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Background, current status and future prospects of transgenic crop plant development

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Resum

Les plantes transgèniques són una part integral de l'agricultura contemporània. Durant l'any 2006 més de noranta milions d'hectàrees de plantes transgèniques van ser cultivades en vint-i-un països. Des de la comercialització de la primera planta transgènica el 1996 els nivells d'adopció d'aquests cultius han augmentat anualment amb percentatges de dos dígits. El desenvolupament i la comercialització de les plantes transgèniques van lligats estretament al comerç mundial, a la globalització, a la disponibilitat de suficient menjar, a la protecció del medi ambient i del consumidor i a la propietat intel·lectual. En aquest article exposem els avenços més recents i les tendències actuals en el desenvolupament dels cultius transgènics i de la seva utilització. També ens fem ressò d'alguns assumptes no científics que s'han de solucionar abans que aquests cultius arribin al màxim del seu potencial, proporcionant una agricultura més sostenible i ecològica. Finalment, ressaltarem la importància de com les plantes transgèniques poden contribuir en la disponibilitat de menjar i en la millora de la pobresa en els països en vies de desenvolupament.

Paraules clau: genòmica, plantes transgèniques, resistència a insectes, sequera, suficiència d'aliments

Abstract

Transgenic plants are now an integral part of contemporary agriculture. Globally, more than 90 million Ha of transgenic crops were grown in twenty-one countries during 2006. Double-digit percentage increases in annual adoption rates have been recorded every year since the commercialization of the first transgenic crops in 1996. The development and commercialization of transgenic plants is closely linked to world trade, globalization, food safety, environmental and consumer protection and intellectual property. In this article, we discuss recent advances and current trends in transgenic crop development and their deployment. We also address some of the non-scientific issues that need to be resolved before these crops can develop their full potential in order to deliver more sustainable and environmentally friendly agriculture. Finally, we will highlight the importance of transgenic crops in contributing towards food security and alleviating poverty in the developing world.

Keywords: drought, food security, genomics, insect resistance, transgenic plants

Crop improvement can be viewed as a continuum that was initiated millennia ago when humans first domesticated, selected and cultivated plants. It progressed through the use of: animal power to make agricultural practices more efficient; Mendelian genetics that has put conventional plant breeding on a scientific footing; machines during and after the industrial revolution; chemical mutagens and ionizing radiation to create novel (useful) variability in existing germplasm; chemical assistance during the Green Revolution in the form of fertilizers and crop protection chemicals; and finally, plant biotechnology. The interesting thing about plant biotechnology and the use of transgenic plants in modern agriculture is that crop improvement can now be accomplished a lot more efficiently and quickly, often eliminating expensive and environmentally harmful practices and chemical inputs from the agricultural production chain. Because of the enormous potential of transgenic plants to revolutionize agriculture, the science and technology of the process have been closely linked to issues of world trade, protectionism, globalization, food safety and intellectual property. It has played into the hands of dubious so-called "environmental" organizations that have their own agendas in trying to block this technology from reaching the people, such as poor subsistence farmers in the developing world, who need its benefits the most.

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Historical background

Plant genetic modification requires efficient, easy gene transfer methods that will be generally applicable to as many plant species and genotypes as possible [1]. This is important once we move from model laboratory plant species, such as *Arabidopsis thaliana* and *Nicotiana tabaccum*, to cultivated crops. Divergence in the molecular mechanisms through which different plants express transgenes, make it necessary to design appropriate genetic constructs with different promoter elements, introns or enhancers, for example in monocotyledonous versus dicotyledonous plants. The delivery and stable integration of foreign genes into plant cells is the first step towards the creation of transgenic plants [2]. Once foreign genes are integrated into the host genome, they need to be translated and transcribed. The resulting gene products, e.g. proteins, enzymes, hormones, etc., need to be stably expressed over subsequent generations in a predictable fashion. Investigations of mechanisms and factors that influence proper transgene expression have been the subject of intense study [3]. Such studies focusing on transgene silencing have resulted in the development of highly sophisticated models that attempt to provide a rational basis for the occasional aberrant expression of transgenes that are integrated into the plant genome. In turn, such experiments form the basis of improved strategies for creating transgenic plants that stably express introduced transgenes. Additional factors that contribute to transgene-expression stability (or instability) are being investigated and such studies are now beginning to reveal the importance of how a transgenic locus is organized following gene transfer [4].

Current trends in transgenic crop development

The development of transgenic technology continues to expand into increasing numbers of crops and introduced traits [5]. The focus remains on the major field crops of soybean, maize, cotton, oilseed rape, rice and potato, with introduced genes conferring herbicide tolerance and/or pest resistance. Second and third generation transgenic plants include plants that are currently in the production, or research and development pipelines and are expected to reach commercialization in the next five to ten years [6]. In addition to “stacked traits” combining insect resistance and herbicide tolerance in the same crop, further traits include food quality and nutritional enhancement (e.g. plants with increased levels of bio-available iron and enhanced vitamin content); productivity increases through the manipulation of physiological (e.g. photosynthetic rate and improved nitrogen uptake) and biochemical (e.g. plants with altered metabolic profiles for non-food applications) characters; tolerances to abiotic stress (e.g. plants that are better able to survive and proliferate under extreme environmental conditions or on marginal soils); development of plants for the production of pharmaceuticals for human and veterinary applications; and plants that contribute to environmentally-friendly agriculture and sustainability to name just a few. Ultimately we are looking at the development of crop plants with enhanced yield potential, better adaptability to water and nutrient uptake, leading to more sustainable and southern hemisphere, concern many plant biotechnologists and policy makers, and this is an area where major, positive impacts of second- and third-generation transgenic crops are beginning to bear fruit, and are expected to contribute even more substantially.

The new frontier: second and third generation transgenic plants

Second-generation insect-resistant transgenic plants with increased potential for durable resistance might result from the deployment of plants expressing multiple insecticidal proteins. We earlier reported the simultaneous introduction of three genes expressing insecticidal proteins, Cry1Ac, Cry2A and Gna, into *indica* rice to control three major pest, rice leaf folder (*Cnaphalocrocis medinalis*), yellow stemborer (*Scirpophaga incertulas*) and the brown planthopper (*Nilaparvata lugens*) [7]. The *Bacillus thuringiensis* (Bt) genes target the leaf folder and the stem borer, and the *Gna* gene targets planthoppers. Transgenic plants were more resistant compared to their binary counterparts. Plants engineered with a fusion protein combining Cry1Ac with the galactose-binding domain of the non-toxic ricin B-chain provide the toxin with additional binding domains, thus increasing the potential number of interactions at molecular level in target insects. Transgenic rice and maize plants engineered to express the fusion protein were significantly more toxic in insect bioassays than those containing the Bt gene alone [8]. They were also resistant to attack by a wider range of insects, including important pests that are not normally susceptible to Bt toxins. The recognition of toxin binding sites in the insect midgut is an important factor determining the spectrum of Bt toxin activity and severity of toxaemia [8 and references therein].

Abiotic stresses, such as drought, represent some of the most significant constraints on agricultural productivity. Transgenic approaches can be used in combination with conventional breeding to create crops with enhanced drought tolerance, and one way in which this can be achieved is through the manipulation of polyamine metabolism. Polyamines are small, ubiquitous, nitrogenous compounds that have been implicated in a variety of stress responses in plants [9]. The link between polyamines and abiotic stress was first documented through putrescine accumulation in response to sub-optimal potassium levels in barley [10]. Since then, a link has been suggested between increased putrescine levels and abiotic stress. Similar phenomena have been described in animals, e.g. during ischemic and post-ischemic responses in neurons [11]. The genetic manipulation of polyamine metabolism has become a valuable tool for studying their physiological roles in plants. Plant polyamine content has been modulated by the over-expression/down-regulation of arginine decarboxylase (*adc*), ornithine decarboxylase (*odc*) and S-adenosylmethionine decarboxylase (*samdc*) [12 – 17]. Over-expression of heterologous *adc* or *odc* cDNAs in plants generally results in the

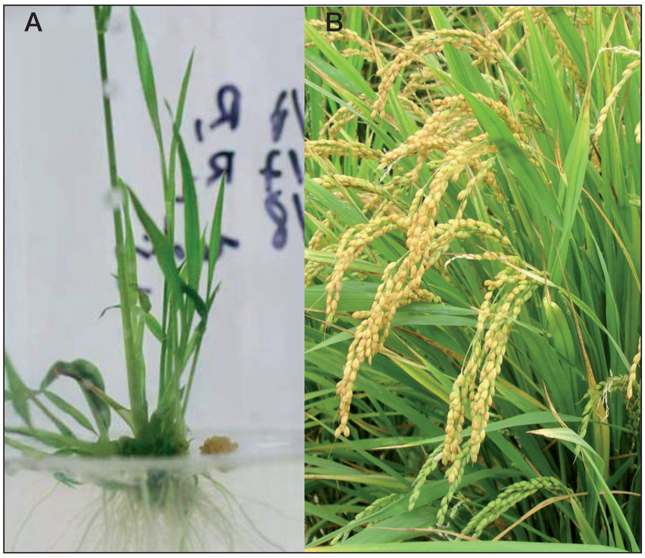

Figure 1. Transgenic rice seedlings (A) and mature flowering plant (B), harbouring the *Datura stamonium* arginine decarboxylase cDNA.

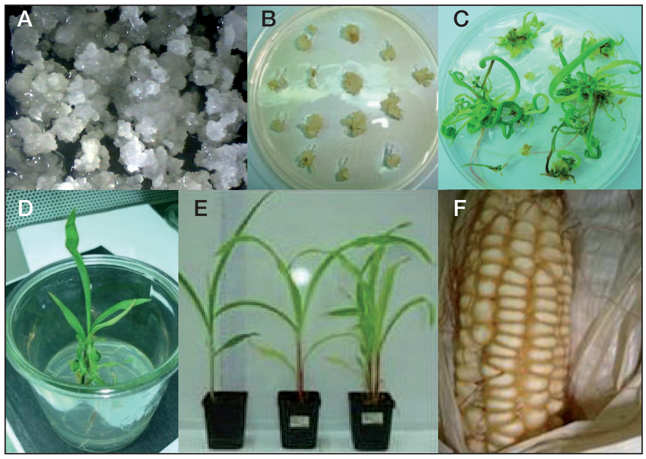

Figure 2. Corn transformation. (A) Corn embryogenic callus; (B) regenerating shoots; (C) shoots on selection media; (D) transgenic corn seedling; (E) transgenic corn plants; (D) seeds expressing HIV-neutralizing antibody.

production of high levels of putrescine [14, 15]. In most cases, this is accompanied by a relatively small increase in spermidine and spermine concentrations. Such findings suggest that the levels of spermidine and spermine are under strict homeostatic regulation. Therefore, the study of plants transformed with genes involved in polyamine biosynthesis may shed light on the importance of polyamines, their role in the acquisition of stress tolerance and relevant stress tolerance mechanisms. We have generated transgenic rice plants expressing the *Datura stramonium adc* gene (Figure 1) and investigated their response to drought stress induced by 20% polyethylene glycol (PEG). We monitored the steady-state mRNA levels of genes involved in polyamine biosynthesis (*Datura adc*, rice *adc* and rice *samdc*) and polyamine levels. Wild type plants responded to the onset of drought stress by increasing endogenous putrescine levels, but this was insufficient to trigger the conversion of putrescine into spermidine and spermine (the agents that are believed to protect plants under stress). In contrast, transgenic plants expressing *Datura adc* produced much higher levels of putrescine under stress, promoting spermidine and spermine synthesis and ultimately protecting the plants from drought. We clearly demonstrated that the manipulation of polyamine biosynthesis in plants can produce drought-tolerant germplasm, and we proposed a model consistent with the role of polyamines in the protection of plants against abiotic stress.

Proteins can be used as diagnostic reagents, vaccines and drugs, and this creates a strong demand for the production of recombinant proteins on an industrial scale. Commercial protein production has traditionally relied on microbial fermentation and mammalian cell culture, but these systems have disadvantages in terms of cost, scalability and safety that have prompted research into alternative production platforms [18]. Despite industry inertia and conservatism, plants have emerged as one of the most promising general production platforms for tomorrow's biologics. Plants allow the cost-effective production of recombinant proteins on an agricultural scale, while eliminating risk of product contamination with endotoxins or human pathogens [19]. Another advantage of the use of plants in recombinant protein production is that vaccine candidates can be expressed in edible plant organs, allowing them to be administered as unprocessed or partially processed material [18]. Four cereal crops – maize, rice, barley and wheat – have been used as seed-based production systems and the first three are being developed commercially. Maize has the highest annual grain yield of the four, moderately high seed protein content and the shortest generation interval, giving the highest potential protein yield per hectare [reviewed in 20]. Maize also benefits from the relative ease of *in vitro* manipulation and transformation, and the availability of several useful seed-specific promoters that can be used to drive transgene expression [Figure 2; 21]. Maize was used to produce the first two commercial plant-derived recombinant proteins, avidin and β-glucuronidase [22]. Maize has also been investigated as a commercial platform by several other parties for the production of a range of pharmaceutical and technical proteins, including recombinant antibodies, vaccine candidates and enzymes [22 –24]. Maize is also the main vehicle for the production of recombinant pharmaceuticals against HIV/AIDS, tuberculosis, diabetes and rabies, within the Pharma-Planta FP6 Integrated project. For more details please see www.Pharma-Planta.org.

Reverse genetics methods for the functional analysis of genes discovered by genome sequencing are well developed. Insertional mutagenesis using T-DNA or transposons is one of the tools for functional analysis to elucidate gene function, but collaborative investments are required for such large-scale efforts targeting elucidation of all gene functions. In several organisms like Drosophila, Caenorhabiditis and even *Arabidopsis*, saturated insertional mutagenesis has been used, first to create a library of insertion mutants and then to select insertions in genes of interest using PCR based screens [25]. However, with larger genomes, saturated mutagenesis is more difficult to achieve, and it is not cost-effective to screen for inserts in a gene for gene approach. The alternative strategy has been to sequence DNA flanking insertion sequences and

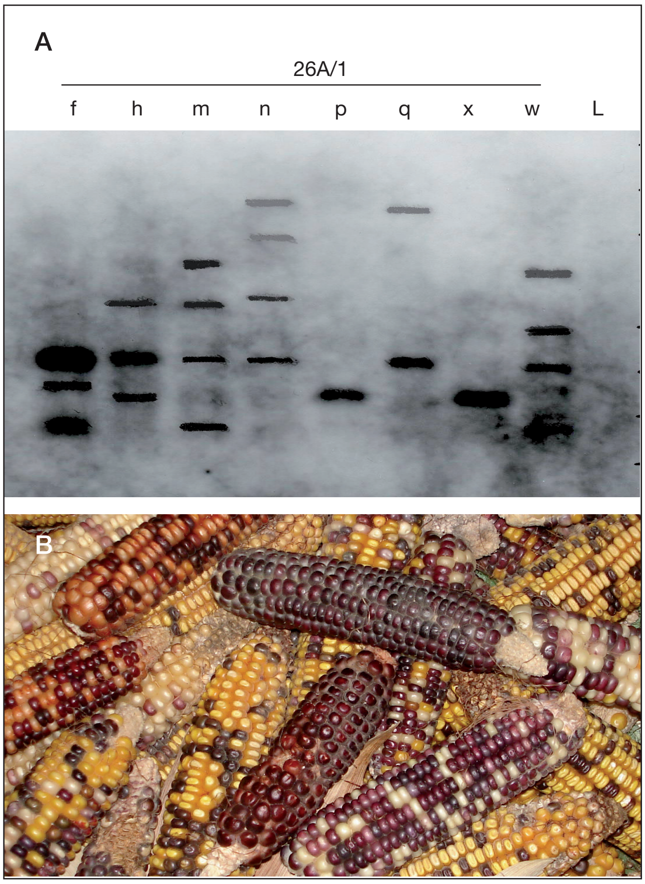

Figure 3. DNA analysis of Ac transposition (A) genomic DNA gel blot analysis of plants containing the Ac transposon; (B) native Ac system in corn and phenotype resulting from transposition.

catalogue these in relation to annotated genes on the genome sequence. Several initiatives to generate large rice insertional mutant collections have been initiated [26] and the databases of insertion flanking sequences are becoming available in the public domain [27; 28]. We took part in a European Consortium starting in 1997 with the aim of developing heterologous transposon mutagenesis strategies for functional genomics in rice [29]. Enhancer trap gene detection constructs using the two-component Ac/Ds transposon system were introduced into japonica rice and a collection of starter lines were developed [30]. In general the Ac/Ds system exhibited high mobility in rice, although inactivation of Ds was observed in later generations (Figure 3). The high frequency of T_1 progeny plants with independent insertions allowed the effective use of the two-component Ac/Ds lines for gene tagging, despite the reduced mobility of Ds in later generations. As the diversity in the T_2 insertion pool appeared to be related to the initial number of different T_0 regenerants and therefore T_1 families propagated, a large population of early generation plants was produced to generate large numbers of independent stabilized insertions. The T_2 and T_3 lines generated thus provide a collection of stable insertions that can be directly used for reverse genetic screenings.

The consortium reported the production of a collection of about 1000 unique flanking sequence tags (FSTs) from japonica and indica rice generated from Ac and Ac-Ds transposon lines for reverse genetics in rice. On comparison to public databases, the FSTs were assigned a position in the rice genome sequence represented by assembled chromosome pseudomolecules. The inserts were distributed fairly evenly over the genome but were higher in gene-rich regions characterized by cDNAs positioned on the chromosomes. The bias of the Ac and Ds transposon inserts for genes was exemplified by the presence of 57% of the inserts in genes annotated on rice chromosomes and 40% present in genes transcribed as disclosed by their homology to cDNA clones. In a screening for inserts in a set of 65 well-annotated transcription factors, including homeobox-containing genes, 4 inserts were found. This high frequency of Ac and Ds inserts in genes suggests that knockout mutagenesis will be efficient and possible with a lower number of inserts than expected. These FSTs and the corresponding plant lines are publicly available through databases and from EU consortium members [31].

Political dimension, impact and regulation

In an ideal world, everyone would have secure access to an adequate supply of safe and nutritious food. This is something that Western societies take for granted, given the seemingly unlimited quantity and variety of fresh produce available on supermarket shelves. In the developing world, however, 840 million people are chronically undernourished, surviving on fewer than 2000 calories per day [32]. Many more people, perhaps half of the world's population in total, suffer from diseases caused by dietary deficiencies and inadequate supplies of vitamins and minerals. It is therefore not surprising that contemporary plant biotechnology stands to benefit huge numbers of people in the developing world, to a much greater extent than consumers in the affluent west. Perhaps this is one of the reasons why well-fed Europeans, who enjoy very high living standards and qualities of life, can afford to reject technologies in the short term, based on ideology and vested economic and political interests.

All transgenic plants are required to undergo thorough and rigorous safety and risk assessment before commercialization. Regulation, amongst other things, is an important component of transgenic crop development and field deployment as it provides *de facto* assurance to the public that all products approved for commercial release, are at least as safe as their non-transgenic counterparts [33].

Intellectual property issues (IPR)

Most countries have legally established that it is reasonable for an inventor to be given a monopoly, or the right to exclude others from the sale or use of the invention for a limited time, in return for a full public disclosure of how the invention has been made. In economic terms, this right of exclusivity is important because it allows the inventor to recover the research investment while not giving competitors free access to the newly created intellectual knowledge [34]. That said, public disclosure of the invention allows competitors to improve on or work around the patent. Intellectual property rights are covered by an international treaty: Trade-Related Aspects of Intellectual Property Rights (TRIPS). The treaty obliges member states to protect, through patents, inventions of any product or process, in any field of technology, assuming such inventions are novel, non-obvious and useful, i.e. capable of commercial exploitation. TRIPS provide grounds for exclusion in granting patent protection on moral, ethical and other grounds. Such exclusions are non-uniform across different territories and this causes great confusion in applying the terms of the treaty. A number of different vehicles exist for protecting plant-related inventions: plant variety protection, US plant patents and utility patents. The process of creating a transgenic plant is a multi-component enterprise and very often, each individual component might be the subject of IP protection. Claims may be directed towards explants used for transformation, the vectors and genetic constructs, transformation procedures, and selectable and/or screenable markers. Early plant biotechnology patents provided inventors with very a broad claim protection. However, a number of these early patents have been challenged in court and some have been overturned. It is now becoming increasingly difficult to obtain a patent with a broad scope of claims.

An issue that is pertinent to IP protection concerns plants and other biotechnology products or processes for humanitarian use, specifically for developing country applications. Precedence exists for at least two examples which pave the way for making IP protection and humanitarian use compatible, rather than mutually exclusive. One example is "Golden Rice", a variety of rice engineered with the vitamin A pathway genes that accumulates beta carotene. The creators of this rice strain were able to convince all the holders of the IP that was used for the creation of "Golden Rice" to donate all the relevant IPs in order to allow the freedom to operate (FTO) for developing countries. The consequence of this agreement is that there are no IP constraints on "Golden Rice" and therefore, this material can be freely distributed to the people who need it most, i.e. people in the developing world who are deficient in vitamin A. The second example is provided by the Pharma-Planta EU project, a consortium of 39 different laboratories in Europe and South Africa working on plant-made pharmaceuticals, focussing on HIV/AIDS, tuberculosis, diabetes and rabies. All members of the project have signed a humanitarian statement which guarantees FTO for the products and the technology derived from the project, for humanitarian use in developing countries.

Concluding remarks

In industrialized countries, the key benefits of transgenic plants include more environmentally friendly and sustainable agricultural production practices obtained by limiting the number of chemical inputs that are required in conventional agriculture.

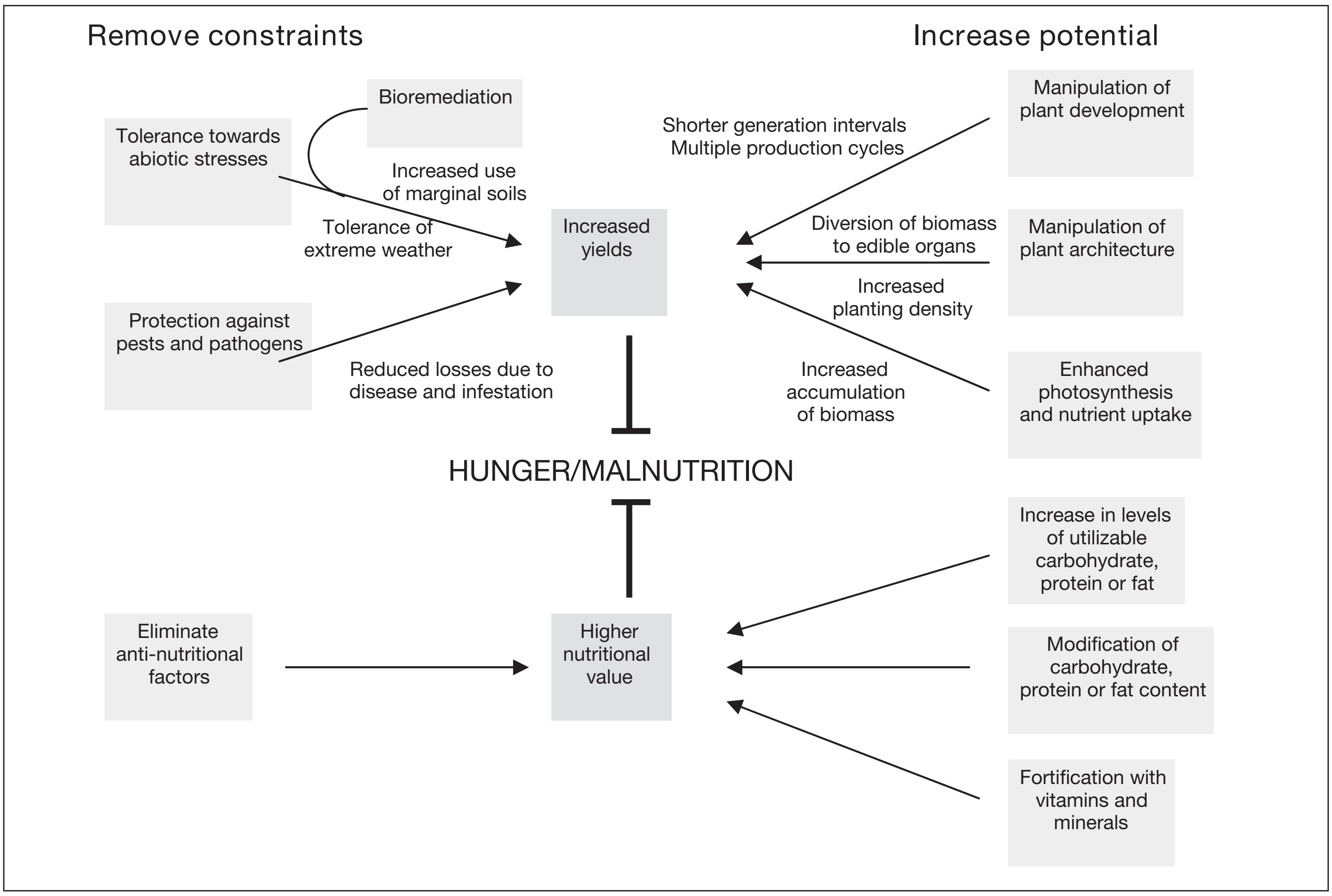

Figure 4. Potential of transgenic plants to contribute towards food security (Christou and Twyman, 2004)

Genetically enhanced crops have the potential to address some of the causes of hunger in the developing world, both directly (by increasing the availability of food) and indirectly (by reducing poverty in developing countries) [Figure 4; 35]. Crop failure due to pests and diseases, could be averted by adopting plants that are resistant to such biotic stresses. The development of plants that are tolerant of extreme environments could allow marginal soils to be brought into agricultural use, and could allow plants to survive periods of drought or flooding. The modification of plant architecture could increase yields and sturdiness by diverting biomass from stems and stalks, to the edible organs. Overall yields could be increased by manipulating photosynthesis, carbon and nitrogen metabolism or modifying plant development to promote early flowering, and multiple growth cycles per year. Exhausted soils could be sown with crops that are better able to extract nutrients, and contaminated soils could be regenerated by plants developed for bioremediation. These measures, in combination with conventional breeding and developments in other agricultural practices, may produce the estimated 50% increase in grain yields required over the next 50 years to cope with the anticipated increase in the global population. Overwhelming evidence has demonstrated the benefits of early generation transgenic crops in terms of increased yields and reduced chemical inputs, despite claims of special interest groups that such early products did not provide any benefits to consumers [36 – 38]. Knock-on effects include improved farmer and consumer health and a cleaner environment. The future security of food-supplies depends in part, on science providing the tools to allow efficient agricultural production, which is sustainable in every sense; furthermore, sustainability of food production has to be considered seriously and not be used as a political slogan by so called "concerned consumers" and environmental or political groups with hidden agendas. Transgenic plants have a twenty-three year track record of success and safety which will become progressively more difficult for opponents of genetic engineering technology, to ignore.

Transgenic plant releases and commercialization are governed by Draconian rules unparalleled elsewhere in any other sector. The European Union in a report following a fifteen-year study (1985-2000) involving 400 public research institutions, at a cost of 70 million Euros stated "... *genetically modified plants and products derived from them present no risk to human health or the environment......these crops and products are even safer than plants and products generated through conventional processes*" [39].

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About the authors

Paul Christou obtained his PhD in organic chemistry in 1980 at University College, London. He then moved to the USA where he joined Agracetus, one of the first Plant Biotechnology companies. In 1994 he moved to the John Innes Centre (JIC, UK) as Head of the Molecular Biotechnology Unit. In 2001 he was recruited by the Fraunhofer Institute to head a new department and assist with the launch of the new institute. In 2004 he became an ICREA researcher professor at the Universitat de Lleida (UdL).

Teresa Capell received her PhD in plant physiology from the Universitat de Barcelona *in 1994. Her research focused on the study of the physiological functions of polyamines with emphasis on abiotic stresses such as drought and salinity. She carried out postdoctoral research at the John Innes Centre (UK) were she became a project leader. In 2001 she moved to the Fraunhofer Institute in Germany where she consolidated her work on abiotic stress and extended her research to functional genomics. She is currently a senior researcher (*Ramon y Cajal*) within the Applied Plant Biotechnology group at the UdL, working in the broader field of genetically enhanced cereals.*

Changfu Zhu was trained as a plant molecular biologist at the Institute of Genetics and Cytology, Northeast Normal University in China, and the Gene Research Center, University of Tsukuba in Japan. He got his PhD degree in 1996, and he subsequently carried out post-doctoral research in three labs engaged in carotenoid molecular genetics and biochemistry from 1997 to 2004. During his tenure at these institutions (Iwate Biotechnology Research Center, Japan; Botanical Institute, University of Frankfurt, Germany; Department of Biological Sciences, City University of New York, USA) he cloned and expressed more than ten different genes involved in the biosynthesis of carotenoid pigments and vitamins. These have been protected through gene registers and through a Japanese patent. He became a full professor in Northeast Normal University in 2004. He is proficient in cloning, gene expression and other sophisticated molecular and biochemical techniques and his research focuses on the engineering of agriculturally important crops to improve their nutritional quality.

Ludovic Bassie started his research activities at the John Innes Centre (UK). During his tenure at the JIC and within the framework of a collaborative project between JIC and CSIC (Barcelona), he obtained his PhD degree in 2004. After post-doctoral research at the Fraunhofer Institute (Germany) he obtained a Juan de la Cierva *post-doctoral position at the UdL. He is in the process of building his own research group within the Applied Plant Biotechnology laboratory, in the department of Producciò Vegetal Ciencia Forestal and also teaching plant molecular biology within the new biotechnology degree at the UdL.*

Ariadna Peremartí studied Biochemistry and received a master in biotechnology from the Universitat de Barcelona*. She joined the Applied Plant Biotechnology group at the UdL in 2005, where she started her PhD studies focusing on the improvement of drought tolerance in cereals. In particular, her research focuses on polyamine metabolism and drought stress. She has been awarded a FPI fellowship by the Ministry of Education and Science (MEC).*

Shaista Naqvi graduated with B.Sc. (hons.) in Agriculture from the University of Agriculture Faisalabad, Pakistan in 2001. She completed a two-year master degree in plant physiology at Quaid-I-Azam University Islamabad, Pakistan in 2004. Tissue culture techniques in Solanaceous plants formed the basis of her dissertation. At present she is carrying out PhD research in the field of nutritional improvement of cereals at the Universitat de Lleida*, Spain. She has been awarded a FPI fellowship by MEC.*

Koreen Ramessar initiated her scientific career in 1997 at the University of Witwatersrand in South Africa where she received her Bachelor of Science degree in biochemistry and microbiology in 2000. She then joined the Council for Scientific and Industrial Research (CSIR) – Biological, Chemical and Food Technologies Division 2001 in South Africa. She worked as junior scientist where she gained experience in maize and tobacco transformation, molecular biology and proteomics research. While employed at the CSIR, she completed her Master of Science degree in Plant Biotechnology at the University of Pretoria (2002-2004). She then enrolled in a PhD program at the Universitat de Lleida, *in the general area of plant biotechnology, where she is involved in the expression of HIV neutralizing monoclonal antibodies in maize seeds. She is funded by the EU FP6 Pharma-Planta project.*

Sonia Gómez is an agronomist engineer who is currently studying for a PhD degree at the Universitat de Lleida*, within the field of plant biotechnology. In particular, her research focuses on the nutritional enhancement of cereal crops with micronutrients (iron, zinc and selenium). She has been awarded an FPI fellowship by the Government of Catalonia.*