QUANTITATIVE GENETICS AND MAIZE BREEDING
DEPARTMENT OF SOIL AND CROP SCIENCES

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Final Report: *Hybrid Corn Breeding and Testing Focused on Improving Yield, Aflatoxin Resistance, Drought Tolerance, Phosphorus and Nitrogen Use*

Investigators

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Project Summary as of June 1

Objective 1: *Publically propose for release at least three inbred germplasm lines and an open pollinated population.*

The publication for the release of three aflatoxin resistant inbred lines (Tx736, Tx740, Tx741), was published

- Mayfield, K.L., F. Javier Betrán, Tom Isakeit, Gary Odvody, Seth C. Murray*, William L. Rooney, and Jean Carlo Landivar. 2011. Registration of maize germplasm lines Tx736, Tx739, and Tx740 for reducing preharvest aflatoxin accumulation. Journal of Plant Registrations. 6:88–94

It is attached and is available online: https://www.crops.org/publications/jpr/articles/6/1/88
A one-page release note is also attached.
Seed from these lines was increased and deposited in the USDA repository and these lines have been crossed to additional elite commercial inbred testers (mostly transgenic) for yield trials both in the College Station summer nursery and the Weslaco winter nursery. They will be trialed next year to fast track getting one or more of these lines into a commercial hybrid.

The open pollinated Texas Argentine Composite (TAC) population was tested in Weslaco and College Station again this year for aflatoxin and yield. In 2010 this population did very well and even obtained up to 240bu/ac in the Texas high plains. This year the open pollinated population did not perform well for aflatoxin or yield in either location due to hot and dry stressful conditions combined with asynchronous flowering. However, 20 F_{3-4} lines derived from this population were tested this year in 54 hybrid combinations and 7 of these beat the population mean by enough to warrant further testing next year. Additionally, based on 2010 yield data alone, 13 of TAC derived lines were self-pollinated to advance to the F_{4-5} in summer nurseries and three of these lines were crossed to new commercial testers in the winter nursery. The new plan to release this population will be as a novel source for new inbred lines adapted to Texas but more data on these lines as hybrids will be necessary.

Objective 2: *Test hybrid testcrosses made in 2010 across multiple environments in Texas.*
Yield and agronomic data has been collected on over 1215 hybrids trialed in College Station (CS) [not including more than 1300 additional hybrids tested in cooperative tests and as part of a USDA grant], 535 hybrids in Weslaco (WE), 39 hybrids in Corpus Christi (CC) and 50 hybrids in Ganado (GA) with BH Genetics. In College Station and Weslaco these tests were planted at the standard time, under standard well watered conditions and included multiple tests, two replicates of either two row or one row plots (primarily based on seed availability). In Corpus Christi, these hybrids were planted late and under dryland conditions to induce aflatoxin and drought stress as one row plots with three replications. The tests included both new hybrids in

their first season (~788-CS; 338-WE; 14-GA) as well as hybrids being tested for a second year and inoculated with *A. flavus* (~277-CS; 99-WE; 39-CC; 26-GA) - numbers may not sum to total because of difficulty in categorizing material (for example in SERAT). In 2011, corn kernels colonized by toxigenic *Aspergillus flavus* were spread between rows at silking for all second year yield trials. Although similar to delivery of atoxigenic strains by growers, this method differs in that conidial inoculum is immediately available for colonization of silks. Subsamples of grain were collected at maturity for agronomic and aflatoxin evaluation of each hybrid.

To maximize opportunities for finding lines and hybrids with both good yield and aflatoxin resistance, the original plan in 2010 was to develop FT-NIRS (Fourier Transformed Near Infrared Reflectance Spectroscopy) calibrations that would rapidly and nearly instantaneously allow us to predict aflatoxin in all hybrids we harvested. In 2010 we subsampled approximately 2400 hybrid plots from the combine over CS, WE, CC locations. However, these FT-NIRS calibrations were unfortunately not accurate enough to be useful, so we saved all samples from 2010 in a cold room until aflatoxin testing could be done using a better strategy. In 2011, we once again subsampled plots from the combine (CS, WE) or hand harvest (CC), but only on the checks and on second year TAMU hybrids that were also grown in 2010 AND had acceptable yield - a total of 869 plots across locations and replicates. In the Fall of 2011 we preliminarily analyzed the yield data from both the 2010 and the 2011 season. Subsamples from all reps of the best yielding plots and the commercial and internal checks were then found (Total – 298; 204 from 2011 and 94 from 2010). The whole grain from these subsamples was scanned into the FT-NIRS (to continue to improve prediction), ground with a Romer mill, scanned into the FT-NIRS again and subjected to the Vicam Aflatest. The results of the second year hybrids which we believe to be the most promising based on yield and/or aflatoxin are presented in Table 1 (non-promising hybrids are not included) but all will require additional balanced testing. Vicam Aflatest measurements showed that 2011 was a much better year for screening hybrids with *Aspergillus flavus* for aflatoxin because of the heat and drought. Samples from 2010 contained much less aflatoxin making it hard to separate those that were resistant from those that escaped infection & colonization or escaped accumulation of aflatoxin so emphasis was made on the 2011 samples. It is important to point out that our hybrid lines did not have the advantages of professional seed treatment, seed corn size screening, or insect resistant traits and therefore the population stands were lower and there was substantially more loss to worms than observed in commercial checks.

In addition to promising second-year hybrids being identified (Table 1), we also identified many very promising first-year hybrids 33 of which exceeded yield of the best second year hybrids (Table 2). Additionally, we gained an appreciation for the new commercial transgenic testers although there were only 42 hybrids in the test crossed to these this year, these new tester hybrids will make up the majority of lines in future years. The major limitation to screening all hybrids is always having enough hybrid seed to put into multi-location-multi-year trials. Therefore, these tests are preliminary and approximate to help us identify what to focus on producing more hybrid seed of. For official release, agreements with companies and publication, more formal tests with additional locations and years will need to be performed – we plan to pursue this in the upcoming years.

Objective 3: *Improve near infrared spectroscopy calibrations for aflatoxin and develop near infrared spectroscopy calibrations for phosphorus and nitrogen using grain from hybrid testcrosses.*
Because the TCPB was not enthusiastic about this objective we have not used additional TCPB funds for this. However, using USDA funds we did scan 298 samples that we tested with the Vicam Aflatest into the FT-NIRS which only took our student workers about 20 man-hours. By continuing this process we hope that this will eventually result in calibrations useful for us to select hybrids from in the breeding program, ***FT-NIRS prediction will likely not be accurate enough for commercial or trade use***, however.

Objective 4: *Make hybrid testcrosses between Texas A&M's best 10 lines from the past few years with commercial testers, and superior publicly available germplasm (ex-PVP's for instance).*
Based on the need to generate rapid impact we developed license agreements with two commercial companies that supply transgenic elite commercial inbred lines. A total of 160 Texas AgriLife lines proven in past yield trials were crossed to 14 new elite commercial testers from these companies and three other testers. We now have now weighed our Summer CS nursery crosses and have sufficient seed from 275 new unique yellow hybrids between different TAMU elite lines and elite commercial hybrids (many transgenic) to test in the 2012 season.

From the results of the 2011 first year yield trials (above) we selected an additional ~50 TAMU inbred lines promising for yield and crossed these with six commercial testers (mostly transgenic and the best of the 14 we used in the CS summer nursery) and ex-PVP lines (commercial inbreds from the 1990's that are freely publically available and we increased seed of in summer). We have shelled our winter nursery crosses and have seed from 171 additional unique hybrids to test in the 2012 season but the quantities have not yet been determined.

We will therefore have between 400 and 450 new hybrids created between elite TAMU material and relevant commercial testers (mostly transgenic) and a few publically available ex-PVP lines to test in at least one location in 2012; many will have seed to test in multiple locations. There should be many outstanding hybrids identified from this objective. This objective is foundational to the success of getting TAMU germplasm into commercial hybrids, and thus grower's fields as quickly as possible.

Objective 5: *Leverage samples from a USDA project in genetic mapping of aflatoxin and drought to include aflatoxin, phosphorus and nitrogen accumulation measurements using NIRS.*
Because the TCPB was not enthusiastic about this objective we have not used additional TCPB funds for this.

Objective 6: *Assist other corn researchers and participate in cooperative corn trials that further corn and aflatoxin research, and increase the visibility of Texas corn.*
We have collected all data (except aflatoxin) from the Southeastern regional aflatoxin trials (SERAT), two USDA led trials (one on aflatoxin, one on yield only), two cooperative trials (one led by Wenwei Xu), one commercial line aflatoxin trial (led by Isakeit), and one inbred line trial for the International Center for Maize and Wheat Improvement (CIMMYT), and an enormous

dryland and well watered trial to test maize lipoxygenase genes/alleles funded by USDA-NIFA (with Kolomiets and Isakeit).

Analysis for two SERAT tests are presented (Table 3) to highlight two promising TAMU lines (one Murray and one Xu). It should be noted that large volumes of seed are needed for SERAT tests and therefore it is usually second or third tier elite lines that we have to enter. Special attention should be paid to Wenwei's line as WXU-9 did outstanding in both irrigated and dryland conditions for both aflatoxin and yield.

Objective 7: *Train/ educate four graduate students and five undergraduates in corn breeding and genetics.*

This summer the program had five graduate students and three undergraduate students being trained in corn breeding and genetics. Without support from the Texas Corn Producers this field based training would not be possible.

Publications made associated with PI Murray's program in 2011:

Mayfield, K., F.J. Betran, T. Isakeit, G. Odvody, S.C. Murray*, W.L. Rooney, and J.C. Landivar. 2012. Registration of maize germplasm lines Tx736, Tx739, Tx740, for reducing preharvest aflatoxin accumulations. Journal of Plant Registrations 6:88-94.

Meeks, M., S.C. Murray*, S. Hague, D. Hays & A. M. H. Ibrahim. 2011 - in press. Genetic Variation for Maize Epicuticular Wax Response to Drought Stress at Flowering. J. Agronomy & Crop Science. doi: 10.1111/j.1439-037X.2011.00495.x

Brummer, E.C., W.T. Barber, S. Collier, T.S. Cox, R. Johnson, S.C. Murray*, R.T. Olsen, R.C. Pratt, and A.M. Thro. 2011. Plant breeding for harmony between agriculture and the environment. Frontiers of Ecology and Environment. 9(10): 561-568.

Boote, K.J., A.M.H. Ibrahim, R. Lafitte, R. McCulley, C. Messina, S.C. Murray, J.E. Specht, S. Taylor, M.E. Westgate, K. Glasener, C.G. Bijl, and J.H. Giese. 2011. Position Statement on Crop Adaptation to Climate Change. Crop Science 51:2337-2343.

Mayfield, K.L., S.C. Murray*, W.L. Rooney, T. Isakeit, and G.A. Odvody. 2011. Confirmation of QTL reducing aflatoxin in maize testcrosses. Crop Science. 51:2489-2498.

Isakeit, T.*, S.C. Murray, and J.C. Wilborn. 2011. Efficacy of Afla-Guard (Aspergillus flavus NRRL 21882) to control mycotoxins on corn in Burleson County, Texas, 2010. Plant Disease Management Reports 5:FC091.
www.plantmanagementnetwork.org/pub/trial/PDMR/reports/2011/FC091.pdf

Isakeit, T.*, S.C. Murray, and K. Mayfield. 2011. Aflatoxin and fumonisin in transgenic corn hybrids in Burleson County, Texas, 2009. Plant Disease Management Reports 5:FC090.

Pietsch, D., J. Blumenthal, S. Murray, and S. Labar. 2011 Corn Performance Tests in Texas. Texas AgriLife Research publication.

Presentations made associated with PI Murray's program in 2011:

Barrero-Farfan, I. D.*, S.C. Murray, D. Pietsch, and S. Labar. 2011. Metanalysis of the texas corn crop testing program. NAPB Annual Meeting; College Station, TX 5/13-25/2011 (poster)

De La Fuente, G.N.*, I. Barrero-Farfan, S.C. Murray, M. Kolomiets, T. Isakeit, and Y.S. Park. 2011 Improving drought tolerance and aflatoxin resistance in maize via altered lipid metabolism. NAPB Annual Meeting; College Station, TX 5/13-25/2011 (poster)

Felderhoff, T.J.*, W.L. Rooney, S.C. Murray, A.Sharma, P.E. Klein, M. Hamblin, and W. Vermerris. 2011. QTLs for energy related traits in a sweet x grain RIL sorghum population. NAPB Annual Meeting; College Station, TX 5/13-25/2011 (poster)

Gaus, T.A. W. Xu, Y. Xue, S. Murray, W.P. Williams, G. Odvody, T. Marek 2011. Exotic Genes From Teosinte for Improving Grain Quality and Yield In Maize. ASA/CSSA/SSA. 10/16-19/2011 (poster)

Hague, S., E. Runge, S. Feagley, J. Aitkenhead-Peterson, C. Morgan, S. Murray, J. Foster, R. Vesey. 2011. Study Abroad Programs In the Department of Soil and Crop Sciences At Texas A&M University. ASA/CSSA/SSA. 10/16-19/2011 (poster)

Mahan, A.*, S.C. Murray, L. Rooney, and K. Crosby. 2011. Analysis of diverse colored maize lines (blue, red, purple) for food corn breeding. NAPB Annual Meeting; College Station, TX 5/13-25/2011 (poster)

Murray S.C.*, G. De La Fuente, T. Isakeit, M.V. Kolomiets, K. Mayfield, G. Odvody, Y-S Park, M.L. Warburton, J.C. Wilborn, W.P. Williams, and G.L. Windham. 2011. Techniques, technologies and approaches to improve maize aflatoxin resistance. Genetics of Maize Disease Workshop. Raleigh, NC. 2/20-23/2011. (invited talk)

Murray, S.C.*, M. Kolomiets, T. Isakeit, and G.D. De La Fuente 2011. Improving drought tolerance and aflatoxin resistance in maize; education, extension, and translational breeding via altered lipid metabolism. USDA awardees meeting - Plant and Animal Genome Conference. (invited poster)

Murray, S.C.* 2011. Effective recombination in plant breeding and linkage mapping populations: testing models and mating schemes. The Maize Genetics Conference. St. Charles, IL.3/17-20/2011. (poster)

Murray S.C.*, G. De La Fuente, T. Isakeit, M.V. Kolomiets, K. Mayfield, G. Odvody, Y-S Park, M.L. Warburton, J.C. Wilborn, W.P. Williams, and G.L. Windham. 2011. Improving pre-harvest aflatoxin resistance in maize: new genetic and phenotypic approaches NCC167 Corn Breeders Meeting. St. Charles, IL. 3/15-16/2011. (talk)

Murray, S.C.* 2011. Effective recombination in plant breeding and linkage mapping populations: testing models and mating schemes. NCC167 Corn Breeders Meeting. St. Charles, IL. 3/15-16/2011. (talk)

Murray S.C.*, G. De La Fuente, M.V. Kolomiets, T. Isakeit, and Y-S Park. 2011. Maize aflatoxin resistance and drought tolerance; testing diverse alleles in the maize lipoxygenase genes LOX4 and LOX5 through association mapping. Genetics of Maize Disease Workshop. Raleigh, NC. 2/20-23/2011. (poster)

Vermerris,W.*, A. Saballos, T. Felderhoff, S.E. Mitchell, W.L. Rooney, S.C. Murray, S. Kresovich, J.F. Pedersen, S. Sattler, and Z. Xin. 2011. Genetic dissection of bioenergy traits in sorghum. DOE awardees meeting. Crystal City, VA. 4/10/2011. (invited talk)

Warburton, M.L.*, W.P. Williams, G.Windham, S.C. Murray, W. Xu, L.Hawkins, C. Daves, and B. Henry. 2011. Phenotypic characterization of a maize association mapping panel developed for the identification of *Aspergillus flavus* and aflatoxin accumulation resistance genes. ASA/CSSA/SSA. 10/16-19/2011 (talk)

Washburn, J. D., S. Murray, B. Burson, R. Jessup. 2011. Dissecting the Genetics of Rhizomatousness: Towards Sustainable Food, Forage, and Bioenergy. ASA/CSSA/SSA. 10/16-19/2011 (poster)

Wilborn, J.C.*, and S.C. Murray. 2011. Calibration development for whole corn grain phosphorus, crude protein, starch and fat content utilizing near infrared spectroscopy. NAPB Annual Meeting; College Station, TX 5/13-25/2011 (poster)

Yan, Y.*,Y.-S. Park, S. Christensen, E. Borrego, X. Gao, G. De la Fuente, K. Mayfield, S.C. Murray, H. Wilkinson, T. Isakeit, W.-B. Shim, R. Meeley, and M. Kolomiets. 2011. Modulating lipid-derived signaling to improve corn traits. NAPB Annual Meeting; College Station, TX 5/13-25/2011 (poster)

Table 1: Hybrids that have been tested for two years in multiple locations. Checks and those with good yield were also tested for aflatoxin. Those bolded look especially promising and certainly want further testing.

Location/ Year →	CC11	CC11	CS11	CS11	WE11	WE11	CC10	CC10	CS10	CS10	WE10	WE10
Second Year New TAMU hybrids	**ppb**	**Bu**	**ppb**	**Bu**	**ppb**	**Bu**	**ppb**	**Bu**	**ppb**	**Bu**	**ppb**	**Bu**
(((B104/NC300)x(CML285/B104))-2-3-BBB/LAMA2002-22-3-BB1)-B*5-1/ *X "SS#1 Tester"*	.	.	338	118	285	160	.	.	***12***	***219***	.	.
(((B104/NC300)x(CML415/B104))-4-2-B-B/Tx760-B-B-B)-B-B-1-B-B-B X "SS#1 Tester"	***82***	***22***	***115***	***150***	***69***	***147***	***20***	***75***	**.**	**.**	**.**	**.**
((B104-1 x Tx714-B-B)-1-4-B-B-B-B/CML161)-B-B-1-B-B-B *X "SS#1 Tester"*	.	.	.	.	.	.	1140	81	26	137	.	.
((B104-1 x Tx714-B-B)-1-4-B-B-B-B/CML161)-B-B-2-B-B-B1 *X "SS#1 Tester"*	.	.	.	.	.	.	47	87	2	142	.	.
((B104-1 x Tx714-B-B)-1-4-B-B-B-B/CML161)-B-B-2-B-B-B1 *X "NSS#1 Tester"*	.	.	817	130	.	.	430	77	26	169	.	.
((B104-1 x Tx714-B-B)-1-4-B-B-B-B/CML161)-B-B-2-B-B-B2 *X "SS#1 Tester"*	.	.	.	.	.	.	148	81	8	159	.	.
((B104-1xTx714-B-B)-1-4-B-B-B-B/CML161)-B-B-1-B-B-B *X "SS#1 Tester"*	363	25	.	.	.	.	.	.	.	.	.	.
((B104-1xTx714-B-B)-1-4-B-B-B-B/CML161)-B-B-2-B-B-B1 X "SS#1 Tester"	***190***	***23***	***160***	***146***	***50***	***158***	**.**	**.**	**.**	**.**	**.**	**.**
((B110xFR2128-B/B104-1/CML343)-BB-11-B*4/LAMA2002-42-BBB)-B*5-1/ *X "SS#1 Tester"*	205	8	227	143	85	173	.	.	.	.	.	.
((CML269/Tx110)/(CML311/Tx110)-1-B-B-B-B/DTPWC8F31-1-1-2-2-BBBB-B)-B-B-3-1-B X "SS#1 Tester"	***58***	***12***	***170***	***157***	**.**	**.**	**.**	**.**	**.**	**.**	**.**	**.**
((CML373/FR825)/(CML269/Tx110)-1-B-B-B-B/CML269/TX114-B-B-B-1-1-B-B-B-B-B)-B-1-B-1-B *X "SS#1 Tester"*	.	.	621	146	.	.	.	.	.	.	.	.
((CML373/FR825)/(CML269/Tx110)-1-B-B-B-B/CML269/TX114-B-B-B-1-1-B-B-B-B-B)-B-1-B-2-B *X "SS#1 Tester"*	.	.	251	158	.	.	.	.	.	.	.	.
((CML450-B/Tx110)-B-3-B-1-B) *X "SS#3 Tester"*	.	.	294	180	.	.	.	.	.	.	.	.
((CML450-B/Tx110)-B-3-B-1-B) *X "SS#2 Tester"*	.	.	170	170	164	167	.	.	.	.	.	.
((LAMA2002-12-1-B/(CML325/B104)-B-1-B-B-B-B)-B-B2-3-2-B-B-B-B)xLH287	.	.	553	156	.	.	.	.	.	.	.	.
((LAMA2002-23-3-B/SCR82-B)-B-B-1-1-B-B-B) *X "SS#3 Tester"*	.	.	587	158	177	157	.	.	.	.	.	.
((Tx114(B73w)-BxCML343/Tx110xPop24)-B-B-B-4-B-B-B-B/CML78)-B-3-B-3-B *X "SS#1 Tester"*	.	.	303	156	.	.	.	.	.	.	.	.
(BS13(S)C8-33-1-B-B-BxTx745)-B-B-B-B-B *X "NSS#1 Tester"*	635	26	.	.	.	.	.	.	.	.	.	.
(CML161/Temp.SSLate(B37,B73,B84)B-81-B-2-B-B-B)-B-B-B-B-B1 *X "NSS#1 Tester"*	346	25	277	156	.	.	.	.	.	.	.	.
(CML269/TX114-B-B-B-1-1-B-B-B-B-B-B)xPHV-63	.	.	263	158	.	.	.	.	.	.	.	.
(CML269/TX130-B-B-B-1-3-B-B-B-B-B-B-B) *X "SS#2 Tester"*	.	.	343	152	.	.	.	.	.	.	.	.
(CML379/CML311-B-1-B-B-B-B/Tx110)-B-1-B-1-B *X "SS#1 Tester"*	90	19	147	149	.	.	.	.	.	.	.	.
(CML450-B/(Tx106-Tx714)-1-1-714-1-1-1-B-B-B-B-B)-B-2-B-3-B *X "SS#1 Tester"*	.	.	.	.	57	177	.	.	.	.	.	.
(CML450-B/(Tx106-Tx714)-1-1-714-1-1-1-B-B-B-B-B)-B-2-B-3-B *X "NSS#1 Tester"*	797	17	303	188	82	165	69	63	.	.	.	.
(CML450-B/Tx110)-B-2-B-1-B *X "NSS#1 Tester"*	285	29	.	.	.	.	.	.	.	.	.	.

(CML450-B/Tx110)-B-3-B-3-B *X "SS#1 Tester"*	.	.	.	.	166	177	.	.	.	.	.	.
(LAMA2002-23-1-BB/LAMA2002-11-1-BB)-B*5-1/ X "SS#1 Tester"	***81***	***16***	***250***	***159***	***146***	***163***	***.***	***.***	***86***	***187***	***.***	***.***
(LAMA2002-23-1-BB/LAMA2002-11-1-BB)-B*5-1/ X "NSS#1 Tester"	***.***	***.***	***.***	***.***	***.***	***.***	***.***	***.***	***2***	***198***	***214***	***150***
(LAMA2002-2-5-B/(CML285/B104)-B-4-B-B-B-B)-B-B2-1-1-B-B-B *X "WHT QPM POL"*	.	.	141	156	.	.	.	.	.	.	.	.
(LAMA2002-46-3-B-B-B-B-B) *X "NSS#1 Tester"*	.	.	243	155	.	.	.	.	.	.	.	.
(LAMA2002-58-3-B-B-B-B-B) *X "SS#2 Tester"*	.	.	102	160	.	.	.	.	.	.	.	.
(LAMA2002-61-2-BB/LAMA2002-53-5-BB)-B*5-1/ *X "SS#1 Tester"*	.	.	153	168	104	159	.	.	.	.	.	.
(Temp.NSSLateB-105-B-B-B-B/CML161)-B-B-B-B-B1 *X "SS#1 Tester"*	.	.	148	138	175	178	.	.	.	.	.	.
(Tx601 x B104-B/FR2128-B x Bord)-2-2-B-B-B-B-B-B-B *X "WHT QPM POL"*	.	.	.	.	.	.	.	.	1	170	.	.
(Tx745/LAMA2002-12-1-BB)-B*5-1/ *X "NSS#1 Tester"*	.	.	.	.	.	.	.	.	20	106	.	.
(TAC) Texas Argentine Flinty Composite-C(1)-6-B1-B X "SS#1 Tester"	***57***	***23***	***253***	***140***	***.***	***.***	***245***	***73***	***.***	***.***	***.***	***.***
(TAC) Texas Argentine Flinty Composite -C(1)-15-B1-B *X "SS#1 Tester"*	.	.	.	.	.	.	417	65	10	161	.	.
(TAC) Texas Argentine Flinty Composite-C(1)-29-B-B *X "SS#1 Tester"*	245	10	213	148	.	.	159	67	.	.	.	.
(TAC) Texas Argentine Flinty Composite-C(1)-36-B2-B1 X "SS#1 Tester"	***.***	***.***	***201***	***134***	***.***	***.***	***.***	***.***	***.***	***.***	***.***	***.***
(TAC) Texas Argentine Flinty Composite-C(1)-37-B-B *X "SS#1 Tester"*	627	15	227	145	.	.	56	72	23	133	79	152
(TAC) Texas Argentine Flinty Composite-C(1)-44-B-B X "SS#1 Tester"	***.***	***.***	***203***	***140***	***116***	***158***	***86***	***66***	***27***	***146***	***76***	***150***
TAMU Released Line Checks												
((Tx736);((Tx772xT246)xTx772)-1-5-B-B-B-B-B-B) *X LH132*	78	14	233	137	89	151	.	.	.	.	.	.
"SS#2 Tester" X ((Tx740);(((LAMA2002-10-1-B-B-B)	.	.	183	159	.	.	.	.	.	.	.	.
(Tx811) *X "NSS#1 Tester"*	1063	20	447	142	333	141	.	.	.	.	.	.
(Tx811) *X "SS#3 Tester"*	.	.	617	153	.	.	.	.	.	.	.	.
Commercial Checks												
Commercial Check #1	148	33	283	158	393	145	.	.	.	.	.	.
Commercial Check #2	***158***	***42***	***223***	***185***	***72***	***174***	***.***	***.***	***.***	***.***	***.***	***.***
Commercial Check #3	***60***	***41***	***253***	***158***	***43***	***181***	***122***	***98***	***2.5***	***146***	***12***	***156***
Commercial Check #4	1533	24	467	154	80	178	.	.	.	.	.	.
Commercial Check #5	135	41	172	151	73	172	.	.	.	.	.	.
Commercial Check #6	.	.	.	.	.	.	343	79	19	85	6.05	162
Commercial Check #7	.	.	.	.	.	.	327	80	71	156	.	.
Commercial Check #8	***277***	***31***	***290***	***178***	***176***	***182***	***.***	***.***	***.***	***.***	***.***	***.***
Commercial Check #9	***217***	***33***	***170***	***189***	***70***	***171***	***.***	***.***	***.***	***.***	***.***	***.***
Commercial Check #10	.	.	.	.	.	.	343	68	0.4	184	4	143
Commercial Check #11	.	.	.	.	.	.	.	.	4	60		
MEAN	**336**	**24**	**285**	**154**	**137**	**165**	**263**	**75**	**20**	**150**	**65**	**152**

Table 2: A total of 541 different hybrids were analyzed with a statistical mixed model that included many tests and locations (CS,WE, CC, GA). 33 (26 TAMU first year hybrids and 7 commercial hybrids) had a yield advantage of 20 bu/ac or greater than the average of all 541 hybrids analyzed.

Pedigree	Bu/Ac Greater than Average of all 2011 plots
CML78xCML270-B-B-B-B-3-B-B-B-B-1 *X "SS#1 Tester"*	35.4
((CML450-B/Tx110)-B-3-B-1-B) *X "SS#3 Tester"*	31.3
Commercial Check #2	30.7
Commercial Check #7	30.3
(LAMA2002-22-1-B-B-B-B/LAMA2002-1-5-B-B-B-B)-2-1-B-1-1 *X "SS#1 Tester"*	29.8
((CML311-B/CI66-B/Tx114(B73w)-BxCML343)-BBB-1-B*6)/(CML78xCML269-B*6)-3-2-BB-1-2 *X "SS#1 Tester"*	28.7
(CML442-B/CML343-B-B-B-B-B-B)-B-B-1-1-B-1 *X "NSS#1 Tester"*	28.6
(((CML326/B104)x(CML285/B104))-2-2-B-B-B-B/(CML288/NC300)-B-9-B1-B-B-B-B-B)-B-2-B-B-2 *X "SS#1 Tester"*	28.5
(LAMA2002-58-3-B-B-B-B-B) *X "SS#2 Tester"*	28.5
(LAMA2002-35-2-B-B-B-B/CG44)-1-3-B-1 *X "SS#1 Tester"*	28.4
Commercial Check #6	27.7
(CG72-B/LAMA2002-20-4-B-B-B-B)-2-2-1-B2 *X "SS#1 Tester"*	27.3
(LAMA2002-22-3-B-B1-B-B/LAMA2002-1-5-B-B-B-B)-2-3-B-1-1 *X "SS#1 Tester"*	27.2
(CML450-B/(Tx106-Tx714)-1-1-714-1-1-1-B-B-B-B-B)-B-2-B-3-B *X "NSS#1 Tester"*	27.1
BH8895VTTP-10sib	26.9
(LAMA2002-58-4-B/(B97/A633)-B-4-B-1-B)-B-B-1-1-B-B-B-B-1 *X "SS#1 Tester"*	26.8
Commercial Check #8	25.8
(CML269xTx110-B*6/CML269/TX130-BBB-4-2-B*6)-2-B-1-2 *X "SS#1 Tester"*	25.4
(LAMA2002-23-3-B/LAMA2002-58-4-B)-B-B-2-3-B-1 *X "SS#1 Tester"*	24.2
((NC300xTx714-B/B104-1/CML343)-2-1-B-B-B-B-B-B/CL-RCY031=CL02410*CML287)B-9-1-1-2-B-B-B)-B-2-B-B-1 *X "SS#1 Tester"*	24.0
(CML379/CML311-B-1-B-B-B-B/Tx110)-B-1-B-1-B-1 *X "NSS#1 Tester"*	23.9
Y21xTx130-B-B-B-B-1-B-B-B-B-1 *X "SS#1 Tester"*	23.8
(CML450-B/(Tx106-Tx714)-1-1-714-1-1-1-B-B-B-B-B)-B-2-B-3-B *X "SS#1 Tester"*	22.8
(RedHybridEar-B-1-2-2-1/RedEar5-2-4-1-4-2)-B-B-1 *X "SS#1 Tester"*	22.6
(LAMA2002-61-2-B-B/LAMA2002-53-5-B-B)-B-B-B-B-B-1-1 *X "SS#1 Tester"*	22.1
(TAC) Texas Argentine Flinty Composite-B-sib-26-B *X "SS#2 Tester"*	21.5
(LAMA2002-22-1-B-B-B-B/LAMA2002-10-1-B-B-B-B)-2-2-B *X "SS#1 Tester"*	21.2

(((B104/NC300)x(CML415/B104))-4-2-B-B-B/LAMA2002-22-3-B-B1)-B-B-B-B-B *X "SS#1 Tester"*	21.0
(LAMA2002-22-1-B-B-B-B/LAMA2002-10-1-B-B-B-B-B)-2-3-B-1-1 *X "SS#1 Tester"*	20.8
Commercial Check #9	20.7
((CML285/NC300)-B-6-B-B-B-B-B-B-B-B/(CML323/NC300)-B-1-1-B-B-B-B-B-B)-2-2-B-1 *X "SS#1 Tester"*	20.4
Commercial Check #3	13.4
Commercial Check #10	8.4
BEST TAMU LINES ALSO IN TABLE 1 (SECOND YEAR OF TESTING WITH AFLATOXIN) FOR COMPARISON ONLY	
((CML269/Tx110)/(CML311/Tx110)-1-B-B-B-B/DTPWC8F31-1-1-2-2-BBBB-B)-B-B-3-1-B *X "SS#1 Tester"*	14.1
(LAMA2002-23-1-BB/LAMA2002-11-1-BB)-B*5-1/ *X "SS#1 Tester"*	7.8
(TAC) Texas Argentine Flinty Composite-C(1)-36-B2-B2 *X "SS#1 Tester"*	5.7
(((B104/NC300)x(CML415/B104))-4-2-B-B/Tx760-B-B-B)-B-B-1-B-B-B *X "SS#1 Tester"*	4.6
((B104-1xTx714-B-B)-1-4-B-B-B-B/CML161)-B-B-2-B-B-B1 *X "SS#1 Tester"*	4.3
(LAMA2002-23-1-BB/LAMA2002-11-1-BB)-B*5-1/ *X "NSS#1 Tester"*	1.5
(TAC) Texas Argentine Flinty Composite-C(1)-44-B-B *X "SS#1 Tester"*	1.0
(TAC) Texas Argentine Flinty Composite-C(1)-6-B1-B *X "SS#1 Tester"*	-2.6 (less than average)

TABLE 3: SERAT multi-rep averages across locations

Pedigree	College Station, TX BuAc	College Station, TX Afppb	Ganado, TX BuAc	Tifton, GA 2011 BuAc	Tifton, GA 2011 Afppb	Starkville, MS Afppb	Alexandria, GA BuAc	Raleigh, NC BuAc	Leesburg, GA BuAc
Murray - (TAC) Texas Argentine Flinty Composite-C(1)-14-B-B/ *X "SS#1 Tester"*	130.1	980	120.4	191	111	159	71.2	102	188
Murray - ***((B104-1xTx714-B-B)-1-4-B-B-B-B/CML161)-B-B-2-B-B-B2*** *X "SS#1 Tester"*	***139.1***	***513***	***124.8***	***197***	***60***	***226***	***61.8***	***109***	***195***
Murray - (TAC) Texas Argentine Flinty Composite-C(1)-15-B1-B *X "SS#1 Tester"*	124.0	943	122.8	178	120	447	61.7	89	182
Murray - ((B104-1xTx714-B-B)-1-4-B-B-B-B/CML161)-B-B-2-B-B-B1 *X "NSS#1 Tester"*	138.9	1250	90.9	162	178	565	37.6	78	134
Murray - ((CML288/NC300)-B-9-B1-B-B-B-B-B-B-B) *X LH132*	**143.5**	**340**	110.8	165	208	152	60.4	82	140
Murray - ((LAMA2002-12-1-B/(CML 325/B104)-B-1-B-B-B-B)-B-B2-3-2-B-B) *X LH132*	131.3	860	.	175	290	151	47.8	75	131
Murray - ((LAMA2002-2-5-B/(CML285/B104)-B-4-B-B-B-B)-B-B2-2-3-B-B) *X LH132*	127.8	463	112.1	163	202	120	50.1	81	132
GP282 x GT603	113.2	757	.	188	90	270	52.1	97	.
Lo964 x GT603	84.5	860	.	173	81	406	48.4	88	.
AT709 x GT601	140.1	750	.	169	106	310	60.4	100	176
GT603XDK888N11Fls3,2141-2-34-B-2-1	114.8	847	.	158	87	289	59.7	89	160
CY1 x NC262B	129.4	1267	.	192	179	520	61.2	107	192
Xu - Tx-WX11-1	144.4	737	129.8	201	152	243	.	116	211
Xu - Tx-WX11-2	141.2	1057	121.8	201	171	1599	.	103	192
Xu - Tx-WX11-3	153.5	693	128.7	229	274	1117	.	118	212
Xu - Tx-WX11-4	**138.6**	**353**	122.1	180	46	84	.	108	189
Xu - Tx-WX11-5	148.8	830	123.6	213	154	354	.	107	208
Xu - Tx-WX11-6	142.6	1130	148	198	556	3443	.	125	206
Xu - Tx-WX11-7	105.8	853	125.3	201	167	388	.	108	187
Xu - Tx-WX11-8	125.1	817	***142.6***	***210***	161	303	.	96	211
Xu - ***Tx-WX11-9***	***162.1***	***373***	***140.3***	***195***	***129***	***107***	.	***108***	***214***
Commercial Check 1	158.1	697	133.7	230	108	826	70.3	129	247
Commercial Check 2	***160.8***	***570***	***131.2***	***220***	***140***	***114***	***78.3***	***120***	***264***
Commercial Check 3	161.7	917	128.3	226	118	159	13.5	117	183
Mean	135.8	786	126.5	188	141	.	58.6	103	194
CV	7.4	53	.	8.8	.	.	19.0	8	9.3

QUANTITATIVE GENETICS AND MAIZE BREEDING
DEPARTMENT OF SOIL AND CROP SCIENCES

NEW Maize Germplasm Lines Tx736, Tx739, and Tx740 For Reducing Preharvest Aflatoxin Accumulations

Aflatoxin is a serious problem to both growers and society causing millions of dollars of losses in grain. Aflatoxin is federally regulated in food over 20ppb (parts per billion or ng g^{-1}). While low aflatoxin corn can be blended with high aflatoxin corn up to 500ppb, corn must be destroyed when over 500ppb. Three corn inbred lines were recently released by Texas AgriLife Research which provide enhanced genetic control of aflatoxin. All three were bred by Texas AgriLife Research in College Station and tested in multiple Texas locations. For aflatoxin screening, plants were heavily inoculated with *Aspergillus flavus* using a silk channel method to provide a "worst case scenario". In a grower's field, where inoculation pressure is substantially less, much lower levels would be observed and these lines would reduce aflatoxin to levels that would likely avoid dockage and could be blended. These inbreds were only tested in combination with a known aflatoxin susceptible stiff stalk inbred. Further testing is being done with modern commercial testers and competitive hybrid yield is expected with the right combination.

Inbred Lines:

Tx736: Cross between Tx772 (known for extremely low aflatoxin levels) and T246 a high-yielding line from Tennessee.

Tx739: Selection from tropical Bolivian hybrids

Tx740: Selection from tropical Bolivian hybrids

Table 1: Inbred trials with Tx739 and Tx740

2004 CS, WE	Aflatoxin ppb
Tx739	83*
Tx740	89*
Check inbred average	638

Table 2: Hybrid trials with Tx736

2006 CS,CC, WE	Aflatoxin ppb	Grain yield (Mg ha^{-1})	Test weight (g L^{-1})	Days to silk
Tx736 x SS	174*	7.75*	82.5	71
Commercial check hybrid average	629	10.06	81.9	71

Table 3: Hybrid trials with Tx739 and Tx740

2005 CS,CC, WE	Aflatoxin ppb	Grain yield (Mg ha^{-1})	Test weight (g L^{-1})
Tx739 x SS	355*	5.30*	75.5
Tx740 x SS	365*	5.54*	76.3
Commercial check hybrid average	812	6.34	75.2

** Significantly different at $p<0.05$*

Registration of Maize Germplasm Lines Tx736, Tx739, and Tx740 for Reducing Preharvest Aflatoxin Accumulation

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ABSTRACT

Maize (*Zea mays* L.) production in Texas, the southern United States, and much of the developing world is constrained by preharvest contamination from aflatoxins—potent mycotoxins produced by the fungus *Aspergillus flavus*. When consumed, aflatoxin can lead to impaired growth, liver cancer, or death of both humans and livestock. Because of these effects, the presence of this mycotoxin is tightly regulated in the U.S. food supply. No complete resistance to *A. flavus* or aflatoxin is known to exist in maize; however, multiple quantitative traits and some sources of resistance have been identified. We propose the release of three maize lines that demonstrate reduced preharvest aflatoxin accumulation. Tx736 (Reg. No. GP-578, PI 662937) is a germplasm line derived from modified pedigree selection of a cross between Tx772, a line with reduced aflatoxin accumulation, and T246, a temperate line with good yield and Texas adaptation, backcrossed to Tx772 once. Lines Tx739 (Reg. No. GP-579, PI 662938) and Tx740 (Reg. No. GP-580, PI 662939) were selected by pedigree methods from S_3 plants selfed out of heterotic groups A, C, and E from Agricomseeds (Santa Cruz, Bolivia). These three lines were field tested as lines per se and as testcross hybrids with introduced inoculation in multienvironmental field trials. In trials, these lines and hybrids had between 30 and 73% lower aflatoxin content than commercial checks. These germplasm lines will serve as unique sources for novel traits and alleles that reduce aflatoxin in elite temperate and subtropical maize.

Maize (*Zea mays* L.) is the largest cereal grain crop produced globally, with production in 2009 exceeding 826 million metric tons (FAOSTAT, 2010). The United States is the largest global maize producer, producing more than 307 Mg with a value exceeding $49 billion in 2008 (USDA-NASS, 2010). Within the United States, Texas was the 12th largest producer of maize, with 0.89 million planted hectares, the largest production for any state outside of the temperate Midwest (USDA-NASS, 2010). Corn production in Texas occurs across very diverse production environments that extend in latitude from 26 to 36° N and range from subtropical production environments in South and Central Texas to temperate ones in the High Plains of the Texas Panhandle.

A major limiting factor to maize production in Texas and the southern United States is chronic preharvest aflatoxin accumulation caused by *Aspergillus flavus*. The pathogen itself rarely causes economic yield losses but can produce up to four different aflatoxins: B1, B2, G1, and G2. Collectively, these compounds are known to cause liver cancer and potentially death (Castegnaro and McGregor, 1998; IARC, 2002). Additionally, chronic exposure to aflatoxin in both humans and livestock can lead to impaired growth and other adverse health effects (Cardeilhac et al., 1970; Lamplugh et al., 1988; Gong et al., 2008). Because of these factors, the U.S. Food and Drug Administration has issued limits on the amount of aflatoxin that can be present in corn: less than 20 ng g^{-1} for that used in human food and less than 300 ng g^{-1} for certain livestock feed (US FDA, 2010). These strict limits, combined with extensive testing, have minimized the presence of aflatoxin in the U.S. food stream. However, these limits also produce significant economic losses to producers where aflatoxin occurs: they are forced to destroy their crops or sell them at a significant loss. The exact economic loss to producers from aflatoxin

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Abbreviations: EPR, ear height to plant height ratio; EH, ear height; PH, plant height.

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contamination of corn is unknown. However, crop insurance payments to Texas producers alone for mycotoxins was $18 million in 2008 (USDA Risk Management Association, unpublished data), and direct losses across the United States have been estimated at $200 million per year (Texas Corn Producers Board, 2010).

Currently there are no known sources of complete resistance to *A. flavus* or aflatoxin accumulation in corn. However, there is a known range of susceptibilities of hybrids to aflatoxin accumulation, and some sources of maize germplasm have been identified that consistently minimize aflatoxin accumulation (Williams, 2006). Tropical germplasm has been identified as a major source of resistance to preharvest aflatoxin accumulation; this resistance is primarily the result of the high disease pressure in tropical areas (Menkir et al., 2008). The pool of tropical germplasm available to screen for aflatoxin resistance is immense, because the genetic base is much broader than the genetic base of maize produced in the United States (Goodman, 1999; Ochs, 2005). From diverse tropical material, germplasm lines with lower amounts of aflatoxins than check lines have been recently identified and released, including Mp313E, Mp420, GT-MAS:gk, Mp715, Tx772, Mp717, TZAR101-TZAR106 (Scott and Zummo, 1990; 1992; McMillian et al., 1993; Williams and Windham, 2001, 2006; Llorente et al., 2004; Menkir et al. 2008).

Reduced aflatoxin accumulation in maize can be associated with multiple traits, which are often tested individually. These include improved husk tip coverage (Odvody et al., 1997), tighter husks (Betrán and Isakeit, 2004), improved kernel integrity (Odvody et al., 1997), harder kernel texture (Guo et al., 1995), improved drought and heat tolerance (Payne, 1992), earlier maturity and better adaptation (Betrán and Isakeit, 2004), and "factors" in the kernels and silks that reduce fungal development or aflatoxin accumulation (Brown et al., 2001; Peethambaran et al., 2010). Quantitative reductions in aflatoxins in tropical material are believed to be the result of combinations of these independent factors. Unfortunately, tropical maize cultivars commonly lack the adaptation and suitable agronomic performance to be used directly in hybrids for U.S. production environments. Specific limitations include delayed flowering and maturity, photoperiod sensitivity, and unacceptably high ear height (Holland et al., 1996; Betrán et al., 2006). This general lack of adaptation and acceptable agronomic characteristics results in lower yield than that produced with temperately adapted maize (Castillo-Gonzalez and Goodman, 1989). Breeding and selection to minimize these negative characteristics is necessary before the true potential of the germplasm can be measured in a temperate environment.

Historically, the Texas A&M and Texas AgriLife Research maize improvement programs have been active in developing maize germplasm with reduced aflatoxin accumulation and have identified multiple sources of resistance, with emphasis on tropical maize (Betrán et al., 2002; Ochs, 2005). The release of the three maize germplasm lines designated Tx736 (Reg. No. GP-578, PI 662937), Tx739 (Reg. No. GP-579, PI 662938), and Tx740 (Reg. No. GP- 580, PI 662939) is proposed based primarily on a consistent reduction in aflatoxin accumulation compared with standard checks in the individual lines per se and in hybrid combinations across multiple Texas environments that include heat and drought stress. Although they are released as germplasm lines, they have the improved agronomics typical of adapted material and thus have the potential to be directly tested in hybrid combinations.

Methods

All lines were developed from breeding crosses using a modified pedigree method of plant breeding. Tx736 is a derived line utilizing a first generation backcross with the pedigree of ((Tx772 × T246) × Tx772)-7-2-B-B-B-B-B-B-B. Tx772 is a yellow maize parent line that was released by the Texas Agricultural Experiment Station in 2003 because it has reduced aflatoxin accumulation (Llorente et al., 2004). T246 is a yellow-grain maize germplasm line with the pedigree Va25 × T204 and was released by the Tennessee Agricultural Experiment Station in 1974 (Gerdes et al., 1993). The single cross Tx772 × T246 was made in the summer of 1996, and the backcross in the summer of 1997. Single ear-to-row progeny selection was then performed for two generations followed by seven generations of selfing two or more individual plants and making balanced bulks of these for planting the following year. Selections for Tx736 were based on desirable ear characteristics (kernel quality and good grain fill), flowering synchrony, and importantly, the absence of *A. flavus*. Balanced bulks were created within a generation when selections of a few ears having a similar phenotype (kernel texture, grain color, and cob color) were bulked together. This method was used because it has the simplicity of a bulk method while still "maintaining genetic variability" through the heterogeneity of the pedigree method (Betrán et al., 2006).

Lines Tx739 and Tx740 were selected from a single population of segregating S_3 plants derived from a mixture of heterotic groups A, C, and E provided by Agricomseeds (Santa Cruz, Bolivia). Although this tropical population was strongly photoperiod sensitive, delayed flowering effects were minimized or eliminated by production in a short-day environment during the fall in Weslaco, TX, as has been done by other researchers (Castillo-Gonzalez and Goodman, 1989). Sixty-three single-row plots were planted on 26 August in the 2002 fall nursery at Weslaco (26°09′48″ N, 97°56′28″ W), where the day length at that time was less than 13 h d^{-1}. Initial selections were made for reduced plant height and plant ear height as well as for desired kernel texture and color (flint kernels and yellow to orange/bronze color). A total of 207 ears were selected and planted ear to row in College Station at the Texas AgriLife Research farm in Burleson County, TX (30°32′48″ N, 96°26′00″ W). Tx739 (LAMA2002-10-1-B-B-B) and Tx740 (LAMA2002-12-1-B-B-B) were subsequently selected on the basis of plant adaptation (plant and ear height, flowering synchrony, and maturity) and kernel characteristics (absence of *A. flavus*, texture, integrity, color) together with their superior performance in testcrosses with temperate testers.

Field Trials

Maize germplasm lines were evaluated in per se evaluations and in hybrid testcrosses. For testcross evaluation, all lines were crossed to LH195 (Corn States, St. Louis, MO). LH195 is a commercially used yellow dent parental line that belongs to the stiff stalk heterotic group (Mikel and Dudley, 2006). LH195 is a good commercial tester across target environments in Texas; it provides adequate separation among testcross hybrids for yield potential and estimation of aflatoxin accumulation (Betrán et al., 2005).

To estimate aflatoxin accumulation, trials were performed on the Texas AgriLife Research farms near College Station, Corpus Christi, and Weslaco, TX and two on-farm trials located near Bardwell and Wharton, TX. Not all of these lines were evaluated in the same trials each year. Each trial consisted of two or three replications arranged in an α-lattice design. To ensure consistent pathogen pressure, the top ears of 10 plants per replication at College Station, Weslaco, Bardwell, and Wharton were inoculated with *A. flavus* using the silk-channel method (Zummo and Scott, 1989). Plants at Corpus Christi were inoculated by placing *A. flavus*–colonized kernels between the rows to allow for natural infection (Odvody, 1997). Inoculated ears were hand harvested, rated for fungal colonization, and bulk shelled. Bulked grain was ground with a Romer Mill (Romer Labs, Union, MO). Total aflatoxin accumulation was estimated with monoclonal antibody affinity fluorescence (AflaTest, Vicam, Watertown, MA).

The grain yield, grain moisture, and test weight were estimated by combine-harvesting plots with a John Deere (Moline, IL) 3300 combine set up for plot harvest by using a HarvestMaster GrainGage H-1 (Juniper Systems, Logan, UT) and adding the grain weights from hand-harvested inoculated ears. Grain yield at Corpus Christi was estimated by hand harvesting all ears in the plot. Plant and ear heights were measured as the height from the soil to the top of the tassel and to the ear node, respectively. The ear height (EH) to plant height (PH) ratio (EPR) was calculated as EPR = EH/PH (Betrán et al., 2005). Days to anthesis and silking was measured from the day of planting until 50% of the plants shed pollen or had silks exposed. Kernel integrity was rated on a scale of 1 to 5 (1 = kernels with good integrity with few kernels broken or damaged, and 5 = kernels with bad integrity, most kernels broken or damaged). Root lodging was measured as the percentage of plants leaning at least 30° off of vertical.

Statistical Analysis

Data were analyzed using the PROC MIXED procedures in SAS v9.2 (SAS Institute, Cary, NC). Orthogonal contrasts were obtained using the Contrast statement in conjunction with the PROC MIXED procedures in SAS 9.2. Aflatoxin accumulation data were transformed (logarithm) to equalize the variances and standardize the data (Betrán et al., 2005).

Characteristics

Tx736, Tx739, and Tx740 were released by Texas AgriLife Research in 2010. Texas Tx736 is a temperate southern/subtropical adapted line that accumulates significantly lower amounts of aflatoxin than commercial hybrid checks when it is evaluated as a testcross with LH195. Across the five environments in 2005, Tx736 testcrosses accumulated 30% fewer aflatoxins than the checks (Table 1). Testcrosses of Tx736 were lower in yield and slightly higher in harvest

Table 1. Analysis of variance mean squares and contrasts of Tx736 testcrosses for aflatoxin, grain yield, kernel traits, height, and flowering in 2005. A trial consisting of 25 entries was evaluated at Bardwell, College Station, Corpus Christi, Weslaco, and Wharton, TX.

	df†	Aflatoxin	Log_{10} aflatoxin	Test weight	df†	Plant height	Ear height	EPR‡	df†	Grain yield	df†	Grain moisture
						Mean squares						
Genotype	24	516672**	0.86***	103.9***	24	466**	10685***	0.003*	24	149.14***	24	172.4***
Environment	4	12114528***	14.38***	159.1	2	17319**	2720	0.007	5	1632.24***	3	1064.5***
Geno × env§	96	245548***	0.21***	35.6	48	199*	3598	0.002	120	115.15***	72	127.6***
Rep(env)¶	10	312780**	0.30**	157***	5	906***	2124***	0.003	12	32.17***	8	42***
Error	240	118387	0.12	33.4	120	129	8107	0.002	280	126.51	186	114.6
						Contrasts#						
		ng g^{-1}	Log_{10} ng g^{-1}	g L^{-1}		——— cm ———		cm cm^{-1}		Mg ha^{-1}		g g^{-1}
Tx736TC		401**	2.23***	74.1		248	89**	0.37*		5.08***		13.9***
Check mean††		575	2.48	72.3		264	110	0.41		6.90		12.1
Test mean		481	2.37	72.1		257	102	0.40		6.14		12.9

*Significant at the 0.05 probability level.

**Significant at the 0.01 probability level.

***Significant at the 0.001 probability level.

†df for aflatoxin and grain moisture were measured at Bardwell, College Station, Corpus Christi, Weslaco and Wharton; df for plant height and ear height were measured at College Station and Weslaco; df for grain yield were measured at Bardwell, College Station, Corpus Christi, Hondo, Weslaco, and Wharton.

‡EPR, ear height to plant height ratio.

§Geno × env, genotype × environment interaction.

¶Rep(env), replicate nested in environment.

#Orthogonal contrast between Tx736 and checks as a whole.

††Mean of the five hybrid checks (P31B13, P32R25, BH8913, DKC69-72, and W4700) included in the trial.

Table 2. Analysis of variance mean squares and contrasts of Tx736 testcrosses for aflatoxin, grain yield, kernel traits, and flowering in 2006. A trial consisting of 20 entries was evaluated at College Station, Corpus Christi, and Weslaco, TX.

	df†	Aflatoxin	Log_{10} aflatoxin	df†	Grain yield	Grain moisture	Test weight	df†	Days to silk
					Mean squares				
Genotype	19	708120*	0.97***	19	7.38**	18***	87.9	19	3.6
Environment	2	6460121***	15.56***	1	263.77***	101.4***	2131	1	5589.7
Geno × env‡	6	187579	0.34*	4	1.76	0.5	1.8	4	1.4
Rep(entry)§	38	301971***	0.23**	19	1.80*	1.6	60.4	19	1.3
Error	113	107677	0.13	73	1.03	1.1	65.5	76	0.9
					Contrasts¶				
		ng g^{-1}	Log_{10}, ng g^{-1}		Mg ha^{-1}	g g^{-1}	g L^{-1}		
Tx736TC		174*	1.97**		7.75***	17.0*	82.5		71
Check mean#		629	2.64		10.06	15.6	81.9		71
Test mean		479	2.34		7.92	15.6	78.3		70

*Significant at the 0.05 probability level.

**Significant at the 0.01 probability level.

***Significant at the 0.001 probability level.

†df for aflatoxin were measured College Station, Corpus Christi, and Weslaco; df for plant height and ear height were measured at College Station and Weslaco; df for other traits were measured at College Station and Weslaco only.

‡Geno × env, genotype × environment interaction.

§Rep(entry), replications nested within entry.

¶Orthogonal contrast between Tx736 and checks as a whole.

#Mean of the two hybrid checks (P31B13 and DKC69-71) included in the trial.

moisture content than commercial checks (Table 1). The test weight was not significantly different between Tx736 testcrosses and the commercial checks. The PH, EH, and EPR measured at three environments were similar to those of the checks; however, EHs and EPRs for the Tx736 testcrosses were reduced compared with those of the commercial checks (Table 1).

In 2006, Tx736 was evaluated for aflatoxin accumulation at College Station and Weslaco. Across the two locations, aflatoxin content in Tx736 testcrosses was 73% lower than the checks (Table 2). Grain yields and moisture were similar to those observed in 2005 (Table 2), and differences were observed between Tx736 and the checks (Table 2). Tx736 testcrosses silked at approximately the same day as the checks (71 d after planting) suggesting that flowering time alone was not the cause of resistance (Table 2). Tx736 flowers approximately 2–3 d later than B73 at College Station. The tassel contains three to four branches and the main branch is bent, although not as severely as the bend observed in Tx772. Leaves are spaced apart, and the top leaves are semierect. Ears of Tx736 have 10 rows of kernels that are more orange than yellow and are semiflint in texture.

Table 3. Analysis of variance and means of Tx739 and Tx740 lines per se for aflatoxin, kernel traits, and flowering in 2003. A trial consisting of 32 entries was evaluated in Weslaco, TX.

	df	Aflatoxin	Log_{10} aflatoxin	Grain texture	Kernel integrity	Days to anthesis	Days to silk	ASI†
		ng g^{-1}						
Entry	18	387491*	1.32**	4.7	1.7***	27***	19***	2.2
Replications	3	433594	1.30*	18.5**	0.7	1	2	2
Bloc(rep)‡	12	124449	0.48	16.0***	0.5	2	2	2.5
Error	42	196668	0.44	3.7	0.3	2	2	2.1
				Test means and separations				
Test mean		343	2	2.2	2.1	70	73	2
$LSD_{.05}$		633	0.95	2.8	0.7	2	2	2
				Entry means				
Tx739		114 ab	2.01 bcdefgh	1.3 a	1.4 ab	72 bc	73 bcd	2 ab
Tx740		76 ab	1.80 abcdef	1.5 a	2.3 cde	74 bc	74 efg	1 a
Tx732§		792 c	2.80 h	3.4 ab	3.8 g	66 a	69 a	3 ab

*Significant at the 0.05 probability level.

**Significant at the 0.01 probability level.

***Significant at the 0.001 probability level.

†ASI, anthesis silking interval.

‡Bloc(rep), blocks nested in replications.

§Tx732 was included as a susceptible check.

When Tx739 and Tx740 were evaluated for aflatoxin accumulation as inbred lines per se, they each accumulated less aflatoxin than Tx732, a susceptible check (Table 3), and less than the average of the other inbred lines in the trial. Tx739 and Tx740 flowered later than temperate lines but at the same time as CML288, a resistant check. When evaluated as testcrosses with LH195, Tx739 and

Tx740 also accumulated less aflatoxin than the commercial checks (Tables 4 and 5). The grain yield was lower than that of commercial hybrids, however, and harvest moisture content increased 2.7–4.0% above the commercial checks. Plants of Tx739 and Tx740 have tassels with five to six branches. Leaves of both lines are wide and have a "tropical" appearance, with the leaves above the ear prostrate. The ears have 12 rows of yellow-orange (Tx739) and orange-red (Tx740) kernels with a flint texture.

Discussion

Several sources of tropical and temperate germplasm with reduced aflatoxin accumulation have previously been released. They include Mp313E, Mp420, GT-MAS:gk, Mp715, Tx772, GT601, GT602, TZAR101-TZAR106, and GT603 (Scott and Zummo, 1990, 1992; McMillian et al., 1993; Williams and Windham, 2001; Llorente et al., 2004; Guo et al., 2007, 2010; Menkir et al., 2008). Each set of released germplasm targeting aflatoxin reduction has provided unique genetic backgrounds, agronomic traits, and adaptations to different environments. The addition of more unique germplasm sources that reduce aflatoxin accumulation in maize will be useful in identifying underlying pathways and will allow the pyramiding of durable sources of resistance.

These lines have many agronomic traits that could serve as bases for reduced aflatoxin accumulation. Examples of these traits include improved husk coverage, increased grain hardness, and maintenance of kernel integrity (Odvody et al., 1997; Betrán et al., 2006). In our tests, Tx739 and Tx740 testcrosses had higher grain moisture contents than the commercial hybrids. This higher moisture content is probably caused in part by a combination of increased husk coverage and a later flowering date. These two traits are more common in tropical maize than in temperate maize; their presence should be acceptable for maize grown in areas with longer growing seasons, such as Texas (Betrán et al., 2006). Although husk coverage was not specifically measured in trials with Tx739 and Tx740, both testcrosses and inbred lines have husks that are tight and extend past the tip of the ear. Tx739 and Tx740 also had improved kernel integrity compared with the commercial checks, which probably reduced the percentage of ears infested with *A. flavus* (Table 5), along with reduced aflatoxin in testcrosses.

All of the inbreds were effective in significantly reducing aflatoxin accumulation relative to the commercial hybrids

Table 4. Analysis of variance and contrasts of Tx739 and Tx740 testcrosses for aflatoxin in 2004. A trial consisting of 32 entries was evaluated in College Station and Weslaco, TX.

	df	Aflatoxin	Log_{10} aflatoxin
		ng g^{-1}	
Genotype	30	444907*	0.67
Environment	1	78117	0.61
Geno × env†	30	241163	0.53
Rep(env)‡	4	459028	1.17
Residual	118	220449	0.52
		Contrasts§	
Tx739		83*	1.64*
Tx740		89*	1.14***
Check mean¶		638	2.47
Test mean		306	2.04

*Significant at the 0.05 probability level.

***Significant at the 0.001 probability level.

†Geno × env, genotype × environment interaction.

‡Rep(env), replication nested in environments.

§Orthogonal contrast between Tx739, Tx740, and commercial checks as a whole.

¶Mean of the five hybrid checks (DKC66-80, DK697, P31B13, P32R25, and LH195/LH210) included in the trial.

Table 5. Analysis of variance, means and contrasts of Tx739 and Tx740 testcrosses for aflatoxin, grain yield, lodging and kernel traits in 2005. A trial consisting of 30 entries was evaluated at College Station, Corpus Christi, and Weslaco, TX.

	df	Aflatoxin	Log_{10} aflatoxin	Grain yield	Kernel integrity	df	Root lodging	Grain moisture	Test weight
		ng g^{-1}		Mg ha^{-1}			%		g L^{-1}
Genotype	28	371020*	0.27	2.23**	2.2***	28	35.4	7.4***	4.9**
Environment	2	2797967***	2.02	817.92***	2.5	1	176.1	13.1	10.5
Geno × env†	56	213737	0.18*	0.96***	0.8	28	22.2	2.1***	1.9***
Rep(env)‡	6	714148*	0.52***	2.40***	2.3**	4	25.2	7.5***	1.5*
Residual	168	191562	0.12	0.44	0.6	112	17.3	0.5	0.5
					Contrasts§				
Tx739		355*	2.38*	5.30*	2.2***		3.5	16.1***	75.5
Tx740		365*	2.32**	5.54*	2.7*		4.7	16.6***	76.3
Check mean¶		812	2.76	6.34	3.4		13	12.6	75.2
Test mean		491	2.51	5.50	2.5		35	14.6	75.9

*Significant at the 0.05 probability level.

**Significant at the 0.01 probability level.

***Significant at the 0.001 probability level.

†Geno × env, genotype × environment interaction.

‡Rep(env), replications nested in environments.

§Orthogonal contrast between Tx739, Tx740, and commercial checks as a whole.

¶Mean of the four hybrid checks (P31B13, P32R25, DKC69-70, and DKC69-72) included in the trial.

that were evaluated under our various inoculated field conditions, which often included drought and heat stress. Testcrosses of Tx736, Tx739, and Tx740 did not show grain yields equal to those of the commercial check hybrids, but each of these lines in testcrosses produced grain with lower aflatoxin accumulation. In Texas, where grain with aflatoxin concentrations of up to 500 ng g^{-1} may be blended with grain having less than 200 ng g^{-1} under an appropriate management plan, the ability for blending would be the difference between selling and destroying grain for producers (Office of the Texas State Chemist. 2010). We expect these sources of germplasm to be useful in programs that are developing high yielding and adapted maize hybrids with consistently reduced aflatoxin accumulation.

Availability

Seed for Tx736, Tx739, and Tx740 will be maintained by the Quantitative Genetics and Maize Breeding Program of Texas AgriLife Research at College Station. Seed of this material has also been deposited in the National Plant Germplasm System but will not be distributed through NPGS until 20 yr after the date of this publication. Seed will be available with a Materials Transfer Agreement from the Office of Technology Commercialization, Texas A&M University System, 1700 Research Parkway, Suite 250, College Station, TX 77845–9548.

Conclusions

Aflatoxin continues to be extremely important in Texas, the southern United States, and many corn growing regions of the world, in part because it is extremely challenging to identify and accumulate genetic components that improve host-plant resistance to aflatoxin accumulation. These three lines, Tx736, Tx739, and Tx740 will serve as sources for quantitative aflatoxin resistance that may be pyramided with other sources of resistance and locally adapted material to ultimately create locally adapted varieties and hybrids with a lower risk of aflatoxin accumulation.

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