INSTITUT DE FRANCE
Académie des sciences

Le monde végétal s'ouvre aux biotechnologies
New trends in plant biology and biotechnology

Colloque international de l'Académie des sciences
Institut de France, 23 quai de Conti – 75006 Paris

15-16 septembre 2008

Résumés / Abstracts

Comité scientifique

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Michel Caboche
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PROGRAMME

Lundi 15 septembre / *Monday, September 15th*

Session 1 Les plantes dévoilent leurs secrets / *Plants Release their Secrets*

8h45- 9h00 **Ouverture du colloque /** *Welcome address*
Jules Hoffmann, Jean-François Bach, Michel Caboche, *Académie des sciences*

SÉQUENCE 1 Les génomes des plantes et leur évolution / *Genomes and their Evolution*

Chair : Michel Caboche, Académie des sciences, UMR INRA/CNRS/UEVE, Versailles

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9h00- 9h30 **La polyploïdie des génomes de plantes** / *The Polyploïdy of Plant Genomes*
Olivier Jaillon, Génoscope, Evry

9h30-10h00 **Les transposons et leur rôle dans l'évolution des génomes de plantes** / *Transposons and their Role in the Evolution of Plant Genomes*
Olivier Panaud, Université de Perpignan

10h00-10h30 **Phylogénie moléculaire et origine des angiospermes** / *Molecular Phylogeny and the Origin of Angiosperms*
Mark W. Chase, Royal Botanic Gardens, Kew, UK

10h30-11h00 **Pause**

SÉQUENCE 2 Les plantes et la biodiversité / *Plants and Biodiversity*

Chair : Christian Dumas, Académie des sciences, URM CNRS/INRA/ENS, Université de Lyon

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11h00-11h30 **Changements climatiques et biodiversité** / *Climatic Changes and Biodiversity*
Pierre Taberlet, Université Joseph Fourier, Grenoble

11h30-12h00 **La diversité des molécules synthétisées par les plantes** / *The Diversity of Molecules Synthesized by Plants*
Jean-Yves Lallemand, Académie des sciences, ICSN (CNRS), Gif-sur-Yvette

12h00-12h30 **Les plantes confrontées à la diversité des pathogènes** / *Plantes Facing Pathogene Diversity*
Stéphane Génin, Christian Boucher, LIPM (INRA, CNRS), Toulouse

12h30-13h00 **Signaux d'interaction entre plantes et insectes** / *Interaction Signals between Plants and Insects*
Gary Felton, The Pennsylvania State University, USA

13h00-13h30 **Les espèces végétales introduites dans le milieu naturel : une menace pour la biodiversité ou une opportunité écologique ?** / *Plants Introduced in Natural Environment : a Threat for Biodiversity or an Ecological Opportunity ?*
Eric Tabacchi, EcoLab, Laboratoire d'écologie fonctionnelle (CNRS, UPS, INPT), Toulouse

SÉQUENCE 3	**Domestication et amélioration des plantes / *Domestication and Breeding of Plants*** ***Chair : Michel Delseny***, Académie des sciences, CNRS, Perpignan
14h30-15h00	**La domestication du maïs** / *Maize Domestication and Breeding* **Michele Morgante**, University of Udine, Italy
15h00-15h30	**L'amélioration d'une céréale, à partir des ressources de sa biodiversité** / *Exploiting Biodiversity to Breed Barley* **Andreas Graner**, IPK Gatersleben, Allemagne
15h30-16h00	**Le Tilling, un nouvel outil pour l'amélioration des plantes** / *TILLING, a New Tool for the Genetic Improvement of Crops* **Abdel Bendahmane**, URGV (INRA, UEVE), Evry
16h00-16h30	**Recherches et production végétale en Inde** / *Research in Crop Biotechnology in India* **Arjula Reddy**, University of Hyderabad, India
16H30-17H00	**Pause**
SÉQUENCE 4	**Production végétale / *Crop Production*** ***Chair : Henri Décamps,*** *Acad*émie des sciences, Laboratoire d'Ecologie fonctionnelle, CNRS, Toulouse
17h00-17h30	**Les semences : un enjeu industriel et économique** / *Seeds, an Important Industrial and Economic Issue for Europe* **Bernard Le Buanec**, ISF Nyon, Suisse
17h30-18h00	**Propriété industrielle et OGM** / *GMOs, Patents and COV* **Nicole Bustin**, CPOV, Ministère de l'Agriculture et de la Pêche, Paris
18h00-18h30	**Les phytosanitaires : les utiliser, les remplacer ou les proscrire ?** / *What Should we do with Agrochemicals : Use Them or Ban Them ?* **Jean-Pierre Décor**, Institut des sciences du vivant, Aventis, Lyon
SÉQUENCE 5	**Science et Société / *Science and Society*** ***Chair : Jean-François Bach***, Académie des sciences, Paris
18h30-19h00	**Quel sens pour le principe de précaution à l'âge de la mondialisation ?** / *What is the meaning of precautionary principle at the globalisation age ?* **Luc Ferry**, Philosophe, ancien Ministre, Paris

Mardi 16 septembre / *Tuesday September 16th*

Session 2 Les OGM / *GMOs*

SÉQUENCE 1 Les OGM apportent-ils des solutions nouvelles à des problèmes anciens ? / *Do GMOs Bring New Answers to Old Issues ?*

Chair : Alain Boudet, Académie des sciences, UMR CNRS/UPS, Toulouse

9h00-9h30	**Les technologies de transfert de gènes, leur histoire et leur avenir** / *Gene Transfer in Plants : from a Breakthrough in Fundamental Research to an Essential Technology for Agriculture, Industry and Environment* **Marc Van Montagu**, IPB, University of Ghent, Belgium
9h30-10h00	**Nouvelles stratégies de protection des cultures contre les insectes** / *New Strategies of Crop Protection Against Insects* **Xiao-Ya Chen**, Shanghai Institute for Biology, China
10h00-10h30	**La protection des plantes contre les virus par technologie OGM** / *Crop Protection Against Viruses by GMO-Based Technologies* **Dennis Gonsalves**, USDA, Hawaï, USA
10h30-11h00	**Pause**
11h00-11h30	**Amélioration de la tolérance au stress abiotique** / *Improvement of Crop Tolerance to Abiotic Stress* **Luis Herrera-Estrella**, National Laboratory of Genomics and Biodiversity, Mexico
11h30-12h00	**Produire de manière durable les acides gras importants pour la nutrition humaine** / *Towards a Sustainable, Land-Based Source of Nutritional Fatty Acids for the Human Diet* **Anthony Kinney**, DuPont Experimental Station, Willmington, USA
12h00-12h30	**Bilan des travaux de la commission de génie biomoléculaire** / *A Survey of the French « Commission de génie biomoléculaire »* **Marc Fellous**, Institut Cochin, INSERM, Paris

SÉQUENCE 2 Doit-on craindre les OGM ? / *Should we Fear GMOs ?*

Chair : Georges Pelletier, Académie des sciences, INRA, Versailles

14h00-14h30	**Evaluation toxicologique et nutritionnelle des OGM** / *Toxicological and Nutritional Evaluation of GMOs* **Gérard Pascal**, *Chercheur émérite à l'INRA, Expert en sécurité alimentaire à l'OMS, Paris*
14h30-15h00	**Les OGM et la santé humaine** / *GMOs and Human Health* **Jean-François Bach**, Académie des sciences, Paris
15h00-15h30	**Dispersion des OGM dans l'environnement et coexistence** / *Release of GMOs in the Environment and co-Existence.* **Joaquima Mésseguer**, Plant Genetics Department, IRTA, Catalunya, Spain

15h30-16h00 **Pause**

16h00-16h30 **Impact environnemental des OGM résistants aux herbicides ou aux insectes** / *Environmental Impact of GMOs Resistant to Herbicides or to Insects*
Klaus Ammann, Delf University of Technology, The Netherlands

16h30-17h00 **Dix ans d'expérience d'un agriculteur sur la culture de maïs OGM résistant à la pyrale**
Claude Menara, Bouglon

17h00-17h30 **Rentabilité des OGM** / *Global Impact of Biotech Crops : Socio-Economic and Environmental effects 1996-2006*
Graham Brookes, PG Economics, Dorchester, UK

17h30-17h45 **Conclusions**
Georges Pelletier

SIGLES

- AFSSA	Agence Française de Sécurité Sanitaire des Aliments (Maisons-Alfort)
- CNRS	Centre National de la Recherche Scientifique
- CPOV	Comité de la Protection des Obtentions Végétales (Ministère de l'agriculture)
- ICSN	Institut de Chimie des Substances Naturelles
- INA	Institut National Agronomique Paris-Grignon
- INPT	Institut National Polytechnique de Toulouse
- INRA	Institut National de la Recherche Agronomique
- IPB	Institute of Plant Biotechnology (University of Ghent, Belgique)
- IPK	Institute of Plant Genetics and Crop Plant Research (Gatersleben, Allemagne)
- IRTA	Institut de Recerca i Tecnologia Agroalimentaries (Catalogne, Espagne)
- ISF	Fédération internationale des semences (Nyon, Suisse) (International Seed Federation)
- LIPM	Laboratoire des Interactions Plantes Microorganismes (Toulouse)
- UEVE	Université d'Evry Val d'Essonne
- UPS	Université Paul Sabatier – Toulouse III
- URGV	Unité de Recherche en Génomique Végétale (Genopôle d'Evry, Essonne)
- USDA	United States Department of Agriculture (USA)

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RESUMES / *ABSTRACTS*

NOTES

DU GENOME DE LA VIGNE A L'HISTOIRE DES POLYPLOÏDIES DANS L'EVOLUTION DES PLANTES A FLEUR

Olivier Jaillon

Génoscope, Université d'Evry, 2 rue Gaston Crémieux CP5706 91057 Evry cedex France

Selon les principales hypothèses, une duplication du génome d'une espèce exposerait son répertoire de gènes à une modification soudaine de ses contraintes. Des adaptations fonctionnelles et des émergences de nouvelles espèces seraient alors facilitées. De grandes radiations comme les téléostéens ou les angiospermes dériveraient ainsi de duplications totales génomiques ou autres polyploïdies, événements cataclysmiques.

L'analyse des premières séquences de génomes de plantes à fleur a révélé une inattendue multiplicité de traces de polyploïdies anciennes. Cependant leur dessein et chronologie au sein des lignées de monocotylédones et dicotylédones sont controversés. La séquence du génome de la vigne *Vitis vinifera* apporte ici des éclairages importants sur l'évolution des plantes à fleur. Ce génome a maintenu une structure remarquablement stable au cours de l'évolution puisqu'il est dépourvu de duplication totale récente et le nombre de remaniements chromosomiques récents y est relativement faible. Le génome de vigne contient toutefois des reliques d'événements très anciens : le contenu haploïde actuel dérive d'une contribution ancestrale de 3 génomes. Cette paléo-hexaploidisation se retrouve aussi chez de nombreuses dicotylédones mais n'est pas détectée chez le riz qui est monocotylédone. L'analyse comparée des différents génomes de plantes permet alors d'établir la chronologie des différents événements de polyploïdisations identifiés dans les plantes séquencées.

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TRANSPOSABLE ELEMENTS AND THEIR ROLE IN THE EVOLUTION OF PLANT GENOMES

Olivier Panaud

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Transposable Elements (TEs) are ubiquitous components of eukaryotic genomes. It is now clearly established that in plants, the activity of TEs are one of the main causes of large genomic changes, leading in some cases to extensive genome size variation (Piegu et al., 2006). One of the primary goals of plant structural genomics is therefore to understand the process through which TEs shape plant genomes both at space and time levels. The availability of the complete genomic sequence of rice *Oryza sativa* has provided the community with the opportunity to conduct for the first time several thorough, genome wide studies of TEs in a small, yet complex plant genome. These studies have lead to the elaboration of a model of plant genome evolution based on the activity of a particular class of TE, the LTR-retrotransposons (Vitte et al., 2005). We will present and discuss this model in the context of the evolution of the plant genus *Oryza* (to which rice belongs). Moreover, we have demonstrated the occurrence of horizontal transfers of LTR-retrotransposons, first among *Oryza* species (Roulin et al., 2008) and more recently among several genera of the *Poaceae* family. This suggests that these mobile elements may spread in the plant kingdom, although the exact mechanisms of such transfers is not yet known.

B. Piegu, R. Guyot, N. Picault, A. Roulin, A. Saniyal, H.R. Kim, K. Collura, D.S. Brar, S.S. Jackson, R.A. Wing, O. Panaud (2006) : Doubling genome size without polyploidization : direct evidence of retrotransposition-driven genomics bursts in Oryza genus. Genome Res, 13: 1262-1269
A. Roulin, B. Piegu and O. Panaud (2008). Evidence of multiple horizontal transfers of the LTR-retrotransposon RIRE1 within the genus Oryza. Plant J. 53: 950-959
C. Vitte and O. Panaud (2005). LTR retrotransposons and plant genome size: emergence of the increase/decrease model. Cytogenetics & Genome Res 110: 91-107

MOLECULAR PHYLOGENY AND THE ORIGIN OR ANGIOSPERMS

Mark Chase

Keeper of the Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3DS PA, UK

Flowering plants (angiosperms) are by far the most diverse and successful of all extant land plants, and they dominate all but the coldest, driest and highest zones on Earth. Even in these harshest areas, they compete well with gymnosperms, which they largely have displaced since about 100 million years ago. Their origins have engendered a great deal of interest and speculation, particularly from Darwin, who observed that this topic as “an abominable mystery”[1] – they are absent from the fossil record until about 125 million years ago (mya), and then they quickly appeared in great diversity. With the advent of molecular systematics, we have learned a great deal about the timing and diversification of the flowering plants[2], and we now can say a great deal about the sequence of appearance of all extant groups. Large-scale efforts along these lines started in 1993 with the paper by Chase et al., using a single plastid gene, *rbcL*[3]. Most recently, efforts to include data from nuclear, single-copy genes and whole-plastid genome sequences have been used to further clarify angiosperms relationships and sort out the remaining problems that were not resolved by sequencing a few genes. Fortunately, these efforts have produced highly congruent results, and in 1998 the angiosperms were the first major group of organisms to be re-classified based largely on DNA sequence data.

Knowing the relationships of all extant groups of flowering plants does not necessarily give many clues to their origins; on the basis of molecular clock experiments, we can say that the angiosperm lineage extends back much further than the first fossils (125 mya) that are clearly angiosperms in the fossil record, to perhaps 180 mya, but there are no fossils that are undisputedly angiosperms from these earlier dates. Clues to the early evolution of flowering plants could be found in evolutionary development studies[4], but these are not particularly well advanced at this point in time. What we can say is that it is clear that there was a burst of morphological diversity around 145 million years ago, at which time all major groups of angiosperms appeared[5], more or less simultaneously. This could be seen a corroboration of Darwin observations on angiosperms origins being a mystery. In addition, ideas about the origins of many families of flowering plants have undergone extensive revision. For example, most botanists before the molecular era considered the orchids to be one of the most recently evolved families; they lack a fossil record and their highly evolved relationships with pollinators makes it difficult to view them as “primitive”. However, based on several lines of evidence, including molecular clock studies, we now believe that the orchids are one of the oldest families of plants – they evolved long before the end of the Cretaceous and co-existed, therefore, with the dinosaurs. This is another line of evidence that supports the idea that modern-appearing tropical ecosystems also evolved some time before the fossil evidence has indicated, again probably before the end of the Cretaceous.

Major implications of this enhanced understanding for relationships are that genetic data can be used for other purposes, such as DNA barcoding[6] (using one or more plastid genes, including *matK*). At the same time, we are seeking to produce a phylogenetic tree based on two gene sequences (including *matK*) for all 13,000 genera of plants, starting with the monocots. The ability to quickly identify plants, even from small fragments, seeds or seedlings, and an improved understanding of the origins of extant biodiversity and why biodiversity is not evenly distributed around the Earth will important outcomes of these studies. When incorporated with models of climate change, both past and present, these phylogentic analyses will become powerful tools that will allow us to better understand the consequences of climate change for the distribution of plants. The study of biodiversity is not merely a quest with the goal of improved understanding; loss of biodiversity has direct consequences for human health and well-being. Ecosystem functioning is important for maintenance of the quality of human life. Of course it provides us with recreation and stimulation, but biodiverse habitats are more stable than those from which species have been randomly removed by extinction. Recently it has been shown that plant species for which seed dispersal occurs via animals are better able to exist in fragmented populations than those that are wind-pollinated[7], which demonstrates that biologically more complex communities can withstand better the effects of climate change and human activity than those with lower levels of mutual dependency. Biodiverse communities will prove to be more resilient than depauperate ones, so it is important to find ways to preserve all forms of biodiversity and thereby maintain the ability of these ecosystems to enrich human life.

1. Darwin, F. & Seward, A. C., eds. (1903) *More Letters of Charles Darwin* (John Murray, London), Vol. 2.
2. Soltis, D. E. & al. (2005) *Phylogeny and evolution of angiosperms* (Sinauer Publishing, Sunderland, Massachusetts, USA)
3. Chase & al. (1993) Phylogenetics of seed plants: an analysis of nucleotide sequences from the plastid gene *rbcL*. *Annals of the Missouri Botanical Garden* 80: 528-580.
4. Frohlich, M. & Chase, M. W. (2007) A dozen years of progress but origin of the angiosperms is still a great mystery. *Nature* 450: 1184-1189.
5. Moore, M. J. & al. (2007) Using plastid genome-scale data to resolve enigmatic relationships among basal angiosperms. *PNAS (USA)* 104: 19363-19368.
6. Chase & al. (2005) Land plants and DNA barcodes: short-term and long-term goals. *Philosophical Transaction of the Royal Society, series B*, 360: 1889–1895.
7. Montoya & al. (2008) Animal versus wind dispersal and the robustness of tree species to deforestation. *Science* 320: 1502-1504.

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CLIMATIC CHANGES AND BIODIVERSITY

Pierre Taberlet

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During the Quaternary, periods of climate stability are not common, and the instability seems to be the rule. However, the current global warming is stronger and faster. The challenge for scientists is to be able to reliably predict the impact of the global warming on biodiversity, i.e. on species distributions. A range of advanced modelling approaches has been used to assess the impact of global change on biodiversity and ecosystems. Such approaches can be illustrated by presenting the impact of climate change on plant diversity in Europe (Thuiller et al., 2005, PNAS, 102:8245-8250).

However, five main limitations remain associated with these approaches:
(i) knowledge and data of past species' distribution is still limited, yet necessary for testing the models in the past before projecting them to the future, (ii) we miss sound estimates of species' long distance migration rates in order to assess whether species will be able to keep pace with rapid global change, (iii) some key assumptions of models, such as niche stability over time and/or space are not well tested, (iv) we need more reliable estimate of uncertainties in model predictions, (v) interactions among species are extremely complex and cannot be taken into account according to the current knowledge. For example, some species adjust their life history characteristics according to the temperature, while some other according to the photoperiod. Finally, I will present the current research in this area.

NOTES

LA DIVERSITE DES MOLECULES SYNTHETISEES PAR LES PLANTES

Jean-Yves Lallemand

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L'homme utilise depuis des millénaires le monde végétal pour se nourrir et se soigner. Il a su trouver dans la diversité naturelle une partie importante de son alimentation comprenant des molécules simples : sucres, acides gras et des biopolymères : polysaccharides protéines. Il a très longtemps utilisé aussi sans en comprendre le rôle des molécules plus rares et plus sophistiquées nécessaires à sa survie comme les vitamines… Il en a découvert et recherché d'autres pour les bienfaits qu'elles apportent par leurs propriétés aromatiques, cosmétiques et surtout médicamenteuses, voire stupéfiantes.

Ce n'est que vers la fin du XIXième siècle, que les premières molécules provenant d'extraits naturels ont été obtenues à l'état pur et les toutes premières structures établies. Une grande diversité moléculaire est apparue avec des molécules aussi différentes que le glucose, la salicine (un précurseur de l'aspirine), le camphre, le terpinéol, l'atropine, la quinine… Les grandes familles des polysaccharides, des poly-phénols, des acides gras, des terpènes et des alcaloïdes ont alors été identifiées.

L'enjeu de découvrir des molécules nouvelles de plus en plus complexes a eu un impact considérable sur le développement de la chimie, des méthodes physiques d'analyse structurale et sur la compréhension des mécanismes biosynthétiques utilisés par les plantes.

Actuellement, on peut considérer que le monde végétal est assez bien connu et compris dans sa diversité moléculaire. Beaucoup de chercheurs restent fascinés par elle (et cela sort largement du cadre du monde végétal) et continuent à explorer la flore de zones mal connues comme les forêts primaires, afin de découvrir de nouveaux squelettes moléculaires et surtout des propriétés biologiques originales. La découverte de molécules pouvant intervenir comme précurseurs avancés et relativement abondants permettant « l'hémi-synthèse » de molécules à très haute valeur ajoutée peut également être un objectif très motivant. Deux exemples historiques seront rappelés concernant les hormones stéroïdiennes et certains anti-cancéreux.

Une question intéressante reste également ouverte sur l'origine de certaines molécules isolées du monde végétal : dans quelle mesure la symbiose plantes-bactéries ou plantes-champignons intervient-elle dans la production de métabolites secondaires de plantes ? La réponse à cette question n'est-elle pas celle de la Nature à la problématique des OGM ?

(1) Molecules that changed the World. K. C. Nicolaou, T. Montagnon. Wiley-VCH, IBSN 978-3-527-30983-2. Cet excellent ouvrage s'adresse à un public scientifique mais non spécialisé et retrace l'histoire des découvertes des molécules importantes et les efforts de synthèse pour les obtenir.

NOTES

PLANTES FACING PATHOGEN DIVERSITY

Stéphane Genin

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Les plantes doivent continuellement se défendre contre différents agents pathogènes, tels les virus, les bactéries, les champignons ou les nématodes. Elles ont développé tout un ensemble de stratégies leur permettant de résister à ces agresseurs. Contrairement aux animaux, chez lesquels la surveillance et la défense immunitaire sont le fait de cellules spécialisées, chaque cellule végétale possède la capacité propre de répondre aux agents pathogènes et d’induire les mécanismes de résistance adéquats.

Les mécanismes de résistance des cellules végétales, très efficaces, sont aussi très coûteux pour la plante. Afin que l’exécution de ces mécanismes ne soit pas délétère pour le reste de la physiologie et du développement de la plante, il est fondamental qu’ils soient régulés très finement, dans le temps et l’espace. Il existe donc non pas une, mais des voies de signalisation menant à la résistance, en connexion les unes avec les autres, formant un vaste réseau extrêmement régulé au sein de multiples voies contrôlant les autres fonctions végétales essentielles.

La lutte contre les bioagresseurs des cultures et le développement de plantes résistantes aux bioagresseurs, que ce soit par la voie de l'amélioration génétique ou par des stratégies biotechnologiques, reposent encore aujourd'hui sur des approches très empiriques. Cela tient en particulier à ce que, malgré des progrès importants durant la dernière décennie, notre compréhension des gènes et des mécanismes régissant les interactions entre les organismes intervenant dans l'environnement biotique des plantes reste encore très limitée. Toute méthode de contrôle de bioagresseurs induit sur les populations cibles une pression de sélection et donc, en retour une évolution de celles-ci qui compromet à plus ou moins long terme son efficacité. La compréhension des mécanismes en jeu doit permettre d’orienter de manière moins empirique la conception et la mise en œuvre de méthodes de gestion visant à une protection intégrée et plus durable des cultures.

Dans un premier temps nous verrons comment les plantes peuvent résister aux attaques d’agents pathogènes : comment elles reconnaissent l’agent pathogène et déclenchent alors, après des processus variés de transduction de signal, un ensemble de mécanismes de défense leur permettant de résister. Nous verrons ensuite comment les pathogènes parviennent à circonvenir ces mécanismes de défense. Nous détaillerons notamment les découvertes récentes montrant que différents pathogènes (fongiques, bactériens, nématodes) ont la capacité d’injecter directement dans le cytoplasme des cellules végétales des protéines effectrices par le biais de structures spécialisées. Ces protéines effectrices vont alors directement inhiber les systèmes de défense de l’hôte et interférer avec les processus métaboliques de la cellule végétale.

NOTES

INTERACTION SIGNALS BETWEEN PLANTS AND INSECTS

Gary W. Felton

Department of Entomology, Penn State University, University Park, PA, USA

At the instant an insect begins to chew on a leaf there is a biochemical cascade of events that precedes changes in plant defense gene expression. I argue that the defense signaling that occurs following this physical injury is ultimately shaped by the interactions of a multitude of potential players—the plant, the insect and their associated microbes. The sheer number of participants at the insect-plant interface is large, yet should not be ignored. In this presentation I will highlight the relative contributions of the respective players: the plant, the insect and the microbes. The major focus of the presentation will include the salivary signals released by insect herbivores during feeding.

NOTES

PLANTS INTRODUCES IN NATURAL ENVIRONNEMENT: A THREAT FOR BIODIVERSITY OR AN ECOLOGICAL OPPORTUNITY?

Eric Tabacchi

EcoLab, Laboratoire d'écologie fonctionnelle, Equipe "Communautés Végétales aux Interfaces Terre-Eau"
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Au cours de son histoire, l'espèce humaine a, volontairement ou non, contribué au déplacement d'espèces d'organismes vivants depuis leur aire de distribution naturelle vers de nouvelles régions du Globe. Les introductions d'espèces se sont considérablement amplifiées au cours des deux derniers siècles, notamment en raison de l'expansion des liaisons intercontinentales. Parmi les espèces introduites, certaines envahissent de nombreux écosystèmes et sont suspectées d'en affecter gravement la structure (biodiversité) et le fonctionnement (production et recyclage de la matière organique, régulation des flux de matière et d'énergie en général), avec souvent des répercussions culturelles et socio économiques graves.

L'objectif de la communication est de souligner l'ampleur du phénomène d'invasion biologique, qui participe aux changements majeurs récents de la biodiversité, mais également de mettre en évidence les biais d'analyse liés à sa perception péjorative inhérente au caractère étranger de l'envahisseur. Jusqu'à quel point les invasions sont-elles une menace réelle pour nos écosystèmes ? Est-il possible d'envisager des mesures d'éradication efficace des envahisseurs? Doit-on se résigner face à ces changements de biodiversité ? Au contraire, peut-on tirer un bénéfice de l'existence de ce phénomène ?

Parmi les espèces introduites et naturalisées, une forte proportion ont été sélectionnées, voire modifiées par l'Homme. Jusqu'à quel point, dans ce contexte, peut-on comparer les espèces végétales envahissantes et les OGM végétaux sur la base de leurs propriétés écologiques ?

NOTES

MAIZE DOMESTICATION AND BREEDING

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Maize is a crop species where the loss of genetic diversity usually associated with domestication is less dramatic than in most other crop species. Cultivated maize is still today characterized by unusually high levels of genetic diversity, that occurs both in the traditional form of single nucleotide polymorphisms as well as in the form of structural variations, i.e large insertion/deletion (indel) polymorphisms. This last form of variation is extremely frequent even among maize breeding lines that are crossed to produce commercial F1 hybrids. Only approximately 50% of the sequences in the intergenic regions are shared among two maize breeding lines such as B73 and Mo17. Recent studies have started to shed light on the origin of such sequence variation outside of the genic regions, pointing mainly to the recent activity of transposable elements of different classes as the cause for the observed differences in sequence composition among maize lines. In addition to the very frequent long terminal repeat retrotransposons, DNA transposons have been shown to be responsible for these polymorphisms. These comprise Helitrons, CACTA and Mu-like elements that are capable of acquiring and piecing together fragments of plant genes and are often expressed. The emerging picture for maize shows that its genome is highly dynamic owing to the activity of transposable elements and that an important component of sequence variation is attributable to recent transposable element insertions. In two well-studied cases of regulatory variation determining phenotypic variation in maize, namely the tb1 (teosinte branched 1, gene involved in domestication that is responsible for the change in the architecture of maize from that of its wild ancestor, teosinte) and the y1 (yellow1, gene coding for phytoene synthase involved in the more recent breeding history of maize that is responsible for the change of color in maize endosperm from white to yellow) loci, it has proven impossible to pinpoint a single nucleotide substitution as the causative mutation. Future analyses of the functional roles of intergenic sequence variation will tell us if we will need to pay more attention not only to genes, but also to the ‘junk’ DNA surrounding them.

NOTES

EXPLOITING BIODIVERSITY TO BREED BARLEY

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Barley a major constituent of European agriculture

Barley (*Hordeum vulgare* L.) ranks world-wide among the most important crop species. Because of its broad environmental adaptability it is grown on all continents, with the European Union being the second largest producer next to Russia.

Historically, barley belongs to the set of neolithic founder crops, whose natural habitats are found within the Fertile Crescent and which have been domesticated some 10.000 years ago (for review see Pourkheirandish and Komatsuda, 2007). Due to its salient importance, cultivated barley was subject to intense selection resulting in landraces. Subsequently, during the past 100 years, cross breeding programmes were performed resulting in modern cultivars.

As a diploid, annual and inbreeding species, barley has been widely used in genetic studies. In single seed descent programs the generation time from planting to harvest can be reduced to about 100 days. Its seven haploid chromosomes represent the basic complement of all species of the *Triticeae* tribe comprising about 20 genera and 300 species, amongst which major crop species such as wheat and rye are found. A large set of molecular tools have been developed over the past 20 years providing the basis for the development of integrated approaches for the genomics based improvement of this crop species (for review see Sreenivasulu et al. 2008).

Towards exploring the genetic architecture of agronomic traits

Key to the development of improved strategies for the genomics-based utilization of genetic resources is the correlation of genetic variation with phenotypic variation. This is best achieved by mapping of agronomic traits either in biparental populations that segregate for a given trait or by using "natural" populations drawing on an approach termed association mapping (for review see Slatkin, 2008). Both procedures are based on exploiting the effect of linkage disequilibrium (LD), which represents a precondition to associate a marker allele with a trait allele by linkage. Potential advantages of association mapping over conventional bi-parental mapping include a higher genetic resolution and a larger number of segregating loci that are represented within an association panel. On the other hand the outcome of an association study requires knowledge on parameters such as population structure, the decay of LD, the effects of selection (for review see Rafalski, 2002).

Association mapping based on candidate genes

To investigate these issues for barley, a set of 224 spring barley accessions has been selected for analyses of phenotypic and genetic diversity, and to investigate association of candidate genes with selected agronomic traits. The genetic background structure of the collection was estimated by using 45 EST-based SSR markers that are equally distributed across the genome. The assessment of population structure was performed by a model-based clustering method implemented in the program STRUCTURE. Two subpopulations were deduced, which by and large correlated to the two morphological subgroups of two-and six-rowed barleys. For phenotypic evaluation field trials were conducted in 2004 and 2005 to score plant height, thousand-grain weight, raw protein content, starch content and flowering time. For all traits broad sense heritabilities exceeded 0.90. For further association analysis, a set of seven candidate genes were selected that play a putative role in grain quality and the regulation of flowering time. As expected, a large degree of variation in sequence diversity, haplotype diversity and LD was detected in the eight candidate genes analyzed. Based on re-sequencing data associations between phenotypic variation and DNA polymorphism in several candidate genes were observed. Data on phenotypic, genotypic and genetic background information were used to build a model for association analysis and to identify genes that contribute to the variation of grain quality traits. Significant associations were detected for flowering time, thousand grain weight, as well as starch and raw protein content.

Conclusions

In the present approach only a limited portion of the phenotypic variation was explained by the sequence variation present in the candidate genes investigated. This is not surprising as our knowledge on candidate genes for quantitatively inherited agronomic traits is still very limited. Hence, a genome-wide scan for DNA polymorphism is expected to better capture the phenotypic variation. The efficiency of such an approach requires a high density of informative DNA markers (mainly represented as single nucleotide polymorphisms, SNPs). At present, there are about 3,000 highly informative SNP markers available for barley to be assayed on high throughput platforms (Rostoks et al. 2006). However, this figure falls far short of the > 3 million million SNPs available for the human genome, which amounts to only half of the size of the barley genome (The International HapMap Consortium, 2007). To facilitate the systematic identification of additional SNPs and to generate a blueprint of the barley genome to be used as a resource for re-sequencing, the International Barley Sequencing Consortium (IBSC) has been incepted in December 2006 (http://barleygenome.org). In a participatory approach it aims at the development of a full genome sequence of this large genome, which will blaze the trail for the systematic utilization of the ample genetic resources barley, which rest in the shelves of *ex situ* genebanks or still thrive on the meadows of the fertile crescent.

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TILLING,

A NEW TOOL FOR THE GENETIC IMPROVEMENT OF CROPS

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In the genomic era, the completion of the sequencing of several plant genomes has enabled the development of reverse genetics strategies, where one first identifies a target gene based on the functional annotation of its sequence, and then proceeds with the phenotypic characterisation of mutant alleles. Several mutagenesis techniques are dedicated to this approach, notably RNAi suppression and insertional mutagenesis, by transposon tagging or *Agrobacterium* T-DNA insertion. Those methods, however, are still mainly based on *Agrobacterium* T-DNA vectors, and thus rely on the ability of a given plant species to be transformed. On the other hand, chemical mutagenesis based on an alkylating agent like ethylmethane sulfonate (EMS); provides an easy and cost-effective way to saturate a genome with mutations.

TILLING (Targeting Induced Local Lesions IN Genomes) uses EMS mutagenesis coupled with a gene-specific detection of single-nucleotide mutations [1]. The success of the TILLING approach relies on the construction of high quality mutant libraries. Ideally, the mutant population is phenotyped so that *in silico* analysis of the mutant lines could be carried out.

We have constructed EMS-mutant population for pea, tomato and melon under controlled conditions and developed a database, UTILLdb, which presents phenotypic data based on visual characterization of M2 plants [2, 3]. A hierarchical categorisation of mutant phenotypes was used to describe the mutant plants. To facilitate the phenotype description, digital images were also recorded.

A critical aspect in implementing the TILLING approach is the choice of mutation detection methods that are designed to screen large stretches of DNA without reducing diagnostic sensitivity or specificity. Previously, we reported the biochemical analysis of five S1 type nucleases from *Arabidopsis thaliana*. We demonstrated that one of them, ENDO1, is a mismatch specific endonuclease, which cleaves with a high efficiency all types of mismatches and has a high sensitivity, detecting one allele in pool of sixty [4]. ENDO1 system could be also exploited in wide range of mutation diagnostics tools [5].

In order to exploit the mutant population using reverse genetics, genomic DNA was prepared from the mutant lines via high-throughput automated protocols and organized in pools for bulked screening. Individuals mutated in the gene of interest were isolated by systematic pool deconvolution. Genes and mutations were integrated in UTILLdb through a web interface, which allows for global analysis of TILLING mutants. This database also serves as a portal for users to request materials or TILLING experiments.

We have selected, for each crop, a list of agronomic traits to be studied by TILLING. These traits have impact on functional properties of the harvested plant for use in food, animal feed, or industrial products. Examples of these agronomic traits include fruit quality in tomato, increase of plant biomass, enhanced disease resistance, and modification of the sex of the plant to facilitate plant breeding [6, 7]. The identification and the characterization of such mutants will be discussed.

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PLANT BIOTECHNOLOGY IN INDIA

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Plant biotechnology in India began in late 80's with initial focus on plant molecular biology and tissue culture studies. Crop plants were chosen as targets for intense investigations and genetic manipulation from the beginning. Most of the research was carried out in public institutions and private institutions entered the field some time in late 90's. Initial focus was on developing regeneration and transformation protocols for major crop plants followed by crop improvement through molecular breeding. Crop Biotechnology Programs have been, and continued to be supported by the Department of Biotechnology, Govt. of India, Indian Council of Agricultural Research and Department of Science and Technology beside several other agencies. Broadly, plant biotechnology research can be discussed under three categories: 1) basic and upstream research, 2) transgenics 3) functional genomics. The current trend is to support research projects of two types: individual group projects and Network projects with few teams of researchers with diverse expertise.

Currently, several research projects are focused on identification of agronomical important genes using biotechnology and genomic tools such as molecular marker development, QTL identification, physical mapping etc., in target crops. Also, a number of investigations are being carried out in major crops on identification of target genes, mapping and functional characterization. Development of transgenic crops for both input and output traits is the most focused area of research across many labs in India. Major targets include food crops, pulses, millets and a few vegetable crops. The transgenic plants developed or used for research in India are listed in Table-1.

Crop functional genomics programs are of both basic as well as applied type and mainly focused on crops where genomic information is complete or partial. These crops include rice, maize, sorghum, pearl millet, tomato, wheat, finger millet and sugarcane. This report is a description of ongoing major projects in crop biotechnology area in India. Further details can be obtained at www.igmoris.nic.in. A partial summary of biotechnology projects on different crops and traits is given in Table- 2.

With regards to GM crops or Biotech Crops, India emerged as number 4 in acreage of transgenic crop plantation(all Bt cotton) reaching about 7 million hectares in 2008. The Bt cotton spread reached to about 80% of all cotton acreage. It is estimated that more than 3 million farmers, most of them having small farm holdings, have planted BT cotton this year. More than 100 Bt cotton hybrids are in the farmers' field already (Table-3) and a thousand or more hybrids are under trials at various levels with all four transgenic events approved as on today (Table-4). The first indigenously developed transgenic BT cotton variety is to be released soon. The Bt event (MON 521) that was originally introduced in 2002 still continues to be a predominant one though three more versions are about to be released. The Bt Brinjal and Bt Okra is about to be released and Bt rice development is at an advanced stage of testing. Other transgenic crops under various stages of development include maize, chick pea, groundnut, sorghum, potato, tomato, rice and Mung bean (Table-5)

Indian plant genomics programs include rice genome sequencing (part of the Chr 11), *Solanaceae* genome sequencing project and also Sugarcane genome sequencing project. Functional genomics projects include rice drought tolerance, salt tolerance, photosynthesis, flower development and yield enhancement. Functional genomic analysis of bacterial blight resistance in rice is a major area of research. These multi-institutional genomics projects were developed on the basis of the core strength in areas which needed to be combined to achieve progress in target areas. Functional genomics program on rice include gene expression profiling during flower and seed development and functional validation of identified genes; Genome-wide transcriptome analyses involving critical stages of reproductive and vegetative development of rice in order to identify genes expressing in specific windows of reproductive development. A network project on identification and functional analysis of genes related to yield and biotic stresses in rice with 12 sub-projects centered around five themes aims at defining genes related to yield and different biotic stresses such as blast, bacterial blight (BB) diseases and gal midge (GM) and brown plant hopper (BPH) pests in rice.

In another network project on salinity and dehydration stress tolerance in rice, several responsive genes and their promoters were cloned, tested and transgenic plants carrying these genes were developed. Gene expression profiling studies in rice grown under different water regimes at various developmental stages revealed sets of coordinately regulated genes associated with stress response. Further, a large number of SNPs associated with regulatory regions of these genes in various drought tolerance and susceptible varieties have been isolated and a partial allele sharing map of regulatory variations at candidate gene loci for drought stress response has been generated. Details will be discussed.

Another network project on development of fully regenerating lines in Indian cotton cultivars, vectors for cotton transformation and transgenics for resistance to bollworms is in progress. Transgenics cotton (Coker 31OFR) have been developed using modified *Cry IA(c)* genes. Seven different constructs were developed where in the *Cry 1A(c)* gene was driven by different promoters namely CaMV 35S double enhancer, a modified 35S

promoter, FMV, MMV double enhancer promoter with or without U leader sequence. A total of 299 independent transgenics in cotton (Coker 31OFR) have been developed using the above constructs.

Identification of molecular markers linked to QTLs for fiber strength and oil content in cotton *(Gossypium hirsutum)* and stress specific genomics of small noncoding RNA in *Brassica sps.,* rice and wheat are being carried out in different labs. Biofortified wheat and maize for micronutrients through MAS and rice for Iron and Zinc are in progress. Overall, research support for plant biotechnology is quite encouraging. New programs such as Small Business Innovative Research Development and public–private partnership projects have been initiated to boost translational research and development of genetically improved crops for target traits.

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Table 1. Transgenic plants developed or used for research purpose

1	*Andrographis paniculata*	29	Brahmi
2	Jute	30	Brassica juncea
3	Maize	31	Brinjal
4	Muskmelon	32	Cabbage
5	Mustard	33	*Capsicum annum*
6	Okra	34	Cardamom
7	Onion	35	Carnation
8	*Oryza sativa(rice*)	36	*Carthamus tinctorius L*. (Safflower)
9	Papaya	37	Cassava
10	Pearl millet	38	Casuarine equisetifolia
11	Pigeon Pea	39	Cauliflower
12	Pomegranate	40	Chickpea
13	*Populus deltoides*	41	Chillies
14	Potato	42	Coffee
15	Ragi	43	Cotton
16	Red gram and Dolichos	44	Cowpea (*Vigna unguiculata*)
17	*Ricinus communis* L. (Castor)	45	*Dianthus caryophyllus*
18	Rubber (*Hevea brasiliensis*)	46	*Eleusine corocana*
19	Sorghum	47	*Eucalyptus tereticornis*
20	Soybean	48	*G. arboreum*
21	Sugarcane	49	Tomato
22	Tea	50	Watermelon
23	*Arachis hypogaea*	51	Wheat
24	Bamboo	52	*Withania somnifera*
25	Banana	53	*Zingiber officinale*
26	Basmati Rice	54	**_Arabidopsis thaliana(model_)**
27	Black gram	55	**Tobacco** (**model**)
28	Black Pepper (*Piper nigrum*)		

Table-2. Crop Biotechnolgy in India: Development of Transgenics

Crop	Disease/pest resistance	Abiotic stress tolerance	Other traits	Major Institutions
Rice	Insects, BLB, SB, Blast	Drought, salt, submergence, heat	vitaminA, antioxidant activity, hepatitis -B	UD, ICGEB, UOH, DRR, TNAU, BI, UASB,MKU, JKSeeds, Naath seeds OU,MSSRI IARI, CCMB, MAHYCO, METAHELIX and other companies
Brassica	insect, pathogen,	---	oil quality	DU, HAU, PAU
Pegion pea	insects, pathogens	---	------	ICRISAT,
Chick pea	insects	---	----	ICRISAT, NIPGR
Black gram	Virus	---	------	MKU
Cardamom	Virus	----	-------	MKU
Groundnut	insect	-----	------	ICRISAT
Sorghum	Phytophera	salt tolerance drought tolerance		NRCS, UASB

NOTES

LES SEMENCES : UN ENJEU INDUSTRIEL ET ECONOMIQUE

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Le mot «semence» à une double signification. Il désigne d'une part un objet, la graine, et d'autre part le génome de la variété qui, en interaction avec l'environnement, définit le comportement de la culture et la qualité des produits récoltés.

Dans sa première acception, les semences étant à l'origine de toute production agricole, elles représentent donc un enjeu économique certain. Sans semences il n'y a pas de production du tout. Ceci est particulièrement vrai pour les pays les moins développés où l'agriculture représente une part importante de la production nationale. Le projet initié par la FAO en décembre 2007 pour fournir des semences à de nombreux pays mets cet aspect en évidence.

Mais au-delà de cette importance basique l'enjeu industriel et économique des semences est considérable, non pas en termes de valeur absolue du marché mais en termes de recherche et de développement et de son impact sur l'agriculture mondiale productrice d'aliments, de fibres, et pour le moment d'agro-carburant de première génération.

En termes de valeur on peut évaluer le marché mondial des semences à environ 35 milliards de dollars US ce qui, de toute évidence, est très faible comparé à son importance stratégique. Cette valeur représente de l'ordre de 1 % de la valeur dégagée par les industries agroalimentaires. L'évolution des marchés nationaux illustre bien l’importance donnée à l'agriculture dans différentes régions du monde. Les échanges internationaux de semences ont également fortement augmenté au cours des 15 dernières années.

Mais l’importance stratégique des semences, c'est-à-dire ici le développement de variétés améliorées, se mesure surtout en regard des défis auxquels nous sommes et continuerons à être confrontés : augmentation de la population mondiale et, en corollaire, la diminution de la surface agricole utile par habitant, augmentation du taux d'urbanisation et accroissement de la demande alimentaire et non alimentaire. Les différents exposés des 15 et 16 septembre montreront l'importance de la recherche pour l'amélioration du potentiel de production, pour la résistance des plantes aux stress biotiques et abiotiques et pour leur composition à des fins alimentaires et non alimentaires. Encore faut-il que cette recherche soit financée et acceptée. On assiste aujourd'hui à une concentration des entreprises semencières, les cinq premières entreprises représentant 45 % du marché mondial contre un peu plus de 20 % il y a dix ans. De plus la recherche se localise essentiellement en Amérique du Nord et, à un moindre degré, en Chine et en Inde alors qu’elle stagne ou décroît en Europe. Il serait important que cet effort de recherche soit mieux réparti.

NOTES

WHAT SHOULD WE DO WITH AGROCHEMICALS: USE THEM OR BAN THEM?

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Agricultural Mission :

Traditionally, delivering edible food (harvest) to ensure :

- Food for the population
- Income for the farmer
- The country's self-sufficiency in food production

And recently, with a minimum impact on the environment.

Means to achieve this mission :

- Growing crops in a favorable ecosystem (soil, temperature, humidity, sunshine)
- Selection of crops that are well adapted to this ecosystem
- Good cultivation practice : suitable varieties, equipment, fertilization...
- Effective protection against pests :
  weeds, insects, nematodes, fungi, viruses...

Pesticides today

The fight against pests is a necessity which has emerged over the centuries with often very rudimentary means. To cope with disasters and to meet the needs of growing urban populations were an absolute necessity whatever the means !

The first tools were :

- For fungicides : mercury salts, sulfur, copper derivatives,
- For insecticides : coal oil, arsenic and lead salts or plant extracts, such as pyrethre or nicotine,
- Sulfuric acid to control broadleaves on cereals : 80 to 120 litres of concentred sulfuric acid diluted in 1000 liters of water were needed for one hectare.

The progress of organic chemistry have contributed to progressively replace mineral products, sometimes highly toxic by more friendly products. In 1946, the launching of phytohormones as active herbicides at 2-4 kgs per hectare was a true revolution !

Nevertheless, the intensive and repeated use of these chemicals has revealed a number of drawbacks mainly ecological such as longer life, excessive mobility in the soil, or side effects on wildlife. In addition some target pests or weeds developed a resistance. Such as sulfonyl ureas, discovered in the eighties that was efficient at the level of few grams per hectare.

The dangerousness of pesticides for human beings was known from the early days but progessively environmental concerns were taken in consideration.

Gradually legislation increasingly binding, not only on criteria of efficacy and low mammal toxicity, was set up to issue authorizations for the application in the fields.

In response, new plant protection molecules, with a minimum impact on the environment, with more effective formulations, have been gradually introduced.

Due to the efficiency and the selectivity of these new products applied doses were considerably reduced.

In addition the introduction of a toxin directly in the crop and seed treatment have allowed in some cases to remove the spray in the fields.

A strong political pressure

In 1987 the Brundtland report proposed the concept of “durable development” as a development corresponding to the present needs without jeopardizing the capacity for the future generations to satisfy their own needs.

Because of the image of pesticides in Europe and especially in France, the first agricultural country, there is an increasing political will to reduce pesticide use. The European law should tighten the conditions for approval of plant protection products. This regulation which must replace to the EC Directive 91/414, is still in the state of project.

The objective is to revise the procedures for evaluating the safety of active substances, to enhance protection of human health, animals and the environment.

Hitherto, pesticides were placed on the market for plant protection products, albeit with the characteristics of toxicity and a risk to the environment, but showing no danger in conditions of application and use.
The project aims to eliminate all products inherently dangerous even if present conditions of use are not risky.
This means, for example, that all neurotoxic insecticides are eliminated such as carbamates and pyrethroïds families.

France launched the Ecophyto 2018 plan which aims "to reduce the use of pesticides by 50% within 10 years if possible".
Reduction of tonnage has no meaning. Impact on the environment is not necessary related to the dose applied. The committee of experts who tried to try to achieve the objective of the Ecophyto plan recommended to generalize better agricultural practices and strengthen monitoring networks of pests. It proposed performance indicators that might be able to measure the decrease of pesticides pressure.

What is the future for pesticides ?

At this level, it is necessary to separate the analysis by domain (herbicide, insecticide and fungicide) and by crop.

For herbicides and for several crops: Ploughing, false seeding and rotation can reduce the herbicide application. Nevertheless one big advantage of the herbicide is to reduce the soil erosion which is an important problem in some countries

As far as insecticides are concerned : the use of auxiliary insects and pheromone trap have to be considered. The concept of GM crops with a toxin can for the medium-long term be more general. Of course the expression of this toxin must be insect specific or to be effective on a parasitic complex related to the crop. Ideally in the plant, the gene expression has to be temporary and tissue specific. However that involves an active research for new toxins and the continuation of studies on transgenic technology.

The fungicide control is the most difficult to solve. In general, a parasitic complex of several fungi have to be controlled. The creation by breeding of plant resistant to fungal attacks will very likely go back to primitive varieties with low nutritional value. Elimination of fungicides is not realistic.

Concerning viruses, there is no chemical likely to control it. In relation to the existing successes, the genetic solution must be advocated.
Its success would make the use of nematicide unnecessary. Indeed,the nematode damage is mainly due to the fact that they are carriers of virus.

Epilogue

For over 50 years, food productivity relied on the phytosantary tool. The use of chemicals is cheap, flexible and efficient. Today, if we want to reduce dramatically their use, we must review a number of basic elements of the current production system and move to another system, integrating alternative methods that many remain to develop.

According to the effort and success of this development, it will leave technical impasses that will affect the profitability and the level of production.

The consequence of the drastic reduction of the use of pesticides in Europe could be, as a first step, importation of food from areas with more favorable climates and less restrictive laws.

Eventually a decline in pesticides is unavoidable. In some cases they cannot be eliminated.
Their decline will depend both on the progress of research and people's ability to pay the price.

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GENE TRANSFER IN PLANTS:

FROM A BREAKTHROUGH IN FUNDAMENTAL RESEARCH TO AN ESSENTIAL TECHNOLOGY FOR AGRICULTURE, INDUSTRY AND THE ENVIRONMENT

Marc Van Montagu

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The discovery of the Ti plasmid of *Agrobacterium* (Zaenen *et al.,* (1974)[1]), and the demonstration that it was responsible for crown gall formation (Van Larebeke *et al.,*1975[2]) initiated the search for the molecular basis of this plant tumour induction. When it was demonstrated that this was a natural event of genetic engineering, whereby *Agrobacterium* was able to transfer, integrate and express a copy of the T-DNA, a segment of the Ti plasmid, it became imperative to attempt to use the *Agrobacterium* system for gene transfer in plants (Nester *et al.,* 2005[3], Wang, 2006 [4]). These discoveries opened the gate to a complete new era of plant sciences and plant engineering. Until that time, new plant varieties could only be obtained by using classical plant breeding methodologies. The new *Agrobacterium*-mediated transformation technology made it possible for the first time to improve crops with genetic information isolated from the genomes of micro-organisms, animals and other plant species. As a result of these early breakthroughs, fundamental research on how plants perceive, react and sometimes overcome abiotic stress (salt, drought, extreme temperatures, and toxic ion concentrations) is progressing well. Although our understanding of stress biology in resistant model plants like *Arabidopsis* is well advanced (http://www.arabidopsis.org/), we are still a long step away from constructing the improved crops needed. Efficient engineering by *Agrobacterium* of desired cultivars and particularly of orphan crops is still a bottle neck. Improvements in *Agrobacterium* strains to remove these barriers are essential. A much better study of soil micro-organisms and plant microbe interactions is also needed, not only for better plant health but for attempting soil reconstructions and fertilisation of arid regions.

Malthus predicted that the population would grow at a faster rate than the world food supply, his prediction, however did not prove true. Thanks to the Green Revolution, through the introduction of input intensive technologies, the production of food per capita on average rose by nearly 0.5% per year from 1961-1999 and this caused the real prices of agricultural commodities to fall by nearly 2% per year over the same period. As the population of the world continues to increase, it will be accompanied by an increase in the demand for food. Since the total acreage planted is no longer increasing, unless new production technology is adopted that supports a sustainable agriculture, with a double or triple yield increase on the same acreage of arable land, such an increase in demand that is not matched by an increase in the supply in the world food market, food security and food price crises are likely to continue, especially in the developing countries (Borlaug, 2002[5]).

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Soaring prices of finite petroleum reserves oblige the chemical industry to realise that biotechnology innovation is not simply a resource for the agrochemical industry but an enormous opportunity for using the best of scientific innovation to build a more sustainable, socially acceptable and environmentally friendlier industry. Petrochemical companies are now beginning to consider the intensive cultivation of some orphan crops (eg: cassava) and the domestication of many wild species (such as *Jatropha* and *Ricinus*) and weeds (such as *Miscanthus*). Progress in molecular genetics is bringing the knowledge and the new tools of Plant Biotechnology to enable this transition. However, the hesitation by governments to encourage this economic development has allowed some political and ideological pressure groups to misinform society on the real benefits of this novel agricultural production. At the same time, underinvestment in Plant Biotechnology has already brought substantial delays in knowledge acquisition and application and has blocked initiatives for prototype development by small and medium enterprises. It did not stimulate our schools of agriculture to embrace this technology. Today, it becomes a global imperative that plant breeders obtain access to these technologies and learn how to construct the high yielding plants industry and society are waiting for. Plant Biotechnology is not only gene engineering but also marker assisted breeding and most importantly, fundamental R&D on the molecular basis of plant growth and development. World agriculture has entered a new, unsustainable, and politically risky period. Agriculture - and the natural resources it depends on - has been overexploited ecologically, has suffered from underinvestment, has recently been exposed to ill-designed bioenergy programs, and has been politically sidelined for too long. It is now at a critical point. Appropriate responses to the food and agriculture price and productivity crises are lacking. A *global initiative for accelerated agriculture productivity is necessary now; such an initiative makes economic sense, is pro-poor and sustainable, and serves security.* The initiative needs political leadership and coordination. There is no effective governance architecture at the global level and national levels to address the matter. Industrialized economies should substantially accelerate their investment in international agricultural research and development (R&D) in cooperation with new players (Von Braun, 2008[6]). To do this, public sector scientists must play their part and must give priority to establishing the appropriate networks between the leading Institutes of the Industrialised World and the best of plant research teams in the Developing Countries, particularly in Africa (Juma and Serageldin, 2008[7]).

Despite all the exciting promise we are witnessing in fundamental research, the best of plant sciences cannot help if we cannot obtain the confidence of society for these technologies. Public sector scientists must explain that the solution of our environmental problems and most of all the building of a sustainable agriculture is only possible by applying plant biotechnology and not by looking backwards to a world with less then 2 Billion people. We have to solve the overregulation which presently stops all introductions of transgenic plants by SME's (Small and Medium Enterprises) (De Greef, 2004[8]). No knowledge based bio-economy can develop without these intermediates and an enabling policy infrastructure. Fortunately, organisations such as the Public Research and Regulation Initiative (PRRI, www.pubresreg.org) offer a forum for the public research sector to be informed about and involved in international agreements relevant to modern biotechnology.

Because fundamental research will continue to drive new innovation for the production of better food, fuels and fibres, such initiatives must be properly financed, appropriate policy infrastructures must be formulated, and public sector scientists must play their part in informing industry, policy makers and society of the real benefits that plant biotechnology offers.

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NEW STRATEGIES OF CROP PROTECTION AGAINST INSECTS

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Insect pests cause great loses to agriculture. Cotton bollworm (*Helicoverpa armigera*) is a generalist herbivorous insect though it mainly attacks cotton plants. Transgenic crops expressing *Bacillus thuringiensis* (*Bt*) toxins have been successful in protecting plants, however, *Bt* proteins are toxic to certain groups of insects (such as lepidopterans) but have little effects on sucking pests. Furthermore, the continuous use of *Bt* crops increases the danger of developing insect *Bt* resistance. Recently, we developed a plant-based RNA interference (RNAi) technology, which shows the potential as a new strategy of insect pest control.

Most cultivars of cotton (*Gossypium* spp.) accumulate gossypol and related sesquiterpene aldehydes in pigmented glands of aerial organs and in epidermal and subepidermal tissues of root. We are interested in gossypol biosynthesis and the role of plant secondary metabolites in plant-insect interactions. In insects, cytochrome P450 monooxygenases are commonly involved in xenobiotic metabolism. By analyzing gene expressions in cotton bollworm midgut after gossypol treatment, we isolated a gossypol-inducible P450 monooxygenase gene, *CYP6AE14*, from *H. armigera*. *CYP6AE14* is mainly expressed in midgut and its up-regulation was required for larval growth when gossypol was supplemented in diet. In order to knockdown *CYP6AE14* expression, we generated transgenic *Arabidopsis* and tobacco plants expressing a hairpin RNA (dsRNA) construct that targets *CYP6AE14* (*dsCYP6AE14*). When the bollworm larvae were fed with *AtdsCYP6AE14-3* leaves, small RNAs of *CYP6AE14* were detected from the midgut two days later, and the *CYP6AE14* transcript abundance in midgut was substantially decreased. As a result of *CYP6AE14* suppression, the cotton bollworm fed on transgenic *dsCYP6AE14* plants showed stunted growth in comparison with the control, and the inhibitive effect of dsRNA-expressing plants became more evident when gossypol was administrated. To determine if other genes of the insect were suppressible by plants that express the respective dsRNA, we tested *H. armigera GST1* gene (EF033109), coding for glutathione-S-transferase. We found that the transgenic plants were also effective in suppressing *H. armigera GST1* expression.

We have introduced *dsCYP6AE14* into cotton plants. In a small scale laboratory test the transgenic cotton plants indeed showed enhanced resistance to *H. armigera*, and the growth of bollworm larvae were drastically reduced when placed on transgenic plants. Selection of more target genes, increase of dsRNA production in an effective form, and testing other insect species are important factors for further development of this new strategy for protecting crops against herbivorous insects.

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CROP PROTECTION AGAINST VIRUSES BY GMO-BASED TECHNOLOGIES

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The approach of pathogen-derived resistance has been used to develop virus-resistant plants for 22 years, with much technical success with a large range of plant viruses and crops. For various reasons, however, only virus-resistant transgenic squash and papaya are actively grown commercially in the US, and none are commercially grown outside of the US. In both crops, the coat protein gene of the target viruses was inserted into the crop to provide resistance to watermelon mosaic Virus II and zucchini yellow mosaic virus in squash and papaya ringspot virus (PRSV) in papaya. This talk focuses on our experience with the development, testing, deregulation, and commercialization of the transgenic papaya in Hawaii and our efforts at technology transfer to other countries. In May 1992, PRSV invaded the Puna area of Hawaii where 98% of the papaya were being grown. Fortunately, we had started research to develop transgenic papaya for PRSV resistance in 1985. By 1992, we had identified a line of resistant transgenic papaya and initiated a field test with R0 clones. In the ensuing six years, PRSV spread rapidly throughout the Puna area. By 1998, papaya production in Hawaii had been reduced by 50% as compared to 1992 levels. In the mean time, the scientific team which consisted of five researchers from University Hawaii, USDA, Cornell University, and the then Upjohn Company worked to field test the transgenic papaya, develop the transgenic line into commercial cultivars, deregulate the transgenic papaya, and finally commercialize the transgenic papaya. All activities were done with very limited research funds. The transgenic 'SunUp' and 'Rainbow' PRSV-resistant cultivars were released for commercial planting in May 1998 and the reclamation of the devastated areas was successful. The resistance of the transgenic papaya is holding up and transgenic papaya now accounts for about 80% of papaya production in Hawaii. Our lab made similar efforts to develop transgenic papaya in cooperation with several countries, including Jamaica, Brazil, Venezuela, and Thailand. Transgenic papaya suitable for the collaborating countries were developed and the transgenic lines have advanced to various levels. In particular, transgenic lines have been extensively tested under confined field conditions in Thailand with excellent results. However, so far efforts by the Thailand Department of Agriculture at deregulation of the papaya in Thailand have not been successful. We are also applying for the deregulation of the Hawaiian transgenic papaya in Japan, which is a significant importer of papaya from Hawaii. Various factors that affect the development and practical deployment of transgenic papaya will be discussed.

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IMPROVEMENT OF CROP TOLERANCE TO ABIOTIC STRESS

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Abiotic stress is a major constraint for plant productivity in both natural and agricultural ecosystems. Drought and poor soil fertility are among the major abiotic factors that reduce plant productivity. Plant varieties with enhanced efficiency for water and nutrient uptake and utilization are urgently needed to cope with the increasing demand for food and feed worldwide, and biomass accumulation for the production of biofuels and other plant-based compounds of industrial processes. In recent years major advances have been made in the understanding of the molecular mechanisms that regulate drought and aluminium tolerance, and nutrient uptake. Global gene expression analysis and functional genomics have allowed the identification of genes that play major roles in these processes. In this paper I will discuss some of the major advances in the understanding of the molecular mechanisms that regulate drought tolerance, phosphate uptake and utilization efficiency and provide some examples as to how this information has been used for crop improvement.

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TOWARDS A SUSTAINABLE, LAND-BASED SOURCE OF NUTRITIONAL FATTY ACIDS FOR THE HUMAN DIET

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Multiple studies have shown that inclusion of long chain omega-3 polyunsaturated fatty acids (LCPUFAs), especially EPA (eicosapentaenoic acid, 20:5) and DHA (docosahexaenoic acid, 22:6), in the human diet can have numerous health benefits including positive effects on cardiovascular and mental health. Fish oils are a rich source of EPA and DHA but there is growing demand for and declining wild fish stocks. In addition, fish oils have objectionable flavors and contaminants that are difficult and cost-prohibitive to remove which limit the applications for fish oil in most foods. Oilseed plants engineered to produce EPA and DHA offer a safe, sustainable and cost-effective alternative to fish oils as a source of LCPUFAs for food ingredients. Towards this end, we have placed novel EPA and DHA biosynthetic pathways into soybeans and other plants, and we have obtained significant amounts of these omega-3 LCPUFAs in the seed oil. The approaches used and recent advances made will be discussed.

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BILAN DES TRAVAUX DE LA COMMISSION DU GENIE BIOMOLECULAIRE (CGB)

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Quatre grands types de dossiers ont fait l'objet de travaux par la CGB depuis sa création en 1987 :

1) L'expérimentation de plantes génétiquement modifiées

La CGB est saisie sur chaque demande d'expérimentation de plantes génétiquement modifiées par le Ministère de l'Agriculture.
Sur ces dossiers, il est demandé à la CGB d'examiner, à un niveau national, les risques pour la santé et l'environnement. En revanche, elle ne se prononce pas sur d'autres aspects, sociaux ou économiques par exemple.

En vingt ans, la CGB a examiné plus de 700 dossiers .Les premiers l'ont été en 1987, dès le début du fonctionnement de la Commission, et ceci jusqu'en 2007.

Les caractères le plus souvent rencontrés sont la tolérance à des herbicides, la résistance à des insectes. D'autres dossiers concernaient une amélioration de la composition en amidon chez la pomme de terre ou de la teneur en sucre chez la betterave, ou l'amélioration des qualités papetières chez le peuplier. D'autres dossiers concernaient la production de molécules pharmaceutiques, comme la lipase gastrique.

2) Mise sur le marché de plantes génétiquement modifiées

Ces dossiers se situent cette fois-ci dans le cadre de procédures communautaires dans lesquelles les Etats membres sont impliqués pour l'évaluation des risques.
Il s'agit d'organismes rendus tolérants aux herbicides ou résistant aux insectes, des systèmes de stérilité mâle (colza, chicorée), de modifications de la couleur (oeillets, chrysanthèmes), de modifications de la composition en amidon (pomme de terre), de la tenue en vase prolongée (oeillets), de la résistance à un virus (melon), de la maturation retardée (tomate).

3) Thérapie génique et vaccins humains

Les essais de thérapie génique et les utilisations de vaccins humains recombinants sont assimilés à des disséminations volontaires dans l'environnement.

Les dossiers examinés concernent certains cancers, un déficit immunitaire, la mucoviscidose, la myopathie de Duchenne, l'artériopathie, l'adénoleucodystrophie.
D'autres dossiers concernaient des vaccins, contre le SIDA, l'hépatite B ou encore shigella. La CGB accorde beaucoup d'importance au produit de thérapie génique lui-même et au vaccin, mais aussi à sa cinétique d'élimination via les liquides biologiques.

4) Vaccins vétérinaires

Les maladies visées ont été, notamment, la maladie d'Aujesky (porc), la rage (renard, chat), la leucose féline (chat), la maladie de Gumboro (poulet), le tétanos et l'influenza équine, ou la grippe aviaire.

Travaux scientifiques de la Commission du Génie Biomoléculaire

La CGB a conduit d'importants travaux de réflexion scientifique, autour de thèmes liés à l'évaluation du risque comme : pratiques agricoles et OGM ; impact sur l'environnement ; évaluation des risques toxicologiques ; la plante et son génome ; OGM et société. Ils ont fait l'objet de publication en ligne (www.ogm.gouv.fr)

Conclusions

Au total, ce sont plus de 900 évaluations qui ont été effectuées par la CGB.
Cette dernière a également contribué à l'orientation des recherches sur les impacts des OGM et a conduit, en parallèle, un important travail scientifique en lien avec l'évaluation des risques. C'est l'ensemble de ces éléments qui lui a permis de construire progressivement une expertise collective et pluridisciplinaire sur les OGM.

EVALUATION TOXICOLOGIQUE ET NUTRITIONNELLE DES OGM
TOXICOLOGICAL AND NUTRITIONAL EVALUATION OF GMOS

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L'Union européenne, la Food and Agricultural Organisation (FAO), l'Organisation Mondiale de la Santé OMS), le Codex alimentarius et l'Organisation de Coopération et de Développement Economique (OCDE) se sont mis globalement d'accord, après plus de 10 ans de réunions et de discussions scientifiques, sur une démarche d'évaluation de risques capable d'assurer la sécurité sanitaire des aliments issus des plantes génétiquement modifiées (PGM).

Cette évaluation de risques de ces aliments destinés à l'alimentation humaine ou animale, consiste en une approche comparative au cours de laquelle on compare l'aliment issu de la PGM à un comparateur issu d'une plante aussi isogénique que possible à cette PGM, dont la consommation est considérée comme sûre. Les éléments clés de cette procédure comparative d'évaluation sont : l'analyse moléculaire de la construction génétique, celle de la composition de la plante, ses caractéristiques phénotypiques et agronomiques. Il s'agit d'identifier les différences entre ces deux sources, différences qui si elles sont mises en évidence, seront évaluées quant à leur conséquences sur la qualité sanitaire et nutritionnelles de l'aliment issu de la PGM ; c'est le concept d'équivalence en substance.

L'objectif de cette comparaison est d'assurer le même niveau de sécurité que celui accepté pour les aliments courants, il ne s'agit pas d'une évaluation toxicologique classique. En effet une telle évaluation est extrêmement difficile, voire impossible dans le cas d'un aliment, ce qui explique que nos aliments de tous les jours n'ont jamais été évalués au plan de leur sécurité sanitaire et qui justifie l'approche comparative retenue dans le cas des PGM.

Cette approche comporte plusieurs étapes qui ont pour objet d'évaluer deux catégories de dangers, ceux liés aux propriétés intrinsèques et aux fonctions des traits introduits intentionnellement et ceux résultant de l'insertion des nouveaux gènes introduits dans le génome de la plante et qui peuvent causer des effets inattendus. Les dangers de la première catégorie peuvent être évalués au cas pas cas, en utilisant des études classiques de toxicologie, *in silico*, *in vitro* et *in vivo* pratiquées sur les protéines nouvellement exprimées, les nouveaux métabolites formés ou les substances toxiques naturellement présentes dont les teneurs peuvent avoir été augmentées par la transformation.

Les dangers de la seconde catégorie sont essentiellement évalués par les comparaisons des paramètres clés entre PGM et comparateur et dans le cas d'un besoin démontré, par des études de toxicité subaigües pratiquées chez le rongeur de laboratoire avec la plante entière ou l'aliment qui en est issu.

Enfin, des études d'alimentarité, pratiquées sur des espèces d'intérêt zootechnique, apportent des compléments en matière de qualité nutritionnelle et d'acceptabilité.

Toxicological and Nutritional Evaluation of GMOs

The EU, FAO, WHO, Codex alimentarius and OECD have established a broadly harmonised safety assessment framework to ensure the safety of whole food and feed derived from GM plants (GMP).
The risk assessment of GMP and derived food and feed follow a comparative approach, i.e. the derived food and feed are compared with their near isogenic counterparts which, because of their history of use are generally regarded as safe to eat. The objective of this comparison is to identify differences which subsequently are assessed with respect to their potential impact on the safety for humans and animals and nutritional quality: this is the concept of Substantial Equivalence. Key elements of the comparative assessment procedure are molecular, compositional, phenotypic and

agronomic analysis in order to identify similarities and differences between the GMP and its near isogenic counterpart which need further evaluation.
The goal of the assessment is to provide the same level of safety as accepted for traditional foods; it is not a classical toxicological evaluation. Such a classical toxicological evaluation is extremely difficult even impossible in the case of a whole food; it is the reason why traditional foods have never been assessed from the safety point of view. This is a justification of the comparative approach.

This approach considers two main categories of hazards, i.e. those related to the intended intrinsic properties and function of the introduced traits and those resulting from insertion of the introduced genes into the plant genome that might cause unintended effects.

The first category of hazards related to the intended properties is taken into account by detailed evaluation and if appropriate, by in silico, in vitro and in vivo safety studies of newly proteins, newly formed metabolites, and of natural toxicants whose level may have been increased as result of gene insertion. This assessment should be done using on a case-by-case basis standardised toxicological methodology designed for assessment of simple chemicals defined substances.

The second category of potential risks may be covered, where appropriate by employing rodent feeding studies on whole GM food and feed.

At the same time it is essential to test the nutritional value for human and animal consumers on target animal species.

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LES OGM POSENT-ILS DES PROBLEMES D'ALLERGENICITE

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Les effets néfastes des OGM sur la santé sont l'objet d'un débat récurrent où s'intriquent des faits scientifiques méritant une analyse objective et des éléments moins rationnels relevant de convictions fondées sur la crainte de l'imprévisible.

Avant d'examiner les risques potentiels des OGM pour la santé, il convient de rappeler que la production d'OGM offre des perspectives majeures utiles à la santé des populations telles que :

- La possibilité espérée de cultiver des plantes résistantes à la sécheresse ou à des conditions salines défavorables dans des régions où le déficit hydrique ou la salinité exposent les populations au risque de dénutrition grave.
- La possibilité de réduire l'utilisation de produits chimiques tels que les pesticides et les herbicides dont l'action délétère pour la santé est plausible sinon probable dans certains cas.
- L'amélioration des qualités nutritives de certains aliments. L'exemple du riz doré illustre bien cet intérêt potentiel majeur des OGM. Le riz décortiqué consommé dans de nombreux pays d'Asie est dépourvu de la provitamine A nécessaire pour éviter une carence nutritionnelle grave affectant de façon sérieuse et irréversible la vision des populations concernées. Le transfert d'un ensemble de gènes permettant la production de cette provitamine dans l'albumen du grain peut permettre de régler ce problème.
- L'utilisation à venir de vaccins et de médicaments produits par des OGM, à faible coût, utilisable dans un premier temps pour les animaux de ferme.

Il convient aussi de rappeler qu'à ce jour, les études épidémiologiques sur une très large échelle, concernant plusieurs centaines de millions d'individus, n'indiquent pas la survenue d'évènements cliniques défavorables liés à l'utilisation des OGM. Certes l'absence de preuves n'est pas la preuve de l'absence d'effets douteux mais l'argument est fort et globalement très rassurant.

En fait, la discussion peut se focaliser sur les points suivants :

En premier lieu et surtout, le risque de réactions allergiques développées contre des protéines codées par le transgène.

En second lieu, le risque d'introduction de résistance aux antibiotiques qui avait été évoqué lorsque des gènes conférant la résistance à certains antibiotique avait été utilisé comme gènes reporteurs pour sélectionner les cellules transformées. Depuis on n'utilise plus ce type de gène reporteur.

Enfin, l'imprévisible, par définition possible qu'il convient de tenter de prévenir par des études toxicologiques réalisées in vivo chez l'animal avec les limites d'interprétation liées aux barrières d'espèces et aux difficultés de prédire des évènements rares.

Nous illustrerons cette discussion par le risque allergique

Un OGM peut exprimer une protéine absente de l'alimentation conventionnelle qui pourrait se révéler allergénique. Les réactions allergiques d'origine alimentaire peuvent en théorie être à l'origine de manifestations cliniques sévères à l'instar des accidents observés après l'ingestion d'un nombre heureusement très limité d'aliments telle que la cacahuète.

C'est ainsi que s'était posé le problème de l'allergénicité pour des plantes enrichies en acides aminés soufrés car exprimant une protéine issue de la noix du Brésil qui s'est révélée allergénique ce qui a été heureusement détectée avant la commercialisation de cette plante.

En fait, ce risque d'allergie sévère est minime même s'il ne peut pas être absolument exclu.

En premier lieu les protéines allergéniques potentielles sont identifiées dans la mesure où elles sont le produit direct du transgène et il est donc possible d'analyser leur pouvoir allergénique chez l'animal, même s'il y a de nouveau une certaine limite d'interprétation à travers les espèces ainsi analysées, mais aussi par tests sérologiques ou cutanés chez l'homme permettant de détecter la production d'IgE spécifique de la protéine codée par le transgène.

En outre le risque d'allergie n'est pas supérieur à celui rencontré par des individus se déplaçant dans des pays où sont cultivées des plantes en dehors de leur pays d'origine.
Le risque n'est pas non plus supérieur à celui auquel exposent les plantes issues d'hybridation selon les méthodes conventionnelles de la génétique. Tout au plus, pourrait-on imaginer que l'introduction d'un OGM pourrait sensibiliser les adultes non exposés dans l'enfance aux néoallergènes. Une publication récente indique que le passage d'allergène dans le lait maternel peut rendre tolérant les enfants à cet allergène mais en tout état de cause, un grand pourcentage d'enfants n'est pas nourri au lait maternel et le caractère général et la preuve de la protection conférés par l'allaitement n'ont pas été apportés.

Enfin et surtout, le risque allergique n'est pas fondamentalement différent pour les allergènes conventionnels que pour des néoantigènes produits par l'homme, qu'ils soient obtenus par synthèse chimique ou par génie génétique. Il faut, en effet, rappeler que le répertoire immunologique qui permet aux être humains de reconnaître une variété considérable d'antigènes est inné et ne présuppose pas en conséquence d'expositions préalables aux antigènes, même si celles-ci peuvent induire une mémoire immunologique. La capacité en l'occurrence défavorable de développer une réponse immunitaire n'est pas plus à craindre pour des néoantigènes que pour des antigènes de l'environnement habituel, elle dépend essentiellement de la configuration tertiaire des antigènes (sans qu'on connaisse les structures les plus potentiellement dangereuses) et la prédisposition génétique à produire une réponse forte contre ces antigènes.

Il faut aussi rappeler que le risque après ingestion de viande provenant d'animaux nourris aux OGM n'expose à aucun risque d'allergénicité dans la mesure où les protéines support de l'antigénécité sont détruites dans le tube digestif de ces animaux.

En conclusion, l'ensemble de ces considérations est tout à fait rassurant, il convient néanmoins, au cas par cas, pour chaque OGM proposé à la consommation de faire tous les tests de toxicologie et d'allergénicité chez l'animal évoqué plus haut et de vérifier sur un échantillon de la population ayant consommé l'OGM l'absence de réaction d'hypersensibilité.

NOTES

RELEASE OF GMOS IN THE ENVIRONMENT AND CO-EXISTENCE

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In recent years, the likelihood of the progressive implementation of genetically modified (GM) plants has given rise to a situation of coexistence that has been subjected to Regulation in Europe since 2003 (Regulation EC 1830/2003). In this document, coexistence is understood as a principle according to which farmers should be able to freely cultivate the crops of their choice, whether genetically modified (GM), conventional or organic. Moreover, Regulation (EC) 1829/2003 on GM food and feed has established an adventitious presence threshold of 0.9% for positive labelling products containing or derived from GM crops. All European countries need to develop national strategies to ensure coexistence (Commission Recommendation, 2003).

Coexistence can be affected by the adventitious presence of one crop with another, which can arise for a variety of reasons. These include seed impurities, sowing equipment and practices, cross pollination between GM and non-GM crops, the presence of volunteers, and product mixture occurring during harvesting, transport and/or storage processes. Of these, cross-pollination is the factor that causes most concern to growers because it is difficult to control and depends on many factors including the type of cultivar and climatic conditions.

Two examples will be shown: maize and rice. Commercial maize cultivars are anemophyllus and so, cross pollination takes place in some extent. Field trials aimed at quantifying the gene flow from GM to non-GM maize clearly show that adventitious presence of GM kernels accumulates in the border and decrease throughout the centre of the field. In general, a separation distance of 20 m is enough to maintain the %GMO below the 0.9% threshold in the yield of the field. In some cases such as small or narrow fields, the separation distance may need to be extended to 50 m. These distances could be decreased if a buffer zone is established or if sowing dates are planed to avoid the flowering coincidence among fields. Recent studies performed in real conditions of coexistence, where GM and non-GM maize fields are sown with different cultivars, with different sowing dates and mixed with other crops confirm the described results. Taking into account that there are not wild and weed maize relatives in Europe, persistence of GM maize in landscape is not probable, and coexistence could be achieved by establishing simple rules that guarantee seed purity, the correct management of sowing equipment and practices and a reasonable separation distance or buffer zone.

European Coexistence regulations for rice will be easier to establish because commercial cultivars are autogamous, being the rate of cross pollination extremely low: i.e. about 0.53% at 1m distance from the pollen source in the prevalent wind direction, decreasing to 0.01% at 5 m distance. So the whole content of adventitious presence of GM rice due to cross pollination in the yield of the field is expected to be lower than the 0.9% threshold. But, in case of rice, there is a weed relative, the red rice, with which cross pollination takes place at rates significantly higher than those encountered within cultivars. So, coexistence regulations should include strategies to control this weed and take into account the environmental impact that transgenic characters may have in increasing the fitness or persistence of this weed.

In conclusion, particular characteristics of each crop have to be taken into account and so, coexistence regulations have to be established in a case by case base.

NOTES

ENVIRONMENTAL IMPACT OF GMOS RESISTANT TO HERBICIDES OR TO INSECTS

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1. The case of herbicide tolerant crops, Application of Conservation Tillage easier with herbicide tolerant crops

The value of reducing tillage was long recognized but the level of weed control a farmer required was viewed as a deterrent for adopting conservation tillage. Once effective herbicides were introduced in the latter half of the 20th century, farmers were able to reduce their dependence on tillage. The development of crop varieties tolerant to herbicides has provided new tools and practices for controlling weeds and has accelerated the adoption of conservation tillage practices and accelerated the adoption of "no-till" practices In the US, most growers are making fewer tillage passes and more than 75% are leaving more crop residue. Today, the scientific literature on "no-tillage" and "conservation tillage" has grown on more than 6500 references, a selection of some 1200 references from the last three years are given in the following link: http://www.botanischergarten.ch/Tillage/Bibliography-No-conservation-Tillage-2006-20080626.pdf.

Several important reviews have been published in recent months, the all tell a positive story regarding the overall impact of herbicide tolerant crops and the impact on the agricultural environment.

2.The Case of Impact of Bt maize on non-target organisms

More than 180 scientific studies are dealing with non-target organism which could be harmed by the cultivation of Bt maize. Observing strictly the baseline comparison with non-Bt maize cultivation, it can be said that there is not a single publication pointing to detrimental effects of Bt maize compared to other maize traits. Four meta studies have been published recently with more or less stringent selection of data published in scientific journals, and all those meta analysis do not show any sign of regulatory problems. Great care was taken to eliminate redundant taxonomic units and multiple development stages of the same species, with a preference of the least mobile development stage, also the datasets are all derived from the same season.

In one particular study (Wolfenbarger) the statistical analysis was not done with the usual original taxonomic units, rather the authors decided to use an additional descriptor, six '*functional guilds*': herbivore, omnivore, predator, parasitoid, detritivores, or mixed. as a wole, database robustnes and sensitivity of the datasets have been thoroughly discussed.

In maize, analyses revealed a large reduction of parasitoids in Bt fields. This effect stemmed from the lepidopteran-specific maize hybrids, and examining the 116 observations showed that most were conducted on Macrocentrus grandii, a specialist parasitoid of the Bt-target, Ostrinia nubilalis. There was no significant effect on other parasitoids, Higher numbers of the generalist predator, Coleomegilla maculata, were associated with Bt maize but numbers of other common predatory genera (Orius, Geocoris, Hippodamia, Chrysoperla, were similar in Bt and non-Bt maize.As a whole, the studies did not reveal any negative effects, confirming for a large amount of data and publications the environmental benefits of the Bt maize tested.

NOTES

GLOBAL IMPACT OF BIOTECH CROPS:
SOCIO-ECONOMIC AND ENVIRONMENTAL EFFECTS *1996-2006*

Graham Brookes

PG Economics UK, effects 1996-2006[1]

This paper provides an overview of the global economic and environmental impacts of biotech crops since their widespread commercial introduction in 1996. The material presented is published in the peer reviewed scientific journal AgBioforum. The paper focuses on the economic impact at the farm level and explores the environmental impact on pesticide usage and greenhouse gas emissions.

Key findings of the comprehensive study include:

- Biotech crops have contributed to significantly reducing the release of greenhouse gas emissions from agricultural practices. This results from less fuel use and additional soil carbon storage from reduced tillage with biotech crops. In 2006, this was equivalent to removing 14.8 billion kg of carbon dioxide from the atmosphere or equal to removing nearly 6.6 million cars from the road for one year;

- Biotech crops have reduced pesticide spraying by 286 million kg (-7.8%: equivalent to about 40% of the annual volume of pesticide active ingredient applied to arable crops in the European Union) and as a result decreased the environmental impact associated with herbicide and insecticide use on the area planted to biotech crops by 15.4% ;

- There have been substantial net economic benefits at the farm level amounting to nearly $7 billion in 2006 and $33.8 billion for the eleven year period. The farm income gains in 2006 is equivalent to adding 3.8% to the value of global production of the four main biotech crops of soybeans, corn, canola and cotton ;

- Of the total farm income benefit, 43% ($14.54 billion) has been due to yield gains, with the balance arising from reductions in the cost of production. Two thirds of the yield gain derive from adoption of insect resistant crops and the balance from herbicide tolerant crops;

- Farmers in developing countries obtained the largest share of the farm income gains in 2006 (54%) and over the eleven year period obtained 49% of the total ($33.8 billion) gains;

- The cost farmers paid for accessing GM technology in 2006 was equal to 28% of the total technology gains (a total of $9.6 billion inclusive of farm income gains ($6.91 billion) plus cost of the technology payable to the seed supply chain ($2.7 billion[1]);

- For farmers in developing countries the total cost of accessing the technology in 2006 was equal to about 17% of total technology gains, whilst for farmers in developed countries the cost was 38% of the total technology gains. Whilst circumstances vary between countries, the higher share of total technology gains accounted for by farm income gains in developing countries relative to the farm income share in developed countries reflects factors such as weaker provision and enforcement of intellectual property rights in developing countries.

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[1] fll report available to download at www.pgeconomics.co.uk
A shorter version will shortly be available in the scientific journal AgBioforum (currently in press). www.agbioforum.org