

Commercial *in vitro* Mass Propagation of Plants: Current Status and Future Investment Prospects

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Summary

Plant tissue culture technology is increasingly expanding due to its possible role in providing part of human need for food and medicine. The technique of micropropagation is applied with the objective of enhancing the rate of multiplication to attain the highest possible number of plants in a short period of time.

Techniques available for *in vitro* propagation, tissue culture requirements, as well as establishments and personnel needs for such programs are outlined. Factors need consideration for establishing a large scale production of *in vitro* plants is also discussed. A survey of commercial companies that utilizes the tissue culture technology around the world, as well as the state of the art in the Arab world is presented. The economic feasibility of investment in mass propagation of plants through tissue culture, in addition to cost-benefit analysis of such project is discussed.

Keywords: Tissue culture; Commercial micropropagation; Investment; Future prospects.

Introduction

Technology became an essential element to evaluate available resources with respect to their utilitarian value, make them available on a sustainable basis for future generations, and convert them into products of economic value. Plant tissue culture is a sun-rise technology that can have a great impact on both agriculture and industry, through providing plants needed to meet the ever-increasing world demand.

Since the *in vitro* produced plants are usually identical with each other (i.e. clones), tissue culture has been effectively used by private and government-owned enterprises for production of plants on commercial scale. According to Bhojwani and Razdan (1996), an estimated one billion plants per year are produced by Micropropagation, in about 50 biotech companies. This number probably doubled during the past 10 years, since more companies were established during this period all the world around (Gupta & Yasuomi, 2006 and Manickam, 2007). Interest in such companies was also generated in some Arab countries like UAE, Saudi Arabia and Egypt; however no commercial companies for plant propagation through tissue culture were established in most of the other countries.

With the turn of the century, the Arab Authority for Agricultural Investment and Development (AAAID) took the initiative to trigger the interest of Arab authorities for plant tissue culture technology. AAAID organized three workshops during 2001 to 2005 in UAE and 2007 in Jordan to focus on the state of the art of plant tissue culture technology as well as exploring possible investments in the Arab countries to meet the ever increasing demand for food and medicines in developing countries.

What is Plant Tissue Culture?

In 1902, Haberlandt came out with the concept of totipotentiality, which, several years later became the cornerstone for plant tissue culture (Razdan, 2002). This con-

cept suggests that each plant cell has the ability to divide and grow into a complete plant, similar to its parent, if the suitable conditions of nutrition, light and temperature are provided. As the years passed, coupled with the discoveries of plant growth regulators and advancement in biological sciences, the process of differentiation and morphogenesis were manifested and applied to various plants belonging to different families and genera (Altman & Loberant, 2000; Onay *et al.*, 2007 and Ramage & Williams, 2003). Today, tissue culture in a simple term refers to the cultivation of plant cells and tissues away from mother plant in a suitable nutrient media under sterile conditions. The cells and tissues divides and differentiates into organs i.e. into shoots, root, leaf, embryo or flower, or complete plant, or they produce a group of undifferentiated tissue called callus which can be induced into root, shoot or complete plant, through manipulation of culture nutritional and environmental conditions. These information were successfully utilized in clonal propagation of plant.

Currently, Plant Tissue Culture (PTC) is used in four major areas including plant breeding and improvement, production of virus-free plants that could serve as a source for propagules in vegetatively propagated crops. In addition, PTC is used in developed countries in the production of pharmaceutical compounds from their natural resource and secondary substance that can not be synthesized in the laboratory in addition to mass propagation of plants (Honda *et al.*, 2001 and Ilan & Khayt, 1997), which could be very useful to the Arab world.

Methods Available for Plant Propagation

Three methods are currently available that can be utilized for mass propagation of plants, each have its advantages and limitations, including the following:

1. Somatic embryogenesis: This depends on stimulation of asexual embryos either directly from cultured organ or indirectly from callus culture derived from cultured organs (Takayama, 2002). A huge amount of embryos can be regenerated from various plant species. Such embryos can be further encapsulated with sodium alginate and treated like synthetic seeds (Re-

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denbaugh *et al.*, 1991). However, wide expansion in propagation by this methods is usually hampered with the possible genetic modifications that might appear, especially if intermediate callus phase is involved.

2. Adventitious bud formation: This method is based on the stimulation of organs (stem, leaf, and root) on callus cultures through manipulation of growth regulators in the medium (Akita and Takayama, 1994). High cytokinin/auxin ratio in the medium favors shoot initiation, while roots can be induced in the presence of high auxin/cytokinin ratio. However, a balanced cytokinin/auxin ratio leads to the regeneration of complete plant. This method is characterized with relatively high number of regenerated plants. Nevertheless, the chances of somaclonal or other variations might be encountered in the developed plants.
3. Enhanced axillary branching in cultured shoot tips and lateral buds: This is the most widely used method in the *in vitro* propagation programs (Hohnle and Weber, 2007). The technique is based on the inhibition of the apical dominance by a cytokinin, followed by stimulation of growth of bud primordia in the axils of leaves within the cultured shoot tip or lateral bud. Shoots developed in this process are severed and serially propagated, then individually rooted. This method is characterized with moderate number of plant production with high genetic stability.

Stages of Mass Propagation of Plants through Tissue Culture

In 1974, Murashige outlined four stages that can be followed in tissue culture, each with specific nutritional and incubation conditions requirements (Murashige, 1974). Such stages are as follows:

1. **Stage I: Initiation stage:** In this stage an explants (i.e. shoot tip, lateral bud, leaf segment, etc.) is surface sterilized and cultured on nutrient medium. The objective of this stage is to obtain a clean or contamination-free cultures that can be used in the following stage, regardless of the amount of growth attained. The nutritional requirements are usually very simple and the cultures are incubated either under light or dark conditions according to the method of propagation.
2. **Stage II: Multiplication stage:** This is the most important stage in any propagation program since it determines the number of produced plants. In this stage, the number of propagules is multiplied by repeated sub- and reculture until the desired (or planned) number of plants is attained. The chemical formulation and physical status of nutrient medium, as well as, the incubation conditions are of prime significance.
3. **Stage III: Rooting stage:** Shoots produced from the previous stage are separated and individually rooted in a relatively high auxin -containing media. In this stage, a good root system is initiated and complete plants are achieved.

4. **Stage IV: Acclimatization Stage:** This stage may be included with the previous stage. Plants developed through tissue culture are heterotrophic, lack cuticle on their epidermis (Adelberg *et al.*, 2000), as well as having non-functional stomates (Czynczyk and Takubowski, 2007). Such plants can not survive the outside unfavorable conditions. Thus, they need hardening and acclimatization, where they receive a special treatment before they can be transferred to the soil in order to stimulate photosynthesis, cuticle development and their stomates starts functioning. Probably this is the most important stage in the whole process. Thus, it should be conducted under proper conditions regarding soil, light, temperature and irrigation.

It is worth mentioning that each specific plant species has its own requirements in all previous stages. The following parameters needs evaluation for each plant considered for propagation through tissue culture:

1. Source of explant.
2. Nutrient media composition and physical conditions at each stage.
3. Incubation conditions regarding temperature, light intensity, duration of light and dark, and humidity.
4. Hardening conditions and transfer to soil.

Investment in Mass Propagation Using Tissue Culture

During the past three decades, several companies were established in different countries around the world, and good amount of money was invested in this respect. Some firms announce the amount of investment, their production scope and annual production capacity, but none of them declare the actual costs of production. Some commercial companies that are currently involved in this business are tabulated here just to give an example on how investment in such projects could be a success (Table 1).

"State of the Art" In the Arab World

PTC was introduced into the Arab world in the late seventies of the last century in Tunisia, Morocco, Iraq and Egypt. Few government-owned laboratories were established in such Arab countries with the hope of introducing this technology to the region during that period, different forms of collaboration and aids were sought to embody this objective. Some transfer of technology was practiced in Egypt, for example, when the USA established a complete tissue culture based program for propagation of strawberry ,as well as supporting Egypt with tissue culture laboratory for the date palms. Another form of collaboration took the form of transferring operational laboratories from the states to the region for production of plant by some investors, and the ownership was turned into the Arab country as happened with Saudi Arabia and Morocco. Some other Arab countries especially north Africa including Morocco and Tunisia, selected the scientific collaboration with France with respect to date palm propagation. KSA sought the assistance of USA and UK in establishing commercial PTC laboratories.

Table 1. Examples of some commercial international companies that utilizes PTC technology for mass propagation (Arab Countries not listed)

No.	Name of Company	Country	Estab. date	Scope of production	Production (million)	Unit price (USD)	Reference
1	Rancho tissue technology	USA	1987	Foliage plants, perennials, tropical, ornamentals	4	0.5-0.8	www.ranchotissue.com
2	Agriforest Biotech. Ltd	Canada	1988	Hostas, shade trees, roses , lilacs	4	1.5	www.agriforestbiotech.com
3	Total of 36 commercial TC units	India	1996	Medicinal plants, banana, sugarcane, orchids	40	1.2	www.indvitrolab.com
4	Tissue-Grown Corp.	USA	1986	Potato, geranium, carnation	7	NA	www.tissuegrown.com
5	DavaoMusatech Corp.	Philip.	1992	Sugar cane, banana, cassava	12	0.9	www.davaomusat.com
6	Phytoculture Ltd.	USA	1986	Potato, orchids, ornamentals	10	1.5	www.phytoculture.com
7	Lapanday Bio Trends	Philip.	1994	Coconut, bamboo, orchids	14	1.6	www.lapandybio.com
8	Watsonville Lab. Galif.	USA	1984	Potato, strawberry	30	1-2	www.watsenlab.com
9	Vitrobio Valenda SL	Spain	2000	Banana, yam, potato	10	1.4	www.vitrobiovalen.com
10	Marionnet GFA	France	1990	Date palm, strawberry, asparagus, raspberry	5	NA	www.marionnet.com
11	MTPM icropagation	India	1997	Forest trees	2	NA	www.dbtmicropropagation.nic.in

The UAE established, in collaboration with FAO in the late eighties an excellent laboratory for mass propagation of date palm.

Most of the Arab countries established PTC research laboratories when well trained senior staff and infrastructure were made available. Senior staff, well trained in various aspects of PTC successfully established laboratories in most of the Arab world including Iraq, Syria, Jordan, Lebanon, Egypt, Kuwait, Qatar, UAE, Tunisia, Algeria and Morocco. Most of these laboratories were completely devoted for research and graduate student studies, but not for commercial propagation.

In general, it can be stated that major activities in the eighties in most Arab world concentrated on developing protocols for possible mass propagation. The nineties of the last century witnessed fine tuning of such protocols. With the turn of the century some laboratories scaled up their production to the commercial level. Most of these laboratories are concentrating the efforts on propagation of selected crops such as date palm, banana, potatoes, strawberries, rootstocks of apple, pear, and citrus, for example. Few other laboratories are also concerned with medicinal plants, ornamental and foliage plants. However lack of information and scarce documentation of information on such activities makes it difficult to assess the feasibility of investment in such activities. The authors attempted to gain information on some Arab firms from the internet regarding their production, amount of money invested in such projects and cost benefit analysis but to no avail. Here it should be emphasized that this dissemination of information and documentation will be of great help in this respect.

Requirements and Establishments Needed for Plant Propagation through Tissue Culture

In order to establish a tissue culture- based project for mass or commercial propagation of plants, the following requirements are needed:

1. Infra-structure consisting of suitable buildings for

plant tissue culture laboratories, green houses for hardening and acclimation as well as a nursery to hold plants until marketing.

2. Well-trained staff in different disciplines of plant tissue culture and related fields to execute the project with different levels of scientific and technical knowledge.
3. A plan for production including plants to be propagated, quantity of production, programming and marketing of products.
4. Small facilities for research and development to support the project for sustainable growth and development.

Based on such information, one might plan his requirements accordingly. In general, the tissue culture facilities include the followings:

1. Preparation lab. : This laboratory is specialized for nutrient media preparation. The preferred size of this lab is around 30 m² to control the movement of personnel and ventilation. The number of such labs is determined by the size of production. More labs can be added if needed.
2. Washing room to wash all glassware used as well as for water distillation and deionization, in addition to autoclaving of nutrient media and disinfections. A 6x6m room is suitable for such purpose.
3. Tissue culture room: this room should be devoted to execute tissue isolation and culture under sterile conditions. The flow of air should be under control to avoid any possible contamination. This room should be provided with one or more laminar flow cabinets according to the size of work.
4. Incubation rooms: These rooms are air conditioned according to the plant needs and provided with light and dark regims to allow most favorable conditions for plant growth and development. Humidity should also be taken into consideration if the project is executed in arid or humid region. It is always preferable to have

several small incubation rooms rather than one large hall to avoid any possible loss in case of infection or electricity failure. A 6x6m size seems a rational size for this purpose.

5. Plastic house and green house equipped with light and temperature controlled regime for acclimation of tissue culture-derived plants. A total area of acclimation facilities in the neighborhood of 600 m² should be sufficient for acclimation of about 50'000 plants at a time. Again, such facilities should be established in small units or compartments to avoid any possible losses due to infection and desiccations. These facilities should be equipped with a good irrigation and mist or fogging system to provide suitable humidity and water needs for the growing plants.
6. Nursery: ten thousands m² of nursery are required to accommodate the plants before marketing. This nursery should be covered with a suitable tent to reduce direct sunlight and prevent desiccation of plants and it should be also provided with a good irrigation or nutrition system as well as stock of pesticides to control any possible infections.
7. A suitable management building for such project is also required.

Amount of Investment Needed

The amount of investment in such project is categorized in the following Table (2).

Table 2. Requirements and their cost needed for investment

No.	Allocation	Estimated cost USD
1	Establishment of building for TC consisting of preparation lab, incubation rooms, tissue culture room, management, stores etc.	200'000
2	Electrical requirements for incubation room, central air conditioning, emergency ac split units, electricity generator.	200'000
3	Laboratory equipments including different number of laminar flow cabinets, balances, ph meters, media dispensers, water distiller and deionizers, autoclaves, ovens, computers etc.	200'000
4	Chemicals, including inorganic salts, growth regulators, amino acids, vitamins, agar, etc. stock amount	200'000
5	Glass ware- different size, numbers and shapes of flasks, culture tubes, jars, beakers, Petri-dishes, pipettes, graduated cylinders etc.	50'000
6	Green house, plastic house and nursery	200'000
7	Vehicles	50'000
8	Scientific visits, conferences and training	50'000
Total: only one million and one hundred and fifty thousand USD.		1.150 million

Production Cost Analysis

The cost of production of plants through tissue culture consists of several parameter including the cost of chemical

compounds used for nutrient media, labor cost (lab, greenhouse, nursery, ...etc), depreciation of apparatus and facilities (buildings and establishments) management and supervision, in addition to other indirect parameters. In order to determine the cost of nutrients medium used in the production, one should calculate the cost of inorganic salts, sugar, vitamins, growth regulator and agar. Prices of such components vary between companies. The cost of individual item could be determined through dividing the price of that substance over its final concentration. For example if the cost of 25gm package of NAA is 300 USD, and this substance is used at a rate of 5 mg/l medium, then the price of 1g equals 12 USD (or 1200 cent). Therefore the price of 1mg =1200/1000=1.2 cents. Then cost of this specific compound in 1 liter medium =5 mgx1.2 cent =6 cents.

Now in order to reduce the cost of production (regarding labor, chemicals, incubation area,... etc.) it is advisable to use Mason jar or Magenta or similar container that can hold 5 explants (or even more) as compared to 1 explant per tube. Accordingly one liter can be dispensed into 40 jars at a rate of 25 ml/jar, which subsequently can be inoculated with 200 explants. Therefore, the cost of NAA (in this case) for each plant =6 cents/200 plant =0.3 cents for that stage. This way, the costs of other medium components can be calculated as shown in Table (3).

Table 3. Cost of media components

No.	Components	Range used	Unit price (USD)	Cost/liter (cent)	Cost/plant (cent)
1	Growth regulator	mg	1-6/g*	4-12	0.3-0.6
2	Sucrose**	30g/l (most common)	1/kg	3	0.15
3	Vitamins	mg	5-10/g*	2-5	0.1-0.25
4	Inorganic salts	mg	10/kg	1	0.05
5	Agar***	7 g/l	100/kg	10	0.5
Total				200-310 cents	1.1-1.5 cents

* If more than one growth regulator or vitamin is used, the cost will be multiplied accordingly, and the price is also variable depending on type of growth regulator or vitamin.

** Table sugar can be used instead of technical or analytical grade sucrose. One kg of sugar is enough to prepare 33 liter of medium.

*** One kg of agar is enough to prepare 142 liter of medium.

This means that the cost of chemical compounds used in the preparation of 1 liter of the medium that contain the above components with one growth regulator and one vitamin ranges between 2-3.1 USD and it is enough to culture 200 plants. Thus the cost of nutrient for each plant is 1.1-1.5 cent/stage. For 3 stages of in vitro propagation the total cost is 3.3-4.5 cents.

The cost of labor, on the other hand, can be calculated according to the monthly salary divided by available working days, given that each individual labor is responsible for culturing 200 jars/day (minimum). Monthly salaries are different in various places. In Iraq, for example, workers in this field receive 200-300 USD per month. This means that the cost of culturing each jar with 5 plants ranges between 4-6 cents. Accordingly the cost of labor per each plant is 0.8-1.2 cents.

Calculation of other costs was conducted in the Tissue Culture Center of the Iraqi Atomic Energy Organization in 2002 for the production of 300'000 plants of date palm, apple and pear root stocks, potato microtubers (unpublished data). The results are listed in Table (4).

Table 4. Analysis of costs other than nutrient medium involved in micropropagation

No.	Source of expenditure	Cost/plant (cent)
1	Utilities (water, electricity, gasoline, lubricants, transportation of labors etc).	1.2
2	Maintenance of cars and incubation rooms including a/c units, florescent lamps and other requirements.	0.7
3	Management (supervision , planning, data analysis, records etc).	0.8
4	Greenhouse (fertilizer, pesticides, soil mixes, pots, beds, plastic covers etc).	0.7
5	Temporary labor in greenhouse, nursery, plastic houses, maintenance workshop and repair.	1.1
Total		4.5

Thus, the total cost of production will be as follows:

1. Nutrient media for the tissue culture stages = 3.3-4.5 cents
 2. Labor cost in tissue culture = 0.8-1.2 cents
 3. Other costs of production = 4.5 cents
- Total cost = 8.6-10.2 cents.

An average cost of 10 cent for plant propagation through tissue culture sounds feasible, when compared to some commercial companies that sales its tissue culture propagated plants for 50-80 cent in test tubes, or 1.5 USD for small potted plant (Rancho Tissue Technology Company, for example). Probably, the cost of production is a bit cheaper in the Arab world when compared to the USA and Europe for example. This could be due to the cheap labor, use of inexpensive and less sophisticated equipments (Chu,1995; Gollagunta *et al.*, 2004 and Herman, 2000), such as computerized instruments, automated systems or use of Robots in tissue culture , as well the use of substitutions (e.g. sugar instead of sucrose) and locally manufactured (rather than expensively imported) chemicals and glassware. Furthermore the cost of land and construction in the region is probably less than those of the USA and Europe.

It is important to mention here that the cost of production is a limiting factor in any commercial tissue culture propagation program. These costs are severely affected by microbial contamination of tissue cultures, accumulation of inhibitory substances in growth medium, verification of plants in tissue culture, transfer to the soil stage and establishment of new plants, recalcitrance of some plant species to in vitro culture, and possible genetic variation that might take place during the process. If such problems are encountered, the cost of production will go up naturally.

Economic Feasibility of Investment in Tissue Culture

Before considering any investment in mass propagation of plants through tissue culture, it is very essential to seriously evaluate the following parameters to make sure that the micropropagation program will be economically viable and sustainable business

1. Size of the market for the plant (plants) to be produced.
2. Calculated requirement for labor and space.
3. Cost of production.
4. Market price of the conventionally produced plants
5. Time of the year at which young plants are required.
6. Costumer preferences.
7. Current changes in the scientific know-how of micropropagation techniques.
8. Accessibility to technology transfer area.

In the Arab world, there is a great demand for a variety of plant species of economical values. Such plants range from date palm, pistachios, apple, pear, grapes, to food crops such as potato, pine apple, banana and strawberry, to foliage and ornamental and forest plants. In any case, one might plan for production of 1 million plants per year, either from specific plant species or combination of few products. As shown from calculation, the average cost of plant production is 10 cents. This cost might increase another 2 cents for deprecation of buildings, greenhouse, laboratory equipments, apparati and machinery, as well as salaries for administrators and scientific leader.

The project can sell its products (plant in a small pot) for suggested price of 1 dollar, an acceptable price when compared to RTT company for example). A total of 1 million dollar will be gained from this sale. If the cost of production (i.e. 1'000'000 plant x 12 cents =12'000'000 cents or 120'000 USD) is subtracted from sale price, the remaining 880'000 USD represents the net profit of the project. Some companies sell their products (like date palm in 2 liter pots) for up to 24 USD per plant, which significantly increases the profit.

It should be mentioned here, however, that the economical production of such projects starts after 3-4 years from establishment (for fast growing crops) or even 6 years for slow growing crops such as date palm. It is necessary to establish stock cultures during the first two years, from which mass propagation can take over.

Expected Outputs

Such project is expected to have direct and indirect outputs as follow:

1. Production of elite plants for local consumption as well as for export with rational price and good benefit.
2. Follow-up with the advancement of science and technology to reduce the gap between the Arab world and developed countries in biotechnology.
3. Development of Arab human resources and training of the national technicians in new fields.
4. Creation of new job opportunities for young or youth generation.

Recommendations

Due to the world wide attention and interest directed towards plant tissue culture technology, the AAAID is highly encouraged to invest in this vital topic. Several advantages are associated with such investment, including large scale production of clonal plants, development of human resources and creation of new jobs.

The AAAID is eligible for preparation of the infrastructure needed for the project in the appropriate site or country, and have excellent experience in management and marketing. The scientific and technical knowledge in the tissue culture field is available, and good Arab scientists are well trained in different disciplines in tissue culture and can help in this respect.

The AAAID is advised to go into such projects, concentrating on some fast growing crops such as banana, potato and rootstock in the beginning to recover part of the capital invested, and then go into more profitable crops such as date palm and pistachios. The elementary plan should concentrate on annual production of one million plants, and increase this production in the years to come.

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الإكثار النسيجي التجاري: الواقع والآفاق الاستثمارية المستقبلية

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الخلاصة

يتزايد الاهتمام بتقانة زراعة الأنسجة النباتية وذلك لدورها المتوّقع في توفير جزء من حاجة الإنسان للغذاء والدواء. وإحدى استخدامات هذه التقانة تنحصر في الإكثار الدقيق للنبات، بهدف زيادة معدّل التضاعف للحصول على أكبر عدد ممكن من النباتات في فترة زمنية قصيرة. تمّت مناقشة أهمّ الطرُق المُتاحة للإكثار الدقيق، فضلاً عن مُستلزمات زراعة الأنسجة من حيث المُختبرات والمُنشآت والعاملين، كما تمّ تحديد العوامل التي يجب أخذها بنظر الاعتبار عند التفكير باستخدام تقانة زراعة الأنسجة في الإكثار الواسع للنبات. لقد تمّ تقديم استعراض سريع للشركات التجارية التي تستخدم هذه التقانة في العالم ووضع التقانة في العالم العربي. شملت المقالة دراسة الجدوى الاقتصادية وحسابات الكلفة والربح للاستثمار في جانب الإكثار الموسّع للنبات بتقانة زراعة الأنسجة.

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