absolute darkness for in vitro tuberization of single node cuttings found that the level of tuberization averaged over all four tested cultivars was initially lower with the 8h photoperiod at 4 weeks but it was similar to the total darkness regime at 8 and 12 weeks and microtubers from all cultivars had a higher mean fresh weight when treated with the 8h photoperiod, although this difference was significant for only three of the four tested cultivars.

From the results of the present work concerning the effect of photoperiod on in vitro tuberization and the results of the above published work, it can be concluded that photoperiod can affect the tuberization process and that it interacts with other factors, including variety, growth regulator additions, irradiance which together affect the endogenous growth regulators controlling tuberization. A similar conclusion was arrived and by Menzel (1985) who suggested that high temperature low irradiance and long photoperiods have similar effect on plant growth and tuber production with all three influences having a common basis, possibly an increased gibberellin content which is inhibitory to tuber formation.

An other factor which was investigated for its effect on in vitro tuberization was the pH of the medium. Testing the initial pH values 4,5,6,7,8,9,10 with two potato cultivars, it was found that in the range between pH 5 and 10 for cv Spunta and between pH 4 and 9 for cv Kennebec no significant difference had showed concerning microtuber productivity and for pH values between 5 and 9 for cv Spunta and between 4 and 8 for cv Kennebec no significant difference had showed

concerning mean fresh weight of microtubers. The change of the pH values of the media after autoclaving and during the tuberization period from the initial values to values ranging between approximately pH 4.8 and 6 for both cultivars at harvest probably explains the good performance of the cultures at the lower and higher initial pH values.

11.1.4 Factors affecting productivity, quality and cost of microtubers in a commercial scale production.

Testing the effect on tuberization of the position of the node on the mother plant, there was not any significant difference between apical and basal nodes in terms of microtuber productivity; the microtubers from the basal nodes were heavier than those from the apical nodes but not sufficiently so to cause problems of quality if all nodes were to be used together for tuberization as would be the case if the plantlets were to be chopped mechanically in commercial scale microtuber production. These results are in agreement with results of Abbot and Belcher (1986) who also found that the position of the node on the initial plantlet stem did not have a significant effect on microtuber formation on the subsequent plantlets.

The effect of the age of the plantlets on the growth and subsequent performance of their nodes was tested with plantlets between 15 & 50 days in age and it was found that the number of the nodes produced increased as the age increased, but there were no significant differences among all tested ages concerning the productivity and mean fresh weights of the microtubers when these nodes were used for tuberization. The plantlets of 30 and 50 days age, however, gave significantly lower proportions of sprouted or elongated microtubers and since these factors determine quality, it would suggest that these ages would be preferable for tuberization.

The fact that the presence of the nodal leaf did not significantly affect microtuber productivity, size or quality (proportion of sprouted and elongated microtubers) gave further evidence that the mechanical chopping of the plantlets before their transfer to the tuberization medium would not cause any problems.

The investigation of the effect of the planting density of explants in the tuberization media on their subsequent tuberization showed that in the range tested (12-36 nodes per 20 ml medium), the exploitation of the medium, as measured by the number of tubers produced per ml of medium, increased as the density increased, and that the microtuber productivity was not affected by this factor, being approximately at the rate of one microtuber per node; however, the mean fresh weight of microtubers, as might be expected, decreased as the density increased. From these results it can be concluded that the required size of the microtubers rather than the productivity is the factor which would determine the planting density of nodes in the tuberization medium and therefore the cost of microtubers.

Comparing the performance of horizontally and vertically grown plantlets cultured for nodal material production, it was found that the plantlets of horizontal growth gave significantly higher numbers of nodes and this probably can be explained by the better nutrient absorption as a result of all of the nodes being in contact with the medium, rather than the basal node only as with the vertical plantlets. This allows, therefore, the use of narrow flat containers which is preferable also because their stackability permits better

exploitation of incubation facilities as well as of medium volume. This again has a beneficial effect on the cost of microtubers production.

From the experiment concerning the use of liquid instead on semi-solid medium for production of nodal material it was found that in liquid medium the plantlets grew faster giving a significantly higher number of more vigorous nodes. This is probably a result of the more efficient absorption of nutrients from the liquid medium over a larger surface area of the explants. Tovar et al (1985) Estrada et al (1986) also used liquid medium for plantlet growth before their incubation for tuberization and Rosell et al (1987) report that plantlets grown in liquid medium were much vigorous than those produced on semi-solid medium, with a well developed root system. Hussey and Stacey (1981) however, reported that the rate of node production was lower in liquid media and this contradiction with the results of this work might be explained by the sinking of small explants in medium of too great at depth. Thus adjusting the depth of the liquid medium in relation to the size of the nodes the advantages of faster and more vigorous plantlets with a subsequent beneficial effect on the exploitation of the medium and cost, can be achieved. The use of liquid instead of semi-solid media also leads to a further decrease costs because the precise positioning of the nodes in each container is not necessary. Special care must be taken, however, during the transportation of the cultures in narrow flat containers such as petri dishes because splashing can lead to microbial contaminations.

When nodes excised from plantlets grown on liquid medium were used for tuberization on semi-solid media on which nodes from semi-solid media gave low productivity, the productivity increased significantly and on all tested media and for both tested cultivars the microtuber fresh weight also increased significantly. These results give further strong evidence that the vigour of the nodes used for tuberization plays a very important role in the tuberization process.

Using semi-solid media of high fluidity (0.3% w/v agar) instead of more normal semi-solid media (0,7% w/v agar(for production of nodal material, the growth of the explants was more rapid and the plantlets were more vigorous. These results can probably be explained by the better contact with the medium, as with liquid media. On these media of high fluidity the contact of nodes even when randomly distributed is excellent, the drowning of the small nodes is avoided and the transportation of the cultures is much more easier than when liquid media are used. The use of these media seems, therefore, to minimize the disadvantages and maximize the advantages of the use of liquid media.

The plantgar polymer was not a suitable substitute for the agar in the media for plantlet growth because it inhibited root formation and the subsequent development of platlets.

11.1.5 Liquid media and semi-solid media of high fluidity for *in vitro* tuberization

With the use of liquid media for tuberization, either applying two-stage or one-stage procedures the upper limit of 100 mg mean fresh weight of microtubers produced on semi-solid media is significantly exceeded and microtubers of 150-500 mg mean fresh weight is the rule for microtubers produced on liquid media. When nodes are cultured for tuberization directly after their excision from the plantlets on liquid media the concentration of BAP must be very low (maximum 0.5 mg $\cdot$ l^{-1}), otherwise callus formation on the explants and inhibition of root formation negatively affects the tuberization process and at the same time distortions appear in the shape of microtubers. When two-stage cultures are used the tuberization medium is applied to well grown rooted plants in liquid media and the disadvantages of using high levels of BAP are avoided. The better exploitation of the nutrients from liquid media possibly helps the explants to produce the necessary endogenous compounds which are responsible for tuberization; this would explain the lack of a need for exogenous BAP for obtaining 100% microtuber productivity, the shortening of the time for tuberization and the large size of microtubers. By using liquid media for plantlet production or tuberization, the cost is reduced by the shortening of the incubation time, the avoidance of the use BAP and agar and the random distribution of the nodes in the medium, possibly

mechanically, and the value of the microtubers would be increased because of their significantly greater size.

Wang and Hu (1982) were the first workers to use liquid media for tuberization. The method involved the layering of whole plants in liquid media supplemented with 10 mg · l^{-1}BAP and 8% (w/v) sucrose; after a four month culture period at 20° C temperature with 8h photoperiod and very low irradiance (100 Lux), 30-50 microtubers weighing up to 10 g were obtained from each 500 ml flask but details concerning the volume of medium, number of explants per flask, mean fresh weight of microtubers and proportion of abnormal microtubers were not given. Hussey and Stacey (1984) reported that microtubers produced in liquid media were larger (up to 200 mg) than microtubers produced in semi-solid media, which is in agreement with the present results, but these microtubers had distorted shapes which could be explained by the high concentration of BAP ($2mg \cdot l^{-1}$) which was used. Tovar et al (1985), Estrada et al (1986), used two successive liquid media for microtuber production, but the procedure, which was very different from that used here, involved additions of GA_3, CCC and BAP, shaking instead of stationary conditions, darkness instead of an 8h day, and the microtubers produced in 50 days, ranged between 50 and 143.5 mg in weight depending on the cultivar. Rosell et al (1987) report also that liquid media invariably induced heavier microtubers than semi-solid media and the process of tuberization on MS or (White-Nitsch-Morel) liquid medium supplemented with 8% (w/v) sucrose, 2mg · l^{-1}BAP, 2 mg · l^{-1} NAA and/or 100 mg · l^{-1} CCC took only two months when nodal cuttings

were cultured at 24-25°C with continuous light of 5.000 Lux in two successive stages.

When semi-solid media of high fluidity (0.3 w/v agar) were used for tuberization in this work, the productivity for both tested cultivars was approximately one microtuber per node which was significantly higher than with medium containing 0.7% agar (w/v) for cv Kennebec but the same high for cv Spunta and the mean fresh weight of microtubers was significantly higher for cv Spunta and statistically equal for cv Kennebec. The size of microtubers in these media was for both tested cultivars more than 120 mg in weight but they were significantly smaller than microtubers obtained in liquid media. The semi-solid media of high fluidity promise high productivity and good size of microtubers, easy distribution of the nodes on the medium, possibly mechanically, lower cost because of the lower quantity of agar, easier transportation when narrow flat containers are used but , when microtubers of higher size are required it would be necessary to use liquid media.

The use of the rice-seed husks as cheaper support material than agar failed to give acceptable results with regard to productivity and size of microtubers when nodes were directly cultured for tuberization. Preliminary studies with vermiculite used as support material gave evidence that this material could be used for <u>in vitro</u> tuberization and that more work on the use of these cheap materials may improve the yield to levels permitting their substitution for the more expensive agar.

11.1.6 Dormancy of microtubers

Hussey and Stacey (1981) reported that *in vitro* microtubers of the cv Arran Pilot germinated immediately when after harvest they were planted in soil, but in their subsequent work (1984) they reported that most microtubers remained dormant for approximately five months after their harvest, irrespective of the storage temperature.

From the more than 10.000 microtubers produced during the present work it was also found that the majority (more than 90%) were dormant for a period of up to four months after harvest. Testing the duration of this period and the percentage of dormant microtubers after harvest for three cultivars cultured under the same conditions it was found that immediately after harvest a proportion of the microtubers ranging from 0 to 40% depending on the cultivar were non-dormant; subsequently the remaining microtubers gradually broke their dormancy until 100% were non-dormant after 95 to 110 days depending on the cultivar.

These results are in agreement with the results of the above authors and also with those of Tovar *et all* (1985), Estrada *et al* (1986), Rossel *et al* (1987) who reported that the microtubers were dormant after harvest. Abbot and Belcher (1986) reported that microtubers from senescing cultures germinated slowly and that short-day grown tubers sprouted more quickly than long-day grown tubers and higher BAP concentrations in the induction medium accelerated germination. Tovar *et al* (1985) and Estrada *et al* (1986), reported that microtubers produced in absolute darkness had

an average dormancy period of 210days instead of the 60 days of microtubers of the same cultivars produced with an 8h photoperiod.

From the results of this work and the reports of the above authors it can be concluded that the majority of microtubers for a period after harvest, depending on factors such as cultivar and tuberization conditions, are dormant and they cannot be planted before the end of this period.

Attempts to break the dormancy chemically by use of gibberellic acid by Tovar et al (1985) gave highly erratic results and these authors suggested that the most reliable and rapid way to break the dormancy is to store the microtubers in the dark in sterile petri-dishes sealed with parafilm at 4° C for between six weeks and ten months. Except for this report, no other works concerning the breaking of the dormancy of the microtubers have been published.

The experiments described in this work were focussed on the need to break the dormancy of microtubers by GA_3 treatment and to produce microtubers in a non-sprouted, , but also non-dormant state for long-term storage at low temperature so that they can be sprouted at any convenient time after harvest. Treating with GA_3 solutions of various concentrations and for various times immediately after harvest or 15 days after harvest or on microtubers wounded at their stolon-site 15 days after harvest, it was found that if GA_3 could be absorbed by dormant microtubers, the dormancy would be broken and it seems that the apparent ineffectiveness of GA_3 applied to dormant

microtubers in previous work was due to the mechanical reasons non absorption.

The strategy of applying GA_3 in the tuberization medium four days before harvest successfully broke the dormancy of the microtubers in proportions of 93 to 100% depending on the cultivar with the result that microtubers actually sprouted before harvest and applying the GA_3 two days before harvest, produced 100% non-dormant but unsprouted microtubers at harvest.

The latter microtubers sprouted 20 days after harvest when they were transferred to planting favourable conditions after storage at 4°C. When microtubers sprouted after their dormancy period or after the breaking of dormancy, the majority of them gave one dominant apical sprout which inhibited the sprouting of the lateral eyes but when this apical sprout was excised from sprouted microtubers which had been treated with GA_3,more of the eyes of the microtubers sprouted, giving evidence that the application of GA_3 succeeded in breaking the dormancy of all of the eyes and not only the dormancy of the apical eye. The strong dominance of the microtubers was also reported by Rosell et al (1986).

11.1.7 Storage of microtubers

The measurements of the storage capacity of the microtubers gave information about the relationship between storage temperature and duration of storage. At 6° C temperature the microtubers can be stored for use for a minimum period of six months; at 4° C, 65-90%, depending on the cultivar, of the stored microtubers of 6 cultivars were kept alive for a period of 20 months after harvest and at 2° C temperature the majority of microtubers of 10 potato clones were kept alive for at least 25 months. Information about the long-term storage of microtubers after harvest is not available from other reports but the above data provide evidence that microtubers would be very suitable for long-term (minimum two years) germplasm storage because they have the properties of the tubers, but being less bulky, they are easier to store, transport and plant in comparison with either tubers or microplants.

11.1.8 Use of microtubers in seed potato industry

The most important use of microtubers when they are produced for commercial purposes, is their use as multiplication material for elite seed-potato production. Wang and Hu (1982) reported such a use of microtubers on a large scale, indicating that 1800kg of virus-free seed-potatoes were obtained from 36.000 microtubers planted in soil and recently Wang et al (1991) also reported that a factory for large scale microtuber production has been established in China which has produced and sold to farmers since 1988 six million microtubers as prebasic seed for further multiplication. The evaluation of the microtubers as plant material and the investigation of factors which could effect the productivity of the plants grown from microtubers were among the main aims of the present work. The evaluation of the microtubers in comparison with alternative plant materials (normal seed tubers, microplants and "small tubers" produced from seed tubers stored at 4°C in dark, for both tested cultivars Spunta and Kennebec, concerned mainly the number of seed-grade tubers produced (minimum length 3cm for cv Spunta and Kennebec and 2 cm only for cv Kennebec), which is the most important measurement for the seed-potato industry, but also the weights of the seed-grade tubers, the total number of tubers and the total weights of tubers. This showed that microtubers and seed tubers gave equal numbers of seed-grade tubers of minimum length 3cm and significantly higher numbers than microplants and "small tubers" for both cultivars and with regard to the numbers of seed-grade tubers of minimum length

2cm for cv Kennebec, microtubers gave significantly higher numbers of tubers than both seed tubers and microplants; concerning the weights of seed-grade tubers, no significant differences were found among all the tested materials for cv Kennebec but for cv Spunta the seed tubers gave significantly higher weights; with regard to total number and to total weight of tubers, no significant differences between microtubers and seed tubers were found for cv Kennebec but for cv Spunta the seed tubers were exceeded significantly.

Wattimena et al (1983) comparing microplants, microtubers and tubers planted in soil found that microtubers and microplants gave statistically equal total numbers of tubers which were higher than those produced by normal tubers. There were no significant differences among the three tested planting materials concerning total yield but the yield of US1A size tubers was significantly higher with normal tubers than with microtubers and microplants, and the yield of US1B size tubers was significantly lower respectively, which means, according to these authors, that micropropagated plants produce smaller-size but a greater number of tubers; the numbers of seed-grade tubers were not given by these authors. There is a contradiction with the present work concerning the productivity of the microplants and microtubers; in the present work microtubers gave significantly higher numbers of tubers of all grades (total or seed grade) than microplants instead of equal numbers as in the work of Wattimena et al, which can be explained by the very small size of microtubers used by these workers (15-30 mg) instead of the larger size (minimum size 100 mg) used in this work. According to McCown

and Wattimena (1987), in a subsequent work, very small microtubers give only one stem which explains the significantly lower number and the weight of tubers produced from microtubers in comparison with microplants and multistem plants grown from seed-tubers when these materials were planted in the field. Haverkort et al (1991) comparing the productivity of seed tubers and microtubers preplanted or not before their planting in the field, have found the seed tubers gave higher yields than microtubers when these microtubers were directly planted in the field but yields within seed grades from plants grown from transplanted microtuber plants were comparable with those of conventional crops as was found in the present work.

When in the present work seed-grade tubers produced from plants grown from microtubers and seed tubers were planted in the field for further multiplication at densities of 100.000 tubers per hectare as recommended for seed potato production, no significant differences were found between these planting materials concerning both numbers of tubers and yield. From the results of this evaluation of microtubers of minimum size 100 mg, in comparison with microplants, seed-tubers and small tubers, strong evidence is given that microtubers can substitute for seed-tubers and microplants in the seed-potato industry in the initial step of elite seed-tubers production.

Concerning the effect on their productivity, of the planting density of the microtubers on a nursery bench, the numbers of tubers produced per plant and their total weights decreased linearly with increasing density between 25 and 100 microtubers per m^2, and at per unit area of nursery bench basis

these values increased linearly with increasing density but similar proportions of larger than 2 cm or 3cm tubers were produced among the three tested densities. These results are in complete agreement with the results reported by McCown and Wattimena (1987) who, testing the planting densities in soil of 7.4, 14.8,29.6,59.2 per m^2 with micropropagated plants, from both microplants and microtubers, found that the yield of tubers expressed on a per plant basis decreased linearly with increasing density, but no significant differences were found concerning mean tuber weight and percentage of US1A class (>113gr) tubers. Focussing the interest on the results of the present work concerning the numbers of tubers per unit area which is the most important value for seed-potato industry, even at the highest density of 100 microtubers per m^2 this value was still increasing and at the same time the proportion of seed-grade tubers was unchanged. This gave evidence that possibly even higher densities of microtubers could be used for elite seed-potato production when they are planted on greenhouse benches.

Planting microtubers of four potato cultivars at a density of 100 microtubers per m^2 in nursery beds in the greenhouse Tovar et al (1985) obtained from 195 to 648 tubers per m^2 depending on the cultivar which is in accord with the 402.5 tubers per m^2 obtained in the present work for the same density of microtubers. Allard and Blake (1991) planting sprouted microtubers of 0.2 -0.6 g in the field in rows 72 cm apart and within-row distances 22,36,51 or 65 cm found that wider spacing gave a highly significant increase in both numbers and weights of tubers harvested per plant and the number of seed-size tubers

per 30cm row
increased with the closer spacing which is in agreement with the results of the present work but these densities are lower than the appropriate ones for use in elite seed-potato production.

The effect of the size of the microtubers was investigated also in this work. Depending on the cultivar and the technique employed for microtuberization, the size of microtubers ranged from 15 mg to more than 500 mg, with this size affecting the cost of production.

In order to investigate the effect of the size of microtubers on the productivity of the plants grown from them, microtubers of fresh weight in four size ranges from 20 mg to more than 220 mg fresh weight, were planted in pots with compost in a comparative trial. From, this trial it was found that the total numbers of tubers and the numbers of tubers of minimum length 2cm did not differ significantly among the four tested size ranges; the numbers of tubers of minimum length 3cm did not differ significantly among the four tested size ranges for cv Desirée but for cv Spunta the two higher size ranges gave significantly higher numbers than the two lower. The total weight of tubers and the weight of tubers of minimum size 2cm did not differ significantly among the microtubers of minimum fresh weight 80 mg for cv Spunta and among the four tested size ranges for cv Desirée; the weights of tubers of minimum length 3cm did not differ significantly among the four tested size ranges for cv Desirée but for cv Spunta the microtubers of minimum size 150 mg gave significantly higher weights than smaller microtubers. Tovar et al (1985) planting microtubers of three sizes (3.3-7.7 mm diameter) in nursery beds in the

greenhouse obtained increasing numbers of tubers with increasing size (427,776,912 tubers of minimum weight 1g per m^2 of bed) although no statistical analysis is available. The microtubers used by these workers were rather similar to the lower two sizes used in the present work, but the results can not be compared accurately because of the different size ranges in the two investigations. Focussing the interest on the number of seed-grade tubers produced, depending on the cultivar it is apparent that there is a minimum size above which this value is not affected significantly.

The question of the dominance of the apical sprout which inhibites the emergence of sprouts from the lateral eyes of the microtubers was examined in the experiments of this work concerning the breaking of the dormancy of microtubers, and also by other workers (Wattimena et al 1983 , Rosell et al 1987), and the effect of this dominance on the productivity of the plants grown from microtubers was determined. From the results of this work it is clear that when the apical sprout is excised from sprouted microtubers, these microtubers give significantly higher numbers of sprouts than the untreated microtubers, and the total numbers of tubers and of seed-grade tubers subsequently produced, are significantly higher than from the untreated microtubers. Focussing on the number of seed-grade tubers, which is the most important consideration for the seed-potato industry, there is strong evidence that the microtubers before planting should be treated by excision of the apical sprout for optimising the number of seed-grade tubers produced.

11.2 Summary and Conclusions

(i) For each potato cultivar and for particular incubation conditions an optimum MS-based medium for productivity, size and quality of microtubers can be determined by testing the factorial combinations of the following concentrations of sucrose and one of the cytokinins BAP, kinetin, zeatin:

sucrose (% w/v): 4, 6, 8, 10

cytokinin ($mg \cdot l^{-1}$): 0, 0.05, 0.5, 2.0, 4.0

When liquid medium is used instead of semi-solid medium, the levels of 2.0 and 4.0 $mg \cdot l^{-1}$ cytokinin may be omitted.

(ii) An 8h photoperiod, 22±1°C temperature, 11.5 $\mu M \cdot m^{-2} \cdot sec^{-1}$ par irradiance can be used successfully for *in vitro* tuberization.

(iii) The initial pH of the MS tuberization medium can be in the range between pH 5 and 8 without any effect on microtuber productivity or size of microtubers.

(iv) Liquid MS media give a better yield in a shorter time, than semi-solid media, either for production of nodal material or for *in vitro* tuberization. Liquid tuberization media are ideal for the production of large microtubers (200-600mg depending on the cultivar).

(v) Flat narrow containers providing good gas exchange, are preferable for production of both nodal material and microtubers.

(vi) Mechanical chopping and distribution of the plant material either on MS liquid media or on MS semi-solid media of high fluidity, can be used for both nodal material production and for *in vitro* tuberization without any problem concerning productivity or quality.

(vii) Photoperiods between 8 and 16 h do not seem to affect microtuber production.

(viii) Inoculation densities of nodes ranging between 0.6 and 1.8 nodes per ml MS semi-solid tuberization medium do not affect microtuber productivity which can be almost one microtuber per node, but the size of microtubers decreases as the density increases.

(ix) The *in vitro* tuberization of potato can be particularly affected by the following factors: cultivar, sucrose concentration, incubation conditions, fluidity of medium, type of containers. For each cultivar there seem to be optimum levels of the remaining factors and when they coexist, the addition of exogenous cytokinin for tuberization seems to be unnecessary; evidence for this conclusion comes from the results of a large scale experiment on microtuber production (data not included) with ten potato cultivars (Majestic, Duke of York, Maris

Bard, Kennebec, Spunta, Jaerla, Desirée and two new clones) in which 1500 nodes of each cultivar were excised from plantlets grown on MS liquid medium, and incubated for tuberization on MS liquid medium supplemented with a high level of sucrose (8% w/v) without growth regulators, gave minimum productivity of 92% and minimum mean fresh weight of microtubers 160mg.

(x) Microtubers can be kept alive for at least two years if, after harvest, they are thoroughly cleaned with water, kept at room temperature for 15 days (for curing) and then stored at 2°C

(xi) Microtubers can be planted at anytime after harvest, without problems of dormancy, if the <u>in vitro</u> tuberization cultures are treated with GA_3 ($10\ m \cdot l^{-1}$) two days before harvest and the microtubers are stored at 2°C.

(xii) Planting microtubers on nursery beds in the greenhouse at a density of 100 microtubers per m^2 for elite seed potato production, gives the optimum exploitation of media, space and greenhouse equipment, and planting at the density of 25 microtubers per m^2 the microtubers give the best yield per plant. Densities range between 25 and 100 microtubers per m^2 affect the number of tubers per plant and per m^2 of the nursery bed, without affecting the proportion of seed-size tubers produced.

(xiii) Concerning the use of microtubers for large scale elite seed potato production, the minimum size of microtubers which gives the best yield depends on the cultivar but the minimum size of 150 mg seems to be ideal for any cultivar.

(xiv) The apical sprout of the microtubers is dominant on the sprouting of the lateral eyes; after the excision of the apical sprout, the microtubers form significantly higher numbers of sprouts and the derived plants give significantly higher total numbers of tubers and number of elite seed-grade tubers.

(xv) Following the above procedure concerning the density of planting of microtubers, the size, the excision of the apical sprout and planting in nursery beds for elite seed potato production, the microtubers can successfully substitute for normal seed tubers or microplants in the seed potato industry.

11.3 Future work

It can now be concluded that the microtubers can successfully substitute normal seed-tubers and microplants for multiplication of valuable virus-tested breeder's material, for elite seed potato production in the greenhouse, for germplasm exchange and germplasm conservation. For use however, in the seed potato industry for elite seed potato production more work would have to be done concerning the productivity of *in vitro* tuberization cultures and the cost of microtuber production.

The first topic for future research could be the mechanization of the procedure, with three main points being investigated: the optimisation of the size and the shape of the containers, the use of mechanical chopping and mechanical distribution of the nodal material on the medium. Containers and machines should designed for use with liquid media for both the nodal material and microtuber production; agar may not be used because of its high price and because liquid-media are better adapted for *in vitro* culture, giving a higher yield and better quality products in a shorter time.

The second topic could concern the breaking of the dominance of the first microtuber formed on the explants in the tuerization cultures. All works concerning tuberization cultures until now have only achieved a productivity, at best of one microtuber per explant where there is more than one node, but potato plants in the greenhouse or in the field produce 4-10 tubers or more depending on the cultivar and the conditions. In the *in vitro* tuberization procedure the first

formed microtuber seems to inhibit the formation of other microtubers on the plantlets and if this dominance could be broken, the productivity of the microtuberization system would increase considerably and the need to cut the explant to single nodes for the tuberization stage would be removed.

The use of the *in vitro* tuberization procedure for other purposes such as the determination of the earliness of potato clones could be of interest in potato breeding because the procedures would be less laborous, less expensive, more rapid and independent of the season and the field conditions.

Investigations into the mechanism of the promotive effect of the sucrose on microtuberization by the use of osmotic agents other than mannitol could be an other topic for future work.

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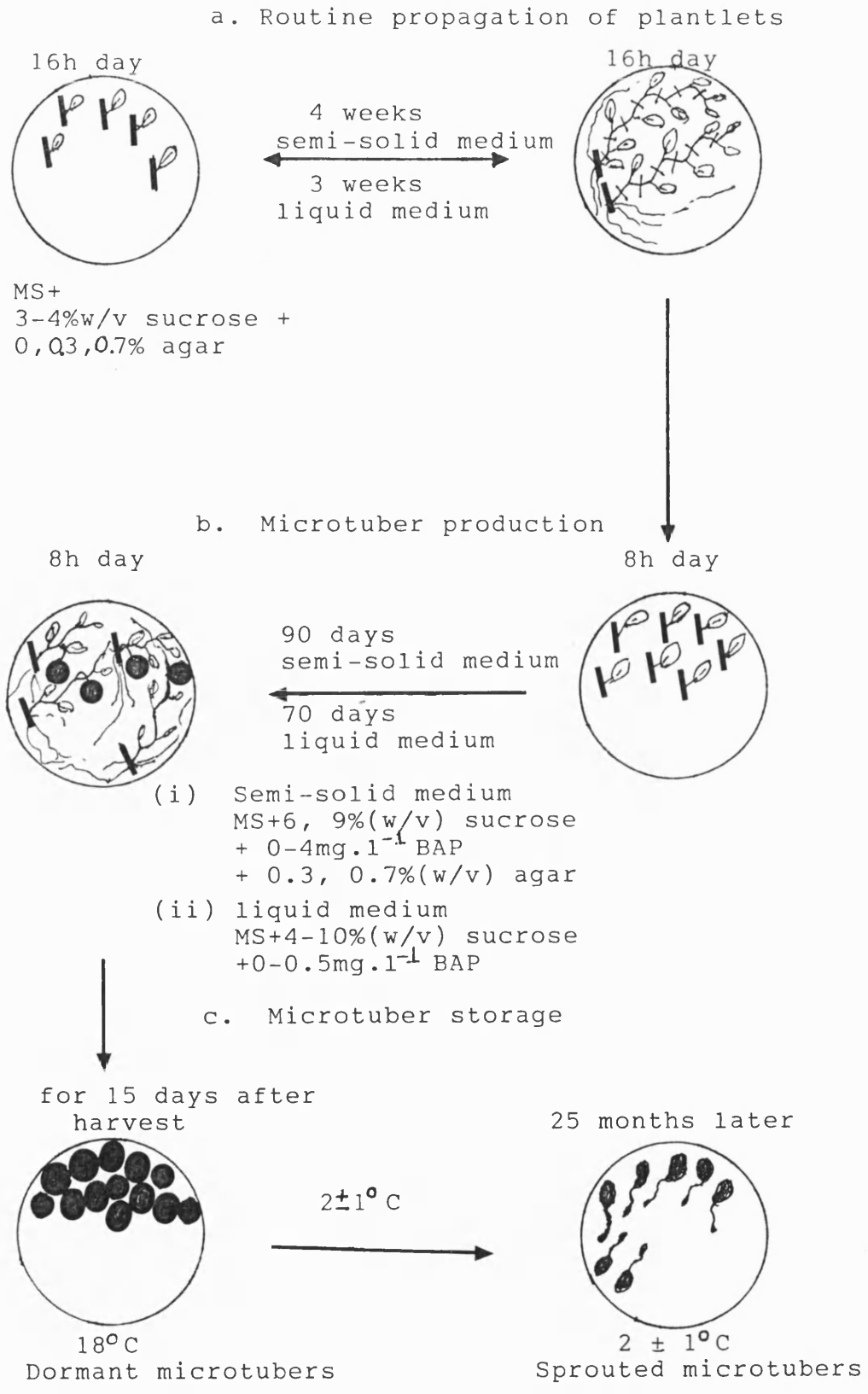
Figure 44. Diagrammatic representation of method used for microtuber production and storage.



APPENDIX I

Composition of nutrient medium

MS (1962)

Components	MS (1962) ($mg \cdot l^{-1}$)
Ammonium Nitrate	1650.0
Boric Acid	6.2
Calcium Chloride (anhydrous)	332.2
Cobalt Chloride. $6H_2O$	0.025
Cupric Sulphate. $5H_2O$	0.025
Na_2-EDTA	37.26
Ferrous Sulphate. $7H_2O$	27.8
Magnesium Sulphate (anhydrous)	180.7
Molybdic Acid (sodium salt). $2H_2O$	16.9
Potassium Iodide	0.83
Potassium Nitrate	1900.0
Potassium Phosphate Monobasic	170.0
Zinc Sulphate. $7H_2O$	8.6
Glycine (free base)	2.0
myo-Inositol	100.0
Nicotinic Acid (free acid)	0.5
Pyridoxine HCI	0.5
Thyamine. HCI	0.1
Grams of powder to prepare 1 litre	4.4

APPENDIX II

Analysis of variance of data of experiments of chapter 3.

(i) The effect of BAP and Sucrose on <u>in vitro</u> tuberization with four maincrop cultivars using semi-solid media and low irradiance. (Figure 3a, 3b)

a) analysis of variance for microtuber productivity.

a_1) cv Majestic. Replicates: 4 (X 10 nodes)

Source	D F	SS	MS	F	F.05
BAP	1	0.42	0.42	0.74	4.41
Sucrose	2	20.10	10.05	17.63	3.55
BAP x Sucrose	2	42.23	21.12	37.05	3.55
Error	18	10.21	0.57		
Total	23	72.96			

Means (10 nodes)		
Sucrose (% w/v)	BAP ($mg \cdot l^{-1}$)	
	2	4
6	9.00 ab	5.00 d
9	8.25 b	9.75 a
12	6.25 c	8.00 b

Lsd at p = .05 : 1.12

a_2) cv. Record. Replicates: 4 (x 10 nodes)

Source	D F	SS	MS	F	F.05
BAP	1	36.0	36.0	52.20	4.41
Sucrose	2	199.0	99.5	142.10	3.55
BAP x Sucrose	2	1.0	0.5	0.72	3.55
Error	18	12.5	0.7		
Total	23	248.5			

BAP ($mg \cdot l^{-1}$):	2	4
Mean	6.25a	5.25b

Sucrose (% w/v):	6	9	12
Mean	6.25b	9a	2c

Lsd at p = .05 : 0.71 Lsd at p = .05 : 0.87

a_3) cv. Pentland Ivory. Replicates: 4 (x 10 nodes)

Source	D F	SS	MS	F	F.05
BAP	1	1.04	1.04	1.37	4.41
Sucrose	2	100.74	50.37	66.30	3.55
BAP x Sucrose	2	5.13	2.57	3.38	3.55
Error	18	13.69	0.76		
Total	23	120.60			

Sucrose (% w/v):	6	9	12
Mean	6.38b	8.5a	3.5c

Lsd at p = .05 : 0.40

a_4) cv. Pentland Squire. Replicates: 4 (x 10 nodes)

Source	D F	SS	MS	F	F.05
BAP	1	2.83	2.83	0.26	4.41
Sucrose	2	40.25	20.13	1.83	3.55
BAP x Sucrose	2	7.42	3.71	0.34	3.55
Error	18	198.00	11.00		
Total	23	248.50			

b) Analysis of variance for mean fresh weight of microtubers
(Figures 4a, 4b)

b_1) cv Majestic. Replicates: 4 (x 10 nodes)

Source	D F	SS	MS	F	F.05
BAP	1	376.4	376.40	184.5	4.41
Sucrose	2	194.1	97.10	47.6	3.55
BAP x Sucrose	2	373.8	186.90	91.6	3.55
Error	18	36.7	2.04		
Total	23	981.0			

Means

Sucrose (% w/v)	BAP ($mg \cdot l^{-1}$) 2	4
6	19.00 c	15.75 de
9	17.5 cd	31.00 a
12	15.00 e	28.50 b

Lsd at p = .05 : 2.12

b_2) cv. Record. Replicates: 4 (x 10 nodes)

Source	D.F	SS	MS	F	F.05
BAP	1	96.0	96.00	18.3	4.41
Sucrose	2	64.8	32.40	6.2	3.55
BAP x Sucrose	2	70.7	35.40	6.7	3.55
Error	18	94.5	5.25		
Total	23	326.0			

Means

Sucrose (% w/v)	BAP ($mg \cdot l^{-1}$) 2	4
4	23.50 b	21.00 bc
6	28.25 a	19.50 c
9	20.25 bc	19.50 c

Lsd at p = .05 : 3.4

b_3) cv. Pentland Ivory. Replicates: 4 (x 10 nodes)

Source	D F	SS	MS	F	F.05
BAP	1	279.8	279.80	40.43	4.41
Sucrose	2	640.8	320.40	46.30	3.55
BAP x Sucrose	2	407.9	204.00	29.50	3.55
Error	18	124.5	6.92		
Total	23	1453.0			

Means

Sucrose (% w/v)	BAP ($mg \cdot l^{-1}$) 2	4
6	36.50 b	32.25 c
9	27.50 d	43.00 a
12	19.25 e	28.50 cd

Lsd at p = .05 : 3.9

$b_{4)}$ cv. Pentland Squire. Replicates: 4 (x 10 nodes)

Source	D.F	SS	MS	F	F.05
BAP	1	367.8	367.80	232.8	4.41
Sucrose	2	130.8	65.40	41.4	3.55
BAP x Sucrose	2	157.9	79.00	50.0	3.55
Error	18	28.5	1.58		
Total	23	685.0			

Means

Sucrose (% w/v)	B ($mg \cdot l^{-1}$) 2	4
6	21.75 d	28.00 b
9	23.00 d	37.55 a
12	25.00 c	27.50 b

Lsd at p = .05 : 1.87

(ii) The effect of light on <u>in vitro</u> tuberization

a) Analysis of variance for microtuber productivity (Figure. 6a, 6b)

a_1) cv Spunta. Replicates: 4 (x 10 nodes)

Source	D F	SS	MS	F	F.05
BAP	4	437.0	109.25	161.00	2.49
Sucrose	2	538.0	269.00	396.00	3.11
Irradiance	1	1.1	1.10	1.60	3.96
BAP x Sucrose	8	150.0	18.75	27.60	2.06
BAP x Irradiance	4	16.0	4.00	5.90	2.49
Sucrose x Irradiance	2	8.4	4.20	6.20	3.11
BAP x Sucrose x Irradiance	8	11.5	1.44	2.12	2.06
Error	90	61.0	0.68		
Total	119	1223.0			

a_1') Comgarison of media at low irrradiance. Replicates: 4 (x 10 nodes)

Source	D F	SS	MT	F	F.05
Media	14	618.5	44.20	51.40	1.92
Error	45	38.5	0.86		
Total	59	657.0			

Lsd at p = .05 : 1.32

a_1'') Comgarison of media at high irrradiance. Replicates: 4 (x 10 nodes)

Source	D F	SS	MS	F	F.05
Media	14	542.25	38.73	75.94	1.93
Error	45	22.75	0.51		
Total	59	565.00			

Lsd at p = .05 : 1.02

a_2) cv Kennebec. Replicates: 4 (x 10 nodes)

Source	D F	SS	MS	F	F.05
BAP	4	276.5	69.10	72.00	2.49
Sucrose	2	590.0	295.00	307.30	3.11
Irradiance	1	67.6	67.60	70.40	3.96
BAP x Sucrose	8	378.3	47.30	49.30	2.06
BAP x Irradiance	4	31.9	8.00	8.30	2.49
Sucrose x Irradiance	2	13.9	6.95	7.20	3.11
BAP x Sucrose x Irradiance	8	37.8	4.73	4.92	2.06
Error	90	86.0	0.96		
Total	119	1482.0			

a_2') Comgarison of media at low irrradiance.
Replicates: 4 (x 10 nodes)

Source	D F	SS	MS	F	F.05
Media	14	615	43.93	42.2	1.92
Error	45	47	1.04		
Total	59	662			

Lsd at p = .05 : 1.45

a_2'') Comgarison of media at high irrradiance.
Replicates: 4 (x 10 nodes)

Source	D F	SS	MT	F	F.05
Media	14	713.25	50.95	74.9	1.93
Error	45	30.75	0.68		
Total	59	744.00			

Lsd at p = .05 : 1.18

b) Analysis of variance for mean fresh weight of microtuberes
Figures 6a, 6b)

b_1) cv Spunta. Replicates: 4 (x 10 nodes)

Source	D F	SS	MS	F	F.05
BAP	4	13080	3270.0	20.80	2.49
Sucrose	2	35200	17600.0	112.00	3.11
Irradiance	1	371	371.0	2.36	3.96
BAP x Sucrose	8	7862	983.0	6.25	2.06
BAP x Irradiance	4	2135	534.0	3.40	2.49
Surcrose Irradiance	2	121	60.5	0.40	3.11
BAP x Sucrose x Irradiance	8	3272	409.0	2.60	2.06
Error	90	14145	157.2		
Total	119	76186			

b_1') Comgarison of media at low irrradiance.
Replicates: 4 (x 10 nodes)

Source	D F	SS	MS	F	F.05
Media	14	28897.25	2064.00	8.67	1.92
Error	45	10712.75	238.06		
Total	59	39610.00			

Lsd at p = .05 : 22

b_1'') Comgarison of media at high irrradiance.
Replicates: 4 (x 10 nodes)

Source	D F	SS	MS	F	F.05
Media	14	32863	2347.4	28.77	1.92
Error	45	3673	81.6		
Total	59	36536			

Lsd at p = .05 : 12.88

b_2) cv Kennebec. Replicates: 4 (x 10 nodes)

Source	D F	SS	MS	F	F.05
BAP	4	7419.0	1855.0	23.22	2.49
Sucrose	2	4505.0	2252.5	28.20	3.11
Irradiance	1	323.5	323.5	4.05	3.96
BAP x Sucrose	8	1773.0	221.6	2.78	2.06
BAP x Irradiance	4	936.8	234.2	2.93	2.49
Sucrose x Irradiance	2	127.4	63.7	0.80	3.11
BAP x Sucrose x Irradiance	8	1392.6	174.1	2.18	2.06
Error	90	7186.7	79.9		
Total	119	23664.0			

b_2') Comgarison of media at low irrradiance.
Replicates: 4 (x 10 nodes)

Source	D F	SS	MS	F	F.05
Media	14	9247.5	660.5	5.8	1.92
Error	45	5104.5	113.4		
Total	59	14352.0			

Lsd at p = .05 : 15.2

b_2'') Comgarison of media at high irrradiance.
Replicates: 4 (x 10 nodes)

Source	D F	SS	MS	F	F.05
Media	14	7061	504.4	6.99	1.92
Error	45	3251	72.2		
Total	59	10312			

Lsd at p = .05 : 12.11

c) Analysis of variance for number of elongated of microtubers
(Figures 6a, 6b)

c_1) cv Spunta.

Source	D F	SS	MS	F	F.05
BAP	4	44939	11235.0	178.9	2.49
Sucrose	2	21105	10553.0	168.0	3.11
Irradiance	1	22277	22277.0	354.7	3.96
BAP x Sucrose	8	29522	3690.0	58.8	2.06
BAP x Irradiance	4	10142	2536.0	40.4	2.49
Sucrose x Irradiance	2	439	219.5	3.5	3.11
BAP x Sucrose x Irradiance	8	5481	685.0	10.9	2.06
Error	90	5652	62.8		
Total	119	139557			

c_2) cv Kennebec.

Source	D F	SS	MS	F	F.05
BAP	4	108035	27009.0	126.2	2.49
Sucrose	2	8461	4230.5	19.8	3.11
Irradiance	1	13846	13846.0	64.7	3.96
BAP x Sucrose	8	10887	1361.0	6.4	2.06
BAP x Irradiance	4	12908	3227.0	15.1	2.49
Sucrose x Irradiance	2	5078	2539.0	11.9	3.11
BAP x Sucrose x Irradiance	8	11710	1464.0	6.8	2.06
Error	90	19258	214.0		
Total	119	190183			

(iii) In vitro tuberization of two first early cultivars using semi-solid media

a) Analysis of variance for microtuber productivity (Figures 7a, 7b)

a_1) cv Maris Bard. Replicates 4 (x 10 nodes).

Source	D.F	SS	MS	F	F.05
BAP	3	9.50	3.2	4.0	2.87
Sucrose	2	10.25	5.1	6.4	3.28
BAP x Sucrose	6	33.25	5.5	6.9	2.37
Error	36	29.00	0.8		
Total	47	82.00			

Means (10 nodes)

Sucrose (% w/v)	BAP ($mg \cdot l^{-1}$) 0	0.5	2.0	4.0
4	10.00 a	9.75 ab	9.50 ab	9.75 ab
6	7.25 c	9.75 ab	10.00 a	10.00 a
9	9.25 ab	6.75 c	8.75 b	9.75 ab

Lsd at p = .05 : 1.29

a_2) cv Duke of York. Replicates 4 (x 10 nodes).

Source	D.F	SS	MS	F.05
BAP	3	132.6	44.20	2.80
Sucrose	2	800.6	400.30	3.19
BAP x Sucrose	6	140.2	23.40	2.30
Error	48	83.6	1.74	
Total	59	1157.0		

Means (10 nodes)

Sucrose (% w/v)	B ($mg \cdot l^{-1}$) 0	0.5	2.0	4.0
4	0 d	0 d	0 d	0 d
6	1.2 d	8.6 ab	9.4 a	9.6 a
9	6.8 c	7.4 bc	8.6 ab	10.0 a

Lsd at p = .05 : 1.68

b) Analysis of variance for mean fresh weight of microtubers
(Figures 8a, 8b)

(cv Maris Bard)

Source	D F	SS	MS	F	F.05
BAP	3	14562	4854.0	11.80	2.87
Sucrose	2	1787	894.0	2.17	3.28
BAP x Sucrose	6	23984	3997.0	9.70	2.37
Error	36	14822	411.7		
Total	47	55155			

Means

Sucrose (% w/v)	B ($mg \cdot l^{-1}$) 0	0.5	2.0	4.0
4	119.75 a	89.25 bc	62.50 cd	65.00 cd
6	65.75 cd	118.00 ab	64.00 cd	71.25 cd
9	130.00 a	69.25 cd	46.75 d	131.25 a

Lsd at p = .05 : 29.11

(iv) In vitro tuberization of cv Bintje using semi-solid media

a) Analysis of variance for microtuber productivity (Figures 9a, 9b)

Replicates: 5 (x 10 nodes).

Source	D F	SS	MS	F	F.05
BAP	3	40.90	13.63	18.8	2.9
Sucrose	1	15.65	15.65	21.4	4.15
BAP x Sucrose	3	44.25	14.75	20.2	2.9
Error	32	23.20	0.73		
Total	39	124.00			

Means (10 nodes)

Sucrose (% w/v)	BAP ($mg.l^{-1}$) 0	0.5	2.0	4.0
6	10.0 abc	9.8 abc	10.8 a	9.0 bc
9	5.4 d	8.8 c	10.2 ab	10.2 ab

Lsd at p = .05 : 1.23

b) Analysis of variance for mean fresh weight of microtubers (Figures 9a, 9b)

Replicates: 5 (x 10 nodes).

Source	D F	SS	MS	F	F.05
BAP	3	1649	549.7	3.56	2.9
Sucrose	1	941	941.0	6.10	4.15
BAP x Sucrose	3	10254	3418.0	22.10	2.9
Error	32	4944	154.5		
Total	39	17788			

Means

Sucrose (% w/v)	BAP ($mg \cdot l^{-1}$) 0	0.5	2.0	4.0
6	88.8 a	73.2 a	61.6 b	29.8 d
9	48.6 bc	43.8 cd	49.4 bc	72.8 a

Lsd at p = .05 : 16.02

APPENDIX III

Analysis of variance of data of experiments of chapter 4

(i) The effect of kinetin on the <u>in vitro</u> tuberization of potato
(Figures 10a, 10b)

a) Analysis of variance for microtuber productivity.
Replicates: 4 (x 10 nodes)

Source	D F	SS	MS	F	F.05
Kinetin	2	20.7	10.35	10.1	3.36
Sucrose	2	4.7	2.35	2.3	3.36
Kinetin x Sucrose	4	7.1	1.78	1.75	2.73
Error	27	27.5	1.02		
Total	35	60.0			

Kinetin ($mg \cdot l^{-1}$) :	0.5	2.0	4.0
Mean	8.83 b	10.00 a	8.17 b

Lsd at p = .05 : 1.11

b) Analysis of variance for mean fresh weight of microtubers.
(Figures 10a, 10b)
Replicates: 4 (x 10 nodes)

Source	D F	SS	MS	F	F.05
Kinetin	2	39241	19621	37.7	3.36
Sucrose	2	8677	4339	8.3	3.36
Kinetin x Sucrose	4	13116	3279	6.3	2.73
Error	27	14069	521		
Total	35	75103			

Means

Sucrose (% w/v)	Kinetin ($mg \cdot l^{-1}$) 0.5	2.0	4.0
4	145.00 b	182.75 a	64.00 ef
6	106.25 cd	91.75 de	79.75 def
9	138.75bc	141.75 b	50.50 f

Lsd at p = .05 : 33.12

(ii) The effect of zeatin on the in vitro tuberization of potato

a) Analysis of variance for microtuber productivity (Figures 11a, 11b)

a_1) cv Spunta. Replicates : 5 (x 10 nodes)

Source	D F	SS	MS	F	F.05
Zeatin	3	0.5	0.17	0.35	2.9
Sucrose	1	0	0	0	
Zeatin x Sucrose	3	1.5	0.5	1.04	2.9
Error	32	15.5	0.48		
Total	39	17.5			

a_2) cv Kennebec. Replicates : 5 (x 10 nodes)

Source	D F	SS	MS	F	F.05
Zeatin	3	55.7	18.60	6.1	2.81
Sucrose	2	125.0	62.50	20.5	3.20
Zeatin x Sucrose	6	115.5	19.30	6.3	2.30
Error	48	146.4	3.05		
Total	59	442.6			

Means

Sucrose (% w/v)	Zeatin ($mg \cdot l^{-1}$) 0.05	0.50	2.00	4.00
4	9.2 ab	7.4 bc	6.4 c	2.2 d
6	10.4 a	7.8 bc	10.2 a	10.2 a
9	9.4 ab	7.4 bc	9.6 ab	9.4 ab

Lsd at p = .05 : 2.22

b) Analysis of variance for mean fresh weight of microtubers
(Figures 11a, 11b)

b_1) cv Spunta. Replicates : 5 (x 10 nodes)

Source	D F	SS	MS	F	F.05
Zeatin	3	5606.6	1868.9	4.84	2.90
Sucrose	1	43.7	43.7	0.11	4.15
Zeatin x Sucrose	3	6759.6	2253.2	5.84	2.90
Error	32	12342.1	385.7		
Total	39	24752.0			

Means

Sucrose (% w/v)	Zeatin ($mg \cdot l^{-1}$) 0.05	0.50	2.00	4.00
6	108.8 c	122.8 b	138.6 a	108.4 c
9	126.0 b	110.4 c	142.0 a	108.6 c

Lsd at p = .05 : 11.33

b_2) cv Kennebec. Replicates : 5 (x 10 nodes)

Source	D F	SS	MS	F	F.05
Zeatin	3	20711	6904.0	14.50	2.81
Sucrose	2	26836	13418.0	28.20	3.20
Zeatin x Sucrose	6	5196	866.0	1.82	2.30
Error	48	22863	476.3		
Total	59	75606			

Zeatin ($mg \cdot l^{-1}$) :	0.05	0.50	2.00	4.0
Mean	87.7 a	51.0 c	99.9 a	68.9b

Lsd at p = .05 : 16

Sucrose (% w/v):	4	6	9
Mean	47.45 b	96.20 a	87.00 a

Lsd at p = .05 : 13.9

APPENDIX IV

Analysis of variance of data of experiments of chapter 5

(i) The effect of photoperiod on <u>in vitro</u> tuberization of potato.

a) Analysis of variance for microtuber productivity (Figure 14)

a_1) cv Spunta

Photoperiod	N	Mean	S D	t	t05
8h	5	10	0	0.417	2.776
16h	5	9.2	1.095		

a_2) cv Kennebec

Photoperiod	N	Mean	S D	t	t05
8h	5	9.6	0.548	0.4	2.776
16h	5	9.8	0.837		

a_3) cv Majestic

Photoperiod	N	Mean	S D	t	t05
8h	4	24.50	0.560	0.88	3.182
16h	4	25.25	1.260		

b) Analysis of variance for mean fresh weight of microtubers
(Figure 14)

b_1) cv Spunta

Photoperiod	N	Mean	S D	t	t05
8h	5	99.6	10.922	0.65	2.776
16h	5	112.6	43.264		

b_2) cv Kennebec

Photoperiod	N	Mean	S D	t	t05
8h	5	165.4	10.90	0.296	2.776
16h	5	156.2	30.73		

b_3) cv Majestic

Photoperiod	N	Mean	S D	t	t05
8h	4	57.75	3.862	0.05	3.182
16h	4	57.50	9.256		

(ii) The effect of the pH of the medium on the <u>in vitro</u> tuberization of potato

a) Analysis of variance for microtuber productivity (Figure 14)

a_1) cv Spunta Replicates: 10 (x 10 nodes)

Source	D F	SS	MS	F	F.05
pH	5	11.9	2.38	2.03	2.39
Error	54	63.1	1.17		
Total	59	75.0			

a_2) cv Kennebec Replicates: 10 (x 10 nodes)

Source	D F	SS	MS	F	F.05
pH	6	30.8	5.13	4.46	2.25
Error	63	72.2	1.15		
Total	69	103.0			

Lsd at p = .05 : 0.959

b) Analysis of variance for mean fresh weight of microtubers (Figure 14)

b_1) cv Spunta Replicates: 10 (x 10 nodes)

Source	D F	SS	MS	F	F.05
pH	5	37910	7582.0	6.63	2.39
Error	54	61719	1142.9		
Total	59	99629			

Lsd at p = .05 : 30.33

b_2) cv Kennebec Replicates: 10 (x 10 nodes)

Source	D F	SS	MS	F	F.05
pH	6	53744	8957.3	16.87	2.25
Error	63	33447	530.9		
Total	69	87191			

Lsd at p = .05 : 20.6

APPENDIX V

Analysis of variance of data of experiments of chapter 6.

(i) The effects of the position of the nodes on the mother plantlet on <u>in vitro</u> tuberization.

Analysis of variance for mean frech weight of microtubers (Figure 16).

Nodes	N	Mean	S.D.	t	t05
$2^{nd} + 3^{rd}$	4	39	1.414	12.25	3.182
$5^{th} + 6^{th}$	4	29	0.816		

(ii) Comgarison of apical shoot tips and nodes as material for <u>in vitro</u> tuberization.

a) Analysis of variance for microtuber productivity (Figure 17).

	N	Mean	S.D.	t	t05
Shoot tips	4	23.25	0.957	1.029	3.182
Nodes	4	24.00	1.414		

b) Analysis of variance for mean fresh weight of microtubers (Figure 17).

	N	Mean	S.D.	t	t05
Shoot tips	4	43.5	3.786	1.35	3.182
Nodes	4	40.00	3.559		

(iii) Effect of age of mother plantlet on *in vitro* tuberization of potato

a) Analysis of variance for microtuber productivity (Figure 18).

Replicates: 4 (x 25 nodes)

Source	DF	SS	MS	F	F05
Age	5	2.75	0.55	0.88	2.77
Error	18	11.25	0.625		
Total	23	14.00			

b) Analysis of variance for mean frech weight of microtubers (Figure 18).

Replicates: 4 (x 25 nodes)

Source	DF	SS	MS	F	F05
Age	5	222.4	44.48	1.77	2.77
Error	18	451.2	25.07		
Total	23	673.6			

c) Analysis of variance for number of sprouted microtubers (Figure 18)

Replicates: 4 (x 25 nodes)

Source	DF	SS	MS	F	F05
Age	5	1323	264.6	8.09	2.77
Error	18	588	32.7		
Total	23	1911			

Lsd at p= .05 : 8.5

d) Analysis of variance for number of elongated microtubers (Figure 18)

Replicates: 4 (x 25 nodes)

Source	DF	SS	MS	F	F05
Age	5	657	131.4	9.3	2.77
Error	18	253	14.1		
Total	23	910			

Lsd at p= .05 : 5.6

(iv) Effect of the presence of the leaf on the nodes on their tuberization ability.

a) Analysis of variance for microtuber productivity Figure 19).

Replicates: 4 (x 25 nodes).

Nodes	N	Mean	SD	t	t05
with leaf	4	24.25	0.957	0.926	3.182
without leaf	4	24.75	0.500		

b) Analysis of variance for mean frech weight of microtubers (Figure 19)

Nodes	N	Mean	SD	t	t05
with leaf	4	48.75	5.188	0.126	3.182
without leaf	4	48.25	6.021		

v) Effect of the planting density of the nodes on in vitro tuberization

a) Analysis of variance for microtuber productivity (Figure 20)

Replicates: 4 (x 10 nodes)

Source	DF	SS	MS	F	F05
Density	5	82.8	16.56	2.25	2.77
Error	18	132.5	7.36		
Total	23	215.3			

b) Analysis of variance for mean fresh weight of microtubers (Figure 20)

Replicates: 4 (x 10 nodes)

Source	DF	SS	MS	F	F05
Density	5	266.35	53.27	152.2	2.77
Error	18	6.25	0.35		
Total	23	272.60			

Lsd at p = .05 : 0.88

(vi) The effect of the orientation of expant growth on the production of nodes.

a) cv Spunta (Figure 21)

a_1) Analysis of variance for nodes.
Replicates: 4 (x 25 nodes).

Container	N	Mean	SD	t	t05
Twyford	4	328.5	19.824	27.84	3.182
Petri dish	4	623.0	7.394		

a_2) Analysis of variance for shoot tips.
Replicates: 4 (x 25 nodes).

Container	N	Mean	SD	t	t05
Twyford	4	383	9.309	29.22	3.182
Petri dish	4	639	14.832		

b) cv Kennebec (Figure 21)

b_1) Analysis of variance for nodes.
Replicates: 4 (x 25 nodes).

Container	N	Mean	SD	t	t05
Twyford	4	389	15.535	20.664	3.182
Petri dish	4	607	14.283		

b_2) Analysis of variance for shoot tips.
Replicates: 4 (x 25 nodes).

Container	N	Mean	SD	t	t05
Twyford	4	468.0	14.606	17.048	3.182
Petri dish	4	641.5	14.177		

(vii) Shoot culture production in liquid medium

a) cv Kennebec

a_1) Analysis of variance for number of nodes (Figure 22)

Replicates: 4 (x 25 nodes).

Medium	N	Mean	SD	t	t05
Liquid	4	606	12.111	26.92	3.182
Semi-solid	4	364	13.292		

a_2) Analysis of variance for mean length of nodes (Figure 22)

Replicates: 4 (x 25 nodes).

Medium	N	Mean	SD	t	t05
Liquid	4	19.5	1.291	8.55	3.182
Semi-solid	4	13.0	0.816		

b) cv Spunta

b_1) Analysis of variance for number of nodes (Figure 22)

Replicates: 4 (x 25 nodes).

Medium	N	Mean	SD	t	t05
Liquid	4	583	8.832	37.3	3.182
Semi-solid	4	320	10.985		

b_2) Analysis of variance for mean length of nodes (Figure 22)

Replicates: 4 (x 25 nodes).

Medium	N	Mean	SD	t	t05
Liquid	4	18	0.816	4.07	3.182
Semi-solid	4	12.5	1.291		

(viii) Use of nodes from shoot cultures grown on liquid medium for *in vitro* tuberization on semi-solid media.

a) Analysis of variance for microtuber productivity of cv Kennebec on tuberization medium: MS+6% (w/v) Sucrose. (Figure 23)

Replicates: 4 (x 10 nodes).

Medium	N	Mean	SD	t	t05
Liquid	4	10.5	1.291	2.64	3.182
Semi-solid	4	9.0	0		

b) Analysis of variance for microtuber productivity of cv Kennebec on tuberization medium: MS+9% (w/v) sucroce at low irradiance. (Figure 23)

Replicates: 4 (x 10 nodes).

Medium	N	Mean	SD	t	t05
Liquid	4	9.75	0.500	1.42	3.182
Semi-solid	4	8.50	0.577		

APPENDIX VI

Analysis of variance of data of experiments of chapter 7.

(i) Use of various containers in two successive liquid media procedure for in vitro tuberization of potato.

Analysis of variance for microtuber productivity Figure 28).

Replicates: 4 (x 10 nodes).

Container	N	Mean	SD	t	t05
Jar	4	25	0	1.36	3.182
Petri dish	4	27	2.944		

(ii) One stage culture for tuberization on liquid medium

a) cv Spunta

a_1) Analysis of variance for microtuber productivity (Figures 29a, 29b)

Replicates: 4 (x 15 nodes)

Source	D F	SS	MS	F	F.05
BAP	2	8.4	4.2	1.45	4.11
Sucrose	3	144.3	48.1	16.6	3.28
BAP x Sucrose	6	111.8	18.6	6.4	2.37
Error	36	104.5	2.9		
Total	47	369.0			

Means

Sucrose (% w/v)	B ($mg \cdot l^{-1}$) 0	0.05	0.50
4	14.50 a	15.00 a	12.00 bc
6	15.00 a	14.75 a	9.75 cde
8	8.00 e	9.00 de	11.00 cd
10	12.00 bc	12.00 bc	14.00 ab

Lsd at p =.05:2.44 (Lsd for % productivity : 16.3)

a_2) Analysis of variance for mean fresh weight of microtubers (Figures 29a, 29b)

Replicates: 4 (x 15 nodes)

Source	D F	SS	MS	F	F.05
BAP	2	27907	13954.00	1.70	4.11
Sucrose	3	198194	66065.00	8.03	3.28
BAP x Sucrose	6	117052	19508.67	2.373	2.372
Error	36	296022	8222.83		
Total	47	639175			

Means

Sucrose (% w/v)	BAP ($mg \cdot l^{-1}$) 0	0.05	0.50
4	293.25 cd	397.50 bc	278.00 cd
6	405.75 bc	266.50 d	389.00 cd
8	439.00 ab	404.50 bc	562.50 a
10	308.50 cd	266.50 d	335.50 cd

Lsd at p = .05 : 130.1

b) cv Kennebec

b1) Analysis of variance for microtuber productivity. (Figures 29a, 29b)

Replicates : 4 (x 15 nodes)

Source	D F	SS	MS	F	F.05
BAP	2	12.38	6.19	4.49	3.36
Sucrose	2	10.05	5.03	3.64	3.36
BAP x Sucrose	4	62.62	15.66	11.35	2.73
Error	27	37.25	1.38		
Total	35	122.30			

Means

Sucrose (% w/v)	BAP ($mg \cdot l^{-1}$) 0	0.05	0.50
4	13.50 cd	16.00 ab	13.50 c
6	14.75 bc	14.25 c	15.00 abc
8	16.50 a	11.75 e	12.00 d

Lsd at p=.05:1.7 (Lsd for % productivity : 11.33)

b_2) Analysis of variance for mean fresh weight of microtubers (Figures 29a, 29b)

Replicates : 4 (x 15 nodes)

Source	D.F	SS	MS	F	F.05
BAP	2	16837	8418.5	4.7	3.36
Sucrose	2	111604	55802.0	31.2	3.36
BAP x Sucrose	4	24323	6080.8	3.4	2.73
Error	27	48264	1787.6		
Total	35	201028			

Means

Sucrose (% w/v)	BAP ($mg \cdot l^{-1}$) 0	0.05	0.50
4	154.25 e	187.75 bcde	192.00 bcde
6	230.00 bcd	172.75 de	244.00 bc
8	244.75 b	335.00 a	351.25 a

Lsd at p = .05 : 61.35

(iii) Semi-solid media of high fluidity for in vitro tuberization

a) cv Spunta

a_1) Analysis of variance for mean fresh weight of microtubers
(Figure 30)
Replicates: 5 (x 10 nodes).

Agar (% w/v)	N	Mean	SD	t	t05
0.7	5	70.0	11.336	3.97	2.776
0.3	5	125.8	29.346		

b) cv Kennebec

b_1) Analysis of variance for microtuber productivity.
(Figures 30)
Replicates : 5 (x 10 nodes)

Agar (% w/v)	N	Mean	SD	t	t05
0.7	5	9.0	0	2.19	2.776
0.3	5	9.6	0.548		

b_2) Analysis of variance for mean fresh weight of microtuber
(Figure 30)

Agar (% w/v)	N	Mean	SD	t	t05
0.7	5	121.8	29.928	1.07	2.776
0.3	5	139.6	14.724		

APPENDIX VII

Analysis of variance of data of experiments of chapter 10.

(i) Comparative trial with microplants, microtubers, small tubers and normal tubers for elite seed potato production.

a) Analysis of variance of number of tubers and total weight of tubers (Figure 35).

a_1) Number of tubers.

a'_1) cv Spunta. Replicates: 10

Source	DF	SS	MS	F	F05
Factors	3	2360.4	786.8	51.76	2.87
Error	36	548.0	15.2		
Total	39	2908.4			

Lsd at p= .05:3.54

a''_1) cv Kennebec. Replicates : 10

Source	DF	SS	MS	F	F05
Factors	2	346	173	35.3	3.36
Error	27	133	4.9		
Total	29	479			

Lsd at p= .05:2.03

a_2) Total weight of tubers

a'_2) cv spunta. Replicates : 10

Source	DF	SS	MS	F	F05
Factors	3	111368	37123	15.5	2.87
Error	36	86089	2391		
Total	39	197457			

Lsd at p= .05: 44.39

a''_2) cv Kennebec. Replicates : 10

Source	DF	SS	MS	F	F05
Factors	2	30134	15067	2.18	3.36
Error	27	186695	6915		
Total	29	216829			

No significant difference.

b) Analysis of variance of number and weight of tubers of minimum fength 3cm (Figure 36)

b_1) Number of tubers of minimum length 3cm
b'_1) cv Spunta. Replicates: 10

Source	DF	SS	MS	F	F05
Factors	3	1041.2	347	37.8	2.87
Error	36	330.4	9.18		
Total	39	1371.6			

Lsd at p=.05:2.75

b''_1) cv Kennebec. Replicates:10

Source	DF	SS	MS	F	F05
Factors	2	136.6	68.3	5.94	3.36
Error	27	310.4	11.5		
Total	29	447.0			

Lsd at p= .05: 3.11

b_2) Weight of tubers of minimum lengh 3cm
b'_2) cv Spunta. Replicates: 10

Source	DF	SS	MS	F	F05
Factors	3	76799	25600	9.83	2.87
Error	36	93722	2603		
Total	39	170521			

Lsd at p= .05: 46.3

b''_2) cv Kennebec. Replicates: 10

Source	DF	SS	MS	F	F05
Factors	2	17933	8966.5	2.13	3.36
Error	27	113929	4219.0		
Total	29	131862			

No significant difference

c) Analysis of variance of number and weight of tubers of minimum length 2cm of cv Kennebec (Figure 37).

c_1) Number of tubers of minimum length 2cm
Replicates: 10

Source	DF	SS	MS	F	F05
Factors	2	312.4	156.2	27.5	3.36
Error	26	147.6	5.68		
Total	29	460.0			

Lsd at p = .05 : 2.19

c_2) Weight of tubers of minimum length 2cm

Source	DF	SS	MS	F	F05
Factors	2	28566.4	14283.2	2.72	3.36
Error	26	136342.6	5243.9		
Total	29	164909.0			

No significant difference

(ii) Tubers produced from plants grown from microtubers for elite II seed-potato production.

a) Analysis of variance of number of tubers and yield. (Figure 38)

a1) Number of tubers. Replicates : 4 (x20 plants)

Source	DF	SS	MS	F	F05
A	1	6202.00	6202.00	48.3	5.12
B	1	163.00	163.00	1.27	5.12
A x B	1	0.06	0.06	0.0005	5.12
Blocks	3	119.00	39.70	0.31	3.86
Error	9	1154.94	128.30		
Total	15	7639.00			

A: Cultivar
B: Plant material

a_2) Yield. Replicates: 4 (x20 plants)

Source	DF	SS	MS	F	F05
A	1	0.08	0.08	0.036	5.12
B	1	3.74	3.74	1.69	5.12
A x B	1	0.78	0.78	0.35	5.12
Blocks	3	4.23	1.41	0.63	3.86
Error	9	19.90	2.21		
Total	15	28.73			

A: Cultibar
B: Plant material

(iii) The effect of the density of the microtubers planted in nursery bench on their productivity (Fugure 39)

a) Analysis of variance of number of tubers
Replicates: 4 (x20 plants)

Source	DF	SS	MS	F	F05
Factors	2	20327	10163.5	41.83	4.26
Error	9	2186	243.0		
Total	11	22513			

Lsd at p = .05: 24.93

b) Analysis of variance of weight of tubers (Yield)
Replicates: 4 (x20 plants)

Source	DF	SS	MS	F	F05
Factors	2	1962217	981108.5	59.36	4.26
Error	9	148750	16527.8		
Total	11	2110967			

Lsd at p = .05 : 205.6

c) Analysis of variance of number of tubers per $1m^2$
Replicates: 4

Source	DF	SS	MS	F	F05
Factors	2	63025.26	31512.6	48.5	4.26
Error	9	5848.05	649.8		
Total	11	68873.30			

Lsd at p= .05 : 40.77

d) Analysis of variance of weight of tubers per $1m^2$
Replicates: 4

Source	DF	SS	MS	F	F05
Factors	2	2007214	1003607	5.62	4.26
Error	9	1608554	178728		
Total	11	3615768			

Lsd at p = .05: 676.2

e) Analysis of variance of proportion of number of tubers longer than 2 cm (%)
Replicates: 4

Source	DF	SS	MS	F	F05
Factors	2	33	16.5	0.26	4.26
Error	9	569	63.2		
Total	11	601			

No significant difference

f) Analysis of variance of proportion of number of tubers longer than 3 cm (%). Replicates : 4

Source	DF	SS	MS	F	F05
Factors	2	89.75	44.88	1.49	4.26
Error	9	271.10	30.12		
Total	11	360.85			

No significant difference

(iv) The effect of the size of microtubers on the productivity of derived plants (Figure 40)

a) Analysis of variance of number of tubers and weight of tubers

a_1) Number of tubers
a'_1) cv Spunta. Replicates: 10

Source	DF	SS	MS	F	F05
Factors	3	60.5	20.2	2.47	2.87
Error	36	294.5	8.18		
Total	39	355.0			

No significant difference

a''_1) cv Desirree. Replicates : 10

Source	DF	SS	MS	F	F05
Factors	3	14.7	4.9	1.63	2.87
Error	36	108.1	3.0		
Total	39	122.8			

No significant difference

a_2) Weight of tubers
a'_2) cv Spunta. Replicates: 10

Source	DF	SS	MS	F	F05
Factors	3	46601	15534	4.13	2.87
Error	36	135252	3757		
Total	39	181853			

Lsd at p = .05 : 55.65

a''_2) cv Desirree. Replicates: 10

Source	DF	SS	MS	F	F05
Factors	3	9734.7	3245	2.95	2.87
Error	36	39635.3	1101		
Total	39	49370.0			

Lsd at p = .05: 30.12

b) Analysis of variance of number of tubers of minimum length 2 cm and of number of tubers of minimum length 3 cm

b_1) Number of tubers of minimum length 2 cm

b'_1) cv Spunta

The same data as a'_1

b''_1) cv Desirree. Replicates: 10

Source	DF	SS	MS	F	F05
Factors	3	11.5	3.83	1.37	2.87
Error	36	100.5	2.79		
Total	39	112.0			

No significant difference

b_2) Number of tubers of minimum length 3 cm

b'_2) cv Spunta. Replicates: 10

Source	DF	SS	MS	F	F05
Factors	3	78.8	26.27	3.58	2.87
Error	36	264.2	7.34		
Total	39	343.0			

Lsd at p = .05 : 2.46

b"2) cv Desirree. Replicates : 10

Source	DF	SS	MS	F	F05
Factors	3	4	1.33	0.81	2.87
Error	36	59	1.64		
Total	39	63			

No significant difference

c) Analysis of variance of weight of tubers of minimum length 2 cm and of weight of tubers of minimum lenght 3 cm

c_1) Weight of tubers of minimum length 2 cm

c'_1) cv Spunta. The same data as a'_2

c''_1) cv Desirree. Replicates : 10

Source	DF	SS	MS	F	F05
Factors	3	9456	3152	2.43	2.87
Error	36	46725	1298		
Total	39	56181			

No significant difference

c_2) Weight of tubers of minimum length 3 cm

c'_2) cv Spunta. Replicates : 10

Source	DF	SS	MS	F	F05
Factors	3	51719	17240	4.68	2.87
Error	36	132536	3682		
Total	39	184255			

Lsd at p = .05 : 55.1

c''_2) cv Desirree. Replicates: 10

Source	DF	SS	MS	F	F05
Factors	3	8787	2929	2.60	2.87
Error	36	40623	1128		
Total	39	49410			

No significant difference.

(v) The effect of the dominance of the apical sprout of th microtubers on the productivity of derived plants (Figure 41)

a) Analysis of variance of total number of tubers

a1) cv Spunta

	N	Mean	SD	t	t05
Untreated microtubers	10	8.8	1.874	6.748	2.262
Treated microtubers	10	18.7	4.244		

a2) cv Kennebec

	N	Mean	SD	t	t05
Untreated microtubers	10	9.4	2.591	7.21	2.262
Treated microtubers	10	23.1	5.425		

b) Analysis of variance of total weight of tubers

b_1) cd Spunta

	N	Mean	SD	t	t05
Untreated microtubers	10	139.1	26.627	1.262	2.262
Treated microtubers	10	156.0	32.917		

b_2) cv Kennebec

	N	Mean	SD	t	t05
Untreated microtubers	10	161.1	29.065	4.294	2.262
Treated microtubers	10	230.1	41.675		

c) Analysis of variance of number of tubers of minimum length 2 cm

c_1) cv Spunta

	N	Mean	SD	t	t05
Untreated microtubers	10	6.3	2.002	3.85	2.262
Treated microtubers	10	9.7	1.947		

c2) cv Kennebec

	N	Mean	SD	t	t05
Untreated microtubers	10	6.5	1.716	6.597	2.262
Treated microtubers	10	14.1	3.213		

d) Analysis of variance of weight of tubers of minimum length 2cm

d_1) cv Spunta

	N	Mean	SD	t	t05
Untreated microtubers	10	136.2	27.466	0.95	2.262
Treated microtubers	10	149.2	33.439		

d_2) cv Kennebec

	N	Mean	SD	t	t05
Untreated microtubers	10	155.7	27.548	3.79	2.262
Treated microtubers	10	213.8	39.897		

e) Analysis of variance of number of tubers of minimum length 3 cm

e_1) cv Spunta

	N	Mean	SD	t	t05
Untreated microtubers	10	4.3	1.636	2.87	2.262
Treated microtubers	10	6.2	1.317		

e_2) cv Kennebec

	N	Mean	SD	t	t05
Untreated microtubers	10	5.0	1.826	5.538	2.262
Treated microtubers	10	8.6	0.966		

f) Analysis of variance of weight of tubers of minimum length 3 cm

f_1) cv Spunta

	N	Mean	SD	t	t05
Untreated microtubers	10	127.9	26.602	0.681	2.262
Treated microtubers	10	137.6	36.375		

f_2) cv Kennebec

	N	Mean	SD	t	t05
Untreated microtubers	10	143.2	33.306	1.666	2.262
Treated microtubers	10	174.7	49.657		

APPENDIX VIII

List of suppliers:

1.	Ms media	Flow laboratories, Irving, Scotland.
2.	Biochemicals	Sigma, Poole, UK
3.	Sucrose	BDH, Poole UK
4.	Plasticware	Sterilin, UK
5.	Agar	Oxoid, UK
6.	Twyfords	Twyford Plant Laboratories, UK
7.	Incubators	Gallenkamp, UK
		Controlla Rodas, Greece.