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GM Diagnostics as an Aid to Strategic Genebank Management

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India has rich genetic diversity in cultivated crops and their wild relatives. It is one of the 12 mega-biodiversity centres and one of the Vavilovian Centres of Origin of Crop Plants. About 29% of flowering plants occurring in India are endemic and three of the 25 hot-spots of biodiversity exist here. While harnessing the benefits of genetically modified (GM) crops, for better yield, nutritional quality and resistance to biotic and abiotic stresses, it is important to safeguard the genetic diversity. Inadvertant and accidental occurrence of GM events may lead to adventitious presence of transgenes in the *ex situ* collections conserved in the genebanks. The genebanks collect, conserve and provide the genetic resources to the breeders, hence maintaining the purity of genetic identity of the germplasm is of critical importance. Therefore, all possible efforts should be made to prevent the unintentional introgression of transgenes into the conserved samples.

Systematic germplasm management procedures and practices vary from crop to crop, in terms of mode of pollination, breeding system, and whether the crop is annual or perennial. The available techniques and procedures do not enable the complete exclusion of unintentional presence of transgenes, in genebank accessions and therefore do not provide an absolute guarantee, without testing every single seed or plant that any given accession is free from transgenes. Only the best management practices and strategies will achieve the required degree of statistical probability to the effect that an accession in the genebank does not include unintentional presence of transgenes.

Global Inititatives

International Maize and Wheat Improvement Centre (CIMMYT) has shared its expertise and advice with the Mexican institutions to formulate guidelines for maize conservation and related aspects and to conduct gene flow studies at Oaxaca and Chipas regions of Mexico. To discuss various strategies for handling the unintentional presence of transgenes in germplasm collections, a workshop to develop policies for addressing the possibility of adventitious presence of transgenes in CGIAR *ex situ* collections was organised by the International Plant Genetic Resources Institute (IPGRI) at Rome in 2004. The workshop aimed mainly to evolve the technical consensus for developing an appropriate approach to handle the probability of unintentional introgression of transgenes into *ex situ* collections in the genebanks. The technical and economic information associated with institutional strategies and practices for collecting, managing and distributing materials to reduce this risk, including screening for GM elements and associated costs so as to formulate an action plan to minimize the adventitious presence of transgenes need to be worked out and shared with the genebanks.

Future Strategies and Thrust Areas

The genebanks need to take proactive steps to determine the risk of unintentional presence of transgenes in *ex situ* collections. Information needs to be compiled addressing the crop-specific guidelines for best genebank management practices. The major area of unintentional introduction of transgenes is the collection and acquisition stage since the genetic resources may have been exposed to gene flow beyond the control of the genebanks. The strategies therefore need to minimize the gene flow of the transgenes at these stages. As part of their risk analysis, when collecting or acquiring new accessions by other means, the genebanks should ensure the following aspects before testing: (i) Whether transgenic events (commercial or research) in the relevant taxa are likely to be present in the area of exploration/collection; (ii) The distance between the collecting site and areas where transgenic events (commercial or research) are being cultivated; (iii) Whether germplasm providers can give adequate documentation of their germplasm management practices with respect to the material in question.

With already conserved germplasm accessions, genebank testing procedures need to be guided by the following criteria: (a) No testing would be required

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when: (i) there are no transgenic events (for commercial or research) in the relevant taxa; (ii) there were no transgenic events (commercial or for research) in the relevant taxa at the time of acquisition (e.g. *Bt* cotton prior to March 2002 in India); (b) there are transgenic events (for commercial or research) present, however, proper management practices have been followed and documented in the management of the accession.

A database on the global and national status of GM research and development for crops need to be maintained by the genebanks.

Issues and Gaps in GM Detection

There are several issues pertaining to the development of methods for GM testing which need to be addressed:

(i) Self certification/disclosure from source countries or personnel

(ii) Obtaining gene sequences for synthesizing probes and primers from the developer of GM crop in case of indigenously developed GM crop and from the importer in case of imported GM crop

(iii) Classifying areas and crops as per the possibility of contamination: certain hot-spots, where the possibility of accidental contamination by transgenic seeds is possible have to be identified

(iv) Cost of detection: less for protein detection in the field, though developmental cost for the kits is high; moderate, if it is for one gene and one sample in case the primer/probe(s) of the transgenes are already available and high if several samples are to be analyzed and it is not known as to which transgene should be analyzed and fresh primer/probe(s) need to be designed and synthesized.

Initiative at National Level

National Gene bank at ICAR-National Bureau of Plant Genetic Resources (ICAR-NBPGR), the second largest genebank in the world, conserves more than 0.4 million accessions conserves of field and horticultural crops and their wild relatives. India being rich in agrobiodiversity, efforts for efficient management of germplasm are being made. For GM-free conservation, efficient strategy is to check the unintentional introgression/adventitious presence of transgenes in *ex situ* collections employing DNA-based GM diagnostics (Tiwari and Randhawa, 2010).

The expertise and capacity for DNA based GM detection has been strengthened and upgraded significantly at ICAR-NBPGR during the last decade. Cost-effective strategies based on GMO matrix, polymerase chain reaction (PCR), real-time PCR, loop-mediated isothermal amplification (LAMP) and multi-target real-time PCR system, which facilitates testing of GM events have been reported by GM detection laboratory. PCR and real-time PCR based diagnostics for *Bt* crops, commercialized (*Bt* cotton) or under field trials (*Bt* okra, *Bt* rice, *Bt* eggplant) in the country, have been developed, which could be employed for monitoring adventitious presence of transgenes in respective crops (Randhawa *et al.*, 2010). To confirm the GM status of a sample irrespective of specific crop and GM trait, a hexaplex PCR-based screening assay targeting marker genes (*aadA, bar, hpt, nptII, pat, uidA*) commonly employed in the GM events, was developed (Randhawa *et al*., 2009). GMO matrix of 141 GM events of 21 crops with 106 genetic elements was developed as a decision support system to check for authorized GM events (Randhawa *et al*., 2014a).

A TaqMan® real-time PCR based multitarget system simultaneously detecting 47 targets for six GM crops was developed (Randhawa *et al*., 2014b). Besides detection of commercialised GM cotton events, the system allows detection of five GM maize events (Bt11, Bt176, MON810, MON89034, TC1507), six cotton events (MON531, MON15985, GFM-cry1A, MON1445, MON88913, Widestrike), rice (Liberty Link), soybean (GTS40-3-2) and wheat (MON71800) using event-/construct-specific assays. LAMP assays were employed to detect commonly employed transgenic elements, *CaMV* 35S and *FMV* promoters, *aadA, nptII* and *uidA* marker genes, and *cry1Ac, cry2Ab2* and *cp4-epsps* genes to check the GM status of the unknown samples (Randhawa *et al.*, 2013; Singh *et al*., 2015). Event-specific LAMP assays have been reported for detection commercialized *Bt* cotton events, Randhawa *et al*., 2015).

To ensure GM free conservation preliminary studies for checking adventitious presence of transgenes in *ex situ* collections of brinjal (150 accessions), cotton (280 accessions), maize (200 accessions), and okra (50 accessions) employing PCR and Real Time PCR based markers were undertaken. The strategy for selecting these

accessions was (i) collection sites in proximity with the regions where field trials of specific GM events of a particular crop were conducted (ii) the year of collection either after commercialisation of the particular crop or the year after which field trials of GM have been conducted. None of the tested accessions of these crops showed adventitious presence of transgenes; Bairwa *et al*., 2016; Randhawa *et al*., 2015; Parimalan *et al*., 2015.

Case studies for monitoring of adventitious presence of transgenes in brinjal, cotton, maize and okra, employing DNA based markers could be used as models for monitoring other major crops with rich diversity or having the Centre of Origin in a particular country, where field trials of GM crops are being conducted in close proximity of those areas rich in their biodiversity.

Meticulously planned introduction of GM crops with effective risk assessment/management strategies and conservation of GM free *ex situ* collections in the National genebanks would ensure purity of conserved germplasm and safeguard the rich biodiversity.

For Further Reading

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