

# Evaluation of Advanced GEM Lines for Multiple Insect Resistance and Fumonisin Concentration

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## PROJECT DESCRIPTION

The overall objective of this project is the development of maize varieties with host plant resistance against the Western corn rootworm (WCR), the European corn borer (ECB), and Fusarium by searching for novel resistance genes and alleles available in GEM base populations. The breeding activities comprise intensive evaluation of GEM germplasm for multiple insect and Fusarium resistance as well as the recombination of GEM germplasm with promising levels of resistance and acceptable yield potential.

## 2006 SEASON - ACTIVITIES

*Overview* - A large set of germplasm was tested in the previous years (2003-2005). The most promising materials were recombined or directly used as base populations for inbred line development (see previous project reports). The summer season 2006 was used to evaluate early generations of newly developed materials, advance selected genotypes, and produce testcrosses for early and late generation testing for summer of 2007 (see *Germplasm Development*). In addition, two experiments were conducted to evaluate experimental hybrids derived from crosses between new GEM lines with promising levels of WCR resistance for their future usefulness as the basis for new breeding populations (Experiment 1) and repeat a diallel experiment to investigate the genetic basis of WCR resistance (Experiment 2, see *Germplasm Evaluation Experiments*).

## GERMPLASM DEVELOPMENT

*WCR-Program 1* - Testcross and *per se* evaluation of GEM-derived S<sub>3</sub> and S<sub>4</sub> inbreds, respectively, led to the identification of ten inbreds with improved WCR resistance and acceptable agronomic characteristics in 2005. All inbreds belong to the non-Stiff Stalk heterotic pool. The selected inbreds were advanced to the S<sub>6</sub> stage in the 2005/2006 winter nursery. It was planned to cross these materials with a series of testers in the summer season 2006. However, the seed quality we obtained from our Hawaii winter nursery was extremely poor. As a consequence the production of the S<sub>6</sub> lines was repeated in the summer nursery and testcross production will be performed by Pioneer in their winter nurseries. In addition, 210 S<sub>5</sub> lines derived from GEM breeding populations UR10001:N1708b (N=28), FS8A(T):N1804 (N=99), AR17056:S1216 (N=8), DKXL212:N11a01 (N=63) were re-evaluated for WCR resistance using plant performance ratings and root lodging percentages. Due to extremely poor performance all inbreds derived from UR1000:N1708b were discarded, whereas for all other evaluated GEM breeding populations about 15% of the inbreds were selected and selfed.

*WCR-Program 2* - Based on their *per se* performance under intensive WCR infestation and agronomic characteristics (earliness, lodging) 479 S<sub>3</sub> lines derived from GEM populations AR16026:N1210 (N=73), FS8A(S):S0907 (N=65), UR13085:N0204 (N=40), and CUBA117:S1520 (N=222), as well as CIMMYT population MIRT\_C5Y (N=79) were selected in 2005. All S<sub>3</sub> lines were re-evaluated for WCR resistance in 2006. A representative subset composed of 93 S<sub>3</sub> lines was crossed to a single tester. The testcrossing was conducted by AgReliant.

*WCR-Program 3* - Three replications (1000 plants/rep) of Cycle 0 of the Illinois WCR Synthetic were planted under WCR trap crop conditions. In each rep 50 individual plants (5%) were selected based on phenotypic appearance and selfed. The S<sub>1</sub> progenies will be evaluated for their WCR resistance in 2007 and selected genotypes will be recombined in 2008.

## GERMPLASM EVALUATION EXPERIMENTS

## MATERIALS AND METHODS

*Plant materials* - The objective of this study (Experiment 1) was to evaluate crosses between newly identified sources of resistance to WCR larvae feeding and to identify the most promising hybrids for base population development with regard to agronomic performance as well as resistance to WCR, ECB, and Fusarium. In the winter nursery 2005/2006, a set of eight newly developed inbreds derived from population DKXL212:N11a01 with improved levels of WCR resistance and good general combining ability was crossed with three sources of WCR resistance derived from GEM breeding population AR17056:N2025 identified in earlier germplasm screens (see previous project reports and Table 1). All hybrids were evaluated for WCR, ECB, and Fusarium resistance in 2006.

In order to learn more about the genetic basis of WCR resistance in maize, we conducted a diallel experiment. Sixty hybrids representing a half diallel without parents derived from crosses between WCR resistant/tolerant (GEM and non-GEM sources) and susceptible inbreds (Experiment 2, “Diallel”) were re-evaluated for WCR resistance and agronomic performance.

*Experimental design* - For all experiments we used a generalized lattice design with two (insecticide protected trials) and three replications (WCR trials in trap crop) and two-row plots. The trials were over-planted and thinned to 25 plants per row, corresponding to 65,000 plants/ha. The experiments and nurseries were planted on April 28, 2006. The WCR treatment was planted in a WCR trap crop area to ensure a high level of infestation. Resistance to WCR larvae feeding was evaluated using root damage ratings (Experiment 2) and the percentage of root lodged plants per plot (all Experiments). All root characteristics were determined for five random plants per plot between July 20 and 28, 2006. The artificial infestation with first generation ECB larvae was performed on July 3 and 5, 2005. Leaf damage caused by ECB larvae feeding was evaluated on July 17, 2006, but surprisingly no noticeable leaf feeding was detectable. Artificial infestation with second generation larvae was performed on August 1 and 2, 2006. Plants were inoculated with Fusarium at flowering time, when silks were visible, in a period between July 20 and August 4, 2006. All experiments were also repeated as yield trials under insecticide protection.

The following resistance traits were determined: (1) leaf damage ratings (LDR) using a 1-9 rating scale, as defined by Guthrie and Barry (1989), (2) stalk damage ratings (SDR) using a 1-9 rating scale, as described by Hudon and Chiang (1991), (3) root damage ratings (RDR) using the

Iowa State 0-3 damage rating scale, (4) number of root lodged plants (RLD) as the percentage of the total plants per plot, and (5) the percentage of ear tissue damaged by Fusarium ear rot. The primary ear of each plant inoculated with Fusarium was hand harvested, dried, and the bulked seed was ground to facilitate the quantification of Fumonisin using the CD-ELISA method. We evaluated the following agronomic characteristics in all ECB infested and insecticide protected trials: (1) plant and ear height in cm, (2) female and male flowering in days, as well as (3) stalk and root lodging in percent. All hybrid evaluations were machine harvested and grain yield in bushels per acre and grain moisture in percent were measured.

*Statistical Analysis* - Analyses of variance were performed on plot means for each experiment. Data were screened for outliers according to the test criterion by Anscombe and Tukey (1963). Data values considered as outliers were treated as missing values in subsequent analyses. For Experiment 2 (“Diallel”) adjusted entry means and effective error mean squares were used to compute the combined analysis of variance. Phenotypic correlation coefficients ( $\hat{r}_p$ ) were calculated among resistance and agronomic traits across all cultivars (Mode and Robinson, 1959). Ward's distance was used to determine the distance between experimental hybrids in Experiment 1 using all resistance and agronomic traits simultaneously. A k-mean cluster analysis was performed for a varying number of maximum clusters ( $0 \leq k \leq 30$ ). The Cluster Cubic Criterion (CCC) and the pseudo-F (psF) statistic were calculated for each k and plotted against k. We selected the smallest k with a local maximum for both statistics as the number of clusters present in our set of genotypes for all subsequent analysis. For the interval  $0 \leq k \leq 30$ , CCC and psF reached a local maximum at  $k = 3$  suggesting three clusters for our germplasm group. For Experiment 1, linear contrasts were calculated to test for significant differences between the three different GEM cultivars used as males and the three cultivar groups identified by the previous cluster analysis. All calculations were done using PLABSTAT (Utz, 2001) and SAS (SAS Institute, 1988).

## RESULTS

*Experiment 1* - For all agronomic and resistance traits highly significant ( $P > 0.01$ ) differences between hybrids were found (Table 1). Between male groups, *i.e.*, all inbreds crossed to the same

male parent, significant differences ( $P>0.01$ ) were detected for the following agronomic traits: PHT, EHT, DMF and DFF (Table 1). Hybrid combinations containing AR17056\_16 as one parent were characterized by low root lodging and reduced Fusarium ear rot. All crosses with AR17056\_4 as a male parent showed a low susceptibility to Fusarium ear rot. No significant differences were found between hybrid groups for ECB stalk damage ratings. It is interesting to note that SDR2 means for all male group were not significantly different from the SDR2 mean of the resistant check carrying the Bt gene active against the ECB. In addition, some hybrid combination showed significantly less stalk damage than the Bt check. A multivariate analysis was conducted taking all traits into account to identify a hybrid subgroup useful for establishing future base populations (Fig. 1). Hybrids in cluster 1 were characterized by moderate yields but good resistance to WCR induced root lodging and Fusarium ear rot (Table 2). Hybrids in cluster 2 combined excellent grain yields with good resistance to root lodging and Fusarium ear rot. Cluster 3 hybrids also showed acceptable root lodging and Fusarium ear rot responses but these were combined with low yields and late maturity. Clusters 1 and 3 showed significantly ( $P<0.05$ ) lower ECB stalk damage ratings than hybrids that were member of Cluster 2. If these results can be substantiated in 2007, hybrids belonging to Cluster 2 combining good agronomic performance with improved levels of resistance to insects and Fusarium will be selfed to form new breeding populations.

*Experiment 2* - In the combined analysis across years, we detected significant ( $P < 0.05$ ) differences between experimental hybrids for RDR and RDL. The RDR means varied between 1.57 (Lo1016xAR17056\_3) and 2.64 (Lo964xCUBA117\_5) with an overall mean of 2.17. The mean root lodging values ranged from 20% (Lo964xB73) to 68% (B64xAR17056\_3) with an overall mean of 42%. Root damage ratings and RDL showed a weak but significant association ( $r_p=0.26^*$ ). We are now in the process of conducting an in-depth analysis of this experiment with the aim of estimating GCA and SCA effects.

#### FUTURE WORK

All WCR breeding programs will be continued in 2007 and selection decision will be made on testcross performance of lines at the  $S_4$  and  $S_6$  inbreeding level. We will repeat Experiment 1

in 2007 to identify hybrids that combine good agronomic performance with resistance to WCR, ECB, and Fusarium. The hybrids will form the bases of new breeding populations. In addition to these materials, 13 new GEM lines derived from seven GEM breeding populations were increased in the summer nursery 2006. These lines will be screened for their level of resistance to WCR, ECB, and Fusarium in 2007. Experiment 2 will be further analyzed and will provide together with QTL and genomic studies, the knowledge bases needed to enhance breeding programs for improving the host plant resistance of maize specifically to WCR.

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Table 1 Means for 24 maize hybrids developed from crosses between GEM derived germplasm evaluated for agronomic characteristics, WCR and ECB resistance traits and Fusarium ear rot in field experiments conducted in Urbana, IL, in 2006.

Genotypes	CL <sup>2</sup>	Traits <sup>1</sup>									
		YLD bu/acre	PHT ----- cm -----	EHT	DMF ----- days -----	DFE	RLD %	SDR1 ----- 1-9 -----	SDR2	ERR %	
<i>Female</i>	<i>Male</i>										
DKXL212.N11a01-02-5-2-2	AR17056_3 <sup>3</sup>	2	173.57	174.5	67.0	79.0	80.5	16.4	5.9	5.6	11.7
DKXL212.N11a01-05-1-2-1	AR17056_3	2	249.46	207.0	85.0	75.0	78.0	17.0	5.8	6.1	11.3
DKXL212.N11a01-05-1-2-2	AR17056_3	3	167.20	191.0	85.5	80.0	82.0	48.5	5.1	4.6	8.2
DKXL212.N11a01-06-1-3-1	AR17056_3	1	182.96	196.0	80.0	75.0	78.0	77.6	4.8	5.6	.
DKXL212.N11a01-09-3-4-2	AR17056_3	3	107.24	203.0	90.0	82.0	83.0	61.7	4.3	4.7	13.2
DKXL212.N11a01-09-3-4-3	AR17056_3	3	100.62	191.0	77.5	80.0	82.0	39.3	4.8	5.0	13.4
DKXL212.N11a01-09-3-4-4	AR17056_3	3	163.34	210.5	89.5	80.0	81.0	.	2.5	2.9	8.3
DKXL212.N11a01-09-3-5-1	AR17056_3	3	126.53	216.5	75.5	80.0	80.0	32.9	4.4	3.6	18.8
	<i>Mean</i>		158.87 <sup>A4</sup>	198.7 <sup>A</sup>	81.3 <sup>A</sup>	78.9 <sup>A</sup>	80.7 <sup>A</sup>	41.9 <sup>A</sup>	4.7 <sup>A</sup>	4.8 <sup>A</sup>	12.1 <sup>A</sup>
DKXL212.N11a01-02-5-2-2	AR17056_4	2	173.74	204.5	85.0	76.5	78.5	39.8	4.9	5.2	4.4
DKXL212.N11a01-05-1-2-1	AR17056_4	2	212.87	206.0	104.0	77.0	79.0	29.5	3.9	5.0	5.4
DKXL212.N11a01-05-1-2-2	AR17056_4	2	169.43	205.5	90.0	79.0	81.0	14.4	6.3	6.5	1.1
DKXL212.N11a01-06-1-3-1	AR17056_4	1	193.57	178.5	65.0	75.0	77.5	57.5	3.2	4.3	7.5
DKXL212.N11a01-09-3-4-2	AR17056_4	1	165.71	196.0	85.5	76.5	79.0	31.0	6.3	2.9	1.4
DKXL212.N11a01-09-3-4-3	AR17056_4	1	158.72	187.0	70.0	79.0	80.0	32.9	2.9	3.0	2.1
DKXL212.N11a01-09-3-4-4	AR17056_4	1	152.89	184.5	72.0	78.5	80.0	28.2	3.1	4.6	1.0
DKXL212.N11a01-09-3-5-1	AR17056_4	1	163.23	198.5	74.0	77.5	78.1	24.5	4.5	4.8	0.3
	<i>Mean</i>		173.77 <sup>A</sup>	195.1 <sup>A</sup>	80.7 <sup>A</sup>	77.4 <sup>AB</sup>	79.1 <sup>B</sup>	32.2 <sup>A</sup>	4.4 <sup>A</sup>	4.5 <sup>A</sup>	2.9 <sup>B</sup>
DKXL212.N11a01-02-5-2-2	AR17056_16	1	147.38	159.0	50.0	75.3	78.1	41.7	4.5	4.4	5.3
DKXL212.N11a01-05-1-2-1	AR17056_16	1	.	164.0	58.5	75.0	77.5	.	4.7	4.7	3.5
DKXL212.N11a01-05-1-2-2	AR17056_16	1	185.37	177.5	70.0	78.0	79.0	10.4	4.3	4.7	6.1
DKXL212.N11a01-06-1-3-1	AR17056_16	1	188.66	169.5	53.5	75.0	77.0	39.1	3.2	6.4	.
DKXL212.N11a01-09-3-4-2	AR17056_16	1	153.81	174.5	66.5	80.0	80.0	35.2	4.4	4.3	3.0
DKXL212.N11a01-09-3-4-3	AR17056_16	1	141.17	164.5	55.0	80.0	80.0	21.7	3.4	4.4	7.8
DKXL212.N11a01-09-3-4-4	AR17056_16	2	224.24	173.0	67.0	75.0	76.0	14.8	5.3	6.2	4.1
DKXL212.N11a01-09-3-5-1	AR17056_16	1	146.65	166.0	60.5	79.0	81.0	.	.	.	.
	<i>Mean</i>		169.61 <sup>A</sup>	168.5 <sup>B</sup>	60.1 <sup>B</sup>	77.2 <sup>B</sup>	78.6 <sup>B</sup>	27.2 <sup>B</sup>	4.3 <sup>A</sup>	5.0 <sup>A</sup>	5.0 <sup>B</sup>

Genotypes	CL <sup>2</sup>	Traits <sup>1</sup>									
		YLD bu/acre	PHT ----- cm -----	EHT	DMF ----- days -----	DFF	RLD %	SDR1 ----- 1-9 -----	SDR2	ERR %	
<i>Checks</i>											
DKC63-78	Commercial	-/-	208.73	206.0	81.0	78.5	78.5	26.3	5.6	7.2	8.8
RX708YG/RW	Commercial	-/-	253.34	196.5	85.5	76.0	77.0	30.4	4.6	5.5	0.0
33D31	Commercial	-/-	207.65	197.0	80.0	76.5	77.5	85.9	3.1	4.9	5.1
34H38	Commercial	-/-	227.35	205.0	84.5	73.0	73.0	.	.	.	.
B73xH84	WCR suscept.	-/-	110.21	184.5	73.0	79.0	82.0	82.9	.	.	.
AgReliant	WCR tolerant	-/-	.	.	.	.	.	30.1	.	.	.
LG2540	Commercial	-/-	.	.	.	.	.	.	4.0	4.4	1.3
RX708	Commercial	-/-	.	.	.	.	.	.	6.1	5.6	1.6
B73xMo17	Fus. suscept.	-/-	.	.	.	.	.	.	6.0	6.2	13.8
34H31	Commercial	-/-	.	.	.	.	.	.	5.9	5.2	4.0
34H35	Fus. resist	-/-	.	.	.	.	.	.	2.5	2.5	4.2
	<i>Overall Mean</i>		173.42	189.2	75.2	77.6	79.1	37.3	4.5	4.9	6.1
	<i>Standard Error</i>		13.76	9.4	7.0	1.0	1.0	11.2	0.8	0.8	2.7
	<i>C.V.%</i>		11.20	7.0	13.0	1.9	1.8	51.0	24.4	22.4	62.4
	<i>Repeatability 1%</i>		77.20	48.8	55.9	67.0	65.6	51.2	33.7	33.5	55.8

<sup>1</sup> YLD = Yield; PHT = Plant height; EHT = Ear height; DMF = Days to male flowering; DFF = Days to female flowering; RLD = Root lodging, SDR = Stalk damage rating (1= no damage, 9 = stalk broken below ear) evaluated for plants manual infested at 1<sup>st</sup> (SDR1) and 2<sup>nd</sup> brood ECB (SDR2) moths appearance; ERR = Ear rot (% damaged ear tissue).

<sup>2</sup> CL = Cluster, revealed by k-mean clustering using all traits applying Ward's distance, see also Table 2.

<sup>3</sup> AR17056\_3 = AR17056:N2025-522-1-B-B-B; AR17056\_4 = AR17056:N2025-728-1-B-B-B; AR17056\_16 = AR17056:N2025 Select # 5-B-B.

<sup>4</sup> Means with different letters are significantly different (P<0.05) in columns.

Table 2 Trait means of clusters identified in a set of 24 maize hybrids developed from crosses between GEM derived germplasm (see Fig. 1) using k-means cluster analysis with a subsequent canonical analysis. Hybrids were evaluated for agronomic characteristics, WCR and ECB resistance traits and Fusarium ear rot in field experiments conducted in Urbana, IL, in 2006.

Trait <sup>2</sup>	N <sup>4</sup>	Mean <sup>1</sup>		
		Cluster 1 <sup>3</sup>	Cluster 2	Cluster 3
YLD	bu acre <sup>-1</sup>	152.32±13.6 <sup>A</sup>	200.55±13.6 <sup>B</sup>	132.99±13.9 <sup>A</sup>
PHT	cm	178.12±3.7 <sup>A</sup>	195.08±6.8 <sup>B</sup>	202.40±5.1 <sup>B</sup>
EHT	cm	66.19±2.9 <sup>A</sup>	83.00±5.8 <sup>B</sup>	83.60±3.0 <sup>B</sup>
DMF	d	77.21±0.6 <sup>A</sup>	76.92±0.7 <sup>A</sup>	80.40±0.4 <sup>B</sup>
DFF	d	78.86±0.3 <sup>A</sup>	78.83±0.7 <sup>A</sup>	81.60±0.5 <sup>B</sup>
RLD	%	36.35±5.5 <sup>AB</sup>	21.97±4.2 <sup>A</sup>	45.61±6.3 <sup>B</sup>
SDR1A	1-9	4.11±0.3 <sup>A</sup>	5.35±0.3 <sup>B</sup>	4.25±0.5 <sup>AB</sup>
SDR2A	1-9	4.50±0.3 <sup>A</sup>	5.76±0.2 <sup>B</sup>	4.17±0.4 <sup>A</sup>
ERR	%	3.79±0.9 <sup>A</sup>	6.32±1.7 <sup>A</sup>	12.36±2.0 <sup>B</sup>

<sup>1</sup> Means with different letters are significantly different (P<0.05) between columns.

<sup>2</sup> YLD = Yield; PHT = Plant height; EHT = Ear height; DMF = Days to male flowering; DFF = Days to female flowering; RLD = Root lodging, SDR = Stalk damage rating (1= no damage, 9 = stalk broken below ear) evaluated for plants manual infested at 1<sup>st</sup> (SDR1) and 2<sup>nd</sup> brood ECB (SDR2) moths appearance; ERR = Ear rot (% damaged ear tissue).

<sup>3</sup> Cluster membership is given in Table 1.

<sup>4</sup> Number of genotypes in the respective cluster.

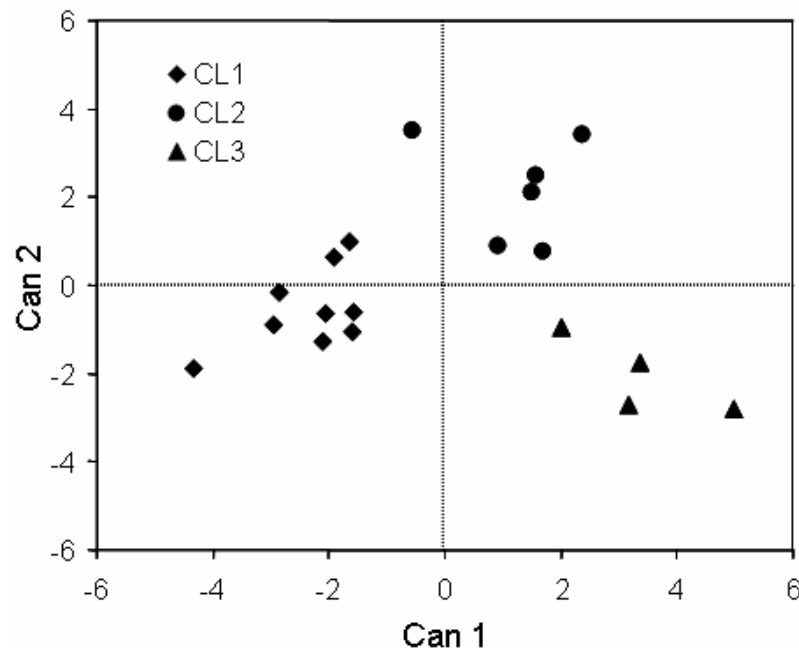


Fig. 1 Associations among 24 maize hybrids developed from crosses between GEM derived germplasm revealed by canonical analysis based on Ward's distance using agronomic characteristics, WCR and ECB resistance traits, as well as Fusarium ear rot evaluations.