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TRANSGENIC CROPS: RECENT DEVELOPMENTS AND PROSPECTS

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ABSTRACT

It is now more than fifteen years since the first transgenic plants were generated experimentally. In that period there have been dramatic advances in our understanding on both basic and applied aspects of plant biology.

Transgenic plant research depends on the availability of procedures for plant transformation. Two types of method for plant transformation exist, the use of *Agrobacterium* as a biological vector for foreign gene transfer, and direct gene transfer techniques, in which DNA is introduced into cells by the use of physical, electrical or chemical means. *Agrobacterium* can be used to transform a wide range of plants, but there are α number of species which are of interest for basic or applied research in which of *Agrobacterium* - mediated transformation is not reproducible or efficient. Using this procedures thousands of transgenic crops have been developed experimentally or field tested, while few of them are currently cultivated world wide, predominately on temperate zone crops and on conditions prevailing in industrial countries offering the potential increasing and improving food production capacity while limiting the use of agrochemicals and protect the environment. The " first generation " of transgenic crops were aimed at improving traits involving single genes. Now we are on the verge of a new step in crop modification, fueled by the rate at which new genes (important for plant growth and development metabolism and stress tolerance) characterised. Transgenic technology has been pivotal in the full spectrum of these new developments, from gene identification to an improved understanding of their regulation, as well as genetic transformation involving more complex transfers of many genes simultaneously. This will further help in managing natural resources like water, soil, e.t.c. in a better way.

Our view of the nature of crop products can also be expected to change in the short to medium term, as plants are exploited for the production of novel compounds such as biodegradable plastics and new pharmaceuticals. However, it is the case that the extent to which the potential of transgenic research is realised will depend on public acceptance. To a significant extent this will require that the biology of transgenics is fully understood, and that a maximum degree of predictability of transgene effect, both phenotypic and genotypic, can be ensured. There is a need for diffusing this technology to tropical plants and adapt it to benefice the small farmer in the developing world were food demands will increase.

To achieve this it requires: to find ways integrating biotechnology research into their national agricultural research activities in one hand horizontal and vertical networking cooperation on the other.

Finally the implication of advancement in this relatively new technology especially in the area of biosafety, production patterns, biodiversity, inertecnal property rights and other critical factors should be sufficiently dismissed and understood.

I. INTRODUCTION

Genetic modification of crops has enabled plant breeders to modify plants in novel ways and has the potential to overcome important problems of modern agriculture. Introduction of genes into plants has been made possible using *Agrobacterium* as a biological vector, and direct gene transfer techniques. *Agrobacterium*-based methods are more efficient and simple but have the disadvantage that are not applicable in every plant species (Christou, 1995). Recent developments indicate that these host-range limitations can be overcome by developing specific plant cell culture procedures and

defining inoculation and co-cultivation conditions (Park *et al.*, 1996). Some important non-host species such as maize and rice have now been stably transformed by *Agrobacterium*. Although plant transformation was initially experimental, the potential of commercialization of new improved varieties was early realized and a fast growing international ag-biotech market has already been formed. New transgenic varieties have been produced that are resistant to pathogens, insects, herbicides, or express novel characters that improve product quality and agronomic traits. The new opportunities to modify plants in novel ways with genetic modification present new responsibilities for safe use to avoid adverse effects on human health and the environment (Dale and Irwin, 1998). Risk assessment studies are integral part in the production and placing to the market a transgenic variety. Different countries have adopted different approaches in biosafety assessment. International harmonization of biosafety standards is an important challenge as we face the international trade of transgenic plant products. The role of international organizations such as OECD and United Nations may be critical towards this goal.

In the following sections we will present the methods and techniques that are utilized in the production of transgenic plants emphasizing recent developments for multiple genes transfers at once; we will consider the major achievements of the new technology and the future prospects and challenges, as well as remaining technological gaps; we will enumerate and discuss some of the possible risks involved in the unrestrained use of transgenic plants and their products; and finally, we will present the current status of the regulatory framework pertaining the field release of transgenic plants.

II. METHODS OF GENE TRANSFER IN PLANTS

Transgenic plant research depends on the availability of procedures of plant transformation. There are two types of effective gene transfer to plants, the first is based on the use of *Agrobacterium* as a biological vector and the second is based on the use of physical, electrical or chemical treatments to introduce isolated DNA into cells alleviating the need for vector use. The latter techniques are commonly termed direct gene transfer methods.

A. Indirect gene transfer using *Agrobacterium* as vector

The most widely used method for the introduction of new genes into plants is based on the natural DNA transfer capacity of *Agrobacterium tumefaciens*. In nature this soil bacterium causes tumor formation (called crown gall) on a large number of dicotyledonous plant species. During this infection a part of the Ti-plasmid of *Agrobacterium*, called T-DNA, is transferred and integrated into the plant genome. This natural capacity made us use this bacterium as a natural vector of foreign genes (inserted into the Ti-plasmid) into plant chromosomes.

Agrobacterium-based and direct gene transfer techniques were developed in parallel, but the former is today the most widely-used method because of its simplicity and efficiency in many plants, although it still suffer limitations in terms of the range of species which are amenable to transformation. These limitations are due to the natural host range of *Agrobacterium*, which generally infects herbaceous dicotyledonous species most efficiently and is less effective on monocotyledonous and woody species (De Cleene and De Ley, 1976). In these plants, direct gene transfer techniques offer the means to establish transformation systems but many of these techniques suffer from a relatively low efficiency of transformation. Attempts are therefore being made to exploit and adapt the relatively simple and convenient *Agrobacterium* system to transform recalcitrant plant species. Recent work has shown that these host-range limitations are not absolute and by developing specific plant cell culture procedures and defining inoculation and co-cultivation conditions, some important non-host species have now been stably transformed by *Agrobacterium*, although there are still many plant species for which *Agrobacterium* transformation is not usable.

The development of reliable transformation protocols for recalcitrant species depends on the establishment of an efficient regeneration procedure, a high transformation rate of the regenerable cells, and an effective selection for regenerating transformed cells (Gheysen *et al*., 1998). The plant genotype is an important factor, which determines both the regeneration capacity and the efficiency of *Agrobacterium* transformation. Equally important is the choice of the bacterial strain, and the external conditions during the preculture and cocultivation of agrobacteria and plant material.

The *Agrobacterium* transformation methods are using two different procedures. The first one is transformation that is dependent on a regeneration procedure while the second is not. The purpose of the regeneration procedure is twofold: it allows the recovery of uniformly transformed shoots and the selection of such shoots. For many plant species, the lack of suitable regeneration method is one of the main bottlenecks in developing a transforming procedure. A particular regeneration method is usually

only efficient with a limited number of genotypes even within a species. Somaclonal variation may also be problematic with some regeneration procedures. Therefore, many efforts have been devoted to the development of regeneration-independent transformation procedures, such as meristem transformation and *in planta* transformation techniques.

The shoot apex has been used in **meristem transformation** as an attractive target for transformation since it contains the meristematic cells from which all the aerial parts of the plant are derived. Because meristems are multicellular organs, primary transformants are expected to be chimeric, consisting of transformed and untransformed sectors. This has two important consequences. First, it does not always result in germline transformation and transmission of the transgenes to the offspring and second, a stringent selection procedure cannot be applied (Gheysen *et al*., 1998). These have as a result this transformation method to be labor intensive and very inefficient. Several reports clearly give evidence for stable transformation that has been achieved through meristem transformation with *Agrobacterium* infection of important crops, such as *Musa acuminata* (May *et al*., 1995), *Oryza sativa* (Park *et al*., 1996). *Oryza sativa* is the first cereal species that has been stably transformed via *Agrobacterium*. Targeting cells of meristem for transformation has, therefore, the advantage that transformed cell lineages, can be obtained without the involvement of a regeneration pathway which involves dedifferentiation and reorganization of cells, so somaclonal variation is not a problem and the transformation is rather genotype-independent. These are the main advantages of an approach like that. Nevertheless, additional manipulations (e.g. hormonal treatments) are necessary to obtain transformants with acceptable frequencies, reintroducing a factor of genotype dependence in the procedure (Gheysen *et al*., 1998).

Over the last decade, several ***in planta* methods** for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana* have been developed that do not involve any tissue culture steps. In the first described procedure (Feldmann and Marks, 1987) imbibed seeds are infected with *Agrobacterium*, allowed to grow into mature plants and finally transformants were identified among the seeds harvested from these plants. Bechtold, *et al*. (1993) inoculated flowering *A. thaliana* plants by vacuum infiltration with an *Agrobacterium* suspension and managed to get transformants at even higher frequencies. Another technique which has been developed recently (Clough and Bent, 1998) is floral dip. It is a simple dipping of developing floral tissues into an *Agrobacterium* suspension. The absence of any tissue culture step (so somaclonal variation does not occur), the simplicity and the relatively high efficiency of the transformation procedure would make such techniques attractive to adapt the technique to other plant species, recalcitrant to regeneration procedure.

B. Direct gene transfer

The development of novel direct gene transfer methodology, by-passing limitations imposed by *Agrobacterium*-host specificity and cell culture constraints, has allowed the engineering of almost all major crops, including formerly recalcitrant cereals, legumes and woody species. Direct gene transfer transformation methods are species and genotype-independent in terms of DNA delivery, but their efficiency is influenced by the type of target cell, and their utility for the production of transgenic plants in most cases depends on the ease of regeneration from the targeted cells, as most methods operate on cells cultured *in vitro*. As direct gene transfer referred methods such as particle bombardment, DNA uptake into protoplasts, treatment of protoplasts with DNA in the presence of polyvalent cations, fusion of protoplasts with bacterial spheroplasts, fusion of protoplasts with liposomes containing foreign DNA, electroporation-induced DNA uptake into intact cells and tissues, silicon carbide fiber-induced DNA uptake, ultrasound-induced DNA uptake, microinjection of tissues and cells, electrophoretic DNA transfer, exogenous DNA application and imbibition, macroinjection of DNA (Barcelo and Lazzeri, 1998· Walden and Schell, 1990). The most significant direct gene transfer methods are presented in Table 1.

Most workers in transgenic plant research are interested primarily in applying a transformation technique rather than in its mechanism of operation, so there is a general wish for technically simple methods which are easily transferred between laboratories and which ideally do not require expensive, specialized equipment. Of the above direct gene transfer techniques, particle bombardment and protoplast transformation are today the most widely used. The former most closely satisfies the criteria of technical simplicity and reproducibility, although it requires a specialized particle gun, the commercial version of which uses relatively expensive consumables. Protoplast transformation can be highly efficient, but demands more complicated cell culture techniques and is limited by the difficulty of regenerating plants. Tissue electroporation is relatively simple, applicable to regenerable tissues and has produced stably transformed plants in several systems after only a relatively short period of development. These results suggest the method should receive further attention to evaluate its potential for wider application. Ultrasound and silicon carbide fiber-mediated techniques are newer methods, which are again technically quite simple. They have been tested in few laboratories and need more

research to determine their limitations. Microinjection and laser-mediated transformation are specialized techniques, which are at present inefficient. Electrophoretic transfer to date does not give us evidence that the gene transfer actually occurs. Whole-plant direct gene transfer methods would be methods of choice for most users, but despite several claims of high transformation efficiencies most critical studies have not produced evidence for integrative transformation (Barcelo and Lazzeri 1998).

Table 1. The most significant methods of direct gene transfer.

METHODS	DESCRIPTION
Particle bombardment	Delivery of DNA into cells using microscopic gold or tungsten particles coated with DNA as carriers accelerated into target cells by gunpowder, gas or air pressure or by electrical discharge (Christou, 1995).
Protoplast transformation	DNA introduction into protoplasts using PEG-mediated DNA uptake and electroporation, liposome (containing plasmid DNA) fusion (Krens et al., 1982;·Caboche, 1990).
Tissue electroporation	Transformation of plant organs or regenerable cell cultrures (Li et al., 1991; D' Halluin et al., 1992)
Ultrasound-induced transformation	DNA uptake into protoplasts, suspension cells, and tissues induced by ultrasound waves (Joersbo and Brunstedt, 1990; Joersbo, 1990; Zhang et al., 1991)
Silicon-carbide fiber or whisker transformation	The fibers perforate cell walls and allow DNA to penetrate the cells (Frame et al. 1994)
Laser-mediated transformation	Laser beams are used to create openings in cell components and organelles allowing DNA insertion (Weber et al., 1989).
Microinjection	Direct delivery of DNA into plant cells using a microsyringe (Schnorf et al., 1991).

Plant genetic engineering is now at a crucial crossroad. The gene transfer constraints appear to have been removed from a number of important crops. Technical problems still remain but they are not insurmountable. The attention of the scientific community is gradually shifting to other areas such as identification and cloning of genes responsible for multigenic traits. The study of genomes (known as "genomics") involves the mapping, sequencing and analysis of genomes in order to determine the structure and function of every gene in an organism. This has already been accomplished in several microorganisms and much effort has been devoted to the complete sequencing of the genome of higher eukaryotes including plants. Information derived from analysis of such data will be used to map entire biochemical pathways, which will be then easier to transfer and incorporate in transgenic organisms. Thus, genomic information can be used to improve important plant traits through genetic engineering, such as high and stable yield and product quality. Utilization of genomics in transgenic technology will also require establishment of routine techniques for simultaneous multiple gene transfer in a single transformation event. In most cases, one or a few genes are transferred to the plant genome along with a selectable marker that facilitates selection of transgenic tissues. Genetic transformation with a single target gene has been used for the production of transgenic crop plants that expressing herbicide tolerance, resistance to fungal, viral and bacterial diseases and insect pests. In addition improved agronomic characteristics have been achieved by manipulating metabolic pathways through overexpression of a specific gene or the use of antisense sequences.

As most agronomic characteristics are polygenic in nature plant genetic engineering will require manipulation of complex metabolic or regulatory pathways involving multiple genes or gene complexes. Redirecting complex biosynthetic pathways and modifying polygenic agronomic traits requires the integration of multiple transgenes into the plant genome, while ensuring their stable

inheritance and expression in succeeding generations. Transfer of multiple genes via *Agrobacterium*-mediated transformation although possible is technically demanding and becomes increasingly problematic as the number of genes and the size of the transferred DNA increases. But this transfer could be achieved through cobombardment in a simple process in which genes carried on separate plasmids are mixed prior to transfer by particle bombardment. In this manner numerous genes can be transferred simultaneously (Figure 1) using a single selectable marker (Chen *et al*., 1998). This will certainly be one of the major future goals of transgenic technology in plants (see also below).

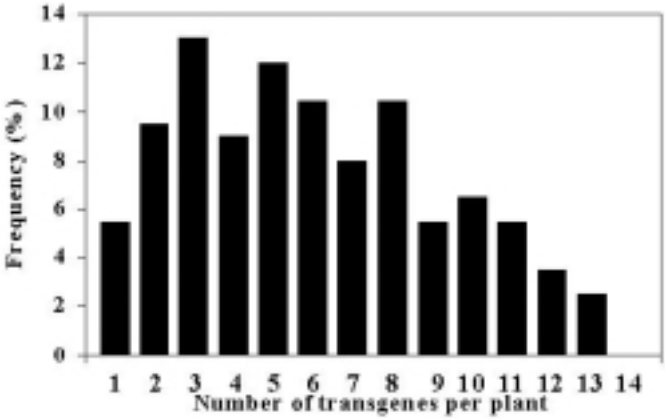

Figure 1. Frequency of transgenic plants containing 1-14 genes.

C. Technological gaps

Although considerable progress in gene transfer technology has already been accomplished, and techniques are consistently improved for more efficient plant transformation, there are several obstacles preventing application of the new technology in certain circumstances. In such cases, transgenic technology needs radical improvement and perhaps reformation of the underlying strategies to enable breeding transgenic varieties in certain plant species and for certain uses. The barriers that new developments in transgenic technology will have to overcome into the near future include:

- The need for genotype independent protocols for regeneration and transformation in a number of mainly tropical vegetables and trees. International organizations should promote networking of interested regional countries and labs to work together towards that goal. Also, companies advanced in transgenic technologies could participate in such networks providing solutions and expertise assistance to overcome difficulties that will arise during these efforts.
- For standing tree populations transgenic technology will face the considerable time that is needed for such trees to be productive. For example breeding olive trees in Mediterranean countries with the new technology will require several years from a successful transformation to a new transgenic plantation fully productive. In such cases, alternatives will have to be devised, that may include grafting of mature trees in the plantation, and *in situ* transformation of mature trees.
- Securing the stability of the transgenic phenotype especially under stress will be of immense importance. There are already examples where significant losses were observed in transgenic varieties grown under adverse environments. Stability of transgene expression can be dependent on the position the transgene has landed in the genome, the number of copies incorporated, the promoter used, the presence of repeated elements or cryptic remnants of transposable elements. Novel techniques allowing precise manipulation of transgene incorporation and advancement of our knowledge on genome function can help to secure stable expression in a wide range of environmental conditions.

III. ACHIEVEMENTS AND FUTURE PROSPECTS

A. Products developed

In conventional plant breeding, genes can be transmitted only by crossing in the same or closely related species. Transgenic techniques have allowed genetic material to be transferred between completely unrelated organisms, so that breeders can incorporate characteristics that are not normally available within a species. The modified organisms exhibit properties that would be impossible to obtain by conventional breeding techniques. Modern biotechnology makes plant breeding programs more effective in two important ways. Firstly it allows transfer of specific genes, incorporating into the new variety only those traits that are wanted. This makes the process of trait transfer faster, more exact, cheaper and less likely to fail than traditional crossbreeding methods. Secondly, it gives breeders the

freedom to incorporate genes from unrelated species into the target plant, a possibility that is unprecedented in plant breeding.

Transgenic methods have been employed over the last 15 years in a number of important crop plants such as maize, cotton, soybean, oilseed rape and a variety of vegetable crops like tomato, potato, cabbage and lettuce. In European Union 1255 field tests involving transgenic plant varieties have been approved. This number has surpassed 5000 field releases (permits and notifications) in the United States. A summary of permits per European Country is shown in Table 2. The commercial production of transgenic crops shows a rapid increase the last few years. The global area (excluding China) of transgenic crops from zero in 1995 has reached 27.8 million hectares in 1998 (Figure 2).

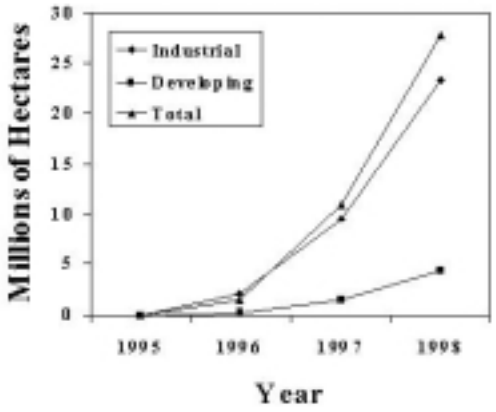

Figure 2. Global area cultivated with commercial transgenic crops (excluding China) from 1995 to 1998. The distribution of the area between industrial and developing countries is also shown. Source: James (1998).

During this period, the larger part of global transgenic crops has been grown in industrial countries with significantly less in developing countries (James 1998). The proportion of transgenic crops grown in industrial countries in 1998 was 84%, slightly less than 1997 (86%), and only 16% grown in the developing countries, with most of that area in Argentina, and the balance in Mexico and South Africa.

The most frequently modified plants form a total of 50 or more plant species is shown in Figure 3. Among these almost 50% of the new varieties are modified maize and oilseed rape plants. The most frequent traits that have been modified using genetic engineering are shown in Figure 4.

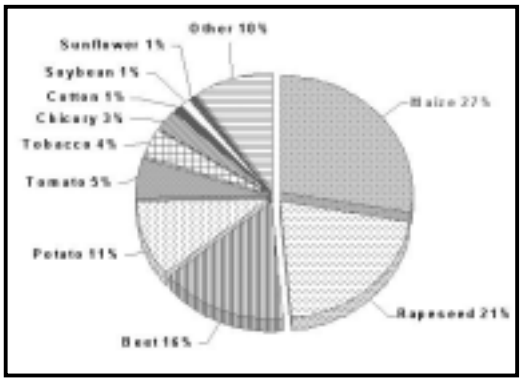

Figure 3. The most frequently modified plants form a total of 50 or more plant species in European Union. Source *http://biotech.jrc.it*

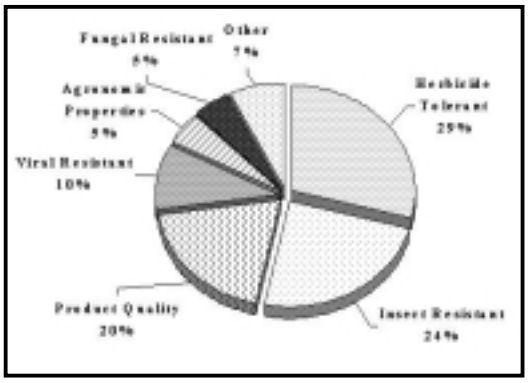

Figure 4. The most frequent traits that have been modified using genetic modification in European Union. Source *http://biotech.jrc.it*

Table 2. Permits for field trials of genetically modified plants in European countries from October 1991 to June 1998. Source: *http://biotech.jrc.it*

Country	Total
Austria	3
Belgium	90
Denmark	30
Finland	13
France	391
Germany	77
Greece	12
Ireland	4
Italy	206
Netherlands	103
Portugal	10
Spain	117
Sweden	35
United Kingdom	164
Total for the European Union	**1255**

The major achievements of transgenic plant technology up to now concern tolerance to insect or disease pests, herbicide tolerance, and improved product quality. A description of the major categories of modified traits with characteristic examples will follow.

Insect tolerance: New varieties of maize, cotton and tobacco, for example, have been developed utilizing a gene from the bacterium *Bacillus thuringiensis* to produce a protein (the Bt protein) that is specifically toxic to certain insect pests including bollworm, but not to animals or humans (Carozzi et al. 1992; Liang et al. 1994). This protein has been used as a pesticide spray for many years. Cultivation of these transgenic plants should help reduce the use of chemical pesticides in cotton production, as well as in the production of many other crops, which could be engineered to contain the *Bacillus thuringiens* gene. For a more detailed discussion on insect tolerance and uses of the transgenic technology for production of pesticidal crops see the chapter by Dr. David Andow in this volume.

Disease resistance: Tobacco mosaic virus (TMV) causes the leaves of some important crop plants to wither and die. Incorporation into the plant of a gene that encodes the coat protein of the virus protects it from disease (Clark et al. 1995). This approach has also been applied to other viral diseases in crops. More progress in development of disease resistant transgenic plants will be seen in the near future. Over the past decade, many efforts were focused on understanding plant-pathogen interactions in molecular terms. This led to the identification of disease resistant plant genes that specify race-specific resistance to pathogens. The tomato disease resistant gene *Pto* for example, confers resistance to the bacterial pathogen

Pseudomonas syringae pv *tomato* carrying the *avrPto* gene. Recently, Tang et al. (1999) reported that overexpression of the *Pto* gene in transgenic tomato plants activated defense responses and conferred broad resistance to several bacterial pathogens.

Herbicide tolerance: Engineering herbicide tolerance in transgenic plants has been accomplished exploiting at least three different mechanisms: overexpression of the target enzyme, modification of the target enzyme, and herbicide detoxification (Tsaftaris 1996). Examples of transgenic plants developed based on each mechanism are following.

Glyphosate is an environmentally more benign, widely used broad-spectrum herbicide. It is easily degraded in the agricultural environment and works by interfering with the EPSPS enzyme system that is present only in plants. Unfortunately, the herbicide kills crop plants as well as weeds. Transgenic plants including maize, soybean, and cotton have been developed, overexpressing an additional copy of the EPSPS gene from *Petunia hybrida* under the strong 35S promoter and exhibiting increased tolerance to glyphosate.

Alternatively, expression of a mutant *Aro A* gene from *Salmonella typhimurium* (which encodes EPSPS) in transgenic tobacco resulted in even higher tolerance to the herbicide than overexpression of the wild-type petunia EPSPS gene (for review see Tsaftaris 1996). This allows farmers to control weeds in transgenic cultivars spraying with glyphosate alone.

A different approach has been applied for development of resistance to the herbicide phosphinothricin (basta). The *bar* gene from *Streptomyces hygroscopicus* or *S. uiridochromogenes* encodes the enzyme phosphinothricin acetyl transferase (PAT), which converts the herbicide to a nontoxic acetylated form. Expression of the *bar* gene in transgenic tobacco, potato, and tomato plants conferred phosphinothricin resistance at up to 10 times the normal application rate of the hebicide in the field (Wohlleben et al. 1988).

Product quality: Transgenic technologies have been used to modify other important characteristics of plants such as starch composition in potato (Tahaka et al. 1998; Lorberth et al. 1998), ripening in tomato (Smith et al. 1990; Klee et al. 1991), lignin content in arabidopsis (Ni et al. 1994), flower vase-life in carnation (Bovy et al. 1995) and explore many new possibilities for uses in agriculture as well as in industry.

B. Future prospects

Major achievements of plant biotechnology are presently limited to traits involving one or a few genes. It will probably require more research before we can manipulate complex traits (such as yield) that are influenced by many genes. However, with newly developed techniques we can now incorporate multiple genes in plant genomes integrating multiple traits.

In addition, advances in structural and functional analysis of higher plant genomes will provide substantial knowledge on important biochemical pathways that are involved in the regulation of more complex characters. This could even enable scientists to identify and transfer entire biochemical pathways from one species to another and incorporate them into new hosts for the benefit of agriculture and/or industry. Eventually it may also be possible to develop crops for non-food uses by modifying traits to make them more suitable for industrial purposes, or to use plants rather than animals to make antibodies for medical and agricultural diagnostic purposes, and delivering vaccines with food in developing countries.

Current research will see the improvement and development of crops for specific purposes. Plants that require less water could be developed for countries with arid climates. Crop plants engineered to be tolerant to salt could be farmed in salt-damaged farmland or could be irrigated with salty water. Crops with higher yields and higher protein values are also possible. Much current research focuses on understanding and developing useful promoter sequences to control transgenes, and establishing precise methods to insert and place the transgene at specific locations in the recipient chromosomes. Much still needs to be done to improve our knowledge of specific genes and their actions, the potential side effects of adding foreign DNA and of manipulating genes within an organism and the problems associated with transgene silencing.

C. Phenotypic stability of transgenic varieties

Transgenic crop plants will only be of value if their phenotype is stable in the field and transmitted faithfully in subsequent generations. Although it is possible to study transgenes with a high level of precision, there is often uncertainty related with inactivation and structural instability of the transgenes. This inactivation is well documented and is most frequently correlated with gene silencing and not loss of the transgene (for review see Tsaftaris and Polidoros 1999). Gene silencing in transgenic plants has been identified as a major obstacle in transgenic technology. Reversal to herbicide sensitivity for example, of transgenic plants bred for tolerance to herbicides could lead to significant loses. From the applied side, gene silencing has come as an unwelcome surprise and is turning out to be a substantial problem. According to Finnegan and McElroy (1994) of 30 companies polled, nearly all reported some problems with unwanted silencing of transgenes. It has been shown that this unwelcome sensitivity due to inactivation of the transgene mediated by methylation, is triggered by stresses like the common agronomic practice of seedling transplantation in the field (Brandle et al. 1995). Thus, it requires closer attention since many crops need transplantation in the field and this imposes a severe stress for the young plantlet. It is clear that some steps that were taken for granted may need to be further investigated for successful commercialization of transgenic crops.

IV. RISK ASSESSMENT AND THE REGULATORY FRAMEWORK

A. Risk assessment

Advances in transgenic technology bring new responsibilities for safe use of transgenic plants for the benefit of humanity and the environment. The objective in risk assessment is to develop safety procedures, proactively rather than reactively. Safety issues are scale dependent and are probably different in small-scale experimental field trials than in large-scale commercial releases. Long-term effects of transgenic plants and their products may be only detectable after large scale or even commercial production of transgenic crops. Thus, one major challenge concerning safety of transgenic organisms is to develop procedures to assess long-term effects on human health and the environment.

Other issues have also been emerged that could be considered in risk assessment. Competitiveness, jobs and investment are thought to be at risk if the technology is not adopted, and potential benefits lost. Another dimension is the issue of consumer choice and rights to reject the technology at the point of sale. In large part this has been the focus of the growing consumer movement in all parts of the world, and especially in Europe.

In addition, much of the commercialization of the new technology relies on a few international companies with capital and power to dominate in the forming market of transgenic plants. The future may well find just a few key multinational industries active in producing recombinant plants to manage plant diseases and to produce agricultural and industrial products leaving the developing countries (which could benefit the most from the new technology) well behind. This can be alleviated if laboratories from developing countries along with advanced ag-biotech companies share knowledge and technology in a network, aiming to the development of improved transgenic varieties of crops that may be of minor commercial value for the companies, but critical as source of food or agricultural income for the developing countries.

A summary of possible risks for human health and the environment, associated with transgenic plants is given below.

Risks for human health

- Formation of new allergens from the novel proteins expressed in the transgenic organism, which could trigger allergic reactions at some stage.
- Creation of new toxins through unexpected interactions between the product of the genetic modification and other endogenous constituents of the organism.

- Dispersion of antibiotic resistance genes used as markers from the genetically modified organism derived food to gut microorganisms and intensification of problems with antibiotic resistant pathogens.

Risks for the environment

- Gene transfer from the transgenic plant to related species as a result of hybridization that could lead to new pests.
- The transgenic plant escapes its intended use and becomes an invader to the natural environment.
- Harmful effects on non-target species with the expression, for example, of insecticide toxins that can kill beneficial as well as targeted insects.
- Development of resistance from the continuous use of the same agent on the target organism.
- Harmful effects on ecosystems when transgenic plant products interfere with natural biochemical cycles.
- Harmful effects on biodiversity if a transgene offers an adaptive advantage in transgenic plants escaped in the area of cultivation or in wild relatives where it could be transferred by cross-fertilization. This is practically important if occurring at the centers of genetic variation of cultivated plants. In addition, biodiversity concerns have been raised for current cultivation systems including many locally adopted varieties if they will be substituted by a few new transgenics.

The process of examining the above risks from the release of a transgenic plant to the environment can provide a framework for risk assessment. Of course, enumeration and listing all the above major questions or possible risks expressed from scientists, consumers, and ecological groups for field testing and commercialization of transgenic plants does not imply that all the above are a concern for all the different transgenics and in different environments. For example, a risk for possible new allergenicity to the consumer could be meaningful, thus requiring testing prior to release, in cases where new genes coding for possibly new allergenic compounds have been cloned into plants. This question should not concern transgenics without such kind of genes cloned.

B. The regulatory framework

The criteria and factors that determine biosafety assessment of transgenic plants vary in different countries. In European Union all plants produced by genetic modification must be assessed (technology based assessment) whereas in the USA and Canada only plants modified with particular genes are regulated (product based assessment). There is considerable debate on the safety guaranteed by the two approaches. The use of *Agrobacterium* as a vector for the transformation process implies that the transgenic plants produced will be regulated under both approaches. However, as other methods avoid *Agrobacterium* and sequences derived from plant pathogens there is considerable difference on the regulatory requirements between North America and Europe. Time and scale differences in commercialization of transgenic crops will also have an effect making one system more "experienced" than the other.

In USA three agencies share the primary responsibility for regulating the genetically modified organisms, whether they be designed for closed systems or for environmental uses. These are the USEPA, the FDA, and the USDA. In addition to federal regulation, several states and municipalities have enacted biotechnology-related legislation, including provisions related to the environmental release of genetically modified organisms. Each of the federal agencies regulating biotechnology is guided in its analysis and decision-making criteria by its specific legislation i.e., the lows passed by Congress charging each agency with specific responsibilities. These laws differ in their mandate as to what populations to consider with regard to adverse effects (e.g., humans, crops, the environment), as well as in their mandate as how to strike a balance between risks and benefits. In addition to its specific legislation, each agency must also adhere to the National Environmental Policy Act (NEPA), which is binding on all federal agencies.

In European Union the regulatory framework on agricultural biotechnology is made of a few European Directives and Regulations (Vega et al 1999). Directive 90/219 issues the regulations covering the contained use of genetically modified organisms and Directive 90/220 issues the regulations covering the deliberate release into the environment of genetically modified organisms. Directive 90/219 has recently been totally revised. The European Commission also presented a proposal (COM/98/0085) for amending the Directive 90/220 so as to harmonize European approaches to the issue. Several Member States have refused to approve commercialization of transgenic plants approved in other Member States in their territories and others have called for a moratorium. The European Parliament's Committee on the Environment, Public Health and Consumers proposed a Europe-wide moratorium on all transgenic crops awaiting authorization to be placed in the market. The Council Regulation (EC) No 258/97 regulates the compulsory indication of the labeling of certain foodstuff produced from genetically modified organisms. The Directive on the legal protection of biotechnological inventions 98/44/EEC regulates issues of intellectual property. For more details on risk assessment and the regulatory framework pertaining transgenic plants see the chapter by Dr. John Beringer in this volume.

Risk assessment needs to have an international dimension and extent beyond the primary country of release or a shared international boundary or a market. International harmonization of regulations and procedures for production, testing, and handling transgenic plants must be a major challenge. Obtaining good scientific data for the long-term effects to the environment will be critical for passing these products from the regulatory framework. This will be even more critical for plants tolerant to different biotic and abiotic stresses. Progress in meeting this challenge will be highly dependent on:

a) how the questions will be formulated

b) the amount and kind of effort that will be devoted towards this goal.

The role of international organizations towards that goal can be critical and meetings and discussions to facilitate this are of immense importance.

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