Riding the tide of biopharming in Africa: considerations for risk assessment

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In the past few years, plant biotechnology has gone beyond traditional agricultural production of food, feed and fibre, and moved to address more complex contemporary health, social and industrial challenges. The new era involves production of novel pharmaceutical products, speciality and fine chemicals, phytoremediation and production of renewable energy resources to replace non-renewable fossil fuels. Plants have been shown to provide a genuine and low-cost alternative production system for high-value products. Currently, the principal plant-made products include antibodies, feed additives, vaccine antigens and hormones for human and animal health, and industrial proteins. Despite the unique advantages of scalability, cost and product safety, issues of politics, environmental impact, regulation and socio-economics still limit the adoption of biopharmaceuticals, especially in the developing world. Plant-based production systems have further complicated biosafety, gene flow and environmental impact assessments with generally genetically modified plants, topics that are already partially understood. This article provides a background to biopharming, highlighting basic considerations for risk assessment and regulation in developing countries, with an emphasis on plant-based vaccine production in South Africa.

Introduction

Molecular plant biotechnology, and genetic engineering in particular, have been around for some time now, and can be said to be mature technologies. To date, the rate of adoption of crop biotechnology has been remarkable with a worldwide increase in hectarage of genetically modified (GM) crops from a few thousand in 1996 to more than 90 million in 2005.[1] Herbicide tolerance and insect resistance were the major traits adopted for single, double or triple gene stacking in soya beans, cotton, canola or maize, with a concomitant improvement in farmers' profit margins and lives. Despite the uncertainty and controversy in Europe and Africa in the past decade with respect to agricultural biotechnology, recent years have seen a policy shift in countries such Spain, Portugal, Germany and South Africa towards GM crops.[1] Lately, biotechnology has gone beyond traditional agricultural products (feed, fibre) to address more complex health-related, social and industrial challenges. The new era involves production of novel pharmaceutical products such as vaccines,[2] speciality and fine chemicals, phytoremediation[3–5] and production of renewable energy resources to replace non-renewable fossil fuels.[1] Unfortunately, these novel ideas and endeavours have brought further complexity to the only partially understood fields of biosafety and environmental impact assessments of GM plants, topics that will be addressed in this article. We also provide a background to biopharming, highlighting basic considerations for risk assessment and regulation in developing countries, with a bias towards vaccine production in plants in South Africa.

The need to find alternative methods to complement microbial and mammalian systems in the production of high-value products such as drugs, enzymes, and antibodies[6] has recently given birth to the concept of 'biopharming', or plant-based pharmaceuticals (PBP). The biopharming industry is projected to be worth $100 billion by the year 2020 (www.molecular-farming.com). Currently, the main products that have been expressed in plants include antibodies, feed additives, vaccine antigens and hormones for human and animal health and industrial proteins.[7–9] Of particular importance has been the development of PBP to find cures or treatments for the so-called 'orphan diseases' including TB, malaria, typhoid and cholera. These are undoubtedly major killers in the developing countries of sub-Saharan Africa, in addition to the ubiquitous and deadly HIV/AIDS.

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Why choose plants to produce vaccines?

According to the World Summit on Children, an ideal vaccine should have the following characteristics: it should be administrable as a single dose, preferably orally; be effective when given near birth; be heat stable; comprise multiple antigens; be effective against diseases which are not current targets; and most importantly, it should be affordable.[10] Several alternative production systems including plant-based vaccines (PBV) are being tested in order to produce these ideal vaccines. To this end, vaccine antigens for a wide range of diseases involving HIV, hepatitis B virus and human papillomaviruses (HPVs) have been effectively expressed in plants.[11] Interestingly, these antigens apparently fold correctly and assemble into quaternary structures similar to those seen in mammalian tissues, are also glycosylated where appropriate, and are highly immunogenic. This contrasts with microbial systems which are unable to simulate the post-transcriptional modification of mammalian cells, and are either unable (if bacteria) properly to glycosylate, or even over-glycosylate the proteins (if yeasts). In addition, indications are that plant-based vaccines might best be administered orally either as only partially purified preparations or even in their raw form, with the plant tissue providing a natural bioencapsulation, thus eliminating the costs and risks of injection and needles.

Research into the best delivery systems for PBVs is still in its infancy. However, using appropriate plant expression organs such as seeds combined with artificial and conventional preservation methods such as air and freeze drying, proteins can be stabilized at room temperature for a long time: this allows increasing vaccine coverage where there is no refrigeration. Because of the relative ease of scalability, the production costs of PBV are estimated to be reduced from $1000–5000 per gram of protein for animal systems to $1–10 in plants,[11] and about 10-fold less when compared to microbial fermentation production costs.[12] For developing countries, this significant cost saving can be used to meet other needs such as improvement of infrastructure for health care delivery and hunger alleviation. Bacterial products almost invariably come contaminated with endotoxins, necessitating meticulous purification, which increases the cost of goods. Finally, PBVs are very safe because they are subunit vaccines made of non-replicating components, and additionally have no risk of viral or other human-transmissible

contamination as can occur with mammalian cell culture products.

Although some 'big Pharma' companies have been sceptical of this technology, relatively huge investments have been made in the industry: for example, Bayer CropScience recently acquired ICON Genetics and Dow AgroSciences is licensing a Newcastle disease virus vaccine for chickens, the first livestock vaccine made commercially, thus increasing competition in the industry. Having said that, it is apparent Africa might not benefit from this new technology unless proper foundations in the form of policies for research and development, regulation and the relevant infrastructure for growing PBVs are put in place.

Production systems

There is an array of plant production technologies that have been successfully used or are being developed for biopharming: they include transient expression (using viral vectors, agroinfiltration, and cell or tissue culture)[13–16] and stable transformants (transgenic plants for *in planta* accumulation[17] or secretion through roots or leaves, as well as transplastomic plants in which genes for the target product are integrated into the chloroplast genome).[18,19]

Until recently, transient somatic expression through agroinfiltration — or the infiltration of plants with suspensions of recombinant *Agrobacterium tumefaciens* — was generally used to verify transformation of the gene expression vector (construct) activity and to validate small amounts of recombinant proteins.[20,21] However, it is now considered a *bona fide* protein expression strategy in its own right, that can yield large amounts of protein.[14] Agroinfiltration has been scaled-up with the help of post-transcriptional gene silencing suppressors such as the plant virus-derived p19 and NSs,[15] to produce commercial yields as high as 100 mg of protein per kilogram of tobacco processed on a weekly basis.[22] In this case the recombinant protein is recovered within a short time with minimum cost. On the other hand, viral vectors have been reported to produce high protein yields in a short time and to allow for mixed infections, but there are safety concerns and construct limitations associated with this approach. The main limitation of transient expression is variability in protein yields among and between batches, and the potentially higher cost of production compared to transgenic production.

Stable transformation systems are based on the incorporation of the foreign genetic material into the plant nuclear or plastid genome, generating transgenic plants. Several methods for transgene delivery have been developed over the years including *Agrobacterium*-mediated transformation, microprojectile bombardment, electroporation and microinjection.[23] The transgene is expressed in the whole plant, specific organ or cell lines over generations, allowing ease of scaling-up and purification. It has become clear, however, that protein expression in the plant cell cytosol often results in low expression and gene silencing. As a solution, transit peptides are used to target nuclear encoded proteins into different subcellular compartment such as the plastids (chloroplasts, etioplasts and chromoplasts), endoplasmic reticulum, oil and protein bodies, vacuole and apoplast.[24,25]

A variation on this concept is the direct incorporation of the transgene into the chloroplast to form transplastomic transgenic lines.[26] Transplastomics have several advantages including high expression levels that can be achieved for nuclear encoded proteins because transgene copy number is high as a result of the many chloroplasts in a typical photosynthetic cell. Furthermore, there is no gene silencing; and multiple genes can be expressed in operons as the chloroplast mechanism is basically prokaryotic, and gene silencing is a eukaryotic phenomenon. Of particular significance is the maternal inheritance of chloroplasts in all higher angiosperms, which helps in transgene containment[22] as transgenes are not transmitted via pollen. However, this system has many of the same drawbacks as prokaryotic expression, including lack of glycosylation and incorrect folding of certain proteins.

Although whole plant systems are the most widely used, cell cultures and 'hairy root' cultures have emerged as recent favourites for the expression of small molecules. With these systems the protein can be either harvested from the tissues directly or from the liquid media after deposition through root exudation or apoplastic plant pathways using the KDEL endoplasmic reticulum targeting sequence. Moreover, comparative targeting experiments have shown that the secretory pathway via the endoplasmic reticulum may produce a 2–10-fold increase in protein yields compared to accumulation in the cytoplasm. This may be explained by the proper oxidation environment, fewer proteases, and abundant molecular chaperons helping to achieve proper protein folding and stability.[27]

In summary, a broad range of technology and plant host and species have been developed for the expression of plant-made biopharmaceuticals. The type of product and crop species are the principal factors influencing the choice of a particular system, as will be discussed below.

Issues in the production of PBP

Despite being one of the most exciting biological innovations of our times, there are numerous obstacles to the successful exploitation of biopharmaceuticals, especially in the developing world. As a result of political, environmental, regulatory and socio-economic issues coupled with pressure from organized environmental non-governmental organizations, or 'greens' as they are commonly called, Africa may miss out on beneficial technologies that could change lives for the better. Until and unless these concerns are properly and decisively addressed, biopharmaceuticals likewise will never be locally produced in Africa, and may never reach the people who need them the most. However, inadequate scientific understanding of the effects of some GM technologies has led to a precautionary approach by most governments of both rich and poor nations. Developing countries, especially in Africa, are susceptible to raw, untested and potentially dangerous technologies from industrial states, and perhaps rightfully tend to protect themselves by putting in place the necessary controls and seek to gain relevant experience before committing themselves to such technologies as biopharming. Some of the issues underpinning the controls, checks and balances are summarized below.

Environmental considerations

Despite the intensive research into the ecological impact of GM plants around the world, our knowledge and understanding is still inadequate.[28,29] There is a fine line dividing the net benefits and environmental risks as clearly demonstrated by the conflicting reports from the different studies on the subject.[30] Consequently, there is immense distrust and doubt among consumers about even some basic scientific facts on GM products. Although most of the concerns are genuine and scientifically sound, some seem to be fruits of consistent misinformation and bad publicity via the media. Nonetheless, the need to address these concerns cannot be overemphasized. The principal environmental risks include detrimental effects on non-target organisms, gene flow to wild relatives or non-transgenic varieties, the inadvertent creation of

weeds, development of resistance to pesticides and tolerance of traditional pathogens, production of novel toxins, recombination of bacteria or viruses to produce new pathogens, impact on agrobiodiversity and crop genetic diversity (see McGeoch and Pringle[31] for references). Studies have been conducted in other countries to understand and adopt mitigation strategies to address these risks but the outcomes may not be easily transferable because of the unique climate and agroecosystems of South Africa and Africa at large. This further strengthens the urgent need for vigorous risk assessments and local interventions.

In short, the possibility of transgene introgression into non-modified counterparts,[32] volunteer plants that may become weedy nuisances, and identity preservation, among others are the main concerns that have led to justifiable attacks on the adequacy of the current frameworks for environmental risk assessment and monitoring. For example, contradictory reports of transgene flow from GM to non-GM maize in Mexico[31,33] have recently fuelled public fears of the technology. Moreover, the recent case where ProdiGene Inc. in the US was fined $3.25 million after their GM corn, which expresses a drug for pig diarrhoea, was discovered in a soybean field has had serious knock-on effects, including discussion of a moratorium on using crop plants for the expression of biopharmaceuticals. Serious questions could be asked in the light of this scandal, such as whether the GM maize volunteer plants could have been discovered in a non-GM maize field. Although our understanding of pollen dispersal and pollen viability has improved over the years, climate change and the effects of strong winds and hurricanes are an additional complicating factor; we need to deal with the various elements of the problem separately. For example, issues of the safe isolation distances between GM and non-GM crops and the allowable threshold of volunteer plants need to be re-visited. In an ideal situation the use of non-food or non-feed crops would be preferred. However, food crops have well-developed transformation protocols, low-cost production and processing and reduced toxicants[22] such that they remain favourite choices for biopharming.[34]

Within the African context, however, other factors such as human migration and illegal seed imports, particularly involving both food and medicinal plants, are more serious challenges for African governments. This is illustrated by the fact that, on average, 1000 to 2000 illegal Zimbabwean immigrants were deported weekly from South Africa in the year 2005 alone, with a presumably higher undetected quotient.[35] To our knowledge, no studies have been commissioned to assess the risk posed by these migrants in terms of the illegal movement of seed. This is a clear indication of the lax and poorly enforced phytosanitary regimes in African countries. It is therefore imperative that the anthropogenic factor be incorporated in risk assessment for PBP. The issues raised above are but a few to highlight the need for serious environmental risk assessments for biopharmaceuticals.

Regulatory and legal issues

The GMO debate in Europe and recent advances in science have made regulation of biopharmaceuticals more complex than ever. On the one hand, regulatory bodies have been confronted with the dilemma of balancing sound scientific principles against public fears. On the other hand, science has not unequivocally declared biotechnologies either safe or unsafe in many cases nor even the conditions in which either suffices. This has caused unnecessary rejection of some useful technologies and delays in producing a regulatory framework for biopharmaceuticals. Nevertheless, there have been two interesting developments in the past three years. First, scientists are beginning to understand better the dynamics of transgene flow in both domestic and wild ecosystems,[36–39] as well as the mechanisms of insect and weed resistance.[40,41] This has helped in making science-based decisions in terms of risk assessment and management. Second, the US Food and Drug Administration (FDA)[42] and European Agency for the Evaluation of Medicinal Products[43] have produced guidelines highlighting specific considerations for producing biopharmaceuticals. These developments are a step in the right direction, but more are still needed, especially from developing countries, in order to harmonize the respective regulatory bodies with those mentioned above. Most governments are in the process of producing, or have just produced, legislation to govern the introduction GMOs, and the respective regulatory bodies have still to survive the test of time and public pressure. For instance, countries like Zimbabwe have a well-developed and comprehensive biotechnology regulatory framework but no GM products have reached the market. The main challenge at government level is to harmonize legislation governing biopharmaceuticals. For example, the Federal Plant Pest Act, Federal Food, Drug and Cosmetic Act, Federal Insecticide, Fungicide and Rodenticide Act and the Toxic Substances Control Act in the United States govern biopharmaceutical product development. The successful adoption of biopharmaceuticals in Africa may be hampered by the complex legal issues that need to be addressed such as patent protection of intellectual property rights (IP) and licensing of patented technology to provide freedom to operate.[44] A progressive policy for rich countries would be to make IP available to developing countries on humanitarian grounds.[2] Whether patent protection benefits the developing world or not is debatable. When developing countries might use others' IP with minimum costs, there is a danger of killing knowledge creation and innovation by promoting copying and imitation.[45] A summary of the important regulatory considerations presented by the FDA as they affect Africa is discussed below.

Choice of host plant

The choice of crop in which the pharmaceuticals are produced has critical implications for the environment. As mentioned above, the use of openly pollinating crop species raises particular concerns about pollen dispersal. Then there is the issue of food versus non-food crops. Basically, the choice of the production platform should be based on the potential allergenicity or toxicity of the molecules being expressed, the method of propagation, and measures of confinement to ensure no inadvertent mixing of bioengineered plant material with human food or animal feed. Any chosen host plant has to be properly described to include growth habit, timing of harvest, and storage. In this respect several plant hosts have been used, including tobacco, maize, alfalfa, rice and tomato.[46] Among the leafy plants, tobacco has been widely used, mainly because it is easy to transform and regulation of transgene expression is well-understood. Being a non-food, non-feed crop, tobacco reduces the risk of contaminating the food or feed chain. The often high nicotine and toxic alkaloids contents may necessitate meticulous purification, reducing the attractiveness of tobacco as a host plant. However, the developed world's growing opposition to smoking, coupled with the well-established agronomic conditions in Africa for the crop, leaves tobacco as the most suitable African candidate for biopharmaceutical production. Its potential is apparent in results from our laboratory, which has recorded protein yields as high as 800 mg per kg (12–17% total soluble

protein) of HPV-16 L1 antigen in transgenic tobacco (J. McLean *et al.* unpublished work). With a yield of up to 2 tonnes per hectare it is possible that a few small tobacco farms could produce enough vaccines for the whole of Africa.

On the other hand, cereals such as maize would be difficult to contain because of the possibility of the escape of transgenic material via pollen dispersal and volunteer plants. These problems are confounded by the fact that maize is the staple food for most of southern and central Africa, making the production of pharmaceuticals in this crop an emotive issue. Having said that, responsibility should be well-defined at all stages of production, harvesting, and transport, with all products being properly labelled. Traceable documentation of the whole production process, quantitative data characterizing the distribution of product in different plant tissues (leaves, roots, stalks and seeds), recombinant DNA vectors and seedbanks should be kept. Extraction and processing should enable efficient concentration of the active compound and separation from the rest of the bulky plant material with minimum concentration of contaminants. Most importantly, all processing procedures should be properly validated and equipment sterilized to the highest standards expected for good manufacturing practice. Moreover, purification processes need to demonstrate the extent of lot-to-lot variation in product yield, and to show sufficient removal of contaminants to provide a consistent product. It would be difficult to satisfy this requirement, however, when using whole plant systems such as field-grown transgenic plants, because of inherent genetic differences and variability in edaphic conditions.

Waste material

According to the FDA guidelines, all in-process wastes (by-products of column wash and diafiltration solutions, for example), rejected material and residual source matter from the purification process should be treated to inactivate the regulated product prior to disposal in a safe and appropriate way. Disposal of this matter should be carried out in a manner to ensure that the product does not enter the human food or animal feed chain. Both primary disposal and secondary use of the material should be approved and well-documented. Interestingly, poor waste management is one of the main challenges of even relatively rich African nations, as witnessed by the high incidence of cholera, *E. coli* infection and typhoid in South Africa,[47] mainly from municipal sewage. One could therefore be justified in concluding that there is no guarantee that waste from biopharmaceuticals will not end up in drinking water. Of greater concern, though, would be the remediation of the problem when it arises.

The way forward

According to Leshner,[48] the success of biotechnology in developing countries depends on adequate scientific and technological cooperation supported by well-developed infrastructure to sustain the science and its progress. With that in mind, proper regulation and enforcement should form the core of the quest for the success of biopharming. Two related strategies can be used to mitigate the risks of the technology: these are confinement and containment.[21] Confinement refers to biological isolation of the plant using techniques involving, for example, buffer zones, cytoplasmic male sterility, transplastomics, self-pollinating species and regulated promoters.[49] On the other hand, containment is the physical isolation of the plant in indoor secure facilities like greenhouses, the maintenance of isolation distance and secretion systems. To this end, the product can be collected in root exudates in hydroponic cultivation, thus minimizing the accidental release of genetically modified organisms expressing antigens or antibodies into the environment. It must be emphasized that identification, assessment and containment/mitigation of biohazards can be achieved only within a strongly enforced, stringent environmental regulatory framework.[50]

The potential benefits of biopharming are enormous but they can be realized only with good policy, exhaustive risk assessments and, most importantly, compliance with management specifications and monitoring of such compliance. Moreover, public education and the broad dissemination of information on GM technology in general should be made a priority so as to raise awareness of the separate risks and provide an informed basis for acceptance or rejection of these technologies. The solutions to the issues raised in this article should help to achieve the safety and purity of the products.[42]

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1. James C. (2005). Preview: Global status of commercialized biotech/GM crops. *ISAAA Briefs* 34-2005. Online: http://www.isaaa.org
2. Ma J.K., Chikwamba R., Sparrow P., Fischer R., Mahoney R. and Twyman R.M. (2005). Plant-derived pharmaceuticals — the road forward. *Trends Plant Sci.* **10**, 580–585.
3. Eapen S., Suseelan K.N., Tivarekar S., Kotwal S.A. and Mitra R. (2003). Potential for rhizofiltration of uranium using hairy root cultures of *Brassica juncea* and *Chenopodium amaranticolor*. *Environ. Res.* **91**, 127–133.
4. Alkorta I., Hernandez-Allica J. and Garbisu C. (2004). Plants against the global epidemic of arsenic poisoning. *Environ. Int.* **30**(7), 949–951.
5. Singh S., Melo J.S., Eapen S. and D'Souza S.F. (2006). Phenol removal using *Brassica juncea* hairy roots: role of inherent peroxidase and H_2O_2. *J. Biotechnol.* 123, 43–49.
6. Sethuraman N. and Stadheim T.A. (in press). Challenges in therapeutic glycoprotein production. *Curr. Opin. Biotechnol.*
7. Fischer R., Stoger E., Schillberg S., Christou P. and Twyman R.M. (2004). Plant-based production of pharmaceuticals. *Curr. Opin. Plant Biol.* 7, 152–158.
8. Webster D.E., Thomas M.C., Pickering R., Whyte A., Dry I.B., Gorry P.R. and Wesselingh S.L. (2005). Is there a role of plant-made vaccines in the prevention of HIV/AIDS? *Immunol. Cell Biol.* **83**, 239–247.
9. Mitchell V.S., Philipose N.M. and Sanford J.P. (Eds) (1993). *The Children's Vaccine Initiative: Achieving the vision*. National Academy Press, Washington, D.C.
10. Walmsley A.M., Kirk D.D. and Mason H.S. (2003). Passive immunisation of mice pups through oral immunization of dams with a plant-derived vaccine. *Immunol. Lett.* **86**, 71–76.
11. Bloomberg News (2005). Syngenta, Bayer bet gene-based drugs can thwart consumer doubts, 27 May 2005. Online: http://quote.bloomberg.com/apps/news?pid=nifea&&sid=adT0ydpQc5Gg
12. Hood E.E. and Woodward J.A. (Eds) (2002). *Plants as Factories for Protein Production*. Kluwer Academic, Dordrecht.
13. Huang Z. and Mason H.S. (2004). Conformational analysis of hepatitis B surface antigen fusions in an *Agrobacterium*-mediated transient expression system. *Plant Biotech. J.* **2**, 241–249.
14. Huang Z., Santi L., LePore K., Kilbourne J., Arntzen C.J. and Mason H.S. (2006). Rapid, high-level production of hepatitis B core antigen in plant leaf and its immunogenicity in mice. *Vaccine* **24**(14), 2506–2513.
15. Voinnet O., Rivas S., Mestre P. and Baulcombe D. (2003). An enhanced transient expression system in plants based on suppression of gene silencing by the p19 protein of tomato bushy stunt virus. *Plant J.* **33**, 949956.
16. Sunil Kumar G.B., Ganapathi T.R., Revathi C.J., Prasad K.S. and Bapat V.A. (2003). Expression of hepatitis B surface antigen in tobacco cell suspension cultures. *Protein Expr. Purif.* **32**(1), 10–17.
17. Tsuda S.K., Yoshioka K., Tanaka T. *et al.* (1998). Application of the human hepatitis B virus core antigen from transgenic tobacco plants for serological diagnosis. *Vox Sang.* **74**,148–155.
18. Koya V., Moayeri M., Leppla S.H. and Daniell H. (2005). Plant-based vaccine: mice immunized with chloroplast-derived anthrax protective antigen survive anthrax lethal toxin challenge. *Infect Immun.* **73**, 8266–8274.
19. Zhang X., Buehner N.A., Hutson A.M., Estes M.K. and Mason H.S. (2006). Tomato is a highly effective vehicle for expression and oral immunization with Norwalk virus capsid protein. *Plant Biotech. J.* **4**, 419–432.
20. Lee M.W. and Yang Y. (2006). Transient expression assay by agroinfiltration of leaves. *Methods Mol. Biol.* **323**, 225–229.
21. Haviv S., Galiakparov N., Goszczynski D.E., Batuman O., Czosnek H. and Mawassi M. (2006). Engineering the genome of grapevine virus A into a vector for expression of proteins in herbaceous plants. *J. Virol Methods* **132**, 227–231.
22. Fischer R. and Emans N. (2000). Molecular farming of pharmaceutical proteins. *Transgenic Res.* **9**, 279–299.

23. Hood E.E. (2004). Bioindustrials and biopharmaceutical products from plants. In *New Directions for a Diverse Planet. Proc. 4th International Crop Science Conference,* 26 September – 1 October, Brisbane. Online: www.cropscience.org.au.
24. Lawrence S.D., Kline K. and Moore G.A. (1997). Chromoplast development in ripening tomato fruit: identification of cDNAs for chromoplast-targeted proteins and characterisation of cDNA encoding a plastid-localised low molecular-weight heat shock proteins. *Plant Mol. Biol.* **33**, 483–492.
25. Kermode A. (1996). Mechanisms of intracellular protein transport and targeting in plant cells. *Crit. Rev. Plant Sci.* **15**, 285–423.
26. Maliga P. (2002). Engineering the plastid genome of higher plants. *Curr. Opin. Plant Biol.* **5**, 164–172.
27. Conrad U. and Fiedler U. (1998). Compartment-specific accumulation of recombinant immunoglobulins in plant cells: an essential tool for antibody production and immunomodulation of physiological functions and pathogen activity. *Plant Mol. Biol.* **38**, 101–109.
28. Ervin D., Welsh R., Batie S. and Carpentier C. (2003). Towards an ecological systems approach in public research for environmental regulation of transgenic crops. *Agric., Ecosyst., Environ.* **99**, 1–14.
29. Hilbeck A. and Andow D. (2004). *Environmental Risk Assessment of Genetically Modified Organisms,* vol. 1: *A Case Study of Bt Maize in Kenya.* CABI Publishing, Cambridge.
30. Kaplinski N., Braun D., Lisch E., Hay A., Hake S. and Freeling M. (2002). Maize transgene results in Mexico are artifacts. *Nature* **416**, 601.
31. McGeoch M.A. and Pringle K.L. (2005). Science and advocacy: the GM debate in South Africa . *S. Afr. J. Sci.* **101**, 7–9.
32. Raven P.H. (2005). Transgene in Mexico maize: desirability or inevitability. *Proc. Natl Acad. Sci. USA* **102**, 13003–13004.
33. Ezcurra E., Ortiz S. and Soberón Mainero J. (2001). In *LMOs and the Environment,* ed. C.R. Roseland, pp. 286–295. Organization for Economic Cooperation and Development, Paris.
34. Castle D. and Dalgleish J. (2005). Cultivating fertile ground for the introduction of plant-derived vaccines in developing countries. *Vaccine* **23**, 1881–1885.
35. IRIN (2006). South Africa–Zimbabwe: government to regularise farm workers. UN Integrated Regional Information Networks. Online: http://www. irinnews.org (accessed 6 March 2006).
36. Pew Initiative on Biotechnology (2003). *Have transgenes–will travel. Issues raised by geneflow from genetically engineered crops.* Pew Initiative on Food and Biotechnology, Washington, D.C. Online: http://www.pewagbiotech.org.
37. Emberlin J. (2000). An assessment of outcrossing in maize, relevant to the proposed decision to add GM maize variety Chardon LL. Evidence submitted at the National Seed List Hearings, October to November 2000, Manchester and London.
38. Thompson C., Squire G.R., Mackay G., Bradshaw J., Crawford J. and Ramsay G. (1999). Regional patterns of geneflow and its consequences for GM oilseed rape. In *Geneflow in Agriculture: Relevance for transgenic crops. Proc. BCPC Symposium* **72**, 95–100.
39. Squire G.R., Rodger S. and Wright G.M. (2000). Community-scale seedbank response to less intense rotation and reduced herbicide input at three sites. *Ann. Appl. Biol.* **136**, 47–57.
40. Bates S.L., Zhao J.Z., Roush R.T. and Shelton A.M. (2005). Insect resistance management in GM crops: past, present and future. *Nature Biotechnology* **23**, 57–62.
41. Zhao J.Z., Cao J., Li Y., Collins H.L., Roush R.T., Earle E.D. and Shelton A.M. (2003). Transgenic plants expressing two *Bacillus thuringiensis* toxins delay insect resistance evolution. *Nature Biotechnology* **21**, 1493–1497.
42. Food and Drug Administration (2002). Guidance for industry: drugs, biologics, and medical devices derived from bioengineered plants for use in human and animals. Online: http://www.fda.gov/cyber/gdlns/bioplant.pdf. Washington, D.C.
43. European Agency for the Evaluation of Medicinal Products. Online: http://www.emea.eu.int/pdfs/human/bwp/076402en.pdf
44. Sweeney P.A. (2002). A warehouse of ideas: Developing and using intellectual property. In *Plants as Factories for Protein Production,* eds E.E. Hood and J.A. Howard, pp. 159–174. Kluwer Academic, Dordrecht.
45. Ashton G. (2006). Intellectual property rules suit the wealthy. Critical health perspectives 2. People Health Movement South Africa, **91**, 127–133. Online: http://www.phmovement.org
46. Menkhaus T., Bai Y., Zhang C., Nikolov Z. and Glatz C. (2004). Considerations for the recovery of recombinant proteins from plants. *Biotechnol. Prog.* **20**, 1001–1014.
47. IOL (2005). Typhoid epidemic: hundreds of cases reported. Independent online, 21 September: Online: http://www.iol.co.za
48. Leshner A. (2002). Science and sustainability. *Science* **297**, 897.
49. Daniell H. (2002). Molecular strategies for gene containment in transgenic crops. *Nature Biotechnology* **20**, 581–586.
50. Pew Initiative on Biotechnology (2003). *Pharming in the Field: A look at the risks and benefits of bioengineering plants to produce pharmaceuticals.* Washington, D.C.