National Library of Canada
Acquisitions and Bibliographic Services Branch
395 Wellington Street
Ottawa, Ontario
K1A 0N4

Bibliothèque nationale du Canada
Direction des acquisitions et des services bibliographiques
395, rue Wellington
Ottawa (Ontario)
K1A 0N4

Your file Votre référence

Our file Notre référence

NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

Canada

THE PRODUCTION AND UTILIZATION OF POTATO MICROTUBERS

By

Yves Leclerc

A thesis submitted to the Faculty of Graduate Studies and Research for the partial fulfilment of the requirements for the degree of Doctor of Philosophy

Department of Plant Science
McGill University, Macdonald Campus
Montréal, Québec
Canada

July, 1993

ISBN 0-315-91659-1

TO MY SON ZACHARIE

THE PRODUCTION AND UTILIZATION OF POTATO MICROTUBERS

Ph.D. Dissertation
Plant Science

ABSTRACT

A protocol is presented for the rapid (28 days) induction of microtubers on micropropagated, layered potato plantlets of 'Kennebec', 'Russet Burbank' and 'Superior' in medium devoid of growth regulators. With this method the addition of coumarin, 6-(2-chloroethyl)-trimethylammonium chloride and 6-benzylamino-purine to the microtuberization medium either had no effect or significantly reduced microtuber weight per plantlet. Increasing the incubation period from 28 to 56 days significantly increased the weight of microtubers per plantlet and the proportion of microtubers heavier than 1 gram. Increasing the volume of microtuberization medium from 50 to 100 ml significantly increased the number of microtubers per plantlet. Microtuber dormancy periods were cultivar-specific and microtubers ≤250 mg had longer dormancy periods as compared to microtubers >250 mg. A positive correlation was established between endogenous abscisic acid levels and microtuber dormancy periods. Microtubers ≤250 mg had lower specific gravity, fewer eyes and produce fewer sprouts than microtubers >250 mg. Microtuber-derived plants were generally single-stemmed. Severe physiological ageing treatment (>2500 degree-days) had no effect on microtuber sprout development, stem number, tuber number and only minimally influenced tuber weight of microtuber-derived plants. Decreasing field in-row planting density from 30 to 10 cm reduced tuber weights and numbers per plant but increased them on a per hectare basis. Economic analysis indicated that optimum planting density varied depending on plantlet cost. The optimum planting density was 10 cm if the cost of plantlet was $0.10 or less, 20 if plantlet cost were from $0.10 and $0.20 and 30 cm for plantlet cost greater than $0.20. A potato seed tuber certification program adapted to the needs and constraints of Egypt is presented.

PRODUCTION ET UTILISATION DE MICROTUBERCULES DE POMME DE TERRE

Thèse de Doctorat
Plant Science

RESUME

Un protocole est présenté pour l'induction rapide (28 jours) de microtubercules à partir de plantules de pomme de terre de 'Kennebec' , 'Russet Burbank' et 'Superior' dans un milieu exempt de régulateurs de croissance. Avec cette méthode, l'addition de coumarine, 6-(2-chloroethyl)-trimethylammonium chloride et de 6-benzylaminopurine au milieu de croissance n'a eu aucun effet ou a réduit d'une manière significative le poids des tubercules par plantule. L'augmentation de la période d'incubation de 28 à 56 jours a accru le poids de microtubercule par plantule ainsi que la proportion de microtubercule pesant plus de 1 gramme. L'augmentation du volume de milieu de microtubérization de 50 à 100 ml, a eu pour effet d'accroître le nombre de microtubercule par plantule. La période de dormance des microtubercules variait de façon spécifique selon le cultivar et les microtubercules ≤250 mg avaient une plus longue période de dormance que les microtubercules > 250 mg. Une corrélation positive fut établie entre le niveau d'acide abscisique des microtubercules et la duré de la période de dormance. Les microtubercules ≤250 mg avaient une gravité spécifique plus faible et possédaient moins d'yeux et produisaient moins de germes que les microtubercules >250 mg. Les plantes issues des microtubercules produisaient généralement une seule tige. L'augmentation sévère de l'âge physiologique des microtubercules (2500 degrée-jours) n'a eu aucun effet sur le développement des germes, le nombre de tige ainsi que sur le nombre de tubercules produits et n'a que faiblement influencé le poids des tubercules produits par plants. Au champs, la réduction de la distance de plantation de 30 à 10 cm entre les plants issus de plantules produits *in vitro* a eu pour effet de décroître le nombre et le poids des tubercule produits par plants mais a eu pour effet d'augmenter le nombre et le poids des tubercules à l'hectare. L'analyse économique des résultats a démontrée que la distance optimale de plantation variait selon le coût de production des plantules. Un programe de certification de la pomme de terre tenant comptes des besoins et contraintes en Egypt est présenté.

ACKNOWLEDGEMENTS

I would like to acknowledge the contribution of my academic advisor Dr. Danielle J. Donnelly, for her support throughout this project and for her help in the preparation of this manuscript. I would like to express my gratitude to Dr. Janet E.A. Seabrook of Agriculture Canada, Fredericton, New Brunswick, who warmly received me as a visiting scientist in her laboratory, and for the most instructive discussions we had during this period. I would like to thank Drs. Warren K. Coleman and Russell R. King of Agriculture Canada, Fredericton, New Brunswick, for their most valuable help and guidance. I would also like to thank François Mercure of Agriculture Canada, La Pocatière, Québec for providing the specific pathogen-tested plantlets. The contributions of Drs. Katrine Stewart and Donald Smith are also acknowledged.

The support of Dean Spaner, Patrick Bullman, Steve Hallet, Francois Bousquet, Micheline Ayoub, Maria-Jose Faria, Martine Korban, Johanne Cousineau and Ribo Deng are acknowledge. I am grateful to Helen Cohen Rimmer, Guy Rimmer, Stewart Leibovitch, Jim Straughton, Stuart Willox, Thomas Mpoulimpassis, Richard Smith and Linda Nelson.

I would like to express my gratitude to my mother, father and brother for the support and help throughout my studies.

Finally, I would especially like to thank my lovely wife Asma Regragui for her tremendous help, support and patience through these years, for without her this would not have been possible.

The financial support of FCAR is gratefully acknowledge.

CONTRIBUTIONS OF CO-AUTHORS TO MANUSCRIPTS FOR PUBLICATION

The following remark concerning the authorship of manuscripts is excerpted from Guidelines Concerning Thesis Preparation published by the Faculty of Graduate Studies and Research:

"the inclusion of manuscripts co-authored by the candidate and others is not prohibited by McGill, the candidate is warned to make explicit statements on who contributed to such work and to what extent, supervisors and others will have to bear witness to the accuracy of such claims before the oral committee. It should also be noted that the task of the external examiners is made much more difficult in such cases, and it is in the candidate's interest to make authorship responsibility perfectly clear."

Chapter 3 is comprised of a manuscript which has been submitted for publication. The co-authors include Dr. Danielle J. Donnelly (my supervisor) and Dr. Janet E.A. Seabrook of the Agriculture Canada, Research Station in Fredericton New Brunswick. Drs. Donnelly and Seabrook provided supervisory guidance and assisted in the manuscript preparation.

Chapter 5 is comprised of a manuscript which has been submitted for publication. The co-authors include Dr. Danielle J. Donnelly, and Drs. Warren K. Coleman and Russell R. King of the Agriculture Canada, Research Station in Fredericton New Brunswick. Drs. Donnelly, Coleman and King provided research guidance and reviewed the manuscript before submission for publication.

Chapter 7 is comprised of a manuscript which has been

submitted for publication. Dr. Donnelly, provided research guidance and reviewed the manuscript before submission for publication.

TABLE OF CONTENTS

Page

ABSTRACT i
RECUME ii
ACKNOWLEDGEMENTS iii
CONTRIBUTION OF CO-WORKERS TO MANUSCRIPTS FOR PUBLICATION .. iv
TABLE OF CONTENTS vi
LIST OF TABLES ix
LIST OF FIGURES xii
CHAPTER 1. INTRODUCTION
1.1 General Introduction 1
1.2 Objectives of the Thesis 3
CHAPTER 2. LITERATURE REVIEW
2.1 Potato Seed Tuber Certification 5
2.2 Potato Seed Tuber Certification in Industrialized Countries 7
2.3 Potato Seed Tuber Certification in Canada: the Example of Québec 12
2.4 Potato Seed Tuber Certification in Developing Countries 15
2.5 Potato Tissue Culture 19
2.5.1 Rapid multiplication (micropropagation) ... 19
2.5.2 Meristem tip culture and virus eradication 22
2.3.3 Microtuberization 25
2.6 Potato Dormancy and its Release 36
2.7 Physiological Ageing 41
2.8 Field Performance of Micropropagated Potato 42

CHAPTER 3. THE INFLUENCE OF GROWTH REGULATORS AND INCUBATION PERIODS ON MICROTUBERIZATION OF PLANTLETS AND NODAL CUTTINGS OF POTATO 48
3.1 Abstract 48
3.2 Introduction 48
3.3 Materials and Methods 50
3.4 Results 53
3.5 Discussion and Conclusions 56
CHAPTER 4. THE EFFECTS OF PLANTLET PRE-CONDITIONING AND SUCROSE AVAILABILITY ON MICROTUBER PRODUCTION 63
4.1 Introduction 63
4.2 Materials and Methods 64
4.3 Results and Discussion 66
CHAPTER 5. MICROTUBER DORMANCY IN THREE POTATO CULTIVARS .. 72
5.1 Abstract 72
5.2 Introduction 72
5.3 Materials and Methods 73
5.4 Results and Discussion 76
CHAPTER 6. MICROTUBER QUALITY AND SPROUT DEVELOPMENT AS AFFECTED BY SIZE, GENOTYPE AND INCUBATION MEDIUM 82
6.1 Introduction 82
6.2 Materials and Methods 83
6.3 Results 86
6.4 Discussion 90
CHAPTER 7. THE INFLUENCE OF PHYSIOLOGICAL AGEING ON THE YIELD FROM POTATO MICROTUBERS 102
7.1 Abstract 103
7.2 Introduction 102
7.3 Materials and Methods 104

7.4 Results and Discussion 105
CHAPTER 8. THE EFFECT OF IN-ROW PLANTING DENSITY AND PLANTING DEPTH ON THE YIELD OF *EX VITRO*-DERIVED PLANTS OF THREE POTATO CULTIVARS 111
8.1 Introduction 111
8.2 Materials and Methods 112
8.3 Results and Discussion 114
CHAPTER 9. ESTABLISHING A POTATO SEED TUBER CERTIFICATION PROGRAM IN EGYPT 120
9.1 Introduction 120
9.2 Discussion 122
9.3 Conclusions 133
CHAPTER 10. SUMMARY 136
CHAPTER 11. GENERAL CONCLUSIONS 148
CHAPTER 12. CONTRIBUTIONS TO ORIGINAL KNOWLEDGE 152
CHAPTER 13. SUGGESTIONS FOR FUTURE RESEARCH 154
CHAPTER 14. REFERENCES 156

LIST OF TABLES

Page

Table 3.1. Microtuber fresh weight and number per original plantlet as affected by cultivar, growth regulator and duration of the incubation period. Each observation represents the average of 72 plantlets 59

Table 3.2. Microtuber fresh weight and number per original plantlet as affected by cultivar, growth regulator and culture type after 56 days of incubation. Each observation represents the average of 36 plantlets (216 nodal cuttings) 60

Table 3.3. Effect of the duration of the incubation period and culture type on the variance of microtuber fresh weight. The tubers of 324 plantlets per treatment were used in this analysis 61

Table 3.4. Effect of the duration of incubation, cultivar, growth regulator and culture type on the frequency distribution (%) of microtubers. The tubers of 36 plantlets per treatment combination were used in this analysis 62

Table 4.1. The effect of environmental pre-conditioning on microtuber weight (mg) and number per plantlet harvested after 4 weeks of incubation 69

Table 4.2. The effect of the quantity of tuberization medium on weight and number of microtubers per plantlet harvested after 4 weeks of incubation 70

Table 5.1. The influence of cultivar and microtuber size on the dormancy period (week, mean ± s.d.) of

microtubers harvested after 28 days of incubation 79
Table 5.2. The influence of cultivar and length of incubation period (28 and 56 days) on the dormancy period (week, mean ± s.d.) 80
Table 5.3. Endogenous abscisic acid (ABA) content of small (≤250 mg) and large (>250 mg, mean ± s.d.) microtubers harvested after 28 d of incubation and correlation analysis of ABA content *vs* duration of the dormancy period of microtubers 81
Table 6.1. the effects of induction medium and microtuber weight on the specific gravity of potato microtubers 93
Table 6.2. The percentage of shoot tips that developed after the excision of buds from apical, lateral and distal eyes of microtubers examined 1 and 2 weeks after explantation 94
Table 6.3. Survival rate (%) of microtubers stored for 8, 12 and 18 months (mo) 95
Table 6.4. The effects of microtuber desprouting on the number of subsequent sprouts ≥10 mm and stems per microtuber-derived plant 96
Table 7.1. The influence of physiological age on sprout number per microtuber, stem number and minituber number and weight per microtuber-derived plants grown in the greenhouse during two different seasons 109
Table 8.1. Yield characteristics and plant height of *ex vitro* plantlet-derived plants grown under three planting densities for two seasons 117

Table 8.2. Tuber size frequency distribution of *ex vitro* plantlet-derived plants grown under three planting densities for two seasons 118
Table 8.3. Cost estimates of tubers produced from EVPD plants grown under three planting densities 119
Table 9.1. Major insect pests and diseases of the potato crop in Egypt 135

LIST OF FIGURES

Page

Figure 6.1. The relationship between microtuber weight and the number of eyes per microtubers. For 'Russet Burbank' R^2=0.9561, $ß_o$=8.9956 and $ß_1$=0.0156; 'Kennebec' R^2=0.9328, $ß_o$=6.3277 and $ß_1$=0.0129; 'Superior' R^2=0.9571, $ß_o$=7.5751 and $ß_1$=0.2123 97

Figure 6.2. Sprout length of microtubers stored at 6 °C for 8, 12 and 18 months 98

Figure 6.3. The position of primary and secondary sprouts on microtubers 99

Figure 6.4. The influence of spatial orientation (apex or distal end up) on the position of sprouts developed from desprouted microtubers of 'Kennebec' .. 100

Figure 7.1. The frequency distribution (%) of minitubers produced from physiologically "young" and "old" microtubers grown in pot trials in the greenhouse during the fall and winter seasons. Physiological ageing did not significantly influence the frequency distribution f minitubers (X^2, p>0.05% for all comparisons) 110

Figure 9.1. Example of potato seed tuber certification scheme adapted to Egypt 134

CHAPTER 1. INTRODUCTION

1.1 General Introduction

The Solanaceae ranks among the most important of the plant families that serve mankind. It includes food plants such as the potato (*Solanum* spp.), tomato (*Lycopersicon esculentum*), pepper (*Capsicum* spp.) and eggplant (*Solanum melongera*), medicinal and poisonous plants (*Datura* spp., *Mandragora officianum* and *Atropa belladonna*) and the "weed" tobacco (*Nicotiana tabaccum*) (Heiser, 1969). There are 162 tuber-bearing *Solanum* species, of which 8 are cultivated (Hawkes, 1978). Among these *S. tuberosum*, a tetraploid, is the only world-wide distributed specie. The others are cultivated in South America, including four diploid, two triploid and one pentaploid species (Hawkes, 1978).

The potato is grown in more countries in the world (79%) than any other food crop except maize (*Zea mays*). The volume of potato production ranks fourth globally after rice (*Oryza sativa*), wheat (*Triticum aestivum*) and maize (Woolfe, 1986). The importance of potato in industrialized countries is well known. However, its importance in developing countries is less widely recognized. The Food and Agricultural Organization (FAO) statistics show that potato production increased by 120 % from 1961-65 to 1986 in developing market economies (FAO, 1990).

The contribution of potato to the human diet is not to be underestimated. For thousands of years potatoes have been used to support the growth and health of populations in the Andean

highlands of South America. The Irish and Scots were dependent on potatoes as their principal source of food from the 17th to the 19th century, up until the devastating potato blight (*Phytophtora infestans*) of 1846-47 (Hawkes, 1978; Woolfe, 1986).

On a per hectare basis, potato yields more carbohydrates than any other food crop, and is second to soya (*Glycine max*) in protein production. The potato proteins are good complements to cereal proteins, being rich in lysine and low in methionine (Woolfe, 1986).

Since potatoes are vegetatively propagated, pathogens are passed from generation to generation and may build up in the population causing serious yield losses. World-wide losses of potato yield through disease have been estimated at 30 % (Wang and Hu, 1985). Certification programs have been established in many countries including Canada, to reduce the level of pathogens in seed tubers, thus increasing their quality. It is possible to obtain plant material devoid of pathogens through shoot tip culture, which is usually preceded by thermotherapy. The specific pathogen tested (SPT) plantlets obtained through thermotherapy and shoot tip culture can then be cloned in vitro and introduced into seed tuber certification programs.

In Quebec, the seed tuber certification program relies on thermotherapy followed by shoot tip culture to obtain SPT plant material. From the SPT material minitubers are induced under semi-controlled environments. These are field-planted on government or private farms for further multiplication. After

three field seasons the seed tubers are sold to certified seed producers who multiply them for one or two additional seasons before sale to commercial growers in the province.

In Québec, the entire process of releasing a new cultivar or "cleaning" and re-releasing an existing cultivar may take up to 7 years, from the time of its reception in La Pocatière to its sale to the commercial potato growers. Our previous work showed that both *ex vitro* plantlet- and microtuber-derived plants produced more tubers than seed tuber-derived plants under Quebec field conditions (Leclerc and Donnelly, 1990). An increased reliance on tissue cultured propagules could significantly enhance the phytosanitary quality of seed tubers and reduce the costs associated with producing potato seed tubers; primarily by reducing the number of field seasons necessary to supply the commercial demand. Microtubers are easier to store, ship and plant than are *in vitro* plantlets; their utilization in seed tuber certification programs would be advantageous. However, the higher production costs of microtubers compared to *in vitro* plantlets and the general lack of information concerning several aspects of their production and utilization of microtubers has limited their use in seed tuber certification programs.

1.2 Thesis objectives

The goal of this research was to optimize the methods of production and utilization of *in vitro* microtubers and micropropagated plantlets. Experiments were designed to address

fundamental questions concerning the production and utilization of microtubers in seed certification programs. The objectives were to:

1. Determine the influence of genotype, growth regulators and incubation period on *in vitro* tuberization of layered plantlets and nodal cuttings. Evaluate how the formation of microtubers on layered plantlets is affected by environmental pre-conditioning of plantlets and by the volume of tuberization medium.

2. Study the effects of growth regulators (coumarin, CCC and BA) incubation periods, microtuber size and the role of endogenous abscisic acid in microtuber dormancy.

3. Determine how several microtuber characteristics including specific gravity, eye number and bud maturity are affected by growth regulators, incubation periods and microtuber size. Study the influence of apical dominance on microtuber sprouting and subsequent stem development.

4. Investigate the effect of physiological ageing on the yield of microtuber-derived plants grown in the greenhouse.

5. Study the effect of in-row planting density and planting depth on the yield of *ex vitro*-derived plants grown in the field.

6. Evaluate the possibility of establishing a potato seed certification program in Egypt based on tissue culture technologies and design a certification program adapted to the particular needs of Egypt.

CHAPTER 2. LITERATURE REVIEW

2.1 Potato Seed Tuber Certification

When the Spaniards introduced the potato to Europe in the 16th century, they selected only a few genotypes from the plethora of wild, semi-cultivated and cultivated species and varieties that existed in the New-World. For more than 150 years only 2 recognizable genotypes were grown; both from the species *Solanum tuberosum* (Salaman, 1949).

In the early and mid 18th century the number of recognizable genotypes under cultivation increased rapidly. The breeding and selection that was done by farmers and agriculturists had only one objective: to overcome the depressing effect of "curl" in the varieties under cultivation. By 1775, the incidence of "curl" was so serious that it threatened the cultivation of potato on the European continent (Salaman, 1949).

The creation of new genotypes through hybridization temporarily eliminated the problem, but after several years of cultivation the "curl" would again affect the new cultivars. Nevertheless, it was a common observation, for over a hundred years, that potato seed tubers derived from plants grown in the highlands gave rise to healthier plants than did seed tubers grown in sheltered enclosures anywhere in the British Isles. From this observation, the foundation for potato seed tuber certification was established and farmers in Scotland could specialize in seed potato production. Nevertheless, it was not

until the pioneer work of Orton early in this century that the "curl" was associated with viral diseases (potato virus Y, M and leaf roll; Salaman, 1949). A number of different viruses were later identified and it was demonstrated that viruses could be spread by green flies (*Aphis* spp.) and aphids (*Myzus* spp.). It was then possible to adopt a scientific approach to potato seed certification. "Healthy" potato seed tubers could be selected and multiplied in areas where vectors were absent or could be controlled. Seed certification programs were established in the early 1920's in Canada and the United States (Wright, 1988) and a few years later in Europe (Salaman, 1949). These certification programs permitted a considerable yield increase but suffered a number of drawbacks: the production of "virus-free" stock of potato clonal varieties depended upon finding an apparently uninfected plant to start the stock. It was not possible to guarantee that seed tubers were actually virus-free. It was not possible to detect and select against the latent viruses PVX (discovered in 1925) and PVS (discovered in 1952).

The major breakthrough in potato seed tuber certification came with the advent of tissue culture and pathogen detection technologies. Using thermotherapy in combination with meristem tip culture Stace-Smith and Mellor (1968) were able to obtain virus-free potato plants of several cultivars. Globally, tissue culture technologies became a permanent feature of potato seed tuber certification programs. This has contributed to the improved field performance of potato seed tubers and was

effective in controlling a number of tuber-borne diseases. In turn, this has promoted the acceptance of tissue culture technologies in seed tuber certification programs (Knutson, 1988).

2.2 Potato Seed Tuber Certification in Industrialized Countries

Tissue culture technologies have been adopted by European and North American seed tuber certification programs. In order to assess the impact of this technology and the degree of similarity or difference between the various programs a survey was conducted by Jones (1988). Questionnaires were sent to 29 North American and 39 European certification agencies and to 9 commercial companies and 4 certified seed growers in the United States. Questionnaires were returned by 22 North American and 14 European certification agencies, 4 commercial companies and 4 certified seed growers. The results showed some differences between the different programs.

In Eurcpe, most programs have developed their own capabilities for producing specific-pathogen tested (SPT) material while in North America most states and provinces programs rely on other agencies or commercial companies to get the SPT material. Pathogen testing is done by each program using a multitude of different tests performed in a variety of ways prior to the rapid multiplication phase. Different plant parts are tested for the detection of pathogens depending on the program; including each initial plantlet (29%), mother plant

(28%), mother tuber flesh (22%) or mother tuber sprout (21%). In North America and Europe, virus indexing was conducted 94% of the time either alone or in combination with other tests such as those for bacteria 84%, Potato Spindle Tuber Viroid (PSTV) 64% and fungi 49% of the time. Virus indexing after the initiation of rapid multiplication was carried out in 92% of the programs. Enzyme Linked Immunosorbent Assay (ELISA) was the most widely used test, followed by mechanical assessment (inoculant rubbed onto indicator plants). Other tests included latex agglutination, chloroplast agglutination and leaf disk assays using transmission electron microscopy. Bacteria-testing prior to rapid multiplication was performed by one or many testing procedures. The most frequently used procedures were nutrient broth, ELISA, immunofluorescence and Richardson solution. PSTV was tested for by 73% of the programs. The most commonly employed test for PSTV in North America were the nucleic acid hybridization test (NAH), polyacrylamide gel electrophoresis (PAGE) and the tomato challenge test. PAGE and the tomato challenge test were the most frequently used tests in Europe. Fungi were tested for by 68% of the programs in North America and 44% of the European programs. The most frequently used assessment media for the presence of fungi were the Richardson solution, nutrient broth and potato dextrose agar.

Ex vitro plantlets were used alone or in combination with other procedures by 90% of North American and 94% of European programs. Stem cuttings were also used in 30% of North American

and 25% of European programs. Five percent of North American and 12% of European programs made use of microtubers in their multiplication scheme.

Continuous propagation of *in vitro* material (not starting in vitro material each year) was used by 40% of North American programs compared with 67% of European programs. Only 1 out of 35 programs surveyed reported more somatic mutation or variants from continual in vitro culture compared to the previous method of seed tuber production (tuber-unit method). For both North America and Europe most programs (89% and 86% respectively) agreed that it was necessary to observe field increase originating from in vitro propagation before releasing seed tuber stock to growers.

An attempt was made to compare yield data between the different programs. The variation in the environmental conditions (greenhouse type, lighting, cultivar, time of year at planting, planting density per pot or hill) made it impossible to draw conclusions. However, it appeared to the author that *ex vitro* plantlets grown in glass greenhouses with high pressure sodium light gave better yields than those transplanted into the field. The European programs surveyed reported a total annual production of 842 000 plantlets of which 300 000 were produced by a single country, presumably Holland. Similarly, of the total 355 450 stem cuttings 98% were produced by a single country. In North America plantlet production totalled 579 700 while stem

cutting production was 44 3000. In addition, 195 000 plantlets were produced by 4 private growers (Jones, 1988).

The advantages to using micropropagated seed tuber stocks was acknowledged by all programs. These included a 10 to 38% yield increase, increased vigour and uniformity and increased ability to withstand and recover from environmental stress.

In North America, seed tuber certification is carried out by various Provincial and State organizations. Each program was expected to supply certified growers with a limited amount of clean planting stock of as many varieties as are of local interest (Addy, 1988). While this system has made significant contributions to the North American potato industry, certification agencies were not considered or expected to be a conduit for the introduction of new technologies. The regulations were often different from program to program. The integration of the North American economy, the national and international seed tuber marketing opportunities and the entry of private firms into the seed tuber certification process will encourage greater uniformity among programs (Knutson, 1988).

The establishment of private firms in the seed tuber certification industry is likely to have a most significant impact. Due to their mandate, State and Provincial certification agencies are not preoccupied by volume production, which tends to make these programs expensive to operate and to maintain. Furthermore, in an attempt to reduce costs to the certified growers, evaluated at $15 000 to $37 000 per ha for

micropropagated seed tuber stock alone (Knutson, 1988), "clean" stocks are generally grown on for several generations in the field before being released to certified growers. This practice increases the risk of pathogen reinfection. The presence of volume production-oriented private firms is likely to reduce the cost of producing basic seed tuber stock. Furthermore, efficient volume production is likely to reduce the time necessary for the release of new cultivars. The introduction of newly patented cultivars developed either through conventional breeding or by genetic engineering techniques will most definitely need a different approach to seed tuber certification. All these changes are likely to modify the role of the existing seed tuber certification agencies.

The increased utilization of tissue culture techniques in the production of SPT seed tubers has lead the Certification Section of the Potato Association of America to recommend standards and guidelines to assure uniform quality of seed tubers produced throughout North America (Johansen et al., 1984).

Two distinct levels of production are involved in developing "basic" seed tuber stock. Level I involves the development of SPT plantlets and level II entails their rapid multiplication. In level I, the "stock" plants and/or tubers from which explants are removed are carefully selected from high yielding clonal lines true to varietal type. To maximize the opportunity to provide a broad genetic base and to avoid selecting a tuber that may carry a genetic mutation, it is recommended that explants be

derived from 10-15 tubers (Johansen et al., 1984). Regeneration from callus is avoided due to the increased risk of somatic mutations. Plantlets derived from each initial explant are screened for the presence of pathogens including bacteria, fungi, viruses and the potato spindle tuber viroid (PSTV). Screening is done as early as possible to avoid discarding large numbers of diseased plants at a later date. The performance of level I seed tuber stock is evaluated annually under field conditions to assess the phenotype and yielding ability of the clonal lines. Before distribution into certified seed tuber programs representative samples of plantlets produced during level II are tested for specific pathogens. Ideally, the plantlets or microtubers are used as entry level stocks moving into a flush-through system where initial material is always being replaced by new SPT material.

Since the implementation of micropropagation techniques in North American and European seed tuber certification programs, yield increases of 10 to 38% have been reported along with increased plant vigour, uniformity and ability to withstand and recover from environmental stress (Jones, 1988).

2.3 Potato Seed Tuber Certification in Canada: the Example of Quebec

Potato seed tuber certification in Canada is regulated by the Seed Act (Department of Agriculture, 1991) and applies to potato seed tubers that are sold or advertised for sale in

Canada, imported into or exported from Canada. There are seven established classes of potato seed tubers which are, from highest class to lowest, a) Pre-Elite, b) Elite I, c) Elite II, d) Elite III, e) Elite IV (Premier Foundation), f) Foundation and g) Certified. Nuclear stock, the entry material of the certification program, is defined as any tuber, plant or vegetative propagule, produced in protected, environmental conditions from tissue culture, that has been subjected to laboratory tests and found free of disease.

Established in 1922, "Le centre de certification et d'épuration" of Agriculture Canada located in La Pocatière is the origin of all potato seed tubers produced in Quebec. The centre's mandate is to produce potato plantlets free of specific viruses (PVA, PVM, PVS, PVX, PVY and PLRV), the PSTV and bacterial and fungal diseases.

On receipt of a new cultivar, the tubers are placed in quarantine and the presence of bacterial wilt (*Corynebacterium sepedonicum*) and PSTV are monitored. If either one of these diseases is detected the material is immediately destroyed. The material is vegetatively reproduced by cuttings and submitted to thermotherapy (Sylvestre and Laganiere, 1981). After 6-8 wk of heat treatment at 37 °C, 20 meristem tip explants (no larger than 0.5 mm) per plant are placed on Murashige and Skoog (MS, 1962) basal medium containing growth regulators. Regeneration is achieved within 6-8 wk. The plantlets are then micropropagated by nodal cuttings. One of plantlet originating from each

meristem tip excised from a single mother plant is acclimatized in a controlled environment and assayed for the presence of potato viruses (PVA, PVS, PVX, PVY^{o}, PVY^{n} and PLRV) and viroid (PSTV) using ELISA and electrophoresis respectively. From the 20 meristem tips 2 plantlets that assayed negative to all tests are selected to form the basal seed tuber stock for the variety and the others are micropropagated and sent to the provincial multiplication farms in Manicouagan.

At their arrival in Manicouagan the in vitro plantlets are kept in quarantine for 1 mo and retested for bacterial wilt before introduction into the "production bank". From there, plantlets are placed in a "conservation bank" (MS, 1962 basal medium containing mannitol) or acclimatized and vegetatively propagated by nodal cuttings under an 18 h day and 100.8 μmol/m^2/s illumination at temperatures above 23 °C. PVX and PVS are indexed at this stage using both an indicator plant and the ELISA method. These cuttings are introduced into a controlled environment for the production of minitubers. This involves 1 wk under 18 h days at 21 °C followed by 8 wk under 10 h day at 15 °C, both under 200.8 μmol/m^2/s photon flux density. A total of 50,000 minitubers from all cultivars can be produced annually. During this production step, the absence of PVA, PVS, PVX and PVY^{o} and bacterial wilt are verified. The minitubers are planted onto 1 ha of land in the Manicouagan region to produce Pre-Elite seed tubers. This region was selected because the climatic and edaphic conditions reduce to a minimum the insect

population that could vector viral and viroid diseases. The following year, the tubers are planted on 3.3 ha of land to produce Elite I seed tubers. In addition to PVX and PVS indexing a sample of the Elite I seed tubers is sent to Florida where they are field-planted and visually observed to assure the absence of any viruses. The third year, the tubers are planted onto 12 ha of land. The Elite II tubers that are harvested are sold to the certified growers who will produce Elite III, Elite IV, Foundation and Certified seed potatoes (Tennier, 1981).

A total of 75,000 verifications are made by provincial inspectors during the 2 year certification period in the certified growers' fields. Checks are done throughout the season, at harvest and during storage. If viruses, viroids, bacterial or fungal diseases are found to be present in excess of the guidelines enforced by "Le centre d'épuration et de certification" of La Pocatière the crop is immediately destroyed.

2.4 Potato Seed Tuber Certification in Developing Countries

The potato seed tuber certification programs in developing countries can be categorized into two distinct groups based on varieties used; those that utilize local varieties and those that utilize European and North American varieties. The countries that utilizes local native varieties and/or improved indigenous types, mainly the Andean countries of South America and India, developed their own certification programs mainly because they

could not import seed tubers. However, these programs have not yet resulted produced sufficient amounts of high quality seed tubers to supply all farmers. For most developing countries, seed tuber certification programs are a recent phenomenon. The first attempts to implement seed tuber certification programs in developing countries took place during the energy crisis in the early 1970's. High petroleum prices forced many countries to lower or completely stop the importation of expensive foreign seed tubers. Attempts to produce potato seed tubers using the traditional European and North American certification systems with their strict quality control regulations, were doomed by adverse biotic and abiotic conditions and technological limitations (Bryan, 1988).

Recent developments in tissue culture and pathogen testing technologies have changed this situation. With the realization that tissue culture, as a maintenance and rapid multiplication process, is not as complicated as earlier believed breakthroughs have occurred. Tissue culture alone or in combination with other methods of propagation is now used successfully in many developing countries of Latin America (Argentina, Brazil, Columbia, Costa Rica, Cuba, Ecuador, Mexico, Peru, Venezuela) and South Asia (Korea, Philippines, Sri Lanka, Thailand, Vietnam). Several other countries are using in vitro transplants, microtubers and/or cuttings to produce improved quality seed tubers (Wang and Hu, 1982; Van Uyen and vander Zaag, 1984; Bryan 1988).

A careful analysis of needs is necessary before embarking on a seed tuber certification program. Good quality seed tubers are a major constraint to potato production in developing countries (Nganga and Potts, 1987; White et al., 1988). For these, farmers are ready to spend 20 to 50% of their production costs to buy seed tubers (Vander Zaag and Horton, 1983). Where subsistence farming predominates, the purchase of seed tubers may be the only production cost other than family labour (Durr, 1980). Seed tuber cost may be a serious constraint to the development of potato production; poor farmers not being able to purchase seed tubers or obtain credit for the inputs required to grow them successfully.

Nevertheless, seed tuber quality is not the only limiting factor to potato production in developing countries. The physiological quality of the tubers, lack of fertilizer, pesticides, and proper soil preparation as well as other agronomic inputs are often more important constraints to high yields. Non-adapted varieties, adverse climates and inadequate or poorly trained research extension and certification personnel are also of concern. Therefore, an analysis of the benefit of using high quality seed tubers should be initiated early in the program to determine the needs of the local farmers.

It is important that seed tuber certification programs be adapted to the particular needs of each country. Certification regulations are of utmost importance. Over-stringent regulations could lead to the rejection of seed lots superior to tubers

currently used by farmers. Nevertheless, a careful systematic testing program must be developed to complement the micropropagation techniques (Bryan, 1988).

The typical developing-world producer grows his potato crop on less than 0.5 ha of land. He is generally isolated and far from the major centers or roads. Adequate storage facilities and distribution systems must be put in place to take into consideration this particular aspect of potato production.

The problems faced by potato seed tuber certification programs in developing countries is frequently not a lack of knowledge of good production practices, but rather the implementation of this expertise given limited resources, bureaucracy, lack of storage facilities and the very small quantities of seed tuber needed by each farmer. In an attempt to overcome these problems a computer model of the production/storage/distribution phases has been produced by White et al. (1988). According to the authors, this program has had a serious impact on the production of certified seed tubers in Burundi and enabled the farm managers to better coordinate the different production steps.

In Egypt, the limited availability of good quality seed tubers at reasonable cost limits the expansion of potato cultivation. The price of seed tubers alone represents 50% of the total production cost (Zaki, 1989). Imported seed tubers used for the winter season are often physiologically too young. Therefore, the full potential of these high quality seed tubers

is not fully exploited (Nganga and Potts, 1987). Furthermore, the same cultivars are used for both the fall (Nili) and winter seasons, although the climatological conditions are very different, the Winter crop being planted under increasing daylength and temperature and the Nili season crop being planted under decreasing daylength and temperature (Nganga and Potts, 1987). The increased availability of better adapted varieties for the winter season, which is the most suitable season for potato cultivation, could lead to significant yield increase.

To be successful the Egyptian Potato Seed Tuber Certification Program must develop an innovative production strategy to lessen the number of field generations. To fulfil this objective the different aspects of potato seed tuber certification must be examined and adapted to the climatological constraints of Egypt.

2.5 Potato Tissue Culture

The potato is the crop plant to which tissue culture propagation techniques have been applied most extensively (Wang and Hu, 1985). It has been described by Espinosa et al. (1986) as a model crop plant for tissue culture.

2.5.1 Rapid multiplication (micropropagation)

Micropropagation techniques are advantageous in the propagation of SPT material. Large numbers of propagules can be

produced under *in vitro* conditions within short periods of time (Wang and Hu, 1985).

Today, nodal cutting techniques are widely used for the rapid multiplication of SPT material. Reports on explant type, media composition and cultural conditions used for the rapid multiplication of potato *in vitro* are numerous (reviewed by Wang and Hu (1985).

Nodal cuttings are generally utilized for the rapid multiplication of potato. Nodal cuttings were grown on a growth regulator-free MS medium (Hussey and Stacey, 1981; Austin and Cassels, 1983) or MS medium containing gibberellins (GA_3, Westcott and al., 1979). The growth of nodal cutting was not stimulated by the addition of cytokinins to the proliferation medium (Wattimena, 1983). Sucrose was generally added at a rate of 30 g/l (Hussey and Stacey, 1981; Estrada et al, 1986) however 20 g/l was also used (Wattimena, 1983). The proliferation medium was variously supplemented with inositol, Ca-pantothenate, thiamine-HCl (Estrada et al., 1986) and amino acids (Hussey and Stacey, 1981). The media were solidified with 7 to 8 g/l agar and the pH adjusted from 5.6 to 5.8 prior to autoclaving (Wattimena, 1983; Estrada, 1986). Incubation temperatures ranged from 22 to 30 °C, the photoperiod from 16 h to continuous illumination and the photon flux density from 20 to 133 $\mu mol/m^2/s$ (Wattimena, 1983; Wang and Hu, 1985; Seabrook, 1987). In a study on the effect of light quality on the growth of nodal segments of 'Caribe' and 'Shepody', a lamp combination of cool-white and

Agrolite fluorescent, giving an illumination of 133 μmol/m^2/s, was optimum based on measurements of leaf number and area, stem length and fresh and dry weights (Seabrook, 1987) .

Other techniques for the rapid propagation of SPT material have been developed. These include the multimeristem, *in vitro* layering and shoot tip culture techniques. In the multimeristem technique developed at the International Potato Centre (CIP) by Roca et al., (1978), excised meristem tips (0.5-0.6 mm) were placed in MS (1962) basal salt medium supplemented with 0.4 mg/l GA_3, 0.5 mg/l benzylaminopurine (BAP), 2 mg/l Ca-pantothenate, 30 g/l sucrose, 7 g/l agar and the pH was adjusted to 5.7 prior to autoclaving. The cultures were incubated at 22 °C, under 12.6 μmol/m^2/s for 5 wk. Plantlets were then transfered to liquid medium containing the same components as described above except for the addition of 0.01 mg/l naphtalene-acetic acid (NAA). One to three plantlets were cultured in 125 ml Erlenmeyer flasks containing 15 ml of medium and incubated on a rotary shaker at 90 rpm under the same environmental conditions as described above. Within 4 wk approximately 14 shoots were produced per plantlet. The shoots were then sectioned into single node cuttings and transferred onto medium containing GA_3 (0.04 mg/l). Plantlets were soon regenerated and could be either transferred to potting mixtures or micropropagated *in vitro* from single node cuttings.

Wang (1977) developed the in vitro layering technique in which repeated layering and subculturing of a single shoot generated approximately 2.5^{17} plantlets in 1 yr. In this

procedure the newly developed axillary shoots were positioned (layered) horizontally onto medium containing MS basal salts, supplimented with 0.01 mg/l NAA and 7 g/l agar. A mass of axillary shoots was obtained. Every 20 d these were sectioned and placed on fresh medium or transferred to potting mixture for *ex vitro* utilization. *In vitro* layering was also used as a preliminary to tuberization *in vitro*.

Goodwin et al. (1980) used shoot tip culture to significantly increase the availability of planting material, generating a 250-fold increase in 4 mo. As many as 40-60 shoot tips were taken from each tuber. Shoot tips of 15-25 mm arising from certified seed tuber pieces were aseptically transferred onto MS medium supplemented with GA_3 (0.1 mg/l). The regenerated plantlets were then used as planting material.

2.5.2 Meristem tip culture and virus eradication

White (1943) was able to successfully subculture TMV-infected tomato roots *in vitro*. When he dissected these roots and tested the various zones using a mechanically inoculated host plant to determine the infectivity of each section, he determined that virus particles inside the growing roots were unevenly distributed. The root apex contained fewer virus particles than the basal part. Limaset and Cornuet (1949) made similar observations on systemically virus-infected plants. They found that the concentration of virus decreased as they approached the apical meristem. In half of the cases no

infection was transmissible from the apical meristems. From these observations, Morel and Martin (1952) postulated that it might be possible to recover virus-free (specific-pathogen tested, SPT) plants from heavily virus-infected plants through the use of meristem tip culture. Since viruses are unevenly distributed in their hosts, it was believed that a sufficiently small piece of tissue might be virus-free. They were indeed able to recover SPT Dahlia (*Dahlia* sp.) from meristem tips derived from heavily infected plants. Using the same procedure, Morel and Martin (1955) reported virus elimination in potato.

Later studies showed that the majority of viruses penetrated meristematic cells. Kassanis (1967) first detected virus particles in apical meristems using transmission electron microscopy (TEM). Potato virus X (PVX) particles were detected by Appiano and Pennazio (1972) in the cells of the apical domes using thin section TEM. The presence of PVX in potato meristems was detected by mechanical inoculation of meristem tips onto indicator plants. This showed conclusively that excised tips contained virus particles at the time they were placed onto culture medium (Pennazio and Redolfi, 1974).

Meristem tip culture, the culture of meristematic dome plus 1 or 2 pairs of leaf primordia measuring from 0.2 to 0.5 mm in length, is generally coupled with thermotherapy. Thermotherapy consists of exposing virus-infected plants to temperatures near the maximum tolerable for a few weeks. For potato, recommended temperatures and duration of treatment vary from 32 to 37 °C for

periods of 1.5 to 13 wk (Mellor and Stace-Smith, 1977). At temperatures above 30 °C virus replication is greatly reduced. Although plants are rarely cured by this treatment, virus concentration is drastically reduced and portions of the plant may be virus-free even though some parts are still infected. New shoots produced during the treatment are more likely to be uninfected or have low virus concentrations. Meristem tips are generally taken from these sections and cultured in vitro (Stace-Smith, 1986). Clearly, virus elimination takes place *in vitro*. The exact mechanism of virus elimination *in vitro* is not known but several explanations have been proposed. According to Hollings and Stone (1964) an inactivation system exists within the apex, whose action is helped by removing the mature portion of the plant. Quak (1977) suggested that virus disappearance could be attributed to contact of the meristem tip with the culture medium. Mellor and Stace-Smith (1977) proposed that enzymes required for one or more steps of viral replication that are generally available for the replication of virus particles in the meristematic dome are unavailable in excised tips. Thus, viral replication is stopped while normal processes of viral degradation continue. By 1980, 48 species had been freed of viruses using a combination of thermotherapy and meristem tip culture (Wang and Hu, 1980).

Chemical substances have been utilized to suppress symptoms or to reduce the virus concentration in plants. Although some reports claimed virus eradication using this method, the results

were contested by other scientists (Mellor and Stace-Smith, 1977). The only encouraging report involving chemotherapy of potato to date, was published by Cassels and Long (1980). They were able to eradicate potato virus Y and cucumber mosaic virus from a large proportion of infected potato explants by adding Virazole (an animal antiviral agent and mutagen) to the culture medium.

2.5.3 Microtuberization

The first report of *in vitro* tuberization (microtuberization) was published by Barker in 1953. Since then, microtuberization has been heavily investigated. Today, microtubers are used as a primary source of SPT material in several seed tuber certification programs throughout the world (Wiersema et al., 1987; Jones, 1988). They constitute the ideal material for international germplasm exchange and are used for medium or long term conservation of germplasm (Budin and Ogluzdin; 1982; McCorran, 1986; Chandra et al., 1988; Kwiatkoski et al., 1988; Lizaraga et al., 1989; Dodds and Watanabe, 1990; Thieme, 1992). Furthermore, recent reports have demonstrated that microtuberization can be used as a breeding tool to screen potato germplasm for early maturity (Lentini et al., 1988) and heat tolerance (Huang et al., 1988; Nowak and Colborne, 1988), to investigate the physiological processes involved in tuber growth (Obata-Sasamoto and Suzuki, 1979; Sattelmacher and Marschner, 1985; Moorby et al., 1990) to study tuber protein gene expression

(Bourque et al., 1987) or for *Agrobacterium*-mediated transformation (Ishida et al., 1989).

The first explants for microtuberization consisted of excised buds from whole plants placed *in vitro* (Barker, 1953; Chapman, 1955: Gregory, 1956). Later, sprouts or stolon segments were used for the induction of microtubers (Palmer and Smith, 1970; Garcia-Torres and Gomez Campo, 1973; Stallknecht and Farnsworth, 1982a, 1982b). With the increased use of micropropagation for the production of SPT material, it became possible to produce large quantities of microtubers using single node cuttings originating from *in vitro* plantlets (Wattimena, 1983). Wang and Hu (1982) were the first to put forward the idea of mass production of microtubers for propagation purposes. The development of a two phase microtuberization system (vegetative propagation followed by microtuber induction) by Estrada et al. (1986) and Meulemans et al. (1986) made mass propagation possible (Akita and Takayama, 1988; Joung et al., 1991). The wide application of this system could have a significant impact on potato seed tuber certification programs. The production of large quantities of potato microtubers could allow a significant reduction in the number of field generations, thus reducing the time necessary to release new cultivars while increasing the phytosanitary quality of the tubers. The use of microtubers instead of *in vitro* plantlets would be advantageous as they are easier to store, handle and do not require any acclimatization treatment.

Potato tuberization is induced in situ by a number of environmental factors; short days (Garner and Allard, 1923), high photon flux densities (Arthur et al., 1930), low night temperatures (Bodlaender, 1960, 1963; Menzel, 1982) and low nitrogen levels (Werner, 1935; Menzel, 1982). Foliar application of abscisic acid (ABA) (Menzel, 1980) and the antigibberellin chlorocholine chloride (CCC) can also stimulate tuberization in vivo (Guanasena and Harris, 1969).

Factors such as environmental conditions, carbon source, growth regulators and retardants and the mineral nutrition particularly nitrogen (N) (Wattimena, 1980; Wang and Hu, 1985; Chandra et al., 1988) are known to influence microtuberization. Discrepancies exist between authors as to which factor or level of a factor is the most effective in inducing microtuberization. Often a factor that was found beneficial in one experiment was found to have no effect or was detrimental in another trial. Wang and Hu (1985) attribute these differences to variations in the cultivars, culture medium, growth regulator, type of explant tissue and the incubation environments used. Explant tissue source was found to affect size and dry matter accumulation of microtubers. Microtubers produced using whole plantlets were larger with lower dry matter content while excised stolons produced comparatively smaller tubers with higher dry matter content (Mitten et al., 1988). The nodal position of single and double node explants influenced subsequent microtuber formation. Poor tuberization was reported with apical single node cuttings,

while double node cuttings showed more frequent tuberization at the upper node (Dasher and Machado, 1988).

Optimum temperatures have been reported to be 8 to 10 °C (Thieme and Pett, 1982), 15 °C (Wattimena, 1983), 20 °C (Wang and Hu, 1982) and between 20 to 26 °C (Lawrence and Barker, 1963; Okasawa, 1967; Palmer and Smith, 1970; Hussey and Stacey, 1981). Temperatures above 35 °C inhibited microtuberization (Palmer and Smith, 1970). Constant optimum temperature was better than alternating high day and low night temperature (Wang and Hu, 1982).

Light requirements are also variable and depend on other culture conditions. The induction of microtubers under total darkness was reported by many authors, including Barker (1953), Mes and Menge (1954), Claver (1956, 1967), Harmey et al. (1966) Palmer and Smith (1969, 1970), Smith and Rappaport (1969), Palmer and Barker (1972), Mingo-Castel et al. (1974, 1976), Stallknecht and Farnsworth (1982a, 1982b), Koda and Okasawa (1983) and Mangat et al. (1984), Schilde et al. (1984) and Estrada et al. (1986). Others have used alternating light and dark (Chapman, 1955; Garcia-Torres and Gomez-Campo, 1973; Tizio and Blain, 1973). Wattimena (1983) used 0, 8 and 24 hr light periods and concluded that the longer the day length the better, while Hussey and Stacey (1981) observed *in vitro* tuberization under 16 and 24 hr light periods, but not under an 8 hr day. They later reported that photoperiod had no effect on microtuberization (Hussey and Stacey, 1984). Pelacho and Mingo-Castel (1991) reported that a

short photoperiod decreased kinetin-induced tuberization of isolated potato stolons, contradicting Wang and Hu's (1985) affirmation that longer photoperiod with higher photon flux density was required when cytokinins were not used. Pearl et al. (1991) reported that exposure of *in vitro* shoots sections to long photoperiods was beneficial to the microtuberization response once the tuberization stimulus had been triggered.

Single node cuttings tuberized more rapidly under total darkness, but greater weights were achieved by using an 8 h photoperiod (Slimmon et al., 1989). Garner and Blake (1989) reported that total darkness stimulated rapid microtuber formation on nodal cuttings when preceded by short days, but not after long day exposure. The exposure of cultures to long photoperiods prior to induction increased the microtuberization response (Chapman, 1955; Garner and Blake, 1989).

Genotypic differences in microtuberization response to daylengths have been reported (Abbott and Belcher, 1986; Marinus, 1990; Sladky and Bartosova, 1990; Seabrook et al., 1993). Photoperiodic pre-conditioning of cultures prior to induction affected the microtuberization response (Garner and Blake, 1989; Ewing and Senesac, 1990; Lakhoua and Ellouze, 1990; Pérennec and Francois, 1981; Seabrook et al., 1993). Genotypes with short critical photoperiod (CPP) tuberized best after long night photoperiod pre-conditioning, while genotypes with long CPP received sufficient induction under short days (Ewing and Senesac, 1990; Lentini and Earle, 1991; Seabrook et al., 1993).

Seabrook et al. (1993) demonstrated that microtuber morphology could be influenced by photoperiodic pre-conditioning.

Photon flux densities reported in the literature ranged from 1.25 $\mu mol/m^2/s$ (Wang and Hu, 1982) to 110 $\mu mol/m^2/s$ (Seabrook, et al., 1993). Marinus (1990) found that microtuberization response to light intensity varied between cultivars. Light quality has been shown to influence microtuberization. The microtuberization of sprout segments was accelerated by exposure to 5 min of red light immediately after excision from the mother tuber (Blanc, 1981). It was later found that the sensitivity to red light varied with the age of the explant (Blanc et al., 1986). The process could be reversed by exposure to far-red light, indicating the possible involvement of a phytochrome system. Additionally, incubating plantlets under blue fluorescent light (400-580 nm) increased the number of microtubers per plantlet compared with incubation under red fluorescent light (600-700 nm) (Aksenova et al., 1989) .

Culture vessel types (Coleman, 1992) and medium gelling agents (Coleman, 1992; Nowak and Asiedu, 1992), but not osmotic concentration of the medium (Lo et al., 1972) affected microtuber yields.

Six to eight percent sucrose was optimum for microtuberization (Lawrence and Barker, 1963; Catchpole and Hillman, 1969; Stallknecht and Farnsworth, 1979, 1982a, Wang and Hu, 1982), but Wattimena (1983) found no difference between 4, 6 and 8% sucrose. Claver (1956, 1977) reported microtuber

induction using 2% sucrose without any additives. In general, increasing the sucrose concentration from 1 to 8 % increased the percentage and earliness of tuberization (Wang and Hu, 1985). However, at concentrations greater than 8% microtuberization was inhibited (Lawrence and Barker, 1963; Catchpole and Hillman, 1969; Palmer and Smith, 1970; Stallknecht and Farnsworth, 1979; Garner and Blake, 1989). Palmer and Smith (1970), Mingo-Castel et al. (1976), Hussey and Stacey (1981, 1984) and Abbott and Belcher (1986) have used 6 % sucrose and cytokinins to induce tuberization. A reliable microtuber production system on medium containing 8% sucrose without the addition of growth regulators was reported by Garner and Blake (1989). Growth regulators failed to induce microtuberization when the sucrose supply was inadequate (Harmey et al., 1966). Sucrose may be the only compound necessary for induction of microtuberization (Gregory, 1956; Ewing, 1985, 1990). Microtuberization was reported on media containing glucose, fructose and maltose (Mes and Menge, 1954; Okasawa, 1967). Glucose and fructose were less effective for induction than sucrose while mannose and mannitol were not inductive (Ewing and Senesac, 1990).

Cytokinin is a promoter of in vitro tuberization; kinetin (Palmer and Smith, 1969a, 1969b, 1970; Tizio and Blain, 1973; Palmer and Barker, 1973; Mingo-Castel et al., 1974, 1976; Forsline and Langille, 1976; Obata-Sasamoto and Suzuki, 1979; Pelacho and Mingo-Castel, 1991), benzylaminopurine (Mauk and Langille, 1978; Wang and Hu, 1982; Estrada et al., 1986), 2iP [6-

(α,α-dimethylallylamino)purine] (Mitten et al., 1988), zeatine riboside (Mauk and Langille, 1978; Koda and Okasawa, 1983) and thidiazuron (Slimmon et al., 1987) have all been used to induce microtubers. The optimum dosage for Kinetin (KIN) was found to be 5 mg/l (23 μM) (Palmer and Smith, 1969b, 1970; Palmer and Barker, 1973; Tizio and Blain, 1973; Mingo-Castel et al., 1974, 1976). Wang and Hu (1982) observed best results with benzylaminopurine (BA) at 10 mg/l (44 μM) for in vitro plantlets. Mauk and Langille (1978) found that the optimum concentration was 3 mg/l BA (13 μM) for in vitro tuberization of stolon apices. Mitten and al. (1988) compared several cytokinins for their microtuberization potential and found that the addition of 2iP to the microtuberization medium gave the greatest number of microtubers. KIN was required only when tuberization occurred in the dark (Wattimena, 1983). The use of cytokinins in combination with ancymidol and coumarin (Wattimena, 1983) or chlorocholine chloride (Kostrica et al., 1985) was shown to increase the microtuberization response.

Gibberellins inhibited microtuberization (Palmer and Smith, 1970; Garcia-Torres and Gomez-Campo, 1973; Parrot, 1975; Stalknecht and Farnsworth, 1982a).

The latest prospect for a natural inhibitor to gibberellins is a compound related to jasmonic acid (Yoshihara et al., 1989). This compound, 12-OH-jasmonic acid (tuberonic acid), produced in potato leaves grown under tuber inductive conditions, had strong inductive potential (Koda and Okasawa, 1988; Koda et al., 1988).

Jasmonic acid (5 μM) increased the number of microtubers per stolon, the tuberization rate and the tuber fresh weight by factors of 2.8, 2.3 and 6.2, respectively, compared with KIN (Pelacho and Mingo-Castel, 1991).

Potato stolon cultures did not require external auxins (Lawrence and Barker, 1963). The addition of auxins at concentration higher than 1 mg/l inhibited microtuberization (Harmey et al., 1966; Okasawa, 1967; Tizio and Blain, 1973; Stallknecht and Farnsworth, 1982a; Mangat et al., 1984).

Abscisic acid (ABA) had no effect or prevented *in vitro* tuberization of cultured sprout sections, stolon tips or potato shoots (Palmer and Smith, 1969a; Smith and Rappaport, 1969; Claver, 1970; Tizio and Maneshi, 1973; Stallknecht and Farnsworth 1982a). The ß-inhibitor-complex (and its main component ABA) was apparently not the "hypothetical tuber initiating factor" in potato stem sections cultured *in vitro* (Tizio and Maneschi, 1973), however, ABA increased the microtuberization rate of cultures incubated in medium containing 2% sucrose (Koda and Okasawa, 1983). The deposition of polymeric aliphatics, aromatics and waxes components of suberized cell walls was markedly stimulated by ABA (Cottel and Kolattukudy, 1982).

In vitro tuberization may be stimulated by some growth inhibitors. Most phenols, an exception was quercitin, had a promotive effect on *in vitro* tuberization of sprout sections at a range of concentration (Paupardin and Tizio, 1969a, 1969b, 1970; Paupardin, 1970 and Tizio and Paupardin, 1971).

Coumarin had a strong promotive effect on microtuberization (Stallknecht, 1972; Stallknecht and Farnsworth, 1979, 1982a, 1982b) and increased the uniformity of microtubers (Joyce and McCown, 1987). Coumarin (25 and 50 mg/l; 171 and 342 μM) was more effective than KIN for in vitro tuberization (Stallknecht and Farnsworth, 1979), but high levels of nitrogen in the medium inhibited the uptake of coumarin. The mode of action of coumarin is different from that of KIN since inhibitors of nucleic acid and protein synthesis significantly reduced tuberization (Stallknecht and Farnsworth, 1982b). Coumarin-induced tuberization was slightly stimulated by KIN but not affected by chlorocholine chloride (CCC), triiodobenzoic acid (TIBA) and succinic acid-2, 2-dimethyl hydrazine (ALAR) (Stallknecht and Farnsworth, 1982a).

Chlorocholine chloride promoted *in vitro* tuberization of isolated potato sprouts (Tizio, 1969; De Stecco and Tizio, 1982), and stem segments (Parrot, 1975) and overcame the delay in tuberization induced by GA_3 (Tizio and Goleniowski, 1985). The optimum concentration was 500 mg/l (3 mM) (Tizio, 1969). Microtuberization was achieved in a broad range of potato genotypes using CCC (500 mg/l; 3 mM) in conjunction with BA (5 mg/l; 22 μM) (Schilde et al., 1982; Estrada et al., 1986), but not with KIN (Palmer and Smith, 1969).

Ancymidol and paclobutrazol (10 μM) stimulated the microtuberization of recalcitrant cultivars (Harvey et al., 1991), while daminozide (Harvey et al., 1991), triadimefon (Chase

et al., 1989), salicylic acid and related compounds (Koda et al., 1992) and solanine-glycoalcaloide (Talukder and Paupardin, 1981) were all found to inhibit microtuber formation.

The effects of ethylene on *in vitro* tuberization have not been well established. The in vitro tuberization of etiolated sprouts was enhanced by 50 ppm ethrel, a ethylene releasing compound (Garcia-Torres and Gomez-Campo, 1973). Ethrel also increased tuber number and counteracted the depressive effects of exogenous gibberellins. In contrast, ethylene applications inhibited *in vitro* tuberization at all concentrations tested (Palmer and Barker, 1973; Mingo-Castel et al., 1976). Ethylene inhibited the promotive effect of KIN and CO_2 on *in vitro* tuberization of stolons (Mingo-Castel et al., 1976).

Studies of the effect of nitrogen (N) on *in vitro* tuberization yielded contradictory information. High nitrogen concentration (60 mM/l) was in general inhibitory and low concentration (2.5 mM/l) stimulatory to coumarin-induced microtuberization (Stallknecht and Farnsworth, 1979). Cytokinin-induced tuberization was not affected by high nitrogen concentration (Palmer and Smith, 1969; Wang and Hu, 1982). Studies with axillary buds from intact plants grown in medium containing 6% sucrose showed that tuberization was improved by adding ammonium nitrate in the range of 16-60 mM/l (Ewing, 1985). Microtuberization of single node segments of etiolated sprouts from old tubers was not affected by adding 40 mM/l ammonium nitrate, if sucrose concentration was ≥4%, but was depressed if

medium sucrose concentration was 2% (Koda and Okasawa, 1983a). Optimal N concentration was 60 mM/l for micropropagated single node cuttings grown on medium containing 4% sucrose (Garner and Blake, 1989). In this system decreasing the nitrate:ammonium ratio to 1:≤2 inhibited microtuberization.

Several aspects of microtuberization production remain to be investigated. Among these, the comparative effects of growth regulators, incubation periods, culture type (plantlets and nodal cuttings) and microtuber quality are particularly of interest for seed tuber certification programs.

2.6 Potato Dormancy and its Release

The potato tuber is a modified stem structure that usually develops below ground as result of the swelling of the subapical portion of the stolon with the simultaneous accumulation of reserve material. It is the current evolutionary outcome of a successful reproductive strategy where a plant adapts to an unfavourable environment by producing distinct vegetative propagules (Coleman, 1987).

For a certain period after their formation, tuber buds are unable to develope under conditions suitable for growth. This physiological state where buds are unable to sprout has been controversially termed dormancy or rest period. Tuber dormancy is thought to begin with the onset of tuberization (Burton, 1963; de Bottini et al., 1982) and end with the resumption of active bud growth, after a slow but continuous period of microscopic bud

growth and development (Burton, 1957; Coleman, 1987). The duration of tuber dormancy proceeds in a cultivar-specific manner with some variation due to seasonal and storage conditions (Simmonds, 1964; Thompson et al., 1980).

Tuber dormancy is thought to be induced and maintained by the β-inhibitor complex (Hemberg, 1985). Tuber formation was accompanied by an increase in endogenous inhibitors and ABA, a major constituent of the β-inhibitor suppressed bud growth (Hemberg, 1965; El-Antably et al., 1967; Nowak, 1977). The onset of rapid bud growth was accompanied by a sudden decrease in ABA concentration, although there was no evidence of a specific threshold concentration of ABA in the tuber below which sprouting occurred (Coleman and King, 1984). The mode(s) of action of the ß-inhibitor in inducing and maintaining tuber dormancy were not well understood due to the fact that its composition was poorly defined (Brenner, 1981) and this remains the case today.

Published information on microtuber dormancy is limited and contradictory. Microtubers exhibited no dormancy period (Hussey and Stacey, 1981), sprouted prematurely (Wattimena, 1983; Ortis-Montiel and Lozoya-Saldana, 1987; Harvey et al., 1991), or had dormancy periods of 1 to 7 mo (Budin and Ogluzdin, 1982; Hussey and Stacey, 1984; Estrada et al., 1986; Joyce and McCown, 1987; Rosell et al., 1987; Thieme, 1992). Uncertainty regarding the dormancy period of potato microtubers has limited their utilization in seed tuber certification programs.

The utilization of external agents for breaking tuber dormancy has been an active field of research since the late 19th century. The substances used for breaking dormancy are wide-ranging in chemistry. However, they often fall within the categories of growth regulators, respiratory inhibitors, sulfhydrils, anaesthetics or end-products of glycolysis (Coleman, 1987).

Exogenous applications of cytokinin broke tuber dormancy and concomitantly reduced the β-inhibitor complex (Hemberg, 1970). This observation was substantiated and the mode of action of cytokinins in breaking dormancy was established (Nooden and Weber, 1978; Stallknecht, 1983). Interestingly, the potential use of cytokinins as dormancy-breaking agents was restricted to brief periods immediately following the beginning and immediately prior to the end of the dormancy period. This variation in the sensitivity of dormant buds to exogenous cytokinin applications as well as changes in the level of endogenous zeatin-like cytokinins according to the specific stage of dormancy indicated that both hormone levels and tissue responsiveness to cytokinins were important in the control of dormancy (Turnbull and Hanke, 1985 a, b).

Potato dormancy can be terminated by exogenous application of gibberellins. Gibberellins may be endogenous regulators of bud dormancy and development, a view which is not consistently supported by all previously published work (Rappaport and Wolf, 1968; Tizio, 1982; Stallknecht, 1983). Gibberellins are known to

regulate the translocation and utilization of tuber food reserves to the developing sprout (Morris, 1966, 1967; Edelman et al., 1969; Dimalla and Van Staden, 1977;). Bailey et al. (1968) demonstrated that both bud and reserve tissues exhibit dormancy. They hypothesized that the storage tissues become responsive to bud-synthesized GA and produce reducing sugars only after the storage tissues are no longer dormant. It was hypothesized that GA regulated reserve mobilization through changes in intracellular compartmentation. In contrast, Clegg and Rappaport (1970) found that applied GA_3 stimulated reducing sugar formation in recently harvested tubers. On the basis of histochemical and quantitative analysis of sprouting tubers it was suggested that the pattern of starch and protein mobilization could be explained in terms of source-sink relationships between tubers and sprouts (Davies, 1984; Davies et al., 1984; Ross and Davies, 1985). However, the factors limiting sink strength of the growing sprouts are yet to be determined (Coleman, 1987).

In a series of experiments, Denny (1926a, b and 1945) evaluated the dormancy-breaking potential of several chemicals. Treatments with ethylene chlorhydrin, potassium thiocyanate, sodium thiocyanate, thiourea, and rindite (a mixture of ethylene chlorhydrin, ethylene dichloride and carbon tetrachloride 7:3:1 v/v) were all found to exhibit dormancy-release properties. Thiourea broke dormancy, inhibited apical dominance and forced the growth of all bud primordia. Glutathione also had dormancy-releasing effect (Guthrie, 1940). Bromoethane was recently

assessed as a dormancy-releasing agent and was as effective as ethylene chlorhydrine with the advantage of low toxicity to humans (Coleman and Coleman, 1986).

Peeling dormant tubers interrupted the dormancy period (Appleman, 1916; Pal and Nath, 1938; Thornton, 1939). Temporary or partial anaerobiosis caused by the reduction of oxygen concentration (Kidd, 1919; Rakitin and Suvorov, 1935; Thornton, 1939; Burton, 1968), increased carbon dioxide or nitrogen (12 to 60%; Thornton, 1939) or the presence of a water film on the tuber (Goodwin, 1966) were effective in terminating dormancy. The physiological basis of this well documented observation is unknown. The absence of accumulation of sugars and the production of volatile end-products of glycolysis, such as ethanol and acetaldehyde under anaerobic conditions have been observed (Samotus and Schwimmer, 1963; Samotus, 1971). The question arises, whether aerobic metabolism is required for maintenance of dormancy, or if there is a positive effect of anaerobiosis on tuber metabolism.

Cutting microtubers in half was effective in releasing dormancy (Ewing et al., 1988). Although the mechanism of dormancy release was not studied, it was speculated that ethylene produced on the cut surface was responsible. The use of rindite was effective in releasing microtuber dormancy (Verhoyen and Givron, 1981).

The general lack of information concerning potato microtuber dormancy limits their utilization of microtubers in certification

programs. The influence of genotypes, microtuber size, *in vitro* incubation period and growth regulators used to promote microtuberization must be determined to optimize the use of microtubers in certification programs.

2.7 Physiological Ageing

The physiological state of potato seed tuber evolves constantly from the onset of tuber formation to their development into plants. To characterize the influence exerted by the physiological state of seed tubers on sprout growth, and subsequent plant growth and yield characteristics, the concept of physiological ageing was introduced by Madec and Pérennec (1962).

The physiological age of seed tubers increases with the chronological age of tubers and with storage temperatures; higher temperatures promoted more rapid aging (Madec and Pérennec, 1956; Pérennec and Madec, 1960; Fischnich and Krug, 1963). Physiological ageing occurred in the presence or absence of a sprout (Ewing and Struik, 1992). High temperatures during the growing season accelerated the physiological ageing of potato tubers (Iritani, 1968; Claver, 1973), but high temperatures after top-kill or removal of tubers from the tops were more important in the acceleration of physiological aging (Van Ittersum and Scholte, 1992). Genotypic variation is a major component in the expression of physiological ageing. Generally, tubers of cultivars that sprouted early in storage tended to become physiologically old more rapidly than cultivars that sprouted

later, but there were a number of exceptions to this rule (Ewing and Struik, 1992).

Tuber yields increased with the physiological age of seed tubers up to a maximum, beyond which they were reduced (Kawakami, 1963). When compared to physiologically younger seed tubers, physiologically older tubers emerged more rapidly, initiated tubers earlier and produced greater yields under a short growing season, but lower yields under longer growing seasons (Toosey, 1964; Iritani, 1968; Wurr, 1978; Allen and Scott, 1980; Pérennec and Madec, 1980; van der Zaag and van Loon, 1987). Adverse environmental conditions before emergence or during the growing season could alter this behaviour (Pérennec and Madec, 1980).

The effects of physiological ageing on the yield of microtuber-derived plants has not yet been determined. A better understanding of the effect of physiological ageing on microtubers would be useful, allowing seed tuber certification programs to use microtubers when these are potentially more productive.

2.8 Field Performance of Micropropagated Potato

The field performance of *ex vitro* plantlet-derived (EVPD) plants has been extensively evaluated but with contradictory results. Stem number per plant was similar for seed tuber-derived (STD) 'Pontiac' plants but significantly lower for EVPD 'Kennebec' plants (Goodwin and Brown, 1980). Tuber number per plant was not significantly different for EVPD and STD plants.

Tuber weight of 'Kennebec' EVPD plants acclimatized for 8 wk prior to field planting was similar to STD plants.

Wattimena et al. (1983) compared the field performance of EVPD and microtuber-derived (mTD) to STD plants and found that stem number per plant was significantly less and tuber number per plant was significantly greater for EVPD and mTD plants than for STD plants. Although total tuber weight per plant was significantly greater at peak flowering time for STD plants, there were no significant differences in tuber weight at the end of the growing season. However, for both cultivars EVPD and mTD plants produced fewer big tubers (US1A) and more small tubers (US1B) than STD plants. EVPD and mTD plants were believed to be physiologically different from STD plants; tuber initiation occurred over a longer period and resorption was less than with tuber-produced plants. Wattimena et al. (1983) observed that single-stemmed micropropagated plants branched vigorously.

Typically, EVPD plants produced single-stemmed plants with extensive bud development (Levy, 1985; Leclerc and Donnelly, 1990). The length of the growing period, and the climatological conditions were also found to affect the multiplication rate and the number of tubers greater than 10 mm in diameter (Levy, 1986). Lengthening the growing season significantly increased the total tuber yield and proportion of larger tubers (Thornton and Knutson, 1986). It is important to take into consideration the length of the growing season and the heat unit accumulation since these parameters were also found to be important in the

recontamination of virus-free stocks (Smith and Storch, 1984). The determination of the optimum growing season length has to take into account both yield and disease-spread factors, since micropropagated plantlets appeared to be more susceptible to infection by PVS and PVY compared with seed tubers (MacDonald, 1987). EVPD plants were found to be more susceptible to early water stress in the field than STD plants probably due to an earlier production of stolons (Leclerc, 1989; Leclerc and Donnelly, 1990).

Acclimatizing plantlets prior to their transfer into the field increased the survival rate from 75% to 98% (Levy, 1986). Protecting the EVPD plants from drought and wind immediately upon planting considerably increased establishment in the field. Studies on the acclimatization and re-establishment of tissue cultured plantlets showed that 5 d under a polyethylene tent, 5 d under a mist of 30 sec every 30 min and either 7 or 14 d under a mist of 60 sec every 30 min yielded the most productive plants (Bourque, 1983). Carbon dioxide enrichment *in vitro* was not found to be beneficial and did not increase growth once the plantlets were removed from culture. Temperatures of 2 to 4 °C were found adequate for storing plantlets for field multiplication while temperatures of 8 to 10 °C decreased the survival rates of plantlets (Allen and Knutson, 1982; Bourque, 1983). Storing plantlets prior to transplanting significantly decreased tuber production and significantly increased plant

height regardless of the storage temperature (Allen and Knutson, 1982).

Cutting *in vitro* plantlets into single or double node cuttings at the time of their transfer into the greenhouse did not significantly affect yield (Levy, 1988) but significantly increased the availability of plant material. There was less variability in the establishment and growth of *ex vitro* cutting-derived (EVCD) plants therefore more accurate yield predictions could be made. In addition, automated and semi-automated planting may be adapted to EVCD plants.

The application of daminozide (0.3 to 0.9 mg/l; 5 to 15 μM), a growth retardant compound found to reduce stem length of tissue cultured potato (Radatz et al., 1962; Wescott, 1981 and Marinus, 1985), improved the survival of plantlets directly transplanted into the field (Sipos et al., 1988). Daminozide caused a delay in tuberization in 'Russet Burbank', but this did not affect tuber-bulking nor plant growth. The application of triazole, a fungicide with growth regulating activities, decreased tuber number and yield of EVPD plants (Chase et al., 1989).

Increasing the volume of the container used during the acclimatization period increased total tuber yield and yield of tubers larger than 35 mm in diameter. So, optimizing the transplant container volume to reduce the greenhouse space requirement while at the same time maximizing yield of EVPD plants is an important issue (Thornton and Knutson, 1986).

A small but increasing number of growers in Canada and the United States are experimenting with on-farm tissue culture with very satisfactory results (Mattive, 1986; Starkel, 1986). The effect of agronomical practices such as planting density and planting depth on the yield of STD plants are well known. This information is readily available to seed tuber and tablestock growers through the culture guides published by provincial agencies. On the other hand, little is known of the effect of these agronomical practices on the yield of *ex vitro* derived-plants. Decreasing the distance between rows from 90 to 45 cm while keeping the distance between plants fixed at 10-15 cm tended to reduce tuber weight but not tuber number per plant (Levy, 1985). Preliminary investigations demonstrated that decreasing the in-row planting density from 45 to 15 cm decreased both tuber weight and number of 'Red Pontiac' (Wattimena, 1983).

Planting *ex vitro* plantlets or microtubers directly into the field could significantly reduce the number of field generations necessary for the production of certified seed tubers. This would allow significant economic gains and would increase the phytosanitary quality of potato seed tubers. To optimize the use of plantlets and microtubers in seed tuber certification programs the effect of in-row planting density and planting depth on the yield of these propagules must be evaluated.

PREFACE TO CHAPTER 3

Chapter 3 is the material contained in a manuscript by Yves Leclerc, Danielle J. Donnelly and Janet E.A. Seabrook submitted for publication to the journal Plant Cell, Tissue and Organ Culture. The format has been changed to conform, as much as possible, to a consistent format within the thesis according to guidelines set by the Faculty of Graduate Studies. In this chapter we described the efforts to increase the productivity of microtuberization systems while reducing medium complexity. To realize this goal experiments were conducted to: a) evaluate the efficiency of coumarin, chlorocholine chloride and 6-benzylamino-purine as promoters of microtuberization, b) evaluate the effect of the duration of incubation period on microtuber yield and c) compare microtuberization productivity of layered plantlets with nodal cuttings.

CHAPTER 3. THE INFLUENCE OF GROWTH REGULATORS AND INCUBATION PERIODS ON MICROTUBERIZATION OF PLANTLETS AND NODAL CUTTINGS OF POTATO.

3.1 Abstract

A protocol is presented for the rapid induction of microtubers on micropropagated, layered potato plantlets in a medium devoid of growth regulators. The addition of 2*H*-1-benzopyran-2-one (coumarin) or (2-chloroethyl)-trimethylammonium chloride (chlorocholine chloride) and *N*-(phenylmethyl)-1*H*-purin-6-amine) 6-benzylaminopurine to the microtuberization medium either had no effect or significantly reduced microtuber weight per plantlet compared with medium containing only 80 g/l sucrose and minimally affected the number of microtubers per plantlet of 'Kennebec', 'Russet Burbank' and 'Superior'. Increasing the incubation period from 28 to 56 d did not affect the number but significantly increased the weight of microtubers per plantlet and substantially increased the proportion of microtubers heavier than 1 gram. Layered plantlets microtuberized more rapidly and produced significantly larger microtubers compared with nodal cuttings.

3.2 Introduction

The first report of *in vitro* tuberization was published by

Barker (1953) who used etiolated sprouts to induce tuberization in medium containing 80 g/l sucrose. Since then the utilization of growth regulating agents to favour tuberization *in vitro* (microtuberization) has been the object of intensive investigation. Among the substances used to induce microtubers, coumarin, (2-chloroethyl)trimethylammonium chloride (CCC) and cytokinins have received most attention (Wattimena, 1983; Wang and Hu, 1985; Chandra et al., 1988).

Cytokinins and coumarin are believe to have strong promotive effects on tuberization, and to constitute part of the tuberization stimulus, either alone or in combination with other substance(s) (Forsline and Langille, 1975; Palmer and Smith, 1969b, 1970; Pelacho and Mingo-Castel, 1991; Stallknecht and Farnsworth, 1982a, b). However, growth regulators failed to induce tuberization when the sucrose supply was inadequate (Harmey, and al., 1966). Sucrose may be the only compound necessary for induction of microtubers (Gregory, 1956; Ewing, 1985, 1990).

A reliable microtuber production method on medium free of any growth regulating agent was reported by Garner and Blake (1989). Nodal cuttings were grown on medium containing Murashige and Skoog (MS, 1962) basal salts with 80 g/l sucrose, and incubated first under a 16 h and then an 8 h photoperiod under 85 $\mu mol/m^2/s$ photon flux density. After 17 wk of incubation each cutting had produced approximately 1 microtuber weighing <200 mg.

Increasing the productivity of microtuberization systems

while reducing medium complexity is a desirable objective. To realize this goal experiments were conducted to: a) evaluate the efficiency of coumarin, CCC and 6-benzylaminopurine (BA) as promoters of microtuberization, b) evaluate the effect of the duration of incubation period on microtuber yield and c) compare microtuberization productivity of layered plantlets with nodal cuttings.

3.3 Materials and Methods

Multiplication of *in vitro* material.

Nodal cuttings of 'Kennebec', 'Superior' and 'Russet Burbank' were grown on modified MS basal salt solution supplemented with 0.4 mg/l thiamine-HCl, 2 mg/l Ca-pantothenate, 100 mg/l inositol, 30 g/l sucrose, 7 g/l agar (Anachemia, Lachine, Quebec) and the pH adjusted to 5.7 prior to autoclaving. Cultures were grown at 22 ± 2 °C under 80 $\mu mol/m^2/s$ cool white fluorescent illumination and 16 h photoperiod and were subcultured every 4 wk.

Microtuber production using layered plantlets.

Microtubers were induced by a modified two-step procedure (Estrada et al., 1986; Meulemans et al., 1986). In the first step, 3 root-severed plantlets with 6 nodes each were layered in 50 ml of propagation medium containing modified MS (1962) basal salt solution supplemented with 0.4 mg/l thiamine-HCl, 2 mg/l Ca-

pantothenate, 100 mg/l inositol, 0.4 mg/l gibberellic acid (GA_3), 0.5 mg/l BA, 20 g/l sucrose, with the pH adjusted to 5.7 in 400 ml plastic containers (Better Plastics, Kissamee, Fla. U.S.A.). This first stage, which promotes vegetative growth prior to microtuberization, significantly increased microtuber weight and number per plantlet compared with plantlets placed directly into microtuberization medium. Cultures were incubated at 22 ± 2 °C under 80 $\mu mol/m^2/s$ cool white fluorescent illumination and 16 h photoperiod. After 4 wk the residual medium was drained off and replaced by 50 ml of microtuberization media containing modified MS basal salt solution supplemented with 0.4 mg/l thiamine-HCl, 2 mg/l Ca-pantothenate, 100 mg/l inositol and either a) 50 mg/l coumarin and 80 g/l sucrose; b) 500 mg/l CCC, 5.0 mg/l BA (CCC-BA) and 80 g/l sucrose and c) a control medium containing 80 g/l sucrose but no growth regulator. Plantlets were incubated at 15 ± 2 °C under 50 $\mu mol/m^2/s$ cool white fluorescent illumination and 8 h photoperiod.

Microtuber production using nodal cuttings.

Three *in vitro* plantlets with 6 nodes each were sectioned into single node cuttings (the shoot apex was discarded) and induced to tuberize in 50 ml of microtuberization medium (media a-c listed above) in 400 ml plastic containers under the incubation conditions described above. Solidified (7 g/l agar) microtuberization media was used since it favoured the tuberization of nodal cuttings in preliminary experiments.

The effect of growth regulators and duration of incubation on the microtuberization of layered plantlets

A factorial experiment, consisting of 18 treatment combinations of cultivars ('Kennebec', 'Russet Burbank' and 'Superior'), growth regulators (coumarin, CCC-BA and control medium) and duration of incubation periods (28 and 56 d), was conducted using a randomized complete block design (RCBD). Seventy-two plantlets per treatment combination were used in this experiment.

Microtuber production using layered plantlets and nodal cuttings.

A factorial experiment consisting of 18 treatment combinations of cultivars, growth regulators and culture types (layered plantlets and nodal cuttings) was conducted using a RCBD. Thirty-six plantlets (216 nodal cuttings) per treatment combination were harvested after 28 and 56 d of incubation.

Data Analysis

Microtuber weights and numbers were compared on a per original plantlet basis (per layered plantlet or per six nodal cuttings). The data was analyzed using the Analysis of Variance (ANOVA) and treatment means were separated by the Duncan's New Multiple-Range Test (Steel & Torie, 1980). The homogeneity of microtuber size was evaluated by analyzing the variance of individual microtuber weights using Log-ANOVA. Microtuber fresh weight distributions were analyzed using the Chi-square test of

independence (Scherrer, 1984).

3.4 Results

The effect of growth regulators and duration of the incubation period on the microtuberization of layered plantlets

Microtuber weight per plantlet was significantly influenced by cultivar, growth regulator, duration of the incubation period, and by both the cultivar-growth regulator and cultivar-incubation period interactions (Table 3.1). After 28 d of induction, 'Superior' had heavier microtuber weights per plantlet than the other 2 cultivars (Table 3.1). Differences in microtuber weights between 'Superior' and 'Russet Burbank' plantlets were no longer significant after 56 d, but both were significantly heavier than 'Kennebec'. Medium containing CCC-BA depressed microtuber weight per 'Kennebec' plantlet compared with the other two media for both incubation periods. Growth regulators did not influence the microtuber weight of 'Russet Burbank' after 28 d, but the medium containing CCC-BA produced a significantly lower microtuber weight per plantlet than the two other media after 56 d. The control medium produced heavier microtuber weights per plantlet for 'Superior' than the media containing coumarin and CCC-BA. The differences between the control and coumarin-containing media were no longer significant after 56 d, but both produced heavier microtubers than the CCC-BA medium.

Microtuber number per plantlet was influenced by the

cultivar and growth regulator treatments while the variance of microtuber weight was influenced by the duration of the incubation period (Table 3.1). Microtuber number per plantlet was significantly less for 'Kennebec' than for the other 2 cultivars after 28 and 56 d of incubation. Growth regulators did not influence the number of microtubers produced per plantlet, except for 'Kennebec' where the medium containing CCC-BA induced fewer microtubers than the other two media. After 56 d of incubation, the CCC-BA medium again induced fewer microtubers per plantlet in 'Kennebec' and 'Russet Burbank', compared with the control medium.

Microtuber production using layered plantlets and nodal cuttings.

After 56 d, nodal cuttings produced significantly lower microtuber weights than layered plantlets when compared on a per plantlet basis (6 nodes), but the combined number of microtubers was significantly greater using nodal cuttings (x=5.9) than for layered plantlets (x=3.6) (Table 3.2). For layered plantlets, the effects of cultivar and inducing agent treatment on microtuber weight and number per plantlet were similar to the results of the previous experiment. For nodal cuttings, 'Russet Burbank' produced a greater microtuber weight per plantlet than 'Kennebec' and 'Superior', while the latter two had similar microtuber weights per plantlet. Microtuber fresh weight per plantlet in 'Kennebec' was significantly lower for medium containing CCC-BA than for the control medium, which produced a

lower microtuber weight per plantlet than the coumarin medium (Table 3.2). For 'Russet Burbank' and 'Superior' medium containing CCC-BA produced a lower microtuber weight per original plantlet than the control and coumarin media. For both layered plantlets and nodal cuttings tuber number per plantlet was not affected by the duration of the incubation period, but microtuber fresh weight per plantlet was significantly greater after 56 than 28 d of incubation (data not shown).

The comparison of the variances of microtuber fresh weights, when produced from layered plantlets and nodal cuttings after 28 and 56 d, showed that the variance increased with incubation period for both types of culture and that it was greater for layered plantlets than for nodal cuttings (Table 3.3).

The Chi-square analysis of the frequency distribution of microtuber fresh weight classified according to 4 categories (≤0.25, 0.25-0.50, 0.50-1.0 and >1.0 g) indicated a significant effect of the length of the incubation period and culture type treatments on the frequency distribution of microtuber weight (Table 3.4). For layered plantlets, increasing the incubation period from 28 to 56 d reduced the frequency of smaller microtubers (≤250 mg) and increased the frequency of microtubers >1.0 g. Growth regulator treatments influenced the distribution of microtuber fresh weights for all three genotypes. For nodal cuttings, increasing the incubation period from 28 to 56 d had a limited effect on fresh weight distribution; nearly all microtubers weighed less than 250 mg. Growth regulator

treatments had no influence on microtuber frequency distribution except for 'Russet Burbank' after 56 d.

3.5 Discussion and Conclusions

The microtuberization system reported here permitted the rapid and extensive tuberization (up to 2 g of microtuber fresh weight per original plantlet) of in vitro plantlets of 'Kennebec', 'Russet Burbank' and 'Superior' in an MS based medium containing elevated sucrose level alone and incubated under an 8 h photoperiod at 15 °C.

The effect of growth regulators and duration of the incubation period on the microtuberization of layered plantlets

The addition of coumarin, CCC and BA to the microtuberization medium of layered plantlets failed to trigger any increase in yield or number of microtubers per plantlet compared with elevated sucrose alone and may have depressed microtuber yield per plantlet to some extent. Coumarin increased individual microtuber weight as indicated by the frequency distribution analysis. Coumarin increased the number of larger microtubers compared with CCC-BA and, to a lesser extent, with the control medium.

Contrary to the results of Hussey & Stacey (1984), the addition of CCC and BA to the microtuberization medium failed to increase the microtuberization response of plantlets compared

with the control medium. The promotive effect of cytokinins and CCC used singly on microtuberization has previously been questioned (Stallknecht, 1985; Ewing, 1990). Under a short photoperiod and cool temperature, the addition of coumarin and CCC-BA to the microtuberization medium of plantlets is unnecessary and may even be deleterious.

Microtuber production using layered plantlets and nodal cuttings.

When layered plantlets and nodal cuttings were incubated for the same period of time, microtuber fresh weight per original plantlet produced from layered plantlets was much greater than for nodal cuttings. After 56 d under an inductive environment the microtuber yield of layered plantlets was 3-5 times greater than for nodal cuttings. Differences in the surface leaf area (site of the perception of the photoperiodic stimulation) and in the surface of plant tissues in contact with the medium are likely responsible for the large difference in microtuber fresh weight between the two types of culture. Favouring vegetative growth of stolon segments and plantlets prior to microtuber induction have been reported to increase microtuber fresh weight (Chapman, 1955; Garner and Blake, 1989). Clearly, greater microtuber yield can be achieved by increased vegetative area of explants cultures prior to microtuber induction.

Although layered plantlets produced heavier microtuber weight per original plantlet, nodal cuttings produced more microtubers. This difference in microtuber number per original

plantlet is most likely due to the expression of correlative inhibition in layered plantlets. When plantlets are dissected into nodal cuttings the hormonal balance of the nodal cuttings is disrupted, promoting microtuberization.

The advantage of the technique outlined here over Garner and Blake's (1989) is that heavier microtubers can be produced more rapidly. One potential drawback of this method is the increased heterogeneity in microtuber weight.

In conclusion, microtuberization of plantlets was achieved rapidly by layering them in propagation medium for 28 d, and then transferring them to modified MS based liquid microtuberization medium containing 80 g/l sucrose under 8 h light period of 50 $\mu mol/m^2/s$ at 15 C. With this method exogenously supplied coumarin and CCC-BA were not necessary for optimal microtuber production. This microtuberization system, a simplified version of the protocol developed by Estrada et al. (1986) and Meulemans et al. (1986), may have significant commercial implications. The use of liquid media, the absence of growth regulators in the microtuberization media and the lower labour input required to handle plantlets could reduce the production cost of microtubers.

The application of this microtuberization system to the study of potato tuberization may prove valuable, since both aspects of tuber formation, i.e. the perception of environmental stimulation and the reaction to this stimulation, can be studied simultaneously *in vitro*.

Table 3.1. Microtuber fresh weight and number per original plantlet as affected by cultivar, growth regulator and duration of the incubation period. Each observation represents the average of 72 plantlets.

Treatments	Microtuber weight (mg)		Microtuber number	
	28 days	56 days	28 days	56 days
Kennebec				
Coumarin	1178 a	2102 a	3.3 a	3.4 a
CCC-BA	792 b	1314 b	2.6 b	2.8 b
Control	1042 a	1992 a	3.4 a	3.4 a
Mean	1004 b	1802 a	3.10 a	3.2 a
Russet Burbank				
Coumarin	1164 a	2292 a	3.6 a	3.9 b
CCC-BA	945 a	1838 b	3.8 a	3.5 b
Control	1026 a	2137 a	4.6 a	4.9 a
Mean	1045 b	2085 a	4.0 a	4.0 a
Superior				
Coumarin	1501 b	2044 ab	4.2 a	4.0 a
CCC-BA	1253 b	1842 b	3.8 a	3.8 a
Control	1624 a	2351 a	4.2 a	4.0 a
Mean	1459 b	2174 a	4.1 a	3.9 a

Significance[1]

Source of variation	Microtuber weight per plantlet	Microtuber number per plantlet	Variance of[2] microtuber weight
Cultivar (C)	***	***	NS
Growth regulator (G)	***	*	NS
C x G	**	NS	NS
Incubation period (I)	***	NS	***
C x I	**	NS	NS
G x I	NS	NS	NS
C x G x I	NS	NS	NS

Means separated by Duncan's New Multiple-Range Test, $p \leq 0.05$.
[1]. NS, *, ** and *** indicate nonsignificance or significance at the α=0.05, 0.01 or 0.001 levels respectively.
[2]. The analysis was conducted by Log-Anova.

Table 3.2. Microtuber fresh weight and number per original plantlet as affected by cultivar, growth regulator and culture type after 56 d of incubation. Each observation represents the average of 36 plantlets (216 nodal cuttings).

Treatments	Microtuber weight (mg) per plantlet		Microtuber number per plantlet	
	Layered shoot	Single node	Layered shoot	Single node
Kennebec				
Coumarin	2146 a	655 a	3.2 a	6.1 a
CCC-BA	1622 b	204 c	2.5 b	5.7 a
Control	1981 a	452 b	3.3 a	6.0 a
Mean	1916 a	446 b	3.0 b	5.9 a
Russet Burbank				
Coumarin	2276 a	840 a	3.5 b	5.9 a
CCC-BA	1801 a	264 b	3.6 b	5.5 a
Control	2202 a	664 a	4.6 a	6.2 a
Mean	2093 a	629 b	3.9 b	5.8 a
Superior				
Coumarin	2093 ab	424 a	4.0 a	6.0 a
CCC-BA	1795 b	194 b	3.6 a	6.0 a
Control	2304 a	366 a	3.8 a	5.9 a
Mean	2064 a	358 b	3.8 b	6.0 a

Significance[1]

Source of variation	Microtuber weight per plantlet	Microtuber number per plantlet	Variance of[2] microtuber weight
Cultivar (C)	NS	*	NS
Growth regulator (G)	***	NS	NS
C x G	NS	NS	NS
Culture type (T)	***	***	***
C x T	***	*	NS
G x T	NS	NS	NS
C x G x T	NS	NS	NS

Means separated by Duncan's New Multiple-Range Test, $p \leq 0.05$.
[1]. NS, *, ** and *** indicates nonsignificance or significance at the α=0.05, 0.01 or 0.001 levels respectively.
[2]. The analysis was conducted by Log-ANOVA

Table 3.3. Effect of the duration of incubation period and culture type on the variance of microtuber fresh weight. The tubers of 324 plantlets per treatment were used in this analysis.

Incubation period (days)	Microtuber fresh weight variance (10^3 mg)	
	Layered shoot	Single node
28	52.3 b	0.9 d
56	161.8 a	1.8 c

Means separated by Duncan's New Multiple-Range Test, $p \leq 0.05$

Table 3.4. Effect of the duration of incubation, cultivar, growth regulator and culture type on the frequency distribution (%) of microtubers. The tubers of 36 plantlets per treatment combination were used in this analysis.

	Size category (g)					
	Layered shoot				Nodal cutting	
Treatments	≤0.25	0.25-0.50	0.50-1.0	>1.0	≤0.25	0.25-0.50
28 days						
Kennebec						
Coumarin	64	20	10	6	100	0
CCC-BAP	59	26	14	1	100	0
Control	70	13	12	5	100	0
Russet Burbank						
Coumarin	54	22	19	5	100	0
CCC-BAP	65	23	11	1	100	0
Control	68	20	10	2	100	0
Superior						
Coumarin	66	15	13	6	100	0
CCC-BAP	66	17	15	2	100	0
Control	61	19	14	6	100	0
56 days						
Kennebec						
Coumarin	35	17	25	23	96	4
CCC-BAP	40	23	29	8	99	1
Control	34	27	20	19	98	2
Russet Burbank						
Coumarin	31	24	23	22	88	12
CCC-BAP	32	28	21	19	98	2
Control	50	18	20	12	99	1
Superior						
Coumarin	34	36	20	10	99	1
CCC-BAP	37	20	26	17	98	2
Control	39	17	23	21	99	1

Significant X^2 values: Period $p \leq 0.001$; Explant type $p \leq 0.001$;
Russet Burbank 30 days: Coumarin (COU) vs CCC-BAP (CCC) p=0.006, COU vs Control (CON) p=0.011, 60 days: COU vs CON p=0.011, Nodal cutting: COU vs CCC p=0.016, COU vs CON p=0.001;
Kennebec 30 days: COU vs CCC p=0.026, CCC vs CON p=0.008, 60 days: COU vs CCC p= 0.026
Superior 30 days: COU vs CCC p=0.02, CON vs CCC P=0.008, 60 days: COU vs CCC p=0.040, COU vs CON p=0.017

CHAPTER 4. THE EFFECTS OF PLANTLET PRE-CONDITIONING AND SUCROSE AVAILABILITY ON MICROTUBER PRODUCTION

4.1 Introduction

The tuberization of cuttings *in vivo* was shown to be influenced by the amount and type of photoperiodic stimuli (Ewing and Wareing, 1978). Photoperiodic treatments of plantlets influenced the *in vitro* tuberization response of nodal cuttings (Garner and Blake, 1989; Slimmon et al., 1989; Lentini and Earle, 1991). The photoperiodic stimulation received prior to the induction of microtubers was retained by the nodal cutting and influenced the microtuberization response (Marinus, 1990; Pérennec and Francois, 1981). Microtuber number and fresh weight was significantly affected by cultivar and pre-conditioning interactions when potato plantlets were pre-treated with different photoperiods (8 and 16 h) prior to microtuber induction (Seabrook et al., 1993). The latter concluded that microtuber production was affected by the maturity group of potato cultivars and photoperiodic regimes *in vitro*.

The concentration and type of sugar in the medium strongly effected microtuberization (Wang and Hu, 1985). The influence of sugar on microtuberization was not related to osmotic effects but rather to carbohydrate availability (Lo et al., 1972). Sucrose at a concentration of 80 g/l, is generally considered to be optimal for *in vitro* tuberization. The effect of increasing the

availability of sucrose by increasing the medum volume has not been investigated.

The objectives of these preliminary experiments were to evaluate the effects of environmental pre-conditioning and increased sucrose availability (without affecting medium osmolarity) on the microtuberization of potato plantlets in order to increase the productivity of the microtuberization system described in Chapter 3.

4.2 Materials and Methods

Microtubers of 'Kennebec', 'Russet Burbank' and 'Superior' were produced from *in vitro* plantlets using the two step microtuberization system described in Chapter 3. The propagation medium contained modified MS (1962) basal salt solution supplemented with 0.4 mg/l thiamine-HCl, 2 mg/l Ca-pantothenate, 100 mg/l inositol, 0.4 mg/l gibberellic acid (GA_3), 0.5 mg/l 6-benzylaminopurine (BA), 20 g/l sucrose and the pH was adjusted to 5.7 prior to autoclaving. Cultures were incubated in 400 ml plastic container (Better Plastic, Kissamee, Fla, U.S.A.) at 22 °C, under 80 $\mu mol/m^2/s$ photon flux density supplied by cool white fluorescent lamps and 16 h light period. The microtuberization media contained modified MS (1962) basal salt solution supplemented with 0.4 mg/l thiamine-HCl, 2 mg/l Ca-pantothenate, 100 mg/l inositol and either a) 80 g/l sucrose, 500 mg/l (2-chloroethyl)trimethyl-ammonium chloride (CCC) and 5.0 mg/l BA or

b) 80 g/l sucrose but no growth regulator. The plantlets were incubated at 15 °C under a 50 $\mu mol/m^2/s$ photon flux density and 8 h light period.

Environmental pre-conditioning of plantlets.

Three root-severed plantlets per container were layered in 50 ml of liquid propagation medium and incubated at 22 °C and 16 h light period (conditions favourable to vegetative growth) for 3 wk. Half the containers were then transferred to environmental conditions conducive to tuber induction; 15 °C and 8 hour light period for 1 wk. The other half was maintained under conditions favouring vegetative growth. Residual propagation medium was drained and replaced by 50 ml of microtuberization media. Both microtuberization media were used in this experiment. Microtubers were harvested after 4 wk. The experiment was conducted using a Randomized Complete Block Design (RCBD). Each replication consisted of 3 containers of 3 plantlets each. The experiments were replicated 4 times in time.

Volume of microtuberization medium.

Root-severed plantlets previously incubated in propagation medium were induced to tuberize in 50 or 100 ml of microtuberization medium containing no growth regulator. Microtubers were harvested after 4 weeks of incubation. The experiment was conducted using a RCBD. Each replication consisted of 3 containers of 3 plantlets each. The experiments

were replicated 4 times in time.

4.3 Results and Discussion

Microtuber weight per plantlet was influenced by the cultivar, the microtuberization medium utilized, the environmental pre-conditioning, and both the cultivar and microtuberization medium, and cultivar and pre-conditioning interactions (Table 4.1). Microtuber weight was significantly heavier when plantlets were pre-conditioned under inductive conditions and were subsequently incubated in microtuberization medium containing CCC-BA, but no differences were observed when plantlets were incubated in the control medium containing only sucrose.

Microtuber number per plantlet was influenced by the cultivar and the microtuberization medium utilized. Plantlets of 'Kennebec' but not 'Russet Burbank' or 'Superior' incubated in the control medium produced more microtubers than plantlets incubated in the CCC-BA medium. Maintaining plantlets under a non-inductive environment increased microtuber number for all cultivar/microtuberization medium combinations, but significant differences were observed only for plantlets of 'Russet Burbank' incubated in the control medium.

The addition of CCC and BA to the microtuberization medium reduced the microtuber weight and number per plantlet compared with the control medium, confirming previous findings (see

Chapter 3). This observation and the fact that plantlets induced to tuberize in medium containing CCC and BA responded differently to environmental pre-conditioning compared with plantlets incubated in microtuberization medium containing sucrose alone indicated that the addition of CCC and BA to the microtuberization medium modified the processes of tuberization.

Microtuber weight and number per plantlet were significantly influenced by the cultivar and the volume of microtuberization medium utilized, and the cultivar and the volume of medium interaction had a significant influence on the microtuber weight per plantlet (Table 4.2). Increasing medium volume from 50 to 100 ml resulted in a heavier microtuber weight per plantlet for all cultivars, but significant differences were detected only for 'Superior'. For all cultivars, microtuber number per plantlet was significantly greater when 100 ml of medium was used.

Pre-conditioning plantlets for 1 wk prior to microtuber induction failed to trigger any increase in the number or weight of microtubers produced in microtuberization medium containing only sucrose. However, microtuber weight was significantly increased when plantlets were pre-conditioned under an inductive environment and incubated in microtuberization medium containing CCC, BA and sucrose. This indicates that the effect of environmental stimulation can be modified by exogenously supplying growth regulating substances into the microtuberization medium, as previously speculated by Wang and Hu (1985). Augmenting the volume of microtuberization medium increased the

number of microtubers per plantlet. It is unclear if 100 ml is the optimum volume for the microtuberization of plantlets under our experimental conditions, but other experiments (data not shown) have demonstrated that totally immersing plantlets into the medium completely inhibited microtuber formation. Optimum medium volume must probably depend on the amount of plant material in the container as well as container type and volume.

Several conclusions may be drawn from these preliminary experiments. Microtuber production (number) can be increased by augmenting the microtuberization medium volume up to an optimum volume, which has not been determined, beyond which tuberization is inhibited. Short (1 wk) pre-conditioning periods did not increase the microtuberization response of plantlets incubated in medium containing sucrose but no growth regulators. The growth regulator composition of the medium can influence the microtuberization response of plantlets to environmental stimulation. Further work is needed to optimize the volume of microtuberization medium and investigate the duration of pre-conditioning treatments.

Table 4.1. The effect of environmental pre-conditioning on microtuber weight (mg) and number per plantlet harvested after 4 weeks of incubation.

	Microtuber weight		Microtuber Number	
	Environmental pre-conditioning		Environmental pre-conditioning	
	Inductive	Non-Inductive	Inductive	Non-Inductive
Kennebec				
CCC-BAP	784 a	579 b	2.5 a	2.9 a
Control	890 a	1068 a	3.1 a	3.7 a
Russet Burbank				
CCC-BAP	1050 a	722 b	3.9 a	4.4 a
Control	996 a	1152 a	4.0 b	5.1 a
Superior				
CCC-BAP	1095 a	885 b	3.5 a	3.8 a
Control	1324 a	1439 a	4.2 a	4.3 a
Significance[1]				
Cultivar (C)		***		***
Microtuberization medium (M)		***		***
C x M		*		NS
Pre-conditioning (P)		*		NS
C x P		***		NS
M x P		NS		NS
C x M x P		NS		NS

Means followed by the same letter are not significantly different at the $p \leq 0.05$ level, Duncan's New Multiple-Range Test.
[1] NS, * and *** indicate non-significance or significance at the $p \leq 0.05$ and 0.001 levels respectively.

Table 4.2. The effect of the quantity of tuberization medium on weight and number of microtubers per plantlet, harvested after 4 weeks of incubation.

	Microtuber weight (mg)		Microtuber number	
	50 ml	100 ml	50 ml	100 ml
Russet Burbank	905 a	936 a	4.8 b	5.8 a
Kennebec	944 a	1051 a	3.7 b	5.3 a
Superior	1671 b	2479 a	4.1 b	5.9 a
Significance[1]				
Cultivar (C)	***		*	
Volume (V)	***		***	
C x V	***		NS	

Means followed by the same letter are not significantly different at the $p \leq 0.05$ level, Duncan's New Multiple-Range Test.
[1] NS, * and *** indicate non-significance or significance at the $p \leq 0.05$ and 0.001 levels respectively.

PREFACE TO CHAPTER 5

Chapter 5 is the material contained in a manuscript by Yves Leclerc, Danielle J. Donnelly, Warren K. Coleman and Russell R. King submitted for publication to the American Potato Journal. The format has been changed to conform, as much as possible, to a consistent format within the thesis, according to guidelines set by the Faculty of Graduate Studies. In this chapter we investigated the effects of cultivar, microtuber size, microtuber incubation period and growth regulators used to promote induction on microtuber dormancy period. The effects on microtuber dormancy period of microtuber bud maturity and endogenous abscisic acid ABA levels in small and large microtubers were also determined.

CHAPTER 5. MICROTUBER DORMANCY IN THREE POTATO CULTIVARS

5.1 Abstract

The relationship between microtuber size, growth regulators used for induction, the duration of the incubation period, level of microtuber bud maturity and abscisic acid content and microtuber dormancy periods of 'Kennebec', 'Russet Burbank' and 'Superior' was evaluated. The length of microtuber dormancy was cultivar-specific and a significant correlation was established between *in vitro* and *in vivo* dormancy periods. Microtubers ≤250 mg had longer dormancy compared with microtubers >250 mg. The dormancy periods of microtubers were not affected by the addition of 2*H*-1-benzopyran-2-one (coumarin), (2-chloroethyl)trimethylammonium chloride (CCC) and *N*-(phenylmethyl)-1*H*-purin-6-amine (6-benzylaminopurine) to the microtuberization media or by the duration of incubation period (28 and 56 d). No differences were observed in the status of microtuber bud development. A positive correlation was established between endogenous abscisic acid levels and the length of microtuber dormancy.

5.2 Introduction

Dormancy refers to the physiological condition inhibiting sprout growth when tubers are placed under environmental

conditions ideal for sprouting. Available information on microtuber dormancy is limited and contradictory. Microtubers have been reported to exhibit no dormancy period (Hussey and Stacey, 1981), germinate prematurely *in vitro* (Wattimena, 1983; Ortiz-Montiel and Lozoya-Saldaña, 1987; Harvey et al., 1991), or have dormancy periods of 1 to 7 mo (Budin and Ogluzdin, 1992; Hussey and Stacey, 1984; Estrada et al., 1986; Joyce and McCown, 1987; Rossel et al., 1987; Thieme, 1992). Uncertainty regarding the dormancy period of potato microtubers limits their utilization in seed certification programs. The objective of this research was to evaluate the effects of cultivar, microtuber size, *in vitro* incubation period and growth regulators used to promote induction on microtuber dormancy period. The effects on microtuber dormancy period of microtuber bud maturity and endogenous abscisic acid (ABA) levels in small and large microtubers were also determined.

5.3 Materials and Methods

Microtubers of 'Kennebec', 'Russet Burbank' and 'Superior' were produced from *in vitro* plantlets using the two-step microtuberization system described in Chapter 3. The microtuberization medium was supplemented with either a) 50 mg/l 2*H*-1-benzopyran-2-one (coumarin) and 80 g/l sucrose, b) 500 mg/l (2-chloroethyl)-trimethylammonium chloride (CCC), 5.0 mg/l *N*-(phenylmethyl)-1*H*-purin-6-amine (6-benzylaminopurine; BA) and 80

g/l sucrose or c) a control medium containing 80 g/l sucrose but no growth regulator. Cultures were incubated at 15°C, with an 8 h light period and 50 $\mu mol/m^2/s$ photon flux density (400-700 nm) supplied by cool white fluorescent lamps. Microtubers were harvested after 28 and 56 d of incubation.

Dormancy period was evaluated in microtubers stored in 1.5 l plastic containers at 20 °C in the dark. A saturated solution of $MgSO_4.7H_2O$ (500 ml per container) was introduced into the bottom of the container, below the rack supporting the microtubers, to maintain the relative humidity constant at 90% (Dhingra and Sinclair, 1985). Microtuber sprout development was monitored on a weekly basis, and the dormancy period was considered to have ended when 80% of the microtubers had at least 1 sprout longer than 2 mm. The dormancy period of microtubers was correlated with the relative dormancy length index of field-grown potatoes (data from Mapplebeck and Asiedu, 1993), where an index of 1 indicated the shortest dormancy period and 10 the longest. The experiments were conducted using a randomized complete block design with six replications in time. Each replication consisted of one apical bud from each of ten microtubers.

Bud maturity was evaluated by excising buds from the apical eye-complex of microtubers and culturing them on a modified Murashige and Skoog (1962) basal salt solution supplemented with 0.4 mg/l thiamine-HCL, 2 mg/l Ca-pantothenate, 100 mg/l inositol, 30 g/l sucrose, 8 g/l agar (Anachemia, Lachine, Qué., Canada) and the pH adjusted to 5.7 prior to autoclaving. Cultures were grown

at 22 °C under a 16 h photoperiod and 38 $\mu mol/m^2/s$ illumination. Sprout length and fresh weight were evaluated after 7 and 14 d *in vitro*. The experiment was conducted using a randomized complete block design with six replications in time. Each replication consisted of buds from 10 microtubers.

ABA was extracted using a solvent partitioning method (Coleman and King, 1984). Composite samples weighing approximately 8 g from about 80 small (ca. 100 mg) or 8 large (ca. 1 g) microtubers were used for each of the extractions. The ABA concentration was determined using a High Precision Liquid Chromatograph (HPLC; Waters, Mississauga, Ont., Canada, U.V. absorbance detector model 441) at 254 nm, equipped with a 15 cm column packed with octadecyldimethylsilyl (5 μm particles, Supelco, model #4693c, Bellefonte, PA, U.S.A.). The mobile phase consisted of water:acetonitrile:acetic acid (750ml:250ml:15ml). The flow rate was 1 ml/min. Under these conditions the retention time of ABA was 8.8 min. Internal ABA standards indicated a consistent recovery rate of 83-87%. Each ABA value represents the average of six extractions. ABA content was measured on both a fresh and a dry weight basis.

Data were analysed by ANOVA, Duncan's New Multiple-Range Test, Pearson correlation coefficient (Steel and Torrie, 1980) and Kendall's Tau (Daniel, 1990).

5.4 Results and Discussion

Microtuber dormancy period was influenced by cultivar and microtuber size but not by the use of growth regulating substances in the microtuberization media (Table 5.1). The dormancy period was shortest in duration for 'Superior', intermediate for 'Kennebec' and longest for 'Russet Burbank'. The dormancy period of microtubers was positively correlated (microtubers ≤250 mg, τ=0.6032 and >250 mg, τ=0.7136) with the relative dormancy period reported for field-grown tubers (L. Mapplebeck and S.K. Asiedu, personal communication). Cultivars having tubers with short dormancy periods *in vivo* also had microtubers with short dormancy periods *ex vitro*.

The dormancy period, measured from harvest to sprouting, of microtubers of fresh weights ≤250 mg was significantly longer (by 2-3 weeks) compared with microtubers having fresh weights >250 mg. Since microtubers were induced over several weeks the larger microtubers were probably those which initiated their development earlier. The longer dormancy periods of small compared with large microtubers probably reflect differences in the age of microtubers at the time of harvest.

Microtubers had longer dormancy periods when harvested after 28 compared with 56 d of incubation, when the dormancy duration was measured from harvest to sprouting. However differences were not significant when the dormancy period was measured from the time of microtuber induction to sprouting (Table 5.2).

The growth of apical buds, isolated from both small and large microtubers and cultured *in vitro*, was not found to differ significantly after 1 and 2 wk in culture (data not shown). It seems unlikely that differences in bud maturity were responsible for the differences in dormancy duration of small and large microtubers.

Endogenous ABA levels, measured on a fresh weight basis, were greater in small than in large microtubers (Table 5.3). The differences were increased when the comparison was made on a dry weight basis. The ABA content of microtubers increased with size, indicating that, as observed with *in vivo* tubers (Krauss, 1981; Koda and Okasawa, 1983b), ABA is either continuously supplied or synthesized in the developing microtubers. Correlation analysis indicated that endogenous ABA levels were positively correlated (R^2=0.5818 to 0.9759) with microtuber dormancy periods of small and large microtubers (Table 5.3). It appears that proportionally higher levels of endogenous ABA are responsible for the longer dormancy periods of small microtubers. High levels of endogenous ABA have been linked with prolonged dormancy periods in field-grown tubers (Coleman and King, 1984). The concentration of both 'bound' and 'free' ABA within potato tubers was greater in the periderm tissues than in the storage parenchyma of the pith (Korableva et al., 1980). Small microtubers have a greater surface to volume ratio so the periderm tissues are proportionally greater than in large microtubers. This could explain the differences in ABA content

of microtubers. The low coefficient of determination obtained with 'Superior' may indicate that ABA is not the only compound involved in microtuber dormancy (Hemberg, 1985). A model has been proposed to explain the dormancy of seeds in which ABA binds to a regulatory protein; the state of this ABA/protein aggregate controls dormancy (Smith et al., 1989).

The physiological processes involved in microtuber dormancy appear to be similar to field-grown tubers. However, the dormancy periods of microtubers were not affected by the duration of the incubation (i.e tuber growth) period, as observed with field-grown tubers (Cho et al;., 1983). Although a positive correlation was established between the dormancy duration and endogenous ABA concentration of small and large microtubers, this does not preclude the involvement of other compound(s) such as regulatory proteins (Smith et al., 1989) or other plant growth regulators (Hemberg, 1985). The low correlation coefficient between dormancy duration and ABA content in 'Superior' described in this study supports this idea and should form the basis for additional studies on dormancy control in tubers.

Table 5.1. The influence of cultivar and microtuber size on the dormancy period (weeks, mean ± s.d.) of microtubers harvested after 28 days of incubation.

Cultivars[1]	Duration of dormancy period: Microtuber size categories ≤ 250 mg	Duration of dormancy period: Microtuber size categories > 250 mg	Relative *in vivo*[3] dormancy duration index
Kennebec y	15.00 ± 0.81 a	12.08 ± 0.72 b	6
Russet Burbank x	16.83 ± 1.41 a	13.25 ± 0.83 b	8
Superior z	14.00 ± 0.59 a	10.75 ± 0.59 b	4

Significance[2]

Source of variation	DF	Mean square	F value
Cultivars (C)	2	41.4	72.91***
Growth regulators (GR)	2	0.1	0.17 NS
C x GR	4	0.2	0.39 NS
Microtuber size (MS)	1	186.5	28.69***
C x MS	2	0.5	0.90 NS
GR x MS	2	0.1	0.17 NS
C x GR x MS	4	0.5	1.05 NS
Error	51	0.6	
c.v. (%)	5.52		

Correlation analysis[4] between τ= 0.6032 and τ= 0.7136 (p=0.0001).

Means followed by the same letter are not significantly different at the P<0.05 level, Duncan's Multiple-Range Test (DMRT).
[1] Means followed by different letters are significantly different at the P<0.05 level (DMRT).
[2] ***, denotes P<0.001; NS denotes non-significant effect.
[3] Data taken from Mapplebeck and Asiedu (personal communication)
[4] Data analysed using Kendall's Tau (τ).

Table 5.2. The influence of cultivar and length of incubation period (28 and 56 days) on microtuber dormancy period (weeks, mean ± s.d.).

	Duration of dormancy from harvest to sprouting		Duration of dormancy from induction to sprouting	
	Incubation period (days)		Incubation period (days)	
Cultivars[1]	28	56	28	56
Kennebec y	12.1 ± 0.7 a	8.2 ± 0.7 b	16.1 ± 0.7 a	16.2 ± 0.7 a
Russet Burbank x	13.2 ± 0.8 a	9.9 ± 0.9 b	17.2 ± 0.8 a	17.9 ± 0.9 a
Superior z	10.8 ± 0.6 a	7.0 ± 0.8 b	14.8 ± 0.6 a	15.0 ± 0.6 a

Significance

Source of variation	DF	Mean square	F value
Cultivars (C)	4	48.5	96.51***
Growth regulators (GR)	2	0.2	0.46 NS
C x GR	2	0.3	0.33 NS
Incubation period (IP)	1	260.7	523.58***
C x IP	2	0.4	0.90 NS
GR x IP	2	0.0	0.06 NS
C x GR x IP	4	0.3	0.75 NS
Error	59	0.5	
c.v. (%)	7.06		

Means followed by the same letter are not significantly different at the P<0.05 level, Duncan's Multiple-Range Test (DMRT).
[1] Means followed by the same letter are not significantly different at the P<0.05 level (DMRT).
[2] ***, denotes P<0.001; NS denotes non-significant effect.

Table 5.3. Endogenous abscisic acid (ABA) content (mean ± s.d.) of small (≤250 mg) and large (>250 mg) microtubers harvested after 28 days of incubation and correlation analysis of ABA content *vs* duration of the dormancy period of microtubers.

	ABA	
	ng/g fresh wt	ng/g dry wt
Kennebec		
≤ 250 mg	55.1 ± 5.5 a	430.9 ± 42.8 a
> 250 mg	38.3 ± 3.7 b	227.3 ± 22.3 b
Correlation coefficient (r)[1]	0.95***	0.98***
Russet Burbank		
≤ 250 mg	177.4 ± 3.5 a	1378.0 ± 27.2 a
> 250 mg	124.6 ± 9.5 b	776.1 ± 59.3 b
Correlation coefficient (r)[1]	0.89***	0.93***
Superior		
≤ 250 mg	123.9 ± 12.5 a	824.3 ± 84.4 a
> 250 mg	101.1 ± 14.1 b	556.4 ± 77.8 b
Correlation coefficient (r)[1]	0.58*	0.73**

Means followed by different letters are significantly different at the P<0.05 level; Duncan's Multiple-Range Test.
[1] Correlation analysis ABA content *vs* duration of dormancy period by Pearson Correlation Coefficient. ***, ** and * indicate significance of the correlation at p=0.001, p=0.01 and p=0.05, respectively.

CHAPTER 6. MICROTUBER QUALITY AND SPROUT DEVELOPMENT AS AFFECTED BY SIZE, GENOTYPE AND INCUBATION MEDIUM

6.1 Introduction

Potato microtubers are increasingly utilized in seed tuber certification programs as a primary source of SPT material and for both short and long-term germplasm storage (Wang and Hu, 1982; Chandra et al., 1985; Tovar et al., 1985; Kwiatkowski et al., 1988; Thieme, 1992). Microtuber induction has been heavily investigated (reviewed by Wattimena, 1983; Wang and Hu, 1985; Chandra et al., 1988) but microtuber characteristics; such as microtuber weight, specific gravity and number of eyes and their effects on storage, subsequent sprout development and the yield of microtuber-derived (mTD) plants have received little attention. Genotype, photoperiod, induction media including nitrogen levels, growth regulators, choice of liquid or solid and brand of gelling agent all influenced the percent dry matter of microtubers produced from nodal cuttings (Wattimena, 1983; Novak and Asiedu, 1992). In general, increased dry matter content occurred in microtubers produced in medium with relatively low nitrogen levels, high coumarin concentrations and in liquid medium (Wattimena, 1983). Gelrite instead of agar and 8 h photoperiod instead of total darkness also promoted greater percent dry matter (Nowak and Asiedu, 1992).

One aspect of microtuber quality that was found to influence

the yield of mTD plants was microtuber weight at planting. Plants grown from heavier microtubers (2.5 g) exhibited greater early plant vigour, foliage ground cover and produced greater total tuber weights than plants from lighter microtubers (0.63 g) when grown under high densities in nursery beds. The greater tuber yields obtained from the larger microtubers related both to greater stem number and vigour (Wiersema et al., 1987).

The objectives of the following experiments were to determine the effects of genotype, growth regulators and microtuber size on microtuber specific gravity, eye number, bud maturity and sprout and stem development from microtubers. The influence of apical dominance on microtuber sprout development was studied. The effects of desprouting microtubers and of storing microtubers under different spatial orientation (apical or distal end up) on apical dominance and sprout development was also investigated.

6.2 Materials and Methods

Microtuber Induction

Microtubers of 'Kennebec', 'Russet Burbank' and 'Superior' were produced using the two-step microtuberization system described in Chapter 3. The microtuberization media contained modified Murashige and Skoog (MS, 1962) basal salt solution supplemented with 0.4 mg/l thiamine-HCl, 2 mg/l Ca-pantothenate, 100 mg/l inositol and either a) 50 mg/l coumarin and 80 g/l

sucrose, b) 500 mg/l chlorocholine chloride, 5 mg/l 6-benzylaminopurine (CCC and BA) and 80 g/l sucrose or c) control medium with 80 g/l sucrose but no growth regulator.

Specific gravity

The specific gravity of small (≤250 mg) and large (>250 mg) microtubers were measured using the weight-in-air/weight-in-water method (Kleinschimdt et al., 1984). Each observation represents the mean of eight replicates per treatment. Each replicate consisted of composite microtuber samples weighing approximately 8 g.

Eye number

The eye (node) number of 200 randomly selected microtubers for each of the treatments were counted using a dissecting microscope at a magnification of 20 X. Buds of the apical eye-complex were considered to belong to distinct eyes when separated by more than 1 mm from other buds.

Microtuber bud maturity

The maturity of buds originating from the apical eye-complex, lateral or distal eyes of small and large microtubers was evaluated by culturing dissected buds *in vitro*. Apical, lateral and distal buds from 40 microtubers per treatment were cultured on modified MS (1962) basal salt solution supplemented with 0.4 mg/l thiamine-HCl, 2 mg/l Ca-pantothenate, 100 mg/l

inositol, 30 g/l sucrose, 8 g/l agar (Anachemia, Lachine, Québec, Canada) and the pH adjusted to 5.7 prior to autoclaving. Cultures were incubated at 22 °C under 50 μmol/m^2/s cool white fluorescent illumination and 16 h light period.

Sprout development

Microtubers were stored in a refrigerator at 6 ± 2 °C in the dark and their survival evaluated at 8, 12 and 18 mo. At these times, sprout number, length (≥1 mm) and position were recorded. Microtubers were then sown in a peat-based potting mixture (Premier Brand, Rivière-du-Loup, Canada) and grown in the greenhouse to monitor their viability. The experiment was conducted four times. Each replicate in time consisted of twenty-five small (≤250 mg) and large (>250 mg) microtubers.

Desprouting microtubers

Microtubers were stored in a refrigerator at 6 ± 2 °C in the dark for 6 mo. At this time the dominant sprout(s) were removed from half the microtubers. Microtubers were then incubated at 20 °C, in the dark for 4 wk. A saturated solution of $MgSO_4.7H_2O$ was used to maintain the relative humidity at 90% as described in Chapter 5. The number of large sprouts (>10 mm) was then recorded and microtubers were sown in a peat-based potting mixture (Premier Brand, Rivière-du-Loup, Canada) and grown in the greenhouse for 4 wk to evaluate the number of stems produced from mTD plants. The experiment was conducted four times. Each

replicate in time consisted of twenty-five small and large microtubers.

Spatial orientation

The effect of spatial orientation on sprout development and apical dominance of microtubers of 'Kennebec' was evaluated by storing microtubers apical or distal end up in moist sand at 20 °C under total darkness. Four weeks after the release of dormancy the number and position of the largest sprouts were recorded and then removed. Microtubers were re-incubated maintaining the same orientation, and the number and position of largest sprouts were recorded after 1 mo. The experiment was conducted 4 times. Each replicate in time consisted of twenty-five small and large microtubers per treatment.

Data analysis

Data was analyzed using ANOVA, Duncańs' New Multiple-Range Test (Steel and Torie, 1980), and Chi-Square Test of Independence (Scherrer, 1980). Non-linear regressions were done using the Marquardt method (Marquardt, 1963).

6.3 Results

Specific gravity

Specific gravity was significantly influenced by cultivar and microtuber weight, but not by the addition of growth

regulators to the microtuberization medium (Table 6.1). Microtubers of 'Superior' had the greatest specific gravity followed by 'Kennebec' and 'Russet Burbank'. For all genotypes, heavier microtubers, weighing >250 mg, had a higher specific gravity than lighter microtubers.

Eye number

The relationship between microtuber fresh weight and eye number per microtuber was best described for all cultivars by the inverse exponential equation; $Y= \beta_0 - \beta_0 (-\beta_1 X)^{-1}$, where Y= number of eyes, X= microtuber weight and β_0 and β_1 were constants (Figure 6.1). For 'Russet Burbank' the coefficient of determination (R^2) was 0.9561, β_0=8.9956 and β_1=0.0156; for 'Kennebec' R^2=0.9328, β_0=6.3277 and β_1=0.0129; and for 'Superior' R^2=0.9571, β_0=7.5751 and β_0=0.2123. Results indicated that the number of distinguishable eyes increased rapidly with increasing microtuber weight, until approximately 250-300 mg, when it plateaued at 8 or 9.

Microtuber bud maturity

No significant differences were observed in the rate of development of shoot tips originating from buds of the apical eye-complex or of the lateral or distal eyes after 1 and 2 wk in culture (Table 6.2). Fewer buds of 'Superior' had sprouted after 1 wk of culture, but differences among genotypes were no longer significant after 2 wk. Microtuber size and induction medium had

no effect on the development of dissected buds.

Sprout Development

Most microtubers produced a single large primary sprout with one to several smaller secondary sprouts (Figure 6.2). Occasionally, more than one large sprout developed on larger microtubers. Total sprout number per microtuber was positively correlated with microtuber weight; Pearson correlation coefficient ranged from 0.3594 for 'Superior' to 0.4440 for 'Kennebec'. After 8 mo storage at 4 °C, the average length of the primary sprouts exceeded 10 mm, while the length of the secondary sprouts averaged approximately 2 mm. After 12 mo storage, the length of the primary sprouts was greater than 20 mm, but the length of the secondary sprouts was less than 4 mm. After 18 mo the primary sprouts averaged nearly 40 mm, but the length of the secondary sprouts did not exceed 7 mm. Storing microtubers at 20 °C favoured the more rapid development of primary sprouts but had no effect on the number of large sprouts or the number and length of the secondary sprouts (data not shown). Significant positive correlations were established between the initial weight of microtubers and length of the primary sprouts after 8, 12 and 18 mo storage. Pearson correlation coefficients ranged from 0.4574 for 'Kennebec' to 0.7122 for 'Russet Burbank'.

Primary sprouts developed more frequently from buds located at the apical eye-complex; fewer than 5% developed from the

lateral eyes and none from the distal eyes (Figure 6.3). Secondary sprouts also developed more frequently from the apical eye-complex. Nearly 10% of secondary sprouts arose from the lateral eyes and fewer than 5% from the distal eye. Primary and secondary sprout position was not influenced by genotype, growth regulator or size.

Microtubers ≤250 mg were more susceptible to desiccation or exhaustion of stored material than larger tubers (Table 6.3). After 12 mo storage at 6 ± 2 °C in the refrigirator the survival rate of these small microtubers was 96, 92 and 91% for 'Kennebec', 'Russet Burbank' and 'Superior', respectively, while that of large tubers was 100%. After 18 mo storage small tubers were no longer viable, while the survival rate of microtubers >250 mg was 75, 67 and 54% for 'Kennebec', 'Russet Burbank' and 'Superior', respectively.

Desprouting microtubers

Removing the dominant sprout(s) of microtubers significantly increased the number of larger sprouts compared with intact microtubers (Table 6.4). This resulted in greater stem numbers per mTD plants 4 wk after sowing. Sprout and stem number were affected by genotype, but not by microtuber induction medium. The beneficial effect of desprouting was observed only with large microtubers, as a large proportion of small microtubers (<250 mg) failed to produce new sprouts once the primary sprout was removed.

Spatial Orientation

Storing microtubers apical or distal end up did not affect the position or number of primary sprouts that developed but significantly influenced the position of the larger sprouts after desprouting (Figure 6.4). Most sprouts that emerged from desprouted microtubers stored apical end up were produced at the apical eye-complex, few sprouts were formed at the lateral eyes and none were formed distally. Sprouts that emerged from desprouted microtubers stored distal end up were more frequently formed at the apical eye-complex but more than 30% of sprouts formed at the lateral and distal eyes.

6.4 Discussion and Conclusions

Among the factors investigated, microtuber size appeared to be the main determinant of microtuber quality. Large (>250 mg) microtubers had greater specific gravity, possessed more eyes and more sprouts developed from them compared with smaller microtubers. The smaller surface/volume ratio of larger microtubers made them less susceptible to desiccation than the smaller, lighter microtubers and prolonged their storage life.

Observations made on *in vivo*-produced tubers showed that mitosis is negligible in the stolon apex once tuberization is initiated (Leshem and Clowes, 1972). Studies on the early microtuber development from stolon nodes cultured *in vitro* revealed a similar behaviour (Peterson and Barker, 1979). The

differences in the number of eyes reported here between smaller and larger microtubers are probably not differences in bud number *per see*, as no new buds are added once tuberization is initiated (Cutter, 1978), but rather to differences in cell enlargement. Light and scanning electron microscopy revealed that the apical eye-complex of small microtubers contained a greater number of buds than that of large microtubers. It would therefore appear that microtubers produced using layered plantlets were initiated on stolons between the eighth or ninth internode from the stolon's apical meristem, as previously reported with *in vivo*-produced tubers (Cutter, 1978).

As microtuber dormancy ended, growth resumed rapidly at one (occasionally two) bud(s) of the microtuber, generally from the apical eye-complex, strongly inhibiting the growth of other buds through apical dominance. As a result, plants originating from microtubers were generally single-stemmed (Wattimena et al., 1983; Leclerc and Donnelly, 1990). Since buds that originated from different microtuber sizes and eye positions were equally functional, the distinct pattern of sprout development observed in microtubers appears to be related to the strong influence of apical dominance in microtubers. Similar observations were reported with small-sized minitubers grown in the field (Thornton and Neundorfer, 1986; Melching et al., 1993). Removing the primary sprout prior to planting partially released the effect of apical dominance for larger microtubers, as observed with field-grown tubers (Hay and Hampson, 1991), but the treatment was

detrimental for smaller tubers. Attempts to release correlative inhibition by storing microtubers distal end up were unsuccessful.

Observation have shown that heavier (>250 mg) microtubers had higher specific gravity, more eyes and produced more sprouts compared to smaller (≤250 mg) microtubers. It would therefore appear advantageous to produce larger microtubers, as they can be stored for longer periods of time and can potentially produce greater yields compared to smaller microtubers.

Table 6.1. The effects of microtuber weight and growth regulators in the medium on the specific gravity of potato microtubers.

	Microtuber weight	
	≤250 mg	>250 mg
Kennebec	1.0471 b	1.0596 a
Russet Burbank	1.0429 b	1.0585 a
Superior	1.0537 b	1.0663 a
Significance[1]		
Cultivar (C)	**	
Growth regulator (G)	0.44	
Microtuber size (S)	***	
C x G	0.82	
C x S	0.11	
G x S	2.06	
C x G x S	0.35	
c.v. (%)	0.67	

Means followed by the same letter are not significantly different at the $p<0.05$ level, Duncan's New Multiple-Range Test.
[1] **, *** denote $p<0.01$ and $p<0.001$ respectively, and α value of non-significant effect.

Table 6.2. The percentage of shoot tips that developed after the excision of buds from apical, lateral and distal eyes of microtubers examined 1 and 2 weeks after explantation.

	1 week			2 weeks		
	Bud origin			Bud origin		
Cultivar	Apical	Lateral	Distal	Apical	Lateral	Distal
Kennebec	62	68	75	75	86	100
Russet Burbank	63	52	75	78	82	88
Superior	55	37	37	62	82	87

Chi^2 test indicated that the origin of buds had no effect on their development.

Table 6.3. Survival rate (%) of microtubers stored for 8, 12 and 18 months (mo).

Cultivar / Storage	Microtuber weight ≤250 mg	Microtuber weight >250 mg
Kennebec		
8 mo	100	100
12 mo	96	100
18 mo	0	75
Russet Burbank		
8 mo	100	100
12 mo	92	100
18 mo	0	67
Superior		
8 mo	100	100
12 mo	91	100
18 mo	0	54

Table 6.4. The effects of microtuber desprouting on the number of subsequent sprouts ≥10 mm and stems per microtuber-derived plant.

	Sprout number		Stem number	
Cultivar	Intact	Desprouted	Intact	Desprouted
Kennebec	1.13	1.17 a	1.02 b	1.14 a
Russet Burbank	1.14 a	1.26 a	1.06 b	1.23 a
Superior	1.02 a	1.07 a	1.02 b	1.19 a
Significance[1]				
Cultivars (C)		*		**
Treatment (T)		*		**
C x T		0.41		1.47
c.v. (%)		3.80		2.68

Means followed by the same letter are not significantly different at the $p \leq 0.05$ level, Duncan's New Multiple-Range Test.
[1] *, ** denote $p < 0.05$ and 0.01 respectively, α value of non-significant effect.

Figure 6.1. The relationship between microtuber weight and the number of 'eyes' per microtuber. For 'Russet Burbank' R^2=0.9561, β_0=8.9956 and β_1=0.0156; 'Kennebec' R^2=0.9328 β_0=6.3277 and β_1=0.0129; 'Superior' R^2=0.9571, β_0=7.5751 and β_1=0.2123.

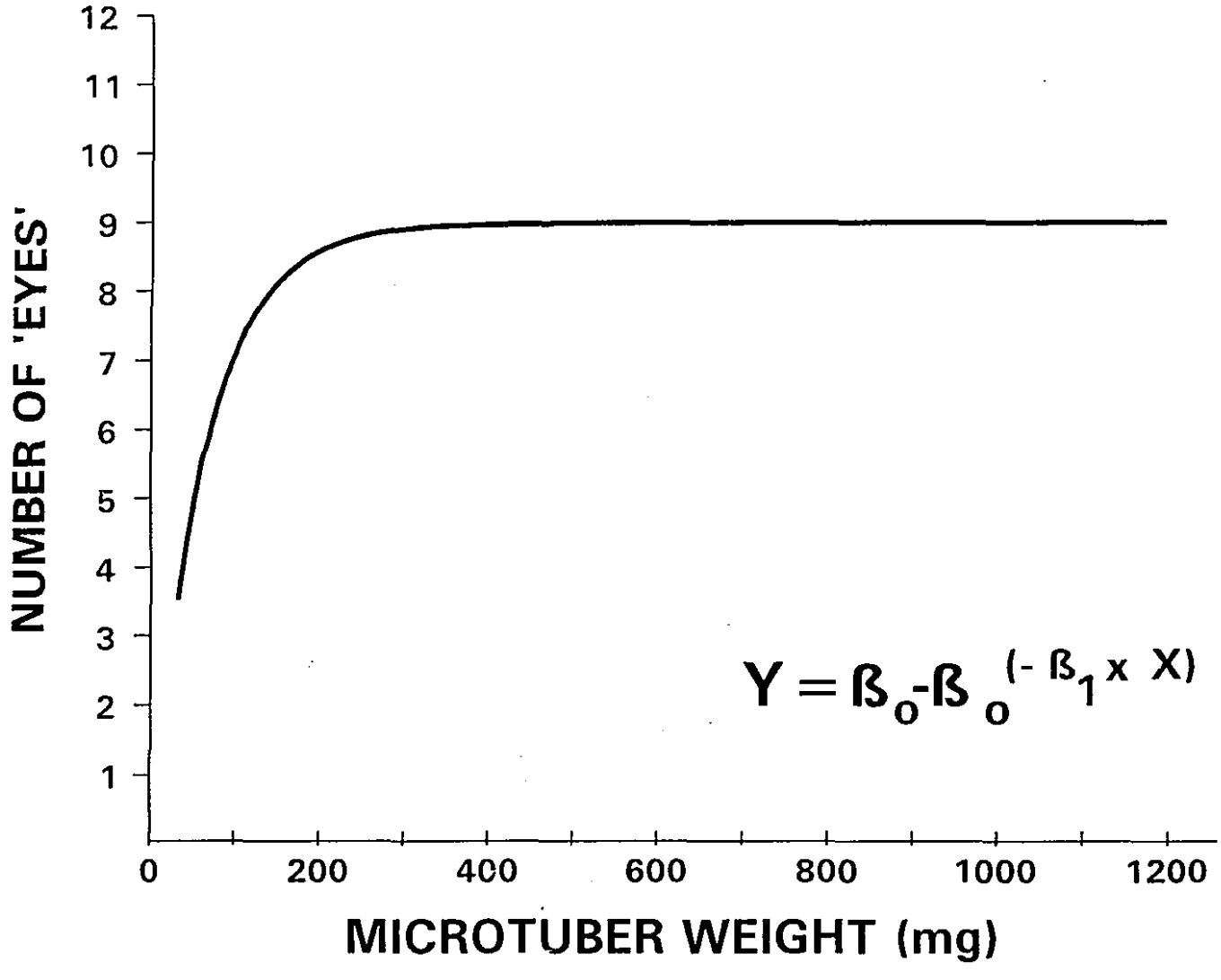

NUMBER OF 'EYES'
12
11
10
9
8
7
6
5
4
3
2
1
0
200
400
600
800
1000
1200
MICROTUBER WEIGHT (mg)
$Y = \beta_o - \beta_o^{(-\beta_1 x X)}$

Figure 6.2. Sprout length of microtubers stored at 6 °C for 8, 12 and 18 months.

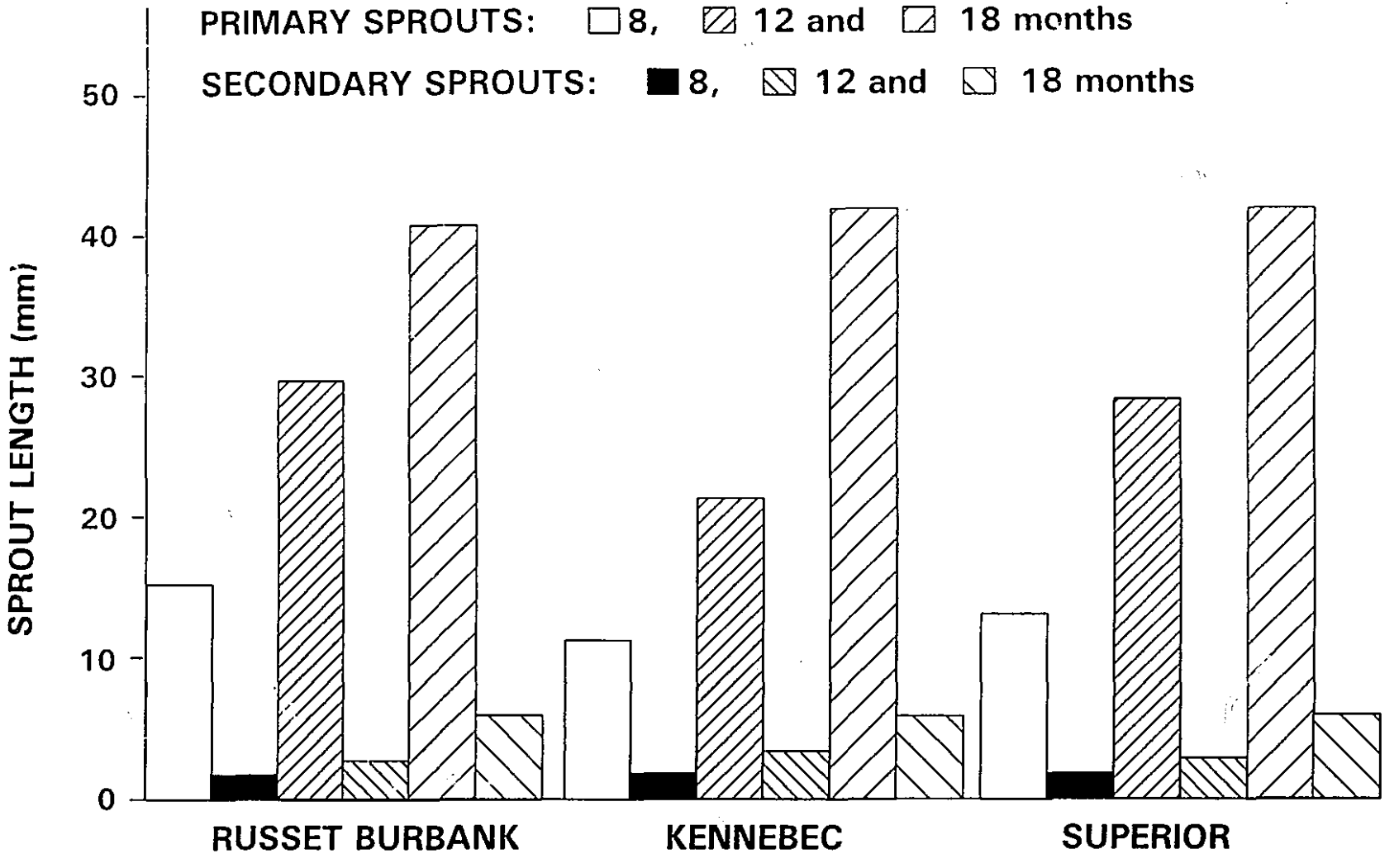

PRIMARY SPROUTS: 8, 12 and 18 months
SECONDARY SPROUTS: 8, 12 and 18 months
SPROUT LENGTH (mm)
50
40
30
20
10
0
RUSSET BURBANK
KENNEBEC
SUPERIOR

Figure 6.3. The position of primary and secondary sprouts on microtubers; apical eye-complex, lateral and distal eyes.

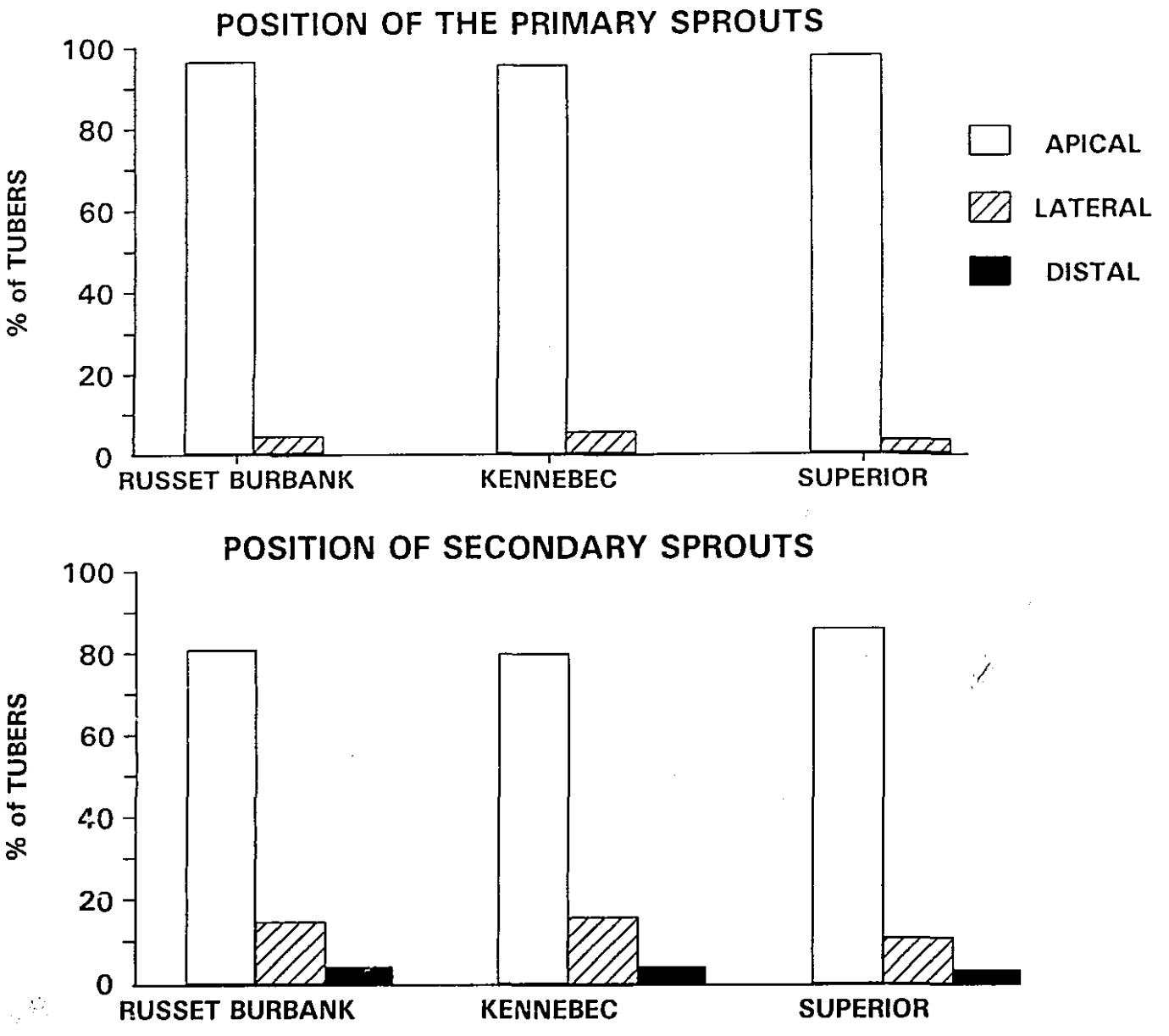
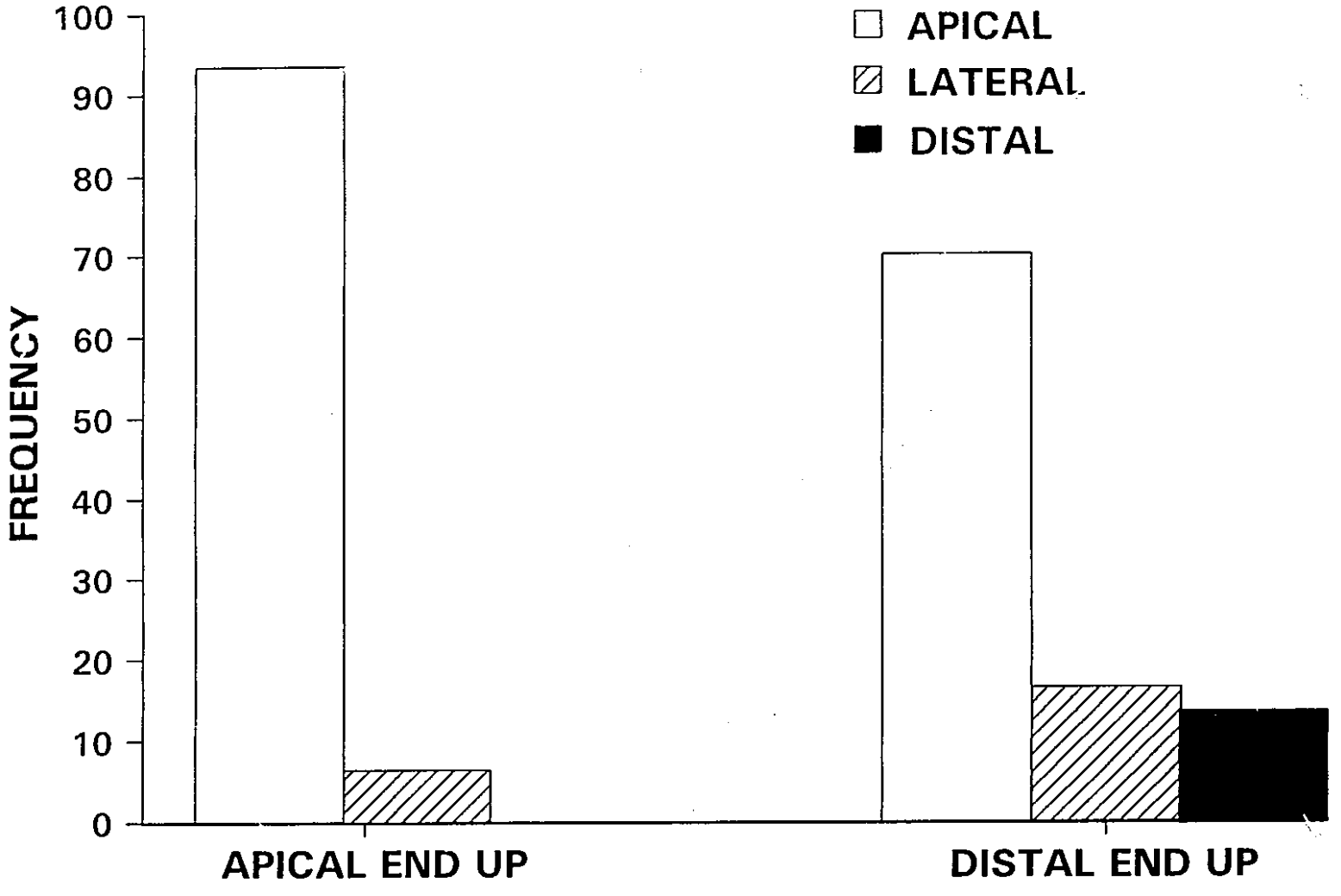

POSITION OF THE PRIMARY SPROUTS
% of TUBERS
100
80
60
40
20
0
RUSSET BURBANK
KENNEBEC
SUPERIOR
APICAL
LATERAL
DISTAL
POSITION OF SECONDARY SPROUTS
% of TUBERS
100
80
60
40
20
0
RUSSET BURBANK
KENNEBEC
SUPERIOR

Figure 6.4. The influence of spatial orientation (apex or distal end up) on the position of sprouts developed from desprouted microtubers of 'Kennebec'; apical-eye complex, lateral and distal eyes.


100
90
80
70
60
50
40
30
20
10
0
FREQUENCY
APICAL
LATERAL
DISTAL
APICAL END UP
DISTAL END UP

PREFACE TO CHAPTER 7

Chapter 7 is the material contained in a manuscript by Yves Leclerc and Danielle J. Donnelly submitted for publication to the American Potato Journal. The format has been changed to conform, as much as possible, to a consistent format within the thesis, according to guidelines set by the Faculty of Graduate Studies. In this chapter the effect of the physiological age of microtubers on the yield from microtuber-derived plants grown in pot trials in the greenhouse was investigated.

CHAPTER 7. THE INFLUENCE OF PHYSIOLOGICAL AGEING ON THE YIELD FROM POTATO MICROTUBERS

7.1 Abstract

Potato microtubers of 'Kennebec' and 'Superior' of two physiological ages were grown in pot trials in the greenhouse. Severe physiological ageing treatment (>2500 degree-days) had no effect on microtuber sprout development, stem number, tuber number and only marginally influenced the tuber weight yielded by microtuber-derived plants compared with physiologically young microtubers (120 degree-days). Microtuber-derived plants were usually, but not always, single-stemmed. The occurrence of multi-stemmed plants was positively correlated with microtuber weight. The close proximity of buds on microtubers is believed to be responsible for the strong apical dominance observed with microtubers. The limited effect of severe physiologically ageing on the plant and yield characteristics of microtuber-derived plants is likely related to the limited volume of storage tissues in microtubers.

7.2 Introduction

The physiological state of potato seed tubers evolves from the onset of tuber formation to their development into plants. To characterize the influence exerted by the physiological status

of seed tubers on sprout growth, and subsequent plant growth and yield characteristics, the concept of physiological aging was introduced by Madec and Pérennec (1962). The physiological age of seed tubers increases with the chronological age of tubers and with storage temperatures (Madec and Pérennec, 1956; Pérennec and Madec, 1960). Upon the release of dormancy, physiologically very young tubers will produce a single unbranched sprout. With increased physiological ageing apical dominance is released, and the number of eyes which develop sprouts increases. Multiple sprouts with greater tendency to branch form at these eyes. With further increase in physiological ageing the sprout branches assume a diagravitropic growth and become stolons which later form tubers at the apex (Ewing and Struik, 1992).

Tuber yields increase with the physiological age of seed tubers, up to a maximum, beyond which they are reduced (Kawakami, 1963). When compared with physiologically younger seed tubers, physiologically older tubers emerge more rapidly, producing plants which initiate tubers earlier and produce greater yields under short growing seasons, but lesser yields under longer growing seasons (Toosey, 1964; Iritani, 1968; Wurr, 1978; Allen and Scott, 1980; Pérennec and Madec, 1980; van der Zaag and van Loon, 1987; Ewing and Struik, 1992). Adverse environmental conditions, before emergence or during the growing season, may alter this behaviour (Pérennec and Madec, 1980).

Microtuber-derived (mTD) plants differed from seed tuber-derived plants; they produced single-stemmed plants, initiated

tubers during a longer period (Wattimena et al., 1983) and were more susceptible to early water stress and high temperatures (Leclerc and Donnelly, 1990). Attempts to modify the development of mTD plants by varying the physiological age of microtubers was never investigated. The objective of this research was to evaluate the influence of physiological ageing on the development and yield of mTD plants grown in the greenhouse.

7.3 Materials and Methods

Potato microtubers of cultivars Kennebec and Superior were induced using a two-step microtuberization system on medium containing 80 g/l sucrose (Chapter 3). Microtubers weighing 50 to 1600 mg were stored at 20 °C under total darkness to release dormancy. The dormancy was considered to be released when at least one sprout per microtuber was longer than 1 mm. After dormancy was released the physiologically "young" microtubers were greensprouted at 20 °C under continuous light, 50 $\mu mol/m^2/s$ illumination, for 2 wk before planting. Physiologically young microtubers accumulated approximately 120 degree-days (> 4 °C) before planting. Physiologically "old" microtubers were obtained by storing microtubers under the same conditions for an additional 6 mo upon the release of dormancy. Physiologically old microtubers accumulated more than 2500 degree-days from the release of dormancy until planting. This severe treatment corresponded to an ageing treatment twice as intense as the most

extreme treatment previously reported in the literature for conventionally propagated seed tubers (90 days at 16-18 °C) (Pérennec and Madec, 1980).

The experiments were carried out in the greenhouse of Macdonald campus of McGill University during the fall of 1991 (October to December) and replicated in the winter of 1992 (February to May). Microtubers were planted at a depth of 5 cm into 6 l plastic pots containing 5 l of peatmoss-based potting mixture (Promix, Premier Brand, Rivière-du-Loup, Canada). The potting mixture was supplemented with 10 cc/l of slow release fertilizer (Osmocote, 19-6-12). High pressure sodium lamps (400 watts; Lumiponic, Boisbriand, Canada) were used to provide a 16 h day for the first month, and a 12 h day subsequently. Greenhouse temperatures were approximately 15 °C at night and 22 °C during the day. Plants were hilled after 20 and 40 d by adding 500 cm^3 of potting mixture per pot. The experiments were conducted using a randomized complete block design with eight replicates. Each replicate consisted of five pots containing one mTD plant in each pot. Plants were harvested after 110 d.

7.4 Results and Discussion

Apical dominance in microtubers was strong, and contrary to what is generally observed with conventionally propagated seed tubers (Kawakami, 1963; Pérennec and Madec, 1980), severe physiological ageing treatment (2500 °C d, >4 °C), did not modify

the expression of the phenomenon (Table 7.1). The large differences in size between microtubers and conventional seed tubers may be responsible for the differences in expression of apical dominance. The buds of microtubers are in closer proximity to one another compared with buds of seed tubers. The developing sprout of microtubers appear to exert a stronger inhibitive influence on neighbouring buds than does the developing sprout of conventional tubers. Multiple sprouting occurred occasionally and was more frequent in 'Superior' than in 'Kennebec'. Sprout number per microtuber was positively correlated with microtuber weight; correlation coefficients were respectively of 0.3595 ($p=0.0035$) for 'Kennebec' and 0.4440 ($p=0.0009$) for 'Superior'.

Microtuber-derived (mTD) plants were usually single-stemmed, and contrary to conventional seed tubers (Pérennec and Madec, 1980) the physiological age of microtubers had no influence on the number of stems per mTD plants (Table 7.1). After 5 wk, no differences were observed in the heights or leaf numbers of mTD plants grown from microtubers of the two physiological ages (data not shown). Multi-stemmed plants were more frequent in 'Superior' than in 'Kennebec'. All multi-stemmed plants originated from microtubers bearing more than one sprout at the time of planting. However, microtubers with more than one sprout did not necessarily produce multi-stemmed plants. A similar fate of sprouts was reported with field-grown tubers (Hay and Hampson, 1991).

The number of minitubers produced per mTD plant was not affected by the physiological age of the microtubers nor by the growing season but was significantly greater for 'Superior' than for 'Kennebec' in both seasons (Table 7.1).

Physiological age of the microtubers had little influence on the weight of minitubers produced in the first trial conducted in the fall (Table 7.1). In the second trial, conducted in the winter, plants grown from physiologically younger microtubers produced greater minituber weights than plants grown from physiologically older microtubers, but the comparison was significant only for 'Superior'. Plants grown in the fall produced a lower minituber weight than plants grown in winter, where higher natural irradiation levels and longer daylength favoured greater yields.

Minituber number and weight was positively correlated with stem number per mTD plant; with correlation coefficients of 0.4668 ($p=0.0010$) and 0.3734 ($p=0.0199$) respectively. Wiersema et al. (1987) reported similar results with mTD plants grown under high density in nursery beds.

The frequency distribution of minitubers was not affected by the physiological age of microtubers but strong seasonal effects were observed (Figure 7.1). In the fall, most minitubers were smaller than 10 g while minitubers produced in winter were much larger.

Physiological ageing is thought to be determined in the storage tissues of tubers (Pérennec and Madec, 1980), but recent

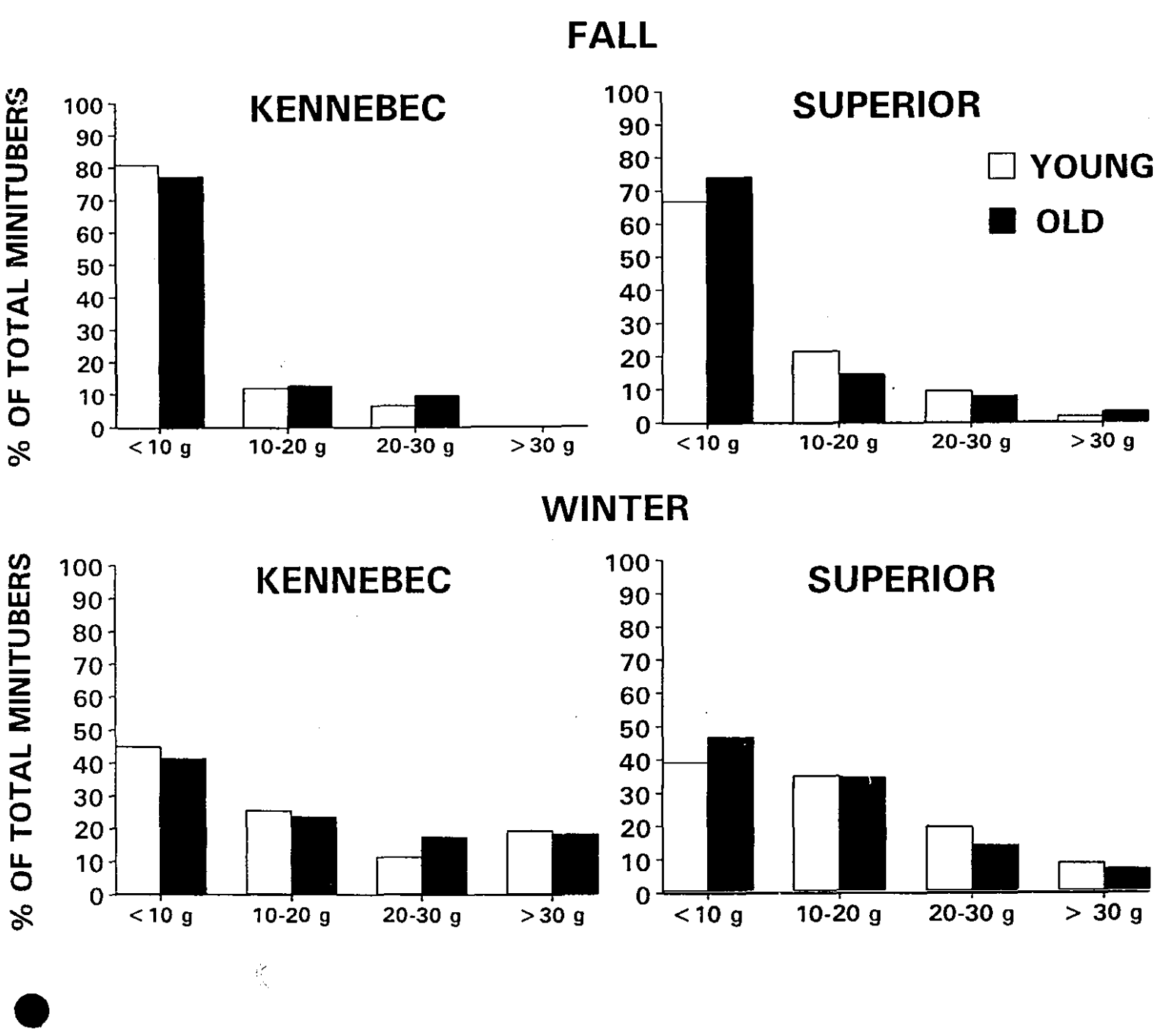
reports indicate that the state of sprouts plays a role in its expression (Scholte, 1989; Allen et al., 1992). The limited volume of microtuber storage tissues may be responsible for the restricted influence of microtuber physiological age on the growth and yield characteristics of mTD plants. Although, microtuber sprout vigour may have been increased by storing the physiologically old microtubers in the light, as demonstrated by Scholte (1989). Physiological ageing had no effect on the expression of apical dominance and only marginally influenced the yield characteristics of mTD plants grown in the greenhouse. Consequently, it appears possible to store microtubers for long periods under adequate conditions with no or few deleterious effects on subsequent yields.

Table 7.1. The influence of physiological age on sprout number per microtuber, stem number and minituber number and weight per microtuber-derived plant grown in the greenhouse during two different seasons.

Season/ Cultivars	Physiological age	Sprout number	Stem number	Minituber number	Minituber weight (g)
Fall 1991					
Kennebec	Young	1.39	1.05	13.7	58.9
	Old	1.33	1.03	11.7	69.3
Superior	Young	1.79	1.24	16.4	72.2
	Old	1.45	1.15	17.5	80.6
Winter 1992					
Kennebec	Young	1.34	1.06	12.7	220.5
	Old	1.25	1.01	11.9	205.1
Superior	Young	1.82	1.28	18.4	266.0*
	Old	1.60	1.24	18.2	240.5

*, indicates a significant difference at the 0.05 level.

Figure 7. 1. The frequency distribution (%) of minitubers produced from physiologically "young" and "old" microtubers grown in pot trials in the greenhouse during the fall and winter seasons. Physiological ageing did not significantly influence the frequency distribution of minitubers (X^2, $p>0.05\%$ for all comparisons).


FALL
KENNEBEC
SUPERIOR
YOUNG
OLD
% OF TOTAL MINITUBERS
100
90
80
70
60
50
40
30
20
10
0
< 10 g
10-20 g
20-30 g
> 30 g
WINTER
KENNEBEC
SUPERIOR
% OF TOTAL MINITUBERS
< 10 g
10-20 g
20-30 g
> 30 g

CHAPTER 8. THE EFFECT OF IN-ROW PLANTING DENSITY AND PLANTING DEPTH ON THE YIELD OF EX VITRO-DERIVED PLANTS OF THREE POTATO CULTIVARS.

8.1 Introduction

Six or seven field generations are necessary to produce sufficient numbers of certified seed tubers in most North American potato seed certification programs. Opportunities exist for innovative agencies, private companies and certified growers to reduce the total number of field generations by implementing volume production strategies in their own laboratories and by planting *ex vitro* material directly into the field (Addy, 1988; Levy, 1985).

Ex vitro plantlet-derived (EVPD) and microtuber-derived (mTD) plants were found to differ in some respects from seed tuber-derived (STD) plants. The EVPD and mTD plants produced single-stems (Leclerc and Donnelly, 1990; Wattimena et al., 1983) initiated tubers during a longer period (Wattimena et al., 1983) and were more susceptible to water stress than STD plants (Leclerc and Donnelly, 1990). EVPD and mTD plants grown in the field consistently produced more tubers than STD plants (Goodwin and Brown, 1980; Wattimena et al., 1983; Levy, 1985; Leclerc and Donnelly, 1990).

The yield characteristics of field-grown EVPD plants were influenced by the between-row plant spacing (Levy, 1985) and by

the in-row plant spacing in preliminary investigations (Wattimena, 1983). The use of growth retardants in the propagation medium, the type of *ex vitro* acclimatization treatment including the duration of the acclimatization period and container volume and the duration of the growing season significantly affected the yield of EVPD plants grown in the field (Thornton and Knutson, 1986; Sipos et al., 1988).

The objective of this research was to evaluate the effect on total tuber yield (number and weight) and plant height of in-row plant spacing and planting depth. An economic assessment was conducted to determine the optimal planting density for EVPD plants in seed tuber certification programs.

8.2 Materials and Methods

Nodal cuttings of specific pathogen-tested (SPT) 'Kennebec', 'Russet Burbank' and 'Superior' obtained from the Agriculture Canada Laboratory in La Pocatière, Québec, Canada, were grown on modified Murashige and Skoog (1962) salt solution supplemented with 0.4 mg/l thiamine HCl, 2 mg/l Ca-pantothenate, 100 mg/l inositol, 30 g/l sucrose, 8 g/l agar (Anachemia, Lachine, Québec, Canada) and the pH adjusted to 5.7 prior to autoclaving. Plantlets were grown at 22 C under 80 $\mu mol/m^2/s$ cool white fluorescent illumination and 16 h photoperiod and subcultured every 4 wk. Plantlets were acclimatized for 3 wk in the greenhouse in clear plastic-covered seedling trays with 125 cm^3

cells containing a mixture of 1:1 peat-based potting mixture (Premier Brand, Rivière-du-Loup):perlite. *Ex vitro* transplants were watered daily. Plastic covers were gradually lifted after 3 d and removed by 5 d.

Field trials were conducted on a sandy loam soil. The EVPD plants had 10-12 leaves at the time of field planting. In the first experiment, treatments consisted of 3 in-row spacings of 10, 20 and 30 cm, while between-row spacing was a constant 80 cm. This corresponded to plant densities of 125 000, 62 500 and 41 500 plants/ha, respectively. The EVPD plants were planted 10 cm above the root stem interface. In the second experiment, EVPD- plants were grown at 3 planting depths of 5, 10 and 15 cm above the root-stem interface, with an in-row spacing of 30 cm. For both experiments, plots consisted of 3 rows of 4 m each. Plants were harvested 100 d after field-planting.

The first experiment was conducted using a randomized complete block design (RCBD) with 3 replicates in 1990 and 4 replicates in 1992. The second experiment was conducted using a RCBD with 4 replicates in 1990.

Yield measurements were evaluated on 2.4 m of the middle row and used to calculate tuber number/ha and metric tons/ha. Tuber number and weight per plant, plant height and tuber size category were measured on eight plants per treatment. The tuber size categories included: Under grade (<30 mm), Grade B (30-45 mm), Grade A (45-80 mm) and Over grade (>80 mm).

The economic assessment was conducted using data published

by the Québec Ministry of Agriculture (Ministère de l'Agriculture des Pêcheries et de l'alimentation du Québec, 1991). The production cost per hectare were evaluated at $1 500/ha, and included the costs of labour ($466), fertilizers ($467), limestone ($23), oats ($30), herbicides ($75), fungicides ($132), insecticides ($60), defoliant ($37) and fuel ($210).

Data were analyzed by ANOVA and Duncan's New Multiple-Range Test (Steel and Torrie, 1980) except tuber size distributions which were analyzed using a Chi-square test for homogeneity of distribution (Scherrer, 1980).

8.3 Results and Discussion

The yield and plant height characteristics of EVPD plants were influenced by planting density and cultivar (Table 8.1) but not by planting depth (data not shown). For both seasons, tuber number and weight per hectare increased as in-row spacing decreased from 30 to 10 cm (Table 8.1). Significantly more tubers/ha were produced when EVPD plants were spaced 10 compared with 30 cm apart. Tuber number/ha was significantly greater at an in-row spacing of 10 compared with 20 cm for all cultivars, except for 'Superior' in 1992. Tuber weight/ha was significantly greater at an in-row spacing of 10 compared with 30 cm for 'Kennebec' and 'Russet Burbank', and at an in-row spacing of 10 compared with 20 cm for 'Kennebec' in 1990. In 1992, significantly greater tuber weights/ha were observed at an in-row

spacing of 10 compared with 30 cm for 'Kennebec' and 'Superior', and for an in-row spacing of 10 compared with 20 cm for 'Kennebec'.

On a per plant basis, tuber number and weight generally increased as in-row spacing increased. Tuber number/plant was significantly greater for all cultivars at an in-row spacing of 30 compared with 10 cm except for 'Kennebec' in 1992. Tuber weight/plant was significantly greater when plants were spaced 30 cm apart compared with plants spaced 10 cm apart. Tuber weight/plant was significantly greater at spacings of 30 compared with 20 cm for 'Russet Burbank' in both years and for 'Kennebec' and 'Superior in 1992. Significantly greater tuber weight/plant occurred for in-row spacings of 20 compared with 10 cm for 'Russet Burbank' in 1990 and with 'Russet Burbank' and 'Superior in 1992. Plant height increased as plant density decreased, but significant plant height increases occurred only between in-row spacings of 30 or 20 cm compared with 10 cm for 'Russet Burbank' and 'Superior' in 1990 and 'Kennebec' and 'Superior' in 1992.

No significant differences were observed in the yield and plant height of mTD and EVPD plants grown at an in-row spacing of 30 cm (data not shown). The results obtained with EVPD plants are therefore likely to apply equally to mTD plants.

Approximately 80% of the tubers produced were of seed tuber Grades A or B. The tuber size frequency distribution was minimally affected by planting density and only affected by the growing season for 'Russet Burbank' at the 20 cm spacing.

Although greater tuber numbers and weights per hectare were achieved by increasing field-planting densities to 125 000 EVPD plants/ha, maximum yields per unit plant were achieved by decreasing the planting density to 41 500 plants/ha. These results are in agreement with the preliminary observations made by Wattimena (1983) on 'Red Pontiac', but differ from Levy's (1985) study in which reduced distance between rows from 90 to 45 cm reduced tuber weight of 'Idit' and 'Orit' but not tuber number per plant.

An economic analysis based on Québec field management costs (Table 8.3), indicated that the optimum planting density varied with the plantlet cost. The optimal field spacing would be 10 cm if the cost of plantlets was $0.10 or less, 20 cm if plantlet costs were from 0.10 to $0.20 and 30 cm for plantlet costs greater than $0.20. Decisions concerning the planting density of EVPD or mTD plants must be economically motivated. The cost per unit tuber must be minimized.

Table 8.1. Yield characteristics and plant height of *ex vitro* plantlet-derived plants grown under three planting densities for two seasons.

Year Cultivar Spacing	Tuber number per ha (10^4)	Tuber weight per ha (T)	Tuber number per plant	Tuber weight per plant (g)	Plant height (cm)
1990					
Kennebec					
10 cm	52.1 a	47.6 a	4.2 b	380 b	75.6 a
20 cm	29.7 b	31.2 b	4.8 ab	498 ab	75.7 a
30 cm	27.9 b	28.0 b	6.7 a	773 a	78.8 a
Russet Burbank					
10 cm	80.8 a	35.9 a	6.5 b	287 c	82.7 b
20 cm	58.3 b	32.4 ab	9.3 ab	518 b	91.1 a
30 cm	44.8 b	27.7 b	10.8 a	665 a	93.8 a
Superior					
10 cm	46.7 a	23.3 a	3.7 b	186 b	30.1 b
20 cm	41.9 b	20.3 a	6.7 a	324 ab	42.5 a
30 cm	23.6 b	18.2 a	5.7 a	436 a	44.7 a
1992					
Kennebec					
10 cm	72.8 a	64.7 a	5.8 a	678 b	36.5 b
20 cm	36.4 b	54.1 b	5.8 a	865 b	38.4 a
30 cm	30.6 b	44.5 b	7.3 a	1067 a	38.7 a
Russet Burbank					
10 cm	54.7 a	42.6 a	4.4 c	341 c	39.6 a
20 cm	38.3 b	36.6 a	6.1 b	586 b	39.6 a
30 cm	34.0 b	37.6 a	8.2 a	902 a	42.0 a
Superior					
10 cm	55.6 a	40.7 a	4.4 b	326 c	15.7 b
20 cm	38.8 ab	37.5 ab	6.2 ab	600 b	20.0 a
30 cm	33.1 b	32.7 b	8.0 a	784 a	20.0 a

Means followed by the same letter within a column are not significantly different (Duncan's New Multiple-Range Test, $p \leq 0.05\%$).

Table 8.2. Tuber size frequency distribution of *ex vitro* plantlet-derived plants grown under three planting densities for two seasons.

Cultivar / Treatment	1990 Tuber size classes				1992 Tuber size classes			
	Under grade	Grade B	Grade A	Over grade	Under grade	Grade B	Grade A	Over grade
Kennebec								
10 cm	5	25	59	10	12	20	57	11
20 cm	7	17	60	16	11	20	55	13
30 cm	5	17	59	19	14	26	47	14
Russet Burbank								
10 cm	8	26	53	12	17	26	47	10
20 cm	4	24	57	15	17	31	37	15
30 cm	5	21	58	18	12	23	50	16
Superior								
10 cm	13	34	47	5	19	38	41	2
20 cm	10	33	49	7	17	45	34	5
30 cm	9	35	43	13	14	39	39	7

The following distribution was found significantly different (X^2, $p \leq 0.05\%$): 1990 vs 1992 for 'Russet Burbank' 20 cm.
Tuber size classes: Under grade (<30 mm), Grade B (30-45), Grade A (45-80 mm) and Over grade (>80 mm)

Table 8.3. Cost estimates of tubers produced from EVPD plants grown under three planting densities.

Densities (plants/ha)	Hypothetical price per EVPD plants ($CAN)					
	0.05	0.10	0.15	0.20	0.25	0.30
125 000	0.013	0.024	0.035	0.045	0.056	0.067
62 500	0.016	0.025	0.031	0.040	0.048	0.055
41 500	0.021	0.028	0.035	0.041	0.047	0.052

CHAPTER 9. ESTABLISHNG A POTATO SEED TUBER CERTIFICATION PROGRAM IN EGYPT.

9.1 INTRODUCTION

In Africa potato production increased by 60% in the last 15 years (FAO, 1990). This production increase is likely to continue. The high yield potential and excellent nutritive quality makes potato a very attractive staple food for countries with rapidly expanding populations. Egypt is one of Africa's largest potato producers; 1.4 million metric tons produced from the winter and fall growing seasons. Yields average 16 tons per hectare (Nganga and Potts, 1988). For the winter growing season, approximately 40 000 tons of potato seed tubers (4-5 10^9 tubers) are imported from the Netherlands at a cost of 15 to 20 million dollars (US). A large portion of the imported seed tubers are planted from December to January and the crop is destined for the export market or stored to be used as seed tubers for the fall growing season. The balance is planted later in the season to supply the local market. In the fall (nili) season planting can begin as early as mid August, if seed tubers were stored in mud-brick structures (nawhellas), and extends to October. The entire fall crop is locally consumed.

The limited availability of good quality seed tubers at reasonable cost limits the expansion of potato cultivation in Egypt. The price of seed tubers alone represents 50% of the

total production cost (Zaki, 1989). Imported seed tubers used for the winter season are often physiologically too young. Therefore, the full potential of these high quality seed tubers is not fully exploited (Nganga and Potts, 1987). The large size of the imported tubers make handling and planting more difficult for unmechanized farmers (Zaki, 1989). Furthermore, the same cultivars are used for both the fall and winter seasons, although the climatological conditions are very different. The winter crop is planted under increasing daylength and temperature and the fall crop is planted under decreasing daylength and temperature (Nganga and Potts, 1987). The increased availability of better adapted varieties for the winter season, which is the most suitable season for potato cultivation, could lead to significant yield increases.

The introduction of true potato seed (TPS) technologies could have a significant impact on potato production in Egypt. However, the heterogeneity of the potato crops produced through TPS makes them inadequate for the processing and export markets. To further self-sufficiency, the development of a potato seed certification program based on clonal propagation would be desirable.

In an attempt to achieve self-sufficiency in potato seed tuber production the Egyptian Ministry of Agriculture, with assistance from the Canada-Egypt-McGill-Agricultural-Response-Project (CEMARP) and the Egyptian Ministry of Agriculture, are in the process of establishing a potato seed tuber certification

program. This program incorporates *in vitro* and greenhouse or screenhouse propagation to generate specific pathogen-tested (SPT) planting material for distribution to Egyptian farmers. The objective of the Egyptian seed certification program in 1991 was to develop the capacity to produce locally 100% of Egypt's annual seed tuber needs within 10 years. Since its instigation, interest in the project has increased considerably. Two governmental laboratories were established; the Groppi Laboratory south of Cairo and the Torel Farm on the desert road near Giza Pyramids. Private tissue culture laboratories are also showing interest in the commercial production of SPT material.

The objectives of this work are to review the different aspects of potato seed tuber certification in Egypt and to discuss the potential roles of governmental institutions and the opportunities for private enterprise in this program.

9.2 Discussion

Potato Seed Tuber Certification Program

The aim of a certification program is to multiply potato seed tubers while maintaining varietal purity of seed stocks and controlling the spread of disease by prescribing tolerances and cultural conditions (Allen et al., 1992). The first mandate of the Egyptian seed certification program is to address the question of rules and regulations that would govern potato seed tuber production in Egypt. Among the aspects that must be

regulated are classes of seed potatoes and their associated quality standards, field production and storage.

In North America and northern Europe seed certification programs generally rely on six to eight generations (field multiplications) to obtain sufficient volumes of seed tubers to supply the commercial growers of tablestock and processing potatoes (Wright, 1988; Withmore et al., 1990; Allen et al., 1992). In warm countries, such as Egypt, large populations of virus transmitting insects are present during the growing season (Marco, 1984). To succeed, the Egyptian Seed certification program must severely limit the number of field generations and practice strict insect control in the field.

Nuclear Stock would be the entry class of the certification program. Nuclear Stock describe any tuber, plant or vegetative propagule, produced in protected, environmental conditions from tissue culture, that has been subjected to laboratory tests and found free of disease and demonstrate freedom from varietal mixture.

Further greenhouse or screenhouse multiplications would be conducted to produce the Greenhouse Elite classes (from one to several generations based on need). Greenhouse Elite I tubers would be produced from Nuclear Stock material or *in vitro* plantlets or microtubers. Greenhouse Elite II or III tubers, would be produced from Greenhouse Elite I tubers or any of the higher classes (i.e. Nuclear Stock material or *in vitro* plantlets or microtubers) if necessary. All greenhouse or screenhouse

propagations should be conducted under strict cultural conditions. The crops would be inspected at frequent intervals during the growing season. Compulsory testing would be done on randomly selected samples of minitubers. The tested material should be SPT. Varietal mixing would not be tolerated in any classes.

Foundation class tubers would be produced in the field from Greenhouse Elite II tubers (or any of the higher classes). No potatoes should have been grown in the fields in question for the previous several seasons (2 years). The crop should be inspected several times during the growing season and found free of varietal mixture and the presence of viral, bacterial and fungal diseases should not exceed prescribed levels. The Foundation class tubers should be grown only in a very isolated area, such as the Mount Katarina area. Previous studies by Marco (1984) have demonstrated that populations of winged aphids in this region are small, are active mainly in the spring, and only until May. If it is not possible to grow Foundation class tubers in an environment of low virus-vector pressure only one field propagation cycle should be permitted in the production of Certified seed tubers.

The Certified tubers would be produced in the field from Foundation tubers (or any higher classes of tubers). No potatoes should have been grown in the fields in question for the previous several seasons (at least 1 year). The crop should be inspected several times during the growing season and no disease should be

found to exceed prescribed levels. Varietal mixing should be as low as possible. Two greenhouse propagations could be conducted every year, in late fall and in winter. However, we recommend that the field crop(s) be grown only during winter, when cool temperatures and low virus-vector pressure are most adequate for potato seed tuber production.

An example of a possible seed tuber certification scheme is given in Figure 9.1. The number of greenhouses and field generations were based on a 10-fold increase per generation. If such a multiplication rate was not realized the number of *in vitro* plantlets and/or microtubers must be increased accordingly. This seed tuber certification program is termed a flush-through system because it is based on the annual input of nuclear stock coupled with the mandatory downgrading of seed lots, each generation, to the lower seed tuber class (Johansen et al., 1984).

The seed tubers should be stored at 4 °C at 90% relative humidity. Potato seed tubers should be inspected periodically during storage, to assure they are being stored adequately and that no pests or diseases have appeared during storage.

A system of tuber grade sizes should be implemented to better evaluate the quality of the seed tubers. Egypt Grade A tubers would include tuber sizes between 20-45 mm which are the ideal size for Egyptian farmers (Zaki, 1989). Egypt Grade B would include tuber sizes between 45-80 mm. Standards should also be prescribed to determine the levels of tuber damage and

malformation acceptable for the Foundation and Certified classes.

A system of official tags should be instated for seed lot identification purposes. The tags, which would be delivered after the final storage inspection, should give information such as the class, variety, tuber grade size and crop certificate number (seed lot number). Tags would also include a statement declaring that the potato seed tubers identified by the certificate number shown on the tag met with the regulations of the Egyptian seed Certification Program at the time of inspection.

Specific Pathogen-Tested Material.

Only specific pathogen tested (SPT) material should be used for *in vitro* propagation. For all desirable cultivars, SPT material should be imported through the International Potato regional office in Egypt as *in vitro* plantlets or microtubers. To assure that SPT material is readily available in Egypt, at least one governmental laboratory should be responsible for *in vitro* maintenance of SPT material. It would be advantageous in the future to maintain the SPT material as *in vitro* microtubers (Kwiatkowski et al., 1988; Dodds and Watanabe, 1990; Thieme, 1992).

In vitro Propagation of SPT Material (micropropagation)

In vitro propagation is done using single node cuttings and *in vitro* microtubers. Single node cuttings are grown on modified

Murashige and Skoog (MS, 1962) basal salt solution supplemented with 0.4 mg/l thiamine-HCl, 2.0 mg/l ca-pantothenate, 100 mg/l inositol, 30 g/l sucrose, 7 g/l agar and the pH adjusted to 5.7 prior to autoclaving (Estrada et al., 1986). Cultures are incubated at 22 °C, 50 μmol/m^2/s cool white fluorescent illumination and 16 h photoperiod.

Microtubers are produced using layered shoots. In a first step, root-severed plantlets (shoots) are layered in propagation medium containing modified MS (1962) basal salt solution supplemented with 0.4 mg/l thiamine-HCl, 2.0 mg/l Ca-pantothenate, 100 mg/l inositol, 0.4 mg/l gibberellic acid, 0.5 mg/l 6-benzyladenine 20 g/l sucrose, 7 g/l agar and the pH adjusted to 5.7. Cultures are incubated at 22 °C, 50 μmol/m^2/s illumination and 16 h photoperiod. After 4 wk the medium is removed and replaced by microtuberization medium containing MS (1962) basal salt solution supplemented with 0.4 mg/l thiamine-HCl, 2.0 mg/l ca-pantothenate, 100 mg/l inositol and 80 g/l sucrose. Shoots are incubated at 15 °C, 50 μmol/m^2/s illumination and 8 h photoperiod (Leclerc et al., 1993).

Both plantlets and microtubers can be used as sources of SPT material for Nuclear Stock production. The advantages of using microtubers over plantlets are that they are easier to store, handle and do not require an *ex vitro* acclimatization period.

Greenhouse and screenhouse propagation of SPT material

The objective is to maximize the production of minitubers;

small sized tubers which do not generally exceed 40-50 g. The prevention of plant recontamination by systemic pathogens is the major concern at this stage. Intensive pest management and roguing practices should be instated at this stage to eliminate diseased plants.

Minituber production is conducted in a light potting mixture (using peat moss, perlite, vermiculite or coarse sand), although pasteurised soil could be used. The potting mixtures should not be re-used for further potato multiplication to avoid the transmission of soil borne diseases. Insect-proof screens are used in the greenhouse to protect the potato plants from pests attacking the foliage, stem or tubers (Table 9.1).

Greenhouse- or screenhouse- grown minitubers can be produced using *ex vitro* plantlets or microtubers (Ranalli et al., 1990; Wiersema et al., 1987) or stem cuttings of *ex vitro* plants (Bryan et al., 1981; Vander Zaag and Escobar, 1990; Van Minh et al., 1990). Minitubers can be produced in pots or in beds under high density planting (Wiersema, 1986).

Seed tuber production in the field

Only certified growers should be allowed to produce Foundation or Certified tubers. These growers should be selected on the basis of geographical location (in relation to virus-vector pressure) of their farm unit, a farm unit being defined as a single or a number of tract(s) of land operating as a single unit and utilizing common equipment, facilities or storage, and

on their capabilities to conform to the prescribed regulations.

For each farm unit the production of Foundation or Certified potato seed tubers should be conducted on a field basis, which corresponds to an identifiable area of land where seed potato of a particular variety and class are planted. The harvested tubers should be stored as individual seed lots (seed tubers of a variety and class grown in a single field) to allow their identification and avoid mixing of varieties and classes.

The phytosanitary conditions under which the crop is grown must be as strict as possible. Pests and diseases must be closely monitored and controlled. Field propagation should ideally be conducted in areas isolated from commercial Solanaceous crops, such as in the Sinai and Eastern desert regions.

Governmental vs private industries: Roles and opportunities

Business opportunities now exist for private firms interested in potato seed tuber production in Egypt. The involvement could include tissue culture propagation, minituber production in greenhouses or screenhouses or Foundation and Certified tuber production in the field. The contribution of private enterprise to the Egyptian Potato Seed Tuber Certification Program will depend largely on their ability to produce large quantities of high quality plant material. Quality control must be intrinsically linked with these propagation efforts and so, the private firms must be strongly linked to the governmental

certification agency. The competitive advantage of a company depends as much on the possibility to go beyond the minimum quality standards imposed by the certification agency as on its production abilities. Clearly, corporate quality standards must surpass those of the certification agency. Besides the sanitary quality of the product, uniformity of age and size, year after year repeatable quality, consumer readiness (ready to be used when planted) and absence of varietal mix are all aspects of quality that companies must deal with to be successful (Addy, 1988).

The government is responsible for the creation and management of the Potato Seed Tuber Certification Program. This governmental certification agency assures that the quality standards of the program are respected by everyone involved in the development of certified seed tubers. Opportunities also exist for governmental institutions in tissue culture, minituber production and field propagation.

Quality Standards

The reason for setting quality standards at every step of the propagation program is to control the disease incidence in the Certified seed tubers. The objective is to assure homogenous quality throughout the program.

Establishing realistic quality standards is one of the major hurdles facing the Egyptian Potato Seed Tuber Certification Program. The establishment of quality standards consists of

determining acceptable limits of viral, fungal and bacterial contamination for every step of the certification program. Quality standards must be strict enough to ensure that the Certified potato seed tubers produced by the program are of good quality, yet they must not be unreasonably strict, since this would lead to the rejection of most of the potato seed tuber being currently produced (Bryan, 1988). A zero-tolerance level of pathogen infection should be instated for the in vitro as well as for the higher Greenhouse Elite classes. For the lower Greenhouse Elite and Foundation and Certified classes the limits of reinfection could increase gradually as tubers move down through the propagation scheme but should be kept as low as realistically possible. The acceptable recontamination level for each step of the propagation program has not yet been established. These recontamination levels should be set by a collegium representing all parties of the Egyptian potato seed tuber industry. Economically, low contamination levels are more expensive to sustain (Knutson, 1988). On the other hand, high contamination levels makes the locally produced potato seed tubers less attractive to farmers. When determining quality standards it must be kept in mind that the objective of a potato seed tuber certification program is to provide to farmers the best quality seed tubers at the cheapest price possible.

The aspect of storage practices in relation to physiological ageing should be addressed by the Egyptian Potato Seed Certification Program as it is an important factor in the

determination of the quality of potato seed tubers (van der Zaag, 1986). The optimum physiological age of tubers will vary from 500 to 2000 degree-days, depending on cultivar and duration of the growing period (Allen et al., 1992). Efforts should be made by the Seed Tuber Certification Program to determine the ideal physiological age for the cultivars of interest. The program should favour the utilization of techniques such as diffuse light storage (Scholte, 1989) to control the physiological quality of potato seed tubers.

Pathogen Testing

The establishment of quality standards implies that potato seed tubers must be tested for all systemic pathogens of concern in Egypt (Table 9.1). Leaving the producers (laboratories, green/screenhouse facilities or the growers) with the obligation of testing their own product will not guarantee a respect for industry standards. For this reason an impartial pathogen testing laboratory under the jurisdiction of the Ministry of Agriculture must be created. The role of this pathogen testing laboratory would be to monitor the quality of the product and to make sure that the program quality standards are respected in the greenhouse, in the field and during storage. The laboratory would be responsible to assure that the rejected product be disposed of in an appropriate manner. It should be noted that the rejection of a potato seed lot does not necessarily imply that it must be destroyed. A rejected seed lot could be

downgraded to a lower class of certification if it met the quality standards of the lower class. The laboratory should also have a mandate to provide strong technical support to farmers and provide educational services.

It is not possible to test all the plant material being produced by a certification program. Sampling strategies must be developed to scientifically evaluate the level of pathogen contamination. To effectively use sampling strategies, a strict account of the accession origin of each in vitro plantlet produced must be kept. Similarly, in the greenhouse or screenhouse and in the field a seed lot identification system must be developed and enforced.

9.3 CONCLUSION

The establishment of a potato seed tuber certification program will have a significant economic impact on potato production in Egypt by reducing the cost of seed tubers. Significant yield increase could be achieved by making high quality potato seed tubers of cultivars better adapted to the Egyptian climate available on the market. It is essential that the rules and regulations put in place by the Egyptian Potato Seed Certification Program be agreed to and respected by all sectors of the potato seed tuber industry. Ultimately, this will be the responsibility of the Egyptian Ministry of Agriculture.

Figure 9.1. Example of a potato seed tuber certification scheme adapted to Egypt. The number of greenhouses and field generations are based on a 10-fold increase per generation. * Indicates that a third greenhouse propagation generation would be required if it were not possible to produce Foundation seed tubers in the field.

CERTIFICATION LEVEL	PROPAGATION GENERATION AND NUMBER OF PROPAGULES PRODUCED
IN VITRO PROPAGATION	(500 000 plantlets or microtubers)
NUCLEAR STOCK	First greenhouse propagation ($5x10^6$ minitubers)
GREENHOUSE ELITE I	Second greenhouse propagation ($5x10^7$ minitubers)
GREENHOUSE ELITE II*	Third greenhouse propagation ($5x10^8$ minitubers)
FOUNDATION*	First field propagation ($5x10^8$ tubers)
CERTIFIED	Second field propagation ($5x10^9$ tubers)

Table 9.1. Major insect pests and diseases of the potato crop in Egypt.

INSECTS WHICH ATTACK FOLIAGE	VIRUSES
Myzus persicae	PLRV
Aulocorthum solani	PVA
Liriomyza trifolii	PVS
Phthorimaea operculella	PVX
Shitocera gregaria	PVY
Epilachna spp.	
Polyphagotarsonemus latus	BACTERIA
Thrips palmi	
	Erwinia chrysanthemi
INSECTS WHICH ATTACK STEMS	*Pseudomonas solanacearum*
Phthorimaea operculella	FUNGI
Agrostis segetum	
Agrostis ipsilon	*Alternaria solani*
	Botrytis cinerea
INSECTS WHICH ATTACK TUBERS	*Collectotrichum coccodes*
	Helicobasidium purpureum
Phthorimaea operculella	*Phytophtora infestans*
Gryllotalpa africana	*Pytium ultimum*
Agrostis spp.	*Rhizoctonia solani*
	Spongospora subterranea
	Streptomyces scabies
	Verticillium albo-atrum
	Verticillium dahliae

(From: Hide and Lapwood, 1992; Raman and Radcliffe, 1992)

CHAPTER 10. SUMMARY

The microtuberization system reported in chapter 3 permitted rapid and extensive tuberization of in vitro plantlets of 'Kennebec', 'Russet Burbank' and 'Superior'. Up to 2.3 g of microtuber fresh weight per original plantlet were produced in an MS-based medium containing 80 g/l sucrose incubated for 56 d under an 8 h photoperiod at 15 °C.

The addition of coumarin or CCC and BA to the microtuberization medium of layered plantlets did not increase the weight or number of microtubers per plantlet compared with elevated sucrose alone and depressed microtuber yield per plantlet to some extent (Chapter 3). Coumarin increased individual microtuber weight as indicated by the frequency distribution analysis. Coumarin increased the number of larger microtubers compared with CCC-BA and, to a lesser extent, with the control medium. Contrary to the results of Hussey & Stacey (1984) who used 2.0 mg/l BA and 500 mg/l CCC, the addition of CCC and BA to the microtuberization medium failed to increase the microtuberization response of plantlets compared with the control medium. The promotive effect of cytokinins and CCC used singly on microtuberization has previously been questioned (Stallknecht, 1985; Ewing, 1990). Clearly, under a short photoperiod and cool temperature, the addition of coumarin or CCC and BA to the microtuberization medium of plantlets is unnecessary and may even be deleterious.

When layered plantlets and nodal cuttings were incubated for 56 d under an inductive environment microtuber fresh weight produced from layered plantlets was 3-5 times greater (from 1.92 to 2.06 g) than for nodal cuttings (from 0.36 to 0.63 g) on a per original plantlet basis. Differences in the surface leaf area, site of the perception of the photoperiodic stimulation, and in the surface of plant tissues in contact with the medium were probably responsible for the large differences in microtuber fresh weight between the two types of culture. Favouring vegetative growth of stolon segments or plantlets prior to microtuber induction also increased microtuber fresh weight (Chapman, 1955; Garner & Blake, 1989). Clearly, greater microtuber yield can be achieved by increased vegetative area of cultures prior to microtuber induction.

Although layered plantlets produced heavier microtuber fresh weight per original plantlet, nodal cuttings produced more microtubers. This difference in microtuber number per original plantlet was most likely due to the expression of correlative inhibition in layered plantlets. When plantlets are dissected into nodal cuttings the hormonal balance at each node is disrupted, promoting microtuberization.

The advantage of our two step microtuberization method over that of Garner and Blake's (1989) is that heavier microtubers can be produced more rapidly, up to 2.3 g in 56 days compared with 0.2 g in 119 days, respectively.

The use of our microtuberization system in the study of

potato tuberization may prove valuable, since two aspects of tuber formation, i.e. the perception of environmental stimulation and the reaction to this stimulation, can be studied simultaneously *in vitro*.

Microtuber weight was significantly increased (by 24 to 45%) when plantlets were pre-conditioned for 1 wk under an inductive environment and incubated in microtuberization media containing CCC, BA and sucrose, but not when the medium contained only sucrose (Chapter 4). The effect of environmental stimulation could therefore be modified by the addition of growth regulating substances in the microtuberization medium.

When the volume of microtuberization medium was doubled from 50 to 100 ml the number of microtubers produced per original plantlet was significantly increased, by 20 to 44 % (Chapter 4).

Microtuber dormancy period was influenced by cultivar and microtuber size but not by the use of growth regulating substances in the microtuberization medium (Chapter 5). The dormancy period was shortest in duration for 'Superior' (14 and 11 wk for small and large microtubers, respectively), intermediate for 'Kennebec' (15 and 12 wk for small and large microtubers, respectively) and longest for 'Russet Burbank' (17 and 13 wk for small and large microtubers, respectively). The dormancy periods of microtubers were positively correlated with the relative dormancy periods reported for field-grown tubers.

The dormancy period, measured from harvest to sprouting, of microtubers of fresh weights ≤250 mg was significantly longer (by

2-3 wk) compared with microtubers having fresh weights >250 mg (Chapter 5). Since microtubers were induced over several weeks the larger microtubers were generally those which initiated their development earlier. The longer dormancy periods of small compared with large microtubers reflected differences in the age of microtubers at the time of harvest. Microtubers had longer dormancy periods when harvested after 28 compared with 56 d of incubation, when the dormancy duration was measured from harvest to sprouting, but differences were not significant when the dormancy period was measured from the time of microtuber induction to sprouting.

The growth of apical buds, isolated from both small and large microtubers and cultured *in vitro*, was not found to differ significantly after 1 and 2 wk in culture (Chapter 5). It seems unlikely that differences in bud maturity were responsible for the differences in dormancy duration of small and large microtubers.

Endogenous ABA levels, measured on a fresh weight basis, were greater in small than in large microtubers. The differences were increased when the comparison was made on a dry weight basis. The ABA content of microtubers increased with size, indicating that, as observed with *in vivo* tubers (Krauss, 1981; Koda and Okasawa, 1983), ABA was either continuously supplied or synthesised in the developing microtubers. Correlation analysis indicated that endogenous ABA levels were positively correlated with microtuber dormancy periods of small and large microtubers.

It appears that proportionally greater levels of endogenous ABA were responsible for the longer dormancy periods of small microtubers. High levels of endogenous ABA have been linked with prolonged dormancy periods in field-grown tubers (Coleman and King, 1984). The concentration of both "bound" and "free" ABA within potato tubers was greater in the periderm tissues than in the storage parenchyma of the pith (Korableva et al., 1980). Small microtubers have a greater surface to volume ratio so the periderm tissues are proportionally greater than in large microtubers.

Among the factors investigated, microtuber size appeared to be the main determinant of quality (Chapter 6). Large (>250 mg) microtubers had greater specific gravity, possessed more distinguishable eyes and more sprouts developed from large microtubers compared with smaller microtubers. The smaller surface/volume ratio of larger microtubers probably made them less susceptible to desiccation than the lighter microtubers and prolonged their storage life.

Observations made on *in vivo*-produced tubers showed that once tuberization was initiated no new buds or internodes were produced (Cutter, 1978). The differences in eye number reported in chapter 6 between smaller and larger microtubers were probably not differences in bud number *per see*, but rather differences in cell enlargement. Light and scanning electron microscopy revealed that the apical eye-complex of small microtubers contained a greater number of buds than that of large

microtubers. It appears that the number of eyes per microtuber is not different between small and large microtubers, but for small microtubers more eyes are located very close together (<0.5 mm) at the apex. Microtubers are initiated on stolons between the eighth or ninth internode from the stolon's apical meristem, as previously reported from *in vivo*-produced tubers (Cutter, 1978).

As microtuber dormancy ended, growth resumed rapidly at one (occasionally two) bud(s) of the microtuber, most frequently from the apical eye-complex, strongly inhibited the growth of other buds through apical dominance (Chapter 6). As a result, plants originating from microtubers were generally single-stemmed (Wattimena et al., 1983; Leclerc and Donnelly, 1990). However, multiple sprouting occasionally occurred, and the phenomenon was positively correlated with microtuber weight (Chapter 7).

Since buds that originated from different microtuber sizes and eye positions were equally functional (Chapter 6), the distinct pattern of sprout development observed in microtubers appears to be related to the strong influence of apical dominance in microtubers. Similar observations were previously reported with small-sized minitubers grown in the field (Thornton and Neundorfer, 1986; Melching et al., 1993). Removing the primary sprout prior to planting partially released the effect of apical dominance for larger microtubers, as observed with field-grown tubers (Hay and Hampson, 1991), but the treatment was detrimental for smaller tubers. Attempts to release correlative inhibition

by storing microtubers distal end up were unsuccessful (Chapter 6).

The physiological age of microtubers had no influence on the number of stems per mTD plants (Chapter 7); these were usually single-stemmed. All multi-stemmed plants originated from microtubers had more than one sprout at the time of planting. However, microtubers with more than one sprout did not necessarily produced multi-stemmed plants. Multi-stemmed plants were more frequent in 'Superior' than in 'Kennebec'. Studies made with field-grown tubers indicated that, similarly, the sprouts present on conventional seed tubers did not necessarily develop into stems (Hay and Hampson, 1991). After 5 wk, no differences were observed in the heights or leaf numbers of mTD plants grown from microtubers of the two physiological ages. The number of minitubers per mTD plant was not affected by the physiological age of the microtubers nor by the growing season but was significantly greater for 'Superior' than for 'Kennebec' in both seasons.

Physiological age of the microtubers had little influence on the weight of minitubers produced in the first trial conducted in the fall (Chapter 7). In the second trial, conducted in the winter, plants grown from physiologically younger microtubers produced greater minituber weights than plants grown from physiologically older microtubers, but the comparison was significant only for 'Superior'. The growing season had a significant influence on the minituber weight of mTD plants.

Plants grown in the fall produced a lower minituber weight than plants grown in winter, where higher natural irradiation levels and longer daylength favoured greater yields.

Stem number was positively correlated with minituber number and weight per mTD plants; with correlation coefficients of 0.4668 ($p=0.0010$) and 0.3734 ($p=0.0199$) respectively. Wiersema et al. (1987) reported similar results with mTD plants grown under high density in nursery beds.

The frequency distribution of minitubers was not affected by the physiological age of microtubers but strong seasonal effects were observed. In the fall, most minitubers weighed less than 10 g while the majority of minitubers produced in winter were heavier than 10 g. Contrary to what was generally observed with conventionally propagated seed tubers (Kawakami, 1963; Pérennec and Madec, 1980), physiological ageing, even a severe physiological ageing treatment (2500 °C d, >4 °C), did not modify the expression of apical dominance in microtubers; most microtubers produced only one sprout regardless of their physiological age. The large difference in the size of these propagules may be responsible for the differences in expression of apical dominance. The buds of microtubers are in closer proximity to one another compared with buds of seed tubers. The developing sprout of microtubers exerts a stronger inhibitive influence on non-growing neighbouring buds than does the developing sprout of seed tubers. Physiological ageing is thought to be determined in the storage tissues of tubers

(Pérennec and Madec, 1980), but recent reports indicate that the condition of the sprouts plays a role in its expression (Allen and Scott, 1980; Scholte, 1989). Although sprout vigour may be increased by storing physiologically old microtubers in the light, as demonstrated by Scholte (1989), the limited volume of microtuber storage tissues may be responsible for the restricted influence of microtuber physiological age on the growth and yield characteristics of mTD plants.

The yield and plant height of EVPD plants were influenced by plant spacing and cultivar but not by planting depth. For both seasons, tuber number and weight per ha increased as in-row spacing decreased from 30 to 10 cm. Significantly more tubers/ha were produced when EVPD plants were spaced 10 compared with 30 cm apart. Tuber number/ha was significantly greater at an in-row spacing of 10 compared with 20 for all cultivars in 1990. In 1990, tuber weight/ha was significantly greater at an in-row spacing of 10 compared with 30 cm for 'Kennebec' and 'Russet Burbank', and at an in-row spacing of 10 compared with 20 cm for 'Kennebec'. In 1992, significantly greater tuber weights/ha were observed at an in-row spacing of 10 compared with 30 cm for 'Kennebec' and 'Superior', and for an in-row spacing of 10 compared with 20 cm for 'Kennebec'.

On a per plant basis, tuber number and weight generally increased as in-row spacing increased. Tuber weight/plant was significantly greater when plants were spaced 30 cm apart compared with plants spaced 10 cm apart. Tuber number/plant was

significantly greater for all cultivars at an in-row spacing of 30 compared with 10 cm except for 'Kennebec' in 1992. Significant differences in tuber number/plant occurred at in-row spacings of 30 compared with 20 cm for 'Russet Burbank' in 1992, while only 'Russet Burbank' in 1992 showed an increased tuber number/plant at an in-row spacing of 20 compared with 10 cm. Tuber weight/plant was significantly greater at spacings of 30 compared with 20 cm for 'Russet Burbank' in both years and for 'Kennebec' and 'Superior in 1992. Significantly greater tuber weight/plant occurred for in-row spacings of 20 compared with 10 cm for 'Russet Burbank' in 1990 and with 'Russet Burbank' and 'Superior in 1992.

Plant height generally increased as plant density decreased, but significant plant height increases occurred only between in-row spacings of 30 or 20 cm compared with 10 cm for 'Russet Burbank' and 'Superior' in 1990 and 'Kennebec' and 'Superior' in 1992.

No significant differences were observed in the yield and plant height of mTD and EVPD plants grown at an in-row spacing of 30 cm. The results obtained with EVPD plants are therefore likely to apply equally to mTD plants.

Approximately 80% of the tubers produced were of seed tuber Grade A or B. The tuber size frequency distribution was minimally affected by planting density and only affected by the growing season for 'Russet Burbank' at the 20 cm spacing.

Our study indicated that although greater tuber numbers and

weights per hectare were achieved by increasing field-planting densities to 125 000 EVPD plants/ha, maximum yield per unit plant were achieved by decreasing the planting density to 41 500 plants/ha. These results are in agreement with the preliminary observations made by Wattimena (1983) on 'Red Pontiac', but partly contradict Levy's (1985) study in which reduced distance between rows from 90 to 45 cm reduced tuber weight but not tuber number per plant.

Decisions concerning the planting density of EVPD and mTD plants must be economically motivated; the cost per unit tuber produced must be reduced as much as possible. The economic analysis based on Québec field management costs, indicated that the optimum plant spacing was 10 cm if the cost of plantlets was $0.10 or less, 20 cm if plantlet costs were from 0.10 and $0.20 and 30 cm for plantlet cost greater than $0.20.

A potato seed tuber certification program adapted to the needs and constraints of Egypt is presented in chapter 9. This programs relies on the mass propagation of micropropagated material followed by a number of greenhouse multiplications. The number of field multiplication(s) is limited to one or possibly two field propagations to reduce to a minimum the risks of pathogen reinfection. The opportunities for private firms are discussed as well as the role of governmental agencies in the certification program. Rules and regulations concerning greenhouse and field production, storage and pathogen testing are discussed in light of Egypt's needs, as well as limitations in

terms of potato seed tuber production.

CHAPTER 11. GENERAL CONCLUSIONS

The results presented in this thesis should have a positive impact on the implementation of microtuber technologies, as an alternative to plantlets, in potato seed certification programs. The microtuberization of plantlets can be achieved rapidly by layering them in propagation medium for 28 d, and then transferring them to modified MS-based liquid microtuberization medium containing 80 g/l sucrose under 8 h light, 50 μmol/m^2/s period at 15 °C. With this method exogenously supplied coumarin, CCC and BA are not necessary for optimal microtuber production. Cultivars differed in their ability to tuberize *in vitro*. Increasing the incubation period from 28 to 56 d increased microtuber fresh weight, while increasing microtuberization medium volume from 50 to 100 ml significantly increased microtuber number per plantlet. This microtuberization system, a simplified version of the protocols developed by Estrada et al. (1986) and Meulemans et al. (1986), may have significant commercial implications. The use of liquid media, the absence of growth regulators in the microtuberization media and the lower labour input required to handle plantlets will help potato seed certification program reduce the costs associated with microtuber production.

Layered plantlets produced a significantly greater microtuber fresh weight, but fewer microtubers per original plantlet compared with nodal cuttings. The effect of a 1 wk

environmental pre-conditioning on microtuberization could be modified by the presence of growth regulating substances in the microtuberization medium.

The physiological processes involved in microtuber dormancy appear to be similar to field-grown tubers in a number of ways. Microtuber dormancy was cultivar-specific; genotypes with longer dormancy periods *in vivo* also had longer dormancy periods when induced *in vitro*. Small microtubers (≤ 250 mg) had longer dormancy periods than larger microtubers when the dormancy duration was measured from harvest to sprouting. Differences in dormancy periods of microtubers appears to be related to variation in the age of microtubers at the time of harvest. Duration of the incubation (growth) period had no effect on the dormancy periods of microtubers, when measured from microtuber initiation to sprouting. Although a positive correlation was established between the dormancy duration and endogenous ABA concentration of small and large microtubers, this does not preclude the involvement of other compound(s) as suggested by Hemberg (1985). The addition of growth regulators to the microtuberization medium had no effect on microtuber dormancy periods. It can be concluded that the dormancy periods of microtubers can be predicted on the basis of genotype and size at harvest. This information will be valuable for seed tuber certification programs and allow them to integrate information on microtuber dormancy into their production schemes.

Size appears to be the main factor affecting microtuber

quality, sprout development and storage life. Larger microtubers (>250 mg/l) had higher specific gravity, more distinct eyes and produced more sprouts compared with smaller (≤250 mg) microtubers. The development of sprouts, and subsequently, stems on microtubers was strongly regulated by apical dominance. Attempts to release apical dominance through desprouting were partly successful for larger microtubers. It appears advantageous to produce larger microtuber, as they can be stored for longer periods of time and can potentially produce greater yields than smaller microtubers.

Physiological ageing had no effect on the expression of apical dominance and only marginally influenced the yield characteristics of mTD plants grown in the greenhouse. The proximity of buds on microtubers favours the expression of apical dominance. The fresh weight of minitubers produced from physiologically old microtubers grown in pot trials in the greenhouse was slightly reduced compared with that of physiologically young microtubers. Minituber number per mTD plant was not affected by the physiological age of the microtubers nor by the growing season. It is therefore possible to store microtubers for long periods of time with little or no effects on their subsequent yield.

Maximum yields per unit area were achieved by increasing the planting density to 125 000 EVPD plants/ha while maximum yield per unit plant were achieved by decreasing the planting density to 41 500 plants/ha. The economic analysis based on Québec field

management costs, indicated that the optimum planting density was 125 000 plants/ha if the cost of plantlets was $0.10 or less, 62 500 plants/ha if plantlet costs were from 0.10 and $0.20 and 41 500 plants/ha for plantlet cost greater than $0.20. This study will help potato seed certification agencies to optimize the use of micropropagated material in the field.

The establishment of a potato seed tuber certification program will have a significant impact on potato production in Egypt by increasing seed tuber availability, reducing their cost and making available to the market cultivars better adapted to the Egyptian climate. To succeed, the Egyptian potato seed certification program must emphasise *in vitro* and greenhouse propagations and limit field propagation to a minimum.

CHAPTER 12. CONTRIBUTIONS TO ORIGINAL KNOWLEDGE

The following are considered to be contributions to original knowledge from the work described in this thesis:

1. An improved protocol was developed for the rapid and extensive tuberization of *in vitro* plantlets in an Murashige and Skoog (1962) based microtuberization medium containing a high sucrose level without the need for chemical inducing agents. In addition, the number of microtubers produced from layered potato plantlets was significantly increased when the volume of microtuberization medium was augmented.

2. This is the first comprehensive report on microtuber dormancy. a) the relative dormancy period of microtubers was found to be cultivar-specific, b) microtubers ≤250 mg were found to have longer dormancy periods than microtubers >250 mg, c) a significant correlation was established between *in vivo* and *in vitro* dormancy periods and d) the involvement of abscisic acid in the dormancy of microtubers was demonstrated.

3. This is the first demonstration of the advantage of increased microtuber size on several important microtuber characteristics, including specific gravity, number of eyes and on sprout and stem development and storage life.

4. This is the first report on the effect of the physiological age of microtubers on sprouting and subsequent yield. Physiological age did not modify the expression of apical dominance in microtubers in contrast to its effect on conventionally produced seed tubers. Physiological age of microtubers did not affect mTD plant development and minituber number and only minimally affected minituber weight.

5. This is the first investigation of the influence of in-row planting density and planting depth on the yield of *ex vitro* plantlets grown in Québec. Tuber number and weight per hectare increased while tuber number and weight per plantlet decreased with increasing plant density. An economic assessment showed that optimal planting density varied according to plantlet cost.

6. This is the first comprehensive discussion of the different aspects of potato seed tuber certification in Egypt.

CHAPTER 13. SUGGESTIONS FOR FUTURE RESEARCH

1. The potential of Jasmonic acid as a promoter of microtuberization should be evaluated and compared to the growth regulator-free microtuberization medium described here.

2. Further work should be done to optimize the volume of microtuberization medium in relation to container size and type. Economic considerations should be employed to determine the optimal volume and container type.

3. Longer pre-conditioning treatments should be studied as a means to increase microtuber number and weight.

4. The application of this microtuberization system to the study of potato tuberization should be further investigated since both the perception of environmental stimulation and the reaction to this stimulation can be studied simultaneously *in vitro*.

4. Research should be conducted in collaboration with engineers to develop a fermentor-type microtuberization vessel in an attempt to further increase microtuber size and reduce their production cost.

5. Additional studies should be conducted in an attempt to evaluate the involvement of other compounds such as regulatory

proteins or other plant growth regulators in the dormancy control of tubers.

6. The possibility of sowing microtubers directly into the field following pre-germination and peat-encapsulation treatments should be investigated.

7. Research on the optimum physiological age of potato tubers grown under the Egyptian climate should be undertaken.

CHAPTER 14. REFERENCES

Abbott, A.J. and A.R. Belcher. 1986. Potato tuber formation *in vitro*. In: Plant Tissue Culture and its Agricultural Applications. L.A. Whithers and P.G. Alderson (eds). Butterworth, London. pp. 113-122.

Addy, N.D. 1988. Opportunities and challenges for private industry. Am. Potato J. 65:221-227.

Akita, M. and S. Takayama. 1988. Mass propagation of potato tubers using jar fermentor. Acta Hort. 230:55-61.

Aksenova, N.P., T.N. Konstantinova and M.K. Chailakhyan. 1989. Morphogenetic effect of blue and red light in the illumination of underground and above-ground potato organs in *in vitro* cultivation. Dolk. Acad. Nauk. SSSR. 305:508-512.

Allen, J.R. and W.K. Knutson. 1982. The effect of cold storage on the growth and development of tissue culture propagated potato plantlets. Am. Potato J. 59:459.

Allen, E.J and R.K. Scott. 1980. An analysis of growth of the potato crop. J. Agric. Sci., Camb. 94:583-606.

Allen, E.J., P.J. O'Brien and D. Firman. 1992. Seed tuber production and management. In: The Potato Crop. P.M. Harris (ed). Chapman & Hall. London. pp. 247-291.

Appiano and Pennazio. 1972. Electron microscopy of potato meristem tips infected with potato virus X. J. Gen. Virol. 14:273-276.

Appleman, C.O. 1916. Biochemical and physiological study of the rest period in the tubers of *Solanum tuberosum*. Bot. Gaz. 61:265-294.

Arthur, J.M., J.D. Guthrie and J.M. Nuwell. 1930. Some effects of artificial climates on the growth and chemical composition of plants. Am. J. Bot. 17:416-482.

Austin, S. and A.C. Cassels. 1983. Variation between plants regenerated from individual calli produce from seperated potato stem callus cells. Plant Sci. Lett. 31:107-114.

Bailey, K.M., I.D.J. Phillips and D. Pitt. 1978. The role of buds and gibberellin in dormancy and the mobilization of reserve materials in potato tubers. Ann. Bot. 42:649-657.

Baker, W.G. 1953. A method for the in vitro culturing of potato tubers. Science 118:384.

Blanc, A. 1981. Action de brefs éclairement de lumière rouge sur la tubérization de tiges de pomme de terre (*Solanum tuberosum* L.) cultivées *in vitro*. Role éventuel du phytochrome sur le mécanisme de la tubérization. C.R. Hebd. Scéances Acad. Sci. 292:137-140.

Blanc, A., J.C. Mery and J. Boisard. 1986. Action des radiations de lumière rouge sur la survie et la tubérisation des germes de pommes de terre cultivés *in vitro*: influence de leur age physiologique. Potato Res. 29:381-389.

Bodlaender, K.B.A. 1960. [The influence of temperature on the development of potatoes]. Mededelingen, Institute voor Biologish en scheikundig Onderzoek van Landbouwegwassen,

112:69-83.

Bodlaender, K.B.A. 1963. Influence of temperature, radiation and photoperiod on development and yield. In: Growth of the potato. J.D. Ivins and F.L. Milthorpe (ed). Butterworths, London. pp. 199-210.

Bogucki S. and D.C. Nelson. 1980. Length of dormancy and sprouting characteristics of ten potato cultivars. Am. Potato J. 57:151-157.

Bottini de, G.A. R. Bottini and R. Tizio. 1982. Physiology of dormancy in potato tubers as related to levels of endogenous regulators. Phyton Rev. Int. Bot. Exp. 42:115-121.

Bottini de, G.A., M. Golenioski and R. Tizio. 1981. Effects of (2-chloroethyl) trimethylamonium chloride upon gibbberellin level in potato plants (*Solanum tuberosum*) and influence of these phytohormones on tuberization in vitro. Z. Pflanzenphysiol. 103:149-155.

Bourque, J.E. 1983. Acclimatization and reestablishment of tissue cultured potato plantlets. Ph.D. Thesis, Colorado State U. Dissertation Abstract 44-08:2297.

Bourque, J.E., J.C. Miller and W.D. Park. 1987. Use of *in vitro* tuberization system to study tuber protein gene expression. *In vitro* Cell. Dev. Biol. 23:381-386.

Brenner, M.L. 1981. Modern methods for plant growth substance analysis. Ann. Rev. Plant Physiol. 32:511-538.

Bryan, J.E. 1988. Implementation of rapid multiplication and tissue culture methods in third-world countries. Am.

Potato J. 65:199-207.

Budin, K.Z. and N.S. Ogluzdin. 1992. Storage of virus-free seed tubers. In: Soviet Agricultural Sciences. Allerton Press Inc., New-York. pp.46-47

Burton, W.G. 1957. The dormancy and sprouting of the potato tuber. Food Sci. Abstr. 29:1-12

Burton, W.G. 1963. Concepts and mechanism of dormancy. In: The Growth of the Potato, J.D. Ivins and F.L. Milthorpe (eds). Butterworth, London. pp. 17-41.

Burton, W.G. 1968. The effect of oxygen concentration upon sprout growth on the potato tuber. Eur. Potato J. 11:249-265.

Burton, W.G. 1972. The response of the potato plant and tuber to temperature. In Crop Processes in Controlled Environment. Proceedings of an International symposium, Glasshouse Crops Research Institute, Littlehampton. A.R. Ress, K.E. Cockshull, D.W. Hand and R.G. Hurd (eds). Academic Press, London. pp.217- 233

Cassels, A.C. and R.D. Long. 1980. The regeneration of virus-free plants from cucumber mosaic virus- and potato virus Y-infected tobacco explants cultured in the presence of virazol. Z. Naturforch. 35:350-351.

Catchpole, A.H. and J. Hillman. 1969. Effect of ethylene on tuber initiation in *Solanum tuberosum* L. Nature 223:1387

Chandra, R. J.H. Dodds and P. Tovar. 1988. *In vitro* tuberization in potato (*Solanum tuberosum* L.).

Newsletter, Intl. Assoc. Plant Tissue Culture. 55:10-20

Chapman, H.W. 1955. Potato tissue cultures. Am. Potato J. 32:207-210.

Chase, C.A., V. Souza Machado, R. Coffin and R.A. Fletcher. 1989. Effect of Triadimefon on in vitro produced potato plantlets. Am. Potato J. 66:509-510.

Cho, J. L., W.M. Iritani and M.W. Martin. 1983. Comparison of methods for measuring dormancy of potatoes. Am. Potato J. 60:169-177.

Claver, F.K. 1967. Influence of long day and temperature on tuberization of shoots and plantlets of potato in vitro. Revist de Investigationes Agropecuarias, Inta, Buenos Aires, Rep. Argentina Series 2, Biologia y Produccion Vegetal, IV. 12:223-230.

Claver, F.K. 1973. In fluence of temperature during the formation of tubers in relation with their incubation state (physiological age) and seed value. Experentia 30:97-98.

Claver, F.K. 1977. The tuberization of shoots of potato and callus culture in vitro. de la revista de la facultad de Agronomia. (3a. epoca), T.XXXII: 11-122.

Clegg, M.D. and L. Rappaport. 1970. Regulation of bud rest in tubers of potato, *Solanum tuberosum* L. VI. Biochemical changes induced in excised potato buds by gibberellic acid. Plant Physiol. 45:8-13.

Cochran, W.G. 1952. The X^2 test of goodness of fit. Ann. Math. Statist. 23:315-345.

Coleman, S. 1992a. The effect of gelling agents on the production of microtubers. New-Brunswick Department of Agriculture. Fredericton, New Brunswick. Internal Document. pp. 2.

Coleman, S. 1992b. The effect of culture vessel on microtuber production in two microtuber induction media. New-Brunswick Department of Agriculture. Fredericton, New Brunswick Internal Document. pp. 3.

Coleman, W.K. 1987. Dormancy release in the potato tubers: A review. Am. Potato J. 64:57:68.

Coleman, W.K. and S.E. Coleman. 1986. The effects of bromoethane and ethanol on potato (*Solanum tuberosum*) tuber sprouting and subsequent yield responses. Am. Potato J. 63:373-377.

Coleman, W.K. and R.R. King. 1984. Changes in endogenous abscisic acid, soluble sugars and proline levels during tuber dormancy in *Solanum tuberosum* L. Am. Potato J. 61:437-449.

Cottle, W. and P.E. Kolattukudy. 1982. Abscisic acid stimulation of suberization. Plant Physiol. 70:775-780.

Cutter, E.G. 1978. Structure and development of the potato plant. In: The Potato Crop. The Scientific Basis for Improvement. P.M. Harris (ed). Chapman & Hall, London. pp. 70-152.

Daniel, W.W. 1990. Applied Nonparametric Statistics. PWS-KENT publishing Company, Boston. pp. 395-400.

Dasher, J. and V. Souza Machado. 1988. In vitro microtuberization in relation to nodal position of potato explant.

Davies, H.V. 1984. Mother tuber reserves as factors limiting sprout growth. Potato Res. 27:209-218.

Davies, H.V. and H.A. Ross. 1984. The pattern of starch and protein degradation in tubers. Potato Res. 27:373-381.

Denny, F.E. 1926a. Hastening the sprouting of dormant potato tubers. Am. J. Bot. 13:118-125.

Denny, F.E. 1926b. Effect of thiourea upon bud inhibition and apical dominance of potato. Bot. Gaz. 81:297-311.

Denny, F.E. 1945. Synergetic effects of three chemicals in the treatment of dormant potato tubers to hasten germination. Contrib. Boyce Thompson Inst. 14:1-14.

Department of Agriculture. 1991. Seeds regulations, amendment. Canada Gazette Part II. 125:3099-3120.

de Stecco, V.L. and R. Tizio. 1982. L'Action du CCC sur la tuberization de germe de pomme de terre cultivé *in vitro* sur une solution minerale exempte de sucre. C.R. Scéances Aca. Sci., Série D. 294:901-904.

Dhingra, O.K. and J.B. Sinclair. 1985. Solutions to maintain a constant humidity in a closed atmosphere. *In*: Basic Plant Pathology Methods. CRC Press Inc., Boca Raton Fla.

Dimalla, G.G. and J. van Staden. 1977. Apical dominance and the utilization of carbohydrates during storage of potato tubers. Ann. Bot. 41:387-391.

Dodds, J.H. 1988. Tissue culture technology: Practical application of sophisticated methods. Am. Potato J. 65:167-180.

Dodds, J.H. and K. Watanabe. 1990. Biotechnological tools for plant genetic resources management. Diversity. 6:26-28.

Durr, G. 1980. Production and utilization of potatoes in Rwanda. Working paper, Social Science Department, CIP, Lima. Peru.

Edelman, J., T.C. Jefford and S.P. Singh. 1969. Studies on the biochemical basis of physiological processes in the potato tuber. Planta 84:48-56.

El-Antably, H.M.M., P.F. Wareing and J. Hillman. 1967. Some physiological responses to d-l abscisin (dormin). Planta. 73:74-90.

Espinosa, N.O., R. Estrada, D. Sylva-Rodrigez, P. Tovar, R. Lizaraga and J.H. Dodds. 1986. The potato: a model crop plant for tissue culture. Outl. Agric. 15:21-26.

Estrada, R., P. Tovar and J.H. Dodds. 1986. Induction of in vitro tubers in a broad range of potato genotypes. Plant Cell, Tiss. Org. Cult. 7:3-10.

Ewing, E.E. 1981. Heat stress and the tuberization stimulus. Am. Potato J. 58:31-49.

Ewing, E.E. 1985. Cuttings as simplified models of the potato plant. In: Potato Physiology. P.H. Li (ed). Academic Press Inc., Orlando, Forida. pp. 153-207.

Ewing, E.E. 1990. Induction of tuberization in potato. In: The

molecular and cellular biology of the potato. M.E. Vayda and W.D. Park (eds). CAB International, Wallingford, U.K. pp. 25-41.

Ewing, E.E. and A.H. Senesac. 1990. *In vitro* tuberization on leafless stem cutting. 11th Triennnial Conference of the European Association for Potato Research. pp. 7-8.

Ewing, E.E. and P.C. Struik. 1992. Tuber formation in potato: induction, initiation and growth. Hort. Review pp.89-198.

Ewing, E.E. and P.F. Wareing. 1978. Shoot, stolon and tuber formation on potato (*Solanum tuberosum* L.) cuttings in response to photoperiod. Plant Physiol. 61:348-353.

Fischnich, O. and H. Krug. 1963. Environmental factors influencing sprout growth and subsequent plant development in the field. In: The Growth of the Potato. J.D. Ivins and F.L. Milthorpe (eds). Butterworth, London. pp. 73-96.

Food and Agriculture Organization of the United Nations. 1990. 1989 FAO Production Yearbook. Vol. 43. FAO, Rome.

Forsline, P.L. and A.R. Langille. 1975. Endogenous Cytokinins in *Solanum tuberosum* as influenced by photoperiod and temperature. Physiol. Plant. 34:75-77.

Forsline, P.L. and A.R. Langille. 1976. An assessment of the modifiying effect of kinetin on *in vitro* tuberization of induced and non-induced tissues of *Solanum tuberosum*. Can. J. Bot. 54:2513-2516.

Garner, W.W. and H.A. Allard. 1923. Further studies in

photoperiodism, the response of the plant to the relative length of day and night. J. Agric. Res. 23:871-919.

Garcia-Torrez, L. and C. Gomez-Campo. 1973. *In vitro* tuberization of potato sprouts as affected by ethrel and gibberrellic acid. Potato Res. 16:73-79.

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

Goodwin, P.B. 1966. The effect of water on dormancy in the potato. Eur. Potato J. 9:53-63.

Goodwin, P.B. and G. Brown. 1980. Field performance of potato shoot-tips proliferated in culture. Potato Res. 23:449-452.

Goodwin, P.B., Y.C. Kim and T. Adisarwanto. 1980. Propagation of potato by shoot-tip culture. 1. Shoot multiplication. Potato Res. 23:19-24.

Gregory, L.E. 1956. Some factors for tuberization in the potato. Ann. Bot. 43:281-288.

Gregory, L.E. 1965. Physiology of tuberization in plants. (Tubers and tuberous roots). Encyclopedia Plant Physiol. Vol XV. W. Ruhland (ed). Springer-Verlag, Berlin. pp.1328-1354.

Guanasena, H.P.M. and P.M. Harris. 1969. The effect of CCC and nitrogen on the growth and yield of two varieties of potato. J. Agric. Sci. 76:33-52.

Guthrie, J.D. 1940. Role of glutathione in breaking of the rest period of buds by ethylene chlorhydrin. Contrib. Boyce

Thompson Inst. 11:261-270.

Harmey, M.A., M.P. Crowley and P.E.M. Clinch. 1966. The effect of growth regulators on tuberization of cultured stem pieces of *Solanum tuberosum*. Eur. Potato J. 9:146-151.

Harvey, B.M.R., S.H. Crothers, N.E. Evans and C. Selby. 1991. The use of growth retardants to improve microtuber formation by potato (*Solanum tuberosum*). Plant Cell, Tiss. Org. Cult. 27:59-64.

Hawkes, J.G. 1978. Biosystematics of the potato. In The Potato Crop. P.M. Harris (ed). Chapman & Hall, London. pp.14-48.

Hay, R.K.M. and J. Hampson. 1991. Sprout and stem development from potato tubers of differing physiological age: the role of apical dominance. Field Crop Res. 27:1-16.

Heiser, C.B. 1969. Nightshade; the Paradoxical Plants. W.H. Freeman, San Fransisco.

Hemberg, T. 1965. The significance of inhibitors and other chemical factors of plant origin in the induction and breaking of rest periods. In: Encyclopedia of Plant Physiology. W. Ruhland (ed). Springer-Verlag: Berlin-Heidelberg-New York. 15:669-698.

Hemberg, T. 1970. The action of some cytokininson the rest-period and the content of acid growth-inhibiting substances in potato. Physiol. Plant. 23:850-858.

Hemberg, 1985. Potato rest. In: Potato Physiology. P.H. Li (ed). Academic Press Inc., Orlando, Forida. pp. 153-207.

Hide, G.A. and D.H. Lapwood. 1992. Disease aspects of potato production. In: The Potato Crop, P. Harris (ed). Chapman and Hall, London. pp.403-437.

Hollings, M. and O.M. Stone. 1964. Investigation of carnation viruses. 1. Carnation mottle. Ann. Appl. Biol. 53:103-118.

Huang, P., P.R. Bohac and J.C. Miller. 1988. Differential response of in vitro cultured potato cultivars in a temperature gradient system. HortSci. 23:754.

Hussey, G. and N.J. Stacey. 1981. In vitro propagation of potato *Solanum tuberosum*. Ann. Bot. 48: 787-796

Hussey, G. and N.J. Stacey. 1984. Factors affecting the formation of in vitro tubers of potato (*Solanum tuberosum*). Ann. Bot. 53:565-578.

Iritani, W.M. 1968. Factors affecting physiological ageing (degeneration) of potato tubers used as seed. Am. Potato J. 45:111-116.

Ishida, B.K, G.W. Snyder and W.R. Belknap. 1989. The use of *in vitro*-grown microtuber disks in *Agrobacterium*-mediated transformation of *Russet Burbank* and *Lemhi Russet* potatoes. Plant Cell Reports. 8:325-328.

Johansen, D.G., Knutson, K.W., S.R. Slack and E.D. Jones. 1984. Recommendations for standards and guidelines for acceptance of tissue cultured potato seed stocks into certification programs. Am. Potato J. 61:368-370

Jones, E.D. 1988. A current assesment of in vitro culture and other rapid multiplication methods in North America and

Europe. Am. Potato J. 65:209-220.

Joung, H., J.S. Koo, J.H. Jeon and J.R. Liu. 1991. Mass production of potato microtubers by noble tissue culture system. Am. Potato J. 68:619.

Joyce P.J. and B.H. McCown. 1987. A system for producing uniform microtubers. Am. Potato J. 64:445.

Kassanis, B. 1967. Plant tissue culture. In: Methods in Virology. K. Maramorosch and H. Koproski (eds). Academic Press, New-York. pp. 537-566.

Kawakami, K. 1963. Age of potato seed tubers affects growth and yield. Am. Potato J. 40:25-29.

Kidd, F. 1919. Laboratory experiments on the sprouting of potatoes in various gas mixtures (nitrogen and carbon dioxide). New Phytol. 18:248-252.

Kleinschmidt, G.D., G.E. Kleinkopf, D.T. Westermann and J.C. Zalewski. 1984. Specific gravity of potatoes. University of Idaho, Cooperative Extension Service Agricultural Experiment Station. Moscow, U.S.A. Current Information Series No.609.

Knutson, K.W. 1988. Implications of new technologies for seed potato certification programs and seed growers. Am. Potato J. 65: 229-235.

Koda, Y. and Y. Okasawa. 1983a. Influences of environmental, hormonal and nutritional factors on potato tuberization *in vitro*. Jap. J. Crop Sci. 52:582-591.

Koda, Y. and Y. Okasawa. 1983b. Characteristic changes in the

levels of endogenous plant hormones in relation to the onset of potato tuberization. Jap. J. Crop Sci. 52:592-597.

Koda, Y. and Y. Okasawa. 1988. Detection of potato tuber-inducing activity in potato leaves and old tubers. Plant Cell Physiol. 29:969-974.

Koda, Y., K. Takahashi and Y. Kikuta. 1992. Potato tuber-inducing activities of salicylic acid and related compounds. J. Plant Growth Reg. 11:215-219.

Korableva, N.P., K.A. Karavaeva and I.V. Metlitskii. 1980. Changes of abscisic acid content in potato tuber tissues in the period of deep dormancy and during germination. Fiziol. Rast. 27:585-591.

Krauss, A. 1981. Abscisic and gibberellic acid in growing potato tubers. Potato Res. 24:435-439.

Kwiatkowski, S., M.W. Martin, C.R. Brown and C.J. Sluis. 1988. Serial microtuber formation as a long term conservation method for in vitro potato germplasm. Am. Potato J. 65:369-375.

Kostrica, P., B. Polreichova and J. Domkarova. 1985. The use of *in vitro* tuber formation for the maintenance of potato genetic resources. Genetica Slechteni. 21:269-278.

Lakhoua, L. and O. Ellouze. 1990. Induction de la microtubérization chez *Solanum tuberosum* variété Spunta. Cinquantenaire de la culture *In vitro*. Versaille (France), 24-25 oct. 989. Ed. INRA, Paris (Les colloques de l'INRA, 12, 51):279-281.

Lawrence, C.H. and W.G. Barker. 1963. A study of tuberization in the potato, *Solanum tuberosum*. Am. Potato J. 40:349-356.

Leclerc, Y. 1989. Acclimatization, field performance and microtuberization of tissue cultured potato *Solanum tuberosum*. M.Sc. Thesis, McGill University, Canada.

Leclerc, Y. and D.J. donnelly. 1990. Seasonnal differences in the field performance of micropropagated potato under a short growing season in Québec. Am. Potato J. 67:507-516.

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

Lentini, Z., R.L. Plaisted and E.D. Earle. 1983. Use of *in vitro* tuberization as a screening system for potato earliness. Am. Potato J. 65:488.

Leshem, B. and F.A.L. Clowes. 1972. Rates of mitosis in shoot apices of potatoes at the beginning and end of dormancy. Ann. Bot. 36:687-691.

Levy, D. 1985. Propagation of potato by direct transfer of in vitro proliferated shoot cuttings into the field. Scientia Hortic. 26:105-109.

Levy, D. 1986. Potato propagation through the use of in vitro proliferated cuttings transferred directly into the field. Am. Potato J. 63:439.

Levy, D. 1988. Propagation of potato by the transfer of transplants of in vitro proliferated shoot cuttings into the

field. Scientia Hortic. 36:165-171.

Limaset, F. and P. Cornuet. 1949. Recherche du virus de la mosaic du tabac dans les meristems de plantes infecte. C.R. Hebd. Scé. Acad. Sci. 228:1971-1972.

Lizarraga, R., Z. Human and J.H. Dodds. 1989. *In vitro* conservation of potato germplasm at the International Potato center. Am. Potato J. 66:253-269.

Lo, F.M., B.R. Irvine and W.G. Barker. 1972. *In vitro* tuberization of the common potato (*Solanum tuberosum*) is not a response to the osmotic concentration of the medium. Can. J. Bot. 50:603-605.

Madec, P. and P. Pérennec. 1956. Influence de l'origine sur le comportement des plants de pommes de terre. Annls. Amélior. Pl. 6:5-26.

Madec, P. and P. Pérennec. 1962. Les relations entre l'induction de la tubérisation et la croissance chez la plante de pomme de terre (*Solanum tuberosum* L.). Annls. Physiol. Végét. 4:5-84.

Madec, P. and P. Pérennec. 1956. Influence de l'origine sur le comportement des plants de pomme de terre. Annls. Amélior. Pl. 6:5-26.

Madec, P. and P. Pérennec. 1962. Les relations entre l'induction de la tubérisation et la croissance chez la plante de pomme de terre (*Solanum tuberosum* L.). Annls. Physiol. Végét. 4:5-84.

Mangat, B.S., G. Kerson and D. Wallace. 1984. The effect of 2-

4-D on the tuberization and starch content of potato tubers produced on stem segments cultured *in vitro*. Am. Potato J. 61: 355-361.

Marco, S. 1984. The possibility of growing seed potato in the area of Mount Katarina in Sinai mountains. Potato Res. 27:431-434.

Marinus, J. 1985. in vitro multiplication of potatoes; descripition of methods and experience in the Netherlands. Centre for Agrobiological Research, Wageningen, Neetherlands. pp. 3-4.

Marinus, J. 1990. *In vitro* tuberization of four potato cultivars under various daylengths and light intensities. 11th triennial Conference of the European association for Potato Research (EARP). EARP Abstracts. pp.7-8.

Marinus, J. and K.B.A. Bodlaender. 1975. Responses of potato varieties to temperature. Potato Res. 18:189-204.

Marquardt, D.W. 1963. An algorithm for least-squares estimates of nonlinear parameters. J. Soc. Ind. Appl. Math. 11:431-441.

Mattive, R. 1986. The grower approach to on-farm tissue culture labs- The success and problems. Proceedings of the 4th Annual Conference of Potato Seed Certification. p.44-46.

Mauk, S.C. and A.R. Langille. 1978. Physiology of tuberization in *Solanum tuberosum* cis-Zeatin riboside in the potato plants; its identification and changes in indogenous levels as influenced by temperature and photoperiod. Plant Physiol.

62:438-442.

McDonald, J.G. 1987. Comparative levels of potato viruses S and Y infection of microplants and tuber-propagated plants in the field. Am. Potato J. 64:517-521.

McMorran, J. 1986. Microtubers: Potato in test tubes. Fourth Annual North American Seed Potato Seminar Proceedings, volume 3.

Melching, J.B., S.A. Slack and E.D. Jones. 1993. Field performance of peat-lite mix encapsulated small minitubers. Am. Potato J. 70:285-307.

Mellor, F.C. and R. Stace-Smith. 1977. Virus free potatoes by tissue culture. In Plant Cell, Tissue and Organ Culture. J. Reinert and Y.P.S. Bajaj (eds). Springer-Verlag, Berlin. pp.616-635.

Menzel, C.M. 1980. The control of tuberization in potato at high temperature: response to gibberellin and growth inhibitors. Ann. Bot. 46:259-265.

Menzel, C.M. 1982. The control of tuberization in potato (*Solanum tuberosum*) at high temperatures. Ph.D. Thesis, University of Queensland.

Mes, M.G. and I. Menge. 1954. Potato shoot and tuber cultures *in vitro*. Physiol. Plant. 7:637-649.

Meuleman, M., J. Dumont, C.S. Anceau and G. Fouarge. 1986. *In vitro* tuberization of potato. Med. Fac. Landbouww. Rijksuniv. Gent. 51/2a:527-531.

Meyling, H.D.G. and K.B.A. Bodlaender. 1981. Varietal

differences in growth, development and tuber production of potatoes. Neth. J. Agric. Sci. 29:113-127.

Mingo-Castel, A.M., F.B. Negm and O.E. Smith. 1974. Effect of carbon dioxide and ethylene on tuberization of isolated potato stolons cultured in vitro. Plant Physiol. 53:798-801.

Mingo-Castel, A.M., O.E. Smith and J. Kumamoto. 1976. Studies on the carbon dioxide promotion and and ethylene inhibition of tuberization in potato explants cultured in vitro. Plant Physiol. 57:480-485.

Ministère de l'Agriculture des Pêcheries et de l'Alimentation du Quèbec. 1991. Le comité de références économiques en Agriculture du Québec. AGDEX 258/821, AGDEX 257/821.

Mitten, D.H., C. Boyes and J. Cucuzza. *In vitro*-produced microtubers of potato. Am. Potato J. 65:492.

Moorby, J., X. Conghua and S. Khuri. 1990. The production and growth of potato tubers and microtubers. Abstract of the 11th Triennal Conference of the European Association for Potato Research. pp. 327-328.

Morel, G. and C. Martin. 1952. Guérison de *Dahlias* atteints d'une maladie à virus. C.R. Hebd. Scé. Acad. Sci. 235:1324-1325.

Morris, D.A. 1966. Intersprout competion in the potato I. Effects of tuber size, sprout number and temperature on sprout growth during storage. Eur. Potato J. 9:69-85.

Morris, D.A. 1967. The influence of light, gibberellic acid and

CCC on sprout growth and mobilization of tuber reserves in the potato (*Solanum tuberosum*). Planta 77:224-232.

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant. 15:473-497.

Nganga, S. and M.J. Potts. 1987. Development of potato in Africa and Asia. Acta Hort. 213:67-90.

Nooden, L.D. and J.A. Weber. 1978. Environmental and hormonal control of dormancy in terminal buds of plants. In: Dormancy and Developmental Arrest. M.E. Clutter (ed). Academic Press, New York. pp.221-268.

Nowak, J. 1977. Biochemical chages in stored potato tubers with different rest periods. II. Influence of the storage temperature and isopropyl phenylcarbamates on enzyme activities. Z. Planzenphysiol. 81:125-133.

Nowak, J. and S.K. Asiedu. 1992. Gelling agent and light effects on *in vitro* tuberization of potato cultivars. Am. Potato J. 69:461-470.

Nowak, J. and D. Colborne. 1989. *In vitro* tuberization and tuber proteins as indicators of heat stress tolerance in potato. Am. Potato J. 66:35-43.

Obatta-Sasamoto, H. and H. Suzuki. 1979. Activities of enzymes relating to starch synthesis and endogenous levels of growth regulators during tuberization of isolated potato stolons cultured *in vitro*. Z. Planzenphysiol. 95:69-75.

Okasawa, Y. 1967. Physiological studies on tuberization of

potato plants. J. Fac. Agric., Hokkaido Univ. 55:267-336.

Okasawa, Y., N. Katsura and T. Tagawa. 1967. Effects of auxin and kinetin on the development and differentiation of potato tissue cultured in vitro. Physiol. Plant. 20:826-829.

Ortis-Montiel, G. and H. Lozoya-Saldaña. 1987. Potato minitubers: technology validation in Mexico. Am. Potato J. 64:535-544.

Pal, B.P. and P. Nath. 1938. Shortening the rest period of the potato (*Solanum tuberosum*). Ind. J. Agric. Sci. 8:777-786.

Palmer, C.E. and W.G. Barker. 1973. Influence of ethylene and kinetin on tuberization and enzyme activity in *Solanum tuberosum* L. stolons cultured *in vitro*. Ann. Bot. 37:85-93.

Palmer, C.E. and O.E. Smith. 1969a. Effect of abscisic acid on elongation and kinetin-induced tuberization of isolated stolons of *Solanum tuberosum* L. Nature 221:279.

Palmer, C.E. and O.E. Smith. 1969b. Cytokinin and tuber initiation in potato *Solanum tuberosum*. Nature 221:279-280.

Palmer, C.E. and O.E. Smith. 1970. Effect of cytokinin on tuber formation on isolated stolons of *Solanum tuberosum* cultured *in vitro*. Plant Cell Physiol. 11:303-314.

Palmer, C.E. and W.G. Barker. 1972. Changes in enzyme activity during elongation and tuberization of stolons of *Solanum tuberosum* L. cultured *in vitro*. Plant Cell Physiol. 13:681-688.

Parrot, F. 1975. Interactions des sels minéraux et de l'acide gibbérellique et du CCC sur la tuberization de fragment de tige de pomme de terre cultivé *in vitro*. Potato Res. 18:446-450.

Paupardin, C. and R. Tizio. 1969a. Action des composé phénoliques sur la tubérization de germe de pcmme de terre cultivé *in vitro*. C.R. Scé. Aca. Sci., Série D. 269:1077-1080.

Paupardin, C. and R. Tizio. 1969b. Présence de composé phénoliques dans des germes de pomme de terre cultivé *in vitro*; comparaison avec les composé phénoliques de plantes intactes. C.R. Scé. Aca. Sci., Série D. 269:1668-1670.

Paupardin, C. and R. Tizio, 1970. Action de quelques composes phenolique sur la tuberization de la pomme de terre. Potato Res. 13:187-198.

Pearl, A., D. Aviv, L. Willmitzer and E. Galun. 1991. *In vitro* tuberization in transgenic potatoes harboring ß-glucoronidase linked to a patatin promoter: effects of sucrose levels and photoperiods. Plant Sci. 73:87-85.

Pelacho, A.M. and A.M. Mingo-Castel. 1991. Effects of photoperiod on kinetin-induced tuberization of isolated potato stolons cultured *in vitro*. Am. Potato J. 68:533:541.

Pelacho, A.M. and A.M. Mingo-Castel. 1991. Jasmonic acid induces tuberization of potato stolons cultured *in vitro*. Plant Physiol. 97:1253-1255.

Pennazio, S. and P. Redolfi. 1974. Potato virus X eradication in cultured potato meristem tips. Potato Res. 17:333-335.

Pérennec, P. and J. Francois. 1981. Influence de la photopériode sur les modalités de croissance et de tubérization de boutures de pommme de terre cultivées *in vitro*. 8th Triennnial Conference of the European Association for Potato research. pp. 3-4.

Pérennec, P. and P. Madec. 1980. Age physiologique du plant de pomme de terre. Incidence sur la germination et répercussions sur le comportement des plantes. Potato Res. 23:183-199.

Pérennec, P. and P. Madec. 1960. Influence du tubercule sur la croissance et le développement du germe de pomme de terre. Annls. Physiol. Végét. 2:29-67.

Peterson, R.L. and W.G. Barker. Early tuber development from explanted stolon nodes of *Solanum tuberosum* var. Kennebec. Bot. Gaz. 140:398-407.

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

Radatz, W., K.H.C. Standke. 1980. Investigations on "minimal growth" of potato in vitro. Plant Growth Regul. Abstr. 6:851.

Raman, K.V. and E.B. Radcliffe. 1992. Insect Pests. In: The Potato Crop, P. Harris (ed). Chapman and Hall, London. pp.403-437.

Rappaport, L. and N. Wolf. 1969. The problem of dormancy in potato tubers and related structures. In: Dormancy and survival. H.W. Woodhouse (ed). Symp. Soc. Exptl. Biol. Cambridge University Press. 23:219-240.

Ratkitin, J.V. and N.N. Suvorov. 1935. The effect of temporary anaerobiosis on the sprouting of young potato tubers. Dokl. Akad. Nauk. SSSR 9:295-297.

Renalli, P., E. Forti, G. Mandolino and B. Casarini. 1990. Improving production and health of seed potatoes stocks in Italy. Potato Res. 33:377-387.

Roca, W.M., N.O. Espinosa, M.R. Roca and J.E. Bryan. 1978. A tissue culture method for rapid propagation of potatoes. Am. Potato J. 55:691-701.

Roest, S. and G.S. Bokelman. 1976. Vegetative propagation of *Solanum tuberosum* L. *in vitro*. Potato Res. 19:173-179.

Ross, H.A. and H.V. Davies. 1985. A microscopic examination of starch deppletion in tubers of cv Maris Piper during sprouting. Potato Res. 28:113-118.

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

Salaman, R. N. 1949. The history and social influence of the potato. Cambridge University Press. Cambridge, U.K.

Samotus, B. 1971. Storage of potato tubers under water. Preliminary investigations. Potato Res. 14:145-149.

Samotus, B. and S. Schiwimmmer. 1963. Changes in carbohydrate

and phosphorus content of potato tubers during storage in nitrogen. J. Food Sci. 28:163-167.

Sattelmacher, B. and H. Marschner. 1985. A simple *in vitro* method to study tuber growth of *Solanum tuberosum*. J. Plant Physicl. 121:23-27.

Scherrer, B. 1984. Biostatistique. Gaetan Morin Editeur, Chicoutimi, Canada

Schilde, R.L., D.N. Espinosa and R. Estrada. 1984. Induction of tubers in vitro and their utilization for storage and distribution of potato germplasm. In: Trienal Conference of the European Association for Potato Research. Switzerland.

Scholte, K. 1989. Effect of daylenght and temperature during storage in light on growth vigour of seed potatoes. Potato Res. 32:214-215 (Abstr.)

Seabrook, J.E.A. 1987. Changing the growth and morphology of potato plantlets *in vitro* by varying the illumination source. Acta Hortic. 212:401-410.

Seabrook, J.E.A., S. Coleman and D. Levy. 1993. Effect of photoperiod on *in vitro* tuberization of potato (*Solanum tuberosum* L.). Plant Cell, Tiss. Org. Cult. In press.

Simmonds, N.W. 1964. The genetic of seed and tuber dormancy in the cultivated potatoes. J. Heridity 19:489-504.

Sipos, J., J. Nowak and G. Hicks. 1988. Effect of daminozide on survival, growth and yield of micropropagated potatoes. Am. Potato J. 65:353-364.

Sladky, Z. and L. Bartosova. 1990. *In vitro* induction of

axillary potato microtubers and improvement of their quality. Biologia Plantarum (Praha). 32:181-188.

Slimmon, T. V. Sousa Machado and R. Coffin. 1989. The effect of light on *in vitro* microtuberization of potato cultivars. Am. Potato J. 66:843-848.

Slimmon, T. V. Sousa Machado, R. Coffin and L. Peterson. 1987. Thidiazuron for *in vitro* tuberization of potatoes. Am Potato J. 67:458.

Smith, J.D., F. Fong, C.W. Magill, B.G. Cobb and D.G. Bai. 1989. Hormones, genetic mutants and seed development. *In*: Recent Advances in the Development and Germination of Seeds. R.B. Taylerson (ed). Plenum Press, New York. U.S.A. pp. 57-69.

Smith, O.E. and C.E. Palmer. 1970. Cytokinin-induced tuber formation on stolons of *Solanum tuberosum* L. Physiol. Plant. 23:599-606.

Smith, O.E. and L. Rappaport. 1969. Gibberellins inhibitors and tuber formation in the potato (*Solanum tuberosum*). Am. Potato J. 46:185-191.

Smith, O.P. and R.H. Storch. 1984. Prediction of potato leafroll virus disease in Maine from thermal unit accumulation and an estimate of primary inoculum. Plant Dis. 68:863-865.

Stace-Smith, R. and F.C. Mellor. 1968. Eradication of potato viruses X and S by thermotherapy and axillary bud culture. Phytopathology. 58:199-203.

Stace-Smith, R. 1986. Virus-free clones through plant tissue culture. In: Comprehensive Biotechnology. Murray Moo-Young (ed). Pergamon Press. Oxford, U.K. pp.169-179.

Stallknetch, F.G. 1972. Coumarin-induced tuber formation on excised shoots of *Solanum tuberosum* L. cultured *in vitro*. Plant Physiol. 50:412-413.

Stallknecht, F.G. and S. Farnsworth. 1979. The effect of nitrogen on the coumarin-induced tuberization of potato axillary shoot cultured in vitro. Am. Potato J. 59:523-530

Stallknecht, F.G. and S. Farnsworth. 1982a. General characteristics of coumarin-induced tuberization of axillary shoots of *Solanum tuberosum* cultured in vitro. Am. Potato J. 59:17-32.

Stallknecht, F.G. and S. Farnsworth. 1982b. The effect of the inhibitors of protein and nucleic acid synthesis on the coumarin-induced tuberization and growth of excised axillary shoots of potato sprouts (*Solanum tuberosum* L.) cultured *in vitro*. Am. Potato J. 59:69-75.

Stallknecht, F.G. 1983. Application of plant growth regulators to potatoes: production and research. In: Plant growth regulating chemicals. L.G. Nickell ed. CRC Press Inc. Boca Raton, Florida. pp. 161-176.

Stallknecht, G.F. 1985. Tuber initiation in Solanum tuberosum: effect of phytohormones and induced changes in nucleic acid and protein metabolism. *In*: Potato Physiology, P.H. Li (ed). Academic Press, New-York. pp. 231-260.

Starkel, R. 1986. On-farm tissue culture. Proceedings of the 4th Annual Conference of Potato Seed Certification. pp. 40-43.

Steel, R.G.P. and J.H. Torrie. 1980. Principles and Procedures of Statistics a Biometrical Approach. McGraw-Hill Company, New-York.

Struik, P.C. and G. Van Voorst. 1986. Effects of drought on the initiation, yield and size distribution of tubers of *Solanum tuberosum* L. cv. Bintje. Potato Res. 29:487-500.

Sylvestre, P. and J. Laganiere. 1981. Etat du développement technique dans la production et la conservation des plants souches de pommes de terre. Phytoprotection. 62:96-100.

Talukder, N.M and C. Paupardin. 1981. Action de la solannine sur la croissance et la tubérisation de fragments de germes de pomme de terre cultivé *in vitro*. C.R. Scé. Acad. Sci. 293:549-552.

Tennier, M. 1981. Technique de multiplication de plants souches de pomme de terre integrees dans un programme a croissance continue. Phytoprotection. 62:101-108.

Thieme, R. 1992. An *in vitro* potato cultivars collection: microtuberization and storage of microtubers. FAO/IBPGR Plant Genetic resources Newsletter. pp. 17-19.

Thieme, R. and B. Pett. 1982. Production of tubers *in vitro* and their use in the establishment of potato store. Arch. Zuechtungsforch. 12:257-262.

Thompson, P.G., F.L. Haynes and R.H. Moll. 1980. Estimation of

genetic variance components and heritability for tuber dormancy in diploid potatoes. Am. Potato J. 57:39-46.

Thornton, N.C. 1939. Carbone dioxide storage XIII. Relationship of oxygen to carbon dioxide in breaking dormancy of potato tubers. Contrib. Boyce Thompson Institute. 10:201-204.

Thorton, M.K. and R. Nundorfer. 1986. Field performance of minitubers as affected by size and greenhouse harvest date. Am. Potato J. 63:458.

Thornton, M.K. and K.W. Knutson. 1986. Effect of transplant container volume and growing season length on field performance of micropropagated potatoes. Am. Potato J. 63:399-410.

Tizio, R. 1969. L'action du CCC (2-Chloroethyltrimethylammonium chloride) sur la tubérization de la pomme de terre. Eur. Potato J. 12:3-7.

Tizio, R. 1982. Fisiologia de la dormicion en tuberculos de papa y sus relaciones con el mecanismo hormonal de la tuberization. Rev. Cienc. Agropecuaries 3:91-105.

Tizio, R. and M.M. Blain. 1973. Are cytokinins the specific factors for tuber formation in the potato plants?. Phyton 31:3-13.

Tizio, R. and M. Goleniowski. 1985. New evidence with gibberellin nature of the "root factor" wich delays tuberization on potato sprout section cultured *in vitro*. C.R. Hebd. Acad. Sci., Serie III. 13:499-502.

Tizio, R. and E. Maneschi. 1973. Different mechanisms for tuber initiation and dormancy in the potato. Phyton 31:51-62.

Tizio, R. and C. Paupardin. 1972. Action de composé phénoliques et de plusieures gibberellines sur la tubérisation de germes de pomme de terre cultivé *in vitro*. Colloques Internationaux du Centre National de la Recherche Scientifique. 193:209-213.

Thurnbull, C.G.N. and D.E. Hanke. 1985a. The control of bud dormancy in potato tubers. Evidence for the primary role of cytokinins and seasonal pattern of changing sensitivity in cytokinin. Planta 165:359-365.

Thurnbull, C.G.N. and D.E. Hanke. 1985b. The control of bud dormancy in potato tubers. Measurement of the seasonal pattern of changing concentrations of zeatin-cytokinins. Planta 165:366-376.

Tovar, P., L. Schilde-Rentschler and J.H. Dodds. 1985. Induction and use of *in vitro* potato tubers. CIP Circular International Potato Center, Lima, Peru. 13:1-5.

Toosey, R.D. 1964. The presprouting of seed potatoes. Factors affecting sprout growth and subsequent yield. Part I and II. Field Crop Abstr. 17:161-168, 239-244.

Van der Zaag, D.E. 1986. Potential demand for seed potatoes in world perspective. 4_{th} Annual North American Seed Potato Seminar Proceedings. Denver, CO. 47-50.

Van der Zaag, D.E. and D. Horton. 1983. Potato production and utilization in world perspective with special reference to

the tropics and sub-tropics. Potato Res. 26:323-326.

Van der Zaag, D.E. and C.D. van Loon. 1987. Effect of physiological age on growth vigour of seed potatoes of two cultivars. Review of litterature and integration of some experimental results. Potato Res. 30:451-472.

Van der Zaag, P. and V. Escobar. 1990. Rapid multiplication of potatoes in the warm tropics: rooting and establishment of cuttings. Potato Res. 33:13-21.

Van Ittersum, M.K. and K. Scholte. 1992. Relation between growth conditions and dormancy of potatoes. Am. Potato J. 63:603-613.

Van Minh, T., N. Van Uyen and P. Vander Zaag. 1990. Rapid multiplication of potatoes: influence of environment and management on growth of juvenile apical cuttings. Am. Potato J. 67:789-797.

Van Uyen, N. and Vander Zaag. 1983. Vietnamese farmers use tissue culture for commercial potato production. Am. Potato J. 60: 873-879.

Verhoyen, M. and D. Givron. 1981. Un schéma de multiplication rapide des pommes de terre sans virus par la production de tubercules *in vitro*. Med. Fac. Landbouww. Rijksuniv. Gent. 46/3:1031-1042.

Wang, P.J. 1977. Regeneration of virus-free potato from tissue culture. In: Plant Tissue Culture and it's Biotechnological Application. W. Barz, E. Reinhard and M.H. Zenk (eds). Proc. Int. Congr. Med. Plant Res. pp.386-391.

Wang, P.J. and C.Y. Hu. 1980. Regeneration of virus-free plants through *in vitro* culture. Adv. Biochem. Eng. 18:61-99.

Wang, P.J. and C.Y. Hu. 1982. *In vitro* mass tuberization and virus-free potato production in Taiwan. Am. Potato J. 59:33-39.

Wang, P.J. and C.Y. Hu. 1985. Potato tissue culture and its application. In Potato Physiology, P.H. Li (ed). Academic Press. Orlando. pp. 503-577

Wattimena, G.A. 1983. Micropropagation as an alternative technology for potato production in Indonesia. Ph.D. Thesis. University of Wisconsin-Madison Microfilms International, Ann Arbour, Michigan. 202 pp

Wattimena, G., B. McCown and G. Weis. 1983. Comparative field performance of potatoes from micro-culture. Am. Potato J. 60:27-33.

Werner, H.O. 1935. The effect of temperature, photoperiod and nitrogen level upon the tuberization in potato. Am. Potato J. 12:274-280.

Westcott, R.J. 1981. Tissue culture storage of potato germplasm. 2. Use of growth retardants. Potato Res. 30:117-120.

Westcott, R.J., B.W.W. Grout and G.G. Henshaw. 1979. Rapid clonal propagation of *Solanum curtilobum* by asceptic shoot meristem culture. In: Biology and Taxonomy of the Solanaceae, J. G. Hawkes, R.N. Lester and A.D. Skelding (eds). Academic Press, London. pp. 377-382.

Weston, L.A. and B.H. Zandstra. 1986. Effect of root container size and location of production on growth and yield of tomato transplants. J. Amer. Soc. Hort. Sci. 111:489-501.

White, G.D. M.J. Potts and G.L.T. Hunt. 1988. A computer aid to potato seed production, storage and distribution in developing countries. Am. Potato J. 65: 551-560.

White, P. R. 1943. A Handbook of Plant Tissue Culture. J. Cattell Press, Lancaster.

Wiersema, S.G. 1986. A method of producing seed tubers from true potato seeds. Potato Res. 29:225-237.

Wiersema, S.G., R. Cabello, P. Tovar and J.H. Dodds. 1987. Rapid seed multiplication by planting into beds micro tubers and *in vitro* plants. Potato. Res. 30:117-120.

White, G.D. M.J. Potts and G.L.T. Hunt. 1988. A computer aid to potato seed production, storage and distribution in developing countries. Am. Potato J. 65:551-560.

Whithmore, J.C., R. Clarke and L. Ewing. 1989. Guidelines for Russet Burbank nuclear seed potato production in Idaho. University of Idaho, College of Agriculture, Moscow Idaho.

Woolfe, J.A. 1986. The Potato in the Human Diet. Cambridge University Press. Cambridge.

Wright, N.S. 1988. Assembly, quality control and use of a potato cultivar collection redered virus-free by heat therapy and tissue culture. Am. Potato J. 65:181-197.

Wurr, D.C.E. 1978. Seed tuber production and management. In: The potato crop, P.M. Harris (ed). Chapman and Hall,

London. pp. 327-352.