

## **GEM Germplasm – Unlocking Maize Diversity for Improving Host Plant Resistance against West Corn Rootworm and Other Biotic Stresses**

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The Western corn rootworm (WCR) is a serious pest of maize in the U.S. and of growing importance in Europe. Soil insecticides, the use of transgenic maize hybrids producing a *Bt* toxin, and the rotation of maize with other crops are successfully used to manage WCR populations. However, the occurrence of WCR populations resistant to insecticides and adapted to cultural practices raises the question of how sustainable transgenic control measures against WCR are. In addition, monogenic resistances, like the *Bt* resistance, might be overcome by the target pest within a short time as amply documented in the literature. It seems, therefore, to make sense to invest in improving the multigenic host plant resistance in corn and to investigate its genetic basis. Secondary infections with *Fusarium verticillioides*, *F. proliferatum*, and *F. subglutinans* occur after European corn borer (ECB) larvae feeding, causing ear rots and contamination with fumonisin, a mycotoxin associated with severe animal and human health disorders. The tolerance of maize germplasm adapted to the Midwest against the ECB larvae was gradually improved over a 60 year period of continuous selection. However, improvements regarding WCR and Fusarium resistance over this time period are small. A major roadblock towards WCR and Fusarium host plant resistant maize genotypes is the apparent lack of genetic variation for these traits in the Midwestern maize pools. This lack of diversity is in stark contrast to the diversity available for improving other quantitative and agronomically important traits, like grain yield, early maturity, or grain composition and emphasizes the need for broadening the genetic basis of the Midwestern maize pools using exotic maize germplasm. The underlying hypotheses of this pre-breeding project are:

- Hypothesis 1** Exotic maize germplasm contains unique resistance genes against WCR, ECB, and Fusarium.
- Hypothesis 2** Genomic regions carrying resistance genes with an exotic origin can be retained in an adapted maize background if inbred development and selection is conducted under adequate WCR, ECB, and Fusarium pressure.

**Objective(s):** The overall goal of this project is the development of maize cultivars, *i.e.*, inbreds and populations, adapted to the U.S. Corn Belt, with improved host plant resistance against WCR, ECB, and Fusarium by broadening the genetic basis for these characteristics using exotic maize germplasm. The specific breeding objectives of this project are to (1) evaluate GEM materials for their resistance against WCR (1<sup>st</sup> selection step), (2) evaluate GEM materials with higher levels of WCR resistance for their resistance against ECB and Fusarium (2<sup>nd</sup> selection step), (3) recombine new GEM lines with improved levels of resistance identified in this and other GEM projects with the goal of combining different resistance mechanisms, and (3) continue the Illinois WCR long term recurrent selection program to develop new maize cultivars with improved WCR resistance.

## General Remarks

The work in the summer season 2008 was divided in germplasm development and germplasm evaluation related activities. Due to cool temperatures and heavy precipitation at the beginning of the 2008 growing season all GEM experiments and nurseries were planted late at the end of May / beginning of June. WCR experiments and nurseries planted in the WCR trap crop established in 2007 at the SoyFace experiment station in Urbana were flooded during the four to five leaf developmental stage. About 75% of the field, which included the nursery and the WCR evaluation experiments of advanced GEM inbreds were submerged for more than four days and the retreating surface water further damaged the experiments.

## Germplasm Development

Due to the loss of the WCR nursery we were not able to successfully advance our materials under high WCR pressure. In the order to minimize the impact of these adverse conditions, we continued the inbreeding process of materials selected in 2007 in the protected nursery and consequently will increase the size of the WCR nursery in 2009. In addition, 32 F<sub>1</sub> hybrids derived from crosses between new GEM lines with increased levels of WCR resistance were selfed to obtain segregating F<sub>2</sub> populations. These hybrids were also crossed with an inducer to initiate the production of double haploid lines.

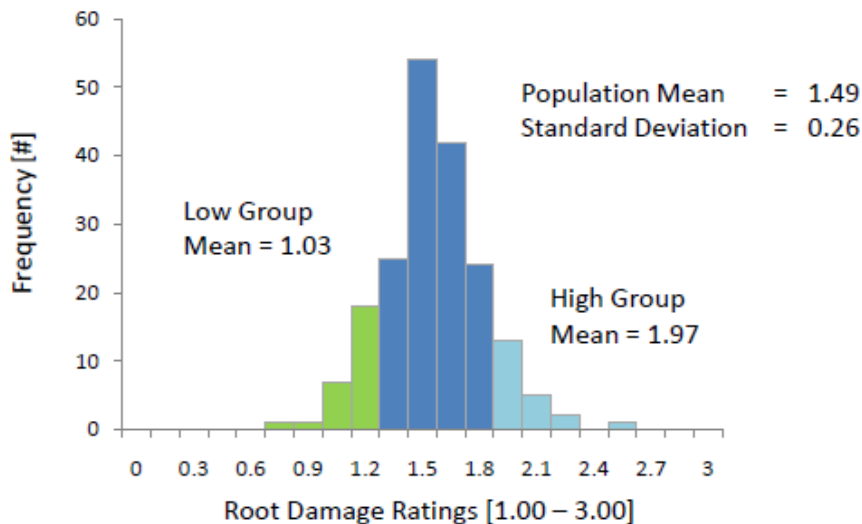
## Germplasm Evaluation

Thirty-two newly developed hybrids were evaluated for their level of ECB resistance as well as agronomic performance in separate field experiments. In order to obtain these hybrids inbreds obtained from populations DKXL212.N11a01 (N=5) and AR17056:N2025 (N=3) were crossed using a factorial design with inbreds obtained from populations BR52060:S0210 (N=2) and NEI19004:S2818 (N=2). All 12 inbreds were found to combine improved WCR resistance with acceptable agronomic performance. For ECB resistance, we found significant ( $P < 0.001$ ) differences between hybrids. However, differences between grain yield means were not significant. In general yields were low ranging from 20 to 127 bushel/acre with a mean of 52 bushel/acre and highly variable (CV=42%). Grain obtained from the ECB infested and protected experiments is currently used for mycotoxin analysis.

It was planned to evaluate a set of ten S<sub>5</sub> inbreds selected for improved WCR resistance from population DKXL212.N11a01 and crossed to three elite testers for WCR and ECB resistance, fumonisin concentration, as well as for their agronomic performance. Due to the loss of the WCR trap crop location we were not able to evaluate these advanced materials for their level of WCR resistance. For grain yield and ECB resistance we found significant ( $P < 0.05$ ) differences between testcrosses (Tab. 1). Among the testcrosses grain yield varied between 115 and 171 bushel/acre with a mean of 138 bushel/acre. The overall testcross grain yield mean was not significantly different from the overall mean of the commercial check hybrids (Tab. 1). Stalk damage ratings among testcrosses ranged from 1.9 to 5.5 with a mean stalk damage rating of 3.64. The difference between overall testcross and check means was not significant. The same experiment was conducted in 2007. A combined analysis revealed highly significant ( $P < 0.001$ )

genotype-by-year interactions and large differences between years. The average test cross yield in 2007 was about 60 bushels/acre higher and grain moisture contents at harvest were significantly lower than in 2008. The differences between results obtained in 2007 and 2008 can be partly explained by the late planting date (end of May) and the testcross specific response to this treatment. In 2008, the testcrossed inbreds showed significant general combining ability effects for ECB resistance. Specifically, inbred DKXL212.N11a01-02-5-2-2-11-1 combined well with the used testers and on average reduced the ECB stalk damage rating by 1.8 (Tab. 2). This inbred also combined well in 2007 with respect to the resistance to WCR larvae feeding and general agronomic performance. This experiment will be repeated under WCR trap crop conditions in 2009.

One goal of this project is also to further evaluate the genetic basis of the improved WCR resistance in materials selected from GEM base populations. One suitable approach is the development of segregating populations for QTL mapping. In a previous project inbred AR17056-16 derived from GEM breeding population AR17056:N2025 showed increased levels of WCR resistance. In close collaboration between the USDA-ARS (Drs. Bruce Hibbard and Ken Dashiell), AgReliant, and the University of Illinois, a population of 193 double haploid lines derived from the cross between AR17056-16 and LH51 was produced and tested in three locations in 2008. The combined analysis across locations showed highly significant differences between double haploid lines with respect to their level of WCR resistance. These results are encouraging and back up the usefulness of the planned molecular marker genotyping of the LH51  $\times$  AR17056-16 derived double haploid population. In order to verify the 2008 results, we will reevaluate the tails of the distribution in 2008 (see Fig. 1).



**Fig. 1** Frequency distribution of root damage ratings obtained for 193 double haploid lines derived from the cross LH51  $\times$  AR17056-16 and evaluated in three locations in 2008. The high and low groups will be reevaluated in 2009.

Table 1 Means for 28 maize hybrids developed from crosses between ten S<sub>5</sub> lines derived from GEM breeding crosses and three testers (provided by Pioneer Hibred, Inc.) evaluated for agronomic characteristics and European corn borer resistance in field experiments conducted in Urbana, IL, in 2008.

Genotype	Tester	Trait <sup>1</sup>						
		Index	YLD bu/acre	MST	PHT ----- cm -----	EHT	ECB 1-9	STG 1-5
DKXL212.N11a01-02-5-2-2-11-1-X	Pioneer SS1	90.87	126.73	24.9 0	268.88	138.40	1.89	2.00
DKXL212.N11a01-02-5-2-2-11-1-X	Pioneer SS2	85.20	122.14	23.3 0	305.31	166.81	1.97	2.00
DKXL212.N11a01-02-5-2-6-1-X	Pioneer SS1	5.25	150.57	24.3 5	263.29	131.14	4.22	1.00
DKXL212.N11a01-02-5-2-6-1-X	Pioneer SS2	38.31	171.06	25.1 5	267.22	112.97	3.80	1.00
DKXL212.N11a01-02-5-2-6-1-X	Pioneer SS3	6.41	150.68	17.9 5	264.97	127.05	4.48	1.00
DKXL212.N11a01-02-5-3-2	Pioneer SS2	46.69	134.26	23.5 0	263.34	115.21	3.03	1.00
DKXL212.N11a01-05-1-2-1-X	Pioneer SS1	-28.15	123.56	27.0 0	268.34	133.43	4.79	1.00
DKXL212.N11a01-05-1-2-1-X	Pioneer SS2	-0.45	134.49	26.9 0	316.68	167.49	4.41	1.00
DKXL212.N11a01-05-1-2-1-X	Pioneer SS3	11.16	120.44	27.6 0	291.66	150.24	3.54	1.50
DKXL212.N11a01-05-1-2-2-1-X	Pioneer SS1	-30.06	136.95	26.4 0	258.09	133.17	5.27	1.00
DKXL212.N11a01-05-1-2-2-1-X	Pioneer SS2	38.11	134.49	25.4 0	300.54	164.77	3.46	1.00
DKXL212.N11a01-05-1-2-2-1-X	Pioneer SS3	66.17	135.30	26.1 5	279.41	161.36	2.75	2.00
DKXL212.N11a01-06-1-3-1-X	Pioneer SS1	-46.80	126.09	23.4 0	271.99	143.64	5.47	1.00
DKXL212.N11a01-06-1-3-1-X	Pioneer SS2	45.00	149.83	22.9 5	282.75	160.92	3.73	1.00
DKXL212.N11a01-06-1-3-1-X	Pioneer SS3	-14.77	118.63	18.4 0	245.82	119.53	4.17	1.00
DKXL212.N11a01-09-3-4-2-1-X	Pioneer SS1	90.84	147.41	25.1 5	263.57	121.37	2.41	2.50
DKXL212.N11a01-09-3-4-2-1-X	Pioneer SS3	-/-	-/-	23.7 0	261.07	122.73	2.03	1.00
DKXL212.N11a01-09-3-4-3-1-X	Pioneer SS1	90.09	169.44	21.8 5	263.90	141.13	3.09	1.50
DKXL212.N11a01-09-3-4-3-1-X	Pioneer SS3	10.62	141.88	23.9 5	261.66	128.88	4.06	1.00
DKXL212.N11a01-09-3-4-4-1-X	Pioneer SS1	50.60	114.68	25.7 5	278.29	127.29	2.59	3.00
DKXL212.N11a01-09-3-4-4-1-X	Pioneer SS3	-18.97	135.47	21.0 0	261.68	134.77	4.71	1.00
DKXL212.N11a01-09-3-5-1-X	Pioneer SS1	3.57	150.96	23.2 5	258.37	125.68	4.44	1.00

DKXL212.N11a01-09-3-5-1-X	Pioneer SS3	14.95	130.53	19.9 5	247.50	109.08	3.52	1.00
31P41	Check <sup>2</sup>	113.3 6	165.61	25.4 5	274.99	135.31	2.48	3.00
33D13	Check	50.87	143.61	21.4 5	267.27	132.50	3.41	1.00
33F85	Check	112.2 2	147.63	23.0 8	268.55	124.76	2.04	1.00
33H26	Check	57.17	147.08	23.2 0	261.11	133.10	3.40	1.50
34P89	Check	133.6 3	170.46	21.2 3	268.14	127.81	2.05	1.33
<i>LSD</i>		-/-	28.2	1.6	14.34	14.26	1.4	0.65
<i>C.V.</i>		-/-	8.5	3.29	2.58	5.16	20.9 1	21.89
<i>Repeatability (%)</i>		-/-	87	89	82	81	67	80
<i>Mean (Checks)</i>		-/-	156.61	23.1	268.13	130.42	2.57	1.65
<i>Mean (without Checks)</i>		-/-	137.53	23.8 2	271.49	136.39	3.64	1.33

<sup>1</sup> Index =  $2 * (YLD/YLD_{check} * 100) - (ECB/ECB_{check} * 100)$ ; YLD = Yield; MST = Grain moisture; PHT = Plant height; EHT = Ear height; ECB = European corn borer damage (2<sup>nd</sup> generation), STG = Stay green.

<sup>2</sup> Checks were used as multiple entries.

Table 2 General and specific combining abilities of five S<sub>5</sub> lines derived from GEM breeding crosses for European corn borer (ECB) resistance. Field experiment was conducted in Urbana, IL, in 2008.

Genotype	Tester						Mean (ECB) <sup>1</sup>	GCA
	SS1		SS2		SS3			
	ECB	SCA	ECB	SCA	ECB	SCA		
DKXL212.N11a01-02-5-2-2-11	1.50	-0.70	2.00	0.68	-/-	-/-	1.75	<b>-1.80</b>
DKXL212.N11a01-05-1-2-1-1	4.00	0.22	3.50	0.59	2.50	-0.91	3.33	<b>-0.22</b>
DKXL212.N11a01-05-1-2-2-1	4.50	0.88	3.00	0.26	2.00	-1.24	3.17	<b>-0.38</b>
DKXL212.N11a01-06-1-3-1-1	5.50	0.05	4.00	-0.58	5.50	0.43	5.00	<b>1.45</b>
DKXL212.N11a01-09-3-5-1-1	4.50	-0.45	-/-	-/-	4.50	-0.08	4.50	<b>0.95</b>
Mean(ECB) <sup>2</sup> / GCA	4.00	<b>0.45</b>	3.13	<b>-0.43</b>	3.63	<b>0.08</b>		

<sup>1</sup> ECB row means are significantly different with P < 0.001.

<sup>2</sup> ECB column means are not significantly different from each other.