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Plant Biotechnology- A Powerful Tool for Development of New Plant Traits

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ABSTRACT: Plant biotechnology (PBT) encompasses a multitude of scientific tools and techniques for screening and genetic manipulation of plants to develop beneficial or useful plant/plant products. The proficiency of these tools and techniques could be augmented by nanotechnological interventions. The novelty and innovativeness of the transgressing discipline of nanotechnology benchmarks the synthesis, characterization, manipulation, and applications of matter at a scale of 1–100 nm such that it digresses from its bulk counterparts manifesting neoteric properties. Nanotechnology has equipped crop biotechnologists to elucidate molecular pathways for plant responses and tailor designer crops by use of nanomaterials (NMs) as vehicles for gene or proteins. Augmenting NMs has led to development of bionic plants that could efficiently capture solar radiations for higher productivity as well as could be multiplexed to behave as sensors. Nanoprobes like semiconductor and metal nanocrystals could be very useful for high-resolution imaging of the plant cell and organs. These nanobioimaging tools could be harnessed to discern dynamics of plant cell's biochemical and physiological responses in real time, and NMs could be used for four-dimensional imaging of plant growth responses. The plant disease theranostics has also been immensely improved, so that there is greater accuracy, speed, and sensitivity for detection of genetics of pests, pathogens, and crop plants including nanobarcodes for identification of genetically modified crops. The plant cell nanofactories could not only perform green and ecofriendly synthesis of NMs but also improve/alter seed germination and plant growth through a plethora of complex NM–biological system interactions.

KEYWORDS: plant biotechnology, ecofriendly, genetically modified crops, nanofactories, nanotechnological, green

I.INTRODUCTION

Current plant biotechnology has developed as a new age of science and technology where production of secondary metabolites, valuable plant genetics improvements, germplasm conservation, and production of large numbers of disease-free and new varieties are preferred. Production of artificial seeds, biopharmaceuticals, plant-made pharmaceuticals, recombinant or other therapeutic proteins, transgenic plants, and plant-made vaccines or antibodies (plantibodies) is part of the current research work in plant tissue culture science. Most of the plant tissue culture industries are exploiting the fundamental property of regeneration of plant cells for rapid production of elite varieties with superior genotypes on a large scale in a comparatively short time. The current plant tissue culture industry is estimated to be worth around US\$150 billion [1,2] (50–60% of the agriculture industry). Annual demand of tissue culture-raised products is about 10% of the total estimated value (US\$15 billion with growth rate of 15%) [1]. Most of the plant tissue culture-based industries are limiting the time-consuming labor or manual processes fully by transforming into more automated and robust technologies. The introduction of such robust and automated technologies has led to rapid multiplication of plantlets at a larger level. In this review, we discuss national and international scenarios of various plant tissue culture-based industries equipped with recent technologies used nowadays to make the process more suitable for large-scale production.

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Plant biotechnology can be defined in many ways, but it is most often the genetic engineering of plants through the use of recombinant DNA. This review focuses on regulatory requirements for genetically engineered (GE) plants. For scientists, particularly public sector and small company scientists, this is the most daunting challenge partially because a simple checklist of tasks does not exist, and even when the developer begins to see the light at the end of the tunnel, the regulators can request even more data and testing. Generally, regulations cover the environmental release of plants produced through genetic engineering. The goals of a regulatory system should be and often are flexibility, science-based rigor, stakeholder-responsiveness, nationally and internationally coordinated, and risk proportional. The review discusses primarily the U.S regulatory system, because the first steps toward regulating GE crops were taken in the United States in the 1970s. The regulatory system has evolved into a system in which regulations are approached on a case-by-case basis.[3,4]

However, a new paradigm is needed for data collection and coordination to simplify and standardize the petition process for federal agencies—fulfilling data needs for crop assessment while ensuring safety of the product. In the end, the ultimate goal of genetic engineering of plants is to benefit society through improvements in agricultural practices, plant health, and plant productivity.

II.DISCUSSION

Plant biotechnology or genetic engineering allows breeders to modify plants in an effort to meet the demands of a fast-growing global community. Many plant biotechnology companies and universities are behind a wave of patent claims on hundreds of genes that confer tolerance to herbicides and biotic and abiotic stresses; genes that improve wood properties for the biofuel and paper and pulp industries; and genes that have important applications in phytoremediation, nutrition, pest resistance, and plant-based pharmaceuticals. The patents cover the development of new crops capable of resisting attack by insects and diseases, and withstanding salt, drought, high and low temperatures, or other environmental changes brought on by global climate change. Worldwide, patent offices have documented thousands of patent applications for genes that confer these characteristics. They have been filed in multiple countries and several of the patents cover many genes across a range of plant species.[5,6]

However, one of the issues relating to the patenting of genetic inventions revolves around the question of whether a DNA sequence can be considered a discovery or an invention. In Europe, the rules of the EPC were recently amended to comply with the EU Biotechnology Directive. These amendments state that biological material isolated from its natural environment or produced by means of a technical process, even if it occurred previously in nature, is patentable. In addition to the need for the sequence to be novel and non-obvious, the process of isolating and characterizing it must also be patentable in order to patent the sequence.[7]

The USPTO takes the position that genes are chemical compounds, albeit complex ones, and, as such, they qualify for potential patenting as compositions of matter. Whereas a naturally occurring product, as it exists in nature, cannot be patented, the USPTO has allowed patents on naturally occurring products that have been purified, isolated, or otherwise altered. For this reason, gene patents have been issued in the U.S. for decades. Additionally, the USPTO produced a set of guidelines arguing that a gene sequence is patentable if it is novel, isolated, its molecular structure is known, and if it has substantive and credible utility. This position is consistent with the European rule amendments; however, on March 29, 2010, the U.S. District Court for the Southern District of New York delivered a serious blow to human-gene patent holders. The opinion, the first round in the closely followed Association for Molecular Pathology v. United States Patent and Trademark Office (widely known as the *ACLU v. Myriad Genetics*) case, invalidates several patents with claims covering genes associated with breast cancer. The result surprised many in the biotechnology industry because the broad ruling, if

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upheld on appeal, would eliminate future gene patenting, as well as effectively make existing gene patents invalid. Weakening patent protection for human genes could lead to a market change for future R&D involving genes not only related to human health, but also to agricultural biotechnology.

The complex nature of gene patents and enabling technologies involved in the development of transgenic plants presents other concerns related to IP rights. These issues are coming to the fore more frequently in developing countries, where no possibilities for gene patenting exist and no PVP laws are in place. For example, India's experience with insect-resistant cotton, transformed with a *Bacillus thuringiensis* (Bt) gene, was first developed by a joint venture between Mahyco, India's largest seed company, and Monsanto. Despite the fact that these varieties were hybrids and, thus, providing at least some protection against farmers saving seed, there were widespread reports of black markets for seeds and clandestine plant breeding to incorporate the Bt gene [8,9]

Another example is herbicide-tolerant soybean, which is the most widely grown transgenic crop in the world. It has become very popular in Argentina, where although genes may be patented, Monsanto was unable to make an application deadline, so its Roundup Ready® (RR) varieties were simply protected by PVP. But much of the RR soybean in Argentina is grown from seed that is saved or informally traded. In Paraguay and Brazil, RR soybean has gained great popularity based on the sale of black-market seed. Because only a small fraction of the RR soybean seed used in these countries is from authorized sources, Monsanto is beginning to pursue legal action for patent infringement against shipments of soybeans from South America that arrive in the ports of European countries, where the technology is patented. The solution currently under discussion is to charge farmers a royalty based on grain sold at harvest, to be collected by grain dealers, processors, or farmer associations. One of the major points of disagreement here is farmers' insistence on their legal right to save seed. Hence, in developing countries, controlling the use of genes and access to transgenic varieties will require the implementation of restrictive IP protection, such as strong laws related to patents and plant breeder's rights, and the vigorous enforcement of these laws, as well as biosafety regulations.[10]

III.RESULTS

Plant biotechnology has experienced exponential development during the past 30 years, facilitated in large part by the capacity of a clever soil bacterium (*Agrobacterium tumefaciens*) to transfer DNA segments into plant cells. This powerful function, harnessed by clever plant biologists during the 1980s, now enables routine delivery of recombinant DNA into plant cells for, among other things, production of recombinant proteins in plants. The use of plants for expression of subunit vaccine antigens, first pursued in the early 1990s, was a natural outgrowth of the emerging technology, coming soon after the groundbreaking demonstration that immunoglobulins could be produced in transgenic tobacco. Interested readers are referred to recent review articles that describe advances in the use of plants for production and delivery of vaccine antigens.

Unfortunately, commercial development of plant-derived vaccine products has been very slow, perhaps due to a very conservative pharmaceutical industry that is hesitant to try new technologies. A hypothesis that drove much of the early work on plant-derived vaccines held that cheap and orally delivered "edible" subunit vaccines could provide protection against infectious diseases in developing countries with limited medical infrastructures. Thus, ingestion of plant material would stimulate protective immunity, mediated by uptake and processing of antigens by the gut-associated lymphoid tissue (GALT). A number of hurdles can interfere with the effectiveness of this approach, including poor stability of proteins in the gut, substantial variability of antigen content in plant tissues, and regulatory concerns for the containment of transgenic crop plants and their use exclusively as pharmaceutical products. These and other issues are discussed in greater detail in

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other papers . Such challenges notwithstanding, most workers and watchers of the field continue to see great value in the use of plants for production of vaccine antigens. Importantly, compelling data on the protective efficacy of orally delivered plant-derived vaccine antigens continue to accumulate, as discussed below in detail in the section on animal vaccines.[11,12]

The approaches for plant-based protein expression include stably integrated transgenes into chromosomes in either the nucleus or the chloroplast, and transient expression using vectors that may use plant virus replication elements to amplify the transgene. We present only a very brief discussion here, and interested readers are referred to excellent recent review articles . Nuclear genes are inherited in a Mendelian fashion, whereas chloroplast genes are passed through progeny only from the maternal parent, since pollen cells are devoid of chloroplasts. Tissue-specific promoters allow foreign proteins to accumulate in seeds . Proteins that accumulate in seeds are very stable to temperature extremes and can be stored for months to years with little loss of protein activity.

During the past decade, the use of rapid transient expression in plants (rather than the slow production of plants with stably integrated transgenes) has increased greatly and represents an ever-growing proportion of plant-based protein expression work. The development of viral vectors has enabled very fast and very high levels of protein expression in plants . *Nicotiana benthamiana*, a tobacco relative ,is the typical plant host, due to its ease of culture and effective support of plant virus replication. Protein expression levels at 1–2 mg per kilogram of leaf mass are often ≥ 10 -fold higher than those obtained with stably transgenic plants, and indicate the potential for purification of plant-produced proteins at costs that are economically competitive with microbial fermentation systems. Moreover, culture of *N. benthamiana* can be readily scaled up to massive levels . Kentucky Bioprocessing, Inc. has developed a large-scale system for plant growth, infiltration with *Agrobacterium*, and purification of recombinant proteins . The convenience of transient plant-based expression enables rapid response to emerging viruses such as influenza and norovirus, as described by Charles Arntzen in his address to the 238th National Meeting of the American Chemical Society . These developments make plant-based expression and purification of vaccine antigens, including virus-like particles (VLP) (Huang et al., 2009; Santi et al., 2008), for mucosal delivery commercially viable.[13,14]

We can also utilize purified antigens for intranasal delivery, which requires more concentrated antigen at lower doses than oral delivery. Nasal immunization induced antigen-specific mucosal IgA responses in salivary glands, respiratory tract, intestines, and reproductive tracts in mammals Moreover, mucosal immunization can prime both mucosal and systemic immune responses . Thus, intranasal delivery of plant-derived vaccine antigens, especially VLP, is a promising strategy, as we discuss further below.

In this review, we will describe the progress in selected areas with mucosal vaccines in human and animal hosts. We focus on studies that involved humans or have shown strong indication of efficacy in preclinical studies.[15]

IV.CONCLUSIONS

While plant biotechnology strategies are now being deployed for a variety of markets, including the production of medicines, improved forest products and pollution control systems (recently surveyed), the largest and most high profile market sector has been the production of genetically modified (GM) food crops, an area where the influence of downstream elements such as governments, regulatory systems, food processors, distributors and end-users/consumers is very clear. Many scientists in Europe have joined the vigorous public debate, primarily in support of the technology as a whole, and few recent plant biotechnology conferences have been complete without a consideration of consumer acceptance of GM crops. In some quarters, the difference encountered in market and regulatory response between the EU and U.S. has generated an erosion of confidence regarding the future growth potential of certain market segments in plant biotechnology.

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Small European biotechnology companies and universities wishing to commercialise plant biotechnology are more vulnerable than the global agro-chemical sector in this regard, being geographically and financially constricted, and relying heavily on public confidence.[16]

Despite this, agricultural biotechnology is currently displaying strong growth, with optimistic projections indicating that the global biotech plant market will increase to US\$20 billion by the year 2010 [13]. The technology is at a critical stage of development, and industry marketers are currently mindful of the historical unfavourable precedent set by food irradiation, where European consumer resistance prevented its immediate commercial application [14]: both technologies involve high volume, low value goods intended for human consumption. The EU agri-food market has also been adversely affected by the BSE crisis and lingering public concerns about antibiotic and growth hormone abuse in agriculture; this may be expected to be exacerbated by the requirement for Europe to honour trade commitments with the U.S. on issues such as the admissibility of growth promoters in beef and the use of bovine somatotropin for dairy herds.[17]

However, despite such difficulties, there are indications that the agricultural commodity sector will indeed be a key biotechnology market for the next millennium, facilitated through the gradual displacement and/or complementation of traditional, chemical-based disease control strategies with biotechnology agents, and the production of GM foods which deliver tangible value to the consumer (especially regarding nutrition and health-promoting properties).

The technical opportunity afforded by monogenic traits, combined with the size of the plant disease control market, and the ability to increase the useful commercial life-time of already successful products, dictated that the agro-chemical sector would choose herbicide/pest tolerant crops as pathfinder products. Herbicides are currently the largest global agro-chemical market, accounting for about 50% of sales, while insecticides make up 30%, with the remaining 20% largely fungicides [15]. In the U.S., there has been rapid market penetration of GM crops: about 28 million hectares were grown in 1998, and the area is expected to be about 60 million hectares by the year 2000 [16]. However, the strong public reaction to herbicide-tolerant GM foods in Europe was unexpected by the industry and bears analysis.

Firstly, it must be noted as a matter of interest that genetically modified tomato pastes have not caused the same degree of public distrust observed for herbicide tolerant crops. While one may conjecture that this is primarily due to the association of products such as Roundup Ready™ crops with a ('chemical') herbicide, it strongly suggests that the market acceptance of future plant biotechnology products will be highly case specific. This will heighten the importance of such factors as accurate and comprehensive market research, product labelling and where appropriate/feasible, crop produce segregation for future product launches.

Secondly, much public concern has centred on potentially adverse environmental effects due to possible transgene transfer. These concerns are now actively being addressed by industry through the development of second generation products which feature either the use of male sterile clones, the use of hybrids to suppress gene transfer by pollen, and the transformation of mitochondria and chloroplasts [17].

Thirdly, the agro-chemical industry failed to discriminate between differences in market factors pertaining to the U.S. and European arenas. The well-documented different eating habits that exist between geographic regions [18] and the differences in how the European public views technology [5] were seemingly ignored by the companies in question. From a marketing perspective, the vociferous adverse public debate may herald the breakdown of the traditionally strong biotechnology industry cohesion which has been observed to date, as individual companies seek to discriminate their technologies from other products or companies with an 'undesirable' image.

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There are other important factors which will accelerate the market for transgenic food crops. World population continues to increase, with a projected minimum doubling of global food demand predicted by 2050, at which point the population will be about 10 billion people [13]. For example, specific projections for agricultural markets imply a future requirement for cereal production to increase by 41%, meat by 63% and roots and tubers by 40% by 2019 [18]. The challenge in producing enough food for the existing population is currently poorly understood by the public. For example, despite an annual investment of US\$32 billion on conventional pesticides, crop pests alone reduce global food production by at least one-third [13]. Increased familiarity with biotechnology and modern agricultural practice through government-funded public education, combined with the public endorsement by agencies such as the World Bank [17], will also act to demonstrate the important social and economic role which biotechnological innovation can play.

While undoubtedly the market for organically produced foods is growing quickly, this form of agriculture is unlikely to displace conventional agricultural practice in producing quality food for the masses. Conversely, the public realisation of the historical dominance of herbicide usage in conventional agriculture is also likely to breed greater acceptance by the markets for technologies which can produce cheap quality food with lower herbicide inputs. In this regard, the benefit of the economic returns from transgenic crops that result from decreased pesticide usage and increased yields must be passed onto the consumer: the Euro has already focused consumer attention on differences in commodity prices between EU member states. Finally, dietary changes in the population and a move to convenience foods which both accompany increased levels of affluence [18] will require the maintenance of the quality and volume advantages offered by biotechnology-aided agriculture.

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