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REVIEW PAPER

History of plant tissue culture

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Abstract Plant tissue culture, or the aseptic culture of cells, tissues, organs, and their components under defined physical and chemical conditions in vitro, is an important tool in both basic and applied studies as well as in commercial application. It owes its origin to the ideas of the German scientist, Haberlandt, at the begining of the 20th century. The early studies led to root cultures, embryo cultures, and the first true callus/tissue cultures. The period between the 1940s and the 1960s was marked by the development of new techniques and the improvement of those that were already in use. It was the availability of these techniques that led to the application of tissue culture to five broad areas, namely, cell behavior (including cytology, nutrition, metabolism, morphogenesis, embryogenesis, and pathology), plant modification and improvement, pathogen-free plants and germplasm storage, clonal propagation, and product (mainly secondary metabolite) formation, starting in the mid-1960s. The 1990s saw continued expansion in the application of the in vitro technologies to an increasing number of plant species. Cell cultures have remained an important tool in the study of basic areas of plant biology and biochemistry and have assumed major significance in studies in molecular biology and agricultural biotechnology. The historical development of these in vitro technologies and their applications are the focus of this chapter.

Keywords Cell behavior · Cell suspensions · Clonal propagation · Organogenesis · Plantlet regeneration · Plant transformation · Protoplasts · Somatic embryogenesis · Vector-dependent/independent gene transfer

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Introduction

Plant tissue culture, also referred to as cell, in vitro, axenic, or sterile culture, is an important tool in both basic and applied studies, as well as in commercial application [1]. Plant tissue culture is the aseptic culture of cells, tissues, organs and their components under defined physical and chemical conditions in vitro. The theoretical basis for plant tissue culture was proposed by Gottlieb Haberlandt in his address to the German Academy of Science in 1902 on his experiments on the culture of single cells [2]. He opined that, to my knowledge, no systematically organized attempts to culture isolated vegetative cells from higher plants have been made. Yet the results of such culture experiments should give some interesting insight to the properties and potentialities that the cell, as an elementary organism, possesses. Moreover, it would provide information about the interrelationships and complementary influences to which cells within a multicellular whole organism are exposed (from the English translation, [3]). He experimented with isolated photosynthetic leaf cells and other functionally differenced cells and was unsuccessful, but nevertheless he predicted that one could successfully cultivate artificial embryos from vegetative cells. He, thus, clearly established the concept of totipotency, and further indicated that the technique of cultivating isolated plant cells in nutrient solution permits the investigation of important problems from a new experimental approach. On the basis of that 1902 address and his pioneering experimentation before and later, Haberlandt is justifiably recognized as the father of plant tissue culture. Other studies led to the culture of isolated root tips [4, 5]. This approach of using explants with meristematic cells produced the successful and indefinite culture of tomato root tips [6]. Further work allowed for root culture on a completely

defined medium. Such root cultures were used initially for viral studies and later as a major tool for physiological studies [7]. Success was also achieved with bud cultures [8, 9].

Embryo culture also had its beginning early in the first decade of the last century with barley embryos [10]. This was followed by the successful rescue of embryos from nonviable seeds of a cross between *Linum perenne* ↔ *Linum austriacum* [11], and for full embryo development in some early ripening species of fruit trees [12]; thus providing one of the earliest applications of in vitro culture. The phenomenon of precocious germination was also encountered [13].

The first true plant tissue cultures were obtained by Gautheret [14, 15] from cambial tissue of *Acer pseudoplatanus*. He also obtained success with similar explants of *Ulmus campestre, Robinia pseudoacacia*, and *Salix capraea* using agar-solidified medium of Knop's solution, glucose and cysteine hydrochloride. Later, the availability of indole acetic acid and the addition of B vitamins allowed for the more or less simultaneous demonstrations with carrot root tissues [16, 17], and with tumor tissue of a *Nicotiana glauca* ↔ *Nicotiana langsdorffii* hybrid [18], which did not require auxin, that tissues could be continuously grown in culture; and even made to differentiate roots and shoots [19, 20]. However, all the initial explants used by these pioneers included meristematic tissue. Nevertheless, these findings set the stage for the dramatic increase in the use of in vitro cultures in the subsequent decades. Greater detail on the early pioneering events in plant tissue culture could be found in White [21], Bhojwani and Razdan [22], and Gautheret [23]. This current article is based on an earlier review by the author [24] (used with permission from Elsevier).

The development and improvement of techniques

The 1940s, 1950s, and 1960s proved an exciting time for the development of new techniques and the improvement of those already available. The application of coconut water (often incorrectly referred to as coconut milk) allowed for the culture of young embryos [25] and other recalcitrant tissues, including monocots. Callus cultures of numerous species, including a variety of woody and herbaceous dicots and gymnosperms, as well as crown-gall tissues, were established as well [23]. It was recognized at this time that cells in culture underwent a variety of changes, including loss of sensitivity to applied auxin or habituation [26, 27], as well as variability of meristems formed from callus [27, 28]. Nevertheless, it was during this period that most of the in vitro techniques used today were largely developed.

Studies by Skoog et al. [29] showed that the addition of adenine and high levels of phosphate allowed nonmeristematic pith tissues to be cultured and produced shoots and roots, but only in the presence of vascular tissue. Further studies using nucleic acids led to the discovery of the first cytokinin (kinetin), as the breakdown product of herring sperm DNA [30]. The availability of kinetin further increased the number of species that could be cultured indefinitely, but perhaps most importantly, led to the recognition that the exogenous balance of auxin and kinetin in the medium influenced the morphogenic fate of tobacco callus [31]. A relative high level of auxin to kinetin favored rooting, the reverse led to shoot formation and intermediate levels to the proliferation of callus or wound parenchyma tissue. This morphogenic model has been shown to operate in numerous species [32]. Native cytokinins were subsequently discovered in several tissues, including coconut water [33]. The formation of bipolar somatic embryos (carrot) was first reported independently by Reinert [34, 35] and Steward [36] in addition to the formation of unipolar shoot buds and roots.

The culture of single cells (and small cell clumps) was achieved by shaking callus cultures of *Tagetes erecta* and tobacco, and subsequently placing them on filter paper resting on well-established callus, giving rise to the so-called nurse culture [37, 38]. Later, single cells could be grown in medium in which tissues had already been grown (i.e., conditioned medium) [39]. As well, single cells incorporated in a 1-mm layer of solidified medium formed some cell colonies [40]. This technique is widely used for cloning cells and in protoplast culture [22]. Finally, in 1959, success was achieved in the culture of mechanically isolated mature differentiated mesophyll cells of *Macleaya cordata* [41], and later in the induction of somatic embryos from the callus [42]. The first large-scale culture of plant cells was obtained from cell suspensions of Ginkgo, holly, Lolium and rose in simple sparged 20-L carboys [43]. The utilization of coconut water as an additive to fresh medium, instead of using conditioned medium, finally led to realization of Haberlandt's dream of producing a whole plant (tobacco) from a single cell by Vasil and Hildebrandt [44], thus demonstrating the totipotency of plant cells.

The earliest nutrient media used for growing plant tissues in vitro were based on the nutrient formulations for whole plants, for which they were many [21]; but Knop's solution and that of Uspenski and Uspenskia were used the most, and provided less than 200 mg/l of total salts. Based on studies with carrot and Virginia creeper tissues, the concentration of salts was increased twofold [45], and was further increased ca. 4 g/l, based on work with Jerusalem artichoke [46]. However, these changes did not provide optimum growth for tissues, and complex addenda, such as yeast extract, protein hydrolysates, and coconut water,

were frequently required. In a different approach, based on an examination of the ash of tobacco callus, Murashige and Skoog (MS) [47] developed a new medium. The concentration of some salts was 25 times that of Knop's solution. In particular, the levels of NO_3 and NH_4 were very high and the arrays of micronutrients were increased. MS formulation allowed for a further increase in the number of plant species that could be cultured, many of them using only a defined medium consisting of macro- and micro-nutrients, a carbon source, reduced N, B vitamins, and growth regulators [48]. The MS salt formulation is now the most widely used nutrient medium in plant tissue culture.

Plantlets were successfully produced by culturing shoot tips with a couple of primordia of *Lupinus* and *Tropaeolum* [9], but the importance of this finding was not recognized until later when this approach to obtain virus-free orchids, its potential for clonal propagation was realized [49]. The potential was rapidly exploited, particularly with ornamentals [50]. Early studies had shown that cultured root tips were free of viruses [51]. It was later observed that the virus titer in the shoot meristem was very low [52]. This was confirmed when virus-free *Dahlia* plants were obtained from infected plants by culturing their shoot tips [53]. Virus elimination was possible because vascular tissues, within which the viruses move, do not extend into the root or shoot apex. The method was further refined [54], and now routinely used.

Techniques for in vitro culture of floral and seed parts were developed during this period [55]. The first attempts at ovary culture yielded limited growth of the ovaries accompanied by rooting of pedicels in several species [56]. Compared to studies with embryos, successful ovule culture is very limited. Studies with both ovaries and ovules have been geared mainly to an understanding of factors regulating embryo and fruit development [56]. The first continuously growing tissue cultures from an endosperm were from immature maize [57]. Plantlet regeneration via organogenesis was later achieved in *Exocarpus cupressiformis* [58].

In vitro pollination and fertilization was pioneered using *Papaver somniferum* [59]. The approach involves culturing excised ovules and pollen grains together in the same medium and has been used to produce interspecific and intergeneric hybrids [60]. Earlier, cell colonies were obtained from *Ginkgo* pollen grains in culture [61], and haploid callus was obtained from whole anthers of *Tradescantia reflexa* [62]. However, it was the finding of Guha and Maheshwari [63, 64] that haploid plants could be obtained from cultured anthers of *Datura innoxia* that opened the new area of androgenesis. Haploid plants of tobacco were also obtained [65], thus confirming the totipotency of pollen grains.

Plant protoplasts or cells without cell walls were first mechanically isolated from plasmolysed tissues well over 100 years ago, and the first fusion was achieved in 1909 [23]. Nevertheless, this remained an unexplored technology until the use of a fungal cellulase by Cocking [66] ushered in a new era. The commercial availability of cell wall degrading enzymes led to their wide use and the development of protoplast technology in the 1970s. The first demonstration of the totipotency of protoplasts was by Takebe et al. [67], who obtained tobacco plants from mesophyll protoplasts. This was followed by the regeneration of the first interspecific hybrid plants (*N. glauca* ↔ *N. langsdorffii*) [68].

Braun [69] showed that in sunflower *Agrobacterium tumefaciens* could induce tumors, not only at the inoculated sites, but, at distant points. These secondary tumors were free of bacteria and their cells could be cultured without auxin [70]. Further experiments showed that crown gall tissues, free of bacteria, contained a tumor-inducing principle (TIP), which was probably a macromolecule [71]. The nature of the TIP was worked out in the 1970s [72], but Braun's work served as the foundation for *Agrobacterium*-based transformation. It should also be noted that the finding by Ledoux [73] that plant cells could take up and integrate DNA remained controversial for more than a decade.

The recent past

Based on the availability of the various in vitro techniques discussed in Subheading 2., it is not surprising that, starting in the mid-1960s, there was a dramatic increase in their application to various problems in basic biology, agriculture, horticulture, and forestry through the 1970s and 1980s. These applications can be divided conveniently into five broad areas, namely: (1) cell behavior, (2) plant modification and improvement, (3) pathogen-free plants and germplasm storage, (4) clonal propagation, and (5) product formation [1].

Detailed information on the approaches used can be gleaned from Bhojwani and Razdan [22], Vasil [74], and Vasil and Thorpe [75], among several sources.

Cell behavior

Included under this heading are studies dealing with cytology, nutrition, primary, and secondary metabolism, as well as morphogenesis and pathology of cultured tissues [1]. Studies on the structure and physiology of quiescent cells in explants, changes in cell structure associated with the induction of division in these explants and the characteristics of developing callus, and cultured cells and protoplasts have been carried out using light and electron

microscopy [76–79]. Nuclear cytology studies have shown that endoreduplication, endomitosis, and nuclear fragmentation are common features of culture cells [80, 81].

Nutrition was the earliest aspect of plant tissue culture investigated, as indicated earlier. Progress has been made in the culture of photoautotrophic cells [82, 83]. In vitro cultures, particularly cell suspensions have become very useful in the study of both primary and secondary metabolism [84]. In addition to providing protoplasts from which intact and viable organelles were obtained for study (e.g., vacuoles) [85], cell suspensions have been used to study the regulation of inorganic nitrogen and sulfur assimilation [86], carbohydrate metabolism [87], and photosynthetic carbon metabolism [88, 89]; thus clearly showing the usefulness of cell cultures for elucidating pathway activity. Most of the work on secondary metabolism was related to the potential of cultured cells to form commercial products, but has also yielded basic biochemical information [90, 91].

Morphogenesis or the origin of form is an area of research with which tissue culture has long been associated; and one to which tissue culture has made significant contributions both in terms of fundamental knowledge and application [1]. Xylogenesis or tracheary element formation has been used to study cytodifferentation [92–94]. In particular the optimization of the *Zinnia* mesophyll single cell system has dramatically improved our knowledge of this process. The classical findings of Skoog and Miller [31] on the hormonal balance for organogenesis has continued to influence research on this topic; a concept supported more recently by transformation of cells with appropriately modified *Agrobacterium* T-DNA [95, 96]. However, it is clear from the literature that several additional factors, including other growth active substances, interact with auxin and cytokinin to bring about *de novo* organogenesis [97]. In addition to bulky explants, such as cotyledons, hypocotyls, and callus [97], thin (superficial) cell layers [98, 99] have been used in traditional morphogenic studies, as well as to produce *de novo* organs and plantlets in hundreds of plant species [50, 100]. As well, physiological and biochemical studies on organogenesis have been carried out [97, 101, 102]. The third area of morphogenesis, somatic embryogenesis, also developed in this period with over 130 species reported to form the bipolar structures by the early 1980s [103, 104]. Successful culture was achieved with cereals, grasses, legumes, and conifers, previously considered to be recalcitrant groups. The development of a single cell to embryo system in carrot [105] has allowed for an in depth study of the process.

Cell cultures have continued to play an important role in the study of plant-microbe interaction, not only in tumorigenesis [106], but also on the biochemistry of virus multiplication [107], phytotoxin action [108], and disease resistance, particularly as affected by phytoalexins [109]. Without doubt the most important studies in this area dealt with *Agrobacteria*, and although aimed mainly at plant improvement (*see* next section) provided good fundamental information [96].

Plant modification and improvement

During this period, in vitro methods were increasingly used as an adjunct to traditional breeding methods for the modification and improvement of plants. The technique of controlled in vitro pollination on the stigma, placenta, or ovule has been used for the production of interspecific and intergeneric hybrids, overcoming sexual self-incompatibility, and the induction of haploid plants [110]. Embryo, ovary, and ovule cultures have been used in overcoming embryo inviability, monoploid production in barley and in overcoming seed dormancy and related problems [111, 112]. In particular, embryo rescue has played a most important role in producing interspecific and intergeneric hybrids [113].

By the early 1980s, androgenesis had been reported in some 171 species, many of which were important crop plants [114]. Gynogenesis was reported in some 15 species, in some of which androgenesis was not successful [115]. The value of these haploids was that they could be used to detect mutations and for recovery of unique recombinants, because there is no masking of recessive alleles. As well, the production of double haploids allowed for hybrid production and their integration into breeding programs.

Cell cultures have also played an important role in plant modification and improvement, as they offer advantages for isolation of variants [116]. Although tissue culture-produced variants that have been known since the 1940s (e.g., habituation), it was only in the 1970s that attempts were made to utilize them for plant improvement. This somaclonal variation is dependent on the natural variation in a population of cells, either pre-existing or culture-induced, and is usually observed in regenerated plantlets [117]. The variation may be genetic or epigenetic and is not simple in origin [118, 119]. The changes in the regenerated plantlets have potential agricultural and horticultural significance, but this potential has not yet been realized. It has also been possible to produce a wide spectrum of mutant cells in culture [120]. These include cells showing biochemical differences, antibiotic, herbicide, and stress resistance. In addition, auxotrophs, autotrophs, and those with altered developmental systems have been selected in culture; usually the application of the selective agent in the presence of a mutagen is required. However, in only a few cases has it been possible to regenerate plants with the desired traits (e.g., herbicide-resistant tobacco) [121], and methyl tryptophan-resistant *Datura innoxia* [122].

By 1985, nearly 100 species of angiosperms could be regenerated from protoplasts [123]. The ability to fuse plant protoplasts by chemical (e.g., with polyethylene glycol [PEG]) and physical means (e.g., electrofusion) allowed for production of somatic hybrid plants; the major problem being the ability to regenerate plants from the hybrid cells [124, 125]. Protoplast fusion has been used to produce unique nuclear-cytoplasmic combinations. In one such example, *Brassica campestris* chloroplasts coding for atrazine resistance (obtained from protoplasts) were transferred into *B. napus* protoplasts with *Raphanus sativus* cytoplasm (which confers cytoplasmic male sterility from its mitochondria). The selected plants contained *B. napus* nuclei, chloroplasts from *B. campestris* and mitochondria from *R. sativus*, had the desired traits in a *B. napus* phenotype, and could be used for hybrid seed production [126]. Unfortunately, only a few such examples exist to date.

Genetic modification of plants has been achieved by direct DNA transfer via vector-independent and vector-dependent means since the early 1980s. Vector-independent methods with protoplasts include electroporation [127], liposome fusion [128], and microinjection [129], as well as high-velocity microprojectile bombardment (biolistics) [130]. This latter method can be executed with cells, tissues, and organs. The use of *Agrobacterium* in vector-mediated transfer has progressed very rapidly since the first reports of stable transformation [131, 132]. Although the early transformations utilized protoplasts, regenerable organs such as leaves, stems, and roots have been subsequently used [133, 134]. Much of the research activity utilizing these tools has focused on engineering important agricultural traits for the control of insects, weeds, and plant diseases.

Pathogen-free plants and germplasm storage

Although these two uses of in vitro technology may appear unrelated, a major use of pathogen-free plants is for germplasm storage and the movement of living material across international borders [1]. The ability to rid plants of viruses, bacteria, and fungi by culturing meristem-tips has been widely used since the 1960s. The approach is particularly needed for virus-infected material, because bactericidal and fungicidal agents can be used successfully in ridding plants of bacteria and fungi [22]. Meristem-tip culture is often coupled with thermotherapy or chemotherapy for virus eradication [135].

Traditionally, germplasm has been maintained as seed, but the ability to regenerate whole plants from somatic and gametic cells and shoot apices has led to their use for storage [22, 135]. Three in vitro approaches have been developed, namely use of growth retarding compounds (e.g., maleic hydrazide, B995, and abscisic acid [ABA]) [136], low-nonfreezing temperatures (1–9°C) [22], and cryopreservation [135]. In this last approach, cell suspensions, shoot apices, asexual embryos, and young plantlets, after treatment with a cryoprotectant, is frozen and stored at the temperature of liquid nitrogen (*ca.* –196°C) [135, 137].

Clonal propagation

The use of tissue culture technology for the vegetative propagation of plants is the most widely used application of the technology. It has been used with all classes of plants [138, 139], although some problems still need to be resolved (e.g., hyperhydricity, abberant plants). There are three ways by which micropropagation can be achieved. These are enhancing axillary bud breaking, production of adventitious buds directly or indirectly via callus, and somatic embryogenesis directly or indirectly on explants [50, 138]. Axillary bud breaking produces the smallest number of plantlets, but they are generally genetically true-to-type; whereas somatic embryogenesis has the potential to produce the greatest number of plantlets, but is induced in the lowest number of plant species. Commercially, numerous ornamentals are produced, mainly via axillary bud breaking [140]. As well, there are many lab-scale protocols for other classes of plants, including field and vegetable crops, fruit, plantation, and forest trees, but cost of production is often a limiting factor in their use commercially [141].

Product formation

Higher plants produce a large number of diverse organic chemicals, which are of pharmaceutical and industrial interest. The first attempt at the large-scale culture of plant cells for the production of pharmaceuticals took place in the 1950s at the Charles Pfizer Co. The failure of this effort limited research in this area in the United States, but work elsewhere in Germany and Japan in particular, led to development, so that by 1978 the industrial application of cell cultures was considered feasible [142]. Furthermore, by 1987, there were 30 cell culture systems that were better producers of secondary metabolites than the respective plants [143]. Unfortunately, many of the economically important plant products are either not formed in sufficiently large quantities or not at all by plant cell cultures. Different approaches have been taken to enhance yields of secondary metabolites. These include cell cloning and the repeated selection of high-yielding strains from heterogenous cell populations [142, 144] and by using enzyme linked immunosorbent assay (ELISA) and radioimmunoassay techniques [145]. Another approach involves

selection of mutant cell lines that overproduce the desired product [146]. As well, both abiotic factors—such as ultraviolet (UV) irradiation, exposure to heat or cold and salts of heavy metals and biotic elicitors of plant and microbial origin, have been shown to enhance secondary product formation [147, 148]. Lastly, the use of immobilized cell technology has also been examined [149, 150].

Central to the success of producing biologically active substances commercially is the capacity to grow cells on a large scale. This is being achieved using stirred tank reactor systems and a range of air-driven reactors [141]. For many systems, a two-stage (or two-phase) culture process has been tried [151, 152]. In the first stage, rapid cell growth and biomass accumulation are emphasized, whereas the second stage concentrates on product synthesis with minimal cell division or growth. However, by 1987 the naphthoquinone, shikonin was the only commercially produced secondary metabolite by cell cultures [153].

The present

During the 1990s, continued expansion in the application of in vitro technologies to an increasing number of plant species was observed. Tissue culture techniques are being used with all types of plants, including cereals and grasses [154], legumes [155], vegetable crops [156], potato [157], other root and tuber crops [158], oilseeds [159], temperate [160], tropical [161] fruits, plantation crops [162], forest trees [163], and, of course, ornamentals [164]. As can be seen from these articles, the application of in vitro cell technology went well beyond micropropagation, and embraced all the in vitro approaches that were relevant or possible for the particular species, and the problem(s) being addressed. However, only limited success has been achieved in exploiting somaclonal variation [165], or in the regeneration of useful plantlets from mutant cells [166]; also, the early promise of protoplast technology has remained largely unfulfilled [167]. Substantial progress has been made in extending cryopreservation technology for germplasm storage [168] and in artificial seed technology [169]. Some novel approaches for culturing cells such as on rafts, membranes, and glass rods, as well as manipulation of the culture environment by use of nonionic surfactants have been successfully developed [170].

Cell cultures have remained an important tool in the study of plant biology. Thus progress is being made in cell biology, for example, in studies of the cytoskeleton [171], on chromosomal changes in cultured cells [172], and in cell-cycle studies [173, 174]. Better physiological and biochemical tools have allowed for a re-examination of neoplastic growth in cell cultures during habituation and hyperhydricity, and relate it to possible cancerous growth in plants [175]. Cell cultures have remained an extremely important tool in the study of primary metabolism; for example, the use of cell suspensions to develop in vitro transcription systems [176], or the regulation of carbohydrate metabolism in transgenics [177]. The development of medicinal plant cell-culture techniques has led to the identification of more than 80 enzymes of alkaloid biosynthesis (reviewed in ref. 178). Similar information arising from the use of cell cultures for molecular and biochemical studies on other areas of secondary metabolism, is generating research activity on metabolic engineering of plant secondary metabolite production [179].

Cell cultures remain an important tool in the study of morphogenesis, even though the present use of developmental mutants, particularly of *Arabidopsis*, is adding valuable information on plant development (see ref. 180). Molecular, physiological, and biochemical studies have allowed for an indepth understanding of cytodifferentiation, mainly tracheary element formation [181], organogenesis [182, 183], and somatic embryogenesis [184–186].

Advances in molecular biology are allowing for the genetic engineering of plants, through the precise insertion of foreign genes from diverse biological systems. Three major breakthroughs have played major roles in the development of this transformation technology [187]. These are the development of shuttle vectors for harnessing the natural gene transfer capability of *Agrobacterium* [188], the methods to use these vectors for the direct transformation of regenerable explants obtained from plant organs [189], and the development of selectable markers [190]. For species not amenable to *Agrobacterium*-mediated transformation, physical, chemical, and mechanical means are used to get the DNA into the cells. With these latter approaches, particularly biolistics [191], it has become possible to transform virtually any plant species and genotype.

The initial wave of research in plant biotechnology has been driven mainly by the seed and agri-chemical industries, and has concentrated on agronomic traits of direct relevance to these industries, namely the control of insects, weeds, and plant diseases [192]. At present, over 100 species of plants have been genetically engineered, including nearly all the major dicotyledonous crops and an increasing number of monocotyledonous ones, as well as some woody plants. Current research is leading to routine gene transfer systems for all-important crops; for example, the production of golden rice [193]. In addition, technical improvements are further increasing transformation efficiency, extending transformation to elite commercial germplasm and lowering transgenic plant production costs. The next wave in agricultural biotechnology is already in progress with biotechnological applications of interest to the food processing, speciality chemical, and pharmaceutical industries.

The current emphasis and importance of plant biotechnology can be gleamed from the last three International *Congresses on Plant Tissue and Cell Culture and Biotechnology* held in Israel in June 1998, in the United States in June 2002, and in China in August 2006. The theme of the Israeli Congress was *Plant Biotechnology* and *In Vitro Biology in the 21st Century*, at the U.S. Congress was *Plant Biotechnology 2002 and Beyond*, and the theme of the last Congress was *Biotechnology and Sustainable Agriculture 2006 and Beyond*. The proceedings for the '98 and '02 Congresses [194, 195], as well as the '06 Congress, were developed through a scientific program that focused on the most important developments, both basic and applied, in the areas of plant tissue culture and molecular biology and their impact on plant improvement and biotechnology. They clearly show where tissue culture is today and where it is heading (i.e., as an equal partner with molecular biology), as a tool in basic plant biology and in various areas of application. In fact, progress in applied plant biotechnology is fully matching and is without doubt stimulating fundamental scientific progress, which remains the best hope for achieving sustainable and environmentally stable agriculture [196]. Indeed, the advancements made in the last 100 years with in vitro technology have gone well beyond what Haberlandt and the other pioneers could have imagined.

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