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

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 at IRRI, 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 those practices that most effectively minimize the probability of unintentionally introducing transgenes, and to identify critical points of risk, so that we minimize the need for unnecessary testing for the presence of transgenes.

The document has been developed following the "Guiding principles for the development of Future Harvest 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 IRRI's genebank 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 genes, is a threat because it changes the genetic identity of the accession. IRRI therefore takes proactive steps to prevent the unintentional introgression of exotic genes, including transgenes, not already present in samples conserved in the genebank. Proper germplasm-management procedures and genebank practices and protocols are followed to ensure the quality and integrity of accessions.

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 the introgression of conventional genes provide an appropriate basis for preventing the introgression of transgenes.

4. Germplasm management procedures at IRRI conform to current best practices for rice. They include procedures and practices that aim to prevent the transfer of genes from sources other than the accession in question. Potential routes for transfer of transgenes from other sources include admixture of seeds and pollination. IRRI's practices are currently under review under GPG2, and may be modified or upgraded following a comprehensive review of risk management and quality management throughout the genebank.

5. Available technical means do not guarantee the complete exclusion of exotic genes, including transgenes, from genebank accessions. In addition, available testing techniques do not provide absolute certainty, 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 a given accession does not include unintentionally-introduced transgenes.

6. The factors contributing to the risk of the introduction of transgenes are assessed here for the following major genebank operations: collection, acquisition, regeneration, distribution, health testing, viability testing, curative treatment, conservation, characterization, evaluation, documentation, and data sharing.

COLLECTION

Risk assessment

Collection of new accessions from *in situ* sources (e.g., farms, marketplaces and wild habitats) is one of the stages at which the genebank is most vulnerable to the unintentional introduction of transgenes, because the germplasm has been exposed to geneflow outside the control of the genebank, and because the risk of misinformation about the status of a collected sample is high.

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 is compounded over fields and seasons, and thus it becomes effectively certain that transgenes will appear at some stage in supposed non-transgenic rice varieties. The outcrossing rate is genetically variable, and is strongly correlated to the extent of the exsertion of the stigma beyond the glume.

The wild ancestors of cultivated rice, *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 the introgression of transgenes into wild rice is much higher.

Numerous transgenic events exist in rice. A few have been grown commercially in Iran, the 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 in is expected to increase. During 2006-2007 there were several reports of transgenes from the 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; thus the risk must be re-assessed beforehand as an essential new component of planning any collecting mission.

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

- The existence and enforcement of regulations on transgenic rice in the country
- The existence and implementation of procedures to monitor the presence of transgenes in the country
- The prevalence of transgenic technologies, germplasm and/or rice crops in the country and region of collection, depending on factors such as public acceptance,

marketing transparency, and the extent to which transgenic varieties meet farmers' needs

- The presence in markets or in aid shipments of imported transgenic varieties not labelled as transgenic, or of imported conventional varieties from high-risk countries such as China, the USA, or 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 transgenic 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 transgenic rice
- Proximity to ports of entry, processing factories or transportation arteries, where volunteer and feral rice plants may be relatively abundant
- Proximity of the collection site to a cross-compatible species (e.g., the proximity of an *O. sativa* collection site to a population of *O. rufipogon* or vice versa)
- Distance to the nearest transgenic field

Risk mitigation

Before any new collection mission is undertaken, a specific review of the level of risk must be carried out for each of the above risk factors.

If the risk of the collection mission is judged to be high or medium, whether across the whole region or in certain locations, funding should be sought to cover the cost of testing some or all of the collected samples. If such funding is not forthcoming, samples should not be collected from locations where the risk is medium or high. If the whole region covered by the mission is considered medium to high risk, the collection mission should be aborted.

If there are known sources of transgenic rice in the collection region, the collecting form used for entering passport data about each sample must include a field for recording proximity to such sources. Similarly, the collecting form must include a field for recording proximity to locations where there is a relatively high risk of the 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 not to be transgenic.

All of any samples that are collected despite a high risk of transgene presence in the collection location, and a subset of any 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 of the seed 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 each of these for the presence of transgenes. Ensure that tests are completed before anthesis (and the consequent possibility of a transgenic plant pollinating other plants in the plot), so that appropriate action can be taken if any 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 informed 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 transgenic 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* sources, acquisition of germplasm from an *ex situ* source (e.g., another genebank or 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 the unintentional presence of transgenes is compounded of the risk of transgene presence or introduction when the provider originally obtained or developed the germplasm, and the risk of introgression during the provider’s management of the sample.

Developing an understanding of the risk 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 the sample

- The presence of transgenes in the area where the provider was managing the sample, including any of the provider's own research and breeding with transgenic rice
- The provider's compliance with good germplasm management practices and with institutional, local and national biosafety standards
- The 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 transgenic status of the sample. To ensure that sufficient information is obtained to make a sound judgement on the level of risk, the provider should be asked to complete following the 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 the 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, which ones?
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 the presence of transgenes in a sample is judged high or medium, the acquisition should be rejected unless the acquisition of the material is of sufficiently high importance to carry out testing, or special funds are available.

Monitoring and containment

Any sample that is accepted despite a high risk of the presence of transgenes, and a subset of any samples accepted despite a medium risk, should be tested according to the same procedure outlined for collection. In the event of a transgene discovery, the provider should be informed and the entire sample, including the MOS, destroyed.

REGENERATION

Risk assessment

Regeneration (i.e., the planting of accessions to produce more seed) is the most critical point during the management of accessions that is 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 with transgenic seed or by pollination by 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 inbreeding annual crops, accessions of cultivated rice are normally regenerated in field plots. This involves risks that are not present where crops are 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 or volunteer plants, or, in the case of pollen from 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, carried into the plot by 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 of transgene introduction 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 expand, and especially if field releases of transgenic rice in the Philippines are approved, the risks will grow. In particular, the frequency of transgenic lines in breeders' plots at IRRI will increase, from currently insignificant to perhaps being a major component in the future.

Factors contributing to the risk of unintentional transgene introduction include:

- Inadequate isolation from sources of transgenic pollen during anthesis
- Incomplete roguing of volunteers in the regeneration plots
- Inadequate prevention of admixture with transgenic seeds during 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-mitigation procedures, already in place as an essential part of standard germplasm regeneration procedures to prevent the introgression of conventional genes from foreign pollen or admixture with foreign seed, are also critical for the exclusion of transgenes:

- Apply growing measures to eliminate seed remaining in 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 within regeneration fields, 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 are most likely to have received 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 that they comply with required standards of cleanliness and care

The following additional procedures are needed, either to specifically 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 transgenic rice and associated control from the regeneration of genebank accessions (see management of transgenic rice under the section on other genebank operations)
- Grow regeneration plots of genebank accessions as far as possible (at least 3km) from any contained field trials[1] of transgenic rice
- Grow a barrier hedge (of 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.
- Plant 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:

[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-scale field trials. In a contained field trial, transgenic rice is grown in the field, but with a series of highly effective spatial and temporal barriers to geneflow, and is subject to frequent inspection by biosafety inspection officers.

- Reduce the density of plants, to about 40cm spacing. This will facilitate 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. Study is needed to determine if it is possible to reduce the proportion of seeds discarded without adversely affecting longevity.

Monitoring and containment

At present, since transgenic rice is not commercially available in the Philippines, and since IRRI is still only at the stage of conducting contained field trials of transgenic 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 transgenic rice in the Philippines become a reality, as transgenes introgress into normal varieties, and as transgenes become a more significant part of IRRI's rice research portfolio.

SEED DISTRIBUTION

Risk assessment

Since it is not possible to guarantee absolutely that there are no transgenes in the genebank at IRRI, there is a non-zero risk that we will unintentionally send transgenic rice to a seed 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 there is no admixture of seeds from different sources, for example due to 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 discussed 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 genebank 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 the material to be certified GM-free, 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.
- 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 provided:

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

OTHER GENEBANK OPERATIONS

Risk assessment

Other genebank operations (including seed health testing, seed viability testing, seed health treatment, conservation, characterization, and evaluation) are treated together here, since the factors contributing to the risk of the 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 through 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 genebank operations must also be carried out in the management of known transgenic rice, generating two distinct categories of risk:

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

Risk mitigation

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

- Transgenic rice should be managed entirely separately from genebank accessions:
    - Different laboratories and other buildings 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 have ever been grown, should be managed like transenic rice, i.e., they should be managed entirely separately from genebank accessions.
- Transgenic facilities (including cold room, drying room, seed handling room, transgenic greenhouses with high-level containment, analytical lab, and 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 transgenic rice from place to place in viable form should be curtailed as much as possible, by:
    - incorporating seed-processing head-houses into low-level containment screenhouses:
    - polishing grain (removing the embryo) before it leaves the genebank for transfer to 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 practices

- 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 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 both inside and out
- The correspondence between seed packet labels and data recording sheets should always be double-checked.

Monitoring and containment

Since the likelihood of the presence of transgenes in the genebank 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 IRRI's policies operate effectively in monitoring and containing transgene introductions during collection, acquisition, regeneration and distribution, this will be sufficient to control the unintentional presence of transgenes under any conditions.