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COMMISSION ON GENETIC RESOURCES FOR FOOD AND AGRICULTURE

RECENT DEVELOPMENTS RELATED TO BIOTECHNOLOGY THAT ARE RELEVANT TO THE ANALYSIS OF THE SURVEY ON THE CODE OF CONDUCT

by

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The Commission has regularly received reports on new developments in biotechnology, in relation to genetic resources for food and agriculture.

This report provides some perspectives on a number of recent advances and debates in biotechnology in relation to the further development of the draft Code of Conduct on Biotechnology.

The study is the responsibility of the author, and does not necessarily represent the views of the FAO or its Member States.

For reasons of economy, the paper is available in the language in which it was prepared.

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I. INTRODUCTION

The purpose of this annex is to report on some recent advances in the field of biotechnology that may be of interest to the Commission. It is not intended to be an comprehensive review of emerging technologies, rather it is meant to provide some perspectives on a small selection of recent advances and relate them to the draft a Code of Conduct on Biotechnology. The report examines three several developments, which:

- Introduce the achievements of, and analyzing the impacts of the completion of outstanding work on the International Genome Sequencing Projects, whose outputs include, sequencing of *Arabidopsis thaliana,* and *Homo sapiens.* These efforts are universally recognized as major breakthroughs that are revealing the intimate structure of heredity, with spin-offs benefits for the conservation and sustainable use of the range of genetic resources for food and agriculture;
- Update recent results in the field of plant apomixis introducing some associated issues;
- Provide a review of the scientific developments in the field of plant plastid transformation as an alternative method genetically engineering crops; and
- Summarize the debate related to the issue of gene flow from GM crops to conventional crops and their wild relatives ("*genetic contamination*") and associated considerations based on two recent workshops.

II. GENOME PROJECTS

As a result of recent advances in engineering technology,[1] work on genome sequencing projects have accelerated beyond expectations over the last three years. Progress was propelled by two separate efforts, with some communication between them. One project was undertaken through a large international public consortia, while the other project was a private sector initiative. The private sector project hoped to produce commercial benefits resulting from the gains in understanding in the field of genomics, especially commercial products to assist the treatment of certain human diseases.

The International Arabidopsis Genome Initiative, a mostly publicly funded[2], was started in 1996 and completed and published[3] in four years. *Arabidopsis thaliana* is the first flowering plant to be fully sequenced. It is a particularly useful plant for geneticists and molecular biologists due to its relatively small genome[4] and short life cycle. The sequenced regions cover 115.4 megabases of the 125-megabase genome, distributed in the five chromosomes extending to the centromeric regions. The Arabidopsis genome contains 25,498 genes encoding a proteome[5], including proteins from 11,000 families, a "tool kit" similar to those encountered in the other two multi-cellular eukaryotic organisms previously sequenced. Also, sequenced was *Drosophila melanogaster* (a fly with 13,601 genes), and *Caenorhabditis elegans* (a nematode worm with 19,099 genes). The amount of coding sequences (exons) represents roughly less than one third of the entire genome, while the inter-genic sequences (introns) account for another 15%. The function of the remaining 56% of the "DNA" is unknown, but is certainly involved in genome physical structure and dynamics plasticity, as well as in gene regulation and overall functioning of the extremely complex mechanisms of life. A putative function has been ascribed to only 17,800 genes (69 %) through comparative genomics. That is, according to sequence similarity to proteins with known functions in all organisms, which can be explained by the highly conservative nature of most basic cell activities, signaling, metabolism, transcription, defense,

[1] capillary electrophoresis for large scale DNA sequencing, robotic technology for chip manufacture , high-throughput mass spectroscopy for structural analysis, and so on

[2] The work was mainly supported by the National Science Foundation Cooperative Agreements, US Departments of Agriculture and Energy, the Kazusa DNA Research Institute Foundation and the European Commission.

[3] The Arabidopsis Genome Initiative: Analysis of the Genome sequence of the flowering plant A. thaliana. Nature *408*, 796 (14 Dec. 2000)

[4] 1.2×10^8, that is two orders of magnitude smaller that the genome of wheat: 1.6×10^{10}

[5] The whole set of proteins encoded by the genome

growth, transport, peptide synthesis etc. However, the function of more than 7,000 genes remains to be determined.

Completion of genome sequencing and gene annotation[6] has involved very precise characterization of the coding regions and permits powerful comprehensive comparisons of conserved processes in all eukaryotes. It facilitate the "identification of a wide range of plant-specific gene functions, and will enable the establishment of rapid and systematic ways to identify genes for crop improvement."[7] Furthermore, comparative genomics offers opportunities to test working hypotheses on the significance of certain phenomena, such as duplication, polyploidization, transposon mutations and introgression of foreign genomes in plant genome evolution. Finally, genome sequencing will provide an important background for understanding the molecular basis of intra-specific diversity[8], which is of utmost importance for crop improvement.

Another sequencing effort has targeted the world's single most important staple crop - rice. The International Rice Genome Sequencing Programme (IRGSP),[9] has been jointed coordinated by the Japanese Ministry of Agriculture and Fisheries[10] and the Monsanto Rice Genome Project group.[11] Other large Multi-national Corporations like DuPont and Novartis are also involved in research programmes aimed at gene isolation based on Expressed Sequence Tags (ESTs).[12] More recently, a newly created Institute of Genomics at Beijing, China, has also published a detailed sketch or rice genome[13]. Research has shown that the rice genome is the smallest amongst the cereals, although it is four times larger than that of *A. thaliana*. Due to the phenomenon of synteny,[14] the full sequence of rice will be useful for gene tagging in related crops: wheat, corn, barley, etc[15]. In fact, a number of *Brassica* genes have already been identified via *A. thaliana*[16].

The Human Genome Project is a very important scientific initiative and is resulting in improved understanding of the human genome. It is also raising ethical questions. The publicly funded genome project (PFP), named International Human Genome Sequencing Consortium,[17] started work in 1990. It is perhaps the largest coordinated molecular genetics project in history involving hundreds of scientists working in at least 25 laboratories worldwide. In 1998, a single private company Celera Genomic, which is based in the United States of America, started a distinctive centralised sequencing effort that challenged the PFP project by utilizing a different approach and using the sequence data made available in the public domain.

Results of the two projects were published in February 2001 in Nature (PFP),[18] and in Science (Celera)[19]. Both projects yielded several impressive and surprising results, especially in relation to the small amount of DNA that is encoding for proteins (gene exons). The results indicates that gene exons

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[6] Identifying the locations and coding regions of genes in a genome and determine what they do

[7] The Arabidopsis Genome Initiative: Analysis of the Genome sequence of the flowering plant A. thaliana. Abstract

[8] Two classes of inter-accession differences were found and located: 25,274 Single Nucleotide Polymorphisms (SNP) and 14,570 Insertions-deletions (InDels).

[9] http://rgp.dna.affrc.go.jp/cgi-bin/statusdb/status.pl

[10] http://rgp.dna.affrc.go.jp/

[11] http://www.monsanto.com/monsanto/biotechnology/background_information/00apr03_rice.html

[12] ESTs are small fragments of genes that have been sequenced and which can act as unique "bar-codes" for each particular gen in one organism. See also C. Spillane, BSP No. 9, para 3.1.

[13] Science, 2002.

[14] The feature of genes and coding regions in general to have similar physical locations on the chromosomes in different organisms.

[15] Messing J. & Llaca V. (1998) Importance of anchor genomes for any plant genome project. Proceedings of the national Academy of Science USA 95: 2017-2020

[16] Lagencrantz U. & Lydiate D.J. (1996) Comparative genome mapping in Brassica. Genetics 144: 1903-10

[17] Full authorship's reference in Nature 409 (2001): 860. initial sequencing and analysis of the human genome

[18] Venter, C. et al. (2001) - Nature 409: 860

[19] Lander E.S. et al. (2001) - Science 291: 1304

account for only 1.1% to 1.5% of the total DNA (according to gene predictions and annotations by both projects). Whereas, noncoding intergenic (intronic) DNA is estimated at 24.4% to 36.4%[20] of the total DNA. Perhaps, even more surprising is the total estimated number of genes found in the genome (26,383 according to Celera) or predicted (<39,000 according to Celera, and (31,000-35,000 according to PFP). These values are significantly smaller than was thought to be the case. Comparison with the other eukaryotic genomes whose sequencing has been completed is indicating a continuum toward high ratios of non-coding versus coding sequences. This may suggest an increase in the complexity of regulatory systems (represented by introns and "junk" DNA), rather than a mere increase in the number of new functions. This inference is not new for biologists that have been investigating integrative approaches to explain the complexity of life[21]. It has been clear to them for many years that the Central Dogma of Molecular Biology,[22] which postulates one function (represented by a protein) for one gene, is only one out of the many possibilities open to the cell machinery. Variations of, or deviations from this dogma include, differential transcription[23], differential mRNA processing[24], post-transcriptional modifications of the primary gene product[25], or even multiple functionality. Much of the genetic material (42% in the human genome, 30% in *A. thaliana*) has no known function, and an unknown proportion of the so-called junk DNA could be involved in highly complex and inter-connected processes that make living organisms functional, and enable them to adapt. The Celera Genomics report, in a final statement indicates the research suggests that there are two fallacies to be avoided[26] First ***determinism***[27], the idea that all characteristics of the person are "hard-wired" by the genome; and second ***reductionism***[28], the view that with complete knowledge of the human genome sequence it is only a matter of time before our understanding of gene functions and interactions will provide a complete causal description of human variability." These views are not shared by the entire scientific community.[29] The same considerations are pertinent to the management of plant and animal genetic resources for food and agriculture, and provide insight (as a pre-requisite for safe and

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[20] According to the highest and lowest extremes in stringency of gene prediction

[21] Savageau, M. (1996) – Integrative Approaches to Molecular Biology. MIT, Cambridge (Mass., USA)

[22] Crick, F. (1958) – Central Dogma of Molecular Biology, Nature 227: 561-3

[23] By which a coding DNA sequence can be transcribed into more than one mRNAs, since more than one "start" or "stop" signals may be present

[24] Alternative spicing, by which the same exons may be transcribed in mRNAs ordered in different sequences in different cells and tissues, resulting in different proteins

[25] By addition of other proteins, complexation with ligands, partial degradation etc, by means of other cell constituents mediated by specific enzymes

[26] *"While few would disagree with the intuitive conclusion that Einstein's brain was more complex than that of Drosophila, closer comparisons such as whether the set of predicted human proteins is more complex than the protein set of Drosophila, and if so, to what degree, are not straightforward, since protein, protein domain, or protein-protein interaction measures do not capture* ***context-dependent interactions*** *[*] that underpin the dynamics underlying phenotype.[..] We have yet to understand the mathematical dependency relating the number of genes with organism complexity* ***[..]****The elements of the [biological] system can be represented by the vertices of complex topographies, with the edges representing the interactions between them. Examination of large networks reveals that they can self-organize, but more important, they can be particularly robust. This robustness is not due to redundancy,* ***but is a property of inhomogeneously wired networks****[*]. [..] [T]here are no "good" genes or "bad" genes, but only networks that exist at various levels and at different connectivities, and at different states of sensitivity to perturbation. [..]This assembly of the human genome sequence is but a first, hesitant step on a long and exciting journey toward understanding the role of the genome in human biology. [..]The next steps are clear: We must define the complexity that ensues when this relatively modest set of about 30,000 genes is expressed. The sequence provides the framework upon which all the genetics, biochemistry, physiology, and ultimately phenotype depend. It provides the boundaries for scientific inquiry. The sequence is only the first level of understanding of the genome. All genes and their control elements must be identified; their functions, in concert as well as in isolation, defined; their sequence variation worldwide described; and the relation between genome variation and specific phenotypic characteristics determined. Now we know what we have to explain. [..]* Venter, op cit.

* Emphasis added

[27] Emphasis added

[28] Emphasis added

[29] Claverie J.M. (2001) – What if There are Only 30,000 human Genes? Science 291: 1255

meaningful manipulation) of the raw matter of heredity, and its relationship with the holistic expression of life.

Efforts are underway to sequence some farm animal species genomes. Studies on humans (and mice as a model animal) and plant genomes, have enhanced exploration of the genomes of economically important animals. New technologies used combination with the information from the human genome sequencing projects and nearly completed genome projects of two plant species as wells the known synteny among very different taxa and even between kingdoms on gene physical locations, is enabling scientists to precisely and quickly map the genomes of farm animals. In the near future, it will be possible to identify genes that contribute to both desirable and undesirable traits in some farm animal species. Most of the research in progress is focussing on the most globally important livestock species, cattle, pig, sheep, chicken and a few fish species. The research is aimed at better understanding key traits of interest to agriculture, mostly quantitatively inherited traits, such as growth, fertility, milk and egg production, and susceptibility or resistance to particularly important diseases. Once key genes have been identified it should be possible to use conventional marker assisted selection (MAS) breeding to introduce desirable traits into pure breeds or crosses. Animal genomics will become increasingly important for understanding and enabling manipulation of genetic variation in production, productivity, product quality, including adaptive fitness and the ability of animals to cope with stressors such as feed and water shortage, infectious and parasitic diseases, and climatic extremes.

The success of the genome projects has been made possible because of advances in specialized technologies including, **bio-informatics** and **proteomics**. **Bio-informatics** is a relatively recent discipline that arose to handle the volume and complexity of data arising from complex research and modeling. In molecular biology, the need for special software packages for data analysis and management arose rapidly and increased dramatically when automated DNA sequencing capacity was developed, including high throughput micro-array,[30] and tandem mass spectrometry.[31] The need for significant data and information capacity is illustrated by the database GenBank[32], which has more than 10^{10} nucleotide single sequences and is growing at a rate of 100% per year[33]. More than 27 millions reads were performed to record nearly 15 billions base pairs (bp) of human DNA[34] (511 folds coverage of the genome) required to reconstruct *in silico* the 2,91 bp consensus sequence of the human genome[35]. Molecular geneticists are now able to search in different databases and compare data for similarities of sequences using a number of software packages that have been designed for these specific needs. There is a pronounced need for enhancing software tools in the domain of functional genomics, a branch devoted to the analysis of gene expression that makes use of DNA chip technology[36]. Thousands of genes can be tracked simultaneously and compared in different cell states in different tissues and organisms. Significant volumes of data and information are required binary in format[37] to enable meaningful comparisons. Analysis usually involves matching results against the

[30] Collins F.S. (1999) – Micro-arrays and macro-consequences. Nature Genetics 21:2-3

[31] By which peptide mixtures are studied in a initial mass spectrometry scan and particular peptides can be fragmented during a second step to generate amino acid sequence information. See: Fields, S. (2001) Proteomics in Genomeland – Science 291:1221-4; Lee, K.H. (2001) Proteomics: a technology-driven and technology-limited discovery science. Trends in Biotechnol. 19:217-21

[32] http://www.ncbi.nlm.nih.gov/HTGS/

[33] Roos, D.S. (2001) Bio-informatics – Trying to Swim in a Sea of Data. Science 291:1260-1

[34] Venter et al. (2001) op cit

[35] A number of algorithms have been developed in order to detect overlapping sequences from hundreds of thousands of cosmid libraries

[36] DNA chips, or micro-array, consist of c-DNA libraries immobilized on solid state grids, usually glass slides. Each individual spot in the grid contains DNA from a single gene that will bind to the messenger RNA (mRNA) produced by the gene concerned. So by liquidizing a sample from a given tissue type, or from the same tissue under different conditions (stresses, pathogen attack), tagging its mRNAs with fluorescent dyes and then exposing the sample to the slide, it is possible to obtain an instant visual read-out revealing which genes were active.

[37] In fact, the signals emitted are only poorly quantitative (the amount of fluorescence isn't linearly correlated to the amount of gene product)

expression signals at different cell states, and then making comparisons through searches on species-specific gene expression databases. All these operations require suitable software. Databases are being constructed for yeast, mouse, and pigs, [38] as well as *Arabidopsis*, rice, maize, and pine. Robust applications for data management and analysis are also required for the study of the primary gene products and proteins that can be expressed at once by a single genome (see below). The understanding of protein function and its relationship with coding and non-coding stretches of the DNA is made possible by analytical tools (amino-acid sequencers, mass spectrometers for steric conformation etc.), and by searching databases that contain comparisons with known proteins and their functions. It is important to note that the present state of genomics research would have been nearly successful achieved without the freedom to use information from GenBank/EMBL/DDBJ"[39]. Policies on access to data and information sharing could be a key consideration in considering the need for and further development of the draft Code of Conduct on Biotechnology.

Proteomics is the second pillar of the "genomic revolution" and it is seen by many as the new frontier in molecular biology research and development. The deciphering of the full-length nucleotide sequences must be followed by gene annotation. That is, genes can be predicted and annotated by their similarities with other genes identified through their protein transcripts. Research is essential to understanding how extremely large and dynamic populations of genes function individually over time, and most importantly, how they interact with each other, spatially and chronologically. Proteomics is includes identification and quantification of proteins, and involves determining the location of proteins, modifications, complex formations and biological activities as a dynamic network. This is a very difficult task as proteins are more complicated molecules than nucleic acids due to their very complex physical and chemical nature, the wide range of bindings they become involved in, and their modularity[40] and plasticity. Moreover, as stated above, a single gene can encode multiple proteins[41]. These complex characteristics result in a proteome[42] estimated to be more complex than a genome. Another reason why proteome science will become increasingly important is that new and powerful DNA chip methods for studying gene expression does not provide essential information about post-transcriptional control of gene expression itself, changes in protein expression level, changes in protein synthesis and degradation rates or protein post-translational modifications. Not surprisingly, recent studies have revealed a lack of correlation between mRNA expression levels and protein expression levels[43], implying that the detailed understanding of the control of gene networks requires information on both mRNA and protein expression levels[44]. The equipment required in advanced proteomics is extremely expensive, and thus not within the budgets of many laboratories or developing countries. As a result, international efforts and consortia are emerging in the public research domain to follow up the Genome Projects. Also, private companies have emerged with ready to make investments in state-of-the-art equipment and services[45]. Understanding of applications of proteomics in agriculture is still at a pioneering stage, whereas potential applications for medicine are progressing rapidly.

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[38] http://www.toulouse.inra.fr/lgc/L444_r.htm

[39] Roos, D.S. (2001), op. cit.

[40] Protein sub-units or domains may be differentially processed and assembled to give rise to functionally distinct entities: see Gerhart, J. & Kirschner, M. Cells, Embryos and Evolution – Blackwell Sci. London, pp. 78, 221

[41] By alternative splicing of the mRNA transcript, by varying translation start and stop sites, by frameshifting during which a different set of mRNA is translated.

[42] See note 6

[43] Selinger, D. et al (2000) RNA expression analysis using a 30 base pair resolution E. coli genome array. Nat. Biotechnol. 18:1262-8; Anderson, L. & Seilhamer, J. (1997) A comparison of selected mRNA and protein abundances in human liver. Electrophoresis 18: 533-7

[44] Hatzimanikatis, V & Lee, K.H. (1999) Dynamical analysis of gene networks requires both mRNA and protein expression information. Metabolic Eng. 1:275-81

[45] For example: GeneProt, a new Swiss company invested some US$n 122m in one year for establishing its European base at Geneva. Nature 410 (2001): 856

Recent breakthroughs in genetics and related disciplines will be extremely valuable for improved use of biotechnology to support crop improvement. It is also reasonable to hope that enhanced knowledge of the most intimate mechanisms governing genotype-phenotype interaction, along with the availability of more powerful and precise manipulating abilities, will increase the value of genetic resources for food and agriculture and will help more effectively utilise agricultural biodiversity to achieve global food security. In this perspective, it is important to underline that research and development have been concentrated on few model genomes, and that additional efforts should be spent for transferring the novel technologies to "orphan" crops and tropical farm animals that are contributing significantly to food security in developing countries. In this respect, a lead role should be given to public research institutions, possibly in partnership with the private sector. This is necessary to ensure the generation of useful products is widely available (databases, molecular kits for MAS and genityping, etc.).[46]

III. ENGINEERING FOR APOMIXIS IN CROP PLANTS

Apomixis is a naturally occurring phenomenon whereby some plant species can produce seeds without fertilization, resulting in off-spring that are identical to the mother plant. The occurrence of apomixis is common amongst wild species. Nearly 10% of angiosperm families are apomicts, within which only 0.1% of species are apomictic. [47] Apomixes is rather rare in crop species and is usually associated with polyploidy. At least nine different types of apomictic mechanisms are known[48].

Molecular tools provide powerful means to investigate the genetics and biology of the different types of apomixis in plants[49], and to isolate the genes that are involved, as well as identity potential transfer in crops. Unfortunately, the understanding of both apomictic and sexual pathways of reproduction is still insufficient to provide understanding of how the genes involved in apomictic reproduction function."[50] There are 3 main options for the introduction of apomixis into sexually reproducing crops: (i). transfer the trait into crops from wild naturally occurring apomictic relatives through a series of backcrossings, (ii) screen sexual crops for apomictic mutants, and (iii) de novo synthesize the apomictic trait directly into crops. The advantages of apomixis are very appealing for breeders and farmers, especially in the developing countries.[51] The main advantages being the ability to fix a desired genotype and also retain hybrid vigour of the F_1 generation in the subsequent seed propagation. Therefore, a successful introduction of apomixis in agricultural crops might result in: a steady creation of new varieties straight from elite genotypes and crosses; one step fixation of genetic recombinants after wide crosses; true breeding of polygenic traits; and elimination of virus transmission in vegetatively propagated crops by using apomictic seedstock. Although some effort has been made recently to create apomictic varieties by means of conventional breeding,[52] the constraints in using such an approach are intuitive: (i) the approach is limited to a few crop species that have apomictic wild relatives (such as wheat, maize, pearl millet,[53] and some tropical forage and fodder crops,[54] (ii) obligate apomicts cannot serve as maternal plants, and breeding of such species is

[46] CGRFA/WG-An-2/00/4, para 54.
[47] Moogie, M. (1992) The Evolution of Asexual Reproduction in Plants. Chapman &Hall Publisher
[48] Crane, F. (2001). Classification of Apomictic Mechanisms. In Savidan et al. Eds. – The flowering of apomixis: from mechanisms to genetic engineering. CIMMYT, European Union, IRD Publishers
[49] van Dijk, P. & van, Damme, J. (2000) Apomixis technology and the paradox of sex. Trends in Plant Science 5(2), 81-4
[50] Savidan, Y., Carman, J.G. and Dresselhaus, T. (2001) Genetic Engineering of Apomixis in Sexual Crops: a Critical Assessment of the Apomixis Technology. In: Savidan *et. al.,* Eds. cit.
[51] C. Spillane (1999) Recent Developments in Biotechnology as they relate to Plant Genetic Resources for Food and Agriculture. Background Study n. 9, FAO/CGRFA; Grossniklaus, U et al (1998). A bright future for apomixis. trends in Plant Science 3(11), 415-6
[52] Hanna, W.W. & Barshaw, E.C. (1987) Apomixis: its identification and use in plant breeding. Crop Science 27, 1136-9
53 Savidan, Y (2001) Transfer of Apomixis through Wide Crosses. In: Savidan Y. et al. (Eds.), cit.
54 Barshaw, E.C. & Funk, C.R. (1987) Apomictic grasses. In: Fehr W.R. (Ed.), Principles of Cultivar Development Vol. 2. NY: Macmillan Publishing Co. pp. 40-82

impossible; and (iii) polyploidy and high heterozygosis further complicate the genetic analysis of off-spring.

While a detailed literature review of the scientific challenges and achievements in the field of apomixis research are beyond the scope of this report, a few issues have been identified. First, it is widely held that there is no single-gene solution to explain the apomixis phenomenon. The identification, characterisation and eventual isolation of genes putatively involved in apomixis is in progress in several laboratories, but progress is slow. Most apomictic species do not belong to the classical model plant species, and therefore, positional cloning is difficult due to the low number of available markers. As a consequence, the development of transgenic apomixis in major crops is not foreseen in the immediate future. Second, benefits that might be expected by the "asexual revolution" (for example, the increased productivity could exceed US$2.5 billion per year for rice[55]) would come at some cost. The major drawback is that an apomictic strategy of reproduction has long-term disadvantages as compared to a sexual approach. One of them being reduced genetic variability, which could affect the adaptive potential of the apomictic variety to changing environments[56]. Whether or not this feature may reduce the durability of such planting material in suitable conditions, as compared with modern sexual varieties is only a matter of speculation. But it is certain that an apomictic crop could not be a substitute for a traditional, heterogeneous landrace in unfavourable conditions, unless it had been selected for those specific environments. Third, the rise of the apomictic transgenics would pose an unprecedented biosafety risk. It is likely that important differences exist between natural apomicts and transgenic ones, and the low occurrence of apomixis in the plant kingdom could be interpreted as the result of evolutionary constraints to such a reproduction strategy,[57] which perhaps led to extinction of "apomictic trials." Knowledge of gene flow between sexual and apomictic populations is very limited and circumstantial. Thus, it is not easy to make predictions on what might be the fate of apomictic genes that escape through pollen from apomictic transgenes. While sexual populations are usually protected from gene introgression from apomictic relatives by incompatibility barriers (since most natural apomicts are triploid or polyploid), in the case of diploid transgenic apomicts, there would be no such barrier to the spread of genes involved in apomixis in wild populations[58].

In addition to technical issues associated with apomictic transgenes, there are likely to be intellectual property issues and constraints. In spite of the 1998 declaration endorsed by a number of scientists in the forefront of apomixis research,[59] the number and dispersion of patent claims on individual genes and/or processes associated with biotechnology powered apomixis is increasing at a rapid pace.[60] Applicants encompass public research institutions and universities in the United States of America, private companies, and international research consortia. However, some major patent holders have declared their commitment to free licensing to developing countries.[61] Nevertheless, the potential of apomixis is so large as to justify even more research efforts and the seeking of suitable solutions to identified issues. Where apomixis technology is in hand, access and deployment of PGRFA would indeed change dramatically resulting from the direct fixation of valuable and favourable cross combinations in the F1. This feature could increase the value of local germplasm in wide crossing programmes both for local adaptation and more broadly.

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[55] McMeniman, S. &Lubulwa, G. (1997). project Development Assessment: an Economic Evaluation of the Poitential Benefits of Integrating Apomixis in Hybrid Rice. Australian Centre for International Agricultural Research.

[56] van Dijk & van Damme (2000), cit.

[57] van Dijk & van Damme (2000), cit.

[58] Asker, S.E. & Jerling, L. (1992) Apomixis in Plants. CRC Press

[59] The Bellagio Apomixis Declaration, available at http://billie.harvard.edu/apomixis

[60] Savidan, Y., Carman, J.G. and Dresselhaus, T. (2001), Table 14.2. cit.

[61] CIMMYT and the Institut de Recherche pour le Developpement (IRD) have reserved the right to distribute all outputs from the project to resource poor farmers without limitations; additional rights are available to Mexico as host country and supporter of the project. Hoisington, D. (2001), Pers. communication

IV. CHLOROPLAST TRANSFORMATION

Plant transformation has been mostly achieved through either *Agrobacterium* trans-infection or through biolistics, that is a bombardment of cultured cells with micro-bullets coated with exogenous gene constructs. The target of both strategies is usually the nuclear genome and its successful outcome has been the stable insertion of foreign genes into the chromosomes in the nuclear genome. As a consequence, transgenes and their "adjuvants" (promoters and marker genes) follow the paternal inheritance and are transmitted through the male gametophytes through meiosis, in the DNA of pollen. This poses well-known and documented cases of " gene escape" of transgenes, and their consequential spread into sexually compatible plants,[62] including non-GM varieties of the same crop growing in the vicinity as well as infecting wild relatives[63]. This has become a collateral risk and concern especially for some traits (herbicide[64] or insect resistance) in the centres of origin or diversity of crops. Moreover, foreign DNA in the pollen is capable of transcription into the gene product, which poses additional hazards to pollinators and other organisms [65] in case of genes encoding for toxins.

The concerns mentioned above may be overcome to a great extent by applying an alternative transformation approach. A number of crop species have been successfully transformed using the chloroplast approach, including tobacco, rice, and potato. Both public and private sector research organisations are involved in this technology, which may have interesting commercial applications, including important non-food and drug[66] production. This approach refers to the introduction of the gene construct(s) of interest into the plastid genome (named *plastome*) located in the plant cell cytoplasm. Using this approach, the gene(s) of interest are introduced into the chloroplast genome (cpDNA) instead of the nuclear genome preventing gene escapes through the pollen. Maternal inheritance of plastomic DNA has been indeed demonstrated for about 200 angiosperm species[67], whereas it is bi-parental in several gymnosperms, and low levels of paternal transmission have been reported in tobacco[68]. Hence, the chloroplast transformation approach is conceptually a suitable alternative strategy for genetic engineering in higher plants providing several advantages in terms of biosafety. First of all chloroplast transformation has great potential for "precision biotechnology", that is, for site-directed insertion of gene "cassettes" within the plastome since they are integrated through recombination of their flanking regions at cpDNA stretches of known complementary sequences. Also, the approach assists in overcoming the random site integration and uncontrolled number of foreign gene copies that is typical of nuclear transformation events. The poor control over transformation events is often the cause of undesired effects such as position effects, variable expression level, and gene silencing. Secondly, since most plastid genes are co-expressed in large "building blocks"[69], the chloroplast approach offers the possibility of insertion and co-expression of multiple foreign genes as operons in a single transformation event[70]. This is not possible in nuclear

[62] Gene flow from transgenic crops to wild relatives has been estimated in the rate of 28-38 % for sunflower and even more than 50 % for strawberry. Data reported in: Daniell, H. (1999), AgBiotechNet vol. 1 (ABN 024). From: King , J. (1996) Could transgenic super-crops someday breed super-weeds? Science 274, 180-1

[63] According to Keeler et al. (see following footnote), the risk of gene flow is tangible for at least 49 out of 60 important crops world wide

[64] Keeler et al (1996) Movement of crop transgenes into wild plants. In: Herbicide-resistant Crops: Agricultural, Economic, Environmental, regulatory, and Technological Aspects – Duke, S.O. Ed. CRC Press, pp. 303-330

[65] The case of toxic effect of pollen from *Bt* mais on larvae of monarch butterfly (Losey et al. 1999. Nature 399, 214), although still disputed, is explanatory of this risk.

[66] Daniell, H. et al. (2001) Expression of the Native Cholera Toxin B Subunit Gene and Assembly as Functional Oligomers in Transgenic Tobacco Chloroplasts. Journal Molecular Biology 311, 1001-9

[67] Corriveau, J.L. & Coleman, A.W. (1988). Rapid screening method to detect potential bi-parental inheritance of plastid DNA and results from over 200 angiosperm species. American Journal of Botany 75, 1443-58

[68] Daniell, H. et al. (1998). Chloroplast transgenic plants: panacea – no! gene containment – yes! Nat. Biotechnol. 16(7), 602

[69] They are named polycistronic expression units

[70] Staub, J.M. & Maliga, P. (1995). Expression of chimeric uidA gene indicates that polycistronic mRNAs are efficiently translated in tobacco plastids. Plant Journal 6, 845-8

transformation where each gene has to be inserted and screened separately[71]. This feature allows for more sustainable and durable genetic deployment of the traits of interest (e.g. gene pyramiding for pest resistance). Thirdly, since the functioning of plastome is different from that of nuclear genome, and rather similar to that of prokaryotic genomes[72], native or "wild" genes of bacterial origin can be inserted without sequence modification, which is necessary in nuclear transformation [73]. Fourthly, since the number of chloroplast genomes is very high (from 5,000 to more than 10,000 per cell) and successful transformation usually leads to homoplasty[74], the expression level of the inserted gene(s) is correspondingly very high, up to several thousands times more than in the case of nuclear transformation. This feature offers the opportunity to pursue high dosage strategies for pest or pathogen resistance and prevent the build up of resistant populations of the target organisms. Finally, the accumulation of gene product is localised where it is actually needed, that is in the green tissues and is not expressed in fruits and other storage organs.

There are also some drawbacks to using of the chloroplast transformation technology including: the need for specific transformation protocols for each species or taxon; generally lower transformation efficiency and consequent higher cost of generation; the need for chloroplast specific promoters; the need of targeted research for the feasibility of the technology for particular traits and its application (e.g. when the gene product should be present in non-green tissues); the possibility of yield drag due to the high expression level of the transgene(s); and the high accumulation of antibiotic resistance gene products used as selection markers. Suitable solutions to these problems may be found through research. Some biosafety concerns have been recently overcome by using a BADH gene isolated from spinach as selectable marker[75].

The chloroplast transformation approach is a very promising and potentially useful technology for genetic engineering applications for crop improvement, which may have multiple advantages over the mainstream nuclear transformation approach. This approach should not be viewed as a panacea for resolving biosafety issues[76] or for achieving productivity objectives. Chloroplast transformation technology seems to be already encumbered by very broad IPR claims.[77] Many protocols for chloroplast transformation are protected through patents. However, the flanking sequences along the cpDNA as well as some of the marker genes are in the public domain. Many native microbial genes may be freely used in the public domain, at least in the countries where the patent protection of micro-organisms and their sub-units is not available for the native, non manipulated ones.

V. GENE FLOW FROM GENETICALLY MODIFIED PLANTS

Gene flow is the phenomenon by which inter-fertile plants within the same taxa, and other compatible species within the same gene pool, exchange genetic information, generally through pollen and seeds, creating new variability that is subsequently exposed to selection pressure. Therefore, gene flow is a major driving force in the evolution of plants.

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[71] De Cosa, B. et al. (1999) Overexpression of the BtCry2Aa2 operon in chloroplasts leads to formation of insecticidal crystals. Nature Biotechnology 19, 1-4

[72] As it is well known, codon preference and usage by transcriptases are different among prokariotic (and plastidic) and eukariotic organisms.

[73] Perlak, F.G. et. al. (1993). Genetically improved potatoes: protection from damage from Colorado beetle. Plant Molecular Biology 22, 313-21

[74] That means sequence homology among the chloroplast genomes

[75] Daniell, H. et al. (2001) Marker free transgenic plants: engineering the chloroplast genome without the use of anti-biotic selection. Curr. Genet. 39, 109-16

[76] Stewart, C. N. & Prakash, C.S (1998) Chloroplast transgenic plants are not a gen flow panacea. Nature Biotechnology 16(5), 401; Daniell, H. & Varma, C.N. Jr (1998) Chloroplast transgenic plants: panacea – no! gene containment – yes! Nature Biotechnology 16(7), 602

[77] Daniell, H. (1999). Universal chloroplast integration and expression vectors, transformed plants and products thereof. World Intellectual Property Organisation, WO 99/10513. The claim seems not to cover BADH gene from spinach as selectable marker.

In recent days, gene flow has attracted the attention of scientists, the media and the public in its novel occurrence as related to Genetically Modified (GM) plants. It has been argued that such exchange of DNA, including genes from very distant organisms or even from man-made gene constructs, might cause hazards to natural plant populations and therefore be a threat to intra-specific, species and eco-system genetic diversity[78]. The likelihood of gene flow from GMOs through pollen and/or seeds is a basic requirement of risk assessment procedures in many countries [79].

The issue suddenly became topical in late 2001, after the publication of a paper in the scientific magazine *Nature*[80], in which the authors claimed evidence of GM contamination in the Southern Mexican state of Oaxaca (more than 2,000 km away from the US border) in the genome of traditional maize landraces. Commercial, and even experimental, cultivation of GM maize is forbidden in Mexico. This report heated up the scientific debate over the impact of GM crops on biodiversity in general and in the centres of origin of those crops in particular. The debate became charged with ideologically-oriented assumptions, while also being biased by economic and trade interests. Were it to be demonstrated that gene flow from genetically modified plants posed hazards to genetic diversity, mega-diverse countries – most of them developing countries – would need to take preventive measures, which could in turn result in a major market failure for the producers of GM seeds world wide.

Under these conditions, the arguments raised by both sides – the supporters and the opponents of GM crops – have been perceived by independent analysts as sometimes scientifically questionable.

While the international legal background that underpins the technical debate in progress on this matter is far beyond the scope of this paper[81], this section is aimed at summarising the content and outcome of the discussion on the impact of gene flow from GM crops to conventional varieties, landraces and their wild relatives at two distinct events. The first workshop was co-organised in early February, 2002 by the author of this paper on behalf of the Scien&Tech Agency of the Italian Ministry of Foreign Affairs [82]. The second one took place soon after at Columbus, Ohio (USA) organised by Ohio State University[83].

The former workshop was intended to facilitate the start up of a smooth international multi-stakeholder discourse on the environmental safety of transgenic crops in their respective centres of

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[78] U.S. National Research Council. 2000. Genetically modified pest-protected plants: science and regulation. National Academy Press, Washington, DC. 263 pp. ISBN 0-309-06930-0

[79] For example, in the European Union (Directive 2001/18/EC, art. 4.3, Annex II, C.2 and D.2) and in the United States of America the assessment of risk associated to gene flow is mainly under the USDA's Animal and Plant Health Inspection Service (http://www.aphis.usda.gov/biotech/)

[80] *Nature* vol. 414, pp. 541-43

[81] The Convention on Biological Diversity stipulates indeed, as far as *in situ* conservation is concerned, that: "*Each contracting Party shall, as far as possible and as appropriate: [..] Establish and maintain means to regulate, manage or control the risks associated with the use and release of living modified organisms resulting from biotechnology which are likely to have adverse environmental impacts that could affect the conservation and sustainable use of biological diversity* […]" (art. 8.g); and also stipulates that: "*The Parties shall consider the need for and the modalities of a Protocol setting out appropriate procedures […] in the field of safe transfer, handling and use of any living modified organism resulting from biotechnology that may have adverse effect on the conservation and sustainable use of biological diversity*" (art. 19.3). The concern is reaffirmed in the Preamble of the Cartagena Protocol to the CBD, where the Parties declare to be "*aware of the rapid expansion of modern biotechnology and the growing public concern over its potential adverse effects on biological diversity* […], also "r*ecognising also the crucial importance to humankind of centres of origin and centres of genetic diversity*". On the other side, the World Trade Organisation has set up the rights and obligations of the member countries as far as trade of any kind of commodities, including GMOs. In the framework of the latter multi-lateral organisation, the refusal by a member country to import GMOs should be supported by scientifically sound evidences and not merely on the base of a precautionary principle.

[82] Istituto Agronomico per l'Oltremare, http://www.iao.florence.it

[83] http://www.ohio.state.edu

origin. The agenda involved the scientific evidences of the impact of gene flow on biodiversity, along with the areas of uncertainty and ignorance, as well as some non-biological topics of concern, including socio-economic issues specifically associated with gene flow from GM sources. The meeting was attended by leading scientists from several countries in the field of biotechnology and environmental risk assessment, along with senior officers of relevant international organisations, outstanding national regulators and policy makers, representatives of farmers' associations and NGOs from the South and the North, scien&tech writers and sociologists.

The Columbus workshop was instead specifically addressed to critically analyse the scientific methods currently available for assessing the ecological and agronomic consequences of gene flow from transgenic crops to wild relatives in a range of crops. The workshop's participants were outstanding experts with a number of US Universities and federal regulatory authorities.

The proceedings of both workshops are now available on the Internet[84]. On a few important points the two workshops reached similar outcomes, namely:

1. Gene flow is generally occurring in wild and crop plants, including transgenic ones, its likelihood and extent is dependant on their reproductive biology, co-presence of inter-fertile stands or populations, and other environmental variables;
2. In some cases (including maize landraces in Mexico), gene flow is intentionally sought by traditional farmers in order to enhance usable diversity[85];
3. There is nowadays no efficient means to completely avoid gene flow between sexually compatible species that occur sympatrically: pollen and seeds disperse too easily and too far to make containment practical[86];
4. The potential for transgenes to be introgressed within wild relatives depend on a range of factors, *inter alia* hybrid fertility, seed dispersal, selection pressure, and on the relative fitness of the hybrid GM progenies, while in the case of landraces, the selection pressure is driven by farmers in the first place, and might follow different patterns;
5. The outcome of successful introgression events is not predictable *a priori*, and its impact on the genetic diversity at the intra-specific, species and eco-system level has to be analysed on a crop-by-crop, transgene-by-transgene, site-by-site approach;
6. While, in the few countries where R&D on agricultural gene-technology has taken place, there is increasing knowledge of the interactions between transgenes and the environment regarding the few crops that dominate the GMO segment of the seed market world wide (maize, soybean, canola, cotton, potato etc.), there is generally a lack of scientific baseline information on these crops when released in different agro-ecosystems (e.g. tropical, savannah, etc.). This ignorance is even more pronounced as far as minor and tropical crops are concerned.
7. Since the geographical specificity of gene/environment interactions is a broadly accepted principle, such insufficient information in most developing countries would make the preliminary risk assessment of transgenic crops in these countries[87] particularly difficult if not actually unfeasible[88].

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[84] *Beyond Oaxaca* - Proceedings of the workshop: "GM crops in the centres of crop diversity - What lesson to be learnt from, and beyond, Oaxaca?" Istituto Agronomico per l'Oltremare Florence, 7 - 9 February 2002, http://biodiv.iao.florence.it/proceedings/oaxaca/rep_oaxaca; *Scientific Methods Workshop*: Ecological and Agronomic Consequences of Gene Flow from Transgenic Crops to Wild Relatives - Meeting Proceedings at: www.biosci.ohio-state.edu/~lspencer/gene_flow.htm .

[85] *Beyond Oaxaca,* report p. 11

[86] Quoted from the background statement of the Ohio workshop

[87] "Whereas food safety research from the North can be used for risk analysis in any other country, cross fertilisation needs to be researched taking the local plant populations into account. The concept of "the botanical files" fills this gap". " Currently, botanists in Eastern Africa are starting to build botanical files for a number of species in their region under the BioEARN programme". N. P. Louwaars, Plant Research International (2002). Contribution No. 19 to FAO electronic conference on Gene Flow.

88 This statement is clearly enounciated in the Florence workshop, whereas it is implictly admitted in the Columbus one, in which "the crops, wild relatives, and regulatory issues we discussed focused on the USA, but much of the workshop was also relevant to **similar** situations in other countries." Proceeding, p. 3. Emphasis added

Technical recommendations were agreed upon in the Florence meeting for preventive measures to be taken in order to avoid gene flow into *ex situ* and *in situ* collections[89].
A point raised at the Florence workshop (which was not in the agenda of the Columbus one) was related to some socio-economic consequences of gene flow from GM crops. Amongst them, the risk of market failure of organic farmers in those countries that have adopted GM crops, along with the discontent of conventional farmers who would be keen to keep their fields free from transgene contamination in order to command premium prices for "GM-free" food[90].
FAO also promoted (May 31-July 5) a third scientific debate in the format of an electronic conference on gene flow, that included the qualified participation of several dozens of research experts and stakeholders[91]. The conclusions of the conference have been posted under the FAO's website at: http://www.fao.org/biotech/logs/C7/summary.htm.

In conclusion, the gene flow discourse has shown another facet of the gap in knowledge and technology between industrial and developing countries, which might jeopardize the so far limited development of appropriate advanced biotechnologies in the latter. This gap is of particular concern due to the global implications of gene technology in contrast to its predominantly national regulation and oversight where "the failure in regulating biotechnology anywhere will harm the industry everywhere"[92].

[89] Oaxaca report, p. 12-13.
[90] Oaxaca report, p. 15.
[91] http://www.fao.org/biotech/forum.asp
[92] David G. Victor & C. Ford Runge (2002) – Farming the Genetic Frontier. Foreign Affairs, Vol. 81 No. 3, pp. 107-21