

FURTHER TESTS AT CIMMYT FIND NO PRESENCE OF PROMOTER ASSOCIATED WITH TRANSGENES IN MEXICAN LANDRACES IN GENE BANK OR FROM RECENT FIELD COLLECTIONS

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El Batán, Texcoco, Mexico—The International Maize and Wheat Improvement Center (CIMMYT) has completed screening an additional 15 Mexican maize landraces from its maize gene bank and determined that none of them carried the common promoter (cauliflower mosaic virus 35S, abbreviated as CaMV 35S) associated with the presence of an introduced gene (transgene).

In mid-October, 2001, a screening of 28 landraces from the gene bank also failed to indicate the presence of the promoter. In addition, CIMMYT tested seeds from 42 Oaxacan landraces (or farmers' populations) that were collected in 2000 for a study on gene flow. Again it was determined that the CaMV 35S was not present in any of the samples. If the promoter had been found (and those results verified), it would indicate that a transgenic maize plant had crossed with a maize landrace, or conventional variety, at some point in the landrace's ancestry.

The screening work at CIMMYT was initiated in response to published reports that transgenic corn had been found growing in the Mexican states of Oaxaca and Puebla (September 27 [Vol. 413] and November 29 [Vol. 414], 2001 issues of *Nature*). To date, all screenings of Mexican maize landraces and varieties at CIMMYT have failed to show the presence of either the promoter or a transgene. Details of both sets of the new screenings are given below.

Germplasm Screening of CIMMYT Gene Bank Materials for DNA Sequence Associated with Transgenics (November 27, 2001)

Seeds of 15 Mexican maize accessions from the CIMMYT gene bank collection were received from Dr. Suketoshi Taba, head of CIMMYT's maize gene bank, on October 1, 2001. Eight of the landrace accessions were from the state of Oaxaca while the remaining seven covered a broad geographic area ranging from Chihuahua in the north to Chiapas in the south. These seeds were germinated and DNA extracted according to the standard protocols of CIMMYT's Applied Biotechnology Center (ABC). DNA was amplified using a primer corresponding to the CaMV 35S promoter, a fragment of DNA found in most commercial transgenic maize and not known to exist naturally in the maize genome (sequence available upon request). DNA was extracted in a bulk of 10 plants, and a total of 50 plants tested per population. 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 (in this case, SSR marker phi076). All positive controls amplified correctly, and no bulk of gene bank maize amplified the CaMV

35S promoter sequence, indicating that in the samples tested, there is no CaMV 35S promoter sequence.

Germplasm Screening of Oaxaca Landraces for DNA Sequence Associated with Transgenics (December 3, 2001)

Seeds of 42 maize varieties were collected by doctoral candidate Gael Pressoir and Dr. Julien Berthaud in farmers' fields in the state of Oaxaca, within a 50-mile radius of the city of Oaxaca, in February 2000. These seeds were germinated and DNA was extracted according to CIMMYT ABC protocols. DNA was amplified using a primer corresponding to the CaMV 35S promoter, a fragment of DNA found in most commercial transgenic maize and not known to exist naturally in the maize genome (sequence available upon request). DNA was extracted from individual plants separately, and DNA from 20 different individuals per population was then mixed into a bulk for amplification. 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 (in this case, SSR marker phi022). DNA of nontransformed maize was also mixed with transformed maize in ratios of 9:1 (nontransformed : transformed); 14:1; and 29:1. In each case, the transgenic sequence successfully amplified, demonstrating the ability to detect a single transgenic plant in a bulk of 30 plants total.

All positive controls amplified correctly. No bulk of a Oaxacan landrace collected from the farmers amplified the CaMV 35S promoter sequence, thus clearly indicating that there was no presence of the CaMV 35S promoter sequence in any of the samples tested.

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