

## Biosafety of RNA silencing and genome editing technologies in crop plants: Malaysian and Australian research perspectives

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**Abstract.** Research in agricultural biotechnology can produce novel solutions to address the ever growing demand for food, feed, renewable materials and renewable energy using increasingly limited resources. Yet research is expensive with long timelines before implementation can disseminate the benefits to society, so there is a need to maximise co-operation and communication between scientists, stakeholders and their governments, to optimise research, its development and the implementation of research outcomes, into mainstream applications. Recognising the impacts of regulations on biosafety, biosecurity and intellectual property policy on strategies for research, senior and early career researchers from two research intensive universities in Malaysia and Australia, held a workshop to identify and to deliberate over two key areas of technology that offer much promise for agriculture, namely RNA silencing and genome editing. A major focus of the workshop was the regulation of new breeding technologies, and how the regulations need to take into account these new technologies. Themes discussed were the need for harmonisation of international legal frameworks and careful use of terminology, standards and guidelines; and the need for good communication and consensus within and between groups of stakeholders and law-makers. This mini-review highlights the deliberations and recommendations from the workshop.

**Keywords:** Australasia, biosafety, biosecurity, genetic modification, RNAi, new breeding technologies

### INTRODUCTION

Agriculture has consistently adopted new technologies to maximise crop productivity, quality and yield. There is little contention that the best available knowledge and resources should be harnessed to address the challenges to agriculture posed by population growth, urbanisation and climate change, while maintaining regard for the environment, and the need for long term sustainability and increased production of food. However, recent increases in fundamental knowledge and the pace of development of new

technologies that can be used to manipulate genes and gene expression that are needed to help meet future food needs has outstripped the capacity and related knowledge to support the formulation and enactment of appropriate guidelines and policies to regulate research, development and its implementation in new crop varieties, in particular in relation to biosafety and biosecurity policy. One obstacle is that there is variation in the terminology used by various national and international agencies and authorities and in the

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ways which they approach regulation of gene technology. Such differences between regulatory agencies, including different stages of maturity of national regulations can also act as a non-tariff barrier to trade, and this has slowed the adoption of new technologies in some jurisdictions.

Agriculture, climate and socio-economic factors differ widely across, and in some cases within, countries and territories. As a result different crops are grown in different regions, and this is reflected in the spectrum of pests and diseases present, which in turn leads to variation in agricultural practices and requirements from new breeding technologies including biotechnology. In the research sphere, there is also wide variation in the capacity, facilities and stage of development of “biotech crops” and technologies, including RNA silencing methods and genome editing (with or without plant genetic modification). The result of these differences has led to the development of inconsistent regulatory frameworks across the Asia-Pacific region, and includes countries with no legal framework for biosafety, some with regulatory frameworks but little or poor enforcement and those with both strong frameworks and good enforcement and compliance.

In response to these challenges and under the auspices of a “IRU-MRUN” joint research programme under the Innovative Research Universities (IRU, Australia) and Malaysian Research University Network (MRUN, Malaysia), senior and early career researchers from the University of Malaya, Kuala Lumpur, Malaysia and Murdoch University, Perth, Australia, held a joint workshop on “Processes for Biosafety of RNA silencing and Genome editing technologies in crop plants: Malaysian and Australian perspectives” by teleconference on the 12<sup>th</sup> October 2016. The workshop aimed to address issues relating to new breeding technologies, in particular gene silencing and genome editing, from both research and implementation perspectives, and comprised six lectures each followed by a question and answer session, and concluded with a roundtable discussion. The workshop participants included Jennifer Ann Harikrishna, Rofina Yasmin Othman, Muhamad Shakirin Mispan, Teo Chee How, Katharina Mebus, Tan Boon Chin, Purabi Mazumdar, Pooja Singh, Lee Wan Sin, Umaiya Munusamy, Lau Su

Ee and Tan Yew Seong from the University of Malaya; and Michael G K Jones, Steve Wylie, Sadia Iqbal, Yong Han, John Fosu-Nyarko, Maria Maqsood, Sharmin Rahman, Jebin Akter, Fareeha Naz and Doug Hall from Murdoch University, and Peter Waterhouse from the Queensland University of Technology, Australia. This mini-review provides a summary of the discussions and recommendations from the workshop.

### **Harmonisation of vocabulary for biosafety legislation and guidelines.**

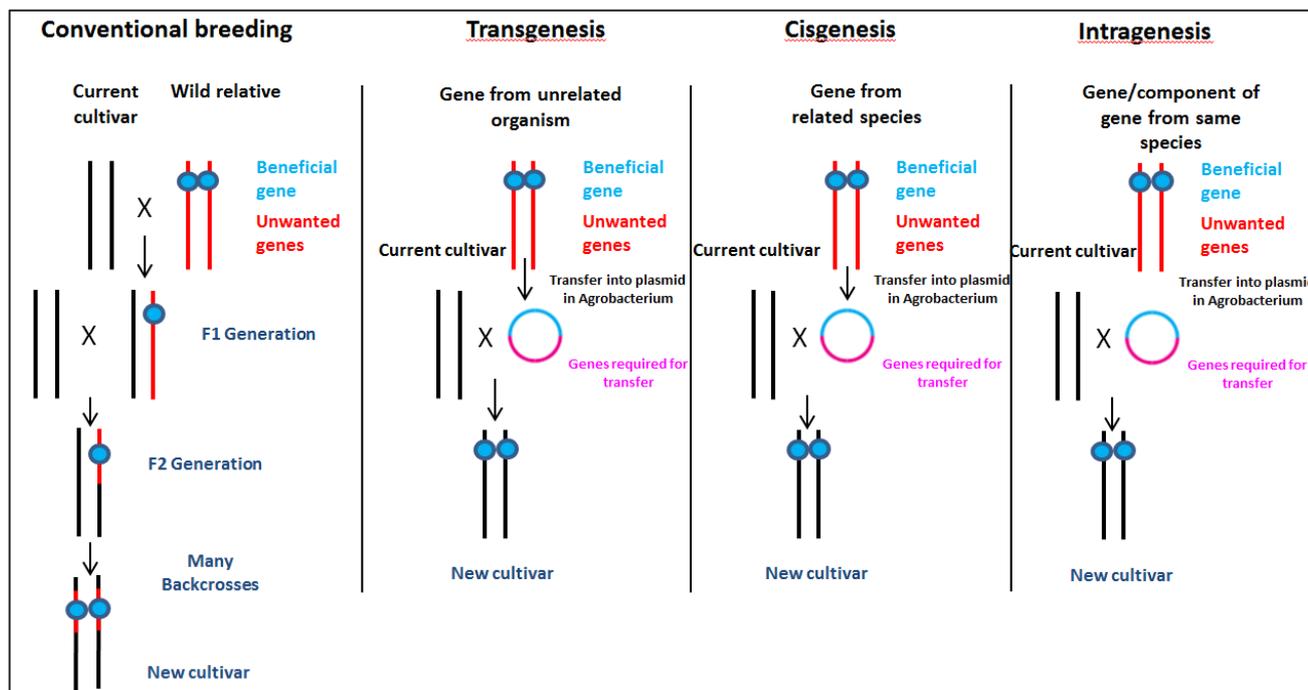
The meeting discussed the differences in vocabulary and terminology used in the various legal frameworks, standards and guidelines for biosafety and biosecurity between different countries and territories. It was suggested that this is a barrier to compliance and collaboration, particularly in relation to transfer of materials across boundaries and which can impact the effective use and development of technology, especially where it is perceived as a barrier to trade or to commercialisation, and which represents a real but unquantified “cost of business”. It was noted that the rapid development of new breeding technologies has led to lack of clarity in the specific terminology among legislators as well as scientists, for example “GMO” (genetically modified organism) and “LMO” (living modified organism) are used to describe the same thing (under different legislations) in Australia and Malaysia respectively (*Gene Technology Act 2000; Malaysian Biosafety Act 2007*). There is also some uncertainty over the terms and definitions to be used for the new breeding technologies, including the definition of “foreign DNA” and technologies such as RNA interference or RNAi (also often termed “RNA silencing” and “post transcriptional gene silencing” for plants), “genome editing” and “synthetic biology”. It is thus important to have regular dialogue between scientists and legislators both within and between different countries both for clarity and for regional harmonisation.

The meeting also discussed the lack of harmonisation between some of the information requested by biosafety guidelines (or what is “nice to know”) and information that represents the real risks based on scientific evidence from study of about 20 years of experience and safe use of genetically modified crops (what we actually “need to know”). A requirement for extensive but

unnecessary information, or duplication of required information, inflates costs and can discourage the use of new technologies.

To clarify terms, the following diagram was used to provide a comparison of breeding technologies, comparing a simplified conventional plant breeding protocol, with

*transgenesis* (in which a gene from an unrelated organism is transferred), *cisgenesis* (in which a gene from a related or sexually compatible species is transferred) and *intragensis* (gene or gene component for the same species).



**Figure 1.** Comparison of breeding technologies. (Source: M. G. K. Jones, expanded from original by J. Dunwell, University of Reading).

It can be seen that there is no difference in the mechanism of transgenesis, cisgenesis and intragenesis, the differences lie in the source of the introduced genetic material. In cisgenesis and intragenesis the transferred genes or parts thereof already exist in the gene pool for that genus or species, and could be introgressed in evolutionary time or by conventional breeding. What these approaches do is to widen the gene pool available for conventional breeding, akin to making crosses from wild relatives or land races in conventional breeding. The benefit of the gene transfer approach is its precision, which enables exclusion of unwanted sequences of unknown and possibly undesirable function, and so could well be regarded as less risky than conventional breeding using wide crosses.

The meeting also discussed industry-agreed definitions of different types of New Breeding

Technologies. When discussing genome editing technologies, it is useful to consider that classical mutagenic approaches (chemical/radiation) have been used for many years to develop a range of crop varieties. Classical mutagenesis generates randomly multiple mutagenised plants, from which undesirable genotypes are excluded and plants with desired characteristics may be selected. Plants selected in this way are grown widely, and include seedless oranges and ruby red grapefruit. In contrast, Oligonucleotide Directed Mutagenesis (ODM) makes use of a specific oligonucleotide to produce a single DNA base change in the plant genome, which similarly does not contain introduced DNA. Site Directed Mutagenesis (SDNs) make use of specific dsDNAses (eg *Fok1*, *Cas9*) and peptides (e.g. ZFNs, TALENs) or more recently oligonucleotides (eg CRISPR/Cas9) that guide

cleavage of both DNA strands at exact sites in the host DNA, and therefore can generate *site specific* mutagenesis, in a much more precise way than the random breaks cause by classical mutagenesis. This is because the natural process of DNA repair makes mistakes in repairing dsDNA breaks. There are variants of SDN technology, in which when oligonucleotides with ends homologous to each side of the dsDNA break are included, then one or more bases may be inserted at the repair site. These SDNs are sub-classified as:

**SDN-1** – non-homologous end joining (NHEJ), in which natural repair mechanisms can result in small nucleotide deletions, additions or substitutions; **SDN-2** – in the presence of an oligonucleotide template with ends homologous to each side of the double-stranded break, homologous end joining (HEJ) can occur, such that one or more bases can be included in the repaired sequence; **SDN-3** – as for SDN-2, but with a longer DNA insert, for example up to a full gene expression cassette.

The question which arises, is where to draw the line in terms of defining what is and what is not a GMO.

In discussing these consequences of new breeding technologies, including the use of guide RNA to modify gene expression or introduce miss-sense/deletion mutants using genome editing, it is clear that some modified plants will not be defined as “transgenic”. From a scientific and safety point of view, crops modified by these methods are no different from plants which would not be regulated under existing biosafety regulations, for example, crops developed using chemical- or radioactivity-induced mutations or where gene expression is modified by environmental factors. Indeed, it can be argued that the new plant varieties developed with these methods present very much lower risks than those produced by random mutation, especially where no new genetic material has been added to the new variety. Indeed, transgenic soybean plants have much less genetic variation from the wild type compared to that between different varieties of soybean or to plants that had been mutagenized (Anderson *et al.*, 2016).

However, there will need to be clarity provided in updated guidelines to cover the new breeding technologies; for example the meeting suggested that no additional methods were

required to assess risk for new technologies, as current protocols are sufficient. Two general principles were agreed, these were: (i) It is not desirable or necessary to develop a “third” class of crop products as a result of genome editing and (ii) It was agreed that plant varieties developed using the new breeding methods should not be differentially regulated if they are similar or indistinguishable from varieties that could have been produced by established breeding methods

As the new technologies are considered by regulators, existing guidelines should be examined and where there are no safety concerns, technologies now regulated should be added to the list of exclusions/exempted methods and materials, based on their track record of safe usage or equivalence to accepted breeding methods. As it is likely that all countries will revise their gene technology regulations to take account of new breeding technologies, such revisions present a real opportunity for dialog and harmonisation of regulations across the Asia-Pacific region with respect to biosafety and biosecurity for crops. The more coherence and congruent the regulations between countries, the more likely risk assessments are to be accepted by other countries, removing the need to repeat assessment studies, which would be especially beneficial to the developing countries in Asia.

The meeting agreed that new strategies of analysis should also be incorporated into the risk assessment process, such as deploying bioinformatics tools that can leverage on the increasing amount of sequence and other biological data that is amassing for many crop plants and varieties to reduce the need for some of the “wet lab” validation. Here again, a regional consensus on the use of risk assessment tools and technology can benefit all parties by sharing of expertise and information.

### Importance of clear communication

The meeting agreed that good communication in matters relating to biosafety and biosecurity is vital to ensure and assist with regulatory compliance and for the reassurance of consumers and stakeholders. Currently there is much inaccurate and misleading information in the press and online which has adversely impacted on the public perception of biotech crops: there are legitimate concerns for farmers in understanding

new technologies, and in particular organic farmers have often adopted an anti-technology stance, even though organic production yields on average 20-50% less than conventional crops, and others at the food production and preparation end often do not understand the technologies. The different groups of stakeholders require different kinds of information, and different approaches are needed to engage these groups in dialogs.

The meeting felt that the general public would mostly have concerns over what may be on their food table, what may be grown near to them and how the crops affect the environment. Since much of the more easily accessed information on GMO is negative and inaccurate, the public needs to be provided with science-based information to correct such misperceptions. Consumer acceptance can have a great influence on the successful introduction of products from biotech crops, although consumer surveys in Asia have also shown that there is generally poor awareness of gene modification technology (ISAAA 2002; Amin *et al.*, 2011; Ismail *et al.*, 2012). Upstream of this, the farmers will have the most exposure to and influence over the choice of crop varieties grown, so they should be engaged both by academic researchers and industry as primary stakeholders.

The meeting suggested that a good model for communication within stakeholders is to consider the approach used in Australia where the public sector research/developers work together at the initiative of private sector stakeholders to find a consensus which is practical, realistic and meets high scientific standards, to assist legislators. The discussion included aspects of avoidance of risk to the environment, avoidance of risk to food supply, ensuring innovation is not restricted and to avoid stifling the development of biotech and trade in biotech goods, especially by small and medium sized industries.

### **Conclusion and Recommendations**

All stakeholders are important for the practical and effective use of new technologies in crops, but scientists can play key roles in this process. Firstly, it is important to have regular dialogue between scientists and legislators both within and between different countries, both for clarity and for regional harmonisation of vocabulary and terminology. Scientists can be a driver for such

dialogue as they are already well networked. Industry should be engaged to form strong consensus before bringing issues and suggesting amendments to lawmakers. At present legislation on GMOs usually encompasses all forms of genetic manipulation, but then excludes those which have been used conventionally and have a history of safe usage. A review of existing guidelines to consider additional exclusions and exempted methods should be carried out and based on the track records of safe usage as well as comparisons with knowledge of genetic variation and horizontal gene transfer which occurs naturally. The periodic revision of gene technology legislation being undertaken now or in the future presents a great opportunity for dialogue and harmonisation across the Asia-Pacific region with respect to legislation on biosafety and biosecurity for crops. With better agreement in regulations between countries, the use of such legislation as potential barriers to trade will be reduced, and this would be of great benefit across the Asia-Pacific region. Finally, as indicated above new approaches should be incorporated where appropriate into the risk assessment process (e.g. bioinformatics tools), reducing the need for some of the “wet lab” validation. Here again, a regional consensus on the use of risk assessment tools and technology can benefit all parties by sharing of expertise and information. Ultimately, the aims are to deploy the best tools to provide the most productive crop varieties to farmers, aided by sensible, evidence-based legislation.

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