

FURTHER TESTS AT CIMMYT FIND NO PRESENCE OF PROMOTER ASSOCIATED WITH TRANSGENES IN MEXICAN LANDRACES IN GENE BANK

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El Batán, Texcoco, Mexico—In January, the International Maize and Wheat Improvement Center (CIMMYT) completed two additional sets of screenings on a total of 39 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). If the promoter had been found (and those results verified), it would indicate that a transgenic maize plant had crossed with a direct ancestor of the sampled maize, at some point in the past.

The screening of landrace accessions in the gene bank 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 the promoter.

In late November 2001, 15 landraces from the CIMMYT maize gene bank tested negative for the presence of CaMV 35S promoter sequence. This followed similar screenings in mid-October, 2001, in which 28 landraces from the gene bank also failed to indicate the presence of the promoter sequence. At that time, CIMMYT also screened seeds from 42 Oaxacan landraces that were collected in 2000 for a study on gene flow. Again it was determined that the CaMV 35S sequence was not present in any of the samples.

Details of both sets of the new screenings are given below.

Set 1: Germplasm screening of CIMMYT gene bank materials for DNA sequence associated with transgenics (January 2, 2002)

Seeds of 14 Mexican maize accessions from the CIMMYT gene bank collection were received from Dr. Suketoshi Taba, head of the CIMMYT Maize Gene Bank. Nine of the landrace accessions were from the state of Oaxaca while five came from northern Mexico. 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

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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 20-40 plants were 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. 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.

Set 2: Germplasms screening of CIMMYT gene bank materials (Zapalote Chico) for DNA sequence associated with transgenics (January 14, 2002)

Seeds of 25 maize landrace accessions were received from the CIMMYT Maize Gene Bank. The accessions were all collected in Oaxaca in 1999 and broadly classified as the race Zapalote Chico. The seeds were germinated and DNA 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 as a bulk (15 individuals 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.

All positive controls amplified correctly. No bulk of an accession amplified the CaMV 35S promoter sequence, thus clearly indicating that the CaMV 35S promoter sequence was not present in any of the samples tested.

Set 1: Test of CIMMYT gene bank materials for the presence of the 35S promoter sequence in maize (January 2, 2002).

Race	CIMMYT accession number	Origin of seeds	Number of seeds tested	Samples amplified 35S
Tablon	347	Jalisco	20	0
--	1946	Nuevo Leon	30	0
Jala	2138	Colima	30	0
Tablon	2230	Jalisco	20	0
Bolita9	2269	Oaxaca	40	0
Zapchi9	2272	Oaxaca	40	0
--	4060	Oaxaca 1970	30	0
--	4060	Oaxaca 1998	20	0
TabPer	5717	Colima	20	0
Conico/Bolita	6040	Oaxaca	20	0
Conico/Bolita	6040	Oaxaca	30	0
Bolita/Pepiti	18031	Oaxaca	30	0
Bolita/Pepiti	18031	Oaxaca	40	0
Bolita	18037	Oaxaca	40	0

Set 2: Test of CIMMYT gene bank materials for the presence of the 35S promoter sequence in maize (January 14, 2002).

Race	Pop. number	Origin of seed	Number of seeds tested	Samples amplified 35S
Zap.Chico	850	Oaxaca 1999	15	0
Zap.Chico	851	Oaxaca 1999	15	0
Zap.Chico	852	Oaxaca 1999	15	0
Zap.Chico	853	Oaxaca 1999	15	0
Zap.Chico	854	Oaxaca 1999	15	0
Zap.Chico	855	Oaxaca 1999	15	0
Zap.Chico	856	Oaxaca 1999	15	0
Zap.Chico	857	Oaxaca 1999	15	0
Zap.Chico	858	Oaxaca 1999	15	0
Zap.Chico	859	Oaxaca 1999	15	0
Zap.Chico	860	Oaxaca 1999	15	0
Zap.Chico	861	Oaxaca 1999	15	0
Zap.Chico	862	Oaxaca 1999	15	0
Zap.Chico	863	Oaxaca 1999	15	0
Zap.Chico	864	Oaxaca 1999	15	0
Zap.Chico	865	Oaxaca 1999	15	0
Zap.Chico	866	Oaxaca 1999	15	0
Zap.Chico	867	Oaxaca 1999	15	0
Zap.Chico	868	Oaxaca 1999	15	0
Zap.Chico	869	Oaxaca 1999	15	0
Zap.Chico	870	Oaxaca 1999	15	0
Zap.Chico	871	Oaxaca 1999	15	0
Zap.Chico	872	Oaxaca 1999	15	0
Zap.Chico	873	Oaxaca 1999	15	0
Zap.Chico	874	Oaxaca 1999	15	0

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