



SPEAKER ABSTRACTS

MEETING

GENETIC BASIS OF UNINTENDED EFFECTS IN MODIFIED PLANTS

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SPEAKER ABSTRACTS

TUESDAY, 14 JANUARY 2014

Unintended effects in genetically engineered plants: what they are and how they are assessed in Canada

Mr. Phil Macdonald, Canadian Food Inspection Agency

The molecular biology of gene function

Dr. Mark Jordan, Agriculture and Agri-Food Canada

The molecular biology of plant genomic changes

Prof. Wayne Parrott, University of Georgia, US

What does it take to bring an ag biotech seed product to market?

Dr. Laura Privalle, Bayer CropScience, US

The roles of backcrossing and other breeding strategies in removing unintended effects caused by the transformation process

Dr. Phil Bregitzer, US Department of Agriculture – Agricultural Research Service

How much of a hazardous substance would have to be produced before it poses a risk?

Prof. Andrew Bartholomaeus, University of Canberra, University of Queensland, Australia

Unintended effects in genetically engineered plants: what they are and how they are assessed in Canada

Mr. Phil Macdonald, National Manager of the Plant and Biotechnology Risk Assessment Unit, Canadian Food Inspection Agency, Ottawa, Canada

The OECD was among the first to articulate some of the core principles for GMO environmental risk assessment such as comparing the modified plant to its unmodified counterpart and concentrating on the properties of the intended trait. This approach forms the basis of the regulatory approach of many competent authorities today. Along with characterizing the intended novel trait, regulatory approaches usually try to assess any unintended consequences of genetic engineering that could result in a risk. Generally, this is achieved by characterizing aspects of the phenotype using qualities such as agronomic properties or nutritional profiles in order to assess whether unintended changes from the genetic engineering may have occurred. With the perspective of almost 20 years of experience with the regulation of genetically engineered plants and advances in genomics, it is time to evaluate the presumptions of the importance of unintended effects that can arise from genetic engineering and place them in the context of many of the changes that occur naturally in plant genomes and during conventional breeding.

The molecular biology of gene function

Dr. Mark Jordan, Agriculture and Agri-Food Canada, Ottawa, Canada

The effects of any given gene are determined by the structure of the gene and its flanking elements. The coding sequence of the gene specifies the protein that is produced. Different proteins will have different effects due to their structure and function with some proteins having many effects. These effects are in part regulated by other aspects of the gene structure such as promoter, 5' and 3' untranslated regions, Kozak sequence and exon/intron boundaries. Mutations in these sequences can alter the way a gene is regulated resulting in changes in protein levels, structure of the protein itself, or changes in temporal or physical location of gene expression. This altered regulation is a result of changes to binding sites of regulatory proteins. In addition, different plant species or genotypes of the same species may differ in genes for regulatory proteins thereby modifying gene expression resulting in unanticipated effects. In some cases these unanticipated effects are beneficial and such altered genes have been selected for by plant breeders. Prediction of unanticipated effects requires knowledge of function as well as of gene regulation. Knowledge arising from the complete genome sequence of crops will allow greater prediction of the effects of particular genes.

The molecular biology of plant genomic changes

Prof. Wayne Parrott, Institute for Plant Breeding, Genetics & Genomics, University of Georgia

Transgene insertion, or undetermined mutations that take place during the plant transformation process, have long been hypothesized to be able to reactivate unknown metabolic pathways, thus possibly leading to the production of toxic end products or their intermediates. As there is no test for unknown substances that may or may not be there, so their presence must be inferred from compositional analysis, and in some cases, whole food feeding studies with rodents or other animals.

One way to evaluate the possibility of reactivating these long dormant (and hence unknown) metabolic pathways is to 1) determine the extent to which insertions and other mutations occur during domestication and breeding, and 2) then determine whether any dormant metabolic pathways has been activated by such mutations during breeding. The advent of molecular tools has made it evident that SNPs are constantly occurring and accumulating at a steady rate. Transposon activity is more common than thought, and other phenomena, such as interorganellar DNA transfer, further contribute to the mutation load.

While cases where breeding has increased levels of known toxins are well documented, there are no reports on the spontaneous appearance of novel toxins previously unknown to exist in a given crop. The likelihood that DNA insertion or other mutation can awaken long dormant pathways is very remote, and there is no reason to believe the likelihood of such occurrence would be higher in a transgenic than in a conventionally bred plant.

What does it take to bring an ag biotech seed product to market?

Dr. Laura Privalle, Bayer CropScience, US

Not every product concept or idea produced through modern biotechnology (aka genetic engineering) results in a crop that gains commercial approval. To understand why, you must first understand the process. The development of a product starts with identifying an agricultural issue that can be resolved through biotechnology. The trait(s) are identified and the gene(s) isolated. After introduction of the gene(s) in the plant genome within a single cell, the cell must regenerate into a fertile plant that will transfer this trait to its progeny and not compromise the crop's agronomic performance. There are many steps involved; failure at any step or lack of commercial feasibility may result in the project's end. It is critical, therefore, to start with many ideas and produce many events to allow identification of the perfect event that meets product specifications. This is not unlike the path that classical breeders take to identify new varieties with desirable attributes. Examples will be presented where potential products have been dropped and the reasons will be discussed. Dossiers/applications are made to regulatory agencies only for products that are safe and registerable.

The roles of backcrossing and other breeding strategies in removing unintended effects caused by the transformation process

Dr. Phil Bregitzer, US Department of Agriculture – Agricultural Research Service

Creating transgenic cereals depends on mutagenic in vitro environments, suffers from imprecise transgene insertions, and generally employs selectable markers. Markers are used intentionally, while the creation of aberrant transgene loci and somaclonal variation (tissue culture-induced mutation) are unintentional, but all are related by the common thread of undesirability in the final product. Minimizing these changes should be the goal when making transgenic modifications. Backcrossing is a simple and powerful method to eliminate changes unlinked to transgenes, as shown in barley by the recovery of recurrent parent performance following transformation. Somaclonal variation can also be minimized by alterations of in vitro conditions. Transgene delivery methods more likely to deliver intact transgenes (e.g., *Agrobacterium* vs. biolistics) are advantageous, but don't address the problem of linked selectable markers and bacterial vector backbone sequences. Harnessing transposition as a tool to deliver "clean" transgenes can produce transgenic plants characterized by stable, highly heritable expression of the transgenic trait, and without selectable markers or vector sequences. Optimized delivery strategies combined with appropriate breeding and testing techniques can substantially reduce unintended changes in transgenic plants.

How much of a hazardous substance would have to be produced before it poses a risk?

Prof. Andrew Bartholomaeus, Therapeutic Research Centre, School of Medicine, Queensland University, & School of Pharmacy, Faculty of Science and Education, University of Canberra

The question reflects confusion around the utility of toxicology studies in GM risk assessment and the value of more robust approaches, particularly safety by design, and in the broadest sense is unanswerable, analogous to asking “how strong is a piece of string?” To address either question, boundaries must be placed around the subject to exclude hypothetical, extreme circumstances in favour of practical, scientifically plausible possibilities. The nature of the risk (population health or individual) and the type of hazard (toxicological, immunological, dietary) must also be delineated. For a high consumption staple food, modest levels of a low toxicity constituent, such as a trans-fatty-acid, may have significant population health implications without presenting a food safety issue in the traditional sense. Very small amounts of an extreme toxin, such as botulinum toxin in food, present an immediate risk, but there is zero risk that such a compound would be formed accidentally just from transgene insertion. Scientifically plausible substances resulting from transgene insertion are those known to be produced by the recipient or donor organisms or related to existing pathways within the recipient organism that are potentially perturbed by the inserted gene or its products. The potential hazards are dependent on the quantities of the substance(s) present in the edible portion of the food, the quantity of that food consumed, and the background levels of intake of the substance(s).

SPEAKER ABSTRACTS

WEDNESDAY, 15 JANUARY 2014 (morning)

HYPOTHESIS-DRIVEN SURVEY OF UNINTENDED CHANGES

Observed Incidences of Unintended Effects

The biology of naturally occurring insertions

Dr. Justin Vaughn, University of Georgia, US

Unintended effects on allergens in carrots and apples

Dr. Thomas Holzhauser, Paul-Ehrlich-Institut, Germany

GM Crop Case Studies

Single gene traits

Dr. Alan Raybould, Syngenta, UK

Hypothesis-driven evaluations of drought-tolerant corn in safety assessments

Dr. Elena Rice, Monsanto, US

Quality traits: altered starch composition in potato

Dr. Christine Wandelt, BASF, Germany

The biology of naturally occurring insertions

Dr. Justin Vaughn, University of Georgia, US

In an inbred species raised under controlled conditions, roughly 3 out every 100 plants from the same parent will suffer a mutation that deletes >3 base-pairs of DNA. This estimate excludes highly mutable microsatellite repeats. Insertions of DNA appear to occur at an equal or greater rate. This rate is likely to increase in outcrossing species in a natural environment. We have investigated the molecular mechanisms underlying these events. Deletions tend to occur as a result of induced double-strand breaks, while insertions result from either transposable element activity or aberrant repair pathways. Nonhomologous end-joining is the dominant pathway of break repair in plants and can result in the promiscuous incorporation of DNA fragments in the nucleus, as might be present during biolistic transformation. Intact transposable elements, which are known to be common source of rapid phenotypic divergence, are rarely observed at these break sites, indicating that isolated breaks do not generally activate such elements. In this regard, breeding techniques that encourage transposable element deregulation, such as cell culture or wide crosses, are more likely to introduce unintended effects than changes in genome structure around a site of transgenesis.

Unintended effects on allergens in carrots and apples

Dr. Thomas Holzhauser, Division of Allergology, Paul-Ehrlich-Institut, Germany

Allergies to food affect approximately up to 4% of the population in Western style countries. Carrot (*Daucus carota*), a vegetable of the Apiaceae family, and apple (*Malus domestica*), a fruit of the Rosaceae family, are among the most prevalent elicitors of allergic reactions to foods in Europe. Allergy to apple and carrot is frequently observed in relation to birch pollen allergy. Additional co-reactivity to mugwort pollen is frequently found in carrot allergy. Both allergenic foods contain pan-allergenic structures, such as proteins of the pathogenesis-related PR10-protein family or profilins. In addition, pan-allergenic isoflavone reductase-like structures are found in carrots and non-specific lipid transfer protein (nsLTP) in apple. Profiles of sensitization to individual allergenic components vary between different geographic regions in Europe, and the severity of allergic reactions may be linked to specific profiles of sensitization, such as to nsLTP. Moreover, allergen isoforms of the PR10-protein family may be inducible upon stress. Thus, genetic and environmental factors may impact the quantitative level of allergens that are present in apple and carrot. Studies on apples and carrots will be presented that describe quantitative differences of endogenous allergen and allergen isoform levels between cultivars and due to the conditions of cultivation, maturity, and post-harvest storage. The highlighted studies describe differences at the level of gene transcription and protein translation. Quantitative studies on pan-allergenic structures in relation to genetic and environmental factors help to understand the extent of unintended effects on allergens due to natural processes.

Single gene traits – Risk assessment as hypothesis testing: identifying essential data on unintended effects of transformation

Dr. Alan Raybould, Syngenta, UK

Regulatory risk assessments for import or cultivation of transgenic crops require studies that look for unintended effects of transformation. Many of these studies are comparisons of the phenotype of the transgenic crop under assessment with that of a non-transgenic comparator. Phenotypic comparison of two crops could be a huge task; for example, in theory one could compare the mRNA, protein, and metabolite profiles of the crops over their whole lives in multiple environments. Given potentially limitless data on unintended effects of transformation, how can we identify which data are essential for risk assessment?

Risk assessments test hypotheses that use of the transgenic crop will not cause unacceptable harm. Corroboration or falsification of such hypotheses helps decision-making. The structure and purpose of risk assessment helps in identifying essential data:

- We require data on potentially harmful differences, not all differences we might be able to detect.
- We require sufficient data to corroborate or falsify our hypotheses with enough confidence to make an acceptable decision.
- We do not necessarily need data on the crop being assessed; our hypotheses may be tested using data from other crops.

These arguments are developed using examples from risk assessments for Bt crops.

Hypothesis-driven evaluations of drought-tolerant corn in safety assessments

Elena Rice, PhD, Monsanto Company, US

Plant breeders have successfully improved maize grain yields despite sometimes very limited knowledge of the genes and gene networks that impact grain yield. Genetic gains in grain yield have been possible largely through 1) creating novel gene combinations that affected the complex gene networks and 2) careful selection process for yield. It is now clear that improvements in grain yield have been associated with significant genetic changes. Biotechnology can aid breeding with continuing increase in grain yield through introduction of specific genes with expected effects on endogenous pathways and phenotypes. However, the use of biotechnology-derived traits, especially to advance yield gains, has been viewed by some observers as a uniquely high source of “unintended effects.” The fact that this has proven not to be the case is driven by two basic considerations:

- i. Commercially viable biotech-traits are selected to introduce small incremental changes in well-established phenotypes known to be associated historically with yield gains in conventional crops; and
- ii. Commercial candidates undergo detailed analyses that include molecular characterization, physiological assessments, metabolite and compositional studies, and extensive field testing.

This presentation details how these considerations were applied to the development of the world’s first biotechnology-derived drought-tolerant corn, DroughtGard.

Quality traits: altered starch composition in potato

Dr. Christine Wandelt, BASF Plant Science Company GmbH, Germany

The presentation will address the safety assessment of potatoes genetically modified for low levels of amylose, which is one of the key starch components in the tuber. The focus will be on applying a hypothesis-based approach to guide the assessment on food and feed as well as environmental safety for a quality trait. Intended, as well as unintended, effects are being considered as part of the analysis of agronomic and phenotypic characteristics, compositional parameters, and in determining the interactions of the potatoes with the environment. Identified changes, both intended and unintended, are assessed for their biological relevance and potential to cause harm. Examples will be given for unintended though predictable effects due to intended changes in the metabolic pathway to achieve the trait. The magnitude of changes in the genetically modified potatoes will also be placed into perspective of the magnitude of changes caused by environmental influences in conventional potatoes.

SPEAKER ABSTRACTS

WEDNESDAY, 15 JANUARY 2014 (afternoon)

SAFETY ASSESSMENT OF CONSEQUENCES OF UNINTENDED CHANGES

Food / Feed Safety

Identification/assessment of possible unintended effect(s) on the overall allergenicity of GM plants

Prof. Jean-Michel Wal, AgroParisTech, France

Revisiting the dormant toxic pathway hypothesis; the use of genomics to evaluate the existence of dormant pathways

Dr. Sue Rhee, Carnegie Institution for Science, US

Food and feed safety of new plant varieties: how to assess unintended changes?

Dr. Esther Kok, RIKILT Wageningen UR, Netherlands

Environmental

Problem formulation

Prof. Alan Gray, Centre for Ecology and Hydrology, UK

Can unintended effects lead to increased weediness / invasiveness?

Dr. Paul Keese, Office of the Gene Technology Regulator, Australian Government

Studying unintended effects confuses the nontarget risk assessment

Dr. Jörg Romeis, Agroscope, Institute for Sustainability Sciences ISS, Switzerland

Identification / assessment of possible unintended effect(s) on the overall allergenicity of GM plants

Prof. Jean-Michel Wal, AgroParisTech, France

The pre-market assessment of allergenicity of GM plants (GMP) should address 2 issues which are currently assessed separately. One is linked with the source of the transgene, i.e., the allergenicity of the newly expressed protein(s). The other is linked with the host, i.e., the possible increased allergenicity of the whole GMP when the recipient is a “common food allergen.” In such case, a qualitative and/or quantitative modification of the pattern of expression of endogenous allergens may result from an unintended effect of the genetic modification. In order to perform a relevant comparison of the allergen repertoire of the GMP versus its conventional comparators, the EU guidance recommends to include “key allergens” in the comparative compositional analysis which is a pillar of the food/feed safety assessment. However, other factors may also indirectly result in an uncertainty on the allergenicity of foods derived from the GMP as compared with conventional ones, e.g., with regards to the actual exposure of particular groups of at-risk consumers.

Based on some case studies, we will present and clarify when and how the evaluation of the overall allergenicity has been performed in GMPs assessed so far. We will then discuss possible perspectives in the evolution of concepts, criteria, and methodology that could be applicable for GMPs to be developed in the near future.

Towards rational assessment of changes in small molecules by leveraging genomics and metabolic network modeling

Seung Yon Rhee, Carnegie Institution for Science, Stanford, CA, USA

Metabolism is a complex phenomenon where thousands of individual reactions are catalyzed by enzymes and regulated at many levels ranging from transcriptional changes of the enzymes to allosteric regulation of the enzymes by metabolites. Metabolism is coordinated at many levels of organization ranging from intracellular compartments to tissues, organs, and whole organisms. Rational and quantitative assessment of metabolic changes in response to genetic modification is an open question and in need of innovative solutions.

We developed a computational pipeline to generate the genome-scale metabolic networks of plant species. The pipeline includes automated prediction of enzymes, reactions, and metabolic pathways from a genome sequence and is highly accurate, transparent, scalable, and flexible. It also includes a semi-automated pipeline to validate the predicted pathways, which has cut-down manual validation time from approximately 1 month to 1 hour. Using the pipeline, we have generated and publicly released metabolic networks of 16 plant and 1 algal species, with 13 new species currently under development.

Using the genome-scale metabolic network of *Arabidopsis thaliana*, we tested the effect of single genetic perturbations of 136 genes (129 knock-out and 7 overexpression lines) by comprehensively profiling the metabolites using 11 analytic platforms including GC-MS, LC-MS, and ESI. Comparison of the metabolite profiling in the network context reveals that the genetic perturbations cause specific and diverse changes to the metabolic network and reveal patterns that are indicative of the functions of the genes that are perturbed.

Emerging tools and resources such as genome-scale metabolic networks, quantitative network modeling, and metabolomics may help assess the effects of genetic modification on metabolism and may facilitate rational assessment of unintended effects of genetic modification on metabolism.

Food and feed safety of new plant varieties: how to assess unintended changes?

Dr. Esther J. Kok, RIKILT Wageningen UR, Wageningen, the Netherlands

There is global consensus on the basic approach for the assessment of unintended side effects of a genetic modification. This assessment will include in all cases a phenotypic and agronomic comparison between the new plant variety and a near comparator that is already on the market and considered as safe. In addition to this for genetically modified (GM) plant varieties, a molecular characterisation and a compositional analysis will form part of the safety assessment. For current GM varieties, this will usually be considered sufficient to conclude on potentially present unintended side effects of the genetic modification. Animal feeding trials with whole foods will in general be too insensitive to provide relevant information with relation to the safety of the new plant variety.

New plant breeding programmes show a trend to the use of a broader range of molecular biological breeding techniques to achieve more complex genetic alterations within the framework of shorter breeding programmes. As a consequence of the shorter duration of the breeding programmes, there will be less time to identify unintended side effects of a breeding strategy. Therefore, it may become increasingly interesting to have more informative analytical methods, such as omics, available that can screen the plant's physiology for unintended effects of the plant breeding programme.

Problem Formulation

Prof. Alan Gray, Centre for Ecology and Hydrology, UK

Because it deals almost exclusively with the phenotype, Environmental Risk Assessment (ERA) for genetically modified (GM) crops considers all plant traits which may have been altered by the transformation, and which have been characterised during the long process of growing trials and product development. ERA thus embraces both intended and 'unintended' effects of transformation. The subject matter of ERA is the collateral effects of cultivating a particular GM crop on a large scale. Problem Formulation provides a way of identifying which of those effects may cause harm and devising a plan to analyse the risk of the harm being realised. It does so by recognising environmental protection goals that may be impacted by cultivating the GM crop, envisioning ways in which they may be negatively affected, and formulating methods for testing the likelihood of such negative effects occurring. Some outcomes of large-scale cultivation, whether negative or beneficial, in a GM or non-GM crop, may be 'unintended' in the sense that it was not the grower's deliberate intention that they should happen. Variable in time and space, these contingent effects are fundamentally different from any intrinsic properties of a GM plant, and are usually captured by the process of problem formulation.

Can unintended effects lead to increased weediness / invasiveness?

Dr. Paul Keese, Office of the Gene Technology Regulator, Australian Government

Can unintended effects lead to increased weediness/invasiveness? Possibly. A common approach is to identify any changes that have occurred and then determine if they are significant: namely, do they fall outside the parameters of substantial equivalence? This can be daunting if we consider unintended effects in the broadest sense as any molecular or phenotypic effect, predicted or unpredicted. Alternatively, we can limit our search to only those characteristics that are relevant to weediness/invasiveness. In Australia, these characteristics have been based on practical experience with more than 1200 major environmental and agricultural weeds in diverse landscapes, including alpine regions, wetlands, rainforests, savannas, and deserts. In response, weed scientists have produced a robust, simple, and validated post-border weed risk assessment protocol that can be readily applied to any plant. This approach examines those characteristics of plants that affect spread and persistence (invasiveness) by considering a plant's ability to establish, survive, reproduce, and disperse. In addition, the assessment examines effects that give rise to adverse impacts depending on the land management goals of the likely receiving environment, such as agricultural production or nature conservation. The relevance of this approach to unintended effects in GM plants will be discussed.

Studying unintended effects confuses the nontarget risk assessment

Dr. Jörg Romeis and Dr. Michael Meissle, Agroscope, Institute for Sustainability Sciences ISS

A common concern among regulatory authorities is the potential for a genetically engineered (GE) plant to have adverse impacts on valued nontarget organisms. This risk is particularly evident for GE plants that express insecticidal proteins. The environmental risk assessment (ERA) is designed to answer specific questions about the potential risk of introducing such plants into the environment. This requires the formulation of testable and relevant risk hypotheses about the likelihood of harmful effects of cultivating the GE plant. It is essential to design robust studies that provide reliable information and result in a high level of confidence in the conclusion drawn. If the novel insecticidal compound has been identified as the stressor of concern, the hypothesis of no effect can effectively be tested under worst-case exposure conditions in the laboratory. To address unintended effects in a study, GE plant tissue is used as test substance, often in multi-trophic experiments. If effects are reported, they might be caused by the insecticidal compound, due to transformation-related effects, or effects derived from the breeding/selection process. The results from such studies can add confusion to the ERA as they are often misleading and the confidence in the conclusions is low.