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Session IV: Identifying and Defining Hazards and Potential Consequences III: Concepts for Problem Formulation and Non-Target Risk Assessment

Ariel ALVAREZ-MORALES*

Dept. of Genetic Engineering, Center for Research and Advanced Studies (Cinvestav), Irapuato, KM 9.6 Libramiento Norte, Irapuato, 36821, Mexico

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Untingdon, UK

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Hilo, USA

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Baorong Lu
Fudan University
Shanghai, China

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Ariel Alvarez-Morales
Centre for Research and Advanced Studies
Irapuato, Mexico

INTRODUCTION

Obtaining a new GM crop variety is a complex and expensive process. Therefore much care is taken to ensure that every step of the process will meet the requirements of the biosafety authorities of the country where the product is intended to be commercialized. Thus, the molecular biology is designed using well characterized, and often already approved, elements such as promoters, terminator sequences, enhancers, markers, *etc*. The transformation process is also carefully monitored to assess the number of copies inserted into the recipient genome, the extent and integrity of the inserted DNA and the precise location of the insertion. Appropriate expression of the inserted gene(s) is also confirmed, as well as the identity, and the safety of the new protein products. The newly generated transgenic plants are then assayed for the new phenotypic characteristics and to ensure that no other change has occurred. All these evaluations are done within the confinements of a well-controlled laboratory, growth chamber or greenhouse. When a field trial is required, all the data previously obtained is presented to the regulatory authorities as part of the product characterization, together with a revision of data from different sources supporting an often theoretical ecological risk assessment. Field trials may then contribute to better product characterization as agronomic performance is evaluated, and may also provide data relevant to understanding the possible impact of the genetically modified plants (GMPs) in the environment. All the information collected through the experimental field releases is then used to write the Environmental Risk

* Corresponding author: aalvarez@ira.cinvestav.mx

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Assessment (ERA) required to request unregulated status. Although the process has been presented in an oversimplified form, it is a complex and demanding scientific exercise aimed at identifying any possible source of risk to human and animal health and to the environment, before the product is commercially released. However, although the laboratory tests may be conclusive, the safety to the environment is usually based on a very low or negligible-risk hypothesis, as all the variables involved in environmental risk assessment cannot possible be evaluated during the field trials.

Therefore, post-market monitoring (PMM) has been proposed as the mechanism to validate the ERA negligible-risk hypothesis, as well as to serve other purposes, such as corroborate agronomic performance, assay trait stability, and measure economic performance and acceptability of the product. All the information obtained through the PMM should then be useful to address any biosafety issues identified, apply risk management procedures, or introduce improvements in future products.

Another case where large scale monitoring of genetically modified products is required is when unintentional or accidental releases occur, and widespread dissemination of the GMP may have taken place. In this case, and contrary to the well-planned PMM, an accidental release of GMPs and the subsequent transgene dispersal associated with this event present a much greater challenge, especially if the time and place of the initial release are unknown, as well as the initial amount and type of GMP involved. Examples of monitoring to address the issues described were presented at the meeting and will now be discussed.

VALIDATING THE ERA NEGLIGIBLE-RISK HYPOTHESIS

The UK farm scale evaluation: Effects of GM herbicide-tolerant crops of farmland wildlife

We were presented with a comprehensive study on the possible impact of the use of genetically modified herbicide tolerant (GMHT) crops on farmland wildlife in the UK. The experiments were designed to assess wildlife diversity in two settings, one in which conventional crops and conventional methods of weeding were being used, and the other where GMHT crops were used together with either glyphosate or glufosinate ammonium. The observations were performed within the cropped fields during one season and in the field margins during two years after the crops had been sown. The final conclusion from this work is that large-scale cropping of GMHT crops will affect UK farmland wildlife. However, it was also clear from the data presented that this conclusion could not be taken as a general rule as, for instance, GMHT maize was shown to actually be beneficial for biodiversity, as the conventional herbicide had more drastic effects on weeds than the herbicides used with the GMHT varieties. Furthermore, it was also recognized that other important parameters affecting the outcome are the cropped area, distribution of GM and non-GM crops, agronomic practices, and very importantly, the natural history of the surrounding environment. Therefore, it seems clear that making a decision as to whether to use GMHT or conventional crops to control a weed problem, will depend largely on the needs of the farm, the economics of the crop, and the commitment of the individual farmer or the community to the preservation of the environment. Furthermore, even if the individual farmer or the community have a strong commitment and desire to preserve their environment, the proper information regarding the possible effect of GM varieties on the environment must be available to them in a suitable form. Thus, the next important step in this type of post-market monitoring, which must be encouraged, is not only to gather relevant and well balanced information regarding the impact of GMHT crops, or any other GM crop, but to distribute this information to the end-user to ensure they have the data required to make sound decisions.

MONITORING FOR AGRONOMIC PERFORMANCE AND PRODUCT DEVELOPMENT/IMPROVEMENT

The science and art in evaluating the potential impact of pleiotropic and unintended effects during the selection of transgenic papaya lines

In this case we were presented with a clear example of how a biotechnology-derived product should evolve by including into the new products elements derived from the knowledge generated by research, as well as elements required to comply with legislation from different countries that are interested in these products. In 1998, two transgenic lines of papaya engineered to confer resistance to *Papaya ringspot virus* (PRSV) were commercially released to growers in the state of Hawaii. These biotechnology products helped an industry on the verge of collapse due to the presence of PRSV to recover and regain its economic potential for the Hawaiian producers.

Initially the transgenic varieties were obtained by expressing the full viral coat protein. Therefore, a major modification engineered into the new products has been the use of non-translatable constructs that are able to confer the same levels of resistance without having an extra protein product, thus eliminating the possibilities of allergenicity, toxicity and other characteristic normally associated with polypeptides. Furthermore, short pieces of coat protein genes have been linked together to confer

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resistance to a wider variety of viruses, while at the same time reducing the chances of recombination and thus formation of new types of PRSVs. An interesting part of this presentation was in relation to the development of papaya resistant to PRSV for the Japanese market. The biosafety legislation in Japan requires that information regarding the sequence flanking the sites of insertion of transgenes be presented. Since the initial products obtained and commercialized in Hawaii were obtained using the gene-gun, several copies of the transgenes had been inserted into the papaya genome. Obtaining the border sequences of the otherwise commercialized and proven varieties, used safely since 1998 has been difficult, so scientists are considering developing an alternative transformation system based on *Agrobacterium,* to reduce the number of insertions and thus make it easier to obtain the border sequences in the transgenic plants. However, it seems ironic in a way that a new transgenic plant may be easier to pass current Japanese biosafety legislation, rather than the already commercialized materials that have a proven record of safety. The author, Dr. Gonsalves, wonders what kind of strategy a developer must follow at the planning stage, in order to produce materials that comply with the different biosafety requirements in different countries. It was argued that since the criteria used to establish biosafety requirements is science-based, it should be possible for different countries to agree on at least the basic and most important requirements that must be fulfilled by a product developer, in order to produce widely acceptable products. This is even more important in South-South collaborations and products develop by academic or public institutions with usually restricted budgets.

MONITORING NON-GM CROPS TO OBTAIN BASELINE DATA FOR THE ERA

Identifying possible environmental hazards from GM rice in China to inform biosafety assessment

The work presented sets an important precedent as to how to approach the problem of whether or not it is possible to introduce a GM crop for which the country is a centre of origin and holds a rich variability in landraces and wild species, some of which are weedy. In this case extensive monitoring was performed on non-GM rice crops to produce a comprehensive scientific assessment of the possible implications of introducing GM rice in Chinese agriculture. The main focus of the work presented was on the analysis of possible effects of the introduction of GM varieties engineered for insect resistance, disease resistance and herbicide tolerance, and these were explored regarding impact on non-target organisms and biodiversity, gene flow and its related hazards, development of resistance to the *Bt* transgene, and other indirect effects.

With respect to possible effects on non-target organisms, the conclusions reached were largely derived from the experience obtained with the extensive use of GM cotton, and although it is considered that the extensive use of chemical pesticides has already had a significant effect on non-targets, and that the use of GM *Bt* varieties may have a less dramatic effect, caution is being exercised regarding the possible flow of this trait to sympatric populations of weedy rice, and with regard to the possible effect on soil organisms such as earthworms. In the case of gene flow, attention has been drawn mainly to the possible consequences of wild, and particularly, weedy rice, acquiring traits such as herbicide tolerance which may create a control problem, or introgression of *Bt* genes into these populations, which would result in the inability to implement preventing measures, such as refuges, to avoid the emergence of *Bt* resistance in insect pests. Furthermore, gene flow has also been acknowledged as a potential trade-related risk, as "contamination" of non-GM varieties, for which there is a valuable market, could have a serious effect. As for the issues regarding resistance to the *Bt* transgene, and besides the problems associated with gene flow mentioned before, it is considered that there is a high risk of developing insect resistance, due to high within-species genetic variation among lepidopteran pests, and also because if *Bt* rice is used by small farmers who are widely distributed in all the rice producing areas in China, to implement and adequately manage preventative measures may not be possible. Other considerations were made, such as the loss of rice biodiversity as a consequence of a widespread adoption of GM varieties, emergence of secondary pests related to the use of *Bt* crops, and changes in the composition of biodiversity as a consequence of the use of herbicide-tolerant varieties.

It is interesting that results from this analysis have fed back into the research laboratories, such that new strategies are being explored that make use of biotechnology to introduce molecular mechanisms to avoid introgression of transgenes into wild rice species. Furthermore, extensive post-market monitoring is being planned to follow the predictions, and explore the real situation posed by the great diversity in landscape, agricultural practices and culture present in China.

MONITORING A LARGE SCALE NON-INTENDED RELEASE OF A GMP

A proposal for a continuous large-scale monitoring program, based on farmers' experience, to detect the possible presence of *cry* transgenes in maize landraces in Oaxaca, Mexico

Monitoring the spread, distribution and persistence of a transgene within the population of maize landraces in

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Oaxaca is a very complicated issue, due to the lack of control over the fate and distribution of the seed derived from landraces. Performing costly analyses to detect transgenes on samples derived from the population of suspected landraces may not lead to any real conclusion unless positive results are obtained. However, recently it has been suggested that the presence of transgenes may be declining, and therefore it may as a result be very difficult and expensive to analyze a large enough sample to convincingly declare that transgenes are not present. Therefore, in order to detect what may be rare instances of transgenes still present in the maize landraces, we were presented with an alternative based on the local knowledge of farmers and the assumption that if transgenes are somehow maintained within the maize population these are most likely *cry* genes used to confer resistance to lepidopteran pests in the original GMPs. The principle of the idea is to establish a system to continuously survey the farms in Oaxaca, trying to identify any possible change that could be attributable to the presence of *cry* genes, such as an unexplained increase in yield, or reports of less damage to crops due to insects, in comparison to the rest of the farmer population. A survey of this type could then identify places or areas that could then be monitored more closely and using the more expensive methods.

Mexico, like most countries, already has in place mechanisms for surveillance used to quickly identify plagues or diseases among crops, and the idea is to use these mechanisms to continuously collect the data required for the proposed surveillance scheme.

Another point raised was the possibility to implement simple and inexpensive monitoring tools such as the use of herbicide or antibiotic solutions, such as kanamycin, to apply to leaves in order to detect herbicide- or antibiotic-resistance phenotypes that could indicate the presence of transgenes.

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