International Conference On
New Plant Breeding Molecular Technologies - Technology Development And Regulation

October 9-10, 2014, Jaipur, India

Report

Department of Biotechnology
Govt. of India

Sponsored By

Department of Biotechnology (DBT),
Ministry of Science and Technology, GOI
&
International Life Sciences Institute-India

In collaboration with

Center For Environmental Risk Assessment
ILSI Research Foundation
&
CropLife Asia

INTERNATIONAL CONFERENCE ON
NEW PLANT BREEDING MOLECULAR TECHNOLOGIES – TECHNOLOGY DEVELOPMENT AND REGULATION

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REPORT

सत्यमेव जयते

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Preface

The New Plant Breeding Technologies (NPBT) have technical advantages over GMOs and have shown good results. Herbicide tolerance in rape-seed and maize, fungal resistance in potatoes, drought resistance in maize, scab resistance in apples and potatoes have been proven with the use of NPBTs. The future of these technologies, however, depends on the decision on how the resulting products have will be regulated.

Extensive studies have been made, for instance, by working groups set up by European Commission and FSANZ. But the scope of regulation is still not well defined. NPBTs do not necessarily involve transfer of entire genes from one organism to another. There is a view that NPBTs should be evaluated concerning the new traits and the resulting new products instead of the technique used to create the new variety. Therefore, NPBTs may not fully qualify as GM and some of the technologies may be exempt from regulation and some others subject to regulation which is simple and takes much less time. Perhaps different approaches will have to be adopted for different technologies.

ILSI-India and Department of Biotechnology, Ministry of Science and Technology, GOI, sponsored a two-day "International Conference on New Plant Breeding Molecular Technologies – Technology Development And Regulation" in Jaipur, India on October 9 and October 10, 2014. The Conference was Co-sponsored by ILSI Research Foundation CERA and Croplife Asia. The Corporate and Institutional Co-sponsors included: Bayer BioScience Pvt. Ltd., BASF India Ltd., Monsanto Holdings Pvt. Ltd., National Seed Association of India, PHI Seeds India Pvt. Ltd., Rasi Seeds Pvt. Ltd., Dow Agro Sciences India Pvt. Ltd., and Ankur Seeds Pvt. Ltd.

It was for the first time that a Conference on NPBT was held in India. The participants included: policy makers, premier institutions engaged in agricultural research and select plant breeders and industry.

The meeting discussed the emerging scientific and technological trends in product development and analysed the emerging elements of regulatory frameworks for food and environmental safety assessment of NPBTs. This Conference was held in two parts. The first day discussed the technical aspects of NPBTs which revealed how close some of the technologies are to GM and how close they are to traditional plant breeding. The second day discussed guidance documents in OECD and EFSA and the regulatory systems in major countries.

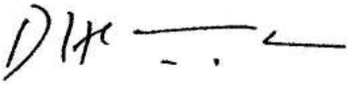
The Welcome address was delivered by Mr D H Pai Panandiker, Chairman, ILSI-India. Dr S R Rao, Advisor, Department of Biotechnology delivered the Opening Address. The technical sessions were chaired by Dr. S R Rao; Dr M S Sheshhayee, Professor, Department of Crop Physiology, University of Agricultural Sciences, Bangalore and Dr B Sesikeran M.D., Chairman, Regulatory Committee For Genetic Manipulation (RCGM).

The Conference was addressed by a number of experts from Argentina, Australia, Canada, China, Europe, India, Japan, South Africa, USA, and OECD.

It was pointed out at the Conference that NPBTs can have three distinct advantages:

- First, the final product does not contain genes that are foreign to species unlike in GMOs and will, therefore, be acceptable to the public.
- Second, the time taken to develop the new products with NPBT will be much less than the time taken by conventional breeding. This will enable considerable saving in costs.
- Third, the regulatory approval procedures if simplified or eliminated will also reduce time and cost to the breeders for pre-commercialization.

Like any other technology, it is the economic advantage of NPBTs that will accelerate their use. Further, it is necessary that there should be a common regulatory approach by all countries. Different regulations by different countries will result in trade disruption and lead to lop sided development of the new technologies. It was underlined at the Conference that some convergence in the outlook for NPBTs should be brought about to facilitate the next phase in policy making in India and possibly other countries. If the regulation facilitates adoption of these technologies, the commercial adoption of these technologies will be fast and wide and promote rapid agricultural development.



(D. H. Pai Panandiker)
Chairman, ILSI-India

SESSION-1

Status Of Science And Technology Advances In Agricultural Biotechnology

Chair

Dr S R Rao, *Advisor, Department Of Biotechnology Ministry of Science and Technology, GOI, New Delhi*

Dr M S Sheshhayee, *Professor, Department Of Crop Physiology University Of Agricultural Sciences, Bangalore*

• Overview – Status Of Science And Technology Advances In Agriculture Biotechnology

By Dr. Peter Kearns

The phrase New Plant Breeding Techniques (NPBTs), which has been applied to these techniques, is one of convenience. They are techniques that can be (or have been) applied in sectors other than plant breeding and at the same time, not all are new. However, they have the potential to make a major contribution to the development of new crop varieties, in fact, already these are examples of how some of these techniques have been used to date. But the future use of a number of these techniques will depend in part on whether and how they are regulated in jurisdictions around the world. All of these techniques depend on sophisticated molecular techniques which to a greater or lesser extent many resemble 'traditional transgenesis' in one way or another. Consequently, there is the possibility that some of these techniques may be subject to existing 'GMO' legislation in one or more jurisdictions.

Current authorisation procedures for GMOs are typically an expensive and laborious process, so the future commercial development of plant varieties derived through the use of NPBTs may be prohibitive. There are four categories of NPBTs:

(i) Those which achieve specific mutations at a targeted site in the genome

- **Oligonucleotide Directed Mutagenesis (ODM):** Applies small mutations to a specific site in the genome.
- **Site Directed Nucleases (SDNs):** Targets a specific site in the genome for small mutations or the insertion of a stretch of DNA and include:
  - ***Zinc Finger Nucleases (ZFNs)***
  - ***Mega Nucleases (MNs)***
  - ***TAL Effector Nucleases (TALENs)***

(ii) Those which result in an end-product free of transgenic material

- **RNA-Dependent DNA Methylation (RdDM)**
  Applies epigenetic changes in the genome: the expression of specific genes can be changed without affecting the genomic sequence.
- **Reverse Breeding**
  Involves an intermediate step were foreign genetic material is present to supress meiosis. No foreign genetic material is present in the end product.
- **Accelerated Breeding**
  In an intermediate step a transgenic approach is used to shorten the juvenile phase of a tree, hence speeding up the breeding process. No foreign genetic material is present in the end product.

(iii) Those which insert genetic material derived from sexually compatible relatives;

- ***Cisgenesis and Intragenesis***
  Introducing genetic material from sexually compatible relatives.

(iv) Those which only affect targeted tissues

— ***Grafting on GM Rootstock***
Introduces transgenes only in the rootstock of a tree.The scion grafted on the rootstock remains free of transgenic DNA.

— ***Agro-Infiltration*:**
Introduces transgenes transiently in a targeted tissue of the plant. It includes:

- **Agro-infiltrations sensu stricto**
- **Agro infection**
- **Floral Dip**

Some Regulatory Examples Of NPBTs:

Rapid Trait Development System (RDTS™) is a SDN by Cibus; a herbicide Canola variety has been approved in Canada; USDA-APHIS, indicated they resemble plants developed through classical mutagenesis. British advisory body ACRE considered Cibus' Canola plants products of classical mutagenesis.

EXZACT™ Precision Technology by DowAgrosciences; relies on zinc fingers; maize varieties have been with decreased levels of the anti-nutrient phytase; USDA-APHIS indicated that plants that contain targeted deletions applied by the cells native repair mechanisms are not considered to be regulated articles since they contain no transgenic sequences.

Cisgenesis apple (from Wageningen) regarded by USDA-APHIS as a regulated article as it involved the use of *Agrobacterium*.

OECD has set up a Working Group on "Harmonisation of Regulatory Oversight in Biotechnology (WG)". The Working Group is consideringemerging issues in harmonization and it is discussing new products and techniques used to produce them. As regards NPBTs the Working Group is gathering information on NPBT and country experience. A Workshop was held and a Questionnaire circulated to gather country information. The responses will help in understanding the products developed using NPBT, understand the techniques, share practical experiences with ERA of products developed using NPBT, identify any new safety issues associated with products and/or with techniques themselves and identify any differences in approaches to ERA between countries. The responses received have revealed that most countries are considering NPBTs. However, most developments are still in research phase.

The most mentioned techniques in the responses are:*Cisgenesis/Intragenesis; ODM; and SDN applications. The m*ost mentioned crops are:*Apple; Potato and Maize. The m*ost mentioned traits are:*Fungal resistance and Herbicide tolerance.*

There is a need for regulatory certainty, including at the international level, if these techniques are to be used without disruptive effects to trade in food and agricultural commodities.

Session-1.1: Mutagenesis – Site Directed Nucleases

A) Zinc Finger Nucleases

By Dr. Matthew Cahill

New breeding approaches focused on targeted genome editing are being developed toaccommodate an increasing demand for complex multi-trait products in the agricultural industry.

The EXZACT™ Precision Technology (Engineering Plant Genomes with ZFNs for Trait Product Development) is a Dow AgroSciences' proprietary technology that facilitates precise changes in plant genomes including point mutations, DNA deletions and targeted gene additions. The technology, based on Zinc Finger Nucleases (ZFNs), is being developed for precision gene addition and gene stacking for production of multi-gene trait products in several crops.

Technology Platform provides multiple methods of genomeengineering using ZFNs:

MUTATIONAL APPLICATIONS	
EXZACT™ Add	Targeted Gene Addition
EXZACT™ Delete	Targeted gene deletion / mutagenesis
EXZACT™ Edit	Targeted Editing (rewriting /mutation) of Genomic Sequence

EXZACT Delete is a targeted mutagenesis technique: EXZACT Delete is simply a rapid, reliable and predictable process for generating targeted mutations in plants compared to traditional breeding and mutagenesis processes.No genetic material is introduced into the genome of the host via genetic recombination. Mutations are known and pre-determined: Unlike random mutagenesis techniques, ZFN are designed to generate mutations only at the predetermined targeted DNA location. ZFNs are absent in final product. No foreign DNA in final product, only native plant DNA and End product is the same as conventional mutagenesis.

EXZACT Edit is a targeted mutagenesis technique: EXZACT Edit is simply a rapid, reliable and predictable process for generating targeted mutations in plants compared to traditional breeding and mutagenesis processes.Mutations are known and predetermined. Unlike random mutagenesis techniques, ZFN are designed to generatemutations only at the predetermined targetedDNA location. ZFNs are absent in final product. No foreign DNA integrated in finalproduct, only native plant DNAand End product is the same asconventional mutagenesis.

EXZACT™ Targeted Gene Addition technique has following benefitsfor trait product development compared to conventional methods:

- Gene addition to a specific genetic locus (safe harbor/high performance)
- *Higher quality events (minimal unintended side effects)*
- *Increased probability of success*
- Targeted analytics, efficient event sorting
- *Reduced cycle times*
- Reuse of a genetic locus, targeting reagents, analytics for new product development
- *Accrued cost savings*
- New gene stacking options for multi-trait product development.

Mutational products have a long history of safe use. Over 3,200 cultivars have been used commercially and are globally adopted. SDNs continue the history of improving crop development through modern targeted mutational applications. SDN-1/-2 allow, for the first time, mutations to be targeted to a specific, desired location in the plant genome.

Traditional gene editing technique involves DNA inserts / mutations introduced randomly in genome. Screening for desirable product is expensive and

time-consuming. As against this EXZACT: DNA changes are at a pre-determined, targeted location. They result in higher quality events with minimal unintended side effects and higher probability of success as also reduced time and cost for trait development.

Conclusions:

- NPBTs are innovative improvements and refinements of existing breeding methods. It is the characteristics of the plant (product) that determines its safety
- The public, private and scientists alike have significant opportunities to employ NPBTs in their breeding programs.
- The adoption of these technologies will be highly dependent on the regulatory requirements imposed on the products produced through NPBTs.
- Non-scientific, unnecessary and non-harmonious oversight / requirements will result in...
  - o *Undue, Costly Burdens.*
  - o *Stifle Innovation.*
  - o *Prevent The Uptake / Limit use of NPBTs.*
  - o *Disrupt Trade.*
  - o *Loss of Public Confidence.*

B) Recent Advances In Precision Genome Engineering: Implications For Crop Improvement

By Dr. Amitabh Mohanty

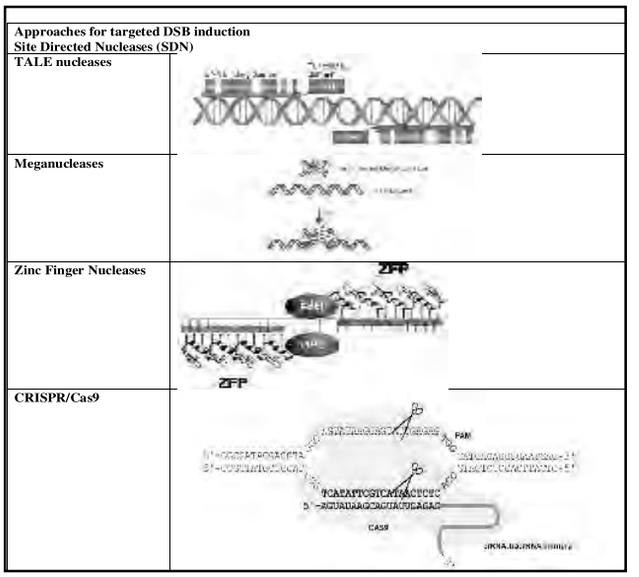
Recent advances in development of tools for precision genome engineering, such as site directed nucleases (SDN) have opened up a plethora of opportunities for plant breeding and genetics. These new breeding technologies (NBTs) facilitate targeted and precise modification of the genome, thereby imparting ability to design and develop a new generation of crops with improved traits. Application of these new breeding technologies has potential to result in significant gains in the area of crop improvement.

C) Targeted Genome Engineering Research

By Dr. Ana Atanasova

Crop improvement by breeders, including the development of new traits, necessitates the continuous creation of new biological diversity, either through crosses of existing plant lines (new combinations of existing genetic variation) or through the generation of new genetic variation through mutagenesis. Through plant genetic engineering, it also became possible to access and introduce into plants new traits not achievable through breeding and mutagenesis.

With the development of the targeted genome engineering technologies, it is now feasible to rationally design the genetics of crops. The basis of current targeted genome engineering approaches is the capacity to induce a DNA double strand break



at a selected location in the genome where the modification is intended. Directed repair of targeted induced breaks allows for targeted genome engineering. Different approaches can be used to achieve targeted breaks, including ZFN, TaleN, Meganucleases and CRISPR/Cas. The application of the targeted DNA double strand break induction and repair technologies to plant improvement requires efficient DNA delivery and cell tissue culture tools to be in place.

Custom designed meganuclease have been used to create a DNA double strand break at a selected location in the cotton genome, and have demonstrated the precise repair of the site through the introduction of functional herbicide tolerance genes.The applications of targeted genome engineering range from targeted non-specific mutation, to precise native gene editing and precise insertion of transgenes.

The precision and versatility of the targeted genome engineering tools will enable their broad application and is a valuable addition to the breeders toolbox for crop improvement.

Site Directed genome engineering applications include:

- Gene function disruption
  - ***gene function discovery***
  - ***trait development e.g. disease resistance***
- Precise integration of sequences
  - ***gene stacking***
- Replacement of endogenous sequences
  - ***gene swapping***
  - ***Improvement of alleles***
- Deletion of sequences
  - ***marker removal***

TALEN-based genome engineering has allowed production of Bacterial blight resistant rice, Powdery Mildew resistant wheat, and Improved soybean oil quality.

Examples of targeted DSB induction and precise repair by HRare:

- Introduction of specific AA substitutions in the acetolactate synthase genes in tobacco conferring herbicide resistance (e.g. Townsend et al. Nature, 2009).
- Targeted disruption of the IPK gene in maize resulting in reduced seed phytate content (Shukla et al. Nature, 2009).
- Targeted molecular trait stacking in cotton to allow linked transmission of transgenes (D'Halluin et al. Plant Biotech J. 2013).

Conclusions:

Directed genome engineering through targeted DSB induction and repair enables precise genome modification.

- It can be applied for both small and large modification.
- It provides a powerful tool for gene function discovery for developing more effective traits for cropsfor trait stacking closed to a preferred locus for removal of undesired sequences.
- It requires efficient DNA delivery methods and efficient cell and tissue culture procedures for many crops still to be further developed or improved.

Session-1.2: Other Technologies

D) The Importance Of Cis- And Intragenesis For (Classical) Plant Breeding

By Prof Evert Jacobsen

Plant breeding is a multidisciplinary scientific activity with tool development as driving force. It is clear from history that availability of genetic variation and selection methods are bottom lines for variety development. The genetic source of traditional plant breeding is restricted to domestication of traits from the so-called 'breeders' gene pool', consisting of crossable sources.

Gene cloning and genetic transformation broadened the available genetic variation to genes from all living organisms. The so-called new genes in genetically modified plants, consist of transgenes, with (chimeric) genes from outside the 'breeder's gene pool', coding for example for herbicide or insect resistance. Transgenic plants needed additional biosafety rules such as Directive 2001/18EC in Europe. However, these rules are, for example, not needed after transformation of the 4, non-engineered, natural four rol-genes from wild-type Agrobacterium rhizogenes. Genetic modification is the first technology that has not been widely accepted in the world by NGOs and consumers. Strict applications of GMO-regulations in the EU are obstructing the development of GM-varieties. These regulations were originally focused on the modification process and on transgenes, originating from non-crossable species.

In the meantime, cloned cisgenes, natural dominant genes from 'breeders' gene pool', and intragenes which are chimeric genes consisting of only functional parts of genes from crossable species, are available. After marker-free transformation of these genes, without using, for example, nptII coding for kanamycin resistance, are providing cisgenic or intragenic crops, respectively, which extend plant breeding with cloned genes coding for traditional traits. From long-term experience, it is clear that traditional breeding with the 'breeders' gene pool' has a history of safe use. Different scientific committees concluded that cisgenic plants are as safe as traditionally bred varieties and that intragenic crop still need the answer of a few additional safety questions.

Intragenesis with, for example, intragenes causing gene silencing, is a powerful tool for mimicking loss of function mutations which are normally recessive. An important observation is that gene silencing in this way is changed in a dominant trait which can be applied directly in existing varieties of all kind of crops. Examples are amylose free starch and bruising resistance in potato by silencing genes coding for GBSS or PPO, respectively. Recently the phenomenon of disease resistance by silencing of susceptibility genes is in focus causing durable disease resistance. Many of these genesare known in Arabidopsis and orthologs are found in all kind of crop plants. In potato silencing of stDND1is not only causing resistance to late blight but also to powdery mildew. So, intragenesis is enabling this new type of disease resistance also in complex crops like tetraploid potato and apple.

Cisgenesis is also a powerful new tool for plant breeding with natural non-engineered genes coding for dominant traits. Examples are: (1) durable resistance to potato late blight and apple scab by R-gene stacking ; (2) the new possibility to come to stacking of monogenic resistance alleles in wheat; (3) engineering of restoration of cytoplasmic male sterility by cloned restorer genes and of altering gametophytic incompatibility by introducing additional S-alleles; (4) increasing phytase activity by gene dosage effect in barley; and (5) the possibility of changing hormone metabolism in (fruit) trees leading to important morphological alterations.

In near future, because of availability of many more cisgenes and intragenes, it is expected that the possibilities of cisgenesis and intragenesis will increase rapidly as next step in plant breeding. The legal interpretation from cisgenesis could become: treatment as non-GMO.

E) Reverse Breeding

By Dr. T.G. (Erik) Wijnker

In 2012 feasibility of reverse breeding has been shown. Reverse Breeding is, a plant breeding technique that, among others, allows systematic generation of chromosome substitution lines: plants in which one or more chromosomes of one accession have been replaced by chromosomes of another accession. Such chromosome substitution lines are great tools for the systematic dissection of complex traits. However, the plants' nucleus does not comprise the whole genome, as also the organelles (mitochondria and chloroplasts) have genomes encoding genes. To allow the study of interactions between the nucleus and organelles recently an easy method has been developed to generate Arabidopsis cybrids: plants that carry the nuclear genome of one plant and the organelles of any other accession. Taken together, these techniques present a unique "toolbox" that provides near full versatility in combining the building blocks of the Arabidopsis genome: its chromosomes and organelles. It is now possible to generate Arabidopsis plants with near any chromosomal composition of the nucleus, and combine such a nuclear genome with any cytoplasmic genome of choice. This allows for completely new strategies to research complex traits. One of these is heterosis: the phenomenon of a heterozygote outperforming its homozygous parents in growth and yield.

Until now, hybrid performance (and heterosis) is usually studied using a reciprocal hybrid, which has shown that maternal, paternal and cytoplasmic effects may all affect hybrid performance. But reciprocal hybrids do not allow for precise testing of these effects independently, as all of these effects may change together in reciprocal crosses. With the above described tools it is these effects can be assessed separately.

F) RNA-Based Tools In Plant Breeding

By Dr. Alexios Polidoros

Facing the world's population rapid expansion with the constraints of limited agricultural land and available inputs (water, fertilizers, plant protection chemicals, energy and machinery), plant breeding must make use of the most advanced technologies in order to increase agricultural production and accomplish its imperative mission to feed the world. RNA-based technologies and tools have played a critical role in understanding organism biology and providing the means to develop science-based methodologies in plant and animal breeding. These technologies encompass transcriptional characterization of genes and genomes, biodiagnostic analysis of genetic diversity, regulatory transcript involvement in plant defense and development, and implementation of RNA tools in genetic engineering and biotechnology.

For breeding elite varieties using transcriptional characterization of genes and genomes, following steps have to be undertaken:

- Ask questions about relationships between specific genes and a condition.
- Use identified gene(s) in genetic modification of target varieties.
- Identify potential ideal genotypes and test their performance.
- Learn the expression 'signature' of different genotypes in a condition.
- Classify genotypes according to their 'signature'.
- Make functional hypotheses about the ideal genotype in the condition.

The applications include: Virus Resistance, Insect Resistance, Pesticide Tolerance, Nutritional Value, Plant Architecture and Gene Stacking (Pyramiding).

RNA-tools have traditionally provided the means to analyze transcriptional characteristics at the single gene level and very efficient methodologies, such as Northern hybridization succeeded by real time RT-PCR, provided the keystone of modern transcriptomics. The study of the complete set of RNAs (transcriptome) encoded by the genome of a specific cell or organism at a specific time or under a specific set of conditions is known as Transcriptomics. The succession to 'omics' technology was enabled by large-scale measurements and aims atthe collective characterization and quantification of all the transcribed genes in a given sample of an organ or tissue at a specific condition. Functional genomics, in particular, is expected to have a tremendous impact in molecular breeding, since knowledge of functional relationships of specific allele combinations can provide the guidelines to design and select elite genotypes.

Transcriptome includes:mRNA, tRNA, rRNA as also ncRNAs (non-coding RNAs), miRNA, siRNA, piRNA, snoRNA and many more being discovered.

Another important RNA-based tool is related to detection of genetic variation in transcript sequences. The so-called Expressed Sequence Tags (ESTs) have played a pivotal role in molecular marker development for detection of genetic variation and gene mapping. Moreover, bio diagnostic RNA-tools include applications in RNA-virus detection and plant protection.

A recent advancement in the RNA world was the discovery of regulatory RNAs that are involved in any aspects of defense and development. These, usually small RNA molecules -microRNA (miRNA) and small interfering RNA (siRNA) – are central to RNA interference (RNAi). RNAi is a process in which RNA molecules inhibit gene expression in a sequence-specific way. Exploitation of this feature has enabled molecular breeders to produce transgenic genotypes expressing double-stranded RNA (dsRNAs) with a sequence in a complementary to a gene of interest, where it is recognized as exogenous genetic material and activates the RNAi pathway. Using this mechanism, breeders can cause a drastic decrease in the expression of a targeted gene. Besides their role in transcriptional silencing dsRNAs are processed to 21-24 nucleotide small interfering RNAs (siRNAs) and guide methylation of homologous DNA loci. This RNA-directed DNA methylation (RdDM) is an epigenetic process first discovered in plants that provides breeders another RNA-tool for targeted interventions in genomes in order to produce elite genotypes.

Biotechnological applications of RNA-tools in plant breeding are diverse, not restricted to transgenic technologies and can be proved equally useful in advancement of basic knowledge of organism function, and development of more efficient methodologies for superior genotype selection.

Sesssion-1.3: Case Studies

• Rice Seed Production Technology (SPT Rice)

By Dr. Valasubhramanian Ramaiah

This technology utilizes naturally-occurring rice gene to produce male sterile parent lines and is an efficient process for male -sterile parent seed increase. SPT Rice provides the solution to better Hybrid Rice in following ways:

- An innovative breeding and hybrid seed production process.
- Eliminates germplasm dependency.
- Improves ability to breed for better hybrids.
- Increases product quality and yield potential.
- SPT process transgenes are reliably and predictably absent in male-sterile female lines or hybrid products.
- Compatible with current hybrid rice production practices.
- Proven and deployed technology in Corn.

• RNAi For Nematode Management

By Dr. Umarao and Prakash Banakar

Globally plant parasitic nematodes are responsible for considerable yield losses amounting to an estimated $157 billion annually. Recognising the limitations of current nematode management practices, there is a pressing need to develop environmentally suitable and sustainable new generation management approaches tailor-made for controlling various plant parasitic nematodes, particularly for the most damaging species of root knot and cyst nematodes. One such approach is by using the genomic information of an organism for exploring genes involved in vital pathways of nematode life and disease cycles that could serve as potential targets for designing transgenic plants with required field tolerance or development of novel nematicidal molecule.

Recent efforts in sequencing of free living nematodes-Caenorhabditis elegans, C. briggsae- and several plant, animal and human parasitic nematodes has resulted in availability of 21 whole genome sequences, EST derived transcriptomes of 62 species comprising more than 679,480 ESTs and 250,000 genes. The momentum also resulted in the completion of sequencing of five important plant parasitic nematodes. There are different avenues for engineering plant resistance and RNA interference is one of them. With the recent advent of gene expression control via small interfering RNA (siRNA) and micro RNA (miRNA) molecules, RNAi based transgenics is becoming the trend to suppress the menace of plant parasitic nematodes (PPNs).

Induction of RNAi by delivering double-stranded RNA (dsRNA) has been very successful in the model non-parasitic nematode, C. elegans, while in PPNs, dsRNA delivery was accomplished by soaking the nematodes with dsRNA solution mixed with the neurotransmitters like resorcinol, octopamine, serotonin etc. Using in vitro dsRNA delivery approaches, down regulation of various housekeeping genes led to reduced parasitic ability, delayed egg hatching, impaired motility, and ability to locate and invade roots, demonstrated in root-knot, cyst, lesion, pine wilt and burrowing nematodes. The success of the in vitro dsRNA ingestion and down-regulation of the target genes inspired the in planta delivery of dsRNA to the feeding nematodes. The most convincing success of in planta delivery of dsRNA to feeding nematodes came from root-knot nematodes.

Limitations of existing nematode management practices have paved the way for RNAi based approach for nematode suppression. Peptide based transgenics produce functional proteins which could have off target effects on non-target organisms but RNAi based transgenics is superior to that as it does not produce any functional proteins and targets organism in sequence specific manner. Although RNAi based transgenics are still in preliminary stage but it offers novel management strategy for the future.

In this endeavour, the IARI laboratory has undertaken identification and validation of several gene targets in M. incognita involved at various stages of disease cycle comprising nematode infection, development and reproduction. These genes have been functionally validated by in vitro RNAi and potential ones expressed inplants for developing nematode resistant brinjal and tobacco.

• RNAi - An Emerging Technology For Developing Virus Resistance In Crop Plants

By Dr. K. Poovannan,Dr. R.M. Packialakshmi,
Dr. M. Saravanakumar and Dr. V. Subramanian

RNA interference (RNAi) is an antiviral defence mechanism naturally present in plants. Development of transgenic plants with virus resistance through RNAi approach is expected to be highly useful for controlling plant viruses which cause potential yield loss in commercially important crops. This is a homology dependent, post transcriptional gene silencing (PTGS) mechanism that mediates silencing of essential functional sequences of target virus. Though there are several reports on RNAi for silencing, utilization for developing resistance against especially DNA viruses in crops is limited. Plant virus belongs to the family Geminiviridae are circular single stranded DNA virus which are affecting many important crops worldwide. Cassava mosaic virus, Okra yellow vein mosaic virus (OYVMV) and Enation leaf curl virus (EnLCuV) and Cotton leaf curl virus (CLCuV) are some of the major plant viruses causing yield loss taken as case study to develop resistance against them since breeding for resistance by conventional approach has limitations.

The PTGS mechanism is exploited in research projects to make hairpin RNAi constructs to develop virus resistance by targeting/silencing number of functional open reading frames of the viruses necessary for its defence. Following developments have taken place:

- Several Transgenic Cassava plants have been developed against Indian Cassava Mosaic Virus (ICMV) and Sri-Lankan Cassava Mosaic Virus (SLCMV) using viral resistant gene(s) separately.
- Cotton Leaf Curl Virus Disease (CLCuVD) caused by CLCuV (Begomovirus) is devastating and becoming serious disease in cotton every year. It causes yield loss up to 80% (Mansoor et al., 2003).RNAi strategy is being used to overcome CLCuV infection. Currently 60 independent transgenic lines have been transformed with CLCuV RNAi 1 gene construct.The Virus challenging assay in transgenic cotton lines will be carried out as per guidelines given by DBT, Government of India.
- Breeding for resistance has limitations in case of Yellow Vein Mosaic Virus and Enation Leaf Curl Virus in Okra. Genetic Engineering has an Alternative. Okra is highly recalcitrant crop for tissue culture based regeneration and genetic transformation due to its highly mucilagenous nature. Highly efficient regeneration and genetic transformation methodology in Okra genotypes has been developed.

Transgenic Plants developed with different gene constructs in Cassava, Okra and Cotton need to be validated for their promising resistance against Target Virus. Bt is a validated system. Virus Resistance through RNAi/PDR is to be established for case to case basis. Targeting Multiple Viruses need to be looked into. Regulatory Guidelines for Virus Resistant Transgenic Crops need to be laid down.

• RNAi In Relation To Rice Development Under Salt And High Tem*perature Stress*

By Dr. Neeti Sanan-Mishra

Increasing population pressure and global climatic change has pressurized urgent need for substantive research to improve yields of crops like rice. However factors like soil salinity and high temperature are emerging as the main environmental factors limiting rice production. Thus, understanding the mechanism of stress tolerance is utmost necessary and important.

The discovery of RNAi has opened a new window in the field of gene regulation and the miRNA (miRs) have emerged as genetic buffers in providing protection against various abiotic and biotic stress conditions. Comparative miR profiling across tissues in rice grown under normal and stressed environments was undertaken to identify the key miR target nodes involved in regulating the rice development in response to salinity and high temperature.

Artificial miR technology has been employed to generate over-expressing rice transgenic lines for functional characterization of selected miRs. The studies would lead to novel outcomes that can be utilized to prepare “smart plants”, which will be an enduring step to fight against abiotic stresses mediated decline in crop yields in rice.

Profiles have been prepared for:

- 1200 rice miRs were expressed on microarray.
- 75 miRs showed variation across NL (PB) and SL (PB).
- Out of these 75 miRs, 33 miRs, 51 miRs and 39 miRs were expressed in NL (PK) SL (PK) and SR(PK) respectively.
- 10 miRs are expressed in response to both biotic and abiotic stresses.
- 79 novel sequences validated.

SESSION 3

Regulatory Guidance /Experiences For New Plant Breeding MolecularBiology Technologies - Legal Frameworks And Scientific Basis For RiskAssessment As Compared To Commercialized GM Crops

• OECD Guidance Documents

By Dr. Peter Kearns

There are many different types of guidance documents available to address topics related to risk/ safety assessment of transgenic crops including a number which have been published by OECD or are in preparation. These documents are equally applicable to address products resulting from advances in science and technology including through the use of New Plant Breeding Techniques (NPBTs).

Some underlying principles are usedin preparation of guidance documents including that of science-based risk/ safety assessment. This principle is common to the regulatory process in many different countries and is, therefore, the cornerstone of efforts to work towards international harmonisation. It is true that there are differences in the practice of environmental risk/ safety assessment as opposed to the safety of foods and feeds. In both cases, there are strong similarities in the information used amongst different jurisdictions as part of a science-based risk assessment. This allows countries to agree on certain set of information used in risk assessments which are often published through 'consensus documents' usually on a crop-by-crop basis. At the same time, other types of documents have been published which include information, relevant to risk assessment, on specific kinds of traits such as herbicide-tolerance and insect resistance. These packages of information can be drawn together to be used in a specific risk assessment.

What causes differences in the GM regulations is not the underlying principle of risk assessment but other factors which get built into the system after the risk assessors finish their work. Risk assessment process for NPBTs will be same. However, in some countries NPBTs may not fall into the regulatory system but in others it may. Science based risk assessment will be same in all countries. Risk assessors speak the same language. Countries exchange information on risk assessment with one another. This avoids duplication, saves time and facilitates trading. There are problems in international trade of GM products but problem is not due to risk assessment but due to other factors.

Risk assessment cannot be discussed in abstract but it has to be product specific that is why step by step approach or case by case approach is adopted for transgenics in agriculture.Risk assessment is done once potential hazard is identified. Look at species in question (any Crop). Look at biology of crop because it will convey how it will behave in environment. Information on Trait is important. Agriculture environment is important.

All OECD documents are consensus documents. They are not by majority or vote but by consensus of all member states.56 science-based consensus documents have been published on:

- Biology and trait
- Microbes
- Emerging issues
  - *Molecular characterization (required in risk assessment of GMOs)*
  - *Low level presence (the document contains how risk basement can be done in case of shipments containing small amount of material from an event in*

product from a country of origin is approved but not approved in the destination country).

- Outreach and information dissemination
  - *Biotrack online*

OECD has set up a Working Group on "Harmonisation of Regulatory Oversight in Biotechnology". Member states have nominated delegates from Ministries or agencies responsible for risk assessment.

This Working Group has pointed out the following with regard to NPBT regulations:

Differences between countries

- New Laws or not
- Regulation endpoints based upon adverse effects or defined risks
- Combined or separate environmental or food/feed safety reviews
- Triggers- novelty, GE/GMO, combination
- Adverse effects
- Number of ministries involved in regulation (and in developing positions for international discussions)

Similarities between countries

- Risk assessment systems
- Biology + trait + environment X interaction
- Use of familiarity
- Comparative
- Step-by-step, case-by-case

Major Outputs of the Working Group are publication of consensus documents by OECD. The OECD has also published following consensus documents on Environmental Risk/Safety Assessment in Plants – paradigm:

- 1986, Recombinant DNA Safety Considerations (The Blue Book), OECD, Paris.
  - *Industrial, agricultural and environmental applications*
  - *Organism, Step-by-step, Case-by-case*
- 1992, Safety Considerations for Biotechnology, OECD, Paris.
  - *Confined Field tests*
- 1993, Safety Considerations for Biotechnology: Scaleup of Crop Plants, OECD, Paris.
  - *Large-scale field tests*

Available electronically at http://www.oecd.org/publications

In OECD there is no consensus on definition of GMOs, LMOs, GEOs, transgenic or rDNA. Country definitions are used.

Biosafety Consensus Documents includes information for use in risk assessment on the biology of crops and traits (familiarity) agreed by authorities. The crop/ trait in agriculture practice is used. It also includes: taxonomy, reproduction, wild relatives – hybridization, center of origin and diversity and weediness. Examples of published biosafety consensusdocuments:

- Crops: maize, soybean, potato, cotton, rice, bread wheat, sugar beet, sunflower, peppers, papaya, cucurbita, brassicas.
- Traits: tolerance to glyphosate herbicide, tolerance to phosphinothricin herbicides, virus resistant through coat protein gene-mediated protection, Bt resistance.
- Trees: Norway spruce, white spruce, poplars, Douglas fir, Sitka spruce, lodgepole pine, Eastern white pine, European white birch, larches.
- Micro-organisms: Acinobacter, Pseudomonas, baculoviruses, Taxonomy in Risk Assessment, Detection.

Food/ Feed Safety Consensus Documents existon**:** Sugar beet, Potato, Rice, Maize, Rice, Wheat, Cassava, Sweet potato, Papaya, Low erucic acid rapeseed, Cotton, Barley, Tomato, Alfalfa, Soy bean, Sugarcane, Grain sorghum. They have information on nutrients, anti-nutrients, toxins etc. which help with food and feed safety risk assessment.

Risk assessment tools for transgenics will work for NPBTs also.

• European Union

By Dr Boet Glandorf

In the EU questions have been raised by companies on the regulatory status of plants obtained by New Plant Breeding Techniques (NPBT). Following these questions, in 2007 an EU expert group was established whose aim was to determine if plants obtained by NPBTs would fall under the definition of a GMO or not.

Working Group On New Techniques

Techniques discussed by the Working Group were:

- Zinc finger nuclease technology
- Oligonucleotide-directed mutagenesis
- Cisgenesis/intragenesis
- RNA-dependent DNA methylation
- Grafting
- Reverse breeding
- Agro-infiltration
- Synthetic biology

The Working Group on New Techniques consisted of experts of 22 EU member states. Its mandate was to evaluate each technique in the context of:

- the GMO definition
- the annexes of the directive
- the most recent available scientific data

Definition of a GMO as per Directive 2001/18/EC is as follows: GMO/GMM defined as "an organism/ micro-organism… in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination".

Annexes in Directive are:

- Non-exhaustive list of techniques that lead to genetic modification.
- Techniques not considered to result in genetic modification (exhaustive list).
- Techniques excluded from the scope of the GMO legislation (exhaustive list). Mutagenesis and cell fusion are included in this list.

The Working Group has held 9 meetings from 2008-2011. It analysed whether NPBTs constitute techniques of genetic modification. If so, it was analysed whether the resulting organism would fall within or outside the scope of the GMO legislation, or was to be excluded. The working group also looked at similarity of the NPBTs to conventional techniques (which are excluded from regulation), to natural processes and gave suggestions for future status of the NPB and their resulting organisms. Important topics of discussion for each of the NPBT were:

- How to interpret: 'Genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination'?
- How to interpret: 'Formation of new combinations of genetic material'?
- How to interpret: 'recombinant nucleic acid molecules'?
- How to assess the transient presence of recDNA?
- What is of importance for the discussion: the offspring of the plants obtained by NPBTs or the intermediate product?

The Working Group finalized its Report in 2011. There was to some extent disagreement in the Working Group whether the NPBTs would fall under the definition of GMO or not. However, there was agreement on future exclusion of most of the NPBTs because similar resulting organisms could be obtained by natural processes or by conventional breeding.

The Report is not yet published. Based on the outcome of the Working Group, the European Commission (EC) has started a legal analysis of the Working Group report. This analysis is not yet finalized and no formal consultation with the Member States has taken place yet. Analysis of European Commission of NPBTs is expected at the end of 2014.

EFSA Opinion on NPBTs

Based on the report of the Working, the European Commission requested the European Food Safety Authority (EFSA) to evaluate the safety of the NPBTs. The questions to EFSA were as follows:

Q1. Determine whether there is a need for new guidance or whether the existing guidance on risk assessment should to be updated or further elaborated, in anticipation of the placing of products on the market through the application of the listed techniques.

Q2. What are the risks in terms of impact on humans, animals and the environment that the techniques could pose? Compare plants obtained by these new techniques with plants obtained by conventional plant breeding techniques and secondly with plants obtained with currently used genetic modification techniques.

The (EFSA) has so far drafted guidance on risk assessment of plants obtained by cis/intragenesis and site directed nucleases.

Conclusions EFSA On Cisgenesis and Intragenesis

- The EFSA GMO Panel considers that the existing EFSA Guidance documents are applicable for the evaluation of food and feed products derived from cisgenic and intragenic plants and for performing an environmental risk assessment and do not need to be further developed.
- It can be envisaged that on a case-by-case basis lesser amount of event-specific data are needed for the risk assessment.
- The Panel concludes that similar hazards can be associated with cisgenic and conventionally bred plants, while novel hazards can be associated with intragenic and transgenic plants.

Conclusion EFSA On Site Directed Nucleases (SDN-3)

- The EFSA GMO Panel considers that the existing EFSA Guidance documents are applicable for the evaluation of food and feed products derived from plants developed using SDN-3 approaches and for performing an environmental risk assessment and do not need to be further developed.
- SDN-3 can be used for site specific insertion of a transgene or of a cisgene. On a case-by-case basis lesser amounts of data are needed, for example when a cisgene is inserted.
- The SDN-3 technique does not differ from transgenesis. The main difference between the SDN-3 technique and transgenesis is that the insertion of DNA is targeted to a predefined region of the genome. Therefore, the SDN-3 technique can minimise hazards associated with the disruption of genes and/or regulatory elements in the recipient genome.

Other Reports

The Joint Research Center (JRC) in EU has drafted a report on the economic effects of NPBTs and on the potential to detect products obtained by NPBTs. This report was published in 2011.

The European Commission indicated that they are drafting a legal analysis on of the NPBT by end of 2014, based on reports of Working Group on New Techniques and of the EFSA opinions.

Cultivation Of Plants obtained
By NPBTs In the EU

Products obtained by NPBTs are only grown in field trials and are not yet commercially cultivated.. Field trials are national decisions and approved by the nations competent authority in an EU memberstate. As regards to existing experience with risk assessment and field trials of plants obtained with NPBTs, so far experience is only obtained with plants obtained by cisgenesis, intragenesis, ODM, SDN and RNAi (OECD survey).

• Australia

By Dr. Michael Dornbusch

Australian legislation that regulates gene technology in came into effect on 2001. At this time the majority of genetically modified plants were produced using *Agrobacterium* mediated transformation to introduce genes that produced proteins conferring traits such as herbicide tolerance or insect resistance.

More recently, a range of other techniques have been developed that utilise the tools of modern molecular biology to modify the genome of plants in a site specific, targeted manner to produce desirable traits. These new plant breeding techniques induce targeted mutations (eg Oligo-directed mutagenesis, meganucleases or zinc finger nuclease versions 1 or 2), introduce new genes in a site specific manner (eg, ZFN 3, other meganuclease techniques) or silence genes (RNAi, RNA directed DNA methylation). Reinsertion of genes/other DNA sequence elements from the same species is also being used (cisgenesis, intragenesis), as well as breeding schemes that involve genetically modified parents only at an early generation. The genetic changes may be stably inherited or they may be transient or not present in the plants that are the end product of the breeding process.

Although the stage of development differs for each of these techniques, their regulatory status has been the subject of discussion in a number of countries. In Australia, definitions in the *Gene Technology Act 2000* and schedules in the Gene Technology Regulations 2001are applied to consideration of whether these new plant breeding techniques are covered by the regulatory scheme. This involves a careful consideration of both the process by which the plants are made and the relevant provisions of the legislation. Those modified plants that are considered to be covered by the scheme would require the appropriate authorisation from the Gene Technology Regulator for work in contained facilities or for release into the environment.

For those that require a licence from the Gene Technology Regulator, the risk assessment process that would be applied would be the same as any other licence application. A new or different risk assessment methodology would not be required. However, the data that might be required for an assessment may differ and could be considered on a case-by-case basis.

Considerations:When considering the regulatory status of each of these new technologies, it is necessary to consider:

- Did it involve gene technology?
    - o *Look in detail at process making the organisms .*
    - o *Definition in the Act and.*
    - o *Schedule 1A regulations- techniques excluded.*
- Is it a GMO?
- Did it inherit traits from a GMO?
    - o *Schedule 1- Is it excluded?*

Some Examples:

SDNs: Using some site directed nucleases as an example. The regulators would carefully look at the processes laid down. See whether foreign or non-homologous genetic material has been introduced at any stage. Examine whether the final plant products has inherited traits from a GMOs made at any stage.

RNAi: Introduced genetic material- regulated. For any techniques, including RNAi – question is whether special RA is required? 19 field trials have been approved since 2002. These include: Poppies, wheat and barley, papaya, ryegrass, cotton

For those plants bred using new technologies which are considered to be covered by the current regulatory arrangements, the regulatory pathway to commercialisation is likely to be the same as the pathway we typically observe.

Risk Assessment Approaches

Even though they may be produced by a technique that may differ from those used to date the same risk analysis principles and approaches would apply.However, the information required may differ and would be considered on a case-by-case basis.The risk assessment consists of the same simple questions.Having asked these simple questions and postulated risk scenarios, those that are considered to have some reasonable chance of causing harm are identified. Identified risks are then characterised in more detail, through qualitative assessment of consequence and likelihood. Risk estimation combines the consequence and likelihood components.The whole process is based on scientific evidence and consideration of uncertainty.Taking RNAi example same risk assessment process- but risk scenarios and hence data required would be different.

The broad considerations for RA are:

A GM plant is a plant, Plants developed using NPBTs are still plants, Phenotype is important for risk assessment, Weediness/invasiveness traits can be identified, Weediness/invasiveness traits encompass all undesirable effects.

In Australia an internationally recognised WRA methodology has been adapted to assess potential weediness. Australian std, is FAO, ISO recognised.

Future work on NBTs

- Independent review of the Act in 2011 considered the coverage of new technologies.
- Government response to the review now finalised.
- The review recommended and all governments supported.
- Investigate regulatory scope to reduce ambiguity and ensure that it keeps up with technology

• South Africa

By Ms. Nompumelelo Mkhonza

Genetically modified organisms (GMOs) have been permitted in South Africa (SA) since 1992, under an amendment of the Agricultural Pests Act, 1983 (Act No. 36 of 1983). To date all activities with genetically modified organisms (GMO's) in South Africa (SA) are regulated by the Genetically Modified Organisms Act 1997 (Act No 15 of 1997) as amended by Genetically Modified Organisms Act, 2006 (Act No. 23 of 2006). The GMO Act aims to ensure that all activities involving genetically modified organisms are carried out in such a way as to limit the possible harmful consequences to human and animal health and the environment. The GMO Act makes provision for the appointment of a Registrar, inspectors and two regulatory bodies i.e. the Advisory Committee, and Executive Council.

South Africa has not classified crops derived from NPBT as GMO or non-GMO,given that SA's experience on NPBT is currently limited to research conducted in research or academic institutions registered under the GMO Act. As a result no crops obtained through NPBTs are currently under regulatory assessment. However the GMO Act uses a process based GMO definition and this might have implications for how NPBT will be regulated.

• USA and Canada

By Dr. Morven A. McLean

In Canada and the United States, as is the case in other countries, the decision to regulate a plant is based on a variety of considerations including: legal, social and scientific. Different jurisdictions will regulate differently based on their existing laws and regulations.

Canada: Canada's biotechnology regulatory framework was formally announced in 1993 following extensive public consultation. It is a product-based approach that focuses on noveltythe properties of the novel product, not the technology used to produce it. It uses existing sectoral legislation and regulatory institutions as described below. Product-specific decision-making is predicated on an evidence-based risk assessment, that is consistent with international guidance and standards (e.g.,OECD, Codex Alimentarius Commission).

Regulatory Agencies for Novel Plants and Novel Foods/Feeds in Canada are:

Health Canada

- Sole responsibility for evaluating the human health safety of all foods
- Authority is under the Food and Drugs Act and Regulations, Division 28
- Defines the regulatory requirements for "novel foods". Novel foods are products that have

never been used as a food; foods which result from a process that has not previously been used for food; or, foods that have been modified by genetic manipulation.

Canadian Food Inspection Agency (CFIA)

- Regulates the importation, environmental release, and use in livestock feeds of "plants with novel traits", which can include genetically engineered plants as well as products of conventional of other breeding techniques.
- Authority derived from: Seeds Act, Feeds Act, Fertilizers Act, Plant Protection Act, Consumer Product and Labelling Act, Health of Animals Act, Food & Drugs Act

A Plant with Novel Traits (PNT) is a plant containing a trait not present in plants of the same species already existing as stable, cultivated populations in Canada, or is present at a level significantly outside the range of that trait in stable, cultivated populations of that plant species in Canada.

Summary: Canada

Canada's existing, product-based regulatory system will continue to apply to novel plants/foods, irrespective of the breeding technique used to introduce a trait or traits.

Biotechnology Framework in the USA

USA has a coordinated framework for biotechnology that was articulated in a policy statement in 1992 which states that U.S. agencies will regulate the products of biotechnology in accordance with their authorities under existing safety regulations. The various agencies involved are:

- Food and Drug Administration
  Center for Food Safety and Applied Nutrition
- Environmental Protection Agency
  Biopesticides and Pollution Prevention Division
- US Department of Agriculture
  Animal and Plant Health Inspection Service

US Food and Drug Administration

USFDA regulates food safety under the Federal Food, Drugs and Cosmetics Act. It is primarily a post-market safety authority. "Novel" foods are addressed through a voluntary, premarket consultation process. While consultation is voluntary safety is mandatory.

US Environmental Protection Agency

USEPA implements the Federal Insecticide, Fungicide and Rodenticide Act and regulates the use of pesticides through a "registration" process. "Plant incorporated protectants" (PIPs) are considered pesticides , for example: Bt proteins; plant viral coat proteins and replicases. USEPA regulates the pesticide, not the plant. It also regulates pesticide residues under Federal Food, Drug, and Cosmetic Act (FFDCA).

Relevant to the topic of new breeding technqiues, the USEPA convened a Scientific Advisory Panel on January 28, 2014 to discuss the topic "RNAi Technology as a Pesticide: Problem Formulation for Human Health and Ecological Risk Assessment". Minutes were published in May 2014:

- http://www.epa.gov/scipoly/sap/meetings/2014/012814meeting.html.

US Department of Agriculture

The Biotechnology Regulatory Service of the USDA APHIS implements the Plant Protection Act and Regulations (7 CFR 340). A genetically engineered plant will be considered a "regulated article" if it meets two requirements:

Produced using genetic engineering (recombinant DNA techniques)

AND

Donor organism, recipient organism, vector, vector agent, is a plant pest

OR

Is an unclassified organism the Administrator determines is a plant pest or has reason to believe is a plant pest

APHIS biotechnology regulations regulate certain GE organisms that may be or are plant pests. If a GE organism is not a plant pest, is not made using plant pests, and APHIS has no reason to believe that

it is a plant pest, then the GE organism would not fall under APHIS' regulatory authority. APHIS examines these situations on a case-by-case basis when a developer asks if a particular plant is a regulated article under the agency's biotechnology regulations.

Summary: United States

For plants produced using new breeding techniques:

- USDA will likely regulate plants with sequences from "pests".
- US EPA will regulate any pesticides in plants.
- FDA will continue to have oversight over food safety. The voluntary consultation process is likely to be requested by developers of novel plants.

Food for Thought

The following questions should be considered regarding regulation of products from NBTs:

- Does a NBT result in a product that falls within the scope of the existing law/regulation?
- If "yes", does the risk/safety assessment paradigm change?
- If "no", does the product raise any new safety concerns (i.e., should it be regulated)?

Related CERA Publication

- Problem Formulation for the Environmental Risk Assessment of RNAi Plants: Conference Proceedings

http://ceragmc.org/docs/cera_publications/pub_08_2011.pdf

• Japan

By Dr. Junichi Tanaka

Following the ratification of the "Cartagena Protocol on Biosafety", Japan enacted the national law called the Cartagena Law in 2003. According to the Cartagena Law, Living Modified Organism (LMO) is defined as "the organism that possesses nucleic acid, or a replicated product thereof, obtained through use of the any of the following technologies (i.e. recombinant techniques)". The Law stipulates that the concerned governmental authorities should carry out scientific risk assessment to evaluate the influence of LMO's on biodiversity before approval of their use. Thereby the LMOs are subject to case-by-case, science-based and product-based evaluation. For instance, the o

ffspring derived from the F_1 seeds produced by Seed Production Technology (SPT) is a good example to understand a product-based evaluation. Japanese regulatory agencies judged that the offspring of these seeds is not GMO as long as foreign genes are eliminated from its offspring — 'null segregant.'

Currently Japan's Ministry of Agriculture, Forestry and Fisheries (MAFF) implements the following NPBT research projects which started from 2013:

1) **Genome editing (CRISPR/Cas9, TALEN and other techniques)**
   The aim of this project is to develop novel genome editing techniques for the improvement of the efficiency of genome editing.

2) **Generation-accelerated breeding using juvenile flowering genes of fruits trees**
   This fast-track breeding technique uses the flowering (FT) gene and this FT gene will be eliminated from final commercial varieties.

3) **Development of efficient recurrent-selection-based breeding system in autogamous crops**
   To establish recurrent-selection-based breeding system in autogamous crops, dominant male sterility and positive/negative selection is essential and these traits should be conferred to autogamous crops like rice. However, these introduced genes should be eliminated from final commercial varieties (= null segregants).

In most NBPTs, e.g. genome editing and improvement of reproductive traits, in plant species, several indispensable genes need to be introduced to the plant genome once, which are subject to regulation under the Cartagena Law. When the foreign genes are eliminated completely from end products (null segregants), then these will not be subject to the Cartagena Law. Japan is presently discussing the evaluation system to prove that a particular end product is null segregant. The most pressing issue is the international harmonization of regulatory oversight that pertain "Null Segregants."

• Argentina

By Dr. Agustina Whelan

In Argentina there are agencies within the structure of the Ministry of Agriculture, Livestock and Fisheries that take part in the assessment and oversee the activities involving genetically modified materials of agricultural use. They are the Biotechnology Directorate, the National Advisory Commission on Agricultural Biotechnology (CONABIA), the National Seed Institute (INASE), the National Service of Agrifood Health and Quality (SENASA) and the Directorate of Agricultural Markets.

CONABIA came to preliminary conclusions regarding whether the derived products from certain New Breeding Techniques are considered GM-plant pursuant to Resolution (SAGYP) Number 763/11 and especially to the GM-plant and event transformation definition contained in Resolution (SAGYP) Number 701/11.

For Cisgenesis, Intragenesis, Floral dip and SDN [(1)]-3, Synthetic Biology and Grafting the resulting product is a GMO according to the regulations in force. With respect to SDN-1 and SDN-2 techniques, the conclusion of CONABIA is that the limited extent of DNA sequence modification in relation to the intended modification means that no new combination of genetic material has occurred. Thus, the resulting product is not a GMO according to the regulations in force. For Reverse Breeding,the resulting product is considered a GMO according to the regulations in force if it carries the transgenes that act over the meiosis. In the case of Oligonucleotide-directed mutagenesis, the resulting product may or may not be a new combination (and therefore fall under the GMO regulations in force) depending on the extent and nature of the modification. For RNA-dependent DNA methylation it is considered that there is no generation of a new combination of genetic material, for this reason, the resulting product is not a GMO according to the regulations in force. Finally, according to agroinfiltration and agroinoculation, therisk assessment should focus on the microorganism carrying the construct of interest (assuming the activity performed falls under our regulation).

The current draft resolution of CONABIA sets out the steps to follow when the applicant uses these techniques. However it is not limited to the techniques described above, thus, it is open to evaluate any other techniques that may come up.

The applicant should submit:

- Background information about the way in which the product is obtained.
- Evidence of the lack of transgenes used transitorily when obtaining the product (if necessary).
- Evidence of the introduced genetic transformations.

CONABIA will determine:

- If the genetic modification into the plant genome does not have enough entity to be considered a new combination of genetic material.
- If it has showed that one transgene transitorily expressed has been removed from the crop to be commercialized (where applicable).
- If the assumptions above are fulfilled; The product is not considered GMO therefore not be achieved by resolution of GMOs.

(1) SDN (Site-directed nucleases): (Site – directed nucleases): Set of techniques that can each be use to introduce the same change of the genome. For example, TALEN, Zinc Finger, Meganucleases. (Maria Lusser and Howard V. Davies).

• India

By Dr S R Rao

The Indian Acts, rules and regulations as well as procedures for handling of genetically modified organisms (GMOs) and rDNA products have been formulated under the Environment (Protection) Act (EPA) 1986 and Rules 1989. The rules in general cover manufacture, use/import/export and storage of hazardous micro-organisms, genetically engineered organisms or cells and came into force from 1993. A set of rDNA guidelines were issued since 1990 covering genetically engineered organisms, genetic transformation of plants and animals, mechanism of implementation of biosafety guidelines, containment facilities under three risk groups. Regulatory framework oversees the development of GMOs including crops from the research stage to large-scale commercial use through three-tier statutory committees.

R&D on NPBT in India is limited to application of RNAi technology. The rules 1989 defines genetic engineering a " the technique by which heritable material, which does not usually occur or will not occur naturally in the organism or cell concerned, generated outside the organism or the cell is inserted into said cell or organism. It shall also mean the formation of new combinations of genetic material by incorporation of a cell into a host cell, where they occur naturally (self cloning) as well as modification of an organism or in a cell by deletion and removal of parts of the heritable material". Therefore, the scientific risk assessment process should not in principle differ to that of current GM crops. The rules 1989 shall apply to "genetically engineered organisms/ micro-organisms and cells and correspondingly to any substances and products and food stuffs, etc., of which such cells, organisms or tissues hereof form part". Thus, the regulations cover both product and process based safety assessment. However, the regulatory bodies recognise that risk assessment should be tailored to the specific implications of new technology or product on food and environmental safety; therefore assessment would be through case-by-case approach.

About Sponsors

Department of Biotechnology, Ministry of Science and Technology, Government of India (DBT)

Website: *http://dbtindia.nic.in*

The setting up of a separate Department of Biotechnology (DBT), under the Ministry of Science and Technology in 1986 gave a new impetus to the development of modern biology and biotechnology applications in India. In more than 25 years of its existence, the department has promoted and accelerated the pace of development of biotechnology in the country. Through several R&D projects, demonstrations and creation of infrastructural facilities a clear visible impact of this field has been seen. The Department has made significant achievements in the growth and application of biotechnology in the broad areas of agriculture, health care, animal sciences, environment, and industry. Necessary guidelines for transgenic plants, recombinant vaccines and drugs have also been evolved. A strong base of indigenous capabilities has been created.

International Life Sciences Institute- India (ILSI-India)

Website: *http://www.ilsi-india.org& http: www.ilsi.org*

ILSI- India is a branch of the International Life Sciences Institute (ILSI) which is a global foundation with headquarters in Washington, D C and 16 regional/country branches in North America, Europe, Japan, China, South East Asia etc. ILSI addresses scientific issues relating to food safety, nutrition, toxicology, agriculture sustainability, biotechnology and environment through its branches and Research Foundation. It has a special consultative status with Food and Agriculture Organization (FAO) of United Nations and is affiliated with World Health Organization as a non-governmental organization.

ILSI-India has been working on agriculture biotechnology issues in the country since 1999. It has organized a number of national and international workshops, conferences, and training programs activities in the country.

CropLife Asia

Website: *www.croplifeasia.org*

CropLife Asia promotes a safe, secure food supply. It helps farmers to grow more abundant supplies of healthy, affordable food while safeguarding the environment and natural resources through access to innovative technologies. Based in Singapore, **CropLife Asia** is part of a global federation representing the plant science industry. In India, CropLife Asia works with partners CropLife India and Association of Biotechnology Led Enterprises-Agriculture Focus Group. For more details, please visit www.croplifeasia.org;www.croplifeindia.org;andhttp://www.agrifocus.org/able-ag/.

Center for Environmental Risk Assessment (CERA), ILSI Research Foundation

Website: *www.cera-gmc.org*

The purpose of the Research Foundation's Center for Environmental Risk Assessment (CERA) is to develop and apply sound science to the environmental risk assessment of agricultural biotechnologies so their contributions to sustainable production of food, fuel and fiber may be safely realized. The Center's research projects are currently focused on genetically modified (GM) plants and, more recently, transgenic arthropods. This scope will be broadened over time to include transgenic animals.

International Life Sciences Institute-India

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