Friday, December 19, 2014

**Final Report I:**

*Advancing Lines, Hybrids and QTL for Yield and Adaptation towards Commercialization*

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**Project Overview**

The overall goals of this project were to: further test and increase seed of the best Texas breeding material which will lead to increased yield and decreased stress losses for Texas producers; to advance new populations to derive the next generation of elite inbreds and hybrids; to develop a set of populations to validate three DNA genetic markers for dramatic yield increases; and to further improve the resources and reputation of Texas corn. Overall it was a tremendously successful summer nursery season with record breaking amounts of seed produced, a very successful summer yield trial season, and a fair to poor winter nursery season. Based on the last few and the upcoming year we will have data to support release decisions of 15 to 25 inbreds, many as inbreds for commercial production and a few as germplasm inbreds.

*Major accomplishments* were plentiful this year. In addition to plentiful seed production and data to support inbred release, we secured two inbred evaluation agreements with a major US multinational and a Turkish company*; others are in the works. This past year, nine graduate students, three visiting professors, and five undergraduates directly worked in the corn breeding program. Two PhD students have taken US industry Corn Breeder jobs in the last two years. We participated and had leadership roles in the Genomes to Fields (G2F) project, initiated by the Iowa Corn Board / National Corn Growers, which is a joint effort by corn researchers throughout the country to identify genes that will improve adaptation. Five peer reviewed publications were published for work supported in part by Texas Corn Producers funding last year. Many high profile presentations were given to the corn research community including Iowa State University, the Donald Danforth Center and the National Association of Plant Breeders. Official inbred line releases are forthcoming.

We thank the Texas Corn Producers Board for supporting this valuable research and want to emphasize that support is critical for the continued maintenance of breeding goals and capacity of the program. This capacity allows us to be competitive in attracting additional resources from federal and state agencies which overall furthers proposed corn research, Texas corn publicity, and gives Texas a seat at the table in future decisions.

*The Turkish company is synergistic and important because they have a climate nearly identical to Texas, they consume more corn then they produce and are a small market (non-competitive with Texas growers), they have a Texas A&M graduate as a research director, they have additional financial and germplasm resources that we can leverage for improving Texas corn, and through them we may have new options to commercialize our lines in the US in the next five to ten years; although we have begun the process for a joint breeding program, the agreement states that all Texas A&M derived material is under our control.

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***Objective 1:*** *Test specific TAMU hybrid combinations that have already been identified as most successful in additional environments and test new hybrid combinations of the best lines; work with industry to develop a path towards rapid commercialization.*

Seed production was good in 2013 and we planted many of our most previously successful hybrids and some new hybrids in a number of locations. Focus made on expanded testing locations of the best hybrids will allow these most outstanding hybrids to be most quickly made available for growers. All location trials in South and Central Texas were better overall than most recent years, owing to adequate rainfall and more temperate weather. Among the most consistent and valuable test data this year was from Charles Rings San Patricio farm with 68% of the variation due to the genetic differences between hybrids, this was a dryland test which helped us to determine which hybrids are better under stress, the only other true dryland test that had some drought stress was Thrall which was also very good (48% due to genetics, 24% due to error, 23% due to a row effect, which suggests some differences in fertilization or weed management). The least consistent test this year was our large Weslaco test (where the genetic variation was ~ 27% of the total and error variation was 21% of the total, range and row effects were ~12% of the total variation) this suggests that there was a lot of variation throughout the field, and/ or combine issues were present; our smaller Weslaco test seemed to avoid these issues and was much better. All College Station tests (large, small, dryland, irrigated) were very good with 42- 75% of the variation due to genetics and 24-48% due to random error.

Although preliminary data analysis is complete we have not had time to review all issues and finalize the analysis, however we can make a number of determinations at this time. The best performing hybrids this year were in large part the same to the previous years, when those hybrids had been tested before. Most hybrids seem to be specialized to irrigated and or dryland locations, irrigated locations generally ranked hybrids similarly and dryland locations generally ranked hybrids similarly. The genotypes that appeared to be outstanding and under both irrigated and dryland conditions across years were hybrids derived from TAMU inbred lines: 'Tx149', 'Tx742', 'Tx775', 'Tx777', 'Tx780' and Checks: DKB64-69, REV28HR20, BH8928VTTP, BH8732VTTP, GA28V81. Overall this preliminary analysis suggests that we have been betting on the right lines as Tx777 hybrids have performed remarkably well under irrigation on a multiple testers including TR8145 and LH195 and very well in most, but not all, dryland locations on SGI890. A few of our lines, notably Tx775 and Tx780 did very well in dryland locations, including on Mr. Charles Ring's San Patricio County farm. Unfortunately, there were three instances of using the non- Roundup Ready version of a hybrid rather than the Roundup Ready version on Mr. Ring's farm and these plots were lost, we did not see this mistake repeated elsewhere.

Overall, in order of planting dates- **Weslaco (Rio Farms):** A 152 entry two row, two replicate test; a 92 entry one row, two replicate test and a 32 entry one row, three replicate test were planted at Rio Farms. **Weslaco (AgriLife):** A 50 entry two row, two replicate test was planted for Dr. Xu. Additionally a 45 entry one row three replicate test of inbreds sourced from collaborators was planted and inoculated as part of an AMCOE project. **BH Genetics**: Two tests were planted by BH genetics: 1) a 50 entry two row, two replicate test in Ganado, 2) and 8 of our best entries were planted in ten locations across the state. These are some of our best opportunities to expand commercial interest in TAMU material. **College Station:** Too many tests were planted in college station to list all of them, a partial list will be given. In field #1 a 154 entry two row, two replicate test and a 60 entry one row, one replicate test were planted and designated as dry land tests. However, due to timely rains the test looks to be outstanding and could be better than some irrigated tests. In field #2, a 193 entry two row, two replicate test, a 60 entry one row, two replicate test were designated as irrigated and planted at the same time; while looking better than last year in this field, the field does not have as good of yield potential as others. Additionally a 160 entry two row, two rep; and a 56 entry and 87 entry one row two rep test were planted of new hybrids. Later plantings in this field including a 49 entry, two row, two replicate test; a 50 entry, two row, two replicate test for Dr. Xu. There are 8 other medium and large tests that were also planted but are mostly for other funded projects. **San Patricio County:** A 60 entry two row two replicate test of Roundup Ready hybrids was planted on Charles Ring's Farm by Dennis Pietsch. However seed on three entries turned out to be non-Roundup Ready (but otherwise the same hybrid as expected) and were killed. This likely occurred from the commercial tester without Roundup Ready being used and was noted incorrectly during processing. **Thrall:** A 71 entry two row two replicate test was planted on Stiles Farm in Thrall cooperatively with Dennis Pietsch. Also a 40 entry demonstration planting was included for field day. **Halfway:** A 50 entry two row two replicate test was planted by Dr. Wenwei Xu.

The summer nursery was successfully harvested and the seed yields were unbelievably high, which is owed to committed and eager students and excellent weather. In total 5136 bags of seed have been shelled and the quantities are much larger than previous years. It appears for the first time we will have more seed than we can test next year for a number of established and excellent hybrids. Most notably, an isolation block with Tx777 as a male produced 26 lbs to 55 lbs on each of 12 different lines including the ones that did so well in this year's trials (TR8145, LH195, SGI890) but also on four new inbred testers from Greenleaf Genetics. This quantity should allow much more extensive testing, which in turn will lead to much more rapid adaptation and licensing by companies. The other hybrids and seed increases were also very successful.

Our Weslaco winter nursery was planted slightly later than normal (Aug 8$^{th}$ and 15$^{th}$) because of the later maturity of our summer nursery. Unfortunately 11" of rain during early plant establishment ruined much of the crossing block, and destroyed our isolation block, so few additional hybrids were obtained. However, many students' projects (in other objectives and for grants other than TCPB) were in a higher part of the field and we did have moderate production which we finished harvesting 12/18/2014.

***Objective 2:*** *Develop and select breeding lines that combine TAMU proprietary best by best genetics with improved seed production capabilities for the Midwest.*

While we are now confident in the best performing TAMU lines (which companies are evaluating and we are pursuing commercialization for) we need to begin to capitalize on this and recycle this elite material by making elite x elite crosses. The hope is that these 'elite x elite' crosses will generate superior yielding inbred lines and furthermore that these new line will be earlier in flowering time to better fit Midwest hybrid seed production systems. This is important because the Midwest is where most Texas hybrid seed is produced; this will ultimately lead to better commercial application of our lines. All elite parent lines already combine well with numerous commercial testers for yield as well as for disease and aflatoxin resistance. 17 segregating $F_2$ populations were planted in our WE13 winter nursery (previous year) from TAMU x TAMU elite crosses and TAMU x ex-PVP (commercial Midwestern lines) and we selfed and selected the earliest and best plants. 190 $F_3$ plots from 6 of these populations were planted and selfed in our College Station summer nursery (CS14). By

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advancing the best ear, and in some cases also advancing the best second or third ear, 272 $F_{2:4}$ plots from these 6 populations were then planted and selfed in our Weslaco winter nursery (WE14), these have been harvested but have not yet been shelled.

**TABLE 1: Crosses for Objectives 2A &2B** $F_1$ hybrids were made in the Weslaco 2012 Fall nursery (WE12), selfed to $F_{1:2}$ in the College Station 2013 Summer nursery (CS13), selfed to $F_{2:3}$ in WE13, selfed to $F_{2:4}$ in CS14 and in Delaware 2014, selfed to $F_{2:5}$ in WE14. These lines will be testcrossed and tested in 2015.

| **Cross** | **Objectives # & Reason(s)** | **Planted $F_{2:4}$ ear to row plots CS14** | **Planted $F_{2:5}$ ear to row plots WE14** |
|---|---|---|---|
| ***1:*** (Tx775 **X** LH82)-B6 | 2nd best yellow NSS, select earliest from LH82 ex-PVP | 37 (1575 seed) | 37 (629 seed) |
| ***2:*** (LH195 **X** Tx773)-B3 | Top 10 yellow select earliest from LH195 SS ex-PVP | 25 (1125 seed) | 32 (544 seed) |
| ***3:*** (Tx777 **X** Tx775)-B5 | $1^{st}$ and 2nd best yellow NSS TAMU lines select earliest | 47 (2115 seed) | 68 (1156 seed) |
| ***4:*** (Tx777 **X** Tx151)-B5 | Best yellow and white TAMU lines, select earliest yellow | 28 (1260 seed) | 42 (714 seed) |
| ***5:*** ((((B104/NC300)x(CML 415/B104))-4-2-B-B-B/LAMA2002-22-3-B-B1)-B-B-B-B-B **X** Tx775)-B4 | Combination of different NSS TAMU line types select earliest | 22 (990 seed) | 31 (527 seed) |
| ***6:*** ( Tx779 **X** Tx780)-B6 | Best dryland TAMU lines, select NSS earliest yellow | 31 (1395 seed) | 50 (850 seed) |

**Additional intermated population now being selfed for inbreds (along with AMCOE and USDA objectives), shown for comparison with what was done in Delaware (Table 2 below).**

| **Cross** | **Objectives # & Reason(s)** | **Planted plots CS14** | **Planted plots WE14** |
|---|---|---|---|
| (Tx740 x Tx772 x Mp715 x Mp313E) - #-# | Early flowering, aflatoxin resistance and yield (supported by AMCOE & USDA) | 1035 kernels, made pooled pollen sibs with Pop#1b | 1904 kernels, mainly selfed |
| (Tx772 x Mp715 x Tx740 x Mp313E) - #-# | Early flowering, Aflatoxin resistance and yield (supported by AMCOE & USDA) | 1035 kernels, made pooled pollen sibs with Pop#1a | 1904 kernels, mainly selfed |

In College Station we know that we cannot separate earliness from photoperiod sensitivity. In other words we can select lines that will flower earlier than the parents, but that could still be unpredictably late in the more Northern US where hybrid seed is produced. In a very large USDA-NIFA project on adapting maize to a

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changing climate, we are part of (PI: Dr. Randy Wisser, University of Delaware) we reciprocally exchange material locally selected from a single tropical population with eight other locations in the US. From recent preliminary results of this study, we have now seen that Central Texas gives us almost no ability to select against unpredictable flowering, where nearly all other US breeding programs can. Even before we had seen these results we had proposed for TCPB to support Dr. Wisser to select from our breeding material in Delaware (this is complete different from the research population we use in the USDA grant). So in addition to what was done in College Station in summer 2014 and advanced in Weslaco in winter 2014, some of the same and similar $F_3$ populations were planted by Dr. Wisser in Delaware and selections and self-pollinations were made on the earliest ~10% to 20% of plants (Table 2). The goal of this was simply to select material that is earlier in a location that can avoid photoperiod sensitivity for Midwest hybrid seed production.

Both the best material selfed in Weslaco (Table 1) and the lines selected by Dr. Wisser in Delaware (Table 2) will be planted to make hybrid testcrosses in 2015, therefore we will collect our first yield data to see how successful this was in 2016.

**TABLE 2**: Populations planted and selected for earliness in Delaware 2014.

| **Population Planted** | **Seed planted** | **Plants Selected** | **Selection intensity%** |
|---|---|---|---|
| ***1:*** (Tx775 **X** LH82)-B6 | 237 | 35 ears | 15% |
| ***2:*** (LH195 **X** Tx773)-B3 | 225 | 45 ears | 20% |
| ***3:*** (Tx777 **X** Tx775)-B5 | 224 | 22 ears | 10% |
| ***4:*** (Tx777 **X** Tx151)-B5 | 220 | 27 ears | 12% |
| ***5:*** ((((B104/NC300)x(CML 415/B104))-4-2-B-B-B/LAMA2002-22-3-B-B1)-B-B-B-B-B **X** Tx775)-B4 | 229 | 28 ears | 12% |
| ***6:*** ( Tx779 **X** Tx780)-B6 | 840 | 69 ears (from 21 of 43 plots) | 8% |
| (Tx740 x Tx772 x Mp715 x Mp313E) - #-# | 788 | 81 ears, made pooled pollen sibs with Pop#1 | 10% |
| (Tx772 x Mp715 x Tx740 x Mp313E) - #-# | 759 | 89 ears, made pooled pollen sibs with Pop#2 | 12% |

***<u>Objective 3:</u>*** *Advance and test populations for important quantitative trait loci (QTL) conditioning yield under Texas conditions.*

We previously identified three genetic markers (quantitative trait loci – QTLs) that each increased hybrid yields by ~5-9 bushels per acre under both dryland and irrigated conditions in a 400 line association mapping diversity panel that was crossed to Tx714 (Barerro et al. accepted). These QTL appear to be segregating in tropical, TAMU breeding and ex-PVP material and if validated they have the potential to increase yield 15-27 bu/ac. Because of high diversity between lines we needed to use a complex statistical analysis, and now must validate that the QTL are real and not statistical anomalies. We created three populations and advanced 400 $F_{2:3}$ lines in summer nursery and planted 527 $F_{2:4}$ lines in winter nursery (Table 3). Parents were chosen because they segregated for the QTL, we already had the crosses in hand (saving a year), and they were likely to also result in new, high-yielding breeding lines.

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These single ear selections from CS14 were planted ear to row and genotyped for the QTL in the WE14 winter nursery. We were successful making testcrosses with the Tx714 tester (95% identical to B73, but selected in TX) in the winter nursery, allowing hybrid validation this summer (2015) on the many that have enough seed. The student in charge of this project, Ms. Yuanyuan Chen, has now successfully developed rapid and successful Kbioscience KASPar SNP genetic markers and scored them in these populations. We measured average plant height, flag leaf height and ear height in the WE14 winter nursery. This was done because these QTL were also significant for plant height in the Barerro et al. study so we could get a preliminary look at the results, and because of Ms. Chen's graduation timeline. The preliminary results (Table 4) are very good and suggest that we will be successful confirming all of these SNPs in at least one population.

**TABLE 3:** Populations planted for validating yield QTL presence in CS14 and WE14.

| **Cross** | **Objectives # & Reason(s)** | **Planted $F_{2:4}$ plots CS14** | **Planted $F_{2:5}$ plots WE14** |
|---|---|---|---|
| ***1:*** (Tx740/NC356)-18 | TAMU line & segregates for two markers | **55 (1925 seed)** | **110 (1870 seed)** |
| ***2:*** (Ki3/NC356)-B7 | Segregates for all three genetic markers | **167 (5845 seed)** | **239 (4063 seed)** |
| ***3:*** (LH82//(LAMA2002-12-1-B-B-B-B/LAMA2002-1-5-B-B-B-B)-3-2-B-1-B3-B)-B5 | TAMU line & ex-PVP LH82 - this may segregate for two of the markers | **178 (6230 seed)** | **178 (3026 seed)** |

**TABLE 4:** Results of three QTL (SNPs) effects on plant and ear height based on phenotypes measured in WE14 winter nursery.
These are very good results considering we used:

1) An off season nursery
2) Inbred lines per se and not hybrids
3) Plant height and not yield

This limitations will be at least partially overcome in 2015 trials.

**QTL1 (SNP1):** Was significant for plant height at **P < 0.01** across only population 2 in Table 3.

| **Genotype** | **LS Means (inches)** | **Number of individuals** |
|---|---|---|
| X:X | 62.5 | 56 |
| X:Y | 61.2 | 64 |
| Y:Y | 59.2 | 86 |
| | | 206 (33 missing) |

**QTL2 (SNP2):** Was significant for ear height at **P < 0.03** across all three populations in Table 3.

| **Genotype** | **LS Means (inches)** | **Number of individuals** |
|---|---|---|
| X:X | 22.5 | 156 |
| X:Y | 21.5 | 144 |
| Y:Y | 21.2 | 182 |
| | | 482 (45 missing) |

**QTL3 (SNP3):** Was significant for plant height at **P < 0.003** across only population 2 in Table 3.

| **Genotype** | **LS Means (inches)** | **Number of individuals** |
|---|---|---|
| X:X | 59.0 | 70 |
| X:Y | 63.2 | 60 |
| Y:Y | 61.4 | 87 |
| | | 217 (22 missing) |

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***Objective 4:*** *Train students and participate in cooperative tests.*

This summer, nine graduate students, and five undergraduates and three visiting professors have worked in the TAMU corn breeding program. Two PhD students have taken US industry Corn Breeder jobs in the last two years. Furthermore, many of the peer reviewed publications were components of the graduate students theses and dissertations and had the students as the first authors.

Multiple cooperative tests were planted, mostly in College Station, Weslaco and Halfway some are under other grants and proposals but TCPB support has been critical to help maintain people and resources to perform these trials.Most notably, the G2F (Genotypes to Field) test.

The G2F project was initiated in mid-2013 by the Iowa and National Corn Boards to translate the maize genome sequence into practical discoveries; how genes affect important traits. These discoveries will result as gains in producer fields through targeted adaptive selection of gene variants. In 2014 each of 23 locations (Figure 1) grew 250 hybrids in 2 replications each (500 plots) and 17 locations grew a separate set of 31 inbred lines in multiple replications. These hybrids included 240 in overlapping sets of inbreds which allowed north and south movement through the use of different exPVP testers plus a common set of 10 checks grown in all locations. All inbreds used in the study have been genotyped with > 1 million genetic markers. Additionally, each location had a weather station measuring air and soil temperatures, humidity, wind speed, solar radiation, and precipitation. We measured yield, moisture, testweight, stand counts, lodging, male and female flowering, plant and ear heights. Through the combination of the weather and trait measurements across locations, the goal is to identify sensitive indicator traits across many environments. Combining these findings with the genetic markers will identify important gene variants for yield and adaptation. A surprising result of this test is the identification of a hybrid from older public lines that handily beat the best commercial check for yield in both replications. Caution is required since it was only a two replicate test but it is an already exciting outcome of this large experiment.

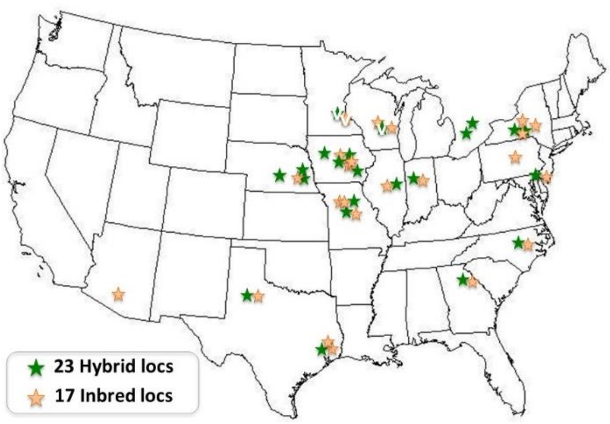


Figure 1: G2F project locations for 2014. Figure courtesy of Dr. Natalia de Leon

The TAMU program was instrumental in producing G2F hybrid testcross seed in our Weslaco winter and College Station summer nurseries. In 2013 Weslaco two separate isolations were grown with LH195 and PB80 as exPVP tester inbreds with approximately 400 inbreds submitted by research programs across the country. The hybrid ears from these isolation blocks were shelled, treated, packaged and sent to three locations for yield trials: Lubbock, TX; Tifton, GA; Raligh, NC; as well as being planted in College Station. Hybrids with additional sufficient seed were sent to Ames, IA and distributed to some of the other 19 locations. In 2014, College Station had one isolation of LH195 with 280 submitted inbreds. This isolation was very successful and we have seed to share with many locations in the upcoming year.

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The G2F project will enhance the ability to predict corn performance and understanding of biology for corn improvement. Additionally, it is networking a broad spectrum of maize researchers throughout the country; developing a public-private partnership; and training programs on how to collect, share, and interpret "Big Data." Importantly, this project places Texas as a player in larger corn breeding research projects and decisions. Our participation ensures relevance of findings and future collaborations to Texas and the Southern US.

Some of the major additional co-operative projects this year included:

- Under our AMCOE proposal four multi-investigator hybrid and inbred yield and aflatoxin trials were planted in College Station and Weslaco and inoculated. In one of these hybrid tests, the Southeast Regional Aflatoxin Trials (SERAT), the same TAMU hybrids proposed for release were also the top yielders in both College Station and North Carolina (Table 3 - not all data has been compiled from all locations yet).
- AMCOE has led to a large multi-state USDA-NIFA aflatoxin project that we first planted this year to examine the interaction between genetic resistance and atoxigenic strains.
- Additional statewide trials were conducted with our second best material by BH Genetics, as we did not have enough seed for our top hybrids. These performed in the middle bottom of the commercial material overall while they were at the top of some tests.
- As explained previously, a very large cooperative test across 8 locations in the US was planted as part of our ATLAS NIFA project http://www.maizeatlas.org/ led by Dr. Randy Wisser.

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