

Two-Tiered Screens of Maize Landraces in CIMMYT Gene Bank and Candidate Landraces for the Collection Indicate No Presence of Transgenes

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El Batán, Texcoco, Mexico—From July through September 2002, CIMMYT's Applied Biotechnology Center screened 65 Mexican landrace accessions from the center's maize gene bank (part of the Wellhausen-Anderson Plant Genetic Resources Center) and 40 Mexican landrace samples being considered for inclusion in the collection, for the presence of transgenes. Samples were collected from 1997 to the present. None of the tested accessions or samples screened positive for the presence of the common transgenic promoter, cauliflower mosaic virus 35S (abbreviated CaMV 35S) or for the *bar* gene (a gene that confers resistance to the herbicide Basta™). A second tier of screening indicated no expression of either the Basta resistance or RoundUp™ resistance genes. These genes are commonly used for both their active traits and as marker genes in Bt maize.

Sampling Methodology

Seed from the 105 accessions and candidate landraces were grouped into 24 bulks by the head of the maize gene bank, Dr. Suketoshi Taba. Bulks comprise a group of landraces or varieties that share criteria based on an established classification strategy. For the latest screens, the landraces were bulked by village or as several villages that possessed comparable topography. Between one and twelve samples, with considerable similarities but also displaying unique agronomic characteristics, were bulked. A finding of a transgene in a bulked sample would mean that the individual samples that constitute the bulk would then be screened to determine the source of the "positive" result.

Analysis

For the first tier screen, the 24 bulks were germinated and DNA extracted according to the standard protocols of CIMMYT's Applied Biotechnology Center (ABC). The DNA was amplified using a primer corresponding to the CaMV 35S promoter and another primer corresponding to the *bar* gene, two fragments of DNA found in most commercial transgenic maize and not known to exist naturally in the maize genome (sequence available upon request). One leaf was taken from each of the 100 plants to represent each bulk and broken down into 10 batches of 10 leaves for DNA extraction, processing, and analysis. In all, 2,400 leaf samples were used to test

the 24 bulks. DNA isolated from a known transformed plant containing the CaMV 35S promoter was run as a positive control. To further ensure that the reactions were working correctly, all DNA samples were amplified using a primer corresponding to a fragment of DNA known to exist naturally in the maize genome. All positive controls amplified correctly, and no bulk of gene bank maize amplified the CaMV 35S promoter sequence or the *bar* gene sequence, indicating that, in the samples tested, neither of these was present.

The second tier screen consisted of growing the plants from the 24 bulks in a greenhouse and spraying them with the herbicides Basta and RoundUp. To survive the treatment, a plant would have to express transgenically-based resistance to the herbicide. One hundred and forty-four (144) plants from each bulk were sprayed with Basta and a like number with RoundUp. No plants survived, indicating that the bulks did not include landraces carrying and expressing either of the herbicide resistance genes.

CIMMYT is currently selecting and contracting an independent laboratory to conduct screens for the CaMV 35S promoter and particular transgenes on approximately 500 gene bank accessions and candidate samples. A portion of these will be landrace materials that have already been screened at CIMMYT, which is being done to verify the Center's own findings to date.

To date, CIMMYT has screened a total of 165 Mexican landrace accessions (most from the State of Oaxaca), 105 samples under consideration for inclusion in the gene bank, and 42 samples collected by CIMMYT researchers as part of their work on gene flow. None have tested positive for the presence of CaMV 35S. Documentation for the earlier screens may be found at <http://www.cimmyt.org/whatisimmyt/Transgenic/index.htm>.

The CIMMYT Commitment

"We at CIMMYT recognize the concern of some of our stakeholders about whether the maize landraces in our gene bank materials are carrying transgenes, and we are proceeding on a number of fronts to address the points in question," states CIMMYT Director General Masa Iwanaga. "First, we are systematically screening gene bank accessions collected since the commercial release of transgenic maize, with an emphasis on Oaxaca—a very important region as far as landraces are concerned and the focal point of studies that reported the introgression of transgenes into Mexican landraces. Second, we have implemented a policy to screen all new landrace samples for transgenes before they can be incorporated into the gene bank collection. To expedite both of these steps, we are committing precious resources to funding additional tests by an independent laboratory. Finally, strict measures are now in place to ensure that no transgenes are transferred into gene bank accessions when they are being regenerated." (See CIMMYT's 17 September 2002 statement ["The CIMMYT Maize Program and Transgenic Maize."](#))

“CIMMYT has long been involved with the conservation of genetic diversity,” says Dr. Iwanaga, “not just in gene banks, but also in situ, in farmers’ fields. In the current controversy over potential impacts of transgenes on the genetic diversity of maize in Mexico, CIMMYT scientists continue to bring their scientific perspectives and expertise to the debate, both through the media and through their participation in numerous scientific fora.

“A number of key questions remain unanswered,” Dr. Iwanaga adds, “and CIMMYT is actively seeking resources to allow our scientists to undertake the studies needed to provide that key information.”