OnLine Journal of Biological Sciences 1 (11): 1106-1111, 2001

Novel Micropropagation System

Zakaria Ahmed, Firoza Akhter, M. Shamsul Haque, Hasina Banu,
M. Motiur Rahman and A. K. M. Faruquzzaman
Bangladesh Jute Research Institute, Manik Mia Avenue, Dhaka-1207, Bangladesh

Abstract: Micropropagation consists of three types of vegetative propagation. It remains a popular research subject and thus continued research is definitely needed to increase the speed with which some plants can be produced giving a superior quality product.

Key words: Micropropagation, morphogenesis, clonal propagation, barriers

Introduction

Micropropagation is a sophisticated technique for the rapid multiplication of plants. It has a great commercial potential due to the speed of propagation, the high plant quality and the ability to produce disease-free plants. It is the art and science of plant multiplication *in vitro*. The process includes many steps--stock plant care, explant section and sterilization, media manipulation to obtain proliferation, rooting, acclimation, and growing on of liners. However, micropropagation is usually done by hand which makes the process cost-intensive and tedious for the workers especially because it requires a sterile work-place. Micropropagation has a great potential in cloning of genetically improved strains for raising new plantations to increase forest productivity (Dunstan and Thorpe, 1986, Boulay, 1987). It true-to-type propagation of a seted genotype using *in vitro* culture techniques. As a concept micropropagation was first presented to the scientific community in 1960 by G. M. Morel producing virus-free Cymbidiums. The necessary tools that made micropropagation a possibility, such as the development of media and an understanding of growth regulators, have been available only since the late 1950s. And it was not until the early 1960s that a generalized culture media was established. The actual establishment of commercial micropropagation as an industry became a reality during the 1970s and 1980s. But, in spite of the micropropagation industry being only 15 to 20 years old, significant progress has been made in the culture of plant tissues and cells *in vitro* and in the experimental manipulation of higher plant parts and cells as microorganisms (Jones and Sluis, 1991; Zimmerman and Jones, 1991).

Micropropagation consists of three types of vegetative propagation: (1) Somatic embryogenesis, in which structures are formed containing a shoot and root connected by a close vascular system (directly analogous to zygotic embryos in regards to histology, physiology and biochemistry), (2) Adventitious shoot production, comprising de novo meristem formation from callus tissue or directly from organized tissues such as epidermal or sub-epidermal cells, (3) Axillary shoot production where axillary buds and meristems give rise to shoots that are excised and used to produce additional such shoots.

Numerous examples of successful applications have been reported for a) meristem culture, b) organogenic micropropagation from undifferentiated tissues, cells or protoplast, c) zygotic embryo culture, d) somatic embryogenesis and e) gametic embryogenesis. Hu and Wang (1983) has received meristem, shoot tip and bud cultures. Meristem cultures have been employed to eliminate virus infection in some asexually propagated species. Meristem and bud cultures have been used commercially for multiplication of some high value genotypes. Zygotic embryo culture has been successfully employed to circumvent post-fertilization, cross-incompatibility during inter-specific transfer of genes among related plant species for many years. Recently embryo rescue techniques have also proven useful in recovery of haploid plants that emerge from chromosome elimination in zygotic embryos following inter-specific crosses.

Regeneration of plants from callus, individual cells and protoplast have involved organogenic or embryogenic differentiation. These techniques have been useful in providing both spontaneous (Scowcraft *et al*., 1987) and mutagen induced genetic variation (somaclonal/gametoclonal/protoclonal variance). Both embryogenic and organogenic differentiation have been involved among examples of plant regeneration following protoplast fusion, genetic transformation, anther culture and micro spore culture. Embryogenesis has played a significant role in recovery of haploid plants from culture anthers or micro spores. Thus, considerable progress in micropropagation technologies is evident and their applications in crop plants are diverse and significant. These technologies have been reviewed frequently during the past decade and the principles of *in vitro* culture are also presented in many good handbooks. The objective is to present here only the novel micropropagation system emphasizing modern techniques of *in vitro* culture. The application of novel micropropagation technologies was studied for plant improvement along with the barriers, both scientific and business related, facing commercial micropropagation. The utility of any technology will ultimately be refted in the relative value of information and products derived there from.

Morphogenesis

The origin of form (morphogenesis) is an area of research with which tissue culture has long been associated. This is the area which micropropagation has made significant contributions both in terms of fundamental knowledge and application. Here morphogenesis is discussed briefly in two aspects.

Organogenesis: In 1957, The classical findings of continue to be guiding principles on *in vitro* organogenesis has been achieved in over 1000 plant species through empirical section of the explant, the medium composition and control of the physical environment (Thorpe *et al*., 1990). Although progress is being made, the determination events are not yet entirely known. In addition to tradition a bulky explants such as cotyledons, hypocotyls and callus cell layers have been used. In all systems examined, the organogenic process begins with changes in a single or small group of parenchyma cells, which then divide to produce a globular mass of cells or meristemoid. These cells can give rise to either a shoot or root primordium.

Embryogenesis: In contrast to organogenesis, which produces a unipolar shoot or root primordium, somatic embryogenesis gives rise to a bipolar structure with a root/shoot axis. Asexual embryogenesis has been reported in over 130 species, including cereals, grasses, legumes and conifers. The process can be divided into two major phases: the induction of cells with embryogenic competence and their subsequent development into embryos. Empirical manipulation of the

explant, medium and culture environment has led to success in a process which is very plastic and may be structurally and / or cellularly different from zygotic embryogenesis (Ammirato, 1983).

Carrot tissues have proven to be very useful in the study of somatic embryogenesis, but to date most of the physiological and biochemical studies have dealt with embryo development rather than the whole process. However, the recent development of a method for setting single cells, which form cell clusters and then somatic embryos in a relatively synchronous fashion (Normura and Komamine, 1985), is allowing for an in-depth examination of the entire process.

Clonal propagation

The use of tissue culture technology in vegetative propagation of plants has become the most widely used application of the technology in agriculture, horticulture and forestry. During the last 25 years it has become possible to regenerate plantlets from explants and / or callus from ornamental plants, food crops, vegetable and condiment plants, fruit and nut crops, medicinal plants and forest trees (Morel and Wetmore, 1951).

Ornamentals: Micropropagation of ornamentals has taken tissue culture out of the lab and into the commercial world (Hyndman, 1987). Today, for the ornamental industry, the use of tissue culture technology is an routine for some plants, as cuttings and other traditional methods are for others. All classes of ornamentals, are propagated by tissue culture methods and many of the newer commercial labs have production capabilities of up to 2,00,000 *in vitro* plantlets per week. In ornamentals, more than in other sectors, chimeras are important. Different foliage plants with variegated leaves that are chimera are micro propagated (*Ficus benjamina* 'Golden King' and 'Golden Princes', *Ficus decora* 'Belgaplant', *Cordyline fruticosa* 'Kiwi' etc.). Probably orchids were the first horticultural plants to be propagated by tissue culture and G. M. Morel introduced the technique of meristem culture as a means of vegetative propagation (Morel and Wetmore, 1951). His method found almost immediate commercial use and a whole new market oriented plant propagation became available that placed orchids within the economic reach of the average person. Today the technique developed by morel is used worldwide and few commercially important genera such as *Paphiopedilium* and *Phalaenopsis* which once remained fairly not feasible to micro propagate are now propagated.

Field and vegetable crops: Micropropagation protocols exist for large numbers of field and vegetable crops. Though once many agronomic crops have been difficult to manipulate *in vitro* and regenerate has been problematic, currently progress in regeneration has been significant, such that most crops can now be regenerated. Of the major field crops, reliable and efficient methods for the regeneration of cereals and other grain species have been available since 1980. This has been made possible by using immature embryos to produce stable regenerable embryogenic callus. The exception is sugarcane, traditionally propagated vegetable and now micro propagated at least for some applications of seedcane field establishment. For other agronomic crops (as well as for sugarcane artificial seeds), the problems are production of thrifty, high-quality somatic embryos, control of somaclonal variation, development of an artificial endosperm (for albuminous species) and optimization of an efficient delivery system. Somaclonal variation can likely be controlled during the callus stage, at least partially, by slowing and directing cell growth. Initial observations with growing alfalfa callus on a high potassium citrate medium have shown marked reduction in variation of subsequent regenerated plants.

For agronomic crops, micropropagation is currently commercial only for sugarcane. Micropropagation for other crops will likely be for development and production of hybrids, with alfalfa, cotton, rice, soybean and sugarbeet being of principal interest. Vegetable crop species have been extensively in tissue culture research (Hyndman, 1987; Morel and Wetmore, 1951). Notable examples include carrot cell culture and embryogenesis research and potato protoplast manipulation and regeneration studies. Many reviews have elucidated the application of cell, tissue and organ culture techniques to vegetable crop species. Specifically, *in vitro* techniques to multiply plants by embryogenesis, organogenesis and non-adventitious shoot proliferation are reported in the literature with many vegetable species.

Fruit, plantation and forest trees: Protocols for the large-scale mass clonal propagation of several members of this heterogenous group currently exist. Initial uses were for small (soft) fruit crops such as strawberry and raspberry and for rootstocks for several tree fruits, especially peach. As methods were developed for more crops and as experience was gained with micro propagated plants, shifts in patterns of usage have occurred (Bhatt and Todaria, 1990).

For both apple and pears, clonal propagation of root stocks and scion cultivar utilizes shoot tips. Several tropical fruit trees can be regenerated via somatic embryogenesis using nuclear tissue or by organogenesis using shoot tips and axillary buds. However, only banana is being exploited commercially. *In vitro* propagation techniques have also been successfully developed for oil, date, coconut, some ornamental palms and peach palm. Micropropagation techniques have been developed for several berry crops including thornless blackberry, raspberry, buebarry and grape. Grape can be regenerated from axillary shoots, adventitious budding and via somatic embryogenesis, but none of these methods as yet allow for mass clonal propagation. Successful micropropagation of forest trees is a relatively recent phenomenon. Plantlets can be produced via organogenesis and somatic embryogenesis in both hardwoods and softwoods and present, protocols exist for over 70 angiosperms and 30 gymnosperms. Several woody species such as poplars, wild cherry, eucalypts, redwood and radiata pine are now commercially micro propagated, while others such as sandalwood, birtch, teak and loblolly pine show promise. Bansal and Chibbar (2000) had achieved a micropropagation of mahua, *Madhuca latifolia* Macb. (Sapotaceae) by culturing excised nodes on Woody Plant Medium supplemented with different plant growth regulators (Jones, 1983; Bansal and Chibbar, 2000).

Pathogen-free plants and germplasm storage

Although these two topics appear to be unrelated, a major use of pathogen-free plants is for germplasm storage.

Production of pathogen-free plants: Crop plants especially those propagated vegetatively, are generally infected with pathogens. Plants infected with bacteria and fungi often respond to treatments with bactericidal and fungicidal agents, but there is no commercially available treatment to cure virus-infected plants. Meristem tip culture have been very useful in the elimination of virus from valuable genetic stocks normally multiplied through vegetative cuttings or tubers. The success of Morel and Martin in eliminating virus from infected dahlia plants in 1952 and from potato plants in 1955 stimulated stem tip culture in many horticultural species. Once isolated, virus-free clones can be maintained *in vitro* to provide disease-free stocks for multiplication through conventional or micropropagation (Hu and Wang, 1983).

Germplasm storage: Traditionally, germplasm has been maintained in the form of seeds, but this method has several limitations. However, the ability to regenerate whole plants from somatic and gametic cells and shoot apices is leading to

their use for storage. Three common *in vitro* approaches have been used, namely growth retarding compounds, low temperature and cryopreservation. A wide variety of growth retarding chemicals, including maleic hydrazide, B995 and ABA have been used to reduce the growth rate of *in vitro* plantlets, so as to increase the time between subcultures. Germplasm can also be stored in cultures at non-freezing low temperatures (1-90°C). At these temperatures the aging of the plant material is slowed down, but not completely stopped. Consequently, subculture of the plant material is infrequent. In contrast to the above methods, most effort during the last 15 years have entered around cryopreservation, where the plant material, after treatment with a cryoprotectant, is frozen and stored at the temperature of liquid nitrogen. More than 100 species have been cryopreserved at present including both monocots and dicots as well as conifers.

Plant modification and improvement

In vitro methods are being used increasingly as an adjunct to traditional breeding methods for the modification and improvement of plants.

***In vitro* fertilization and embryo culture:** The technique of controlled *in vitro* pollination on the stigma, placenta or ovule can be used in several ways. These include the production of interspecific and intergeneric hybrids, overcoming sexual self-incompatibility and the induction of haploid plants. Embryo, ovary and ovule culture have been used in overcoming embryo inviability, monoploid production in barley and in overcoming seed dormancy and related problems. Embryo abortion is a common problem in breeding programs, due to failure of endosperm development. By aseptically culturing the embryo (or in some cases the ovary), this problem can be overcome. Interspecific and intergeneric hybrids of many important crops have been obtained by this embryo rescue approach. One novel use of embryo culture has been in the production of monoploids and double monoploids in barley. The causes of seed dormancy are varied, but excision of the embryo is often enough to allow germination, as in the case of the immature embryos of orchids, which led to the first commercial application of tissue culture technology (Murashige and Skoog, 1962; Manzanera and Pardos, 1990; Morel and Wetmore, 1951; Knauss, 1976; Hyndman, 1987). Embryo culture has also proven useful in reducing the breeding cycles of new varieties, in cases where long dormancy and/or slow growth of the seedlings resulted in long breeding seasons.

Haploidy: Natural haploidy has been know for a long time, but it was the studies on *in vitro* induction of androgenesis or the direct production of haploid embryos from anthers in the early 1960s, that led to renewed interest in this phenomenon. Haploids may be used to facilitate the detection of mutations and recovery of unique recombinants, since there is no masking of recessive alleles (Bajaj, 1983; Ammirato, 1983). The requirements for successful androgenesis or gynogenesis must be determined empirically. Direct regeneration, rather via a haploid callus is the usual route, but only a low percentage of the explants respond positively. At present, much more success has been achieved with androgenesis than gynogenesis, perhaps due to the earlier success with the former. Nevertheless, the latter has allowed for haploid production in certain genera for which anther culture is not an effective technique. Androgenesis has been reported in some 171 species, of which many are important crop plants (Hu and Zeng, 1984) and gynogenesis in 15 species. Androgenic haploids have been integrated into breeding programs as homozygous diploid lines. In china, this method has led to the development of new varieties of rice, tobacco and winter wheat and new breeding lines of maize and sugarcane (Hu and Zeng, 1984).

Somaclonal variation: In addition to the variants/ mutants obtained as a result of a setive agent in the presence or absence of a mutagen, many variants have been obtained through the tissue culture cycle itself. These somaclonal variants, which are dependent on the natural variation obtained in a population of cells, may be genetic or epigenetic, and are usually observed in the regenerated plantlets (Barve and Mehta, 1993; Morel and Wetmore, 1951). Many of the changes observed *in vitro* regenerated plants have potential agricultural and horticultural significance. These include alterations in plant pigmentation, seed yield, plant vigour size, leaf and flower morphology, constituents of essential oils, fruit solids and disease tolerance or resistance. Such variations have been observed in many crops including wheat and triticale, rice, oats and maize, sugarcane, tobacco, tomato, potato, and celery. The above types of variation obtained from somatic cells and protoplast can also be obtained from gametic tissue. One of the major potential benefits of somaclonal variation is the creation of additional genetic variability in co-adapted, agronomically useful cultivars, without the need to resort to hybridization. This method could be valuable if *in vitro* section is possible, or if rapid plant screening methods are available.

Use of protoplast: Plant protoplast can be routinely produced from many species using leaf mesophile cells and cell suspensions and mixtures of cell wall degrading. These protoplast have been used in fundamental as well as applied studies. The number of species that can be regenerated from protoplast is steadily increasing.

Protoplast fusion: This has been suggested as a means of developing unique hybrid plants, which can not be fused by chemical and physical means. Production of unique somatic hybrid plants is limited by the ability to regenerate plants from isolated plant protoplast. Most success has been limited to model plants from the genera *Nicotina, Petunia, Datura* and *Hyoscyamus*. Only recently some success has been achieved in Gramineae; but none with grain legumes, although regeneration from protoplast of several forage legumes has been achieved. Protoplast fusion can be used to produce unique nuclear-cytoplasmic combinations and to transfer cytoplasmically controlled male sterility between breeding lines. To date this has been achieved in *Nicotina, Petunia* and *Brassica napus*. Populations of regenerated plants arising from protoplast fusion contain more variability than comparable populations of plants produced by sexual hybridization. Much of the research carried on to date has been directed towards the production of novel hybrids, not breeding line development. To make the technology more valuable for the production of new crop varieties emphasis must be placed in this area.

Vector-independent gene transfer: Genetic modification of plants is also being considered through uptake of DNA organelles and single cell uptake in protoplast. Genetic transformation through DNA uptake require DNA from one source to be taken up, incorporated into the recipient cell in a stable form and that the genetic information encoded in the foreign DNA is expected as new stable characteristics. One of the most recent developments for vector less transfer is the use of the particle gun or high velocity micro projectile technology. Here DNA is short through the cell wall and into the cytoplasm on the surface of small metal particles that have been accelerated to speed of several hundred meters per second (Klein *et al.*, 1987). In this method stable transformed tobacco and soybean plants have been produced without going through protoplast.

Vector-mediated gene transfer: The use of *Agrobacterium* as

a tool for gene transfer has progressed very rapidly since the first published report of stable transformation. Although the early transformation experiments utilized plant protoplast, regenerable organ such as leaves, stems and roots have subsequently used with many dicot plants (Gasser and Fraley, 1989). In addition to vectors based on *Agrobacterium tumefaciens*, others have used *Agrobacterium rhizogenes* plasmids. Recently progress in this area has been so rapid that genetically engineered soybean, cotton, rice, rape, sugerbeet, tomato and alfalfa crops are expected to enter the market place before the end of this decade.

Micropropagation of aroids

Some years ago, Mr. David Burnett of Sydney, Australia became the first author to bring a very rare and beautiful aroid, *Alocasia guttata var. imperialis* N.E. Brown, from the brink of obscurity back into the horticultural limelight by using a plant tissue culture technique known as micropropagation. He then made propagules available to the collectors around the world. In Burnett's landmark work on the *Alocasia* (Burnett, 1984; Morel and Wetmore, 1951), he describes the colourful history of this remarkable species and how it had become so rare that it was only known to exist in a single collection. It was very rare in it's native Borneo as well . The micropropagation of *Alocasia guttata var. imperialis* N.E. Brown using a procedure known as shoot tip culture was successful (Lloyd and McCown, 1980). Since that time, a once very rare species is now available and enjoyed by many collectors of aroids. From this experience many people learned what a powerful tool tissue culture can be for the conservation and preservation of endangered aroids and how very advantageous the technique can be to the collector of rare aroids as well (Morel and Wetmore, 1951).

An aroid, *Amorphophallus konjac* K. Koch, was actually the first monocotyledon to be reported in the literature as having been successfully tissue cultured (Hyndman, 1987; Morel and Wetmore, 1951). In fact, the authors reported the first successful propagation of an *Amorphophallus* by tissue culture in that same paper with the production of adventitious shoots from callus derived from tuber tissue grown on a defined salts medium to which B vitamins and a synthetic auxin were added. Comparable results with *Sauromatum guttatum* Schott were mentioned as well (Hyndman, 1987).

Since those early years in plant tissue culture history, numerous reports about the micropropagation of aroids have appeared in the literature. Generally, these reported techniques can be applied to related species and genera (Hyndman, 1987; Morel and Wetmore, 1951). The hobby tissue culturist can apply these techniques quite effectively to the propagation of rare aroids. Alternatively, an aroid collector could contract a willing commercial laboratory to propagate a limited number of plants. When participating in a micropropagation program, the aroid collector must observe one important caution: the demand for collectors' aroids is very small, as compared to commercial cultivars, therefore, the propagation of only a very few high quality specimens should be contemplated. When this one caution is observed, the advantages of aroid micropropagation include: (I) the production of potentially disease free plants, (ii) the conservation of endangered species and rare cultivars, (iii) the potential of creating new cultivars, and (iv) the international shipment of aroids with much reduced quarantine restrictions.

Producing disease free aroids : Hartman (1974) was the first to report the use of micropropagation for the purpose of producing disease free aroids. He carefully dissected out the shoot tips, which consisted of the apical meristem and a leaf primordium and subsequently cultured them on a chemically defined medium. He was able to produce Caladium bicolour, *Xanthosoma sagittifolium* and *Colocasia esculenta* plants free of dasheen mosaic virus. In 1981, the Agricultural Research Center, Apopka, Florida, released an improved variety of *Dieffenbachia maculata* cv. Perfection given the cultivar name of 'Perfection-137B'. These same micropropagation techniques can be used quite effectively to get disease free plants in other species of aroids as well. Just because an aroid is micro propagated does not mean that it is automatically disease free, or somehow immune to diseases. Micro propagated aroids are no different from any other aroid in their ability to resist diseases. They may only be considered to be disease free if they have been specifically tested for the pathogen in question.

Potential of creating new cultivars: Marcel Lecouffe (1981) explained how he had discovered a new cultivar of *Caladium humboldtii* which he named 'Marcel' from a micro propagated population. When the first author was commercially micropropagating *Alocasia* 'Black Velvet', a new *Alocasia* ,probably from Borneo, he discovered an individual plant among the larger population of clones that had larger and thicker leaf laminae, thicker petioles and more robust growth overall. Additionally, there are a number of new syngonium cultivars in the foliage plant industry that are mutations from tissue cultured *Syngonium podophyllum* 'White Butterfly'. Several patented and trademarked cultivars having red pigmentation in their leaves are produced by Agric. Starts, Inc., a micropropagation company in Apopka, Florida. All of these aroids are examples of micropropagation serving as a source of new cultivars. The mechanism of this phenomenon is not entirely understood. It is generally referred to as somaclonal variation (Hyndman, 1987; Morel and Wetmore, 1951; Larkin and Scowcroft, 1981).

With all of these advantageous uses of micropropagation for the aroid collector, This propagation technique is not a cure-all for the problems that might be set plants. For example, a particular species that is difficult to propagate by more conventional methods such as by cuttings or by seed, may be just as difficult to micro propagate. Micropropagation is more likely to produce less desirable plants due to somaclonal variation than it is to produce uniquely desirable new cultivars.

Shoot tip culture

Many trees in the family Rosaceae have been propagated by tissue culture. Primary attention to date has focused upon those grown commercially for fruit. Cherry, plum, peach, almond and both scion and rootstock cultivars of apple have been multiplied *in vitro* (Abbott and Whiteley, 1976; Jones and Hopgood, 1979; Jones *et al.*, 1977; Jones *et al.*, 1979; Tabachnik and Kester, 1977; Hyndman, 1987). Established cultures of the plum rootstock `Pixy" and the cherry rootstock `F 12/I' have achieved a hundred-fold multiplication in 9 months (Jones and Hopgood, 1979). Cox's Orange Pippin apple explants produced 10 shoots per culture 6 weeks after subculturing (Abbott and Whiteley, 1976). Shoot tip cultures of the rootstock clone M26 each produced between 20 and 42 shoots after 18 weeks in culture (Lloyd and McCown, 1980; Jones *et al.*, 1977). Shoots which had been produced in tissue culture were stimulated to form roots by transferal to a culture medium similar to the shoot multiplication medium, but lacking the cytokinin. This medium contained 0.1 mg/l IBA (indolebutyric acid) which stimulated rooting of up to 97% of the M26 shoot tip cuttings within 6 weeks (Morel and Wetmore, 1951; Jones and Hopgood, 1979; Hyndman, 1987). This technique has also been successfully applied in the rooting of cultured shoot tips of five apple scion cultivars (Jones *et al.*, 1979) and a similar technique was described for tissue cultured almond clones (Tabachnik and Kester, 1977; Hyndman, 1987). Multiple shoots are seen to arise from a basal mass, which actually represents small, unexpended

shoots rather than callus. Little, if any, callus has been observed in any of the cultures examined to date. Shoot multiplication appears to proceed primarily by lateral shoot growth, although formation of adventitious shoots cannot be ruled out. In some cases, lateral shoots have been observed in active growth as close as one or two expanded internodes distance from the shoot tip. The conditions of the tissue culture environment thus appear to circumvent apical dominance and result in a mass of shoots each producing more shoots. The ability to mass propagate *Amelanchier* through tissue culture adds further evidence that plants within the Rosaceae should respond similarly to the *in vitro* environment (Morel and Wetmore, 1951; Hyndman, 1987).

Barriers in micropropagation

Scientific barrier: Continued research is definitely needed to increase the speed with which some plants can be produced giving a superior quality product. There is often inadequate research in the development of protocols for proliferation, rooting, acclimation and reestablishment. Transplanting problems still exist. The ability to successfully take a plant, especially woody plants, through the four stages of micropropagation and consistently produce healthy, saleable plants still remains a challenge and requires coordinated planning. Testing for the presence of pathogens unfortunately is not a routine procedure and many laboratories produce pathogen-free plants by coincidence rather than by design. There is a great need for increased automation, as the majority of the costs associated with micropropagation are related to hand labour (Zimmerman and Jones, 1991). Research funding, both private and public, needs to be increased if scientific barriers are to be resolved in a timely manner. The success of micropropagation is clearly dependent on maintaining the genetic integrity of a propagule to guarantee clonal identity. Practices that do not guarantee the maintenance of clonal identity, e.g., regenerative process, need to be avoiding until such time that the causes of somaclonal variation are fully understood and controllable.

Business-related barriers: In the early years, micropropagation firms sold to finishing nurseries and expected nursery operators to educate themselves as to the handling and marketing of quality micro propagated plants (Jones and Sluis, 1991; Standart-de Metsenaere, 1991). This is changing. Micropropagation firms now a days aligned with a nursery to better ensure the high quality of the finished plants. Successful micropropagation requires a strategy to produce a large number of quality plants using very labour-intensive protocols based on a highly trained labour force. But the ability to produce a large number of high-quality plants does not guarantee success (Jones and Sluis, 1991). Micropropagation must compete with conventional propagation, which often produces at a lower cost. Finally, it is also sensitive to prospects/constraints of general agriculture and horticulture (Standart-de Metsenaere, 1991).

Micropropagation is extremely labour-intensive with nearly half (46%) of the costs attributable to labour. Research and development accounts for only 1%! Cost reductions have been difficult because the cost for labour is directly associated with the transfer and maintenance of culture. One solution is to move to lower-cost labour sites, but there are problems with this strategy, including perishability of product, costs associated with long distance communication and unreliable electrical power sources. Part of the answer clearly lies in automation.

This brief review of micropropagation system illustrates part of the ground work, since the field is vast and multi disciplined. The various systems discussed here are all being used in crop plant modification and improvement. Researchers in universities, government and industrial laboratories are all working in this area. But, why is this necessary? The increased emphasis on sustainable agriculture and an increasing world population, coupled with the continued lass of prime agricultural land to housing and industry, signify that we will have to fed, clothe and house more people than ever existed in the history of mankind. To do that we have two option. Either we have to cultivate new land or increase per unit crop yield. In real sense the first one is not possible. Then the alternative is to increase productivity and to do so micropropagation and other biotechnological systems are essential. Last of all, has micropropagation delivered all that it promised? Probably not. Micropropagation is a young industry with an excellent future and it will give the answer to many (if not all) problems associated with conventional propagation and other plant improvement techniques.

References

Abbott, A. J. and E. Whiteley, 1976. Culture of Malus tissues *in vitro*. I. Multiplication of apple plants from isolated shoot apices. Sci. Hort., 4: 183-189.

Ammirato, P. V., 1983. Embryogenesis, pp: 82-123. In: D. A. Evans, W. R. Sharp, P. V. Ammirato and Y. Yamada (eds.). Handbook of plant cell culture, 1, Macmillan, New York.

Bajaj, Y. P. S., 1983. *In vitro* production of haploids. In: D. A. Evans, W. R. Sharp, P. V. Ammirato and Y. Yamada (eds.). Handbook of plant cell culture, vol. 1, Macmillan, New York.

Bansal, Y. K. and T. Chibbar, 2000. Micropropagation of *Madhuca latifolia* Macb. Through nodal culture. Plant Biotechnology, 17: 17-20.

Boulay, M., 1987. In: Green C. E. *et al.* (Edi.) Plant tissue and cell culture. Alan R. Liss Inc. New York, pp: 367-382.

Barve, D. M. and A. R. Mehta, 1993. Clonal propagation of mature elite trees species of *Commiphora wightii*. Plant cell Tiss. Org. Cult., 35: 237-244.

Bhatt, B. P. and N. P. Todaria, 1990. Vegetative propagation of tree species of social forestry value in Garhwal Himalaya. J. Tropical Forest Sci., 2: 195-210.

Burnett, D., 1984. The cultivated Alocasia. Aroideana 7(3 and 4).

Chase, A. R., 1981. Perfection-137B, a pathogen-free section of *Dieffenbachia maculata* derived through tissue culture. Circular S-280, Florida AES, IFAS, University of Florida, Gainesville.

Dunstan, D. I. and T. A. Thorpe, 1986. In: Vasil, I. K. (Ed.), cell culture and somatic cell genetics of plants, 3, 223-241. Academic Press, Inc. USA.

Hu, C. Y. and P. J. Wang, 1983. In: Handbook of plant cell culture, crop species. Sharp, W. R., Evans, D. A., Ammirato, P. V. and Yamada, Y. (ed.), 1, pp: 177-227. MacMillan, New York.

Hu, H. and J. Z. Zeng, 1984. Development of new varieties via anther culture. In: Handbook of plant cell culture Ammirato, P. V., D.A. Evans, W.R. Sharp and Y. Yamada, (ed.),3:65-90. MacMillan, New York.__

Hartman, R. D., 1974. Dasheen mosaic virus and other phytopathogen eliminated from caladium, taro and cocoyam by culture of shoot tips. Phytopath., 64: 237-240.

Hyndman, S. E., 1987. The advantages of tissue culture for the aroid collector. Aroideana, 10: 7-10.

Jones, J. B. and C. J. Sluis, 1991. Marketing of micro propagated plants. In: Debergh, P. C. and R. H. Zimmerman, (ed.), pp: 141-154. MacMillan, New York.

Jones, O. P., 1983. *In vitro* propagation of tree crops. In: Plant Biotechnology, 139-159, Mantell, S. H. and Smith, H. (Edi.), Cambridge Uni., Press.

Jones, O. P. and M. E. Hopgood, 1979. The successful propagation *in vitro* of two rootstocks of Prunus: the plum rootstock.

Jones, O.P., M.E. Hopgood and D. O. Farrell, 1977. Propagation *in vitro* of M26 apple rootstocks. J. Hort. Sci., 52:235-238.

Jones, O.P., C.A. Pontikis and M.E. Hopgood, 1979. Propagation *in vitro* of five apple scion cultivars. J. Hort. Sci., 54:155-158.

Knauss, J. F., 1976. A tissue culture method for producing *Dieffenbachia maculata* cv. Perfection free of fungi and bacteria. Proc. Florida State Hort. Soc., 89: 293-296.

Klein, T. M., E. D. Wolf, R. Wu and J. C. Sanford, 1987. High-velocity micro projectiles for delivering nucleic acids into living cells. Nature, 327: 70-73.

Lloyd, G. and B. McCown, 1980. Commercially feasible micropropagation of mountain Laura (*Kalmia latifolia*) by use of shoot tip cultures. Comb. Proc. Int. Soc., 30: 421-427.

Larkin, P.J. and W.R. Scowcroft, 1981. Somaclonal variation -- a novel source of variability from cell cultures. Theor. Appl. Genet., 60: 197-214.

Morel, G. and R.H. Wetmore, 1951. Tissue culture of monocotyledons. Am. J. Bot., 38:138-140.

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

Manzanera, J. A. and J. A. Pardos, 1990. Micropropagation of Juvenile and adult *Quercus suber* L., Plant Cell Tiss. Org. Cult., 21: 1-8.

Normura, K. and A. Komamine, 1985. Identification and isolation of single cells that produce somatic embryos at high frequency in a carrot suspension culture. Plant Physiol., 79:988-991.

Scowcroft, W. R., R. I. S. Brettel, S. A. Ryan, P. A. Davis and M. A. Pallotta, 1987. Somaclonal variation and genomic flux. In: Green, C. E., D. A. Somars, W. P. Hackett and D. D. Biesboer, (edi.), Plant Tissue and cell culture. pp. 275-286. A. R. Liss, New York.

Standart-de Metsenaere, R. E. A., 1991. Economic considerations. In: Debergh, P. C. and R. H. Zimmerman, (ed.), pp: 123-140. Kluwer Academic Publ., Dordrechi, Netherlands.

Thorpe, T. A., I. S. Harry and P. P. Kumar, 1990. Application of micropropagation to forestry. In: Debergh, P. C. and R. H. Zimmerman, (edi.), Micropropagation. Kluwer Academic Publ., Dordrechi, Netherlands.

Tabachnik, L. and almond and almond-peach hybrid clones *in vitro*. Hort Sci., 12:545-547.

Zimmer man, R. H. and J. B. Jones, 1991. Commercial micropropagation in North America, In: Debergh, P. C. and R. H. Zimmerman, R. H. (ed.), pp: 173-179, Micropropagation. Kluwer Academic Publ., Dordrechi, Netherlands.