PROPOSED POLICIES TO ADDRESS THE POSSIBILITY OF THE UNINTENTIONAL PRESENCE OF TRANSGENES IN THE T.T. CHANG GENETIC RESOURCES CENTRE, IRRI (2007)

Contents

PREAMBLE

This document sets out proposed policies to address the possibility of the unintentional presence of transgenes in the T.T. Chang Genetic Resources Centre, in particular to:

- minimize, as far as possible, the probability of unintentionally introducing transgenes into the collection;
- establish an effective programme of testing for the presence of transgenes; and
- establish an effective response strategy to deal with the case that a transgene is discovered in supposedly non-transgenic rice.

The strategy to be followed by IRRI is to adopt practices that most effectively minimize the probability of unintentionally introducing transgenes, and to identify critical points of risk, so that we minimize the need to test unnecessarily for the presence of transgenes.

The document has been developed following the "Guiding principles for the development of CGIAR Centres' policies to address the possibility of unintentional presence of transgenes in *ex situ* collections", published and adopted by all CGIAR centres in 2005 following an Expert Workshop on "Technical issues associated with the Development of CGIAR policies to address the possibility of adventitious presence of transgenes in CGIAR ex situ collections".

BACKGROUND

1. In the management of germplasm, IRRI embraces the following overarching principles: ethics, transparency, accountability, risk analysis and quality control.

2. The purpose of the genebank at IRRI is to collect, conserve and make available rice genetic resources. The maintenance of the genetic identity of each accession in the genebank is an overriding objective; a key element of this is to prevent the unintentional introgression of genes from other accessions or other sources. Introgression of exotic genes, whether transgenes or conventional, is *per se* a risk because it changes the genetic identity of the accession. IRRI therefore takes proactive steps that aim to prevent the unintentional introgression of exotic genes, including transgenes, not already present into samples conserved in the genebank. Proper germplasm management procedures and genebank practices and protocols to ensure quality and integrity of accessions are followed.

3. Transgenes and conventional genes are subject to the same underlying biological processes of mutation, geneflow, introgression, recombination and natural selection. Therefore, best practices for preventing introgression of conventional genes provide an appropriate basis for preventing introgression of transgenes.

4. Germplasm management procedures in IRRI conform to current best practices for rice. These best practices include procedures and practices that aim to prevent the transfer of genes from sources other than the accession in question. Routes for transfer by other sources include admixture of seeds and pollination. These practices are currently under review under GPG2, and may be modified / upgraded following based on a comprehensive review of risk management and quality management throughout genebank management.

5. Available technical means do not permit the complete exclusion of unintentional presence of exotic genes, including transgenes, in genebank accessions. In addition, available

testing techniques do not provide an absolute guarantee, without testing every single seed or plant that any given accession is free of transgenes. However, best practices in genebanks will achieve a high degree of statistical probability that an accession does not include unintentionally present transgenes.

6. Factors contributing to the risk of presence of transgenes are assessed here for the following major genebank operations: collecting, acquisitions, regeneration, distribution, testing health, testing viability, curative treatment, conservation, characterization, evaluation, documentation, data sharing.

COLLECTING

Risk assessment

Collecting from *in situ* conditions (farms, market places, wild habitats) is one of the stages at which the genebank is most open to unintentional introduction of transgenes, because the germplasm has been exposed to geneflow outside the control of the genebank, and because of the risk of misinformation about the status of a collected sample.

Cultivated rice (*Oryza sativa* and *O. glaberrima*) is predominantly inbreeding. Thus the risk of transgenes appearing in conventionally-bred varieties by natural introgression from GM varieties is lower than for outbreeding crops at a similar stage in the development of transgenic varieties. However, the low probability of introgression between two rice crops compounds over fields and seasons, and thus it becomes effectively certain that transgenes in rice will appear at some stage in supposed non-transgenic varieties. Outcrossing rate is genetically variable, and is strongly correlated to the extent exsertion of the stigma beyond the glume.

The wild ancestors *O rufipogon* and *O. longistaminata* are predominantly outbreeding. Hybrid swarms between *O. rufipogon* and *O. sativa* are common where the wild species is found close to farmers fields, and the rate of outcrossing between the two is high. Thus the probability of introgression of transgenes to wild rice is much higher.

Numerous transgenic events exist in rice. A few have been grown commercially in Iran, USA and China, and have been field tested in Bangladesh, India and the Philippines. The numbers of transgenes grown and the number of countries they are grown is expected to increase. During 2006-2007 there have been several reports of transgenes from USA and China appearing spontaneously in a number of countries in Europe and Africa, and moving between varieties. Thus already there is a non-zero probability of transgenes being present in the field even in countries that do not permit transgenic rice. The probability obviously varies between countries and is expected to increase, but must already be considered non-zero in every country that imports rice.

The general risk of transgenes rice appearing in non-GM varieties in a particular country or region depends on a number of factors:

- The existence and enforcement of regulations on GM rice in the country
- The existence and implementation of procedures to monitor the presence of transgenes in the country
- The prevalence of GM technologies, germplasm and/or rice crops in the country and region of collection, depending on factors such as public acceptance, marketing transparency, the extent to which GM varieties meet farmer’s wishes

- The presence in markets or in aid shipments of imported GM varieties not labelled as GM, or of imported conventional varieties from high-risk countries such as China, USA and Iran.
- The presence of outbreeding wild relatives such as O. rufipogon in habitats close to rice crops
- The degree of informal seed exchange between farmers

Within a country or region, the specific risk at a site of collection also depends on other factors such as:

- Proximity to research and development facilities and field testing sites where GM rice is knowingly studied
- Proximity to research and development facilities and field testing sites that frequently introduce new germplasm for testing and are therefore at higher risk of unknowingly introducing GM rice
- Proximity to ports of entry, processing factories or transportation arteries, where the volunteer and feral rice plants may be relatively abundant
- Proximity of the collected sample to a cross compatible species (e.g. the proximity of a sample of *O. sativa* to a population of *O. rufipogon* or vice versa)
- Distance to the nearest GM field

Risk mitigation

Before any new collecting mission is undertaken, a specific review of the level of risk must be undertaken for each of the above risk factors. Since the risk must be considered non-zero in every country that imports rice, and since it varies between countries and is expected to increase with time, a new risk assessment must be taken before each mission, even on repeat missions to the same region. The result of the specific risk assessment must be used to guide planning of the collecting mission.

If the risk is judged to be high or medium, whether generally across the whole region or specifically in certain locations, then funding for the collecting mission should be sought to test some or all collected samples. If such funding is not forthcoming, samples should not be collected from locations where the risk is medium to high; and if the whole region covered by the mission is considered medium to high risk, the collecting mission should be aborted.

If there are known sources of GM rice in the collecting region, the collecting form used for entering passport data about each collected sample must include an item for recording proximity to such sources. Similarly, the collecting form must include an item for recording proximity to locations where there is a relatively high risk of unintentional presence of transgenes.

Monitoring and containment

If the risk of transgene presence is high, the sample should be treated as transgenic and contained under GM regulations until it is demonstrated to be not transgenic.

Every sample collected despite a high risk, and a subset of samples collected despite a medium risk, should be tested according to the following procedure:

- After completion of relevant import and phytosanitary procedures, use a subset to establish a regeneration plot.

- Put aside the remaining seed for long-term conservation as the “Most Original Sample” (MOS). This sample should not be used again except in exceptional circumstances.
- Take leaf samples from every parental plant in the regeneration plot, and test all for the presence of transgenes. Ensure that tests are completed before anthesis (and the consequent possibility of pollinating other plants in the plot), so that appropriate action can be taken if a transgene is detected (see below)
- If no transgene is detected, use the harvested seed as the basis for the accession
- In the case of any future need to revert to the MOS, test all seed used from the MOS

If a transgene is detected in any plant, the authorities where the sample was collected should be collected and a decision jointly made on appropriate further action. Possible actions include:

- Destroy the plot and the MOS
- Destroy only the transgenic plant(s) and keep only the non-transgenic plants as the basis for the accession
- If permissible under any intellectual property rights associated with the detected transgene, separate the transgenic plant(s), for conservation as a GM rice in its own right either instead of or in addition to the non-transgenic plants.

ACQUISITION

Risk assessment

As with collecting germplasm from *in situ* conditions, acquisition of germplasm from an *ex situ* source (e.g. another genebank, a breeder, university, or research institute) also represents a significant critical risk point, again because the germplasm has been exposed to geneflow outside the control of the genebank and because of the risk of misinformation. In this case, the risk of unintentional presence of transgenes compounds the risk of presence when the provider originally obtained or developed it, with the risks of introgression during the provider’s management of the sample.

Knowledge of the risk also depends on the provider’s ability to provide relevant information. This includes information on the origin of the material, on standards and procedures followed to assess risks, maintain genetic integrity and monitor transgene presence, and on compliance with relevant biosafety and genebank management standards.

Factors contributing to the risk include:

- The presence of transgenes in the region where the provider obtained it
- The presence of transgenes in the environs where the provider was managing the sample, including the provider’s own research and breeding with transgenic rice.
- The provider’s compliance to good germplasm management practices and to institutional, local and national biosafety standards.
- Ability of the provider to assess, manage and document the unintentional presence of transgenes.

Risk mitigation

Before any rice is acquired from an *ex situ* source, the potential provider should be asked to provide a declaration on the GM-status of the sample. To ensure sufficient information is obtained to make a sound judgement on the severity of the above risk factors, the provider should be asked to complete following questionnaire:

1. Is the sample transgenic (yes / not tested / tested with negative result)?
2. If you have tested the sample and failed to detect transgenes,
    a. What procedure did you use to detect transgenes?
    b. What minimum frequency of transgenes can your test procedure detect, with what confidence?

If you have not tested the sample, please answer the following questions:

3. Did you breed the sample yourself?
4. If you did not breed the sample,
    a. When did you obtain it?
    b. From where did you obtain it?
    c. Did you acquire it with any statement or analysis of the presence of transgenes at the time of acquisition?
    d. What do you know about the likelihood of intentional or unintentional presence of transgenic rice in the region of the provider at the time of acquisition?
5. Does your organization knowingly handle transgenic rice?
6. If your organization does knowingly handle transgenic rice, what procedures do you follow to keep transgenic rice separate from non-transgenic rice?
7. What other potential sources of transgenic rice might there be, known or unknown, in the region where you work?
8. Do you follow documented best practices for germplasm management? If so, what?
9. What institutional, local and national biosafety standards do you follow that are relevant to the risk of presence of transgenes

If the risk of presence of transgenes in a sample is judged high or medium, reject the acquisition, unless (a) special funds are available to test or (b) acquisition of the material is of sufficiently high importance to test.

Monitoring and containment

Every sample accepted despite a high risk, and a subset of samples accepted despite a medium risk, should be tested according to the same procedure outlined for collecting. In the event of the discovery of transgenes in a sample, the provider should be informed and the sample and MOS destroyed.

REGENERATION

Risk assessment

Regeneration (i.e. growing plants of accessions to produce more seed) is the most critical point during the management of the accession under the control of the genebank. Some genetic change during regeneration is inevitable. Like any gene, transgenes may be incorporated into an accession during regeneration by admixture of transgenic seed or by pollination with transgenic pollen. The seed or pollen may be from a known transgenic line or from a line containing an undetected transgene.

In common with other annual inbreeding crops, accessions of cultivated rice are normally regenerated in field plots. This involves risks that are eliminated from crops regenerated in isolation chambers:

- pollen may be introduced from neighbouring plots in the regeneration field, or from nearby plots or fields outside the control of the genebank; the pollen may come from deliberately-sown plots or from volunteer plants or, in the case of wild relatives, from nearby wild habitats;
- volunteer plants may develop within the plot from seed left in the soil from the previous season's harvest, or from seed flowing in to the plot in irrigation streams, or dropped from equipment or clothing used during sowing and plot management.

Wild rice species, as potentially invasive species, are grown only in a contained screenhouse. For these species, risks will be low, provided good management practices are followed in the screenhouse. The only potential source of transgenic pollen would be the few cases of *O. sativa* or *O. glaberrima* grown in the screenhouse to meet special cultural needs.

As the development and use of transgenic rice develops, and especially if field releases of transgenic rice in the Philippines are approved, the risk will increase. In particular, the frequency of transgenic lines in breeders' plots at IRRI will increase, changing from insignificant at the moment to possibly one of the major factors in the future.

Factors contributing to the risk include:

- Inadequate isolation from sources of transgenic pollen during anthesis
- Incomplete roguing of volunteers in the regeneration plots
- Inadequate prevention of admixture of transgenic seeds throughout the regeneration process, for example through inadequate cleanliness of clothing or equipment.
- Inadequate management of and security against human factors such as vandalism and mislabelling

Risk mitigation

The following risk-mitigating procedures, already in place as an essential part of standard germplasm regeneration procedures to prevent the introgression from foreign pollen or admixture with foreign seed, are also critical for the exclusion of transgenes:

- Apply cultural measures to eliminate remnant seed from the soil
- Ensure scrupulous cleanliness of all equipment at all times, including the complete removal of seed from any item of equipment after processing each accession

- Ensure scrupulous personal cleanliness, including the removal of seed from all items of clothing after processing each accession
- Where approximate times from transplanting to anthesis are known, alternate between early- and late-flowering accessions, such that there is an interval of at least two weeks between the flowering of adjacent plots.
- Discard the outermost rows of each plot at harvest, as they contain most foreign pollinations
- Filter irrigation water to ensure that seeds cannot move from plot to plot during irrigation
- Ensure full training or constant supervision of labourers, to ensure they comply with required standards of cleanliness and care.

The following additional procedures are needed, either specifically to address the case of transgenic rice or to provide a higher level of risk mitigation than provided for in current recommended procedures:

- Completely separate the regeneration and handling of known GM rice and associated control from the regeneration of genebank accessions (see management of GM rice under other genebank operations)
- Grow regeneration plots of genebank accessions as far as possible (≥ 3km) from any contained field trials[1] of GM rice
- Grow a barrier hedge (tall sorghum or tall maize) around the regeneration field
- Maintain a 150m barrier between the regeneration field and any other rice plots; the barrier should be grown to a different crop, not left fallow.
- Grow regeneration plots one month earlier than the nearest plots of other rice

The following procedures may also be desirable for further risk mitigation, but their efficacy has not been adequately studied:

- Reduce the density of plants, to about 40cm spacing. This will improve the detection and removal of volunteer plants. However, the implication for seed quality needs to be assessed – the larger yield per plant may, for example, reduce the uniformity of seed maturation.
- Delay harvest. The current procedure, harvesting at "physiological maturity", is reported to improve seed longevity in storage, but results in a large proportion of immature seeds that have to be discarded manually. Can we reduce the proportion discarded without adversely affecting longevity?

[1] Philippine biosafety procedures for GM crops include "contained field trials" as a stage in biosafety testing, after testing in contained facilities and prior to full field-scale trials. In a contained field trial, GM rice is grown in the field but with a series of highly effective spatial and temporal barriers to gene flow, and subject to frequent inspection by biosafety inspection officers.

Monitoring and containment

At present, since GM rice is not commercially available in the Philippines, and since IRRI is still only at the stage of conducting "contained field trials" of GM rice, implementation of the above mitigation procedures is considered sufficient to reduce the risk of introducing transgenes in regeneration plots to a level where monitoring is not necessary.

However, this conclusion must be reviewed regularly. It is likely to change within a few years as full field testing and commercial growing of GM rice in the Philippines becomes a reality, and as transgenes introgress into normal varieties and transgenes become a more significant part of IRRI's rice research research portfolio.

SEED DISTRIBUTION

Risk assessment

Since it is not possible to guarantee that there are no transgenes in the GRC at IRRI, there is a non-zero risk that we will unintentionally send GM rice to a recipient.

Risk mitigation

The procedures for "other genebank operations" described below apply equally to seed distribution. Good laboratory practice must be followed during the preparation of seed for distribution, to ensure that seed packets are not mislabelled and that, and that there is no admixture of seeds from different sources, for example by inadequate standards of personal cleanliness or cleaning of equipment between successive samples. Staff and labourers must be fully trained or constantly supervised to ensure good laboratory practice.

Implementation of all risk mitigation procedures for all processes analysed in this document will, by reducing the likelihood of the unintentional presence of transgenes in the genebank, reduce the likelihood of unintentionally distributing transgenes to others.

Monitoring and containment

Since the likelihood of the presence of transgenes in the GRC is low, no programme of testing is currently envisaged to monitor the presence of transgenes in rice samples distributed to others.

If a requestor asks for a GM-free certificate, then the following responses will be offered:

- If the material has not been tested, the following statement will be made:
  "To the best of our knowledge the sample is GM free. It was developed and produced solely by conventional methods, without the application of genetic modification technologies, and in isolation from any known source of potential contamination by genetically modified varieties. It has been managed in accordance with a series of protocols to minimize the likelihood of the unintentional presence of transgenes, as described at *(URL)*"
- If the material has been tested, the following statement will be made:
  "The seed has been sampled from material that has been tested for the presence of *(transgene(s))*. Within the limits of detection by the test, the sample is certified free of the said transgenes)"

If a requestor asks for tests to be conducted, this will be offered at the requestor's expense. Two levels of testing may be offered:

- Testing one subsample to the requestor's required degree of certainty, and sending a separate subsample
- Using a subsample to regenerate a new generation, and testing every parental plant in that subsample.

DOCUMENTATION AND DATA SHARING

Risk assessment

Inaccurate documentation may lead to incorrect decisions on handling accessions.

Risk mitigation

The primary mitigation is to ensure accurate documentation. Procedures and protocols for improving data accuracy are currently under review in IRRI, both as an institute-wide assessment and specifically for the GRC within the context of GPG2. These include the improvement of automated data validation as well as the improvement of individual working habits. The process of recording and encoding data must be double-checked.

OTHER GENEBANK OPERATIONS

Risk assessment

Other genebank operations (testing health, testing viability, curative treatment, conservation, characterization, evaluation) are treated together here, since factors contributing to unintentional introduction of transgenes are essentially the same; all involve handling seeds.

Contributing factors include:

- Mislabelling of seed packets
- Mixing of seeds from different sources, for example by inadequate standards of personal cleanliness or cleaning of equipment between successive samples
- Inadequate quality control, documentation of procedures or training and supervision of staff

The same operations are required for the management of known GM rice, generating two distinct categories of risk:

- The risk of mislabelling or admixture from known GM rice
- The risk of mislabelling or admixture of undetected transgenes in a supposedly non-transgenic genebank accession

Risk mitigation

A major element of mitigating risk is to ensure that GM rice remains separate from genebank accessions:

- GM rice should be managed entirely separately from genebank accessions.

    - Different buildings and laboratories should be used;
    - Staff and administration should be independent, to ensure that transgenic material is not accidentally transferred by staff moving between transgenic and genebank facilities;
    - Transport routes (for example between laboratory and field, or between laboratory and screenhouse) should be independent and non-overlapping
- Control plants, and any other plants harvested in any containment facility in which transgenic plants were ever grown, should be managed like GM rice, i.e. they should be managed entirely separately from genebank accessions.
- GM facilities (cold room, drying room, seed handling room, transgenic greenhouses with high-level containment, analytical lab, transformation lab) should be organized as far as possible into a single unit with controlled access and the highest level of containment, and with no external transport routes between facilities.
- Movement of GM rice from place to place should be curtailed as much as possible in viable form:
    - Incorporate seed-processing head-houses into low-level containment screenhouses: grain should be polished (removing the embryo) before leaving the facility for transfer to the adjacent analytical labs

Good laboratory practice in genebank operations, already undertaken to prevent mislabelling and admixture, is equally essential to prevent the spread of undetected transgenes within the genebank:

- Staff and labourers must be fully trained or constantly supervised to ensure good laboratory practice.
- All processes and workflows must be fully documented and implemented
- All equipment must be kept scrupulously clean; and in particular all remnant seed must be removed and disposed of after completing each process
- Personal cleanliness must be scrupulously observed, including the cleanliness of clothes and hair; and in particular clothing and hair should be checked for the seeds after completing each process
- Working areas must be well demarcated so that there can be no seed admixture between groups working on different accessions
- All seed packets should be labelled inside and outside
- The correspondence between labels on the seed packet and data recording sheets should always be double checked.

Monitoring and containment

Since the likelihood of the presence of transgenes in the GRC is currently low and all accessions were acquired before transgenic rice was created or tested, no programme of testing is currently envisaged to monitor the presence of transgenes during these genebank operations. This is considered unlikely to change: if the policy operates effectively monitoring and containment of transgene introductions during collection, acquisition, regeneration and distribution will be sufficient to control unintentional transgene presences under any environment.