GPG2, Activity 2.1.2

Guidelines for the detection of the unintentional presence of transgenes in potato germplasm accessions at CIP's genebank.

1. Collection

Activity:
Cultivated potato is collected *in situ*, e.g., in farmers' fields and markets.

Source(s) of risk:
Transgenic varieties have been released and produced on a commercial scale only in the USA and Canada. These were NewLeaf varieties by NatureMark, a subsidiary of Monsanto, available between 1996 and 2001. These transgenic varieties are no longer commercialized at significant scales. No export of unprocessed potatoes took place from those countries.

Transgenic research materials have been field trialed in many countries around the world. However, collection of potato germplasm at CIP has been almost exclusively of native and commercially-released varieties from Andean countries. In Peru, Argentina, Chile, and Bolivia, transgenic research materials have been trialed in the field, but in well-controlled environments at very small scales. None of the transgenic research materials were released or persisted in the field. The materials were not of interest to farmers and are not known to confer competitive advantage or greater fitness. The engineered traits were resistance to viral and bacterial diseases. Only virus resistance proved effective, and this trait is generally not recognized by farmers.

Risk management:
No cultivated potato was collected by CIP in Argentina, Bolivia, Chile, Peru or the USA at the time of commercial cultivation of transgenic varieties (Table 1). All cultivated potato samples acquired by CIP during this period originated from germplasm conserved by research institutions (see section 2 on acquisition and introduction).

In conclusion, the risk of the unintentional presence of transgenes in collected cultivated potato samples is low and therefore collected materials do not need to be tested.

2. Acquisition and introduction

Activity:
In addition to collected materials, cultivated potato germplasm is also acquired from *ex situ* sources (centers, genebanks, individuals, scientists, private growers, seed companies, etc). Both collected and acquired material is grown in the greenhouse and later in the field in order to score morphological descriptors and perform taxonomic identification. This work can take more than two years depending on the material and the level of characterization required.

Source(s) of risk:
Potatoes, including transgenic research materials, are clonally propagated in the greenhouse and *in vitro*, sometimes in the same facilities by the same staff, which may constitute a source of mixtures in the materials acquired by CIP. Morphological differences and labels are normally used for precise identification. However,

morphological discrimination is of limited value when only plant tissue cultures or tubers are transferred to CIP. Mislabeling is also possible, although minimized in responsible institutions. CIP has experienced such identity errors in handling conventional varieties both at the acquisition and conservation stages.

Gene flow through pollen is a rare event in modern varieties as these are based on germplasm from Chilotanum groups (i.e. *S. tuberosum* ssp. *tuberosum*), which are adapted to long-day conditions and often have male sterility. However, the possibility cannot be entirely disregarded. The risk of volunteer plants from modern varieties depends on the variety itself and field management practices. The mixing of tubers is also possible when materials are harvested simultaneously. Mixtures may also be created by acts of vandalism, but require an understanding of the potential for harm and how to cause it.

Risk management:
Between 1994, when the first transgenic research in potato began, and 2004, the date of its most recent acquisitions, CIP acquired cultivated potato germplasm as tubers or *in vitro* material from the *ex situ* sources listed in table 2. Of these, none of the research groups providing accessions to the CIP genebank were developing transgenic potatoes at the time of the transfer of the material. Other groups in these institutions were involved in transgenic research but these were physically separated. For future acquisitions, it is advisable to inquire about any current transgenic research at the providing institution and its physical separation from genetic resource conservation.

There have been no commercial field trials of transgenic potatoes in or near the fields and facilities where the collected and acquired materials were handled. CIP did carry out one research field trial close to its experiment station in Huancayo between November 1997 and December 1997 using a transgenic potato (Desiree cv.) with a proteinase inhibitor gene. The field was of a small size surrounded by a maize row acting as a plant barrier. No genebank material was present on the experiment station at the time of this trial. Although Desiree cv. flowers poorly or not at all in Huancayo, any stems with floral buds were manually removed throughout the trial to avoid pollen transmission. Extreme care was taken at harvest: a single field harvest was carried out to eliminate the possibility of mixing, and all residues were burned *in situ*. The harvested tubers were stored separately and subsequently brought to Lima for research analysis.

In Huancayo, Desiree cv. has a very limited dispersal capacity. It usually does not flower, but tuberizes early and produces few large tubers. The probability of natural survival of volunteer plants is estimated to be very low, as reported by potato experts and the available literature. Desiree has a short growing cycle of 90 days; leftover tubers may germinate after harvest when tuber dormancy is broken, but these volunteer plants will die when exposed to the frosts that occur from June to August. Desiree has no resistance to frost and the proteinase inhibitor gene appears unlikely to increase frost resistance. Desiree is also susceptible to late blight disease, which results in reduced tuber production and eventually kills diseased plants. The disease occurs in repeated cycles, reducing the chances of survival of Desiree volunteers. The fields in this area are generally left fallow or are not used for potato for 5 to 10 years between potato crops, long enough for frost to eliminate any possible volunteer plants.

The possibility of vandalism can be disregarded because staff members were not aware of the potential impact of mixtures with genebank accessions, as transgenic crops were not controversial in Peru until very recent years.

The possibility of tubers being taken by staff or farmers can also be discarded. In Huancayo, Desiree shows no attractive traits that may act as incentives to farmers to use it locally: it has a low and underdeveloped canopy, plant senescence is too early, it is susceptible to diseases, and it produces only few tubers, with low dry matter content.

In conclusion, the risk of the unintentional presence of transgenes in potato material provided to CIP from *ex situ* sources is low, and the risk of the unintentional introduction of transgenes to potato material characterized in the greenhouse or in the field at CIP prior to its introduction into the genebank are low and therefore genebank materials that have gone through this process do not need to be tested.

3. Regeneration

Activity:
Cultivated potato is periodically regenerated from its conserved stage *in vitro* by tissue culture or cryopreservation, and in tuber collections. The latter are obtained from field and greenhouse activities and consist of material in transitory status prior to introduction into the genebank's *in vitro* collection.

Source(s) of risk:
Mixtures between genebank accessions and transgenic research materials are possible where there is inadequate physical separation. In CIP, until 2001, the genebank and transgenic research materials were propagated and stored in the same laminar flow chambers and growth and conservation rooms, contributing to this risk. Vandalism could also have been a source of mixtures.

Risk management:
A sample of 1,235 accessions from the potato genebank was screened for the presence of transgenes in 2004. The results indicated no evidence for the presence of transgenes in the accessions. The sample was randomly selected and represents almost one third of all the genebank accessions of cultivated potato.

In conclusion, there is a moderate risk of the unintentional presence of transgenes in potato material regenerated from its conserved stage *in vitro* at CIP, and therefore those genebank materials that have not yet been screened may need to be tested for the unintentional presence of transgenes.

4. Characterization

Activity:
Potato genebank accessions are characterized for highly heritable physical, morphological and molecular characteristics. Some of these evaluations take place in the greenhouse and others in the field.

Source(s) of risk:
In the field, pollen fertilization is theoretically possible although, an extremely rare event in modern varieties. Tuber mixtures at harvest may occur through simultaneous harvest or volunteers. An act of vandalism would also be possible.

Risk management
Regardless of the level of risk, the characterized material is not returned to the genebank. The risk of the unintentional introduction of transgenes through characterization of potato material from the CIP genebank is low, and therefore characterized materials do not need to be tested.

5. Conservation

Activity:
As mentioned above under Regeneration, cultivated potato is conserved *in vitro* by tissue culture or cryopreservation, and in tuber collections. The latter are obtained from field and greenhouse activities and consist of material in transitory status prior to introduction into the genebank's *in vitro* collection.

Source(s) of risk:
Mixtures between genebank accessions and transgenic research materials are possible where there is inadequate physical separation. Since 2001, the genebank and transgenic research materials are propagated and stored in two fully separated buildings under the responsibility of different teams of technicians and researchers.

Risk management
Due to the physical separation, the conservation activity presents no risks of inadvertent introduction of transgenes into the genebank.

In conclusion, the risk of the unintentional presence of transgenes in genebank as a result of the conservation activity is low and therefore genebank materials do not need to be tested.

6. Testing (health, viability, transgenes)

Activity:
The detection of relevant pathogens and pests is an important procedure for genebank material. It takes place in specialized greenhouses and tissue culture facilities.

Source(s) of risk:
The tested material is never returned to the original accession in the genebank, so there is no source of risk.

Risk management
There is no risk of the unintentional introduction of transgenes through potato material submitted to testing for the presence of pathogens and pests.

7. Health treatment

Activity:
Potato accessions found to contain viral pathogens are heat-treated.

Source(s) of risk:

Meristem culture and heat-treatment took place in the same facility used for transgenic research material prior to 2001, so it is possible that mixtures may have occurred either accidentally or deliberately.

Risk management
As mentioned above, almost one third of the total genebank accessions of cultivated potato were screened for the presence of transgenes in 2004, and none were observed.

The risk of the unintentional presence of transgenes introduced during treatment in potato material conserved *in vitro* at CIP is comparable to the risk of the presence of transgenes introduced during conservation, i.e. moderate. Therefore the unscreened genebank materials may need to be tested for transgenes.

8. Evaluation

Activity:
The potato genebank accessions are regularly assessed for performance in the greenhouse and in the field.

Source(s) of risk:
The material evaluated does not re-enter the genebank and therefore does not present a source of risk of the introduction of transgenes to the genebank.

Risk management
There is no need to develop testing as the material does not re-enter the genebank.

9. Distribution

Activity:
Genebank accessions are propagated from the *in vitro* conservation collection and distributed.

Source(s) of risk:
The propagation of genebank accessions and transgenic research material took place in the same facility prior to 2001, so there is a possibility that mixtures may have occurred either accidentally or deliberately.

Risk management:
Since one third of the genebank collection has tested negative for the presence of transgenes and there has been no report of the discovery of new traits corresponding to existing transgenic events from distributed genebank materials, the likelihood of the unintentional presence of transgenes in distributed materials is considered to be low. However, it may be advisable to check all materials available for distribution before they are sent out, as this is relatively inexpensive and easy to implement by direct PCR. CIP has the expertise to perform such testing.

10. Documentation

Activity:
The organized collection of records that detail the structure, purpose, operation, maintenance, and data requirements of the genebank, including acquisition

documentation (including MTA and IP documents) and accession maintenance documentation (including of initial sample size) for genebank accessions.

Source(s) of risk:
The potato genebank does not currently hold transgenic potato varieties, so there is no risk of sharing incorrect information that could lead to the distribution of transgenic varieties under the documentation of non-transgenic materials.

Risk management
No risk-management procedure is required, as there are no risks in this case.

Table 1: Transgenic potato varieties that received regulatory approval for commercialization including release into the environment. Data extracted from the AgBios database (www.agbios.com).

Events	Company	Description	Regulatory approval
ATBT04-6 ATBT04-27 ATBT04-30 ATBT04-31 ATBT04-36 SPBT02-5 SPBT02-7	Monsanto	Colorado potato beetle resistant potatoes produced by inserting the *cry3A* gene from *Bacillus thuringiensis* (subsp. *tenebrionis*).	Canada: 1997 USA: 1997
BT6 BT10 BT12 BT16 BT17 BT18 BT23	Monsanto	Colorado potato beetle resistant potatoes produced by inserting the *cry3A* gene from *Bacillus thuringiensis* (subsp. *tenebrionis*).	Canada: 1995 USA: 1995
RBMT15-101 SEMT15-02 SEMT15-15	Monsanto	Colorado potato beetle and potato virus Y (PVY) resistant potatoes produced by inserting the *cry3A* gene from *Bacillus thuringiensis* (subsp. *tenebrionis*) and the coat protein encoding gene from PVY.	Canada: 1999 USA: 1999
RBMT21-129 RBMT21-350 RBMT22-082	Monsanto	Colorado potato beetle and potato leafroll virus (PLRV) resistant potatoes produced by inserting the *cry3A* gene from *Bacillus thuringiensis* (subsp. *tenebrionis*) and the replicase encoding gene from PLRV.	Canada: 1999 USA: 1998

Table 2: The institutions and the nature of the research groups that provided germplasm to CIP between 1994 and 2009, either as tubers or *in vitro* plants.

Country	Institution	Nature of research group
Argentina	INTA Balcarce	Potato breeders and geneticists
Chile	INIA Remehue	Potato breeders and geneticists
Bolivia	PROINPA	Potato breeders and geneticists
USA	USDA University of Wisconsin	Genetic resources and taxonomy
USA	University of Idaho	Potato breeders and geneticists
USA	USDA Nat. Germ. Resources Lab.	Genetic resources and taxonomy
USA	Cornell University	Potato breeders and geneticists
USA	North Dakota State Seed Department	Genetic resources and taxonomy
USA	Virginia Poly Institute and State University Department of Horticulture	Potato geneticists
Canada	University of Toronto	Potato breeders and geneticists
Canada	Potato Research Center - Agri. Food Canada	Potato breeders and geneticists