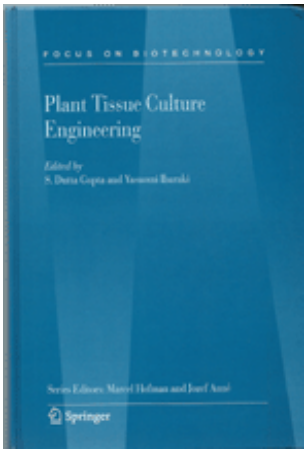

Integrating Automation Technologies With Commercial Micropropagation

An economic perspective

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Abstract

Significant advances have been made in micropropagation technologies at both the laboratory and greenhouse levels which offer promise for future mechanization. Several mechanized or automated processes are already seen in commercial practice. This chapter will discuss the various types of mechanization with an emphasis on commercial applications throughout the world. Sections will be devoted to the individual technologies followed by specific analysis of the advantages and disadvantages of the systems relative to commercial implementation. Batch fermentation coupled with bud cluster growth habit modification, and robotics, guided by vision systems, in both laboratory and greenhouse applications will be reviewed. Significant factors key to the commercialization of automation will be discussed, including: 1) bacterial contamination management, starting with culture indexing; 2) economics, especially as regards seasonal production and competitive labor; and, 3) growth habit modification strategies, focussing on bud clusters versus linear growth habits. Potato tissue culture illustrates the complexity of the economic and biological issues, internationally, and will be discussed in depth as an example of a species which can be micropropagated either via linear growth habit, suitable for robotic manipulations, or as a bud clusters, suitable for indiscriminate chopping.

Keywords: micropropagation, biofermentation, bioreactors, cost accounting, robotics

1. Introduction

Replacement of the people who do micropropagation work in laminar flow hoods, with equipment of any kind, is neither technologically simple nor readily economically achievable. The fundamental fact remains that the human eye-hand-brain combination is both highly sophisticated, technologically, and incredibly inexpensive, certainly when considered on a global scale. Consequently, commercial micropropagation companies in both Europe and North America have followed the path of lower costs to those countries for which the infrastructure, such as reliable power supplies, and logistics, such as political stability and transportation issues, are favorable.

Cost accounting needs to take into consideration many factors which are not always obvious at the onset of a project; in the case of micropropagation these include risk assessments, refinement of protocols, and employee training. Aseptic culture systems are vulnerable to bacterial, fungal and even insect contaminants which can destroy the

plantlets, as well as to genetic and epigenetic shifts, which can seriously impair their quality. The transfer of the operation from human to mechanical means can differentially affect each of these factors. The costs of maintaining a high level of genetic purity and the risk of contamination must be factored into the long-term costs of mechanized systems. The history of micropropagation has created a legacy of sudden, disastrous plantlet losses, the magnitude of which have cooled the ardor of all but the most hardy researchers. Likewise, the financing and funding of various companies and projects has been erratic, often resulting in a lack of continuity and instability; as evidenced by ventures such as Plant Genetics, of the United States, based on scale-up of somatic embryogenesis [1], ForBio, of Australia, focussed on elite tree micropropagation using robotics [2] and Osmotek, of Israel, a supplier of plastics for biofermentation and liquid culture systems.

2. Biological Parameters

2.1. The plants growth form affects mechanized handling.

The list of plants which can be grown in vitro is broad and covers many genera [3,4]; nonetheless, the vast majority of plant species are not able to be economically micropropagated, due either to technical difficulties in tissue culture or to their expense relative to standard propagation by seed, cuttings, tubers or bulbs. The growth form of a plant can be significantly modified in vitro by the use of plant growth regulators and environmental controls, so that a plant which normally grows as a linear vine, such as a potato, can become either a linear, straight-stemmed plantlet, such as is commonly seen in test tubes, or a dense compact cluster of buds, such as is possible in liquid culture using ancymidol, or even a linear microtuber, as can occur under certain environmental conditions. Other genera naturally grow as rosettes, and inducement of axillary bud growth results in dense masses of tiny shoots (see the Limonium plantlets in Figures 1 and 2). For example, in the case of potato and carnation, the preferred growth form for robotic access and separation has been linear; the plantlets can be grown upright, then laid flat for cutting (see Figures 3 and 4) or they can be grown in shallow plastic boxes with domed tops and repeatedly hedged, as was described by Aitken-Christie for pine [5]. Both of these methods are effective in increasing access for mechanical handling of the plantlets. Potatoes can be grown in liquid culture as nodes [6,7,8] as bud clusters [9,10,11] or made into microtubers [6,8,12,13] or even somatic embryos [14]. However, none of these methods have been scaled up to millions of plantlets due to two barriers: 1) economics: the potato industry is based on tuber seedpieces costing less than a penny apiece, and 2) size issues: in North America, field conditions dictate that the tuber seedpiece will not be replaced by anything smaller than a greenhouse minituber for many years to come.

Historically, commercial micropropagation was based on enhanced axillary bud break; overcoming the natural apical dominance with cytokinins and other factors to encourage lateral buds to grow out into shoots; this increases the number of shoots per culture per month, the multiplication rate. However, if strategies with lower multiplication rates, for example, straight stemmed, unbranched shoots, give significant advantages to mechanization, then branching options may been to be reexamined within the new framework. Multiplication rates of greater than 10-fold per month can be achieved in tissue culture (see Figures 1 and 2); however, these may not contribute significantly to reducing the end-product cost if the labor for singulation/rooting is thereby greatly increased (see Table 1) and/or the quality of the plantlets decreased.

Somatic embryogenesis continues to be a highly attractive biological strategy for large-scale production research, despite the difficulties, although full automation still appears to be years away. The largest ongoing operations based on this technology appear to be in the forestry sectors, where manual handling of the output embryos is still the norm, dramatically raising final costs [15]. Even if the embryos cost next to nothing apiece and can be made in the hundreds of thousands, a single manual handling step, such as singulation or planting, can make the system economically prohibitive [16]. The genetic component within species regarding the ability to form somatic embryos can be significant

[14] as is the potential for loss of genetic fidelity in the pre-embryoid tissues. Genetic testing technologies are assisting vegetable breeding companies in confirming the true-to-type characteristics critical for seed parents (Rijk Zwaan, personal communication) and the existing automation of PCR testing of cotyledon discs could enable future monitoring of somatic embryo-derived plugs; however, the automated somatic seed concept still appears many years off [17,18].

On an international scale, another biological method for micropropagation, known as bud clusters, has gained widespread acceptance both for its potential application to many species, as well as its obvious physical compatibility with mechanical handling [19]. One method developed by the late R. Levin [20] and M. Ziv [11,21,22], combines the bud cluster growth form, either in liquid culture or on agar, with a simple fixed-blade mechanical cutting device, such as a grid of blades, allowing the clusters to be mechanically subdivided into up to 100 pieces with one operation; this has been shown to work in potato, lilies and several other crops.

The most common media typically used for induction of bud cluster growth patterns involve the use of liquid culture, a gibberellin inhibitor, such as ancymidol, and an axillary bud growth promoting agent, such as the cytokinin benzylaminopurine. The bud cluster induction treatment needs to be repeated serially for several subcultures to establish the formation of true clusters. It is difficult to scale up to commercial levels in liquid culture systems, due to hyperhydricity [21] and bacterial contamination problems. While this avenue of production research has great potential for long-term production in high volumes of quality plantlets, the difficulties remain problematic and the limitations, especially for commercial laboratories, remain significant. Several major genera of plants already in mass propagation via tissue culture are quite amenable to the liquid bud cluster construct, as they readily form a densely compact mass of basal proliferation and are tolerant of high humidities, liquid environments and mechanical damage. These will probably be propagated in increasing numbers over time using biofermentation approaches.

Researchers in several crops and from several countries are scaling up the bud cluster system [11,19,21,22,23,24]. Basically, cluster culture involves the reduction of the tissue culture plantlet to a compact mass of leafless, highly branched, short masses of buds; there is little or no callus proliferation or adventitious bud formation. These organized bud clusters are then maintained in a multiplication mode as long as necessary for production of sufficient numbers to meet the goals of the project. When it is time for finishing the plantlets, the pressure of the cytokinin/growth retardant combination is removed and the shoots grow out into their normal morphology.

Currently the most advanced commercial biofermentation systems in application are based on the incubation of cultures in a redesigned biofermentation vessel consisting of a five or ten liter autoclavable plastic bag, similar to a medical medium or serum bag, complete with input and output ports. Implementation of this technology is being intensely pursued by at least two major high volume laboratories in North America. Rather than being an automated system, biofermentation of bud clusters is actually still an operator-assist method, the subcultures are still carried out by hood operators; the vessels combined with bud clusters greatly increases the productivity of the operator and hence significantly reduces the cost per plantlet, while still benefiting from human decision-making. Attempts to automate these systems further have not yet been realized, but are nearing. The bag fermentors, equipment and supplies facilitating the production of plantlets, bud clusters, somatic embryos and other propagules in liquid fermentation was commercially available prior to 2004, but the withdrawal of the manufacturer currently makes the development of liquid bud cluster systems less accessible to smaller operations.

Liquid culture systems are, in general, more difficult to stabilize, maintain and commercialize than simple agar-based standards. Humidity must be carefully managed for maintenance of consistent medium volumes and component concentrations. In smaller vessels, the variability between vessels can be dramatic. When propagation is transferred

from agar-based to liquid many factors in the medium itself will need to be adjusted. In some cases this can amount to starting from scratch, never an attractive option for the tissue culture propagation laboratory.

Many plants do not take easily to being submerged in liquid. To overcome problems such as hyperhydration, deformed growth, insufficient cuticles and other side effects of oxygen depletion and underwater growth, enhanced oxygenation of the solution, and timed, temporary immersion rather than full-time exposure to the liquid environment can improve the quality of plantlets substantially [25,26]. However, intermittent flooding, while clearly of benefit to many species, is cumbersome and even more prone to difficulties with contamination, so challenges remain [21].

2.2. Microbial contaminants hinder scale-up.

Microbes present much more of a threat to the mass propagation of plants in vitro than they do in greenhouses. Normally harmless, airborne organisms, such as molds, yeasts and otherwise unheard of bacteria [27,28,29,30,31], become lethal to plantlets in the micropropagation environment, simply by overwhelming the cultures. Internal bacteria, some of which can be quite significant, can be carried at extremely low populations for years without detection [32].

Plant tissue culture originated in tightly capped, glass culture vessels using very small tissues, such as meristems, which had no capacity to produce sufficient photosynthate for growth and development. Consequently, sugars were required in the medium, and sugars are used in nearly all of todays commercial laboratories, including our own. Plants do not normally require extraneous sugar for growth and development; the artificial conditions of restricted gas exchange, low light levels and high humidity, incur the need for sugar in tissue culture media. While true meristems, embryos, protoplasts and other tissues certainly require carbohydrate sustenance from without; micropropagated plantlets are fully capable of supporting themselves. The micropropagation industry has paid heavily for its reliance on sugar, both from the severe restrictions on automation and mechanization research resulting from the extreme requirements for sterility in any process involving sugar-based production, and from the plantlet losses during transitioning due to weaknesses in the epidermal tissues and root systems [33,34,35,36,37,38]. Photoautotrophy has clearly been demonstrated to produce healthy and vigorous plants, but it has not been fully incorporated into production laboratories.

Photoautotrophy, which clearly reduces the bloom of microorganisms and which equally clearly promotes healthy plantlet growth, has not been an easy goal to attain at the commercial level, in part due to the reluctance to aerate the culture vessels, thereby risking contamination, which can be a very real problem, and in part due to reluctance to spend significant funds on facilities and culture vessel modifications. The requirements for environmental controls and modified vessels are somewhat stringent in order to achieve true parity on a production scale. Cutting corners, while still permitting improvements in plant performance, do not help with bacterial control in automation research, as even a little sugar in the medium will support very vigorous microbial populations. Although green plantlets conduct photosynthesis while in tissue culture, the rates are often low and reliance on sugar is high, even in the greenest plantlets. Still, it is logical that photoautotrophy or at least enhanced photomixotrophy [25,38] will become standard for standard types of commercial propagation in vitro.

Culture indexing, whereby plantlets or tissues are assayed for the presence of internal, or non-obvious, bacteria is commonly practiced using several standard media which encourage bacterial growth, such as nutrient broth and potato dextrose agar. While culture indexing is important in agar-based systems, it is critical for liquid-based systems, where contamination can overtake the cultures within a matter of days, or even hours.

Sterility is critical to maximum batch size, as a greater percentage of the plantlets produced are at risk when more explants are in a single vessel. Obviously, if plantlets are subcultured in test tubes, and 1% of the explants are contaminated, then 1% of the

plantlets are lost; however, if 50 plantlets are subcultured into each culture vessel, a 1% contamination rate quickly adds up to many more plantlets being lost.

Antibiotics and bactericides, such as hydrogen peroxide and sodium hypochloride, have been added to culture media to kill bacteria, or at least inhibit their growth [30]. Other strategies, such as refrigeration, filtration or ozonation of the recirculation medium, have been implemented to a lesser degree [39,40].

3. Physical Parameters

Several physical parameters can be re-examined for potential modifications or options which may favor new automation or mechanization technologies. Physical constraints which have been accepted as fixed for standard parameters may need to be modified in order to make new systems feasible. For example, the benefits of automation on final cost-per-unit may ultimately outweigh the subsidiary input costs of using more expensive culture vessels. The benefits of photoautotrophy may outweigh the outlay of expenses for culture room modifications.

3.1. Culture Vessels

The physical parameters of the micropropagation system begin with the choice of culture vessel. The culture vessel either permits ready access or hinders it; it allows varying degrees of gas exchange and clarity, and it has an impact on plantlet growth and quality. Many factors come into play when choosing a vessel for commercial propagation. Inexpensive culture vessels which impede operators are, in fact, far more costly than slightly more expensive culture vessels which streamline labor. From a materials-handling perspective, glass is heavy, awkward and requires washing, an added expense. From an access perspective, test tubes are seriously limiting, and operators can rarely handle more than 800 per day; but test tubes retain their usefulness in many applications, including culture initiations and germplasm maintenance. Culture vessels may be designed specifically with an automation device in mind, as is the case with most robotic applications [41,42].

The choice of culture vessel is also important to controlling contamination losses: the larger the vessel, the greater the number of plantlets which are lost with each introduced contaminant. Consequently, the use of larger vessels typically requires ultra-clean laboratories, incurring additional facilities costs [43].

In addition to the higher multiplication rates attainable in 10L liquid culture bags, these vessels have good accessibility throughout the subculture cycle, and daily operator productivity, can be increased substantially as a result.

3.2. Physical orientation of explants for subculture or singulation

Over the past 20 years, many different concepts for the mechanization or automation of micropropagation have been envisioned; originally, mechanical approaches were based on either robotics with computer imaging, for cutting of straight stemmed cultures (potatoes, trees, sugarcane, carnations)[41,42], or adventitious regeneration approaches, which are combined a blender approach to cutting of tissues, with species such as ferns. Subsequently, researchers studied the semi-automated production of artificial seeds using somatic embryos [2,7,18].

Each of these systems had its drawbacks and limitations. For mass regeneration systems, the phenotypic and genotypic changes of somatic embryos were problematic in crops which required a high degree of uniformity [17]. For robotic cutting systems, there were few suitable crops needed in the volumes required to amortize the high costs of the initial production line and its maintenance, and there were ongoing issues of low speed relative to the human operator. In the case of somatic seed, commercial efforts still had a heavy reliance on operators at the final stages of singulation and sorting.

Bud clusters are physically compatible with random, or spatial, mechanical cutting equipment in the multiplication stages, as there are so many buds in various stages of development that damage to a certain percentage of them is bearable. Once true bud clusters have been created, subdividing the clusters by means of mechanical, fixed blade cutting devices becomes feasible [9,22,24]. For potatoes, even operator-assist devices,

such as grid blades (similar to French fry cutters) can greatly increase efficiency, as essentially 25-36 subdivisions can take place with one cut. Resterilization of the grid blades over the course of the day is not any more cumbersome than resterilization of forceps and scalpels, but the cost of multiple tools and handling the cutting devices is slightly more expensive and awkward.

3.3. Gas phase of the culture vessel impacts automation.

Plantlets grown under conditions of reduced humidity, reduced ethylene, adequate carbon dioxide and adequate oxygen perform better during the transitioning period, which is instrumental to elimination of the tissue culture rooting stage. The choice of vessel influences the amount of gas exchange possible between the sterile interior and ambient, or external air. Currently, biofermentation using temporary immersion or nutrient film delivery techniques, rather than full submersion, can provide environments that are highly favorable to the plantlet in terms of both photosynthetic activity and epidermal function.

Innovations in photoautotrophy are accompanied with greater understandings of the effects of light spectra and intensity on the quality of plantlets [44,45,46]. Research into chopper light may allow significant savings in cooling costs, as well as decrease electrical costs for lighting.

Greenhouse operations have been adding carbon dioxide to the plant environment for years. Increased carbon dioxide in the growth room (at 2-4x ambient levels) can enhance the performance of plantlets even on sugar-based media, especially when culture vessels are well vented. Advances in porous filters and tapes (i.e. 3M Micropore tape) have enabled the venting of many previously sealed containers.

4. Economic Parameters

For any new technology, such as automation of micropropagation, the primary indicator of its commercial potential is its projected impact on the cost of the plantlet. While true cost accounting is a complex and multifaceted task that is required for ongoing operations and fine decision making [47], it can be simplified for the purposes of preliminary evaluations. For this purpose Table 1 was designed to permit comparison of various factors, such as labor daily costs and multiplication factors; it is a model only, each major crop group within each commercial laboratory requires its own analysis for accurate cost accounting.

4.1. Baseline Cost Models

The total payroll of micropropagation laboratories is typically over 65% of the monthly budget; however, this does not give an accurate picture of the pyramid of costs linked to each hood operator hour. Costs need to take into account all aspects of the operation, so one simplistic approach, used by several laboratories including ours, is to take the total monthly outlays and divide them by the parameter being evaluated, for example hood operator hours per month (excluding medium preparation, dishwashing and other non-hood activities), for an average cost per hour of the hood work. Although this is overly simplistic, it is useful for evaluating the impact of various systems. For standard agar-based systems, the fully loaded hood operator rate is ranging between $27 and $42 per hour, which is actually 20-25% of the total monthly expenses. This figure can then be divided by the average annual plantlet output per hour for a very rough general cost per plantlet. The average annual plantlet sales per hood operator are on the order of 200,000 plantlets for laboratories producing steady volumes of a spectrum of standard ornamentals; this number converts to approximately 100 sellable plantlets per hour per operator over the course of a typical 2,000 hour year. Using this average number, with the average hourly cost of $35 per hour, gives an average plantlet cost of US$0.35 apiece, a figure which will then require a sales price of US$0.45 or above per plantlet rooted in vitro (Stage IIIb). In fact, very few plantlets in the United States or Europe are sold for much less.

Given these high costs, and correlating high prices, it is easy to see why micropropagation is increasingly taking place in India, Singapore, South Africa, China and Eastern Europe, where direct wages can be as low as US$0.40-50 per hood operator hour; less than US$2.00 per fully loaded hood operator hour, giving an estimated final

plantlet cost of US$0.03 (see Table 1).

4.2. Economics of Operator-Assist Strategies

While automation research is extensive and ongoing at university and government levels [16,21,48,49,50,51,52] some work is actually accessible to commercial laboratories.

A person can perform a typical cut-and-place operation, without any additional steps, in 3 seconds, which translates into a theoretical maximum of 9000 per operator day (7.5 working hours). This simple calculation reveals that the hood operator is seriously under-optimized, from the standpoint of the inherent skills in the human complex of hand-eye-brain, which are essential to the tasks of: 1) explant analysis for selection of the cuts, 2) making the cuts with minimal damage, and, 3) sorting and placing the explants in new media. We have seen one operator reach 8000 potato node cuttings in a single day, when supplied with ideal plantlets grown in 25x150(mm) petri dishes at 20 nodes per dish; in this case, plantlets were hedged, i.e., cut in place, they were not removed from the agar in order to be cut. The workstation was expanded to a full eight foot hood, or 24 square feet of workspace, sufficient for the entire load of inputs and outputs. We have also seen daily rates with potatoes of 8000 with 2-person teams using electric knife hedging systems (one operator shears 2 cm. long microcuttings onto a paper towel roll while a second operator selects and places the cuttings into fresh medium, with the assistance of a foot-pedal operated conveyor). Realistically, it is difficult to maintain operator rates over 3000 for many weeks on end, as people prefer to work at a lesser level of concentration for the pay scale typical of micropropagation jobs. Subsequently, on a sustainable monthly basis, operator performance rates for average tissue culture laboratories stabilize at around 1200 per day. This is similar to equivalent tasks, such as hand grafting of vegetable transplants (J. Boskermolen, JOBU plastics, personal communication) and transplanting of 288 cell seedling flats to 4 inch (10 cm.) pots on conveyor lines (L. Oki, UCD personal communication).

It seems probable that sustainable operator rates of 2400 plantlets per day are attainable with operator-assist strategies at slightly elevated pay rates. However, some degree of alleviation of the cut-and-place operations is usually necessary for most operators to sustain performance over long periods of time.

4.3. Organization of the approach to rooting: in vitro or ex vitro.

Table 1 is based on a single one-to-one handling step by a person; a single step can be costly, but if more than one direct step is needed, costs can dramatically increase. If the single one-to-one manual step takes place in the greenhouse, or aseptically, costs are less than if the handling occurs under sterile conditions. Research aimed at elimination of the final rooting stage in vitro has an immediate impact on costs, which, while not as dramatic as automation, is still very worthwhile. The combination of semi-automated sterile fermentor production with plantlets that can be separated and planted in the greenhouse has many advantages for the commercial laboratory. Bud masses can be shipped from foreign laboratories more readily than plantlets can, and certainly more readily than once they are in soil. Mechanization of the greenhouse planting of tissue cultured plantlets is clearly already functioning on a commercial scale. Final refinements in technologies which singulate, or unitize, plantable explants from fermentation vessels will bring micropropagation to the next level of commercialization.

4.4. Economics of New Technologies

The general consensus of the micropropagation industry regarding new technologies is that the growth of cultures in liquid temporary immersion systems can drive the price down by 50%, after a somewhat significant learning curve, and, in the hands of skilled personnel can drop the price by as much as 67%. However, there is a significant increase in the degree of precision and sterility required throughout the production process and sudden losses, due to slight variations in medium, environment or other parameters, can still be costly and disruptive.

Overlays [53,54] and hedging [5] are still very viable ways of bringing costs down while maintaining quality and true-to-type characteristics of non-callus based propagation

systems [17]. These have the added advantage of being adaptable to many species and compatible with both high volume and low volume applications.

Operator assist mechanisms, such as mechanical grafting equipment, are not always commercially successful. Vegetable transplants in the Netherlands are grafted, or stinted, by hand despite the existence of mechanized equipment. The vegetable seedlings are selected on a wide variety of criterion, then matched, scion to rootstock, cut and clipped; the grafts are slightly more successful with trained personnel, working with the clips manually, than they are with existing machinery, and there is insufficient financial incentive to stay with the mechanized grafting equipment. In micropropagation work, a similar degree of refinement exists in decision-making at the operator level; consequently, it seems logical that the very last step to be mechanized will be the singulation of difficult species.

5. Business Parameters

In addition to the biological, physical and economic parameters, there are business parameters to be considered in determining if new technology can be applied commercially. Every business evaluates its production options for the products it sells; importation and outsourcing, either via establishment of foreign operations or importation of multiplied clusters or clumps of shoots, or even importation of entire plantlets, is on the rise in industrialized nations and can be expected to increase substantially over time. Automation, mechanization and other strategies for cost controls will be employed by foreign operations as well and can be expected to benefit the global industry as larger markets open up.

In the case of potato, the United States produces less than 7% of the worlds crop [55], even so, literally billions of seedpieces are planted annually in the United States [56], and the cost of each commercial grade seedpiece, which consists of a minimum of 50 grams of tuber with 2-3 eyes, is less than $0.01 apiece (US$7.50/cwt800 seedpieces). An average potato seedpiece is more than 50 times the size of an average microtuber. The entire commercial crop in the United States technically requires less than 400 first field generation acres, or 8 million minitubers, which are produced from approximately two million plantlets, on a highly seasonal basis in specialized greenhouses. The number of plantlets required for production of elite minitubers, which are the starting point for the North American potato industry, are orders of magnitude less than is required for justification of automation in the laboratory. A closely monitored, multi-year certification process is in place to minimize the reintroduction of potato diseases, and this is used to bring down the high cost of each minituber derived from laboratory plantlets. Frito-Lay, the major purchaser and manufacturer of potato minitubers in North America, established a state-of-the-art modified hydroponic warehouse for production of Technitubers a hybrid between sterile microtubers and ultrasmall (1.5 gm.) minitubers, using a system enabling harvest of an average of 100 tiny tubers per plant. Even this operation, which produced 15 million tubers in one year, required only 150,000 plantlets from the laboratory, easily producible by hand. Unfortunately, Technitubers proved too expensive and too small for acceptable field performance in the United States, and production ceased in 2003. Similar facilities in regions of the world where field seed cannot be kept free of diseases for more than a few years, such as India and China, may be able to justify microtubers, or ultrasmall tubers. Even in these countries, the few hundred thousand potato plantlets required to make these tubers are easily produced manually.

Perhaps that the future role of micropropagation-oriented laboratories in the industrialized nations will be to expand their expertise into creation of improved tissue culture systems and interface between foreign operations and in country greenhouse plant and plug operations with quality, biological systems development, germplasm isolation and maintenance and disease management functions.

5.1. Volumes per Cultivar

Significant reasons for micropropagation include: 1) propagation when other

methods are too slow or too expensive, 2) to increase new cultivars rapidly, 3) to modify the growth form, for example to increase branching and fullness, and 4) to maintain and distribute elite stock plants for propagation [57].

For any crop to be considered a candidate for micropropagation, the value-added benefits need to economically offset their higher costs relative to the alternative propagation methods. One of the primary advantage of tissue culture over other methods of vegetative propagation, such as cuttings, is that the cleanliness of the starting culture, in terms of bacteria, viruses, fungi and insects, can be ascertained, and, once indexing and eradication procedures have been completed, large populations of elite stock can be produced without significant risk of recontamination. Consequently, micropropagation is also used for germplasm maintenance and early stock build up of vegetatively propagated crops such as lilies and potatoes, where many additional generations of field increase are used to bring the costs down to the requisite levels of only pennies apiece.

Generally speaking, crops with a single primary cultivar sellable year-round in numbers of totaling over 10 million units per year are excellent candidates for robotic automation, as it exists today. The advantages of robotic or fully automated tasks in micropropagation hinge on high volume, year-round and nonstop operation.

5.2. Seasons

The second most significant factor in economic assessment of a new production technique, mechanical or otherwise, is the practicality, or adaptability, of the system to the crops and situations encountered in actual production cycles, product mixes and annual seasons of the commercial laboratory. Very few laboratories in any country are built around a season-less production of a single crop. There remain a few examples where a nearly steady production of over 5 million plantlets per year of a single cultivar, or its closely related derivatives, are in demand in the United States; these industry standards include: Spathiphyllum Petite, Syngonium White Butterfly, and Nephrolepsis Boston ferns.

5.3. Cost Reduction Targets

Robotic plantlet production will have a major impact on the industry when costs can be driven down to 25% of the current pricing for manually equivalent operations and when new markets become accessible which are currently out of reach. Examples of such markets include: more advanced generations of elite stock programs, high cost seed transplants, elite tree cultivars, and specialty fruits and vegetables. Each and every application meets competition in the marketplace, so pricing is of the essence in bringing robotics to the micropropagation industry. The hardware for robotic applications is in existence, and has been for many years. What is really needed is readily modifiable software to enable the industry to adapt the functions of the cut and place style robots to various needs over the course of the year and over the course of shifts in the marketplace. Pick and place robots themselves are basically hands, the technical difficulty lies in the presentation of the tissues and in seeing and programming the control of the operations. The second issue for robotics/software remains cost; the cost per microcutting handled needs to drop to at least 25% of the cost of a human handling operation in order to drive the shift away from people and towards mechanized handling.

For example, in elite stock programs, each step in cost reduction increases the volumes approximately ten-fold. Each generation of potatoes is roughly fifteen times the acreage of its predecessor [55] with a price per planting unit dropping nearly 50% in the first field year. To assert a market acceptance at ten-fold volumes, minitubers or field plantable equivalents, must perform equally to the larger field-grown seedpieces for 1/3 of the price. A micropropagated verbena plantlet selling for US$1.00 is needed in only small quantities; the same product at US$0.15 would have a ten-fold increase in market size. However, in both of these examples, the market exists for literally only a few months of the year. From a business perspective, profitability does not increase if the market size

increase is offset by reduced sales price, i.e., the net profitability of selling 1 million plantlets at US$0.60 may not be much different from the profitability of selling 10 million plantlets at US$0.06. For many segments of the elite stock plant market, there may be no great volume increases for intermediate price reductions. At the end of the fiscal year, from the business perspective, all of the additional work of bringing such a change to fruition may not have resulted in greater financial gains.

Automation and mechanization will most logically enter the micropropagation arena from the greenhouse transplanting end [58,59,60]. The final cost of the greenhouse plant sold is impacted directly by one-on-one handling steps: the two most obvious steps are the final tissue culture singulation step and the planting of singulated units into soil. Looking at the greenhouse industry it is clear that robotics have made significant inroads in elite plant plug production.

An illustrated web site with a virtual tour depicting an automated tissue culture planting and growing facility can be found at www.pothosplant.nl (under Company: Product Routing) for the company Pothos Plants, B.V. in the Netherlands. Robots as large as fork lifts move 2m. x 5m. benches of mechanically sorted plantlets in soil plugs from the planting station, through the hardening facilities (large multileveled, artificially lit, computer controlled rooms) to the greenhouses, where automated watering, feeding and spraying ensure that very few people ever need to enter. Smaller robots have additional, specialized tasks. Vision screening systems assist in grading the plantlets and transitioned plugs, ensuring that entire benches of homogeneously sized plants are produced. Plug trays are mechanically filled and transported. Empty benches are mechanically moved to sterilization areas. The facility, operational since 2001, brings in plantlets from tissue culture facilities around the world and then plants, hardens, grows, and ships nearly 80,000 plants per day, using a production crew of only 25 people, an efficiency at least five times that seen in non-automated greenhouses. This style of thinking will clearly produce the types of laboratory systems that will bring tissue culture micropropagation to its next level of productivity. This company, although it is closely linked to a micropropagation lab in the Netherlands, VitroCom, still makes use of all its options in tissue culture plantlet procurement, including significant importation and foreign liaisons, and sees no immediate prospects for automation in the laboratory (P. Olsthoorn, personal communication).

For some species, the singulation, rooting and planting steps can be combined, for example, when unrooted shoot clumps are separated unto individual units at the time of planting While this slows down the planting, it still is much less expensive than singulation under sterile conditions in the laboratory. Production is also advanced with systems where plantlets are singulated, or separated from the parent plantlet, under sterile conditions, but not rooted in vitro. Hedging, or the multiple harvest, of nodes or shoots from a base that is maintained in one container for many passages, can be used for hardy species in production of unrooted microcuttings that proceed directly to greenhouse planting [34]. Another, highly productive technique in this category is overlaying of the multiplying cultures with a rooting medium [53,54]. In this case, explants are subcultured onto multiplication medium and, after numerous shoots have been induced, overlaid with a rooting medium (or an elongation medium, depending on the species). The statice shoot clusters in Figure 1 were produced with an overlay of multiplication medium, resulting in a 50-fold multiplication factor after 10 weeks, as shown in Figure 2. Since the medium overlay is very quick and easy, the labor for increasing the culture base is decreased.

Cultivars required year-round in volumes of at less than one million per year make better candidates for mechanization than automation, and this is where the greatest opportunities currently exist for less glamorous, but perhaps more practically useful, and more economically viable, operator-assisted systems. The price of entry, or start-up costs, is significantly lower and the flexibility is significantly greater for many of these systems.

The original assumptions of automation engineers, as regards the ultimate pricing of the end products targeted, sometimes have overlooked their essential competition:

laboratories in low labor-cost countries. Consequently, automation systems designed to produce plantlets at a cost of US$0.15 apiece in the industrial countries of Europe and North America were made obsolete by importation.

Even in countries with lower labor costs, plantlets are still higher than seedlings or vegetative cuttings, and therefore, operator assisted mechanization is as attractive in those countries as elsewhere. The largest example of robotic production was set in the Monsanto/ForBio joint venture: Monfiori Nusantara, in Indonesia, for the production of elite tree cultivars. Propagation targets were for tens of millions of tree plantlets within a few key genera: notably Eucalyptus and teak. With the 2001 liquidation of ForBio in Australia, the Indonesian venture, as well as the Singapore facility and other locations internationally where the equipment had been set up, stopped using the robotic units and nothing appears to remain of these robots in production. The fact that these robotic stations are sitting in warehouses around the globe, with no current financial encumbrances beyond supplies and software updates, attests to the difficulty of establishing viable production systems at the technical level using simple robotics.

6. Political Parameters

Even in micropropagation, international politics play a role. Governments determine trade priorities and fund, either directly via grants, or indirectly, via favorable taxation and trade agreements, various sectors of their economies. The European Union has established a favorable position on micropropagation: Thus, for a sustainable and competitive agriculture and forestry in Europe, in vitro culture is essential: it is a prerequisite for the successful application of plant breeding by biotechnological methods, for the rapid introduction of improved plants in the market and it offers unique possibilities for the production of plants of superior quality.

The high costs of labor in the EU, needed for the skilled manual labor inherent in the current processes of micropropagation, present a major economic obstacle if in vitro culture is to be fully exploited. Currently, labor accounts for 60-70% of the costs of a plant produced in vitro. Thus, in the EU the competitiveness of the plant-based industries is compromised. Furthermore, the benefits that may be achieved through tissue culture are being applied successfully only to a limited number of crops, because many crops are unresponsive to tissue culture.

The action focuses on two strategies to increase competitiveness of the European in vitro plant production industry: Theme 1) the development of high-tech micropropagation methods which reduce labor input and, Theme 2) the production of plants of superior quality compared with the plants that are usually produced in tissue culture.[61].

7. Conclusions

Micropropagation is still an industry in its infancy; costs are too high to compete in the marketplace effectively and consequently, the volumes necessary to make full use of advanced automation technologies are often lacking. If automation can be developed which decreases the direct cost per plantlet, by 50%, i.e., from US$0.35 to US$0.17, then certain markets will open up and volumes of plantlets sold will increase. Likewise, if flexibility and software advances permit users to modify robotic production lines in house, then greater numbers of varieties and species can be run through a single line, allowing more compatibility with the existing framework of micropropagation laboratories and their product mixes and annual fluctuations.

Photoautotrophic culture systems offer significant hope for the future. As production volumes from biofermentation and other large scale handling systems become increasingly reliable, the industry will gain a higher degree of credibility in the eyes of large volume plant producers and additional inroads into markets with stringent requirements for delivery times and volumes can be made.

Historical emphasis of micropropagation research has been on multiplication rates; however, these are not, in fact, the primary cost controlling factors. Once a minimum increase of 3.5-4 fold per subculture has been established, operator daily productivity

contributes far more to the final cost; consequently, optimization of the operator throughput rates can yield significant benefits.

Full automation and mechanization research needs to focus on dropping the price by nearly an order of magnitude, while establishing reliability and throughput quality, in order to drive the micropropagation industry to its next level.

8. Aknoweldgements

The author wishes to acknowledge the long term support and years of stimulating exchange of both extremely creative and extremely practical ideas with three key individuals: the late Dr. Robert Levin, who was instrumental in the development of many of the technologies outlined in this chapter and a champion of commercial micropropagation, Rodney Kahn and Donald Griffey who contributed similarly creative inputs without the ability to publish due to commercial restraints.

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11. Tables

Table 1. Model of cost per plantlet, as influenced by various factors.

www.PothosPlants.nl	robotic transplanting and growing of plantlet plugs
www.ChopperLight.de	color shift lighting for plant growth modification

Multiplication Rate (xx)	Table 1A. Vary the Plantlet Cost by Tissue Culture Systems						
	Daily Hood Operator Rates						
TC Systems:	600/day	900	1200	1500	1800	2100	2400
Standard (b)	Cost fixed at $35/hr fully loaded (a)						
2x	$0.933	$0.622	$0.467	$0.373	$0.311	$0.267	$0.233
3x	$0.700	$0.467	$0.350	$0.280	$0.233	$0.200	$0.175
4x	$0.622	$0.415	$0.311	$0.249	$0.207	$0.178	$0.156
5x	$0.583	$0.389	$0.292	$0.233	$0.194	$0.167	$0.146
6x	$0.560	$0.373	$0.280	$0.224	$0.187	$0.160	$0.140
7x	$0.544	$0.363	$0.272	$0.218	$0.181	$0.156	$0.136
8x	$0.533	$0.356	$0.267	$0.213	$0.178	$0.152	$0.133
9x	$0.525	$0.350	$0.263	$0.210	$0.175	$0.150	$0.131
Advanced (c)							
10x	$0.519	$0.346	$0.259	$0.207	$0.173	$0.148	$0.130
20x	$0.491	$0.327	$0.246	$0.196	$0.164	$0.140	$0.123
30x	$0.483	$0.322	$0.241	$0.193	$0.161	$0.138	$0.121
40x	$0.479	$0.319	$0.239	$0.191	$0.160	$0.137	$0.120
50x	$0.476	$0.317	$0.238	$0.190	$0.159	$0.136	$0.119
60x	$0.475	$0.316	$0.237	$0.190	$0.158	$0.136	$0.119
70x	$0.473	$0.316	$0.237	$0.189	$0.158	$0.135	$0.118

Loaded Cost Per Hour (US$)	Table 1B. Vary the Plantlet Cost with Global Labor Costs						
	Daily Hood Operator Rates						
TC Systems:	600/day	900	1200	1500	1800	2100	2400
Standard (5x fixed)							
$35/hr (d)	$0.583	$0.389	$0.292	$0.233	$0.194	$0.167	$0.146
$25/hr	$0.417	$0.278	$0.208	$0.167	$0.139	$0.119	$0.104
$15/hr	$0.250	$0.167	$0.125	$0.100	$0.083	$0.071	$0.063
$ 5/hr	$0.083	$0.056	$0.042	$0.033	$0.028	$0.020	$0.021
$ 2/hr	$0.033	$0.022	$0.017	$0.013	$0.011	$0.010	$0.008
Advanced (30x fixed)							
$35/hr	$0.483	$0.322	$0.241	$0.193	$0.161	$0.138	$0.121
$25/hr	$0.345	$0.230	$0.172	$0.138	$0.115	$0.099	$0.086
$15/hr	$0.207	$0.138	$0.103	$0.083	$0.069	$0.059	$0.052
$ 5/hr	$0.069	$0.046	$0.034	$0.028	$0.023	$0.020	$0.017
$ 2/hr	$0.028	$0.018	$0.014	$0.011	$0.009	$0.008	$0.007

a. Fully Loaded Cost per Hour includes both direct and indirect costs: facilities, utilities, materials, freight

b. Standard Tissue Culture (TC) includes: axillary branching, nodal (straight stem)

c. Advanced Tissue Culture (TC) includes: somatic embryos, adventitious, overlay, hedge, biofermentation

d. $1-2=lesser developed nations (China), $5-15=developing nations, $25-35=industrialized nations (US, Europe)

12. Figures

Figure 1. Axillary branching in statice using liquid medium additions.

Figure 2. Subculture of Figure 1 explant into 50 plantlets for rooting.

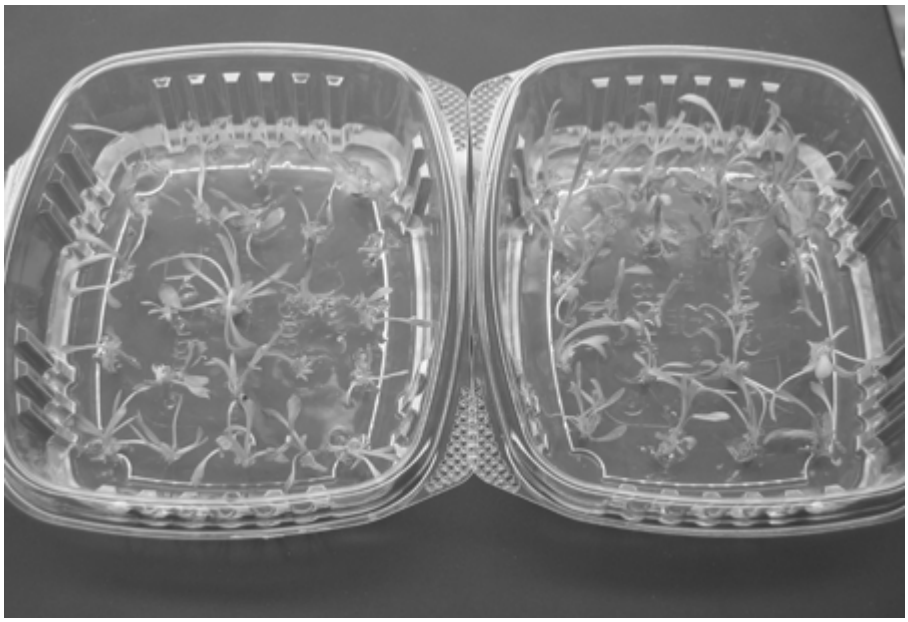

Figure 3a. Cassette style (square petri dish) of potato culture at 2 weeks.

Figure 3b. Cassette style of potato culture at 4 weeks.