

Crop Biotechnology. Where Now?

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Nature Biotechnology organized a conference in London on Agbiotech 99: Biotechnology and World Agriculture (November 14–16, 1999). The conference focused entirely on crop biotechnology and covered both societal and scientific aspects. Below is an account of the more important issues raised by the speakers and the audience.

THE BIGGER PICTURE

General Societal Issues

Western Europe has become resistant to food produced from genetically modified (GM) crops. Almost all of the European supermarket chains have removed GM crops from their own brands. There have been extremely active protests from a number of non-governmental organizations, including Greenpeace, Friends of the Earth, and Christian Aid. The EU and its member states are backing down from the approval of GM crops. The protests are spreading around the globe to the U.S. (Fig. 1) and other actual or potential producer countries. The conference tried to identify some of the causes for this.

Sir Robert May (Office of Science and Technology, London; for detailed views of May and the Office of Science and Technology on GM crops, see www.dti.gov.uk/ost/ostbusiness/index.htm) pointed out a taxonomy of his concerns, the major ones being food safety, transfer of genes from crops to wild species through cross-hybridization, and the effect of crop husbandry on impoverishing the countryside. The general agreement from critics and proponents alike was that, although there is a need to be careful, there are no major concerns about food safety of GM products, and that the U.K., the U.S., and other countries have put into place effective regulatory procedures that are much more rigorous for GM than for non-GM foods. For some crops in some regions (but not for a whole range of others such as wheat, maize, and rice, over most of their global distribution), cross-hybridization of crops with wild species does occur. This occurs for both GM and non-GM versions—the question is, does it matter? Clearly, research is needed to establish this, and some results were presented by Detlef Bartsch (Aachen University of Technology, Germany) on gene flow from sugar beet to wild beet populations (Bartsch et al., 1999; Pohl-Orf et al., 1999). His experience suggests that it is not easy to demonstrate that accession of transgenes by wild relatives would provide a selective advantage for the recipient in the wild.

Benedict Haerlin (Greenpeace, Berlin) outlined the reasons why Greenpeace opposes the introduction of GM crops. These include issues related to politics and to our scientific knowledge about the crops. He expressed concern that genetic engineering creates unprecedented new genetic life forms, that genetically modified organisms (GMOs) cannot be recalled, that DNA exchange is not understood, and that the long-term evolutionary effects of introducing GMOs were unknown. He proposed that we proceed on the basis of what is not known rather than what is, and invoked the strictest use of the precautionary principle. Although he did not wish to say that Greenpeace would never accept GMOs, the organization has such a long list of questions that he saw little chance of these being satisfied in a decade or two. How many of these questions can ever be answered without large-scale experimental field releases, which Greenpeace also opposes, is completely unclear.

He expressed the view that the problems of GM crops and agriculture should not be left for technocrats to solve. Rather, Greenpeace feels that the big picture is political. Its concerns are the industrialization of agriculture, the movement of people from rural to urban areas, unlimited growth rather than sustainable growth, public good versus private business, and genetic engineering allowing agrochemical companies to “mutate” into life science companies. However, it was not clear that GMOs have had much to do with many of these issues, most of which predate the technology. In contrast, I and many others think that GMOs have the promise to address some of the real concerns raised by Greenpeace and others.

Greenpeace clearly believes that the majority of the public is of its opinion. It was therefore welcome that Richard Braun (BIO-LINK, Worb, Switzerland) was present to describe the Swiss experience and the results of the one democratic vote on biotechnology, which resulted in a large majority favoring the continuation of biotechnology in Switzerland. This was due in large part to the scientists eventually waking up to the need to engage the public in a debate and even to take to the streets to demonstrate their convictions. Braun emphasized that the dichotomy between the apparent distrust of science and the con-

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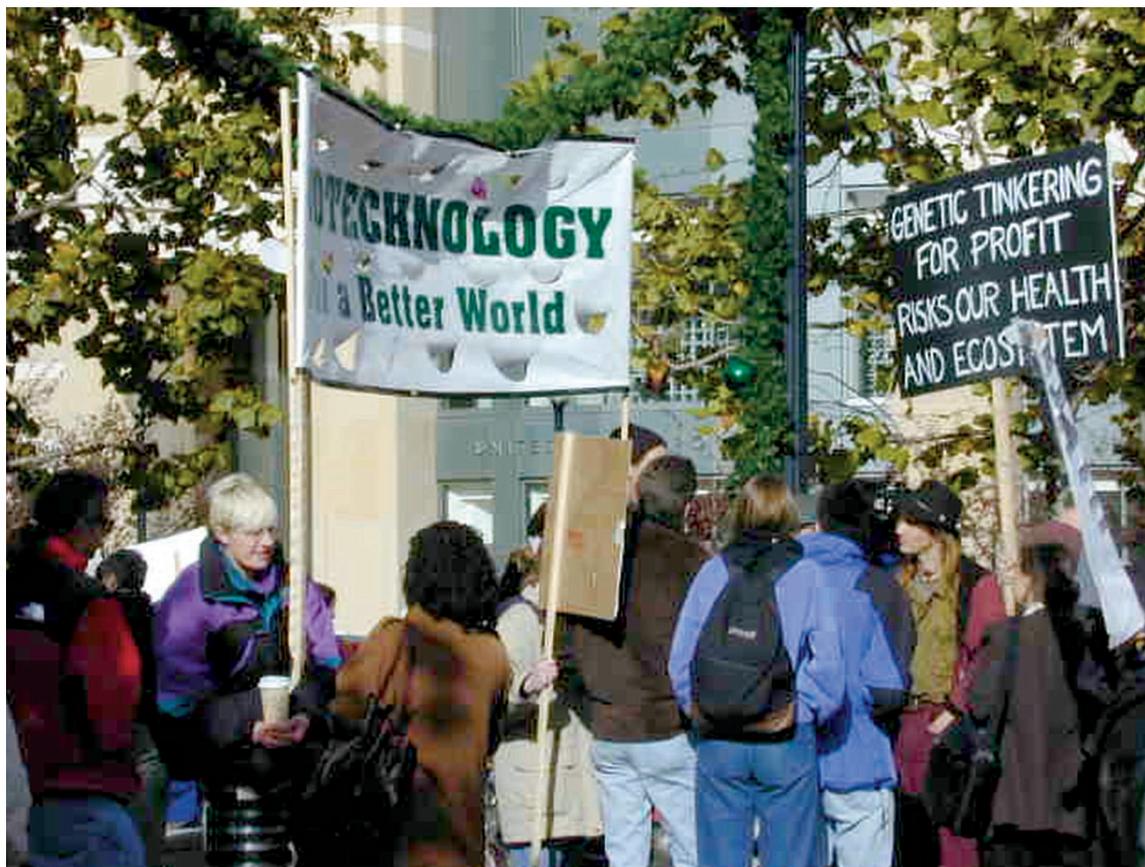


Figure 1. Anti-GMO demonstrations come to the U.S. On December 13, 1999, the FDA held a hearing in Oakland, California, on the labeling of food produced from genetically improved organisms. This hearing was accompanied by protests from activists opposed to biotechnology and genetically modified plants. Professor W. Gruissem from University of California, Berkeley, organized San Francisco Bay area plant scientists into a show of support for biotechnology research. They distributed information on the benefits and risks of the use of genetic engineering in agriculture and food production. The initiative received strong attention from the media, resulting in more balanced reporting (photo by Cynthia Waters).

tinued improvement in human well-being needs to be countered by scientists making a more active contribution to the social debate.

Robert May pointed out that the practice of agriculture had often been bad news for the rest of the natural world, and that we need to take care that GM crops do not make this worse. Brian Johnston (English Nature, Tauton, UK; www.english-nature.org.uk) developed this theme in more detail. He listed a range of concerns relating to GM crops that are cross-fertile with weed species or tolerant to wide spectrum herbicides. However, he emphasized that there is the potential to develop GM crops that would contribute to a more sustainable agriculture. The problem may turn out to be that developing such crops may not provide commercial profit and therefore would need to be developed with public money. Undoubtedly, there is concern that this powerful technology is being used to further commercial aims at the expense of the public good. As May said, the first Green Revolution was achieved by the non-profit sector, but this one is being achieved by industry. The challenge will be to see that the potential

public benefits of the technology are delivered in both the developed and developing world, even where they do not provide an attractive target for commercial research. Although much discussed during the 3 d of the conference, this issue was not resolved. In any event, success is likely to depend on sufficient funds being made available to public sector research organizations and on technology transfer from multinational companies to the public sector.

No technology is perfect, and the benefits and drawbacks of every technology are relative. GM crops need to be judged against the conventional technology they replace. Ben Miflin (IACR-Rothamsted) summarized some of the first analyses of the effect of GM crops in cultivation in the U.S. Surveys over time and place do not make direct comparisons of GM and non-GM crops. However, growers of GM cotton containing a gene for an insecticidal toxin derived from *Bacillus thuringiensis* (Bt) use one-half as much insecticide as growers of conventional cotton. It has been estimated that total insecticide use in cotton in 1998 was around 1,000 tons less than that used before Bt cotton was intro-

duced (James, 1998; Gianessi and Carpenter, 1999). In addition, Munkvold et al. (1999) and Windham et al. (1999) have shown that the grain levels of mycotoxins (aflatoxin and fumonisin) produced by fungi that infect the grain following damage to the ear by corn borers is far less in certain Bt maize lines. Mifflin argued that, in ignoring these benefits and trying to ban such crops, the non-governmental organizations such as Greenpeace are selling the public short, and that their opposition to GM crops may have more to do with its publicity value in their broader political campaign.

Scientists are also not immune from neglecting to carry out the appropriate comparisons. The work of Losey et al. (1999) on the monarch butterfly was discussed a number of times. Both Ian Denholm (IACR-Rothamsted) and Ian Baldwin (Max Planck Institute for Chemical Ecology, Jena, Germany) pointed out that it was an all too obvious result: Bt toxins have been known to be toxic to Lepidopteran species for a very long time, and sprinkling these toxins (in maize pollen or some other form) over the monarch butterfly's food will cause a toxic response. The same would be true if the milkweed had been dusted with dead bacterial cells containing the Bt toxin (the insecticide used by organic farmers) or with conventional synthetic chemical insecticides. The questions are: (a) does it happen in the field and (b) which method of insect control is least harmless to wild life? Bt crops may come out best in a proper comparative study.

The introduction of GM crops has also led to entirely new approaches to the commercial launching of a pesticide product. The requirements of producers and farmers to develop and implement strategies to combat the development of insect resistance to Bt crops is, as Denholm argued, a very significant step forward in resistance management. Such a requirement is not in force for conventional insecticides nor for the use of organic Bt sprays (for recent developments in requirements for refugia, see www.epa.gov/pesticides/biopesticides). The development of new insecticidal principles and their deployment in crops offers further opportunities for overcoming resistance (Schuler et al., 1998).

Developing Countries

There was widespread discussion on the application of biotechnology in developing countries. Most people agreed with the recent conclusion of the Nuffield Council on Bioethics that "The moral imperative for making GM crops readily and economically available to developing countries who want them is compelling" (www.nuffield.org/bioethics/publication/modifiedcrops/rep0008132.html). Unfortunately, as one of the speakers pointed out, the invited speakers on this topic were middle-aged white men from the West. If another conference is convened, the organiz-

ers should address this lack of balance (for the views of a female African scientist, see Wambugu, 1999). It is not appropriate for the well-fed and rich West to push its capitalist, socialist, or environmentalist philosophies onto the developing world. As Luis Herrera-Estrella (Centro de Investigacion y Estudios, Guanajuato, Mexico), the one scientific speaker from a developing country, forcibly pointed out, "non-governmental organizations, representing environmentalist philosophies of the well-off, are prepared to sacrifice the needs of the people of the developing world in the name of 'Nature,' pretty much in the same way as 400 years ago, the 'conquistadores' sacrificed the lives of the Native Americans in the name of 'God'." (Herrera-Estrella, 1999).

The facts were communicated well by several speakers. The world's population, which reached 6 billion in October 1999, is estimated to reach around 8 billion in 2020; the majority of this increase is likely to occur in the cities of the developing world. Currently, there is around 0.25 ha of agricultural land per person in the world; however, whereas a Kenyan uses 0.1 ha, a Canadian has around 1 ha. By 2020, the world average will be down to about 0.17 ha. Despite improvements over the last 50 years, some 800 million people have insufficient food, and over a billion people, particularly women and children, are affected by specific nutrient deficiencies (iron, vitamin A, etc.) (www.fao.org; www.unfpa.org).

Although the world may be able to produce enough food, particularly if Eastern Europe and the former Soviet Union make full use of their agricultural resources, the problems of politics, poverty, and distribution are still likely to prevent the needy from being fed. As Willy De Greef (Novartis Seeds, Basel) emphasized, there needs to be a distribution of technical knowledge to enable and enhance production close to the centers of need. The Consultative Group on International Agricultural Research (CGIAR) institutes (www.cgiar.org) have been very successful (but not sufficiently) in doing this in the past with the first Green Revolution. Gordon Conway (Rockefeller Foundation, New York) made his well-known case for a second Doubly Green Revolution (Conway, 1999; Conway and Toenniessen, 1999). This revolution would involve both more productive crops and the application of modern ecology in the form of integrated pest management to conserve natural resources and the environment. Biotechnology can play an important part in improving crop performance, and Conway provided a number of possible objectives, including improving photosynthesis to increase crop yield potential, improving stress tolerance (especially on marginal land), and improving nutrition (e.g. rice to provide vitamin A, see below). In this revolution, the input of the farmers themselves is crucial in the planning and implementation of the research.

There was general agreement that the needs of the developing world probably will not be addressed by commercial organizations. This is especially true for those crops that are of minor importance in the West. However, multinationals can play an important role in the transfer of technology to the CGIAR institutes or to national governments via organizations such as ISAAA (www.isaaa.org). There are already success stories in which such transfers have taken place and some crops are already in commercial production, but more needs to be done (James, 1997; www.isaaa.org/Flyer.htm). Conway also argued for companies not to enforce patent rights in developing countries and to rely, when possible, on plant variety rights. He congratulated Monsanto for shelving the development of the so-called "terminator" technology.

SCIENTIFIC DEVELOPMENTS

Crop Protection

Most of the GM crops in production at the moment have modified crop protection characteristics, chiefly protection against insects and from competition (herbicide tolerance). While there are some commercial virus resistant crops, there are further possibilities for improvement, especially in relation to the plant diseases that prevail in developing countries. Progress, particularly in using pathogen-derived resistance, was reviewed by Roger Beachy (Donald Danforth Plant Science Center, St. Louis; Beachy, 1997). A more difficult challenge has been to engineer resistance to a range of fungal pathogens. Nevertheless, considerable progress has been made recently in understanding the genetics of the interactions between fungi and plants. Jonathan Jones (John Innes Centre, Norwich, UK) described advances in the isolation of resistance genes and also their molecular structure (Hammond-Kosack and Jones, 1997; Noel et al., 1999; Parniske and Jones, 1999). *Arabidopsis* carries around 100 resistance genes, whereas crop plants may have two to three times this number. Many have been used in conventional breeding by introgression from wild sources. This is a slow process, and the durability of a single resistance gene is short when deployed in monoculture. The molecular description of the resistance genes should enable them to be moved more rapidly, either by marker-assisted breeding or by transformation into crops. It should also enable a range of different resistance genes to be assembled in different transgenic lines of the same cultivar so as to allow mosaics of resistance genes to be used within a single field. The understanding of the interaction of the Leu repeat regions of resistance genes with the recognition proteins of the pathogen, allied with techniques of gene shuffling and in vitro evolution, opens up opportunities to produce new resistance gene variants.

Whereas agrochemicals have been successfully used against biotic stresses, they have had little effect in protecting crops against abiotic stresses. There are

indications that biotechnology may be more suited to achieve this goal. One of the widest ranging stresses in world agriculture is pH, with some 40% of arable land being too acidic and another 20% too alkaline for optimal crop production. Acidic soils lead to metals such as aluminum becoming toxic, and to nutrients such as phosphate becoming deficient. Herrera-Estrella described an approach to engineering stress resistance based on the observation that certain acid-tolerant plants excrete organic acids that chelate and trap aluminum in the rhizosphere (De la Fuente-Martinez and Herrera-Estrella, 1999; Herrera-Estrella, 1999). Generating maize plants that overexpress cytosolic citrate synthase leads to excretion of organic acids by their roots. In experiments using plants in pots, these plants were found to have enhanced tolerance to toxic concentrations of aluminum and an increased capacity for phosphate uptake. Although Herrera-Estrella has sufficient material to conduct field tests, he pointed out that such GM field experiments in Mexico have been blocked under pressure from Greenpeace and other environmental groups.

Water is probably the crop resource that is in shortest supply and this condition will worsen. In addition, the quality of the water used for irrigation will decline because of a greater salt load. Because plants need to have their stomata open to take up CO₂ for carbon fixation, they lose water continuously through transpiration. This water needs to be replaced by the uptake of water from the soil. Can plants be created that lose less water in times of water deficit and yet carry out photosynthesis and grow? Julian Schroeder (University of California San Diego, La Jolla, CA) discussed research elucidating the genetics of some of the steps in the opening and closing of stomata. Stomata are regulated by major signal cascades involving abscisic acid, cytosolic calcium, protein kinases and phosphatases, potassium channels, and farnesyl transferase (Ichida et al., 1997; Allen et al., 1999). Several of the genes involved have been cloned, and transgenic plants have been made. The results hold out the hope that modifications in stomatal control may be made that would favor more efficient water use.

End-Use Qualities

The recent development that was most widely mentioned during the conference was that of the β -carotene-rich, yellow rice created by I. Potrykus, P. Beyer, and colleagues (Fig. 2). Ingo Potrykus (Swiss Federal Institute of Technology-ETH, Zurich) described the science behind this advance and some others in his laboratory (Ye et al., 2000). Rice endosperm does not contain any provitamin A (β -carotene). Theoretically, four enzymes complete the pathway from geranylgeranyl pyrophosphate to provitamin A, and genes for these enzymes were isolated from *Narcissus* and *Erwinia*. These genes were



Figure 2. Golden rice. Milled rice is deficient in important nutrients, causing serious public health problems among people in many countries of Africa, Asia, and Latin America, who rely exclusively on rice as their staple food. The introduction of a combination of transgenes into rice enabled the transgenic rice plants to synthesize β -carotene, the precursor to vitamin A. Although plants synthesize β -carotene in other tissues, it is not normally made in the endosperm, so “golden rice” could not have been produced with traditional breeding techniques (courtesy of Peter Beyer).

combined in transgenic rice, and some stable lines with yellow endosperms were produced. Biochemical analysis confirmed that the color was due to provitamin A. This was present in sufficient amounts such that a typical Asian rice diet using these lines would provide the daily requirement.

Iron deficiency has also been approached by a multigene strategy in which genes for phytase, ferritin, and a Cys-rich metallothionin-like protein were transferred and expressed in rice endosperm. Current lines have around twice as much iron as the wild type. This modified rice now has to go through the normal biosafety and agronomic tests prior to release into Asian fields. These are significant hurdles. However, there may be more obstacles, as de Greef noted that the EU rice buyers, who buy only a small proportion of Thai rice, had notified Thailand that its rice risked rejection if any of it was found to contain GM material. Thus, the EU and the powerful multinational buyers of cereals are exerting pressure that could block the introduction of what he described as “one of the most significant biotechnological developments of the last decade.”

The major harvested products of plants are polysaccharides, particularly starch. Lothar Willmitzer (Max Planck Institute of Molecular Plant Physiology, Golm bei Potsdam, Germany) discussed approaches

he and his colleagues have taken to modify carbohydrate metabolism. They have made transgenic plants that produce modified starches that might be useful in industry. Producing such starches in plants removes the need for certain chemical modifications that have environmental side effects (Lloyd et al., 1999). However, to achieve this has not been easy and the detailed story shows many examples of transgenic plants that failed to behave in the manner predicted from the biochemical hypotheses on which the choice of transgenes were based (e.g. Veramendi et al., 1999). The research has also led to identification of proteins and genes in starch granule breakdown that were not known nor predicted by biochemistry.

Biopharming is a term used to describe the use of transgenic plants to produce pharmaceuticals. Mitch Hein (EPIcyte Pharmaceutical, San Diego, CA) described the production of antibodies in plants. The first success led to the synthesis in tobacco leaves of antibodies effective against dental caries. Subsequently, it has been possible to synthesize high-affinity monoclonal secretory antibodies that can prevent microbial infection in humans. The technology can thus be considered for other immunotherapeutic uses, especially in mucosal tissue (Ma et al., 1999). Plants could be particularly valuable as commercial production systems for antibodies that are needed in large amounts. Current pharmaceutical production is expensive and limited; the total Western hemisphere fermentation production capacity is 500 kg. If antibodies are to be used for prophylaxis, production would need to be in excess of 5,000 kg/year. Plant production systems could provide this, but the current limitation is devising suitable purification procedures. Hein identified a number of potential targets, such as contraception and sexually transmitted herpes.

Improved Transformation

Although there have been a range of commercially successful transgenic crops, the technologies used for transformation have been relatively crude. There is a need to improve the efficiency of transformation, to limit the presence of unnecessary genes in the products, to direct insertion to specific sites, and to give more control over when, where, and how much expression of the transgene occurs. Not all of these topics were addressed, but Nam-Hai Chua (Rockefeller University, New York) described improvements in transformation based upon the utilization of genes promoting endogenous hormone production under the control of chemical signals. One system uses the *ipt* (isopentenyltransferase) gene from the Ti plasmid of *Agrobacterium tumefaciens* to increase cytokinin levels, leading to the generation of shoots from transformed plant cells (Kunkel et al., 1999). However, these shoots retain the shooty phenotype and result in sterile plants. Using a construct in

which the *ipt* gene is only active in the presence of a chemical inducer, the transformation and early cultivation of the cultures is carried out in the presence of the inducer. When shoots have formed, the inducer is removed and whole fertile plants can be recovered with high efficiency. The use of chemical induction cassettes in conjunction with the CRE-lox recombination system (Ow, 1996) has allowed Chua and colleagues to trigger the removal of transferred DNA from transgenic plants. This has considerable potential for crop biosafety through the ability to remove transgenes, which are no longer needed, before the product reaches the fields and the market.

Pal Maliga (Waksman Institute, Rutgers University, Piscataway, NJ) pointed out that using the plastid genome as a site for transgenes has several advantages over the nuclear genome. These include transfer of the genes only via the female line (for many but not all plant species), thus preventing the movement of the transgene to wild species via pollen; very high levels of expression (so far up to 20%–25% of total cellular protein); and targeted homologous recombination into the plastid genome. The technology is challenging and only works routinely with tobacco, although Maliga is optimistic that it will soon work well in rice (Maliga and Nixon, 1998; Khan and Maliga, 1999).

Markers and Crop Improvement by Non-Transgenic Methods

Recombinant DNA technology has given rise to a range of methods that allow the genome to be tagged with DNA markers (Karp et al., 1997; Davis et al., 1999; Vuylsteke et al., 1999). Some of these methods and the new developments that may be expected, were reviewed by Mark Zabeau (University of Gent, Gent, Belgium). The crucial drive is to provide systems that can be automated using robots, chips, sophisticated analyses (e.g. MALDI-TOF), and computers so that large populations of plants can be handled in a cheap and routine manner. Based on the discussions at the conference and elsewhere, it would seem that considerable success is on the way. Such technology has a number of uses, the most important being the identification of agronomic trait loci and their movement into adapted cultivars using marker assisted breeding. This method of crop improvement is not (currently) subject to criticism and even received the vocal approval of Haerlin. Once loci have been identified, map-based cloning can be used to isolate the genes involved. Marc van Montagu (University of Gent) pointed out that this could be of value to mine for genes of importance (e.g. those responsible for the synthesis of pharmacologically active compounds) from exotic species.

The practical value of marker technology was exemplified by the talk of Susan McCouch (Cornell University, Ithaca, NY), in which she described an

experiment done with rice involving scientists in many centers in different countries, which was designed to discover quantitative trait loci important in crop performance and to recombine favorable alleles at those loci. *Oryza rufipogon*, a wild relative of rice, was crossed with three elite cultivars adapted to different growing environments in China, Korea, and Columbia, and 300 backcross lines were derived. These lines were tested in a wide range of environments and evaluated for 12 key agronomic traits. Several lines showed superior performance to the parents (Xiao et al., 1998). Subsequent marker analyses showed that, surprisingly, not all of the favorable alleles were in the adapted cultivar. *O. rufipogon* contributed alleles that were consistently associated with improvements in yield, quality, maturity, and plant height. This work, together with other examples, suggest that reservoirs of genetic diversity that reside in the wild relatives of crop species may contain numerous alleles that can provide the key to future increases in the productivity of a number of crops (Tanksley and McCouch, 1997). The great advantage of this approach is that it overrides preconceived notions of what lines or which traits might be valuable in a cross, instead allowing the genes to “speak for themselves.” This is a humbling notion for scientists, and McCouch found considerable difficulty in getting breeders interested in participating in the analysis of a cross involving such an apparently useless parent as *O. rufipogon*.

The Big Problem/Opportunity

Tremendous opportunities for crop improvement are likely to arise as a result of the complete sequencing of plant genomes. This is well under way and due to be completed soon for Arabidopsis (Lin et al., 1999; Mayer et al., 1999); a rice genome sequence should not be far behind. The information that we have on plant synteny (Fig. 3), particularly for the grasses (Gale and Devos, 1998), should ensure that the results are useful for a much wider range of plants than those sequenced. The problem is in deriving, in a timely way, knowledge of those genes that are important for crop function or for the synthesis of high-value molecules from the great mass of sequence information that is being generated.

Geoffrey Duyk (Exelixis Pharmaceuticals, San Francisco) outlined some general approaches to the problem both from a broad life science perspective and from the perspective of his own company. The innovative drive of the technology is important in that it has already reduced the cost of sequencing 10-fold since the beginning of the human genome project, and Duyk expects another 10-fold reduction in the future. Automated, high-capacity technologies and informatics have been recruited to the biological research laboratory to deal with the sequence information generated and the opportunities that it pre-

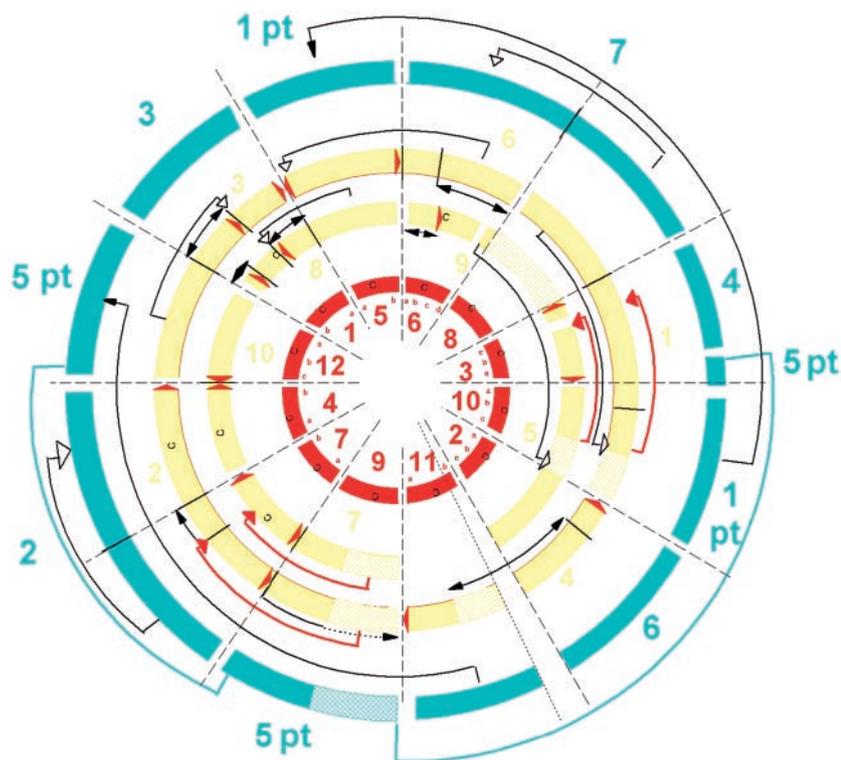


Figure 3. Synteny. A single cereal consensus map demonstrates synteny. Each circle represents the chromosomal complement of the three major cereals, with rice (12 chromosomes) in the center, maize (two sets of five chromosomes), and wheat (seven chromosomes) on the outside. The genomes are arranged relative to rice, and it has been necessary to invoke only a few transpositions and inversions to reflect the evolutionary differences that have arisen over the past 60 million years. Gene content and gene order have been so conserved that radii will pass through different versions of the approximately 25,000 genes we expect to find in cereal crop species (courtesy of Mike Gale).

sents. His view is that this is leading to a shift in the paradigm of research as the gathering and presentation of data has become an end in itself, resulting in a dissociation of data acquisition from classic hypothesis-based research. As this process gains speed, many more genomes, particularly of important pests and pathogens, will be targeted. Exelixis is particularly focused on insects and nematodes in the hope of aiding the discovery of novel insecticides and nematocides.

John Ryals (Paradigm Genetics, Research Triangle Park, NC) discussed his company's approach to identifying new target sites to aid in the discovery of crop protection chemicals based on high-throughput DNA sequencing, high-throughput reverse genetics, and knowledge-based computer systems. Reverse genetics is based on generating transgenics overexpressing cDNAs in the sense and antisense orientation, and then analyzing their phenotype. The generation of mutants by this or any other approach (such as tagging) is likely to be one of the limiting steps. Phenotypic analysis will be automated as far as possible. The approach is to generate and digitize images of the plants, to conduct biochemical profiling via HPLC/mass spectroscopy for around 5,000 molecules of under 5,000 D, and to interrogate the database iteratively. This is likely to generate huge amounts of data that will need to be stored and accessed. A primary focus is thus on data management. It is estimated that 10 terabytes will be generated in the analysis of *Arabidopsis*. Such a program needs a large amount of resources focused on the

ultimate objectives. Although the major multinationals were not represented at the conference, it is likely that similar resource-intensive approaches are being used by them.

An alternative company approach was described by Guy della-Cioppa (Genomics Biosource Technologies, Vacaville, CA). His company has developed a plant expression system based on RNA viruses such as the tobacco mosaic virus (Kumagai et al., 1995; Della-Cioppa and Grill, 1996; Baulcombe, 1999; Koo et al., 1999). These virus vectors are sprayed on a growing plant and rapidly multiply, giving very high expression of their inserts, which can be in either the sense or the antisense orientation. This approach allows the identification of genes whose expression leads to a marked change in phenotype in the leaves (for example, infection with a phytoene synthase insert rapidly leads to yellow plants); it may be more limited in cases in which the gene controls early development or does not function in leaves.

The major public sector initiatives were represented by Michel Caboche (Institut National de la Recherche Agronomique, Versailles, France). Unfortunately, for various reasons there were no speakers from the U.S. or other European countries. France has set up the Genoplante project. This is a 5-year scientific program, involving private and public laboratories and funding, which will share resources and data. So far, around 80 projects have been funded. The goal of the project is to find a way to co-operate with other genome projects. Its main goals are to develop expertise, infrastructure, and competitive-

ness in plant genome analysis. The program has two components, a generic part that focuses on model genomes (*Arabidopsis* and rice), and a more commercial part that will analyze the genomes of major crops (wheat, maize, oilseed rape, and sunflower) and their synteny with the model species. The program aims to identify genes and alleles useful for molecular breeding by positional cloning and candidate gene approaches. Caboche sees the commercial approach generating industrial property rights and new biotechnology companies, but the program does not include the generation of GM crops. Results from the generic part will be published and placed in a Genoplante database. Within the *Arabidopsis* program, the French projects (Camilleri et al., 1998; Desprez et al., 1998) have, or aim to produce, 5,000 non-redundant expressed sequence tags, a yeast artificial chromosome library and physical map, and 50,000 T-DNA mutant lines produced by vacuum infiltration. Caboche outlined how some of these resources are being utilized within his own laboratory in the analysis of lipid metabolism in seeds.

CONCLUSIONS

Science and Society

It is my opinion that plant research is vital for the future of a world in which the human species has to reach a balanced and sustainable relationship with the rest of nature and its environment. We are increasing in population and this population is likely to have higher expectations in food provision and in longevity. All of this means a rapid increase in the demand for food, feed, fiber, and fuel. On the other side of the equation, we are rapidly depleting our fossil fuel reserves, and there are severe limitations on the amount of land and water available for agriculture. In addition, political problems and distribution costs suggest that we need, as far as is possible, to enable the bulk of food to be produced close to where it is needed. Eventually, if that does not happen, the hungry will move in large numbers to where there is food. The political and social consequences of a failure to meet the legitimate demands of the developing world will eventually be serious for the developed world.

There is a view that, since the West is chiefly suffering from too much rather than too little food, it can afford to put biotechnology aside and GM crops could only be used in the developing world. There are a number of dangers in this. First, there is the argument of resource efficiency: using more land, materials, and energy than we need to produce food is a misuse of resources. Second, the technology and know-how are being developed with commercial and public resources in the West, and if the technology is blocked, the flow of funds for research will inevitably diminish severely as shareholders and the public ask why money should be spent on developing this ap-

parently unwanted technology. Third, the food supply chain is international and the decisions as to what is grown are taken by a few people in charge of the major supermarkets and commodity traders; they are more likely to support a GM-free chain for the richer customers rather than a special GM chain for the poorer customers. Finally, globalization of pressure groups is leading to universal opposition to GM crops. Thus, there is a danger that the research and development of solutions that GM crops could provide will slow or stop, despite the needs of millions of people for sufficient locally produced food. In the words of de Greef, "a food shortage caused by an empty R&D pipeline in the long-term is lethal, preventable, and immoral."

The conclusion of Braun and others is that, if scientists believe in the importance of their research and the technologies deriving from them, then they have to engage in the political process, to confront the challenges of the Green activists, to engage society in the debate, and, if necessary, take to the streets with banners. Because the outcome will most affect the young scientists, their careers, and their dependents, and because they are the ones most likely to be acceptable to society (Farmelo, 2000), it is crucial that they are motivated to participate in the debate before it is too late. Reversing the present situation in Europe will not be easy, as supermarket chains and processed-food producers such as Gerber will need a believable case to put to the public as to why they have changed their minds on the inclusion of GM crops in their food products. However, failure to reverse the situation will jeopardize the future of plant science research and those involved in it as well as those likely to benefit from it.

Crop Improvement

The scientific goal for biotechnology and world agriculture is the improvement of the genetics of our crops. The big challenge for biological research in general, and crop improvement in particular, is how to get the most valuable knowledge the quickest from the explosion in DNA sequence information. A large-scale genomics approach is chiefly being followed in industry, but to some extent is also being tried in national and international programs. It will undoubtedly yield many benefits, but also has many limitations. It tends to be driven by data rather than hypotheses (see Duyk's comments above), which means that it may be linear and unprioritized (i.e. start with the first available sequence and work out the function of the genes one by one). It also faces difficulties in that many important crop traits depend on the interaction between a number of genes or may be encoded by multiple copies of the same gene. In addition, plants are renowned for their ability to compensate physiologically. Thus, generating large populations of plants in which single genes are either

knocked out or overexpressed is unlikely to reveal the genetics of processes subject to these phenomena; generating multiple combinations will probably be numerically impossible. Even when the function of a gene sequence has been correctly identified, there remains the problem of finding the better or best alleles. For these reasons, I have argued elsewhere that the current thrust, which is largely based on a genocentric view, needs to be balanced by a matching emphasis on a phenocentric approach (Miflin, 2000).

A phenocentric view of crop improvement starts from the viewpoint that farmers cultivate phenotypes, i.e. crop performance is determined by the interaction of genotype and environment. While the farmer can do something to ameliorate the environment, crop improvement mainly depends on plant breeders assembling the best combination of alleles of the genes governing the key traits. The modified varieties are then widely tested to determine to which environments they are adapted. The development of marker technology, as exemplified in the work presented by McCouch, has now opened up the ability to identify many of the loci, genes, and alleles that are most important in crop improvement. It allows interactions between genes to be identified and it enables the mining of new favorable alleles from wild sources. Its crucial limitation is the ability to measure the phenotypic trait sufficiently reliably and accurately. It is also dependent on the existence of sufficient variation in the genetics of key traits in crossable species; where there is no difference between alleles at a locus, the importance of that locus in the process is unlikely to be revealed (e.g. the genes important in determining the rate of photosynthesis in wheat, in which there is little variation; Evans, 1993).

Physiology and biochemistry provide the third approach to identifying genes for crop improvement. In some cases, knowledge of biochemical pathways can lead to the identification of candidate genes, and the subsequent transgenic plants behave as expected (see the developments described by Herrera-Estrella and Potrykus). In contrast, most of the developments in trying to change carbohydrate metabolism (as described by Willmitzer) have led to the conclusion that we did not understand the biochemistry as well as we thought we did, or that compensation overrides the genetic changes. Nevertheless, much useful information has been gathered and, slowly, useful changes are being achieved. Sound physiological knowledge can also be highly useful in developing screens to probe the many populations of sequence-defined mutants that are being generated. An example of this in the past is the success of the screen devised by Somerville and Ogren (1979) in identifying many of the key steps in the pathway of photorespiration and determining their genetic control. Current technology will make this approach easier and more fruitful. All that re-

mains is to devise the requisite "smart" screens to probe the physiological phenomena of interest.

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