Potato Microtuber Production and Performance: A Review

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ABSTRACT

Almost half a century has passed since *in vitro* tubers (microtubers) were first described in potato, but their adoption as a seed propagule has been uneven globally. Consensus is lacking regarding optimal production practices for microtubers and their relative productivity in relation to other propagules for minituber production. There is significant uncertainty regarding the utility of microtubers for evaluation of agronomic characters. However, the application of microtubers in germplasm conservation is widely accepted. Microtubers are produced *in vitro* in a plethora of different growing systems with varying environment, media constituents, and storage intervals. Many of the interactions between growth parameters *in vitro* and subsequent productivity appear to be genotype-specific. Accordingly, microtubers come in different sizes, have different dormancy requirements, and differ widely in relative growth potential and productivity. Despite these differences, there is evidence for strong analogies in growth responses between field-grown tubers and microtubers. The use of microtuber technology in seed tuber production, breeding programs, germplasm conservation, and research appears to have enormous potential. This review discusses microtuber production, yield and performance, *in vitro* screening, and germplasm storage and exchange.

RESUMEN

Casi medio siglo ha transcurrido desde que los tubérculos *in vitro* (microtubérculos) fueran descritos en papa por primera vez, pero su adopción como propágulos semillas ha sido irregular a nivel mundial. Falta consenso con respecto a las prácticas optimas de producción de microtubérculos y su relativa productividad en relación con otros propágulos para la producción de minitubérculos. Existen dudas significativas en relación con la utilidad de los microtubérculos para la evaluación de caracteres agronómicos. Sin embargo, el uso de microtubérculos para la conservación del germoplasma está ampliamente aceptado. Los microtubérculos son producidos *in vitro* en una infinidad de sistemas de crecimiento con medio ambiente variado, diferentes constituyentes de los medios de cultivo e intervalos de almacenamiento variados. Muchas de las interacciones entre los parámetros de crecimiento *in vitro* y la subsiguiente productividad parece ser específica para el genotipo. En consecuencia, los tubérculos vienen en diferentes tamaños, tienen diferentes requerimientos de latencia y se diferencian ampliamente en lo que respecta al potencial de crecimiento relativo y a la productividad. A pesar de estas diferencias, existe evidencia de fuertes analogías en la respuesta de crecimiento entre los tubérculos obtenidos en el campo y los microtubérculos. El empleo de la tecnología de microtubérculos en la producción de semilla, programas de mejoramiento, conservación del germoplasma e investigación parece tener un potencial enorme. Esta revisión analiza la producción de microtubérculos, rendimiento y comportamiento, tamizado *in vitro* y almacenamiento e intercambio de germoplasma.

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Abbreviations: acetylsalicylic acid (ASA), 6-benzylaminopurine (BAP), (2-chloroethyl) trimethylammonium chloride (CCC), day/night cycle (d/n), fresh weight (FW), gibberellic acid (GA), N-isopentenylaminopurine (2-iP), 6-furfurylaminopurine (Kn), tetcyclasis (TET), Murashige and Skoog basal salt medium (MS), naphthalene acetic acid (NAA), photosynthetically active radiation (PAR)

INTRODUCTION

Although *in vitro* tubers (microtubers) were described in the mid-20th century (Barker 1953; Mes and Menge 1954) microtubers remain enigmatic in many respects and have generally been under-utilized as a propagule. For example, uncertainty surrounds microtuber production practices, their role in the seed tuber industry, and their potential use for evaluation of agronomic characters. The objective of this review is to present a summary of available information in these areas. We examined the use of microtuber production technology in the seed tuber industry including choice of initiation propagules, inducing agents, and commercial scale-up. We summarized what is known of microtuber performance in comparison with other propagules that are used in the seed tuber industry. We also explored the utility of microtubers for evaluation of agronomic characteristics and in germplasm storage and exchange. It is hoped that this synthesis of information will lead to increased understanding of microtuber production and increased awareness of their potential for *in vitro* screening and conservation. A previous review examined the validity of microtubers as a model for tuber research (Coleman et al. 2001).

MICROTUBER PRODUCTION

Many countries lacking isolated and vector-free growing areas that permit the production of quality potato seed tubers consider microtuber technology a vital component of seed potato production. These countries include Taiwan (Wang and Hu 1982), South Korea (Joung et al. 1994), Italy (Ranalli et al. 1994b), the Philippines (Rasco et al. 1995), South Africa (Venter and Steyn 1997), and many others. In other countries, microtubers are one of several propagules favored during early certification stages for seed tuber production. This is true in Europe, North America, and several South American countries, with their long-established certification programs. Microtubers may also provide a solution in countries where the availability of high-quality seed tubers forms a constraint due to explosive increases in new potato growing areas, such as China, India, and other parts of Asia (FAO 1995; Maldonado et al. 1998).

Microtubers are utilized for minituber (small tubers produced from *in-vitro*-produced propagules) production in greenhouses or screenhouses and, less commonly, are directly field-planted. Wherever microtuber and minituber production technologies have been implemented, they have halved the field time necessary to supply commercial growers (3 or 4 years compared with 7 or more years), and greatly improved seed tuber quality (fewer viral, bacterial, fungal problems).

Microtubers have been described as merely an alternative propagule to plantlets for which production techniques do not contribute, or contribute very little, to further multiplication (Struik and Wiersema 1999). With increasing understanding of factors affecting tuberization, both induction and subsequent weight accumulation, the overall productivity of both microtuber and minituber production systems is likely to increase. There is a long list of variables affecting plantlet growth and microtuber induction and growth. Some of these variables interact with one another and with genotype. The most critical decisions involve choice of initiation propagule, microtuber inducing agents including environment and medium components, and economies of scale.

Choice of Propagules for Microtuber Production

Of the numerous propagules available for microtuber production, the most common include single-node cuttings (small stem sections each with one main axillary bud and subtending leaf) and layered shoots (horizontal placement of plantlets with roots and shoot apex removed) (Wang and Hu 1982). The purpose of cutting individual single-nodes from micropropagated plantlets is to increase the percentage of nodes that eventually microtuberize. However, the production of single-node cuttings is a labor-intensive process. Many variables affect growth and microtuberization efficiency from single-node cuttings, including minor variations in stem length (Papathanasiou et al. 1994). Establishment of single-node cuttings usually is followed by shoot or plantlet production prior to induction. Consequently, layering (horizontal positioning of a shoot with six to ten nodes, shoot tip and roots removed), which is less tedious, appears to be a more efficient approach to establishing cultures for microtuberization. Layered shoots microtuberized more rapidly and produced larger microtubers than single-node cuttings placed into stationary cultures with growth regulator-free MS (Murashige and Skoog 1962) basal medium containing 8% sucrose under 8/16 h d/n cycle at 50 $\mu mol\ m^{-2}s^{-1}$ and 15 C (Leclerc et al. 1994). About 20% of the microtubers produced exceeded 1 g fresh weight (FW) each after 2 months in inductive medium.

Less commonly used, but very promising, source materials include recycled microtubers (McCown and Joyce 1991;

Khuri and Moorby 1996) and sprouts from cold-stored seed tubers (Hoque et al. 1996). Microtubers considered too small for field-planting (< 3 mm diameter) were recycled back into tissue culture once they became non-dormant. These propagules initiated new plantlets more quickly than single-node cuttings (Khuri and Moorby 1996). The plantlets, in turn, produced microtubers more rapidly than did plantlets from single-node cuttings. Along similar lines, cold-stored seed tubers formed successive populations of sprouts that were surface-disinfested for use as source material for microtuberization (Hoque et al. 1996). The physiological age of the source tubers can have an impact on subsequent *in vitro* microtuberization. For example, single-node segments isolated from 'Kennebec' tubers demonstrated earlier and greater microtuberization rates when the source tubers were physiologically older (Villafranca et al. 1998).

Microtuberization

Environmental features implicated in microtuber induction are light and temperature, while medium components implicated in induction include sucrose, nitrogen, growth regulators, and natural products. Microtuber-inducing agents are discussed in the following sections. Factors influencing subsequent microtuber growth and dry matter accumulation cannot always be separated readily from those promoting induction and are less well understood. These are listed in Table 1.

TABLE 1 — *Key factors promoting increased microtuber size or fresh weight when applied throughout microtuberization—during induction and subsequent growth (A) or following induction during the growth phase (B).*

Key Factor		Reference
Increased nitrate:ammonium Increased nitrogen:carbon levels	(A)	Chen and Liao, 1993; Avila et al., 1998
Presence of light (cultivar specific)	(B)	Slimmon et al., 1989; Gopal et al, 1998
Increased temperature (25 C)	(B)	Akita and Takayama, 1994b
Increased sucrose availability	(B)	Yu et al. 2000

Microtuber-inducing Agents In Vitro: Environment

Light—Microtuberization efficiency increased when micropropagated source plants were grown under long days (16/8 h d/n) compared with short days (8/16 h d/n), followed by microtuber induction under short days or continuous darkness (Seabrook et al. 1993). For example, decreased daylight from long to short days promoted earlier (Garner and Blake 1989) or more numerous (Wang and Hu 1982) microtubers and increased microtuber size (Seabrook et al 1993). It is interesting that change from either long days (Slimmon et al. 1989) or short days (Dobranszki and Mandi 1993) to continuous darkness also promoted microtuber induction in some cultivars. However, change in day length or short day cycles did not always enhance tuberization (Hussey and Stacey 1984). The promotive effects of short photoperiod on induction were less apparent when cultures were grown under strongly inductive conditions of elevated sucrose and cytokinins (Abbott and Belcher 1986). Microtuberization response varied with the relative maturities of the cultivars tested and appeared to be partly controlled by photoperiod (Lentini and Earle 1991; Seabrook et al. 1993). Photoperiod also affects dormancy duration. Short photoperiod (8 h) significantly reduced the dormancy duration of microtubers compared with growth in the dark (Coleman and Coleman 2000).

Microtuberization was faster in the dark, but the percentage of nodes tuberizing (Wang and Hu 1982; Wattimena et al. 1983; Ortiz-Montiel and Lozoya-Saldana 1987; Forti et al. 1991) and microtuber fresh weights of some cultivars (Slimmon et al. 1989; Gopal et al. 1998) increased in the light compared with continuous darkness. Curiously, the presence of low light (6-12 $\mu mol\ m^{-2}s^{-1}$) and a short photoperiod (8-10 h) increased the number of eyes on the microtubers of some cultivars compared with growth in the dark (Gopal et al. 1997, 1998). The increased eye number and shorter dormancy of green compared with white microtubers may explain the better field performance (tuber yield) of the green microtubers (Gopal et al. 1998).

Temperature—While temperatures of 20 to 25 C promote micropropagated plantlet growth, temperatures are generally lowered (15 to 18 C) for microtuber induction (Wang and Hu 1982; Leclerc et al. 1994; Akita and Takayama 1994b). As noted for photoperiod, interactions of temperature with medium sucrose (Koda and Okazawa 1983) and growth regulators (Levy et al. 1993) affect *in vitro* tuberization. These relationships are complex. Koda and Okazawa (1983) found an inter-

action between incubation temperature of 25 C (but not 10 or 30 C) and medium sucrose concentration. Microtuber induction increased at 25 C as the medium sucrose concentration increased from 2% to 8%. Growth regulator effects, especially anti-gibberellin agents, were more important than temperature or other factors in promoting tuber induction from single node cuttings (Levy et al. 1993).

Microtuber-inducing Agents In Vitro: Medium Constituents

Sucrose—The most critical stimulus for tuber formation in intact potato plants is attributed to sucrose (Wang and Hu 1982; Abbott and Belcher 1986 [reviewed by Ewing and Struik 1992]). Sucrose is essential *in vitro* for its osmotic effect (Khuri and Moorby 1995), as an energy source, and, at higher concentrations, it may have a role as a signal for microtuber formation (Perl et al. 1991; Simko 1994; Struik and Wiersema 1999). To maximize microtuber induction, sucrose levels are increased from the 2% to 3% commonly used for micropropagation up to 8% to 9%, regardless of growth regulators (Abbot and Belcher 1986; Garner and Blake 1989; Forti et al. 1991). Sucrose levels above 8% are not beneficial; no yield differences were found when induction took place using 8% to 14% sucrose (Chandra et al. 1992). Once induced, microtuber growth rates were dependent on sucrose availability; growth rates were limited by sucrose hydrolysis to glucose and fructose (Yu et al. 2000).

Nitrogen and nitrogen/carbon ratio—Some cultivars are more sensitive than others to total nitrogen levels or relative concentration of nitrate:ammonium in the medium during micropropagation (Avila et al. 1998) or microtuberization (Garner and Blake 1989; Sarkar and Naik 1998). Low nitrogen in the micropropagation and microtuberization media was best for microtuberization (Stallknecht and Farnsworth 1979; Wattimena 1983). Total nitrogen and nitrate:ammonium levels were used effectively to improve preconditioning, induction (Charles et al. 1995; Zarrabeitia et al. 1997; Sarkar and Naik 1998), and microtuber growth (Chen and Liao 1993; Sarkar and Naik 1998). Reduced ammonium levels during source plant preconditioning resulted in increased numbers and better synchronization of subsequent microtuber induction (Charles et al. 1995; Zarrabeitia et al. 1997; Vreugdenhil et al. 1998). Increased nitrate:ammonium (50 mM nitrate:10 mM ammonium compared with 40 mM nitrate:20 mM ammonium) promoted microtuber growth (Chen and Liao 1993). Most (82%) of microtubers in the higher nitrate:ammonium medium weighed >1.0 g compared with only 22% weighing >1 g in the lower nitrate:ammonia medium after 2 months growth. Changes in total N or ammonium:nitrate ratios of the medium impact on the relative N:C levels (Chen and Liao 1993; Avila et al. 1998). The latter is important in determining carbon use and is reflected in the amount of microtuber dry matter accumulation.

Growth regulators and natural products—There are concerns within the potato seed tuber industry that prolonged exposure to growth regulators during micropropagation or microtuberization may unnecessarily risk somatic change. Growth regulator use has led to such changes or carry-over effects in other plant systems (Garner and Blake 1989; Bizari et al. 1995). Microtuber formation in the presence of BAP and CCC was associated with tuber anomalies in cultivar Desiree, including round instead of elongated shape, reduced number of eyes, larger lenticels, and thinner, less well-organized periderm, compared with microtubers formed in growth regulator-free medium (Nasiruddin and Blake 1994). Accordingly, some propagators rely entirely on environmental inducing agents (such as reduced photoperiod or lowered nitrogen levels or increased nitrate:ammonium ratio), in conjunction with increased medium sucrose levels. However, many others employ growth regulators (cytokinins including BAP, 2-iP, and Kn, auxins including NAA) and/or chemical growth retardants (alar, ancymidol, CCC, coumarin, fluridone, TET).

The literature describing the utility of cytokinins and growth retardants for induction is contradictory, partially because different cultivars, propagules, and incubation conditions were employed. Plantlets 1 month old with several nodes generally produced from one to four microtubers (average of approximately two; consistent with Garner and Blake [1989]) usually at the basal nodes. Exogenous cytokinins may stimulate this process (Levy et al. 1993), promoting both microtuber initiation and growth (Lian et al. 1998), although this is cultivar-dependent and they may only stimulate growth not induction (Gopal et al. 1998). Since apical dominance is completely eliminated in single-node cuttings, exogenous cytokinins were not necessary for microtuberization and 50% to 75% microtuberization occurred in the presence of ancymidol (5 mgL^{-1}). Ancymidol and TET improved synchronization of microtuber induction, without disturbing starch synthesis or patatin gene expresssion (Perl et al. 1991; Vreugdenhil et al. 1994). While CCC increased tuberization in the presence of BAP (Hussey and Stacey 1984; Tovar et al. 1985; Estrada et al. 1986; Rosell et

al. 1987; Lillo 1989), it retarded microtuber development (Lian et al. 1998). The use of growth retardants, which usually suppress GA synthesis, is not always stimulatory to induction. Growth retardants may act to stimulate microtuberization only under weakly inductive conditions such as in the absence of medium sucrose or under long days (Stecco and Tizio 1982; Vecchio et al. 1994) and on a cultivar-specific basis. Growth retardants inhibited tuberization in some cultivars that were able to microtuberize in their absence (Harvey et al. 1991). There are many natural products implicated in the induction process, but much of this information is preliminary and contradictory. For example, tuberonic acid, its glucoside or precursors, especially methyl jasmonate have been implicated in tuber induction (Koda et al. 1988; Yoshihara et al. 1989; Kiyota et al. 1996). Castro et al. (2000) studied tuber development *in vitro* to elucidate the role of jasmonic acid (JA). JA promoted microtuber formation in unrooted but not in rooted cuttings. This suggested that synthesis of GAs by roots somehow antagonized JA action in rooted cuttings. JA may interact with endogenous GAs in stolon meristems to effect tuber formation. However, as JA was found at high concentrations in young tuber periderm (Abdala et al. 1996), an exclusive role for JA in the tuberization mechanism was discounted (Castro et al. 2000)

Commercial Scale-up

Productivity of a culture system can be measured based on the number of microtubers per original node placed in culture and the final harvested microtuber size or fresh weight yield. Where this information was available, information on original node numbers, yields, and culture duration were included. However, it was not easy to compare the relative productivity of different published microtuber production systems. Often there were too many variables for ready comparison, including the cultivars used, medium and environmental conditions, and culture duration.

Relatively small, stationary containers have yielded sufficient microtubers (30-50 microtubers of 200-300 mg per 100 shoots in 4 months) for successful international application (Wang and Hu 1982). However, commercial-scale microtuber production has begun to evolve away from small stationary containers to rotating culture systems, and larger (8- to 10-L) fermentors or bioreactors. In addition, there is a trend toward optimization of up to four distinct production stages (Akita and Takayama 1988, 1993, 1994a, 1994b; Charles et al. 1995; Hulscher et al. 1996; Ziv and Shemesh 1996). These production stages include (a) preconditioning of source plants; (b) pre-induction of propagules derived from the source plants; (c) induction of microtubers on plant material derived from the pre-inductive growth phase; and (d) growth of microtubers.

Fermentors (vessels containing plant material surrounded continuously, or at intervals, with liquid nutrient solution) and bioreactors (vessels containing one or more layers of plant material held on screens or porous substrates subjected to nutrient mist and aeration cycles of varying duration) have been described recently for commercial-scale microtuberization. Theoretically, these can be used to more efficiently increase potato shoot mass and induce the formation of microtubers more synchronously and in greater numbers than in stationary cultures (Akita and Takayama 1988, 1993, 1994a, 1994b; Hulscher et al. 1996; Hao et al. 1998). Ideally, all axillary buds in the unit would respond at the time of induction to form microtubers that would subsequently enlarge to a suitable size for harvest. A limited number of patent applications for large-scale microtuber production have been filed in the USA and elsewhere (Table 2). In reality, commercial scale-up from stationary cultures has been problematic. Some similarities and differences among published methods are outlined below.

Microtuber production in 8- or 10-L airlift-type jar fermentors was described by Akita and Takayama (1988, 1993, 1994a, 1994b) and Hulscher et al. (1996). Micropropagated plantlets were used as a source of 100 single-node cuttings (Akita and Takayama 1994b) or inoculated directly into fermentors (Hulscher et al. 1996). Nodal cuttings were pre-induced for 4 wk during growth into 15- to 20-cm-long shoots under continuous diffuse light (9.4 umol $m^{-2}s^{-1}$ or 2.5 Wm^{-2}) at 25 C (Akita and Takayama 1994a, 1994b). Generally, pre-induction refers to the production of plantlets from the original single-node cuttings used to inoculate the culture vessel. In some cases, inclusion of chemical agents is used to stimulate subsequent tuber induction. For example, plantlets were pre-induced over an 8-wk interval on medium containing the carotenoid biosynthetic inhibitor fluridone (unspecified amount) at 20 C, under a 16/8 h d/n cycle ($9Wm^{-2}$) (Hulscher et al. 1996). Microtubers were induced in the dark on medium containing elevated sucrose (8% or 9%). Reduced temperature (17 instead of 25 C) during induction affected both microtuber number and fresh weights; weights increased when temperatures were returned to 25 C following 2 wk of induction (Akita and Takayama 1994b).

Intermittent medium volume changes (temporary immersion or partial immersion) can be done in a variety of ways; in special containers or using a separate reservoir to store medium. Specific volumes of medium from the reservoir are pumped into the culture container at intervals for various lengths of time. Yields improved with continuous diffuse light (0.9 Wm^{-2}) and intermittent medium volume changes during a microtuber induction and growth period of 6 wk (Akita and Takayama 1994b). Ten-liter fermentors yielded 500 to 960 microtubers of uniform weight with about 18% dry matter (w/w). Induction occurred on 20- to 25-cm shoots in medium with 2.5 mgL^{-1} BAP and 0.02 mgL^{-1} NAA at 20 C over 5 to 10 wk (Hulscher et al. 1996). Varying the surface level of the induction medium (from 8 to 2 L), extending the microtuber growth interval, and refreshing the medium at intervals, all increased yields. Microtuber numbers were 1653+/-50 per unit with a total fresh weight of 1420 g and with 30% of the microtubers > 1 g several months after induction began.

Temporary immersion systems were adapted for potato microtuberization at CIRAD in France using a culture vessel attached to a separate reservoir called a "double RITA" (Teisson and Alvard 1999). These culture vessels permit repeated air pressure-based medium evacuation into a reservoir at intervals during the day. Teisson and Alvard described productivity in their 1-L system after 10 wk; average of up to three microtubers per original node and 90 microtubers per vessel, 50% of which were above 0.5 g. The temporary immersion system described by Jimenez et al. (1999) was similar and also convincingly productive. For the cultivars used, Desiree and Atlantic, each single-node cutting averaged three microtubers after 9 wk in culture in 4-L vessels. In 10-L containers, cv Atlantic averaged 2.6 microtubers per single-node cutting and 1.3 g fresh weight per microtuber. The recommended immersion intervals were 5 min every 3 h with eight immersions per day.

Small plastic containers (1 L), rotated slowly (1 rpm) and covered with air-permeable membranes (Akita and Ohta 1998), or rotated (0.5 rpm) and equipped with ports for forced air flow (50 mL min^{-1} during shoot growth, 150 mL min^{-1} for microtuberization) (Yu et al. 2000) simplify nutrient distribution and aeration. Rotation-based intermittent immersion pro-

TABLE 2 — *Key patents[1] describing microtuber production technology.*

Patent number[2]	Issued (d/m/yr)	Title	Inventor(s)	Applicant(s)	Brief Summary of Invention
EP0388109	19/09/90	Potato production	Joung, H. et al.	Korea Inst. of Science and Tech.	Cv. Superior shoots layered in culture; "microtuberogenic" cultures selected and microtuberized in Petri dishes on solid culture medium.
US05047343	10/09/91	Microtuber propagation of potatoes	Joyce, P.J. , and B.H. McCown	Wisconsin Alumni Res. Found.	Microtuber is used to form a shoot complex; shoot tip necrosis is induced and tuberization occurs on multiple shoot axes.
US5498541	31/05/95	Method for producing C. Sluis	Oka, I. , and	Japan Tobacco Inc.	Plantlets cultured on high sugar (8% w/v) for 1-4 weeks in light, microtuber production in the dark.
US5854066	26/03/97	Process for producing potato microtubers	Oka, I.	Japan Tobacco Inc.	Micropropagation under low sugar, high light levels, exogenous ethylene; microtuber production under high sugar, low light, no ethylene.
US5862626	26/01/99	Process for producing	Onishi, N., K. Hayashida, K. Mamiya	Kirin Beer Kabushiki Kaisha	Micropropagation followed by microtuber formation in alternating light and dark with CO_2 at 0.1-30% (v/v).
WO0005942	10/02/00	Culture container and process for producing potato microtubers by using the same	Chen, Z., Y. Geng, Z. Hu, L. Wang, X Deng, T. Zhang	Chen, Z., Y. Geng, Z. Hu, L. Wang, X Deng, T. Zhang, Inst. of Genetics Chinese Acade.	Specialized container with lid, several ports. Process involves plantlet production from cuttings in shallow medium, under light, with intermittent ventilation; microtuber production in dark, with intermittent ventilation.

[1]Compiled from www.uspto.gov/patft/index.html and http//ep.espacenet.com/

[2]When patents were held in more than one country the patent number reflecting the earliest date of issue was selected.

moted yields in these units that were improved over static cultures. One hundred microtubers were produced per unit in 200 mL of medium (Akita and Ohta 1998). Although microtuber numbers were greater, their dry matter contents were reduced over those described in airlift-type fermentors. Optimal microtuber sizing (>0.5 g) was achieved through frequent medium renewal. Medium renewal is believed to compensate for the rapid sucrose hydrolysis that may limit microtuber growth (Yu et al. 2000). These slowly rotating containers seem relatively inexpensive and simple compared with airlift or immersion-type fermentors. At the moment it is not possible to directly compare yields. All have merit for various research purposes and potential for commercial microtuber production.

A nutrient mist bioreactor was described by Hao et al. (1998). In this unit 30 single-node cuttings, derived from micropropagated plantlets, were supported on each of three layers of screen. Pre-induction for 10 d to favor shoot growth (approx. PAR value of 25 μmol $m^{-2}s^{-1}$, 12/12 d/n cycle) resulted in 3- to 4-cm shoots. Pre-induction doubled the number of single-node cuttings (98%) that subsequently formed microtubers, shortened and synchronized induction (to 1 wk) and increased microtuber uniformity and size. Induction occurred in medium with 8% sucrose and 10 mgL^{-1} BAP (5 min of mist, 10 min of aeration, followed by 2 h off with 5-10 min for the mist to clear), at 18-20 C in the dark for 4 wk. Reported yields were much less than those described using fermentors, and similar to yields in small stationary systems (about one microtuber per node). However, the system appears promising; optimization of media and growing conditions for each of the four microtuber production stages may greatly boost productivity in the bioreactor system.

YIELD AND PERFORMANCE

Microtubers vs Plantlets for Minituber Production

Microtubers are commonly harvested aseptically or are fungicide-treated, dried for a time or suberized in the dark at 20 C in open flats, then cold-stored in refrigerators (4-5 C) to meet dormancy requirements. Greening *in vitro* (16/8 h d/n under 30 μmol $m^{-2}s^{-1}$ for 10 d) prior to harvest may reduce shrinkage and improve sprout emergence following storage (Naik and Sarkar 1997). Microtubers are easier to transport and handle than plantlets and are less delicate, so require less aftercare, when planted in a greenhouse or screenhouse (Wang and Hu 1982; Hoque et al. 1996). If microtubers are compared directly with whole plantlets, for essentially the same number of propagules, microtuber production takes 1-2 months longer than production of plantlets and microtubers must be harvested, stored, and their dormancy needs met before they can be planted (Ahloowalia 1994). For these reasons Ranalli (1997) questioned their advantage over plantlets for minituber production, but this point has been very difficult to resolve. Microtuber productivity comparisons are confounded by the many variables involved in their production, storage, pre-emergence treatment, and cultural practices following planting. Of particular import are microtuber size, number of eyes, physiological age, and dormancy-breaking treatments. Comparisons with other propagules are equally confounded by a number of factors including differences in plantlet production, time in culture, genotype (Ahloowalia 1994), size of cutting (whole plantlets vs nodal cuttings), nodal position (Ali et al. 1995), and after-effects of nutrient and growth regulator levels.

With respect to yield in nursery beds, microtubers produced similar total fresh weights, but fewer minitubers than plantlets did; yield on a per stem basis was greater for the plantlets at two planting densities (Wiersema et al. 1987). When microtubers of 10- to 28-mm diameter were planted at a density of 100 m^{-2} the yields were greatest for microtubers that had been stored longest (7 months storage, 500 minitubers m^{-2}) and least for those stored for the shortest time (3 months storage, 200 minitubers m^{-2}) (Molet 1991). Results were similar for microtubers in the 7- to 9-mm range; larger, physiologically older microtubers out-produced smaller or younger microtubers (Désiré et al. 1995) in high-density nursery beds. Microtuber yields were similar to stem cuttings (Ahloowalia 1994); the latter outperformed whole plantlets. For example, stem cuttings outperformed plantlets in a greenhouse minituber production system (three to five times greater yield) but were not compared with microtubers (Ali et al. 1995).

Microtubers vs Other Propagules for Field Tuber Production

Yield comparisons between microtubers, plantlets, minitubers, and conventional seed tuber pieces in the field did not always agree. Yields from plantlets were less (Haverkort and van der Zaag 1989) or similar (Wattimena et al. 1983; McCown and Wattimena 1987; Leclerc and Donnelly 1990) to yields from 40- to 60-g whole seed tubers. Disagreement exists

regarding the practicality of directly field-planting microtubers. Some have found it easy to adapt them to large-scale mechanized field-planting (reviewed by Naik et al. 1998). In other cases, directly field-planted microtubers were not found to be practical; crop development was too slow. However, when pre-planted in a greenhouse and transferred to plastic mulch in the field, larger microtubers (>0.5 g) significantly outyielded plantlets and gave comparable yields to seed tubers for late, but not early maturing cultivars (Haverkort et al. 1991). For later-maturing cultivars, plants from microtubers formed greater amounts of foliage before tuber induction, resulting in greater yields than the early cultivars. Physiologically older microtubers also performed better in the field than younger ones. This was apparent for late but not early cultivars (Ranalli et al. 1994a). Small microtubers (0.090-0.120 g) yielded far less tuber fresh weight than minitubers or seed tubers (Ranalli et al. 1994b). However, total tuber number per m^2 was quite high (160) at close row spacing (60 cm). There have been disturbing reports of increased sensitivity in transplants or minitubers to potato viruses S and Y transmission or spread compared with plants from field grown tubers (McDonald 1987). Boiteau et al. (2000) suggested that these concerns have been partly responsible for the limited use of *ex vitro* transplants. However, their research demonstrated that plant origin has very little to do with aphid colonization, which is more influenced by factors such as relative plant height, surface features and maturity. This report should lead to renewed interest in the use of *ex vitro* propagules and minitubers in the seed tuber industry.

Microtuber vs Minituber Productivity

Researchers in Taiwan (Wang and Hu 1982) reported 36,000 microtubers per 10 m^2 within 4 months in stationary cultures. These microtubers produced 1,800 t seed tubers in three field seasons, enough for national needs. Private companies claim annual productivity of up to 50,000 microtubers (0.2 to 1.0 g) per 10 m^2 per year (reviewed by Haverkort and van der Zaag 1989). The establishment rate (approx. 93%) and productivity (three to six, average is approximately five minitubers) of these microtubers in greenhouse production systems is excellent (Rasco et al. 1995). When directly planted to the field, claims of 10-fold increases in number have been made (reviewed by Haverkort and van der Zaag 1989), although this increased level in the field is usually associated with larger propagules such as minitubers (Rasco et al. 1995). While they did not indicate where their figures were derived, Haverkort and van der Zaag (1989) claimed that production cost comparisons for certified seed derived from microtubers or minitubers were similar to that from pre-basic seed after three to four field multiplications while disease status is likely to be improved. Comparative field performance (but not relative costs) of plantlets, microtubers, minitubers and small tubers was reviewed by Struik and Wiersema (1999). They concluded that optimal choice of production system is determined by many variables. Most critical are field performance in a given site (especially season duration), required amount of pre-basic seed, disease pressure, and costs and availability of specialized facilities.

Microtuber and minituber production technologies are both evolving rapidly. Predictions were that the relative costs of microtubers and minitubers were eventually likely to favor microtubers. The rationale was that microtubers were produced aseptically, and automation would reduce the costs (Haverkort and van der Zaag 1989). We tend to agree with these predictions since microtuber production efficiency has improved dramatically. Any breakthrough technology that could increase microtuber size has the potential to completely eliminate the role of minitubers. However, new hydroponic systems have substantially lowered the costs of minituber production compared with early greenhouse or screenhouse production (especially in temperate climates where greenhouse costs are high and screenhouses can be used for only one crop per year). Hydroponically grown minitubers, such as the Technituber® (Austrialian proprietary technology) are produced under stringent sanitary conditions in high-density plantings. Minitubers are harvested at intervals from plants growing in nutrient film (Gable et al. 1990). Alternatively, many successive crops can be grown per year on rockwool slabs using short (70-90 d) production cycles (Lowe 1999). Unfortunately, we have not been able to compare the costs of minituber production in these proprietary systems with conventional production in greenhouses or screenhouses.

Optimizing Microtuber Performance

The contributions of the mother tuber to plant growth and subsequent tuber production have been extensively discussed (Ewing and Struik 1992) and characteristics applied to small seed tubers (<50 g) would seem to apply equally to microtubers. Small mother tubers exhibit slow initial growth rates, including higher shoot/root ratios. Like other transplants, microtubers tend to sprout at a single eye. There is a general

tendency for these plants, with few main stems, to exhibit increased axillary branching. When photoperiod is short relative to the critical photoperiod for the genotype, premature tuberization may occur.

Optimizing performance of microtubers of all sizes must include efforts to improve physiological aging treatments (Ranalli et al. 1994a). Physiological aging is affected by genotype, storage interval and degree of dormancy and affects sprouting vigor and emergence. Both total degree-days and timing of degree-day accumulation during storage are important in determining physiological age. Following treatments for physiological aging, microtubers can be field planted or planted in greenhouses in plug trays, treated like bedding plants, hardened-off and transplanted mechanically to the field. Transplants reduce the effect of initial tuber weight and take greatest advantage of a short production cycle in the field (Haverkort et al. 1991). Yield can be further increased by transplanting under floating plastic film as well as careful irrigation and fertilization (Ranalli 1997).

Clearly, there are many potential remedial measures available to increase the productivity of microtubers. In addition, various treatments recommended to increase minituber production from plantlets may prove equally useful for microtuber-derived plants. These include bacterization using a pseudomonad sp. (Nowak et al. 1999), planting into mycorrhiza-inoculated medium (Niemira et al. 1995, 1996) or foliar application of plant growth regulators such as paclobutrazol (Bandara and Tanino 1995), or cytokinins (Caldiz 1996).

IN VITRO SCREENING FOR AGRONOMIC CHARACTERS

Potato plant breeders may typically evaluate tens to hundreds of thousands of seedlings each year. This testing is usually done in the field over several different location years. These evaluations are "cumbersome, labor intensive, and time consuming" (Gopal and Minocha 1998:67). One concern is that small sample sizes (one plant) and microenvironment effects may contribute to sampling error and inaccuracy in determining potentially useful agronomic qualities (Gopal and Minocha 1997). The utility of *in vitro* screening of putative transformants, at least as an initial screening mechanism for virus resistance, was demonstrated with potato (Russo and Slack 1998). Sampling for ELISA testing from putative virus resistant transformants was as effective as from greenhouse-grown *ex vitro* transplants and was considerably faster and less costly.

In vitro multiplication of advanced selections may alleviate some constraints resulting from restricted material present in the early generations and may lead to more rapid cultivar release. Alsadon et al. (1988) and Lentini (1988) first indicated, based on a few cultivars, that tuber yield and related characteristics could be evaluated *in vitro* and would reflect *in vivo* performance. Gopal and co-workers have investigated the possibility that crops raised from plantlets or microtubers could contribute to effective selection (Gopal and Minocha 1997; Gopal et al. 1997). They evaluated 18 potato genotypes by comparing a seed tuber crop that was increased over a 2-year period with microtuber-derived crops. Selection in the greenhouse using microtuber-derived crops was very effective for characteristics including plant height, stem habit, tuber color, and general impression. It was only moderately effective for internode length and leaf characteristics, stem pigment, plant vigor, and tuber shape. They concluded that selection could be done at the microtuber crop level for general impression and all of the important tuber characteristics (color, shape, yield, and average weight).

Gopal and Minocha (1998) then evaluated 22 genotypes which were compared in the field, over two spring and two fall seasons and under eight growing conditions *in vitro*. Tuber and stem color, number of eyes, and relative yield characteristics could be evaluated directly on microtubers grown in some, but not all, *in vitro* media, photoperiod, and flux density conditions. Genotypes could be more reliably evaluated *in vitro* than *in vivo*; error mean squares *in vitro* were much lower than genotype mean squares *in vivo*. Microtuberization in the dark gave microtubers with more realistic colors than those induced in the light, which tended to have greenish skin color, although eye-colors were pigmented normally. For some criteria, such as number of eyes and tuber weight, 50% of genotypes could be safely discarded based on microtuber evaluation. Other criteria, including plant vigor, foliage senescence, and tuber yield, were not as efficiently selected *in vitro*. Naik et al. (1998) compared 37 cultivars divided into early, medium and late maturing groups for yield components *in vitro* and in the field. Microtuber number was more important than weight in determining yield in the field; perhaps partly because microtubers were presprouted and planted in the greenhouse for 3 wk prior to field planting. Naik et al. (1998) concluded that *in vitro* yield performance was not an accurate measure of field performance.

To increase the efficacy of *in vitro* screening, environmental conditions should be investigated in culture that will

maximize the expression and simulate the phenotypic expression found in *in vivo* conditions (Gopal and Minocha 1998). The same principal may apply to *in vitro* screening for abiotic stress tolerance. Abiotic stress tolerance often has not been evaluated *in vitro*, although the potential has been demonstrated. The use of microtubers for heat tolerance screening has been suggested based on a limited number of cultivars (Nowak and Colborne 1989). Also, Gopal and Minocha (1998) indicated that such screening was used to help identify a putative heat tolerant genotype. Screening for salinity (NaCl) tolerance was done *in vitro* using single-node cuttings and microtuber bioassays, which agreed and were supported by field-lysimeter results (Zhang and Donnelly 1997).

GERMPLASM STORAGE AND EXCHANGE

Factors that have contributed to the increasing importance of microtubers include recognition of their storability, phytosanitary advantages, and ease of transport (Dodds 1988). As a back-up to micropropagated potato plantlets most germplasm repositories now keep cold-stored microtubers (Altoveros et al. 1996). For germplasm distribution and exchange microtubers demonstrated several advantages compared with plantlets (Tovar et al. 1985; Estrada et al. 1986; Ranalli 1997). The choice of propagule for transport is determined by availability of requested germplasm, and the proposed end-use when the material goes from the repository to another facility. The additional expense of producing microtubers may be warranted when medium-long term storage or transport is involved (Dodds 1988; Veramendi et al. 1998; Struik and Wiersema 1999). Microtubers can be stored at room or fridge (4 C) temperatures for many months (Dodds 1988). Alternatively, in vitro shoots or plantlets with attached microtubers may be held under conditions suitable for either multiplication or conservation for medium-term storage (Veramendi et al. 1998) or in the dark at 4 to10 C for up to several years (Kwiatkowski et al. 1988; Fletcher et al. 1998). Light-induced greening was examined for its effect on storage behavior in 16 genotypes (Naik and Sarkar 1997). Greening promoted shorter dormancy (Gopal et al. 1997) and improved microtuber storage, possibly due to increased thickening or suberization of the periderm. Greening was also associated with increased resistance to pests and disease (Percival et al. 1998).

CONCLUSIONS

Microtubers are a critical part of seed tuber production programs in many countries where isolated vector-free production areas are not available (e.g., Bangladesh, Italy, Philippines, South Africa, South Korea, Taiwan, among others). They are also important components, along with plantlets and minitubers, for seed tuber production programs in Europe, North America and some South American countries. They are likely to become especially important in the most rapidly expanding production areas in China and India. The implementation of microtuber production systems have contributed to improved self-sufficiency, reduced number of field generations in seed tuber production, and reduced disease incidence in commercial plantings. In some cases, microtuber use has led to dramatic increases in quality of seed tubers, which have resulted in huge yield increases (e.g., 35 tha^{-1} up from 20 tha^{-1} in south Korea; Hoque et al. [1996]). Overall, microtubers are an important tool for the seed potato producer; providing an alternative to plantlets for direct planting, conservation, and exchange.

The past decade has seen major advances in microtuber production efficiency including scale-up to fermentors and bioreactors. These systems are primarily experimental, but commercialization has begun (i.e., Osmotek, Israel). The size and dormancy issues that constrained the use of microtubers for field planting a decade ago (Jones 1988) have partly been overcome (e.g., Akita and Takayami 1994b; Coleman and Coleman 2000). Furthermore, there appears to be a strong and consistent analogy for induction, growth and development, and agronomic characteristics, even abiotic stress tolerances between microtubers and field-grown tubers (Coleman et al. 2001). This serves to underline the importance of microtubers as a tool for basic research as well as a propagule for seed tuber production and conservation systems.

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