ACCLIMATIZATION, FIELD PERFORMANCE AND MICROTUBERIZATION
OF TISSUE CULTURED POTATO (Solanum tuberosum L.)
cv. Russet Burbank.

by

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A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfilment of the requirements for the degree of Master of Science

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ACCLIMATIZATION, FIELD PERFORMANCE AND MICROTUBERIZATION OF TISSUE CULTURE POTATO (*Solanum tuberosum*) cv. Russet Burbank.

M.Sc. Plant Science Tissue Culture

ABSTRACT

The persistence and contribution of in vitro-formed roots to the establishment of tissue cultured *Solanum tuberosum* 'Russet Burbank' plantlets were examined. In vitro-formed roots persisted and developed ex vitro. They significantly enhanced the development of transplants during the acclimatization period. However, rooted and root-severed transplants were not significantly different in yield and plant growth characteristics after 100 d in the field. The field performance of ex vitro transplants and seed tuber-derived plants were compared for two consecutive years under a growing period of 106 d. The total tuber weight of ex vitro transplants failed to equal that of seed tuber-derived plants. Interestingly, tuber number per plant was significantly higher and mean tuber weight lower for ex vitro transplants resulting in large differences in the frequency and yield distribution of tubers produced by the two types of propagules. Ex vitro transplants were apparently more susceptible to early water stress but less affected by high seasonal temperatures than seed tuber-derived plants. Significant differences were found in total microtuber biomass and size but not in number when the International Potato Center (CIP) and the single node methods of microtuber induction were compared. These differences did not translate to significant differences in plant growth or yield characteristics after 100 d in the field.
L'ACCLIMATATION, LE RENDEMENT ET LA MICROTUBERIZATION DE LA POMME DE TERRE (*Solanum tuberosum*) cv. Russet Burbank PRODUIT IN VITRO.

M.Sc. Plant Science Culture de tissus

Résumé

La persistance ainsi que la contribution des racines de pomme de terre (*Solanum tuberosum*) ’Russet Burbank’ formées in vitro furent étudiées. Les résultats ont démontrés que les racines produisent in vitro persistent et contribuent de façon significative à l'accroissement du développement des plantules lors de l'acclimatation. Cependant, le rendement au champs ainsi que les caractéristiques des plantes provenant de plantules avec ou sans racines étaient semblables après 100 j de croissance. Le rendement au champs des plantules provenant de la culture in vitro ainsi que celui des plants issus de tubercules furent étudié durant deux saisons. Sous une courte période de croissance de 106 j, le poids total de tubercule produit par plante fût inférieur pour les plantes issues de la multiplication in vitro. Il est cependant intéressant de noter que le nombre total de tubercule par plante produisent fût supérieur et le poids moyen des tubercules inférieur pour les plantes issues de la multiplication in vitro. Les fréquences de distribution et de poids des tubercules étaient significativement différentes pour les deux modes de propagation. Les plants issus de la multiplication in vitro furent apparament plus susceptible à la sécheresse tôt dans la saison que les plantes provenant de tubercules. La comparaison des méthodes du noeud simple (single node) et du Centre International de la pomme de terre (CIP) pour la microtubérisation a fait ressortir des différences dans le poids total et le poids moyen des microtubercules produisent mais pas dans le nombre. Ces différences n'ont pas résultées dans des différences de rendements au champs après 100 j de croissance.
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CHAPTER I

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 belladona) and the "weed" tobacco (Nicotiana tabacum) (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 a 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, 1987).

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 Scottish were
dependent on potatoes as their principal source of food from the 17th to the 19th century, up until the devastating potato blight (*Phytophthora infestans*) of 1846-47 (Woolfe, 1986; Hawkes, 1978).

On a per hectare basis, potatoes yield more carbohydrates than any other food crop, and are second to soya (*Glycine max*) in protein production. The potato proteins are good complements to cereal proteins, being rich in lysine and low in sulfur-containing amino acids.

Since potatoes are vegetatively propagated, pathogens may be 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 the quality of seeds.

It is possible to obtain plant material devoid of pathogens through shoot tip culture, which may be supplemented by thermotherapy and/or chemotherapy. The specific pathogen tested (SPT) plantlets obtained through 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 virus-free plant material. Once shoot tips have grown into well developed plantlets they are transferred into pots under controlled environments. Cuttings from these mother plants are placed under tuber-inducing conditions and tubers are harvested after 9 weeks. Fifty thousand tubers are produced annually and field planted in
the Manicouagan region to obtain Pre-Elite tubers. After 2 more years of field propagation Elite II seed tubers are harvested and sold to private seed producers where they are multiplied for 2 more years before release to the certified potato growers. The entire process of releasing a new cultivar may take up to 7 years, from the reception in La Pocatiere to the release. The use of micropropagated material could substantially reduce the length of time needed to assure supply to the producers or to release a new cultivar.

The ultimate objective of this study was to evaluate micropropagated plantlets and microtubers (in vitro produced tubers) as alternatives to the conventional mode of production for the potato seed tuber industry in Quebec. In preliminary experiments the contribution of in vitro-formed roots to the acclimatization of micropropagated plantlets was determined. The field performance of ex vitro plantlets and microtubers were evaluated under a short growing season and the effects of seasonal differences on field performance were noted. Finally, two methods of microtuber induction were assessed.
CHAPTER II

LITERATURE REVIEW

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.

A) Callus culture

In the early days of tissue culture it was thought that adventitious plantlet regeneration from callus culture could be useful for the rapid propagation of plant material. However, this idea was abandoned due to the genetic heterogeneity of the regenerated plants (Earle and Demarly, 1982 and Bhojwani and Razdan, 1977). Nevertheless, in vitro regeneration of plantlets from somatic cells can be a useful tool in mutation breeding of potato crops (Wang and Hu, 1985). In vitro callus culture of potato was first reported by Steward and Caplin (1955). However, they along with Chapman (1955), Anstis and Northcote (1973) and Pollock and Rees (1975) were not able to regenerate adventitious organs from their potato callus cultures. Although sporadic regeneration on large size explants had been reported (Svoboda, 1964; Okasawa et al., 1967 and Yamagushi and Nakajima, 1974) the first protocol for potato plantlet regeneration was published by Wang and Huang (1975). Later, Roest and Bokelman (1976) demonstrated the possibility of producing adventitious shoots on callus of petiole and leaf blade explants. Methods for
regenerating shoots and roots from callus culture vary among species, cultivars and donor tissue. These were reviewed extensively by Wang and Hu (1985).

B) Anther culture

One of the major obstacles to the use of true potato seeds (TPS) is the heterogeneity of the progeny. The cultivated potato is a tetraploid. Reduction of the ploidy level to diploid and then to haploid could open avenues for the production of pure homozygous lines through chromosome doubling (Wang and Hu, 1985). The conventional way of reducing the ploidy level of potato is through female parthenogenesis, as described by Van Breukelen et al. (1975). According to Wang and Hu (1985), anther culture could advantageously replace conventional methods because more monoploids are obtained from the male than the female gametophyte. Anther culture could potentially apply to all potato species. Potato plantlets regenerated from anther culture are homozygous diploid and tetraploid since chromosome doubling takes place spontaneously during culture, avoiding the necessity for colchicine treatment. Wenzel and Urig (1981) obtained potato clones through anther culture that expressed high levels of resistance to nematodes and viruses. This demonstrates the potential use of anther culture in resistance breeding.

Unfortunately, potato responds poorly to anther culture as compared to most Solanaceae (Wang and Hu, 1985). Major genotypic differences in the ability of anthers to respond to culture were demonstrated by Mix and Sixin (1983) and Jacobson and Sopory (1978). Sopory (1977) noted that clones of dihaploid plants that were not especially bred and selected to respond to in vitro
conditions showed poor growth when placed in culture. The developmental stage of the pollen grain at the time of in vitro culturing was important for expression of androgenesis (Sutherland, 1974). The uninucleate stage was most responsive to anther culture in potato (Dunwell and Sutherland, 1973; Sopory et al., 1978). The general aspects of anther culture were reviewed by Bajaj (1983).

C) Protoplast culture

The first potato protoplasts were isolated from tuber tissue by Lorenzini (1973). Complete regeneration of plantlets from protoplasts was first achieved by Shepard (1975). Since then regenerants from potato protoplasts include: tetraploid commercial cultivars, dihybrid clones, monoploid lines and other potato species (Wang and Hu, 1985). Intergeneric somatic hybrids between potato and tomato were constructed by Shepard et al. (1983). Interspecific somatic hybrids of cultivated potato and S. chacoensis (Butenko and Kusko, 1979; cited from Wang and Hu, 1985) and between dihaploid S. tuberosum and S. nigrum (Binding et al., 1983) have been achieved. Protoplast culture offers interesting avenues in mutation breeding, genetic manipulation and somatic hybridization.

D) 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.

In the multimeristem technique developed at the International Potato Center (C.I.P.) by Roca et al., (1978),
excised shoot tips (0.5-0.6 mm) were placed in Murashige and Skoog (MS) (1962) medium containing GA\(_3\) (0.4 mg/l), BAP (0.5 mg/l) and NAA (0.01 mg/l) and incubated on a rotary shaker at 90 rpm. Within 4 weeks approximately 14 shoots were produced per explant. 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 propagated 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 year. In this procedure the newly developed axillary shoots were positioned horizontally on the agar surface until a mass of axillary shoots were 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 cultures to significantly increase the availability of planting material, generating a 250 fold increase in 4 months. 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 excised, surface sterilized and transferred onto MS medium supplemented with GA\(_3\) (0.1 mg/l). The regenerated plantlets were then used as planting material.

Nodal segments have also been used as means for rapid multiplication. The explants are taken from in vitro-derived
shoots, from greenhouse-grown plants or from tuber sprouts (Wang and Hu, 1985). The culture medium can be either growth regulator-free MS medium (Hussey and Stacey, 1981) or MS medium containing GA$_3$ (0.5 mg/l) (Westcott and al., 1979).

E) Meristem tip culture

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 host plant to determine the infectivity of each section, he noticed 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 virus particles were detected in the apical meristems. From these observations, Morel and Martin (1952) postulated that it may be possible to recover virus-free 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 virus-free Dahlia from heavily infected plants. Later Morel and Martin (1955) reported virus eradication in potato. However, later studies showed that the majority of viruses were penetrating meristematic cells. Kassanis (1967) first detected virus particles in apical meristems using electron microscopy (EM). Potato virus X (PVX) particles were detected by Appiano
and Pennazio (1972) in apical domes of potato cells using thin section EM. The presence of PVX in potato meristems was detected by mechanical innoculation of meristem tips on indicator plants (Pennazio and Redolfi, 1974). There is then conclusive evidence that excised tips contain virus particles at the time they are placed on the culture medium. Since 48 species have been freed of viruses using meristem tip culture (Wang and HU, 1980), it is clear that virus elimination takes place in vitro. The exact mechanism of virus eradication 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.

Although meristem tip culture has proven to be successful in eradicating some viruses, it does not permit the inactivation of all viruses so it is generally coupled with thermodiyapy and/ or chemotherapy. Thermotherapy consists of placing a virus-infected plant at temperatures near the maximum tolerable for a few weeks. For potato, recommended temperatures and duration of treatment varies from 32 °C to 37 °C for periods of 1.5 to 13 weeks (Mellor and Stace-Smith, 1977). At temperatures above 30
OC 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 especially likely to be uninfected or have low virus concentrations. Meristem tips are generally taken from these sections and cultured in vitro (Stace-Smith, 1986).

In chemotherapy chemical substances are utilized to suppress symptoms or to reduce the virus concentration in plants. Although some reports have claimed virus eradication using this method, these results have been 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) in the culture media. It must be noted though that Virazole is considered to be a mutagen.

POTATO CERTIFICATION

A) General guidelines

The increased utilization of tissue culture techniques in the development 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 stock. Level I involves the development of disease-free plantlets and level II entails their rapid multiplication. In level I, the "stock" plants and/or tubers from which explants are initiated 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 stock is evaluated annually under field conditions to assess the phenotype and yielding ability of the clonal lines. Before distribution into certified seed programs representative samples of plantlets produced during level II are screened to assure pathogen-freedom. Ideally, the plantlets and microtubers are used as entry level stocks moving into a flush-through system where initial material is always being replaced by new SPT material.

B) Potato seed certification in Quebec.

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

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 the diseases is detected the material is immediately destroyed. The rest of the material is vegetatively reproduced by cuttings and submitted to thermotherapy (Sylvestre and Laganier, 1981). After 6-8 weeks of heat treatment at 37 °C, 20 meristem tip explants (no larger than 0.5 mm) per plant are placed on MS medium containing growth regulators. Regeneration is achieved within 6-8 weeks. The plantlets are then micropropagated by nodal cuttings. One plantlet originating from each meristem tip excised from a single mother plant is acclimatized in a controlled environment and assayed for the presence of most potato viruses using Enzyme Linked Immunosorbent Assay (E.L.I.S.A.) and electrophoresis for PSTV. From the 20 meristems 2 plantlets that assayed negative to all tests are selected to form the basal seed 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 medium containing mannitol) or acclimatized and vegetatively propagated through cuttings under 18 hr d and 8 Klux illumination at temperatures above 23 °C. PVX and PVS are indexed at this stage.
using both a host plant and E.L.I.S.A.. These cuttings are then introduced into a controlled environment for the production of minitubers. This involves 1 wk under 18 hr d at 21 °C followed by 8 wk under 10 hr d at 15 °C, both under 16 Klux fluorescent light intensity. A total of 50,000 minitubers from all cultivars can be produced there annually. During this production step, the absence of PVX, PVS and bacterial wilt are verified. The minitubers are planted onto 1 ha of land in the Manicouagan region. This region was selected because the climatic and edaphic conditions reduce to a minimum the insect population that could vector viral diseases to potato plants. The following year, the Elite I tubers are planted on 3.3 ha of land. In addition to PVX and PVS indexing a sample of the Elite II tubers are sent to Florida where they are field planted and visually observed to assure the absence of any viruses. The third year, Elite II tubers are sown onto 12 ha of land. The Elite III tubers that are harvested are sold to the certified growers who produce foundation and certified seed potato (Tennier, 1981).

A total of 75,000 verifications are made by provincial inspectors during the 2 yr certification period in the certified growers' fields. This is 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 Pocatiere the field is immediately destroyed.
FIELD PERFORMANCE OF MICROPROPAGATED POTATO

Field performance of potato from tissue culture was evaluated by Goodwin and Brown (1980) and Wattimena et al. (1983) with contrasting results. Goodwin and Brown (1980) compared the field performance of greenhouse-rooted ex vitro shoots with foundation stock tubers. Stem number per plant was similar for ex vitro transplants and seed tubers of 'Pontiac' but significantly lower for transplants of 'Kennebec'. Tuber number per plant was not significantly different for ex vitro transplants and tubers. Tuber yield of 'Kennebec' plants grown from ex vitro transplants acclimatized for 8 wk prior to field planting was similar to that of plants from conventionally grown seed tubers. As for 'Pontiac', no plants from culture approached those from seed tubers for tuber yield, but plants yielded more from ex vitro transplants acclimatized for 8 wk than 6 wk prior to field planting. Wattimena et al. (1983) compared the field performance of plants grown from in vitro produced tubers (microtubers) and in vitro produced shoots rooted in the greenhouse (microcuttings) to plants grown from seed tubers of 'Norland' and 'Red Pontiac'. Stem number per plant was significantly less and tuber number per plant was significantly higher for plants grown from microtubers and microcuttings compared to plants grown from seed tubers of both cultivars. Although total tuber weight per plant was significantly higher at peak flowering time for seed tuber-produced plants, there were no significant differences in tuber weight in either cultivar at the end of the growing season between plants produced from...
tubers and microtuber- or microcutting-produced plants. However, for both cultivars ex vitro plants produced fewer big tubers (US1A) and more small tubers (US1B) than plants grown from seed tubers. To explain the increase in tuber number per plant for ex vitro microcuttings, Wattimena et al. (1983) suggested that these plants were physiologically different; tuber initiation occurred over a longer period and resorption was less than with tuber-produced plants. Wattimena et al. (1983) observed that single-stem micropropagated plants branched vigorously. After 1 mo no differences in vine growth were evident between ex vitro and seed tuber-derived plants. Levy (1985) found that all ex vitro plants developed one vigorous sturdy stem, some of which branched vigorously at the soil creating "rosette type" multi-stem plants. To explain the differences in stem number per plant between their results and Goodwin and Brown's (1980) they argued that Goodwin and Brown counted the branches as stems, thus finding no differences in stem number per plant between ex vitro transplants and tuber-grown plants. Levy (1986) compared the yield of in vitro-proliferated cuttings transferred directly into the field to those that were acclimatized prior to field planting. The survival rate of cuttings directly transferred into the field ranged from 17 to 75 % while the survival rate of acclimatized cuttings was 98 %. Protecting the plantlet from drought and wind immediately upon planting considerably increased plant establishment in the field (Levy, 1985). The length of the growing period, the distance between plants and the climatological conditions were all found to affect the multiplication rate and the number of tubers greater than 10 mm
in diameter. Levy (1988) compared the field performance of ex vitro plantlets to plants originating from single or double node cuttings of in vitro plantlets rooted in peat. He found that this method could increase the material available for planting thus increasing the overall multiplication rate. The author also reported an increase in the establishment of these transplants in the field, as well as a greater uniformity in growth compared to ex vitro plantlets.

Bourque (1983) studied the acclimatization and reestablishment of tissue cultured 'Russet Burbank' and 'Norland' and found that 5 d under a poly 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. Carbon dioxide enrichment in vitro was not found to be beneficial and did not increased growth once the plantlets were removed from culture. A temperature of 2 °C was found to be adequate for storage of plantlets for rapid multiplication and low temperature was found to enhance subsequent growth. A light intensity of 2 Klux (25.2 μmolm⁻²s⁻¹) and a 16 d were found to promote high survival rates in storage.

Thornton and Knutson (1986) studied the effect of container volume and length of growing season on tuber production and yield of ex vitro 'Centennial Russet' and 'Russet Burbank' plantlets. They found that increasing the container volume increased total tuber yield and yield of tubers larger than 35 mm in diameter. Leslie et al. (1986) found a similar correlation between container volume and early yield of tomato. The advantage of
using larger cells is probably due to the increased root
development and reduced root binding (Knavel, 1965). Optimizing
the transplant container volume to reduce the greenhouse space
requirement while maximizing yield of ex vitro potato plantlets
becomes an important issue. Lengthening the growing season
significantly increased the total tuber yield and yield of larger
 tubers (Thornton and Knutson, 1986). Since the main purpose of
growing micropropagated plants has been to provide disease free
seed tubers (Johansen et al., 1984), one should take into
consideration the length of the growing season and the heat unit
accumulation. These parameters were also found to be important
in the recontamination of virus free stocks (Smith and Storch,
1984). Therefore, determination of the optimum growing season
length has to take into account both yield and disease-spread
factors. These become even more important in light of McDonald’s
(1987) results. He compared the reinfection levels of PVS and
PVY in daughter tubers of conventionally propagated potato
plants and ex vitro plantlets of four cultivars. The use of
micropropagated plantlets to produce seed tuber stock appeared to
increase the risk of infection with both viruses although the
results for tuber infection with PVY was significant only 1 year
out of 2.

IN VITRO TUBERIZATION

Potato tuberization is induced in situ by a number of
environmental factors; short days, high light intensity, low
night temperature and low nitrogen level. Foliar application of
abscisic acid (ABA) and the antigibberellin chloroethyltrime-
thylamonium chloride (CCC) can also stimulate tuberization in vitro (Wang and Hu, 1985). The conditions inducing tuberization in vitro are often different than those in situ, indicating that physiological reactions of in vitro plantlets differ considerably from those of in situ plants. Since the first report of in vitro tuberization by Barker (1953) a number of factors have been identified as affecting tuberization in vitro. Often a factor that was found beneficial in one experiment was found to have no effect or was detrimental in another trial. These differences can be explained by variations in the cultivar, culture medium, growth regulator, type of explant tissue and the incubation environment (Wang and Hu, 1985).

The optimum temperature for in vitro tuberization appears to be quite variable. Thieme and Pett (1982; cited by Wang and Hu, 1985) induced their microtubers at 8 to 10 °C, while Okasawa et al. (1967) found these temperatures to be inhibitory. Wattimena (1983) determined that 15 °C was the optimum tuber-inducing temperature but Wang and Hu (1982) found it to be 20 °C and Hussey and Stacey (1981) determined that it was between 20 and 25 °C. The light requirements are also variable and depend on the other culture conditions. Lawrence and Baker (1963) were able to induce tuberization only in complete darkness. 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. For the different experiments, light intensities ranged from 0.8 Klux (10.8 µmolm⁻²s⁻¹) (Hussey and Stacey, 1981) to 0.1 Klux (12.6 µmolm⁻²s⁻¹) (Wang and Hu, 1982).
According to Wang and Hu (1985) different photoperiod optima are due to the presence or absence of cytokinins in the medium. When cytokinins were not used longer photoperiod with higher light intensity was required. Wang and Hu (1982) found that 8% sucrose was optimal for in vitro tuberization, while Wattimena (1983) found no differences between 4, 6 and 8% sucrose. These differences might be due to increased photosynthesis in Wattimena’s experiment since the light intensity was greater. In general, increasing the sucrose concentration from 1 to 8% increased the percentage and earliness of tuberization (Wang and Hu, 1985). However, at higher sucrose concentrations tuberization was inhibited (Palmer and Smith, 1970 and Lawrence and Barker, 1963). Cytokinin is a promoter of in vitro tuberization; Wang and Hu (1982) observed best results with benzylaminopurine (BAP) at 10 mg/l for in vitro plantlets. Mauk and Lingille (1978) found that the optimum concentration was 3 mg/l BAP for in vitro tuberization of stolon apices. Wattimena (1983) found that kinetin (KIN) was required only when tuberization occurred in the dark. Neither auxins nor gibberellins (GA_3) were necessary for in vitro tuberization and both were inhibitory when used in high concentrations i.e. > 1 mg/l for auxin and > 2.5 mg/l for GA_3 (Stallnecht and Farnsworth, 1982). In vitro tuberization may be stimulated by some growth inhibitors. Coumarin (25 mg/l) was more effective than KIN for in vitro tuberization (Stallnecht and Farnsworth, 1979, 1982), 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 while, KIN-induced tuberization was not affected by these compounds (Stallnecht and Farnsworth, 1982; Palmer and Smith, 1962). Cycocel (CCC), triiodobenzoic acid (TIBA) and maleic hydrazide (MH) were found to induce in vitro tuberization (Tizio and Blain, 1973 and Parrot, 1975), but CCC was inhibitory to KIN-induced tuberization (Palmer and Smith, 1969). Coumarin-induced tuberization was not affected by CCC, TIBA and succinic acid-2, 2-dimethyl hydrazine (ALAR) (Stallnecht and Farnsworth, 1982). Mingo-Castel et al. (1974, 1976) found that CO₂ was stimulatory to in vitro tuberization of stolons as its absence was found to be detrimental to microtuberization. Although some reports have shown ethylene to have stimulatory effects on in vitro tuberization (Stallnecht and Farnsworth, 1982 and Garcia-Torres and Gomez-Campo, 1973, cited by Wang and Hu, 1985) others have demonstrated its inefficiency (Palmer and Barker, 1973) or its inhibitory effects on microtuberization (Mingo-Castel et al., 1974, 1976). High nitrogen concentration is in general inhibitory and low concentration stimulatory to coumarin-induced microtuberization. Cytokinin-induced tuberization is not affected by high nitrogen concentration (Wang and Hu, 1982).

To date, the focus has been on the development of protocol to induce in vitro tuberization. It now becomes important to evaluate these different methods in terms of productivity; the number, weight and size of the microtubers produced are particularly important for field production.
IN VITRO PRODUCED LEAVES

Plantlets cultivated in vitro are subjected to environmental conditions that differ from natural field conditions. The epigenetic adaptations of tissue cultured plantlets prohibit direct transfer to greenhouse or field conditions. A period of acclimatization, in which the environmental factors are gradually modified to resemble natural conditions, is necessary to assure good survival of ex vitro plantlets. Plantlets in vitro are totally dependant on the exogenous carbon source in the medium (Grout and Aston, 1977a, b). Chlorophyll content, carbon dioxide fixation and light-induced oxygen evolution (Hill reaction) of plantlets are lower than seedlings of a comparable age (Grout and Aston, 1977a, b). Donnelly and Vidaver (1984b) found that CO₂ uptake by leaves of tissue cultured red raspberry was relatively low for a wide range of light intensities (2 to 6 Klux; 25.5-75.6 µmolm⁻²s⁻¹) compared to field control plants. Pigment content was higher in plantlets incubated at lower (2 to 4 KLUX; 25.5-50.4 µmolm⁻²s⁻¹) compared to higher light intensities. By tagging leaves produced in vitro, they found that after 1 mo of acclimatization the persistent leaves made up 30% of the transplant leaf area but contributed less than 10% of the CO₂ uptake. These leaves were shown to be net respirers at higher light intensities (6 or 9 KLUX; 75.6 or 113.4 µmolm⁻²s⁻¹). Although persistent leaves showed an increase in dry matter accumulation after 1 mo acclimatization, this was at the expense of more actively photosynthesizing newly formed leaves. Photosynthetic inability may be partly explained by anatomical abnormalities of tissue cultured plantlets. The chloroplasts of
sweetgum plantlets were flattened and lacked internal membrane development, while palisade parenchyma were poorly differentiated (Wetzstein and Sommer, 1982). Relatively low light intensity, CO₂ limitation and the presence of exogenous carbon probably limit photosynthesis in vitro. Grout and Millan (1985) demonstrated that in vitro-developed leaves of strawberry were incapable of developing significant photosynthetic ability during ex vitro acclimatization. Chlorophyll content of persistent leaves decreased sharply 21 d after transfer to acclimatization conditions. Lee et al. (1985) examined the effect of quantum flux on photosynthesis and chloroplast ultrastructure of sweetgum (Liquidambar styraciflua). They found that culturing plantlets under higher light intensity increased leaf thickness and density. Chlorophyll content was decreased by high light intensity but at lower light intensity chloroplast membranes were not differentiated into grana stacks and internal membranes were irregularly arranged. However, net photosynthesis was significantly greater at medium (200 μmolm⁻²s⁻¹) than at high or low light intensity (315 and 50 μmolm⁻²s⁻¹). Net photosynthesis, chloroplast development, chlorophyll content and starch granules were greater for greenhouse-grown seedlings than tissue cultured plantlets at the same light intensity, showing that photosynthetic apparatus in vitro is affected by factors in addition to light intensity.

Leaves produced in vitro show a number of anatomical changes compared to greenhouse- and field-grown plants. Poor epicuticular wax formation (Grout and Aston, 1977a; Sutter and
Langhans, 1979; Fushigami et al., 1981), reduced stomatal control (Sutter and Langhans, 1979 and Brainerd and Fushimi, 1981), smaller and thinner leaves caused by less compact arrangement of palisade and mesophyll cells and altered palisade cell shape (Donnelly and Vidaver, 1984a) are among the abnormalities generally encountered. These changes increase water stress and are responsible for high mortality rates of ex vitro plantlets (Earle et al., 1975; Boxus et al., 1977 and Broome et al., 1978) and poor survival of in vitro-produced leaves during acclimatization. Donnelly and Vidaver (1984b) tagged in vitro-produced leaves of red raspberry (Rubus icaea) and found that more than half of these died within 1 mo of transferring plantlets to soil. Grout and Aston (1977b) also observed this pattern of leaf die-off with cauliflower.

IN VITRO-FORMED ROOTS

The necessity of rooting shoots in vitro over rooting in soil is still a controversial issue. There are a number of objections to rooting shoots in vitro:

1) The in vitro rooting process contributed at least 35% of the total cost of in vitro-produced plants (Deberg and Maene, 1980), 56% of the total cost of Begonia and Ficus (Donnan et al., 1978 and 75% of the total cost in the case of lily production (Anderson and Meager, 1978).

2) According to Debergh and Maene (1980), there is a delay in growth when in vitro-rooted shoots are transferred to soil, due to the difficulty in inducing a functional root system with root hairs in vitro. These authors claimed that the in vitro-produced
roots died and new roots started to develop after about 2 wk ex vitro.

3) Rooting done under a constant hormone regime is not considered to be optimal as exogenous auxins are generally required for root initiation but inhibit root elongation.

4) Roots are often damaged during the transfer to soil, increasing the chance of root or stem diseases.

Grout and Aston (1977a) studied the anatomy of the shoot-root interface and water transfer from root to shoot of adventitious cauliflower plantlets. They found no evidence of direct transfer of water from roots to shoots. Their anatomical study showed that the zone of transition between roots and shoots was the site of anatomical abnormalities. In regenerated plants, the xylem tissue formed a closed system across the base of shoots prior to root formation. However, in their production system the basal part of regenerating shoots formed callus at the site of root formation. It is then not surprising that poor vascular connections were achieved. No abnormalities were found in the xylem of the transition zone between the roots and the stem of axillary shoot proliferated red raspberry (Donnelly, personal communication). In vitro rooted transplants of three Rubus species were hardier at 1 mo post transplant than those rooted in soil (Donnelly et al., 1980).

For micropropagated potato shoots both in vitro and ex vitro rooting are used. A number of root-inducing media have been proposed. In some cases, rooting was accomplished at the same time as stem elongation (when the cytokinin:auxin ratio was low) in others, a rooting stage was necessary. In Wang and Hu’s
(1982) in vitro "layering" medium, where only NAA was added, adventitious roots developed spontaneously. The same was true in Hussey and Stacey's (1981) nodal multiplication medium where the last subculture was done in stationary petri dishes containing liquid medium instead of the agar medium generally used for multiplication of nodal cuttings. Roca et al. (1978) and Goodwin and Brown (1980) media both contained high levels of cytokinins that prevented adventitious root formation on in vitro shoots. To induce rooting, Goodwin and Brown (1980) transferred their shoots to a growth regulator-free medium containing casein hydrolysate. Roca et al. (1978) transferred their nodal cuttings onto a MS (1962) modified medium containing GA$_3$.

For rooting micropropagated shoots on a large scale ex vitro, Goodwin and Brown (1980) found that it was preferable to place the shoots directly into potting mixture at low temperature (18 °C). McCown and co workers (Wattimena, 1983; Wattimena et al., 1983) developed the following large scale micropropagation protocol. Ex vitro shoots were individually transferred into non sterile peat-perlite plug trays. Ex vitro transplants rooted in 1 wk were hardened without the tent for an additional 2 wk in the greenhouse and then transferred into a cold frame for 1 wk before field planting. They now complete the rooting and hardening procedures in 10 d (personal communication, McCown; cited in Wang and Hu, 1985). Acclimatizing transplants in a plug tray system facilitates mechanical field planting (Hornton and Knutson, 1986).
CHAPTER III

THE CONTRIBUTION OF IN VITRO-PRODUCED ROOTS TO THE ESTABLISHMENT OF EX VITRO PLANTLETS

INTRODUCTION

The necessity of rooting shoots in vitro is still a controversial issue. Debergh and Maene (1981) observed a growth delay when in vitro-rooted shoots (plantlets) of ornamentals were transferred to soil. The in vitro-produced roots died and new roots started to develop 2 wk after plantlets were transferred to soil. This was attributed to the difficulties inducing a functional root system possessing root hairs in vitro. In vitro-formed roots of *Acacia koa* on agar-solidified medium showed poor vascular system development and lacked root hairs (Skolman et al., 1978). These abnormalities were attributed to the poor gas exchange in solidified medium. Ex vitro *Salpiglossis sinuata* survived poorly when roots grew adventitiously from callus (Hughes et al., 1973) as did carnation (*Dianthus caryophyllus*) (Davis et al. 1977). However, Donnelly et al. (1980) found that plants from axillary shoots of 3 *Rubus* species proliferated and rooted in vitro were hardier at 1 mo post transplant than those rooted in soil.

Although a number of studies have shown the increased cost associated with rooting in vitro (Anderson and Meager, 1978; Donnan et al., 1978 and Debergh and Maene, 1981), it is currently unclear whether in vitro-formed roots persist or function efficiently ex vitro. To the best of our knowledge nothing is known on the persistence and contribution of in vitro-formed
roots of potato plantlets ex vitro. The objectives of this study were to monitor the persistence of in vitro-produced roots after transplant and to determine their contribution to the acclimatization, early growth and yield of tissue cultured potato plantlets (Solanum tuberosum 'Russet Burbank'). The potential benefits of auxin application at transplant time were also assessed for potato.

MATERIALS AND METHODS

Nodal cuttings of 'Russet Burbank' were grown in modified Murashige and Skoog (MS, 1962) salts and vitamin solution (Thiamine 0.4 mg/l, Nicotinic acid 0.5 mg/l, Pyridoxine 0.5 mg/l and Glycine 2.0 mg/l), containing 0.8% agar and 3% sucrose (pH 5.7) under 37.8 µmol m⁻² s⁻¹ (3 Klux) cool white fluorescent illumination, 16 hr light period at 25 °C for approximately 4 wk (Fig. 1). Plantlets were then rinsed thoroughly, blotted dry and their fresh weight and leaf number recorded. The roots of half the transplants were severed at the base of the stem (Fig. 2) and the root fresh weights recorded. Transplants with and without roots were transferred into clear plastic covered seedling trays with 125 ml cells containing a mixture of 1:1 promix (Premier Brands): perlite. Slow release osmocoat (19-6-12) fertilizer and dolomitic limestone were added to the medium at a rate of 30 cc/l. Transplants were watered every other day with a weak (ca. 0.5 g/l) 20-20-20 fertilizer solution. After 5 d the plastic covers were gradually lifted and by 7 d they were completely removed (Fig. 3). One hundred and twenty transplants (60 rooted and 60 root-severed) were acclimatized on a growth bench under
NOTICE

THE QUALITY OF THIS MICROFICHE IS HEAVILY DEPENDENT UPON THE QUALITY OF THE THESIS SUBMITTED FOR MICROFILMING.

UNFORTUNATELY THE COLOURED ILLUSTRATIONS OF THIS THESIS CAN ONLY YIELD DIFFERENT TONES OF GREY.
Figure 1. Potato plantlets cultured on MS (1962) salt and vitamin solution containing 0.8% agar and 3% sucrose after approximately 3 wk in culture.
Figure 2. Rooted and root-severed transplants prior to transfer into clear-plastic covered seedling-trays.
Figure 3. Ex vitro transplants during acclimatization in a seedling tray with (top) and without the clear-plastic cover (bottom).
300 \mu\text{molm}^{-2}\text{s}^{-1} (23.8 \text{Klux}) cool white fluorescent illumination, 16 hr light period at room temperature. Half the transplants were harvested after 2 wk and the rest after 3 wk. At harvest the variables measured included: plant fresh weight (PFW), plant dry weight (PDW), root fresh weight (RFW), root dry weight (RDW) and total leaf area (TLA). The later was evaluated by determination of the fresh weight of 10 leaf blade disks of known area (25 replicates) and the total leaf weight. The experiment was conducted using a completely randomized design with five replicates.

Forty transplants (20 rooted and 20 root-severed) were acclimatized in a growth chamber (Conviron model E15, Controlled Environments Inc.) under 120 \mu\text{molm}^{-2}\text{s}^{-1} (9.5 \text{Klux}) cool white fluorescent illumination, 16 hour photoperiod at 25 \degree\text{C} for comparison purposes and harvested after 2 wk. The experiment was conducted and analysed as described earlier.

In further growth chamber experiments with groups of 40 similar transplants, half of the rooted and half of the root-severed transplants were treated with Stim-root #1 or #2 (0.1% or 0.4% IBA, Plant Propagation Lte. Inc.) rooting powder and harvested after 3 or 4 wk. All auxin experiments were conducted using a 2 by 2 factorial in a completely randomized design with 5 replicates.

Visual observation were made on rooted and root-severed transplants to monitor possible structural or morphological differences between the root systems.

A convariance analysis was conducted for every trial because of the heterogeneity of plantlet weight at transplant. When
found significant the effect of initial weight of transplants (IWT) and the initial number of leaves (INL) was removed for the ANOVA analysis.

In our tissue culture system nodal cuttings rooted within a few days. The primary roots branched into secondary and tertiary root systems. Root hairs were present when root growth occurred on the surface of the medium, but not in the solidified medium. In vitro-formed roots possessed a green pigmentation that was still apparent many weeks after transplantation to soil. Presence of pigmentation was used to monitor the persistence of in vitro-formed roots. The acclimatization period was defined as being the time elapsed between the transfer of plantlets to soil to the initiation of the exponential growth phase. The beginning of the exponential growth phase was defined as a weekly doubling of growth.

The field trial was conducted at the E. A. Lodds experimental farm of Macdonald College in Ste-Anne de Bellevue on clay loam soil where alfalfa had been growing for the two previous seasons. No other fertilizer treatment were applied. The alfalfa was ploughed-down in the fall and again just before planting. Eighty transplants (half rooted and half root-severed) were acclimatized in the greenhouse for 3 wk and transferred into an outdoor cold frame for an additional 1 wk before field planting on May 27th 1988. Plants were spaced 45 cm apart in 80 cm rows with one guard row between each treatment. Transplants were irrigated when transferred into the field and for the next few days but no irrigation was subsequently given. Weed control
was done manually and rotenone powder used to control Colorado Potato Beetles (*Lepinotarsa decemlineata*). Harvesting was done September 9th after 100 d. This simulates a very short growing season since risks of pathogen reinfection are known to increase as the season progresses (Smith and Storch 1984). Variables measured included plant height (PH), total plant fresh weight (TFW) which included the tubers, plant fresh weight (PFW) without the tubers, plant dry weight (PDW), plant tuber weight (TW) and tuber number (TN). The experiment was conducted and analysed using a randomized complete block design with 4 replicates with 8 sampling units each.

RESULTS

The covariance analysis indicated a significant influence of IWT on the early development of ex vitro transplants (Table 1). Initial weight of transplants positively influenced PFW, FWG, PDW, RFW, RDW and TLA of transplants for 2 and 3 wk under both controlled environment conditions. By 4 wk, the effect of IWT on transplant growth was no longer apparent. There was no significant effects of INL on plant growth after 2, 3 and 4 wk under growth chamber and after 3 wk under growth bench conditions. There was a significant influence of initial number of leaves on plant fresh weight, fresh weight gain and total leaf area after 2 wk under growth bench conditions. In vitro-formed roots had a significant influence on the acclimatization and early growth of transplants. Rooted transplants acclimatized under growth bench conditions showed significantly higher values of plant fresh and dry weight, fresh weight gain, root fresh and dry weight and total leaf area after 2 and 3 wk than root-severed
Table 1. Results of the covariance analysis showing the influence of the initial weight of transplants (IWT) and the initial number of leaves (INL) on the development of ex vitro transplants. The experiment was conducted using a completely randomized design with 5 non-real replicates.

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<tr>
<th></th>
<th>GROWTH BENCH&lt;sup&gt;1&lt;/sup&gt;</th>
<th>GROWTH CHAMBER&lt;sup&gt;2&lt;/sup&gt;</th>
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<td>2 WEEKS</td>
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<td>IWT INL</td>
<td>IWT INL</td>
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<td>PLANT FRESH WEIGHT</td>
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<td>FRESH WEIGHT GAIN</td>
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<td>PLANT DRY WEIGHT</td>
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<tr>
<td>ROOT FRESH WEIGHT</td>
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<tr>
<td>ROOT DRY WEIGHT</td>
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<td>**</td>
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<tr>
<td>TOTAL LEAF AREA</td>
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</table>

** and * indicates a significant influence of the covariable on the dependant variable at the 0.01 and 0.05% level respectively and -- indicates that there is no influence of the covariable.

1. Each non-real replicates of 6 plants each.
2. Each non-real replicates of 4 plants each.
Table 2. Effects of in vitro-formed roots on the development of ex vitro transplants with roots (+root) or root-severed (-root) under controlled environment conditions. Each experiment was conducted using a completely randomized with 5 non-real replicates.

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<th>3 WEEKS</th>
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<th>4 WEEKS</th>
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<td>+ROOT</td>
<td>-ROOT</td>
<td>+ROOT</td>
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<td>+ROOT</td>
<td>-ROOT</td>
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<tr>
<td>PLANT INITIAL WEIGHT (mg)</td>
<td>283</td>
<td>162</td>
<td>228</td>
<td>188</td>
<td></td>
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</tr>
<tr>
<td>PLANT FRESH WEIGHT (mg)</td>
<td>1023a</td>
<td>593b</td>
<td>1728a</td>
<td>1104b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FRESH WEIGHT GAIN (mg)</td>
<td>740a</td>
<td>431b</td>
<td>1500a</td>
<td>913b</td>
<td></td>
<td></td>
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<tr>
<td>PLANT DRY WEIGHT (mg)</td>
<td>84a</td>
<td>46b</td>
<td>141a</td>
<td>88b</td>
<td></td>
<td></td>
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<tr>
<td>ROOT FRESH WEIGHT (mg)</td>
<td>169a</td>
<td>70b</td>
<td>245a</td>
<td>138b</td>
<td></td>
<td></td>
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<tr>
<td>ROOT DRY WEIGHT (mg)</td>
<td>24a</td>
<td>10b</td>
<td>28a</td>
<td>15b</td>
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<td></td>
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<tr>
<td>TOTAL LEAF AREA (cm²)</td>
<td>15.6a</td>
<td>8.9b</td>
<td>26.4a</td>
<td>17.5b</td>
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Means followed by the same letter within a row for each harvest date are not significantly different (p < 0.05%).

1 Each observation represents the mean of 5 replicates of 6 plants each.
2 Each observation represents the mean of 5 replicates of 4 plants each.
transplants (Table 2). Values of plant fresh and dry weight and fresh weight gain were ca. 40 % higher and root fresh and dry weight were ca. 50 % higher for rooted compared to root-severed transplants for both trials. Growth measurement values were similarly influenced under growth chamber conditions. Plant fresh and dry weight and fresh weight gain were ca. 20 % higher and root fresh and dry weight and total leaf area were ca. 50 % higher for rooted compared to root-severed transplants after 2 wk. Growth measurement values were ca. 40 % higher for rooted compared to root-severed transplants after 3 wk. At 4 wk the values of PFW, FWG, PDW and RDW were ca. 20 % higher for rooted transplants where RFW and TLA were not significantly different than root-severed transplants.

Visual observation of rooted transplants at harvest confirmed the persistence of green, in vitro-formed roots. New root formation which was not green apparently originated from the in vitro-formed roots. Occasionally de novo root formation also occurred higher on the stem on and between nodes.

Auxin application on rooted and root-severed transplants had a negative effect during early growth (Table 3). Transplants treated with 0.1 % and 0.4 % IBA and harvested after 3 and 4 wk respectively showed significantly lower values for most growth measurements. Only RFW of transplants harvested after 4 wk did not differ significantly. Two distinct phases characterized the growth rate of transplants under growth chamber conditions (Fig. 4). During the first 2 wk ex vitro transplant growth was in a latent phase as transplants were acclimatizing.
Table 3. Effects of IBA treatment on the development of ex vitro transplants under controlled environment conditions. Each observation represents the mean of 5 replicates of 4 observations each.

<table>
<thead>
<tr>
<th></th>
<th>3 WEEKS(^1)</th>
<th>4 WEEKS(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-AUXIN</td>
<td>+AUXIN</td>
</tr>
<tr>
<td>PLANT FRESH WEIGHT (mg)</td>
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<td>1516b</td>
</tr>
<tr>
<td>FRESH WEIGHT GAIN (mg)</td>
<td>2270a</td>
<td>1261b</td>
</tr>
<tr>
<td>PLANT DRY WEIGHT (mg)</td>
<td>200a</td>
<td>120b</td>
</tr>
<tr>
<td>ROOT FRESH WEIGHT (mg)</td>
<td>334a</td>
<td>213b</td>
</tr>
<tr>
<td>ROOT DRY WEIGHT (mg)</td>
<td>41a</td>
<td>24b</td>
</tr>
<tr>
<td>TOTAL LEAF AREA (cm(^2))</td>
<td>24a</td>
<td>13b</td>
</tr>
</tbody>
</table>

Means with the same letter within a row for each harvest date are not significantly different (p < 0.05 %).
1. Stimroot #1 (0.1 % IBA).
2. Stimroot #2 (0.4 % IBA).
Figure 4. Ex vitro growth rate of rooted and root-severed transplants under growth chamber conditions (120 μmolm⁻²s⁻¹, 16 hr light period at 25 °C.)
Table 4. Field performance of rooted and root-severed transplants. Each observation represents the mean of 4 replicates of 8 plants each.

<table>
<thead>
<tr>
<th></th>
<th>ROOTED</th>
<th>ROOT-SEVERED</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLANT HEIGHT (cm)</td>
<td>86a</td>
<td>89a</td>
</tr>
<tr>
<td>TOTAL FRESH WEIGHT (g)</td>
<td>1371a</td>
<td>1461a</td>
</tr>
<tr>
<td>PLANT FRESH WEIGHT (g)</td>
<td>832a</td>
<td>882a</td>
</tr>
<tr>
<td>PLANT DRY WEIGHT (g)</td>
<td>117a</td>
<td>119a</td>
</tr>
<tr>
<td>TUBER WEIGHT (g)</td>
<td>536a</td>
<td>585a</td>
</tr>
<tr>
<td>TUBER NUMBER</td>
<td>10.2a</td>
<td>8.6a</td>
</tr>
</tbody>
</table>

Means followed by a the same letter within a row are not significantly different (p < 0.05 %).
The plant fresh weight increased by only 50% in 1 wk. This period was followed by an exponential growth phase commencing after the 2nd wk. Plant fresh weight increased by 4 and 3 times respectively for rooted and root-severed transplants between the 2nd and 3rd wk. After 4 wk ex vitro PFW was 30 times greater than IWT. This growth spurt indicated that the transplants had fully acclimatized to their new environment.

The comparative field performances of rooted and root-severed transplants harvested after 100 d in the field showed no significant differences for growth (PH, TFW, PFW and PDW) or yield (TW and TN) characteristics (Table 4).

DISCUSSION

The acclimatization period for ex vitro potato transplants lasted about 2 wk under growth bench and growth chamber conditions. The acclimatization period and early growth rate was influenced by IWT but not INL and was characterized by a slow, but increasingly more rapid growth rate. Acclimatization was considered complete when transplant growth rate exceeded a doubling each week. This occurred between the 2nd and 3rd wk ex vitro. At 4 wk the IWT no longer influenced transplant growth rate, the growth of transplants was so extensive that the advantage of a heavier initial weight at transplant time was no longer apparent. While INL did not affect the acclimatization and early growth rate of transplants, leaf number only approximates leaf area, which was not measured, and which is probably more important to ex vitro acclimatization.

Roots of potato formed in vitro did not cease to grow and
did not die after transfer from culture contrary to the observations of Davis et al. (1977) and Debergh and Maene (1981) on ornamental species. In vitro-formed potato roots formed the basis for new secondary root development throughout their length and through continued root extension. There were no apparent structural or morphological differences between the root systems of rooted- and root-severed transplants up to 4 wk ex vitro other than the differences in pigmentation. Rooted transplants grew significantly more quickly than root-severed transplants thus roots clearly played a role during acclimatization and soon after. The slope of the growth rate was sharper for rooted compared to root-severed transplants over the entire interval in which they were compared. However, acclimatization occured between the 2nd and 3rd wk for both rooted and root-severed transplants. Differences in transplant growth rate between the growth bench and the growth chamber conditions may have been the result of the higher (and possibly more stressful) light intensity in the growth bench environment.

Although in vitro-formed roots may undergo modifications to adapt to the ex vitro environment, such as the acquisition of root hairs, it appears that these occur readily. The influence of in vitro-formed roots was greatest during early growth ex vitro and not as clear once plantlet growth accelerated. Preliminary results indicate that after 4.5 wk ex vitro there were no differences in growth rate of rooted and root-severed transplants. This supports the results of the field comparison where no differences were found between rooted and root-severed transplants in plant growth (PH, TFW, PFW, and PDW) or yield (TN
and TW) characteristics.

In conclusion, in vitro-formed roots of potato plantlets were persistent, and elongated to form secondary and tertiary roots with root hairs ex vitro. Furthermore, in vitro-produced roots significantly influenced the early growth rate of transplants and may prove to be a significant asset for some tissue cultured species. In vitro-formed roots could also confer significant advantages to ex vitro plantlets under stressed environment.
INTRODUCTION

Potato micropropagation techniques are now routinely used in both industrialized and in developing countries to provide pathogen-tested planting material to local farmers (Gang and Hu, 1982; Bryan, 1988; Dodds, 1988 and Knutson, 1988). Micropropagated plantlets or microtubers can be used to produce minitubers (Wiersema et al., 1987), cuttings (Van Uyen and Vander Zaag, 1983) or can be planted directly into the field (Goodwin and Brown, 1980; Wattimena et al., 1983 and Levy, 1985, 1986 and 1988).

Field performance evaluated by yield (tuber weight and number per plant) was similar for 8 wk old 'Kennebec' microcuttings and conventional seed tubers (Goodwin and Brown, 1980). No differences in total tuber weight per plant were detected between microcuttings, pre-germinated microtubers and conventional seed tuber-derived plants but stolon tuber and numbers were found to be superior for ex vitro material (Wattimena et al. 1983). Ex vitro plants were thought to be physiologically different from conventionally propagated seed tuber-derived plants; tuber initiation occurred during a longer period and resorption was less in ex vitro plants (Wattimena et al., 1983). In addition, micropropagated plants were apparently more susceptible to recontamination by potato viruses S and Y than conventionally propagated potato (Macdonald, 1987).
To the best of our knowledge, no trials have been conducted to determine the field performance of ex vitro material in Quebec. It was predicted that under a short growing season total tuber weight of ex vitro plants would be inferior to conventional seed tuber-derived plants (Wattimena et al., 1983). Although the effects of environmental stress on yield and tuber distribution of conventionally propagated potato are well known (Ewing, 1981; Struik and Van Voorst, 1986; Mackerron and Jeffries, 1988 and Mackerron et al., 1988), little is known of climatic effects on ex vitro propagates besides the fact that stress reduces the multiplication rates i.e. the number of tubers produced (Levy, 1986 and 1988).

The objective of this research was to gather information on comparative field performances of tissue cultured potato plantlets, microtubers and conventionally propagated seed tubers under a short growing season in Quebec. Meteorological data was collected to determine the effects of environmental factors on ex vitro and conventional seed tuber-derived plant growth, yield and tuber distribution.

MATERIALS AND METHODS

Nodal cuttings of 'Russet Burbank' were grown in modified Murashige and Skoog (MS, 1962) salts and vitamin solution (Thiamine 0.4 mg/l, Nicotinic acid 0.5 mg/l, Pyridoxyne 0.5 mg/l and Glycine 2.0 mg/l) containing 0.8 % agar and 3 % sucrose, pH 5.7 under 37.8 μmolm⁻²s⁻¹ (3 klux) cool white fluorescent illumination with 16 hr light period at 25 °C for approximately 4 wk. Microtubers were induced as described by Estrada et al.
(1986) in MS salts and vitamin solution containing 500 mg CCC, 14 mg/l BAP and 80 % sucrose. On transplant, plantlets were washed thoroughly and transferred into clear plastic-covered seedling trays with 125 ml cells containing a mixture of 1:1 promix (Premier Brands):perlite in the greenhouse. Slow release fertilizer (Osmocote 19-6-12) and dolomitic limestone were added to the medium at the rate of 30 cc/l. Transplants were sprayed and watered every other day with a weak (ca. 0.5 g/l) 20-20-20 fertilizer solution. After 5 d the plastic covers were gradually lifted and by 7 d they were removed. After 3 wk ex vitro transplants were placed into an outdoor cold frame for an additional week. After ca. 60 d at 4 °C microtubers were placed into clear plastic-covered seedling trays. When microtubers had germinated the plastic cover was removed. The microtuber-derived plants were treated and acclimatized as indicated for plantlets. After 5 wk in the greenhouse microtuber-derived plants were transferred into an outdoor cold frame for 1 wk prior to field-planting.

Potato field trials were conducted on clay loam soil where alfalfa had been growing for the two previous seasons. The alfalfa was ploughed-down in the fall and again just before planting. Plants were spaced 45 cm apart in 80 cm rows with one guard row between each treatment. Transplants were irrigated when transferred into the field and for the next few days but no irrigation was given subsequently. Weed control was done manually and rotenone powder used to control Colorado Potato Beetles (Leptinotarsa decemlineata).
Figure 5. Partial view of the experimental plot showing ex vitro plantlets and microtubers 1 wk after transfer to the field.
In the first experiment, carried out in 1987 and repeated in 1988, the performance and yield of ex vitro transplants and conventional seed tubers were compared. Transplants were approximately 25 cm tall and possessed about 15 leaves (Fig. 5). Ex vitro plantlets possessed stolons at the time of transfer to the field (June 6th in 1987 and on May 24th in 1988).

Harvesting was started on September 15th in 1987 and Sept 3rd in 1988 after 106 d of growth. This simulated a very short growing season since risks of pathogen reinfection are known to increase as the season progresses (Smith and Storch, 1984) (Fig. 6).

Since the main reason for using micropropagated material is to provide pathogen-tested propagates (Johansen et al., 1984) it was considered advisable to shorten the growing period to simulate a reduced-risk season and assure high quality planting material.

At harvest, plant height (PH) was measured from the collar to the top of the plant. Total plant biomass (TPB), plant fresh weight (PFW), plant dry weight (PDW), leaf fresh weight (LFW), leaf dry weight (LDW), stem fresh weight (SFW) and stem dry weight (SDW) were also measured. The dry weight was obtained by oven-drying the material at 75°C for 24 hr. Tuber weight per plant (TW) and tuber number (TN) were recorded. At harvest tubers were measured and distributed into 5 size classes: A (0-25 mm), B (25-50 mm), C (50-75 mm), D (75-100 mm) and E (> 100 mm) and the total weight of each class measured. The experiment was conducted using a randomized complete block design with 3 replicates in 1987 and with 4 replicates in 1988, with 8 sampling units each, and analysed using an analysis of variance.
Differences in tuber number and yield were analysed using a Chi-square test for homogeneity of distributions.

In a second experiment, conducted in 1988, ex vitro plantlets and microtubers were field-planted on June 8th and harvested on September 17th after 100 d. The microtuber-derived plants were slightly smaller than the plantlets and measured approximately 15 cm in height with 10 leaves. Both plantlet- and microtuber-derived plants possessed stolons at the time of transfer. The experiment was conducted using a randomized complete block design with 4 replicates with 8 sampling units each. Variables measured included PH, TPB, PFW, PDW, TW and TN. Results were analysed using an analysis of variance.

Meteorological data were collected daily at the E.A. Lodds research station of Macdonald College by Water and Soil Conservation Inc. and included precipitation and maximum and minimum daily temperature. This information was used to monitor the climatic seasonal differences in 1987 and 1988.

RESULTS

Plant growth and yield characteristics

Plant growth and yield characteristics were influenced by the type of planting material and the climatic conditions. In 1987, but not in 1988, PH and TPB were significantly greater for seed tuber-derived plants than ex vitro transplants (Table 5). No differences were seen between propagates in total plant, leaf or stem fresh and dry weight during the two seasons. However, the values for these parameters as well as plant height were significantly greater in 1988 than in 1987.
Table 5. Comparison of plant growth and yield characteristics of ex vitro plantlets and seed tuber-derived plants of 'Russet Burbank'.

<table>
<thead>
<tr>
<th>PLANT CHARACTERISTICS</th>
<th>1987</th>
<th>1988</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLANT HEIGHT (cm)</td>
<td>60.4</td>
<td>67.9</td>
</tr>
<tr>
<td>TOTAL PLANT BIOMASS (g)</td>
<td>1467.4</td>
<td>1995.0</td>
</tr>
<tr>
<td>PLANT FRESH WEIGHT (g)</td>
<td>709.7</td>
<td>674.0</td>
</tr>
<tr>
<td>PLANT DRY WEIGHT (g)</td>
<td>111.1</td>
<td>98.9</td>
</tr>
<tr>
<td>LEAF FRESH WEIGHT (g)</td>
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<td>330.0</td>
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<td>LEAF DRY WEIGHT (g)</td>
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</tr>
<tr>
<td>STEM DRY WEIGHT (g)</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>YIELD CHARACTERISTICS</th>
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</tr>
</thead>
<tbody>
<tr>
<td>TUBER WEIGHT (g)</td>
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</tr>
<tr>
<td>TUBER NUMBER</td>
<td>18.9</td>
<td>12.4</td>
</tr>
</tbody>
</table>

**, * indicates a significant difference at 0.01 and 0.05 level respectively.
1. Indicates a significant difference (p < 0.05%) between seasons.
2. Each observation represents the mean of 3 replicates of 8 plants each.
3. Each observation represents the mean of 4 replicates of 8 plants each.
Ex vitro transplants were found to be significantly taller than conventional propagates in 1988. Tuber weight was significantly greater and tuber number significantly less for seed tuber-derived plants than ex vitro transplants. Tuber number was 34% less in 1988 for ex vitro transplants and 20% less for seed tuber-derived plants compared to 1987 (Table 5). There was a difference in stem development between plants derived from the two types of propagates. Seed tuber-derived plants produced two to six unbranched stems. Ex vitro plants had single-stems and were characterized by development of most of the axillary buds over the entire length of the stem. This resulted in an increased number of branches and leaves on ex vitro plants compared to conventional plants.

The frequency and weight distributions of ex vitro and seed tuber-derived plants were found to be significantly different in 1988 (Table 6). Micropropagated plants produced more class A, B and C tubers but fewer tubers in the D and E classes (Table 6). The tuber weight distribution followed a similar pattern. The average weight of tubers from ex vitro plants was higher for classes A and B but seed tuber-derived plants produced heavier classes C, D and E tubers.

In 1988, as in 1987, frequency and weight distributions of both types of propagules were significantly different (Table 6). Plants from culture produced more class A, B, C and D tubers but fewer class E tubers than plants from seed tubers. Thus, weight from ex vitro plants were larger for the A, B, C and D classes and smaller for class E. Average tuber weight of all classes was equal or higher for seed tuber-derived plants.
Table 6. Frequency distribution (FD), weight distribution (WD) and average tuber weight (ATW, g.) of all tubers produced from micropropagated and conventional seed tuber-derived plants.

<table>
<thead>
<tr>
<th></th>
<th>PLANTLETS</th>
<th>SEED TUBERS</th>
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<th>SEED TUBERS</th>
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<tr>
<td></td>
<td>FD</td>
<td>WD</td>
<td>ATW</td>
<td>FD</td>
</tr>
<tr>
<td>A</td>
<td>33</td>
<td>2</td>
<td>3</td>
<td>20</td>
</tr>
<tr>
<td>B</td>
<td>27</td>
<td>14</td>
<td>24</td>
<td>13</td>
</tr>
<tr>
<td>C</td>
<td>22</td>
<td>32</td>
<td>64</td>
<td>21</td>
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<tr>
<td>D</td>
<td>12</td>
<td>27</td>
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<tr>
<td>E</td>
<td>6</td>
<td>25</td>
<td>179</td>
<td>23</td>
</tr>
</tbody>
</table>

All distributions were found significantly different ($X^2$, $p < 0.05\%$) between treatments and year.
Tuber size classes: A= 0-25 mm, B= 25-50 mm, C= 50-75 mm, D= 75-100 mm and E= > 100 mm.
In 1988, fewer small (A) and large (D and E) and more middle (B and C) sized tubers were produced for both types of propagules.

Secondary growth protuberances and regrowth of tubers were encountered in 1988 on both types of propagules. No differences were noticed in the frequency and amplitude of the phenomenon.

The growth, yield and the growth habit of ex vitro plantlets and microtubers were not found to differ significantly. Although plant height, total plant biomass, plant fresh weight, tuber number and weight were all numerically higher for microtuber derived plantlets these differences were not statistically significant (Table 7). It is interesting to note that the 2 wk planting delay over the first experiment resulted in a significant reduction of total plant biomass, plant fresh weight and tuber yield. Some of this reduction in tuber yield may be attributed to the shorter growing period.

Climatic conditions

Large differences in precipitation pattern occurred in the 1987 and 1988 field seasons (Fig. 7). In 1988 the first month of growth was characterized by a severe drought; only 16 mm of rain fell and all of it in 1 day. During the rest of the season precipitation was irregular and totaled 264 mm. In 1987 rainfall was more evenly distributed and abundant, totaling 367 mm.

Mean maximum and minimum temperatures differed between seasons (Fig. 7). In 1987 the mean maximum temperatures occurred at 5 wk with lower peaks at 7 and 11 wk. Minimum temperatures increased, reaching a maximum at 5 wk, with secondary peaks at 6, 7 and 11 wk. In 1988 the mean maximum temperature curve showed a peak at 3 wk followed by a sudden decrease at 5 wk and then mean
Table 7. Comparison of plant growth and yield characteristics of ex vitro plantlets and microtubers. Each observation represents the mean of 4 replicates of 8 sampling unit each.

<table>
<thead>
<tr>
<th></th>
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<th>MICROTUBERS</th>
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</thead>
<tbody>
<tr>
<td><strong>PLANT CHARACTERISTICS</strong></td>
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<td></td>
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<td>PLANT HEIGHT (cm)</td>
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<td>1423.0a</td>
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<td>PLANT FRESH WEIGHT (g)</td>
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<td>843.0a</td>
</tr>
<tr>
<td><strong>YIELD CHARACTERISTICS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TUBER NUMBER</td>
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<td>11.8a</td>
</tr>
<tr>
<td>TUBER WEIGHT (g)</td>
<td>536.2a</td>
<td>599.8a</td>
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</table>

Means followed by the same letter within a line are not significantly different (p < 0.05)
Figure 7. Meteorological data of 1987 and 1988 showing the differences in precipitation and temperature patterns.
maximum temperatures higher than 25 °C which remained for a 7 wk period, with a peak of 32 °C at the 10th wk. The mean minimum temperature curve followed a similar pattern. Overall mean minimum temperatures in 1988 were lower or equal to those of 1987 for the first 7 wk growth period but were continuously higher after this point.

DISCUSSION

For both years of field trials, the tuber weight of ex vitro 'Russet Burbank', a late maturing cultivar grown under a short season, failed to equal the weight of conventional seed tuber-derived plants, as predicted by Wattimena et al. (1983).

The number of tubers per stem is known to be affected by water stress from the time of emergence onward but not from the onset of tuberization. The amplitude of this phenomenon was correlated with the duration of the stress period (Mackerron and Jeffries, 1986). Since micropropagated material began to form stolons right after ex vitro transfer the differences in TN observed between propagates were probably due to the fact that ex vitro plantlets were submitted to a longer period of water stress during this critical time. Ex vitro plants bore a higher number of tubers per stem since they produced only single-stemmed plants. It might be possible to further increase the yield on a per ha basis of ex vitro-derived plants by increasing the planting density to reach the number of stems per ha equal to the level of seed tuber-derived plants.

As observed by Wattimena et al. (1983) ex vitro transplants produced more small tubers and fewer large ones than seed tuber-derived plants. This resulted in significantly different
frequency and weight distributions for both types of transplants. Overall, micropropagated plants produced smaller tubers for all size classes. The high seasonal temperatures and low precipitation in 1988 resulted in an increased yield of smaller sized tubers (> 50 mm) for both types of propagules. A similar response was observed by Mackerron and Jeffries (1988) for conventional potato when these were grown under irrigated and non-irrigated field conditions. Although the yield of smaller sized tubers increased in 1988 there was a drastic decrease in frequency and weight distribution of class A tubers, particularly for ex vitro transplants. This could be attributed to the early water stress that significantly reduced tuber number.

Although tuber weight of ex vitro plants failed to equal that of seed tuber-derived plants under a short growing period, ex vitro propagates produced more tubers. As tuber number is more important than weight, the indications are that ex vitro material, both plantlets and microtubers, could be utilized to the great advantage of the seed tuber industry in Quebec. Furthermore, the preliminary indications obtained on early water stress indicate that it may be possible to increase the yield efficiency by modifying the watering regime, thereby reducing the number of marginally sized tubers. Sufficient irrigation should be given to ex vitro transplants at the time of planting to avoid significant reduction of tuber number.

Although the main effect of the climate was to reduce the mean tuber size, as reported by Mackerron et al., (1988) on irrigated and non-irrigated fields, the mean weight of class A
tubers increased significantly probably due to the reduction in tuber number.

The climatic conditions reduced yields in 1988 compared to 1987 for both seed tuber-derived plants (-30%) and ex vitro transplants (-15%). Although yield reduction of seed tuber-derived plants may be partly accounted for by differences in seed source and storage conditions differences in the physiological response of micropropagated plants to climatic stress is indicated. High temperatures are known to affect potato in a number of ways; reducing photosynthesis and increasing foliar respiration (Burton, 1981), reducing the allocation of photosynthates to the tubers (Gregory, 1965 and Marinus and Bodlaender, 1978) and causing secondary growth protuberances on tubers (Bodlaender et al. 1964). The relatively high temperatures recorded in 1988 clearly reduced partitioning of photosynthates to the tubers as indicated by significantly increased values for plant growth characteristics (PH, PFW, PDW, LFW, LDW, SFW and SDW) and significantly decreased tuber yield compared to the previous year. The relatively lower precipitation of 1988 contributed to this yield reduction. Water stress is known to reduce yields (Robin and Domingo, 1956).

Wattimena et al. (1983) observed that although 'Norland' and 'Red Pontiac' micropropagated plants had only one emergent stem, no differences in vine growth were visually evident after 1 mo due to vigorous stem branching. In our experiment the growth habit of ex vitro 'Russet Burbank' plants was different than that of seed tuber-derived plants. This was clearly demonstrated by the plant height differences observed between both types of
propagates; ex vitro plants being smaller than seed tuber-derived plants in 1987 and taller in 1988. This suggests that ex vitro stems develop in a different pattern and are influenced differently by high temperatures. Meyling and Bodlaender (1981) observed that growth characteristics of different European cultivars were differentially influenced by environmental factors. Meyling and Bodlaender (1981) related the response to differences in varietal earliness. Preliminary experiments conducted in 1988 with 'Norland', an early maturing cultivar, indicate that response of ex vitro transplants to environmental stress is equally cultivar dependant.

Preliminary indications showed that ex vitro transplants differed from seed tuber-derived plants in their response to the length of the growing season, early water stress and high temperature. More research is needed to determine the causes of these differential environmental responses.
EVALUATION OF TWO METHODS OF MICROTUBER INDUCTION

INTRODUCTION

Interest in utilizing microtubers (in vitro produced tuber) in potato seed tuber certification programs is rapidly increasing worldwide. Although more expensive to produce than micropropagated plantlets, microtubers are easier to handle, store and distribute (Dodds, 1988; Wiersema et al., 1988 and Rosell et al., 1988). Microtuber utilization in a seed tuber certification program can be especially valuable in areas where short growing seasons restrict transplantation of ex vitro plantlets to certain periods of the year (Dodds, 1988).

Many different protocols exist for microtuber induction (Palmer and Smith, 1969; Stallknecht, 1972; Lozoya and Dawson, 1981; Wang and Hu, 1982; Wattimena et al., 1983; Hussey and Stacey, 1984 and Estrada et al., 1986). Some of these methods were compared in relation to the size and number of microtubers produced (Ortis-Montiel and Lozoya-Saldana, 1987). However, comparative data is lacking on the field performance of microtubers generated using different methods. The objective of this study was to compare the productivity, quality and field performance of 'Russet Burbank' microtubers produced using the method developed at the International Potato Centre (CIP) by Estrada et al. (1986) which has become the international standard, and the single node method of tuberization (Hussey and Stacey, 1984) using the media composition of Wattimena et al.
(1983). The single node method was selected because it was not part of the study of Ortiz-Montiel and Lozoya-Saldana (1987) and had not been adequately evaluated.

MATERIALS AND METHODS

Nodal cuttings of 'Russet Burbank' were grown in test tubes containing 15 ml of modified Murashige and Skoog (MS, 1962) salt and vitamin solution (Thiamine 0.4 mg/l, Nicotinic acid 0.5 mg/l, Pyridoxine 0.5 mg/l and Glycine 2.0 mg/l) containing 0.8% agar and 3% sucrose adjusted to a pH of 5.7. Cuttings were incubated under 37.8 μmolm⁻²s⁻¹ (3 Klux) 5.7 cool white fluorescent illumination with 16 hr light period at 25 °C for ca. 4 wk. The regenerated plantlets were trimmed of roots and terminal leaves forming 6-node sections.

A. In the CIP method the 6-node sections were placed into 50 ml of liquid medium containing the MS (1962) salt and vitamin solution supplemented with 0.4 mg/l GA₃, 0.5 mg/l BAP, 0.01 mg/l NAA, 2% sucrose at pH 5.7 in 250 ml erlenmeyer flasks. Sections were shaken at 60 rpm on a rotary shaker under 12.8 μmolm⁻²s⁻¹ (1 Klux) illumination with 16 hr light period for 10 d. The propagation medium was then replaced with 50 ml of induction medium consisting of MS (1962) salts and vitamins supplemented with 5 mg/l BAP, 500 mg/l CCC, 8% sucrose and the pH set at 5.7. Cultures were incubated at 22 °C under continuous dark for 40 d (Fig. 8).
Figure 8. Microtuber induction using the CIP (top) and single node (bottom) methods approximately 20 d after induction.
B. In the single node tuberization method 6-node sections were further divided into single nodes to leave equal stem tissue on either side of the node and introduced onto 50 ml of MS (1962) salt and vitamin solution supplemented with 6% sucrose, 3.4 mg/l kinetin and 0.7% agar at pH 5.7 in 250 ml erlenmeyer flasks. Cultures were incubated at 15°C in continuous dark for 50 d (Fig. 8).

At harvest, total microtuber weight per flask, individual microtuber weight and microtuber number per flask (the multiplication rate) were recorded. Each experiment was repeated twice and conducted using a completely randomized design with 10 replicates. Data was analysed using analysis of variance.

After a dormancy-breaking interval of 60 days at 4°C in the dark, microtubers produced from both the single node and CIP methods, having an average weight of 51.7 and 189.2 mg respectively were transferred into clear plastic-covered seedling trays with 125 ml cells in the greenhouse. The potting mixture contained 1:1 promix (Premier Brands):perlite with slow release fertilizer (Osmocote 19-6-12) and dolomitic limestone added at the rate of 30 cc/l. When the microtubers had germinated the plastic covers were removed. Plants were watered every other day with a weak (ca. 0.5 g/l) 20-20-20 fertilizer solution. The height (PH) and number of leaves (NL) of the microtuber-derived plants were recorded after 5 wk. Plants were then transferred to an outdoor cold frame for an additional 1 wk and subsequently field planted on May 27th 45 cm apart in 80 cm rows 10 cm deep. Hilling was done after 4 wk.
Plants were harvested on September 3rd after 100 d. This simulated a very short growing season since risks of pathogen reinfection are known to increase as the growing period lengthens (Smith and Storch, 1984). At harvest plant height (PH), total plant biomass (TPB) and tuber weight (TW) and number (TN) were recorded. The experiment was conducted using a randomized complete block design with 4 replicates of 6 plants each. Data was analysed using an analysis of variance.

RESULTS

The CIP method of microtuber induction yielded a larger total tuber weight per flask (812.7 mg) than the single node cutting method (238.4 mg) (Table 8). However, the mean tuber number per flask was not significantly different between the CIP (4.1) and the single node cutting method (4.5). Microtubers produced by the CIP method were significantly larger, as indicated by the total tuber weight per flask (812.7 mg) mean tuber weight (208.5 mg), number of tubers per flask > 100 mg (3.2) or > than 10 mm (0.5), compared to the single node tuberization method, with about 25% of the total tuber weight per flask (208.5 mg) and mean tuber weight (54.4 mg) and few tubers > 100 mg (0.4) or > 10 mm (0) (Table 8).

The non-destructive measurements done on microtuber-derived plants after 5 wk of growth in the greenhouse (Table 9) showed a significant difference in plant height between the CIP (11.3 cm) and the single node (10.3 cm) microtuber-derived plants but no differences in leaf number per plant (8.9 and 8.5 respectively).
Table 8. Comparison of the yield and microtuber characteristics of the CIP and single node methods of microtuber induction. Each observation represents the mean of 20 replicates.

<table>
<thead>
<tr>
<th></th>
<th>CIP</th>
<th>SINGLE NODE</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOTAL MICROTUBER WEIGHT (mg)</td>
<td>812.7a</td>
<td>238.4b</td>
</tr>
<tr>
<td>PER FLASK</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NUMBER OF MICROTUBERS PER FLASK</td>
<td>4.1a</td>
<td>4.5a</td>
</tr>
<tr>
<td>AVERAGE MICROTUBER WEIGHT (mg)</td>
<td>208.4a</td>
<td>54.4b</td>
</tr>
<tr>
<td>MICROTUBERS PER FLASK &gt; 100 mg</td>
<td>3.2a</td>
<td>0.4b</td>
</tr>
<tr>
<td>MICROTUBERS PER FLASK &gt; 10 mm</td>
<td>0.5a</td>
<td>0.0b</td>
</tr>
</tbody>
</table>

Means followed by the same letter within a row are not significantly different (p < 0.05).
Table 9. Comparison of the early development and field performance of microtubers produced using the CIP and single node methods of microtuber induction.

<table>
<thead>
<tr>
<th></th>
<th>CIP</th>
<th>SINGLE NODE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AVERAGE MICROTUBER WEIGHT (mg)</strong></td>
<td>189.2</td>
<td>51.7</td>
</tr>
<tr>
<td><strong>AFTER 5 WEEKS IN GREENHOUSE ¹</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PLANT HEIGHT (cm)</td>
<td>11.3b</td>
<td>10.3a</td>
</tr>
<tr>
<td>NUMBER OF LEAVES</td>
<td>8.9a</td>
<td>8.5a</td>
</tr>
<tr>
<td><strong>AFTER 100 DAYS IN THE FIELD ²</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PLANT HEIGHT (cm)</td>
<td>86.9a</td>
<td>87.4a</td>
</tr>
<tr>
<td>TOTAL PLANT BIOMASS (g)</td>
<td>1354.4a</td>
<td>1493.2a</td>
</tr>
<tr>
<td>TUBER WEIGHT (g)</td>
<td>550.6a</td>
<td>600.5a</td>
</tr>
<tr>
<td>TUBER NUMBER</td>
<td>12.5a</td>
<td>11.1a</td>
</tr>
</tbody>
</table>

Means followed by the same letter are not significantly different (p < 0.05).

¹ Data analysed using analysis of variance.
² Experiment conducted using a randomized complete block design with 4 replicates of 6 sample units each.
After 100 d of field growth no differences were found in plant or yield characteristics between plants originating from microtubers produced using the CIP and single node tuberization methods (Table 9).

The CIP- and single node microtuber-derived plants showed similar values of PH (86.9 cm and 87.4 cm respectively), TPB (1354.4 g and 1493.2 g respectively), TW (550.6 g and 600.5 g respectively) and TN (12.5 and 11.1 respectively).

DISCUSSION

Despite the fact that the two step CIP method results in a much greater plant biomass per flask prior to tuber induction than the one step single node method, only microtuber size and weight and not the multiplication rate per flask were affected.

Furthermore, the large differences in microtuber weight generated by the two methods did not translate to differences in plant or yield characteristics in the greenhouse or in the field. This supports the results of Rosell et al. (1987) who did not find differences in vigor between plants derived from microtubers of 203 and 359 mg mean weight. On the other hand, Wiersema et al. (1987) observed differences in early plant vigor and ground cover between microtubers of 0.63, 1.25 and 2.50 g. While increased stem number occurred in plants derived from larger microtubers this apparent increase in vigor resulted in greater mean tuber biomass but not greater SPT (specific pathogen-tested) tuber number (Wiersema et al., 1987).

It is clear from our results and those of Rosell et al. (1987) and Wiersema et al. (1987) that the emphasis in microtuber
production should be given to maximization of microtuber number, rather than microtuber size. As no differences in the multiplication rate in vitro were detectable between the two induction methods and since no differences in the number of potential SPT tubers were seen in plants derived from different size microtubers the choice of a microtuber induction method should be based on microtuber number and the cost of production. The first step of the CIP method is undoubtedly a powerful procedure to produce large quantities of plant material, but we feel that for microtuber induction this plant material should subsequently be divided into smaller sections to increase the multiplication rate.
CHAPTER VI

SUMMARY AND CONCLUSIONS

Studies conducted on the early growth rate of ex vitro potato transplants have enabled us to propose a definition for the acclimatization period: time elapsed between the transfer of plantlets to soil to the initiation of the exponential growth phase, the beginning of a weekly doubling of growth. In our system ex vitro 'Russet Burbank' transplants were acclimatized after approximately 2 wk post transplant.

The initial weight of transplants was found to affect their growth rate during the first 3 wk of growth but not beyond this point. In vitro-formed roots significantly enhanced the growth rate of transplants during and for at least 2 wk after the acclimatization interval. In vitro-formed roots not only survived transfer to soil but elongated and formed secondary and tertiary root systems with root hairs. This clearly demonstrates that in vitro-formed roots are able to acclimatize and can contribute significantly to the early growth of transplants ex vitro. This is the first documented evidence that roots formed in vitro are functional after transfer. This is of particular importance for tissue cultured species with slow growth rates or those that show poor rooting ex vitro.

In contrast, initial leaf number was not found to have a significant influence on transplant growth rate. Auxin applications at transplant time were not found to be useful for potato. Rooted and root-severed transplants did not exhibit
differences in plant growth or yield characteristics after 100 d in the field.

Field performance comparisons between ex vitro transplants and seed tuber-derived plants were conducted for two consecutive years. Total tuber biomass of ex vitro transplants failed to equal that of conventionally propagated potatoes. However, ex vitro transplants produced significantly more potential seed tubers than seed tuber-derived plants under a short growing period (106 d). The frequency and weight distributions of tubers formed by the two types of propagates were different. Ex vitro transplants produced significantly more small sized tubers and fewer large sized tubers than seed tuber-derived plants. Average tuber weight was less for ex vitro transplants than for conventionally propagated potato for each of the 5 size categories. This confirms the results of Wattimena et al. (1983) with 'Norland' and 'Red Pontiac' grown under a normal season. No significant differences were found in the fresh and dry weight of plants, leaves or stems at harvest time. However, plant height and growth habit in the field differed between the two types of propagates. Ex vitro transplants had a unique appearance. They produced a single stem with extensive axillary bud development unlike seed tuber-derived plants which had multiple stems. The significance of this is unknown. The two types of propagates differed in susceptibility to environmental stress. Ex vitro transplants were apparently more susceptible to early water stress in the field than conventionally propagated plants. This increased susceptibility to water stress may be due to an earlier
production of stolons on ex vitro transplants. Seed tuber-derived plants were apparently more affected by high summer temperatures in the field than ex vitro transplants as indicated by the large yield reduction observed in 1988. Reasons for this are as yet unknown. Differences not were found between ex vitro transplants and microtubers in plant growth or yield characteristics. The decision to use either ex vitro transplants or microtubers should be based on a cost/benefit criteria since no differences were found in yield between both types of propagates.

The single node and CIP methods of microtuber induction generated differences in microtuber weight and size but not number. The CIP method produced a larger total microtuber biomass with larger microtubers than the single node tuberization method. The large differences in the initial weight of microtubers did not translate to differences in growth or yield characteristics of field plants harvested after 100 d. This indicates that emphasis in microtuber production should be given to number rather than size of microtubers. Emphasizing number rather than size may help reduce the cost of microtuber production by optimizing the available plant material.

Direct field planting of ex vitro plantlets or microtubers could be advantageous to the potato seed tuber certification industry in Quebec. This could significantly reduce the time required to release a new cultivar or make available specific pathogen-tested seed tubers to farmers. In times of strong international competition this could prove to be an important asset to Quebec potato growers. Ex vitro planting material
should also interest the potato canning industry since tissue cultured potato produced a significantly larger number of small sized tubers than conventionally propagated potato.

The direct use of micropropagated material in seed certification could enhance the competitiveness of the Quebec seed certification industry. A large scale assessment is necessary to make possible the implementation of these techniques by certified seed growers in the province.
CHAPTER VII

CLAIM OF ORIGINALITY

The following points describe original contributions to knowledge:

1. This is the first documented evidence that in vitro-formed roots are functional after ex vitro transfer.

2. A new definition for ex vitro acclimatization is proposed based on the growth rate of transplants rather than on anatomical modifications.

3. This is the first report of field performance of ex vitro plantlets and microtubers in Quebec.

4. We obtained evidence that in vitro plantlets and microtubers are more susceptible to early water stress than seed tuber-derived plants.

5. It was shown that large differences in microtuber weight do not translate to differences in the field performance of microtuber-derived plants.
CHAPTER VII

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