

2008 NCCC-167 Annual Report  
North Central Corn Breeding Coordinating Committee

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Annual Report prepared by Sherry Flint-Garcia, USDA-ARS Columbia, MO.

## Meeting Program

**2009 NCCC-167 Meeting  
Allerton Conference Center, Monticello Illinois  
March 10-12, 2009**

### **Tuesday, March 10**

3:00 PM-6:00 PM Registration

6:00 PM Dinner

7:30 PM Tom Brutnell, Cornell University – Genetic mechanisms of shade avoidance

8:30 PM Discussion

### **Wednesday, March 11**

7:00 AM- 8:30 AM - Breakfast

Research presentations: Genetics and Development

The Genetic Architecture of Vegetative Phase Change  
Candice Hansey, Eric Riedeman, Rajan Sekhon, William Tracy, Natalia de Leon,  
and Shawn Kaepler\*

Variation for compositional attributes of maize plant parts across hybrids and  
associations between plant development stages  
Candice Hansey\*, Aaron Lorenz, Natalia de Leon

Quantitative Genetics at the Molecular Level: A Root Metabolomics Case Study  
Andrew Hauck\* and Martin Bohn

10:00-10:30 AM -Break

Research presentations: Breeding for agronomic traits

Current Trends in Corn Production and the Seed Corn Industry in China  
Dave Mies\*

Breeding maize for enhanced silage quality: aspects of stover and grain  
improvement  
Gustafson, T.J.\*, J.G. Coors, and N. de Leon.

12:00 PM -2:00 PM Lunch, Individual meetings and discussion

Research presentations: New Resources

The Grin – Global project  
Candy Gardner\*

Development and Characterization of Maize-Teosinte Introgression Libraries  
Sherry Flint-Garcia\*, Christopher Bottoms and Mike McMullen

3:00-3:30 Break

Research presentations: Grain Quality

Translating Biochemistry to Breeding for High Carotenoid Traits in Maize  
Catherine Kandianis\* and Torbert Rocheford

Breeding High-Methionine Corn for organic poultry producers  
Walter Goldstein\*

Populations selected for grain methionine content exhibit altered allelic  
composition at loci involved in methionine biosynthesis  
Adrienne Moran Lauter, Nevzat Aydin and Paul Scott\*

Breeding for Desirable Thinner Pericarp Thickness and Favorable Ear Traits in  
Fresh Market Waxy Corn Germplasm  
Eunsoo Choe, Department of Crop Sciences, University of Illinois

6:00 Dinner

Research presentations: Yield components

Breeding, Genetic, and Genomic Approaches to Improve Yield and Starch  
Concentration in Maize  
Sofia Silva, Department of Crop Sciences, University of Illinois

Pollen Shed Delay  
Forrest Troyer\*

Is Harvest Index Related to Maize Productivity?  
Aaron J. Lorenz, Timothy J. Gustafson, James G. Coors and Natalia de Leon\*

**Thursday, March 12**

7:00 AM-8:30 – Breakfast

8:30 – 10:00 AM – NCCC-167 Business meeting

10:00 AM Break, check out, adjourn

12:00 Lunch

\* Indicates presenting author

## Meeting Attendance

### Researchers/Faculty/Staff

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Tom Brutnell	Cornell Univ.	tpb8@CORNELL.EDU
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## Presentation Abstracts

### The Genetic Architecture of Vegetative Phase Change

Candice Hansey, Eric Riedeman, Rajan Sekhon, William Tracy, Natalia de Leon, and Shawn Kaepler\*

University of Wisconsin- Madison (\* = presenting author)

Juvenile to adult transition, termed vegetative phase change, is a trait with substantial variability in maize. Juvenile vegetative tissues have a 1  $\mu\text{m}$  cuticle, epicuticular wax and aerial roots, lack of epidermal hairs and bulliform cells, and have lateral buds that can form tillers. Adult vegetative tissues, in contrast, lack epicuticular wax and aerial roots, and have a 3  $\mu\text{m}$  cuticle, epidermal hairs, bulliform cells, and lateral buds that are either absent or can form ears. The variable proportion of the plant that is juvenile has implications for traits of commercial interest. For example, juvenile leaves are more digestible by ruminant animals, and more susceptible to certain insect and disease pests. Timing of the juvenile to adult transition is heritable and varies substantially in maize populations and among maize varieties. We have characterized the timing of juvenile to adult transition in approximately 4000 lines of the Buckler Nested Association Mapping population and in a collection of inbred lines. Substantial variation was identified in this material with the earliest transitioning genotype having the fourth leaf as the first adult leaf and the latest transitioning genotype having the 14<sup>th</sup> leaf as the first adult leaf. While there was some correlation between maturity, leaf number, and transition leaf, there are QTL that could clearly be identified as controlling timing of transition independent of maturity and leaf number. Information on quantitative trait loci and correlations with stover quality will be presented.

## Variation for compositional attributes of maize plant parts across hybrids and associations between plant development stages

Candice Hansey\*, Aaron Lorenz, Natalia de Leon

University of Wisconsin- Madison (\* = presenting author)

The quality of maize stover for use as a biofuels feedstock could be improved through selective breeding and genetic manipulation. Understanding the genetic relationship of stover composition between different plant parts and plant developmental stages would facilitate the genetic improvement of stover quality. Our objectives were to determine the relative contribution of individual plant parts to whole plant yield and composition as well as assess the relationship of plant part composition between different stages of development with whole plant yield and quality at physiological maturity. An experiment was conducted to evaluate the compositional attributes of leaf blade, leaf sheath, stalk, cob, midrib and husk fractions of 23 hybrids. Twenty of the hybrids were derived from a factorial mating design between a set of high-quality inbred lines (W601S, W602S, W603S, W604S and W605S) and four standard inbred testers (HC33, LH244, LH332 and TR725). A brown-midrib hybrid, a Leafy hybrid, and a commercial grain hybrid were also evaluated. Plants were harvested and dissected into the aforementioned parts at four developmental stages corresponding to V3, V12, R3 and R6. Tissues were dried and ground for compositional analysis, which included neutral (NDF) and acid detergent fiber (ADF) and in vitro ruminal digestibility. In addition, dry matter weights for each component were taken at the R6 stage. Evaluations were conducted at two locations on field plots arranged in a randomized complete block design with two replications. Preliminary results for NDF and ADF indicate significant differences among hybrids for most plant parts. Stalk was the largest fraction of whole plant dry matter at R6 and stalk ADF was associated with whole plant ADF at R6. For stalk ADF, no association was found between early developmental stages (V3 and V12) and R6, suggesting that phenotyping for quality at early stages of development is not indicative of quality at R6. There are significant plant part by entry and plant part by location interactions at R6. This indicates that the relationship between quality of specific plant parts or developmental stage and quality of whole plants at physiological maturity will change based on the hybrid being evaluated and the growing environment.

## **Introduction and Background**

Over the last several years, a number of plant studies have utilized high throughput analysis methods, such as expression profiling, protein quantization, and metabolic profiling, on plants to obtain large data sets of molecular phenotypes on a variety of genetic backgrounds (see Young and Udvardi, 2008). For example, metabolic profiling has been used to observe the effects of mutated genes (Feihn et al., 2000) and genetically map quantitative metabolite traits in a population of introgression lines (Schauer et al., 2006). Despite such prevalence and interest in these approaches, there has been relatively little work to reconcile the kind of data obtained with these methods to plant breeding theory. There are many examples of breeding for traits identified with a rapid assay, such as spectroscopy or UV-chromatography, from the last several decades, but adaptation of the newer profiling approaches has not been intuitive. The molecular traits that should be improved are not initially defined, as they are generally not the desired end product themselves. In other words, the final overall desired phenotypes are known, but the quantitative molecular phenotypes that contribute to them are not, requiring some association between them to be made. The compensating advantage of profiling techniques is the ability to accurately determine the abundance and identity of a large variety of molecules, on the order of one hundred to tens of thousands, depending on the methods and molecules. The challenge is to discover ways to coherently interpret and apply these kinds of data, which are now a highly available resource. This will require basic research into the range and conditions of molecular variation, nature of inheritance, and their relationships to the traditional agronomic traits of interest.

About two decades ago the introduction of inexpensive genetic markers had a profound impact on the methods of plant breeding. Similar improvements in DNA chips, sequencing, and mass spectrometry could represent another fundamental advance. This possibility derives from the understanding that a plant's reproductive productivity depends on a multitude of prior biochemical and physiological responses to environmental conditions and the feasibility of gathering such information. Indeed, the capability to acquire tens to thousands of molecular phenotypes per genetic background, tissue, developmental time point, and environment combination provides a mind boggling number of potential options for collecting data to select on. Obviously, many challenges remain before widespread practical implementation of "omics" based breeding can proceed. Critically, we must determine how omics phenotypes answer the fundamental questions of plant breeding. These questions include: "What are the molecular traits that should be selected for, their variance in my populations, and the accuracy of measurements of them?", "Is there suitable variation to perform selection on and are the genetic effects inherited in a familiar way?", and "How does the trait under selection influence other traits of interest?" Simple answers for all of these straightforward questions are elusive with omics data. The kinds of relationships that exist between such information and primary traits need to be investigated for a variety of conditions. Conventional analyses appear less suited to the nature of

this kind of multivariate data, which has substantial correlation and can change rapidly in response to various stimuli, including the process of sampling and tissue preparation. Moreover, the interrelationship between variables, especially with metabolites, implies that selection on any one of them may result in simultaneous changes to others, influence the steady state or dynamic properties of the system as a whole, and introduce unknown risks of undesirable behavior. In the case of improving metabolic systems, simultaneous selection of many molecular traits towards rationally chosen goals determined from a comprehensive understanding of network relationships might not be just an ideal, but rather a requirement for functional application. Since the relationships between genetic backgrounds and metabolome characteristics are essentially unexplored in maize, we decided to perform a simple experiment to investigate these issues. We compared vigorous hybrids with their less vigorous inbred parents to begin identifying distinguishing features between more productive and less productive metabolic networks and pursue answers to the fundamental questions of plant breeding for profiled molecular traits..

## **Experimental Design and Methods**

A half diallel design plus a set of two inbreds and their reciprocal hybrids were employed to investigate the relationship between genetic background and root metabolic profiles. The inbred parents include flint (3), dent (2), and iodent (1) inbreds, resulting in combinations of inter- and intrapool hybrids (Figure 1). Seeds from the eight hybrids and six parental inbreds were germinated *in vitro* using germination paper rolls. Five seeds per entry were placed on individual pieces of germination paper and rolled up. The fourteen rolls per experimental replication were divided equally between two 2.5 L containers filled with 750 ml distilled water and 20ml of a 2.5g per L Captan solution, which was also used to pre-moisten the rolls. All of the containers were simultaneously incubated at 28 degrees Celsius and 100% relative humidity for 8 days in the absence of light. The experimental design was an incomplete block design with three replications. The germination paper rolls represented plots and the two containers per replication were regarded as blocks. Two pairs of seedling roots were harvested into liquid nitrogen and ground, generating two bulked samples per germination paper roll, for all replications on the same day, with the goal of acquiring a total of six biological replications per entry.

Metabolites were extracted from freeze dried root tissue by incubating 15mg in 1.5ml 80% methanol overnight at 4 degrees Celsius. The methanol solution was removed, filtered, and dried down, and the samples were submitted to the Roy J. Carver Metabolomics Center at the University of Illinois at Urbana-Champaign for derivitization and Gas Chromatography - Mass Spectrometry (GC-MS). Relative levels of 159 confidently identified metabolites were provided for each of the 79 samples submitted. Metabolite abundances were reported in relative terms, rather than absolute, due to the unavailability of quantitative standards for all detected molecules. Relative levels are calculated by comparing the detected signal intensity of a chemical standard with the intensities of identified metabolites. Metabolites related to a wide variety of metabolic pathways were identified (Table 1), but metabolism involving sugars or amino acids is particularly well represented.

## Results and Discussion

The shotgun metabolomic approach employed provides a snapshot of observed metabolites at the time of tissue harvest, not experimentally determined flux through pathways. It should also be noted that the estimates are valid only for the entry, time point, tissue, and environmental conditions sampled, which are quite limited in scope in our experiment. However, the goal for this project was not to make future predictions about metabolite levels, but to determine the nature and proportion of variation in metabolite abundances that is attributable to the genetic composition of entries. The metabolite profiles of entries often displayed cases of both consistency and sharp variation (Table 2). In the second case, instances of reproducible variation for a metabolite could generally not be partitioned to effects from the paper rolls (plot), container (block), or replicate of the experiment (environment). This feature of strikingly increased or decreased abundances for certain metabolites in a subset of replicates was observed for all entries. Since we do not have a statistical measure at this time to identify these cases strictly, our working definition of this feature encompasses instances where two or more replicates of an entry with different environments have average differences with the other replicates in the group of approximately 1.5 fold for highly abundant metabolites or 2 or 3 fold for the least abundant ones. Repeated instances of this behavior for a particular metabolite can be correlated with similar effects at other metabolites, though the correlations can be entry dependent. These metabolomic perturbations could result from some post-harvest effect, although it is interesting to note that all samples show some amount of this behavior for some subset of metabolites.

Comparing results of inbreds with hybrids also indicates the role of factors unconsidered at the onset of the experiment. For example, many of the hybrids have levels of core metabolites below the detection threshold, while the inbred lines have abundant amounts. This might be explained by noting the differential growth and development rates between inbreds and hybrids. Although both were harvested at the same time, the hybrid entries had greater lateral root quantity and length, by visual inspection, and may be significantly more advanced developmentally, even at this early stage, resulting in a greater consumption of initial seed resources. This distinction makes it more difficult to compare inbreds and hybrids metabolically without a time series analysis.

The structure of the root metabolite data set is typical of similar current high-throughput approaches to biology, containing information on a large number of correlated variables and a fewer number of observations. These factors preclude most multivariate analysis methods typically used on phenotypic data. Additionally, univariate methods are unattractive since metabolic phenotypes at the molecular level can change rapidly, information on co-variation with other metabolites provides more confident estimates, and results from shotgun style studies lend themselves to interpreting results of biological interest in terms of network pathways. To this end, we endeavored to evaluate some alternative statistical methods to see which ones may be more informative.

One common method for dealing with high dimensional data with few observations, in applications such as Near Infrared Spectroscopy, is called Partial Least Squares or Projection to Latent Structures (PLS). PLS is implemented with an iterative algorithm involving matrix decomposition that extracts summarizing factors, like the more familiar Principal Components method, which maximize the covariance between the independent and dependant variables. For

each iteration, variance is partitioned into a factor and removed, leaving residuals that are passed for the next round of partitioning to use. If the information explained by a factor fails a certain threshold, it is discarded and the process concludes. Each resulting factor is orthogonal, having no overlap of information with other factors, and assigns a value to every Y group that is a weighting of many X variables. The total Y variation that the factors explain combined is reported as R-square.

We can perform a discriminate form of this analysis with entry as a qualitative Y trait and plot several of the factors to see if the information contained in the metabolite data is sufficient to distinguish the different genetic backgrounds represented in the diallel. Initial results obtained using SAS proc PLS showed separate clusters of replicates for most entries when plotted with three factors, so enhanced methods were sought. The metabolite data was next processed using SIMCA-P software and an Orthogonal PLS Discriminate model (OPLS-DA). Orthogonal PLS has the additional feature of compartmentalizing variation in the X data that is unrelated to the Y. With this method, a portion of variation from environmental effects and noise is removed to improve the quality of the factors and facilitate their interpretation (Trygg and Wold, 2002). With this approach, 13 factors and 2 orthogonal components were identified. Investigating the factors revealed that each one primarily discriminated several of the lines, thus plotting only three of the factors in three dimensional space may not fully resolve all fourteen of the entries into independent clusters. Nevertheless, many of the factors provide good separation of most of the entries (Figure 2). For the purposes of plant breeding, such an approach might be useful for identifying a more functional or predictive form of diversity than genetic differences.

Another suitable approach for our data involves estimation of the partial correlations between all of the metabolites. When attempting to determine the statistical association between two variables, partial correlations has the advantage, compared with correlation, of removing the influencing effects from other variables. A conventional implementation of partial correlation analysis will not work with data with fewer observations than variables, but alternative means are available. A package for R, Genenet, uses a shrinkage based estimate of partial correlations and a local *fdr* multiple testing adjustment to determine relationships between variables in datasets with fewer observations than variables (Opgen-Rhein and Strimmer, 2007). The results from this program are then used to construct a Gaussian Graphical Model (GGM), which visualizes the connections between metabolites with the most significant partial correlations. GGMs do not lend themselves to clear interpretation or obvious application, since actual biochemical network is not reconstructed and information on the entirety of the metabolome cannot be acquired from shotgun approaches at this time, but the graphs may be useful for determining roles and relative importances of identified but uncharacterized metabolites. The R code also generates some statistics for partial variances and partial regression coefficients of the metabolites that we might be able to use for better estimates with more traditional methods like comparison of means and contrasts.

## **Conclusions**

The metabolic profiles of our maize genotypes contain sufficient information to distinguish each entry. This implies that allelic diversity has a discernable influence on the metabolic characteristics of primary root tissue, which likely contributes to the final root phenotype. Possible examples of metabolite traits inherited with simple additive effects were identified by comparison of hybrids with their inbred parents, but require additional data to

confirm. Apparent coordinated effects were observed in replicates of each entry that spanned different subsets of metabolites. Partial correlations are a way to construct a relationship network more confidently that includes metabolites without assigned chemical structures, however methods to validate the findings need to be explored. The data showed particular differences between inbreds and hybrids that raise questions about the assumptions involved with a direct comparison that cloud interpretations of heterosis. Additional methods of analysis appropriate for this kind of data are being pursued. Previously, improvement for desired secondary metabolites has taken place using intensive biochemical analyses, but not direct selection on the transcriptome, proteome, or metabolome as a whole towards an omic ideotype. Connections between molecular profiles and traditional agronomic traits need to be established before such an approach can be applied. The evaluation of a sufficient number of contrasts between vigorous and non-vigorous material may provide a framework for future omics based breeding efforts. Currently, the main limitations of omics experiments are the financial resources required for satisfactory replication and the challenge of confidently identifying instances of technical error versus natural variation. “Ome” wide selection is the culmination of the application of functional genomics to plant breeding.

**Acknowledgements** – We thank Prof. A.E. Melchinger (University of Hohenheim, Germany) for providing the seed of the materials used in this study.

## References

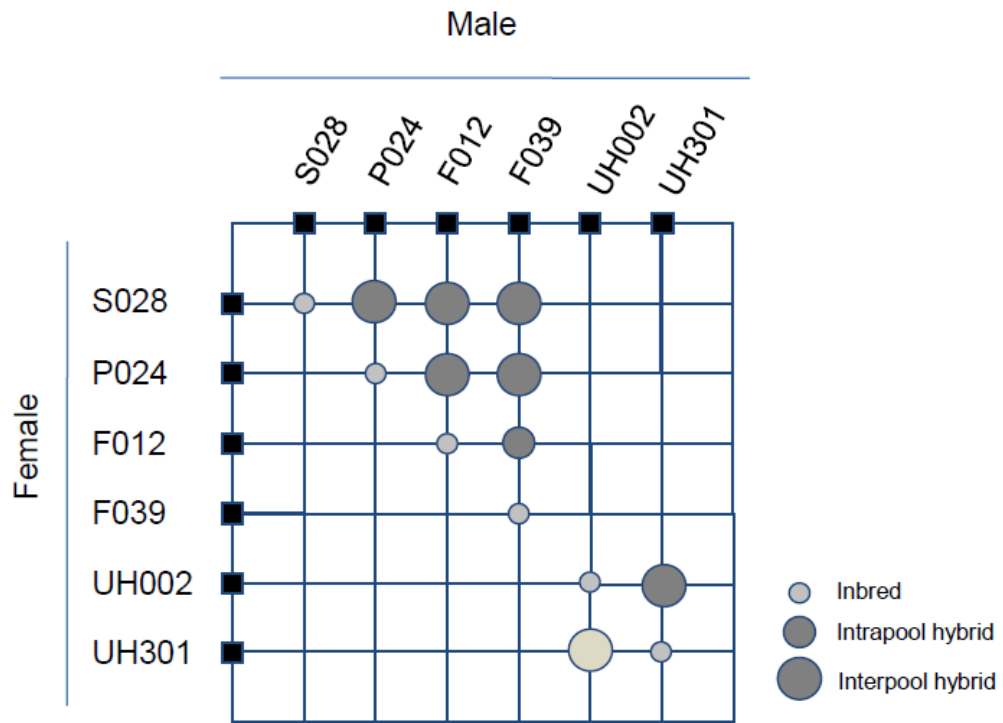
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**Table 1** Major pathways represented by profiled metabolites. Some metabolites were assigned to more than one pathway, others were not included.

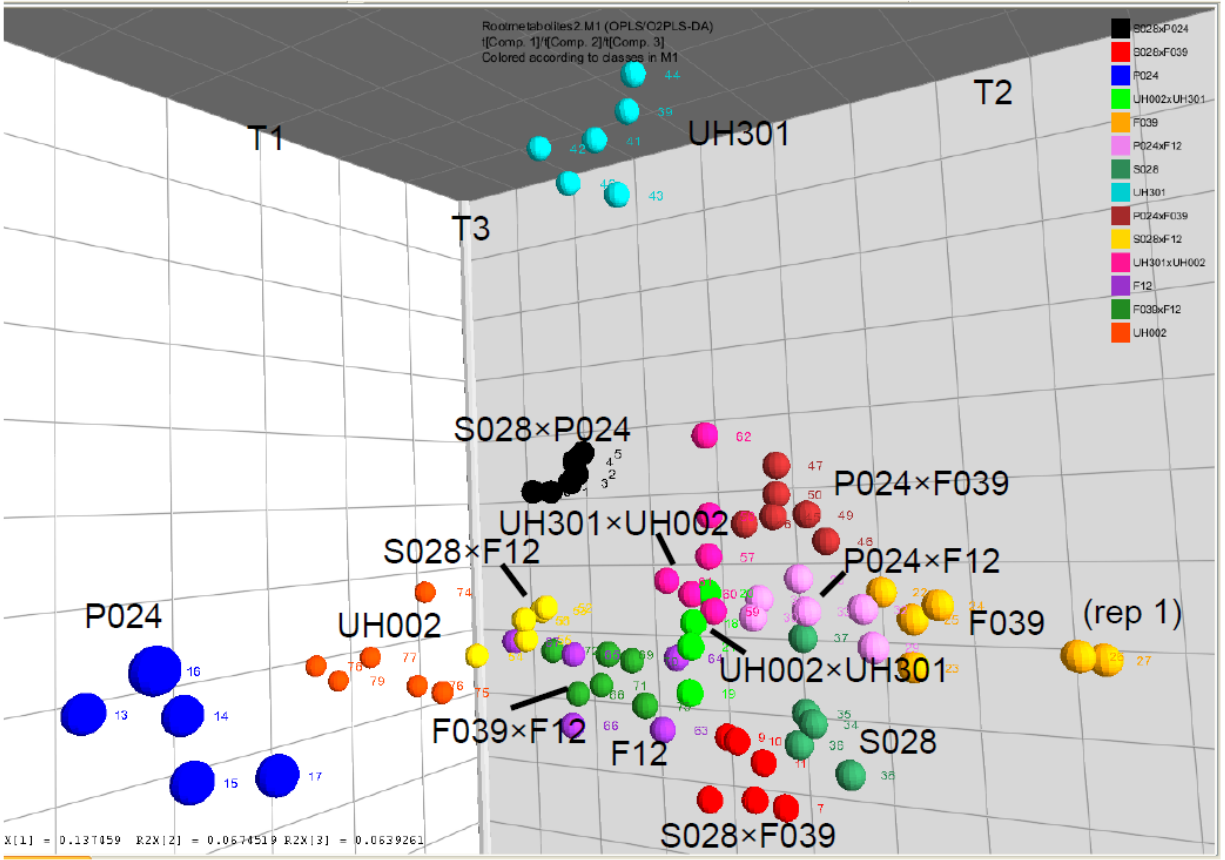
Aspect of Metabolism	# of related metabolites
Sugar / Starch	40
Amino Acid	38
Phosphotransferase System	13
Phenylpropanoids	12
Fatty Acids	8
Glyoxylate and dicarboxylate	7
Urea	7
Alkaloid synthesis	7
Ascorbate	6
Central Metabolism	6
Pentose Phosphate Pathway	6
Purine	6
Pyrimidine	6
Glycerolipid/phospholipid	5
Steroid	5

**Table 2** Relative abundances of selected metabolites for a inbred-hybrid triplet. Quantities of metabolites for replicates of the inbreds are consistent across replications in most cases. Two of the hybrid's replicates show drastic correlated effects across many amino acids and the additional metabolites GABA and sucrose. One of the S028 replicates shows a reduced sucrose level and a replicate of P024 has a greatly increased level, but both lack the correlated response to other metabolites as observed in the hybrid. Note the consistent values of arabinose in replicates and the reduced level in the hybrid compared to the inbreds. The value of zero for glutamine in P024 should be interpreted as being below the sensitivity of the analysis, rather than as an absolute.

Env.	Entry	Alanine	Asparagine	Lysine	Met.	Phen.	Proline	Tyrosine	Cysteine	Glut.	Arabinose	GABA	sucrose
1-1	S028	19.3	181.8	5.2	21.5	4.8	1.5	26.1	1	21.7	19.6	23.9	4.3
2-2	S028	13.6	210.1	5.3	23.4	6	1.7	27.2	1.3	21	25.6	26.2	817.3
2-2	S028	17.2	186.2	5	20.8	5.7	1.9	22.1	1.7	17.1	25.2	30.5	975.7
3-1	S028	22.6	193.3	6.2	27.1	8	1	22.7	1.7	24.2	24.3	20	813.6
3-1	S028	15.9	199	5.2	27.2	5.2	1.2	25.7	1.6	15.9	24.4	26.9	899.1
1-2	S028xP024	<b>96.6</b>	<b>96.6</b>	<b>88.3</b>	<b>47.9</b>	<b>47.9</b>	<b>28.7</b>	<b>98.6</b>	2.6	8.6	3.1	<b>313.4</b>	<b>10.8</b>
1-2	S028xP024	35	84.1	9	80.1	13.5	1.7	31.2	1.2	10.2	4.9	34.4	838.6
2-1	S028xP024	24.4	129.3	6.1	69.2	10.8	1.4	22.7	0.8	9.7	3.8	35.5	887.3
2-1	S028xP024	<b>114.1</b>	<b>16.7</b>	<b>55.4</b>	<b>41.4</b>	<b>40.9</b>	<b>19.4</b>	<b>91.4</b>	1.1	9.1	2.7	<b>178.2</b>	<b>13.8</b>
3-1	S028xP024	23	110.8	10.3	61.8	14.2	1.8	29.9	1	8.3	3.2	25.7	1148.6
3-1	S028xP024	25.3	125.5	9.2	63.6	12.9	1.6	35.4	1.2	11.6	3.4	34.2	1122.4
1-1	P024	13	9.6	2.7	17.3	1.9	5.6	69.4	1.2	0	17.6	114.9	10.1
2-2	P024	13.3	12	2.6	16	1.5	6.9	51.1	1.1	0	12.1	8.6	9.3
2-2	P024	14.2	17.5	2.3	15.6	2.6	5.8	55.8	3.5	0	13.1	6.5	9.4
3-2	P024	16.7	13.5	2.6	11.4	1.7	6.2	57.5	1.5	0	10.5	8.6	126
3-2	P024	15	19.1	2.8	11.9	1.3	5.5	46.2	1.5	0	10.7	8.1	9.6



**Fig 1** Maize Genotypes and crossing design used in this study.



**Fig 2** SIMCA visualization of entry clusters discriminated using the three most significant OPLS factors. Each sphere is a replicate of an entry with SIMCA assigned observation numbers beside them. For these factors, replicates of the reciprocal hybrids derived from crosses between UH002 and UH301 were not well segregated and entry F12 is similar to two of the hybrids it is a parent of. The isolation of two replicates of F039 from the same germination paper roll from the others along the T2 factor could be explained by environmental effects.

Breeding maize for enhanced silage quality: aspects of stover and grain improvement.

Gustafson, T.J.\*, J.G. Coors, and N. de Leon.

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Genetic variation for forage yield and silage quality in maize has been observed, and breeding programs have subsequently been established to improve those traits. The Wisconsin Quality Synthetic (WQS) is a population in our silage breeding program that has undergone four cycles of S<sub>2</sub> topcross recurrent selection for silage yield and quality. We evaluated whole plant and stover yield and quality for each cycle of WQS *per se* as well as their testcross performance when crossed to two commercial Stiff Stalk type testers. Whole plant and stover yields increased through selection in the population *per se* and in testcrosses. Whole plant quality increased in the population *per se* and testcrosses while there was no change in stover quality. Thus indirect selection on a whole plant basis may not be effective to improve stover quality. Increases were seen in whole plant starch content even though ear percent has not changed, suggesting an increased kernel content which was confirmed by a significant increase in kernel yields with no increase in cob yield. This increase in kernel yields has a major impact on silage quality as starch from the kernel is a high energy source. Variation in ruminal starch degradability however has been documented. We performed two separate studies evaluating the variation and heritability of kernel vitreousness and hardness which have both been correlated to starch degradability. Significant variation was seen in multiple Corn Belt dent populations for these kernel traits. Positive correlations were found for kernel vitreousness and hardness between early and late generation inbreds as well as between inbreds and respective testcrosses. This indicates that early inbred selection for kernel vitreousness and hardness would positively impact the kernel starch degradability of hybrids produced with those inbreds.

## Development and Characterization of Maize-Teosinte Introgression Libraries

Flint-Garcia, S.A.<sup>1,2\*</sup>, Bottoms, C.A.<sup>2</sup>, McMullen, M.D.<sup>1,2</sup>

<sup>1</sup>USDA-ARS Columbia, Missouri    <sup>2</sup>University of Missouri, Columbia, Missouri

Genetic analysis and improvement of crops relies on variation in genes controlling agronomic traits. In maize, artificial selection during domestication starting 7500 years ago and/or modern plant breeding over the last century has diminished this critical genetic variation. This is especially true for key genes responsible for traits that define differences between maize and its wild ancestor teosinte (*Zea mays ssp. parviglumis*). We have developed ten teosinte introgression libraries, each derived by backcrossing a different parviglumis accession into B73. Eight of these libraries, comprising 640 maize lines at the BC4S2 stage, were characterized with 768 SNP markers to define the introgressed teosinte chromosomal regions. Each line contains an average of 3 chromosomal segments encompassing ~4% of the teosinte genome. The development and evaluation of these maize-teosinte insertion libraries will enable us to evaluate allele series, test the impact of domestication on trait variation, and reintroduce valuable genetic variation into maize germplasm.

## Translating Biochemistry to Breeding for High Carotenoid Traits in Maize

Catherine Kandianis<sup>1</sup> and Torbert Rocheford<sup>1,2</sup>

<sup>1</sup>Department of Crop Sciences, University of Illinois

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Combined use of association mapping and linkage analysis is highly effective in the identification of loci governing quantitatively inherited traits. In this strategy, the underlying genetic basis and function of QTL can be tested through a hypothesis driven approach that draws upon biochemical information from model species. Results from the linkage mapping experiment can then be reconsidered in the context of a larger biochemical system. Understanding of the genetic architecture controlling carotenoid concentrations in maize endosperm has been enhanced by this strategy through the identification of two QTL, *LCY $\epsilon$*  and *CrtR-B1* (Harjes, 2008; Yan, 2008) that significantly affect the synthesis and conversion of carotenoids within the pathway. Previous QTL analyses indicated that a locus in bin 9.07 significantly affected carotenoid traits in multiple populations. On the basis of the QTL effect and location, we proposed that genetically controlled degradation processes could be the contributing factor and tested *ZmCCD1*, a maize homolog of the carotenoid cleavage dioxygenase family. A polymorphism in the promoter of *ZmCCD1* was found to associate with changes in total carotenoid concentration as well as lutein. The allelic series discovered in this process was used to design allele-specific markers for use in a linkage mapping analysis of carotenoid QTL in the A619 x SC55 F<sub>2:3</sub> population. Results indicate that the position of a significant and large effect for lutein and total carotenoid concentrations lies at the *ZmCCD1* map location, and reveals that the allele associated with a strong degradation effect is dominant. Interactions of *ZmCCD1* with known loci *LCY $\epsilon$*  and *CrtR-B1* are evaluated in the context of statistical epistasis and kernel development.

## Pollen Shed Delay

A. Forrest Troyer

DEKALB, ILLINOIS. University Of Illinois, Urbana

Pollen shed delay, Silking Anthesis Interval (SAI), occurred in a cool, late season

Corn has an imperfect flower. The female flower becomes the ear, and the male flower is the tassel. Darwin pointed out that silk delay after pollen shed is normal in corn varieties to facilitate cross pollination, which increases plant vigor. The term silk delay also explains what happens during drought (moisture stress) at flowering time. Fresh corn silks are 90% water; thus, they are sensitive to water availability. Corn breeders' selection against silk delay at high plant densities has been useful to increase hybrid corn's drought stress tolerance. Growing degree heat units to pollen shed is normally very stable with a much lower coefficient of variation than heat units to silk. Upright leaves have become more popular in commercial hybrid corn during the last 40 years. I noticed some very unusual flowering of corn while pollinating in my nursery during this late, cool, 2008 season in northern Illinois. This is about heat units and flowering in corn.

My breeding starts are typically backcrosses of related, elite inbred lines. I grew 1600 plants each of six backcross pedigrees involving four different elite inbred backgrounds at 60,000 plants per acre, including alleys. I self-pollinated the earliest, strongest silking 10% of the plants in each population. This year many plants silked strongly, and I had to wait a day or two or three or more days for the tassel to shed before pollinating. That's very unusual. The pollen shed delay plants had their tassels tightly encased in the uppermost one or two leaves of the plant. All of my plants had ligules. When I "unwrapped" these tassels, the tassels felt cool and damp; they were water cooled by plant transpiration. I had never seen plants and felt tassels like these at flowering time. This year I saw and handled about a thousand plants with delayed pollen shed.

My nursery was planted April 24 and emerged evenly in about 10 days. We were 30% short of heat units in May, and June was normal. The crop remained late the entire season; we never caught up. The July 7, 2008 Illinois Weather & Crops vol. 29 no. 19 shows corn tied with 2002 for shortest plant height in the last 11 years. August, 4 no. 23 shows corn two weeks behind the 5-year average for dough stage. We had a cool, late season with timely, ample rainfall—seven inches in July on our farm. My corn grew very tall. I pollinated inbred plants that appeared to be too tall and too late for northern Illinois; yet, on those same days, I drove by hybrid corn fields on the way to work that had not yet flowered. It was a very unusual season. We had higher than average yields; several experiments on the farm averaged well over 200 bushels per acre.

Is there a lesson? Yes: The delayed, late-shed tassels that were encased in a leaf or two indicate heat units must warm the tassel per se to cause pollen formation and dissemination. The plant is sufficient receptor of heat units to develop the plant, the female flower, and tassel, but the tassel per se evidently must receive heat units to develop and shed pollen.

I learned later that Mr. Michael Toohill of Midwest Seed Genetics in Bloomington, IL visited several farmers' hybrid corn fields with pollen shed delay in central and northern Illinois. He told me they all pollinated well and yielded well. Spring seasons like northern Illinois in 2008 are probably rare.

## **Is Harvest Index Related to Maize Productivity?**

Aaron J. Lorenz, Timothy J. Gustafson, James G. Coors and Natalia de Leon\*

Department of Agronomy, Plant Breeding and Plant Genetics Program, University of Wisconsin-Madison, 1575 Linden Dr., Madison, WI, 53706

The use of maize (*Zea mays* L.) stover as a feedstock for cellulosic biofuels production will create demand for maize hybrids with greater stover yield. Grain yield is expected to remain the most critical trait and continue to drive hybrid sales, requiring that any increases in stover yield be made without sacrificing grain yield potential. The objective of this review was to determine the relationship between grain yield, harvest index, and stover yield in order to illuminate the potential for increasing both grain and stover yield through breeding. In contrast to what has been observed in other crops, gains in maize grain yield over time in the U.S. Corn Belt have been accompanied by increases in stover yield. Where recurrent selection on grain yield has been most successful, stover yield also increased while harvest index has been relatively stable. The opposite situation has been observed in tropical germplasm, where gains in grain yield have been associated with increasing harvest index and relatively constant biomass yield. We expect that stover yield of Corn Belt hybrids will continue to increase along with grain yield, resulting in future hybrids capable of producing both more food and biofeedstock for energy production. If maize breeders pursue selection for increased stover yield, we found no evidence to suggest that breeding for stover yield will necessarily reduce rate of gain in grain yield.

## Business Meeting Minutes

**March 12, 2009**

State Representatives Present: Martin Bohn (U. of Illinois), Sherry Flint-Garcia (USDA-ARS; Missouri), Shawn Kaeppler and Natalia de Leon (U. of Wisconsin), Richard Pratt (U. of Ohio), Paul Scott and Candice Gardner(USDA-ARS; Iowa)  
Administrative Advisor: B. Tracy (U. of Wisconsin).

Committee Chair, Paul Scott called the meeting to order and presented an outline of items to be discussed.

### 1. 2008 Official Minutes

A draft of the 2008 minutes was sent to the email list serve on April 8, 2008 and was open for comments and corrections. The official minutes were posted on website. A motion to accept the minutes was made (Rich Pratt) and seconded (Martin Bohn), and the minutes were approved unanimously.

### 2. Treasurer Report

The treasurer, Marcelo Carena (NDSU), was absent due to winter weather. Paul Scott presented the budget situation to the group.

Previous balance	0.00
2009 meeting cost	- 14,041.08
Incoming funds as of 3-12-09	12,840.52
(\$8,385.20 from registration fees)	
(\$4,455.32 donated by T. Rocheford)	
<hr/> Balance	<hr/> -1,200.56

The cost of the 2009 meeting at Allerton was very expensive as compared to prior meetings. The low attendance was partially attributed to the increased cost, and contributed to our inability to pay for the meeting through registration fees. Fortunately, surplus funds from the 2007 "Future of Maize Genetics" meeting were donated by Torbert Rocheford to help offset some of the deficit. In Marcelo's absence, we could not assume that carrying a negative balance in the account was acceptable.

### 3. Administrative Advisor Report

Bill Tracy informed the group of report requirements and upcoming deadlines.

- The 2009 meeting report is always due 60 after the meeting. This report consists of an attendance list, abstracts from presentations, the minutes, and state experiment station reports. The secretary is responsible for filing this report.

- The mid-term report is already overdue (due date of Dec. 15, 2009). Because of an oversight, our group did not receive notice that this report was due. Rich Pratt volunteered to write the mid-term report.
- The current project expires in 2011, and will be up for renewal in 2010. The due date for the renewal is in December 2010. A writing committee was already appointed at the 2008 meeting (Bill Beavis and Rich Pratt).

#### **4. Corn Breeding Executive Committee (CBEC)**

Bill Tracy reported that the ad hoc Executive Committee (appointed at the 2008 NCCC167 meeting in Baltimore, MD) met in June at the SCCC-80 breeding meeting. Only 2-3 members of the ad hoc committee were present; therefore, discussion was tabled until the 2009 NCCC167 meeting.

We decided to design the Corn Breeding Executive Committee (CBEC) after the Maize Genetics Executive Committee (MGEC): members serve a 5 year term, elections occur just before the maize meetings, runoff elections are held between the primary nominees, members rotate on and off the committee in a staggered fashion, leaving sufficient member overlap for continuity and institutional memory. A point was made that our group was too small to be support rotation on and off the CBEC. The conclusion – in order for our group to be sustained and worthy of an executive committee, we need to make the corn breeding group larger by being more inclusive.

Paul pointed out that it is the responsibility of the CBEC to define our group. We need a mechanism for appointing CBEC members.

Shawn pointed out that our group is too small to have a strong voice (i.e. to pursue funding to get what we need). We need a fairly sizeable executive committee.

Potential resources for identifying new corn breeding community members include: IL Corn Breeders School attendee list, 2006 Corn Breeding Survey (administered by M. Bohn), Maize BioList (maintained by M. Sachs), Maize Genetics Steering committee list (2009 Committee Chair: Steve Moose). The CBEC should obtain access to these lists, send a solicitation email, and add positive responders to the Corn Breeding Community.

The executive committee should define the bylaws, mimicking those of the GEM or MGEC.

The group present decided to appoint a new Provisional Executive Committee to begin implementation of the following:

- Define Bylaws and Mission Statement of the CBEC.
- Define the Community via email
- Develop a new webpage independent of the NCCC167 webpage. This is to make the group more inclusive (not regionally defined).

Nominations were held for the Provisional Executive Committee. Elections were held, and the following were elected:

- Martin Bohn
- Paul Scott
- Sherry Flint-Garcia
- Travis Frey (Industry Representative)
- Tom Brutnell (to provide advice from the MGEC)

The Provisional Executive Committee will initiate these steps before field season begins.

## **5. Election of Officers**

In an email, Treasurer-Secretary Marcelo Carena requested splitting the Treasurer-Secretary office into two separate offices. Because he is not able to regularly attend the meeting, he felt it would benefit the group to elect a regular attendee as Secretary. A motion was made (Rich Pratt) and seconded (Natalie de Leon) to split the offices, and the motion was approved unanimously.

Nominations were made for the post of Secretary, for the term ending in 2011. Sherry Flint-Garcia was elected by unanimous vote.

Nominations were made for Chair-Elect (will be Chair of the 2011 meeting). Shawn Kaeppler was elected by unanimous vote.

Former Chair-Elect Liz Lee becomes the new Chair, and will host the 2010 meeting in Guelph, Ontario.

## **6. Regional Yield Trials**

In 2008, the 100-300 Maturity Group conducted their yield trials, though no one from that group was present to present their results.

There was not enough interest in the 400-600 Maturity Group to conduct trials in 2008.

The 700-800 Maturity Group conducted trials in 2008. Rich Pratt distributed the yield trial results.

Some people were still interested in the testing program, so those interested in participating will communicate with others in the same Maturity Group to coordinate trials on a yearly basis.

A motion was made and seconded to adjourn the meeting, and Meeting was adjourned. A large number of NCCC-167 attendees then drove to St. Charles, IL to attend the Maize Genetics Conference.

## Discussions regarding the “Future of Corn Breeding”

During the meeting at large and the business meeting, the topic of the Future of Corn Breeding was discussed.

Following is an informal report of these discussions:

1. From the Maize Genetics Community 2007 Allerton Report:
  - A. Short term planning (1-3 years)
    - Current sequencing/annotation completed, additional map based sequencing efforts initiated.
      - Sequencing is complete, annotation is in progress, additional sequencing has been initiated.
    - Centralized databases with increased funding.
    - Indexed reverse genetic resources finalized, current mutagenesis libraries indexed with new technologies.
    - Expression platforms and information tools selected and developed.
    - Transformation capacity enhanced in public sector, continuous support mechanisms for public transformation resolved.
  - B. Long term
    - Databases and stock center capacity enhanced, coordinated, and supported
    - Create map based sequences of other inbreds, race, and teosintes
    - Phenomics initiative underway, with large scale multi dimensional phenotyping capabilities for multiple environments
    - Maize scientific community will be large, diverse, and interactive.
2. From the 2009 NCCC167 Corn Breeders Meeting:
  - A. Discussions of the Genetics Community Short Term Planning list (above):
    - Maize genetics community is focused on databases. GRIN Global (germplasm resource information network) is an effort from Candice Gardner that is relative to plant breeding. What other databases can be integrated into the information resource?
      - Maize GDB is already listed by the maize genetics group.
      - Paul recommends putting together an online pedigree based database, such as the “Compilation of North American Germplasm.”
      - Rich said that this group had, in the past, worked to characterize phenotypes of inbreds and germplasm. These data are currently on paper, so isn't accessible inside or outside of the group.

- Paul is interested in making phenotype data from multiple environments available.
  - Need to discuss definition of phenotyping with maize genetics group: “What is a Phenotype?” Our breeding group doesn’t agree that a phenotype is a result of a one-gene knockout.
  - Rich says we are still doing regional yield trials, but no longer developing inbreds. The inbreds were evaluated for disease reactions, physiology, etc across environments, data that would still be useful today.
- Candice asks what the goals of our group are and how we can focus our objectives toward those goals. Once the objectives are identified, then we can identify our needs. Is the priority of this community to get funding in order to do more science?
- In 2007, the short term goals were to get the tools ready so work could move forward in a standardized fashion.
  - Tom – the objectives/needs/priorities change over time; need to reevaluate regularly.
  - Rich - One way to standardize breeding would be to standardize how phenotypes are scored (e.g. on a 1 to 10 scale for a given disease, sometimes 1 is no disease, sometimes 10 is no disease).
  - Paul - overall goal is to improve the science of breeding.
  - Shawn - the goal is to produce genotypes that will perform well in specific environments (gxe).
  - Rich - we have moved past simple commodity maize to creating diverse specialized types, such as those for industrial products including biofuels. This is an opportunity to redefine the goals of plant breeding to reflect this.
  - Rich – Breeding is integrative work.
  - Walter - plant breeding is an applied science, but there is now more money for basic/theoretical science than applied science. He is concerned that breeding is so poorly funded that it is on the way out.
  - Travis - maize breeding is getting quantitative, we are past simple traits. This requires more collaborations. We need funding that will allow geneticists and breeders to work together in a beneficial way.
  - Martin - we need to have tools that allow us to capture information from maize geneticists then bring the information to the field.
  - Candice - we want to secure the basis for personnel/students and develop tools to improve maize.
  - Tom – Push the “quantitative genetics” component. Plant breeders already have sound skills in statistics; quantitative genetics=breeding=statistics. Engineering algorithms exist to work for breeding. - speaks again to collaborations, need bioinformaticists to develop tools and databases
  - Paul - Pioneer has a pedigree based database of their germplasm. We need a similar database for the public.
  - Martin – All NSF projects are required to make their data publicly available. Our germplasm is in the public domain, so the related phenotypic information

should be available to all. This will require databases for pedigree and data storage.

- Travis - Gramene already exists, could have germplasm info added including phenotype and microarray data.
- Tom – the Arizona microarray group was a repository of array data , but because demand decreased they went bust . The new expression technology is Solexa genotyping.
- Bill Tracy - Geneticists are following a clear advancement of research. Breeders are experiencing a big paradigm shift . It used to be that we were simply trying to advance a trait (in order to design a genotype for a specific trait or environment, breeders did it by selection). Now, we are being asked to use far more information than before (How do we use genetic and genomic tools to do this?). Need to have a more clearly defined goal. How are we going to get the traits to the field, integrating new tools with selection. The private sector has figured this out, but the public hasn't. Is this a capacity issue? Maybe we need to ask for more money to increase our capacity?
- Shawn - says the capacity to collect phenotypes is diminished. Knowledge of phenotypes, particularly agronomically relevant traits, often cut across a wide range of phenotypes like disease resistance and standability.
- Martin - Europe and Australia have phenotype initiatives, working to find phenotypes that are predictive, helping get from the lab bench to the field.
- Travis - what is the goal of maize breeding in the public sector that won't overlap with the goals of industry so the public can get funding? The public breeders need more collaboration with industry, but they shouldn't compete with industry because the public would lose.
- Dave – We need a database integrating QTLs, maps, and genes. If a plant breeder has a QTL, it would be helpful to be able to search in a database: What is near that QTL that might be affected if selection is targeted to that QTL? This is related to linkage drag.
- Paul - problem with maize genome data bases now is that they are wide but not deep. We may have QTL info in some populations, but don't know how they might react when moved to another population. The B73 sequence will become more useful to breeders when we have other genotypes sequenced. Communication gap between genetic data base people and breeders.

B-- Discussions of the Maize Genetics Long Term Planning list (above):

- Bill – This list is much more appealing to breeders.
- Walter – There are differences in objectives and implications. Geneticists are science driven; working to advance the science. Breeders seem to have more of a social impact; they are working to improve the situation of humanity. Perhaps we are service driven rather than science driven. The objectives of breeding programs often have important social implications like improving nutrition or the environment. Our goals are beyond science, beyond papers.

- There was general objection to “rather than science driven” ..... better to say “Scientific and social research”
- Paul - perhaps we need to better define our group. Are we scientists? Who do we serve? Clearly our objectives are societal
- Shawn - the objective depends on the granting agency. Some like CREES care about the social aspects.
- Travis - every grant asks what is the applicability of the science, but most grants don't really study the applicability during the research. Breeders study the applicability, make the science reality.
- Tom - How to distinguish what the public breeders are doing from what industry is doing? Is it more than public breeders being the “poor cousins” of the multinationals?
- Candice – There is general skepticism of public citizens toward scientists and government. They don't want us to waste our resources and they want us to take their interests seriously.
- Andrew – The public takes risks in science that the private sector won't.
- Travis – but the private sector has the money to take the risks and the public doesn't!

**Illinois Agricultural Experimental Station  
Department of Crop Sciences  
University of Illinois  
Urbana, IL 61801**

**Annual Report NCCC-167**

**- 2008 -**

**Faculty of the corn breeding and genetics group at the University of Illinois**

- Martin O. Bohn
- Dick Johnson
- Rita H. Mumm (since 08-2008)
- Torbert R. Rocheford (joined Purdue faculty 08-2008)

**Emeritus Faculty**

- J.W. Dudley
- R. Lambert

**Our Objectives**

- Improve grain yield and value-added traits of corn.
- Improve agronomic characteristics of corn including resistance to disease and pests.
- Provide educational opportunities for students that prepare them for careers in plant breeding, genetics, and plant pathology.
- Provide continuing educational opportunities for commercial corn breeders.

**Program Highlights**

- The Illinois Plant Breeding Center was established (see <http://plantbreeding.illinois.edu>). Dr. Rita Mumm is the director of this new center.
- The Illinois Maize Breeding and Genetics Laboratory (IMBGL) organized the 44<sup>th</sup> Illinois Corn Breeders' School held in Champaign, Illinois, March 3-4, 2008. This CBS was attended by more than 200 corn breeders and geneticists from the public and private sector. Highlight of the CBS was an Open Forum and Panel discussion on "How Public and Private Sector Can Effectively Work Together to Enhance Support for Maize Breeding and Genetics Education and Translational Research". The CBS also featured a mini session on heterosis. More detailed information is available at the Illinois corn breeding and genetics laboratory (IMBGL) internet site (<http://imbgl.cropsci.illinois.edu/index.html>).
- Research results of the Illinois corn breeding and genetics group, including new findings on maize host plant resistance to western corn rootworm larvae feeding, maize root complexity, and cell wall composition, maize tassel and ear architecture, as well as kernel quality, including starch, protein, oil, and vitamin contents, were presented at several national and international conferences, including the 45<sup>th</sup> Illinois Corn Breeders' School (Champaign, IL, March 2008), Annual NCCC-167

Meeting (Baltimore, MD, March 2008), Maize Genetics Conference (Washington, DC, March 2008), DIABR-ACT – Harmonize the Strategies for Fighting *Diabrotica virgifera virgifera* (Goettingen, Germany, May 2008), Joint Meeting of “Gesellschaft fuer Pflanzenzuechtung e.V.” and “Deutsches Maiskomitee e.V.” (Stuttgart, Germany, June 2008), Agronomy Day of the College of ACES/UIUC (Urbana, IL, August 2008), 2008 Annual ASA-CSSA-SSSA Meeting (Houston, TX, October 2008), International Conference on Conventional and Molecular Breeding of Field and Vegetable Crops (Novi Sad, Serbia, November 2008), Germplasm Enhancement in Maize Annual Meetings (Chicago, IL, December, 2008), and at multiple departmental seminar series (University of Wisconsin, University of Illinois, National Taiwan University)

- The maize breeding program has currently five PhD students and four MS students.

## Publications

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- Dudley JW. 2008. Epistatic Interactions in Crosses of Illinois High Oil × Illinois Low Oil and of Illinois High Protein × Illinois Low Protein Corn Strains. *Crop Sci.* 48:59-68.
- Flint-Garcia A, Dashiell KE, Prischmann D, Bohn M, Hibbard B. Conventional screening overlooks resistance sources: Rootworm damage of diverse inbred lines and their B73 hybrids is unrelated. *J Econ. Entomol.* *Accepted.*
- Gray M, Sappinton T, Miller N, Moeser J, Bohn M. Adaptation and Invasiveness of Western Corn Rootworm: Intensifying Research on a Worsening Pest. *Annual Rev. Entomol.* *In print.*
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- Moose SP and Mumm RH. 2008. Molecular plant breeding as the foundation for 21st century crop improvement. *Plant Physiology* 147: 969-977.
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- Warburton M, Reif J, Melchinger A, Frisch M, Bohn M, Xianchun X, Crossa J, Franco J, Hoisington D, Pixley K., and Vasal S. 2008. Trends in genetic diversity in CIMMYT’s non-temperate maize germplasm. *Crop Sci.* 48:617-624.
- Wassom JJ, Mikkilineni V, Bohn MO, and Rocheford TR. 2008. QTL for Fatty Acid Composition of Maize Kernel Oil in Illinois High Oil × B73 Backcross-Derived Lines. *Crop Sci.* 48:69-78.
- Wassom JJ, Wong J, Martinez E, King J, DeBaene J, Hotchkiss J, Mikkilineni V, Bohn MO, and Rocheford TR. QTL Associated with Maize Kernel Oil, Protein, and Starch Concentrations; Kernel Mass; and Grain Yield in Illinois High Oil × B73 Backcross-Derived Lines. *Crop Sci.* 48:243-252.

# NCCC167 Minnesota Station Report

## March 2008 – February 2009

Rex Bernardo  
*Department of Agronomy and Plant Genetics*  
*University of Minnesota, St. Paul, MN*

### Mission and Program Description

My mission at the University of Minnesota is (i) to discover new ways of breeding corn, (ii) to breed corn for new uses, and (iii) to educate future plant breeders. My current research focuses on exploiting cheap and abundant molecular markers in corn breeding, breeding for dwarf corn, and the prospects of breeding corn for both grain yield and stover quality for cellulosic ethanol. My work involves theoretical research, computer simulation, molecular marker analysis, and field experimentation.

### 2008 Program Highlights

- Published a major invited review entitled *Molecular markers and selection for complex traits: Learning from the last 20 years* in *Crop Science*. Plant breeders have learned how to routinely map quantitative trait loci (QTL) but the great majority of QTL remain unexploited in breeding programs. I speculated that future applications for complex traits will likely focus on predictive methodologies for marker-based selection prior to phenotyping and for marker-based selection without QTL mapping. These applications will take advantage of cheaper costs of genotyping than of phenotyping.
- Conducted research on the choice of generation for inducing doubled haploids in maize. Previous studies have suggested that the low frequency of recombinants in doubled haploids may reduce the response to selection. From simulation studies, I found that selection responses were up to 4-6% higher when doubled haploids were induced from  $F_2$  plants instead of the usual  $F_1$  plants. If year-round nurseries are used and new  $F_1$  crosses for inbred development are initially created on a speculative basis, the development of doubled haploids from  $F_2$  rather than  $F_1$  plants should not cause a delay in inbred development.
- Patricio Mayor (Ph.D. student, funded by his company Syngenta Seeds) and I continued our studies on the joint use of doubled haploids and molecular markers in maize breeding. We found that doubled haploid populations are most useful in genomewide selection and marker-assisted recurrent selection for traits controlled by many QTL and with low heritability but have limited value over  $F_2$  populations in detecting major QTL for introgression. Patricio and I have also submitted a manuscript that describes an empirical evaluation of the doubled haploid + marker-assisted breeding schemes that we have investigated.

- Completed the phenotypic analysis (M.S. thesis of Magan Lewis) and the marker analysis (part of Ph.D. thesis of Robenzon Lorenzana) for our USDA-funded research on *Strategies for using molecular markers to simultaneously improve corn grain yield and stover quality for ethanol production*. Magan found high heritabilities and favorable genetic correlations among traits and concluded that there is no genetic impediment to breeding corn for both grain yield and stover quality for ethanol. Benzon did not find any major QTL for any of the stover quality traits and found that predictions of genotypic value were more accurate if the predictions were based on all available markers rather than only the markers with significant effects. The research has now transitioned to marker-based selection for grain yield, agronomic traits, and stover-quality traits. This research on marker-based selection will be part of Jon Massman's Ph.D. thesis.
- Recruited two new students in addition to Jon Massman. Cathrine Ziyomo (Zimbabwe) is a Ph.D. student supported by an AgGrad (Methodist Church) fellowship. Cathrine will work on the breeding and genetics of both N-use efficiency and drought tolerance in maize. Christopher Schaeffer (Minnesota) is an M.S. student supported by a Pioneer Hi-Bred Plant Breeding Fellowship. Chris will be working on response to selection for hybrid grain yield and agronomic traits in dwarf corn, as well as forage quality of dwarf corn.
- Bruno Poupard, a visiting scientist in my program, finished his research on machine-learning methods in marker-assisted selection. Bruno found that machine-learning methods are not any better than statistics-based methods that are already in place for marker-based selection.

#### **Publications (\* indicates primary authorship)**

1. Lorenzana\*, R.E., and R. Bernardo\*. 2008. Genetic correlation between corn performance in conventional and organic production systems. *Crop Sci.* 48: 903-910.
2. Wong\*, C.K., and R. Bernardo\*. 2008. Genomewide selection in oil palm: Increasing selection gain per unit time and cost with small populations. *Theor. Appl. Genet.* 116: 815-824.
3. Bernardo\*, R. 2008. Molecular markers and selection for complex traits: Learning from the last 20 years. *Crop Sci.* 48: 1649-1664.
4. Bernardo\*, R. 2009. Genomewide selection for rapid introgression of exotic germplasm in maize. *Crop Sci.* 49: 419-425.
5. Mayor\*, P.M., and R. Bernardo\*. 2009. Genomewide selection and marker-assisted recurrent selection in doubled haploid versus F<sub>2</sub> populations. *Crop Sci.* (accepted).
6. Bernardo\*, R. 2009. Should maize doubled haploids be induced among F<sub>1</sub> or F<sub>2</sub> plants? *Theor. Appl. Genet.* (submitted).

7. Lorenzana\*, R.E., and R. Bernardo\*. 2009. Accuracy of genetic value predictions for marker-based selection in biparental plant populations. *Theor. Appl. Genet.* (submitted).
8. Mayor\*, P.J., and R. Bernardo\*. 2009. Doubled haploids in maize breeding: One- and two-stage phenotypic selection versus marker-assisted recurrent selection. *Theor. Appl. Genet.* (submitted).
9. Lewis\*, M.F., R.E. Lorenzana, H.-J. G. Jung\*, and R. Bernardo\*. 2010. Potential for simultaneous improvement of maize grain yield and stover quality for cellulosic ethanol. *Crop Sci.* (submitted).

# NCCC167

## University of Missouri Station Report for 2008

Sherry Flint-Garcia  
USDA ARS, Columbia, Missouri

### Mission and Program Descriptions

Maize research at the in Columbia Missouri (USDA ARS and University of Missouri) is primarily genetic in nature. Although there is currently no active corn breeding program, several programs have applied projects that require large-scale field trials. These include Mike McMullen (USDA ARS), Sherry Flint-Garcia (USDA ARS), Bruce Hibbard (USDA ARS), and Georgia Davis (University of Missouri, Plant Sciences).

Mike McMullen investigates how selection has shaped molecular diversity in maize, and relates molecular diversity to functional phenotypic variation. As part of an NSF grant entitled "The Molecular and Functional Diversity of Maize," he has developed the 5000 recombinant inbred line "Nested Association Mapping (NAM)" population derived from 25 inbred lines chosen to capture the greatest amount of genetic diversity. Joint linkage and association mapping will permit high-power and high-resolution genetic dissection of quantitative traits for maize.

Sherry Flint-Garcia utilizes the maize domestication model to identify genes of agronomic importance. Objectives include 1) examining the impact of artificial selection on kernel protein and amino acid composition; and 2) more broadly, diversifying the genetic base of maize by utilizing landraces and teosinte, the ancestor of maize. To accomplish these objectives, she has created introgression (or near isogenic line -NIL) libraries of a number of teosinte and landrace accessions in the B73 background.

As part of an NSF grant entitled "The Genetic Architecture of Maize and Teosinte," Mike and Sherry will fine map genes for flowering time, plant height, and kernel quality, and examine epistatic interactions for these traits. Allele series will be examined and trait predictions will be tested

Bruce Hibbard works on Western Corn Rootworm chemical ecology and host plant resistance, including 1) Identifying, developing and releasing corn germplasm with native resistance to the rootworm; 2) Determining the mechanism(s) of resistance and its inheritance; and 3) Investigating the biology, pest/ host interactions and chemical ecology of the rootworm, especially as they relate to resistance management.

Georgia Davis conducts functional genomics of biotic and abiotic stresses: 1) Fungal resistance in maize with emphasis on mechanisms and sources of resistance to *Aspergillus flavus* and aflatoxin production; 2) Insect resistance in maize, specifically fall armyworm and southwestern corn borer and their relationship to juvenility associated traits; and 3) Maize root architecture in relation to drought response.

Other active maize researchers in Columbia and/or the ARS Plant Sciences Research Unit include: Jim Birchler (Biological Sciences), Ed Coe (professor emeritus), Toni Kazic (Bioinformatics), Gerry Neuffer (professor emeritus), Kathy Newton (Biological Sciences), and Mel Oliver (Research Leader for the ARS Plant Genetics

Research Unit). Ivan Baxter joined ARS located at the Danforth Center in St. Louis, with plans to work on maize genetics beginning in summer 2009. The Division of Biological Sciences is currently recruiting two maize researchers in their department.

## **2008 Program Highlights**

### *Mike McMullen:*

To access the extensive genetic variation present in maize inbred lines, Mike and ARS scientists at Ithaca NY and Raleigh NC have developed a new genetic resource to enable joint linkage-association analysis called nested association mapping (NAM). To form the NAM population, 25 extremely diverse inbred maize lines were crossed to the B73 reference line, and 5000 recombinant inbred lines were created. The NAM genetic map consists of 1106 loci, and has a total genetic length of 1400 cM making it the most powerful genetic resource for the dissection of complex agronomic traits for any species. The NAM population has been phenotyped in multiple environments for up to 30 traits. As an example of the power of NAM, the analysis of flowering time has resulted in identification of ~ 50 loci controlling the variation in flowering time across maize inbred lines. The NAM population has been deposited in the Maize Genetic Stock Center and has been adopted by the maize community as the central genetic resource for trait characterization.

### *Sherry Flint-Garcia:*

Sherry has developed ten introgression libraries of teosinte, each derived by backcrossing a different teosinte accession into B73, an elite maize inbred line. Eight of these libraries comprising 640 maize lines have been characterized with molecular markers to define which chromosomal regions are from the teosinte parent. Each line contains an average of 3 different chromosomal segments encompassing ~4% of the teosinte genome. A subset of five populations was evaluated for disease resistance and other agronomic traits. The development and evaluation of these maize-teosinte insertion libraries will enable geneticists to test hypotheses regarding the impact of domestication on corn improvement, and breeders to reintroduce valuable genetic variation into maize germplasm for very specific genomic regions.

### *Bruce Hibbard:*

Transgenic corn that controls the corn rootworm is a viable alternative to insecticides for managing the most economically important pests of corn. The agricultural industry has adopted a high dose/refuge strategy as a means of delaying the onset of insect resistance to transgenic crops, but the Bt corn products developed for corn rootworm control have been introduced with less than high-dose. Bruce has developed colonies of the western corn rootworm that survived the Bt product in the greenhouse along with an unselected colony. After three generations of full larval rearing on Bt corn, rootworm larvae survived equally on Bt and susceptible corn in greenhouse trials. In dose-response assays with the protein from the Bt corn on artificial diet, the dose of toxin required to kill half the population was 22-fold greater for the selected colony than the unselected colony. After six generations of greenhouse selection, larval recovery on Bt corn as a ratio of larval recovery on susceptible corn in

the field was 11.7-fold greater for the selected colony than the unselected colony. Reciprocal crosses of the selected colony with the unselected colony suggest that several genes of small effect and multiple mechanisms are likely involved in conferring the observed resistance. The results suggest that rapid response to selection is possible in the absence of mating with unselected beetles, emphasizing the importance of effective refuges for resistance management.

## Relevant Publications

Behle, R.W., **B.E. Hibbard**, S.C. Cermak, and T.A. Isbell. 2008. Examining *Cuphea* as a potential host for western corn rootworm (Coleoptera: Chrysomelidae) larval development. *J. Econ. Entomol.* 101:797-800.

Buckler Iv, E.S., Yu, J., Holland, J.B., **McMullen, M.D.** 2008. Genome-wide complex trait dissection through nested association mapping. *Genetics.* 178:539-551.

**Hibbard, B.E.**, Y.M. Schweikert, M.L. Higdon, and M.R. Ellersieck. 2008. Maize phenology affects establishment, damage, and development of the western corn rootworm. *Environ. Entomol.* 37: 1558-64.

Meihls, L.N., M.L. Higdon, B.D. Siegfried, T.A. Spencer, N.K. Miller, T.W. Sappington, M.R. Ellersieck, and **B.E. Hibbard**. 2008. Increased survival of western corn rootworm on transgenic corn within three generations of on-plant greenhouse selection. *Proceedings of the National Academy of Science.* 105: 19177-19182.

Oyediran, I. O., B. W. French, T. L. Clark, K. Dashiell, and **B. E. Hibbard**. 2008. Prairie grasses as hosts of the northern corn rootworm (Coleoptera: Chrysomelidae). *Environ. Entomol.* 37:247-254.

Sanchez-Villeda, H., Schroeder, S., **Flint Garcia, S.A.**, Guill, K.E., Yamasaki, M., **McMullen, M.D.** 2008. DNA align editor: DNA alignment editor tool. *Bioinformatics.* 9:154.

Šimic, D., M. Ivezic, I. Brkic, M. Brmez, I. Majic, T. Ledencan, J.J. Tollefson, and **B.E. Hibbard**. 2008. Environmental and genotypic effects for western corn rootworm tolerance traits in American and European maize trials. *Maydica:* 52-425-430.

Yamasaki, M., Schroeder, S., Sanchez-Villeda, H., Gaut, B., **McMullen, M.D.** 2008. Empirical analysis of selection screens for domestication and improvement loci in maize by extended DNA sequencing. *The Plant Genome.* 1(1):33-43.

## Impact

Maize research at Missouri may be described as ranging from basic biology to applied quantitative trait analysis. Members of the four large-scale projects focus on trait dissection for corn rootworm, drought, insect, and disease resistance, all traits that impact maize production. All four projects also utilize diverse germplasm (inbred lines, landraces, and teosinte) to identify germplasm with novel alleles for trait improvement. Although Missouri does not have a corn breeding program per se, we are making great strides in understanding the genetic basis of traits of economic value while evaluating diverse germplasm that is critical for diversifying the genetic base of corn.

## **New York State Report for NCCC167**

Margaret Smith, Cornell University

In 2008, we started off with generally dry weather in May that helped with getting the corn crop in the ground in a timely manner. Temperatures were a bit cool but overall conditions for planting were good. Temperatures were above long-term averages around the state in June and July, with some record-breaking heat in the central region of the state in early June. Rainfall was above average for June and quite a bit above average for July. The Champlain Valley in June and Central Lakes area in July were especially wet. A storm with very high winds passed through northern New York in mid July, knocking down large trees in the Chazy area. Seasonal or slightly cool temperatures prevailed in August, with rainfall about average in much of the state. Conditions were a bit dry in the St. Lawrence Valley and a bit wet in the western part of the state. September was generally a bit warmer than long-term averages and tended to be dry. The southeast corner of the state was wet, probably due to effects of tropical storm Hanna early in the month, which also caused lodging in the Hudson Valley. Winds from the edges of Hurricane Ike caused stalk lodging pressure around central and northern parts of the state, particularly at Sackets Harbor. As we moved into October, temperatures were a bit cool and conditions were wetter than long-term averages, slowing grain dry-down and harvest operations. The last week of October brought considerable rain and snow. A foot of snow on the hurricane-lodged corn near Sackets Harbor caused some losses. With cool conditions continuing into November, much corn around the state stayed standing in the field later than usual and harvest continued slowly. By early fall, rust and eyespot were common in central and northern parts of the state, and anthracnose leaf blight and gray leaf spot were present in central and southern parts of the state. Northern leaf blight and European corn borer were noticeable at isolated locations.

Overall, there were 1.13 million acres of corn planted in the state. Of this total, 445,000 acres were harvested as silage and the state average silage yield was 20 tons/acre, which topped the previous record of 18 tons/acre in 2006. Another 640,000 acres were harvested as grain. Despite the challenging harvest conditions, the state average corn grain yield was reported as 144 bu/acre – 15 bu/A above the previous state record grain yield from 2006!

# 2008 North Dakota State University Report

## NDSU Corn Breeding Program

### **BRIEF SUMMARY OF RESULTS**

During 2007-2008, nine corn products were developed and released as new sources of early maturing and genetically diverse germplasm. These new cultivars were requested by 21 public and private institutions so far. The project has achieved the goals proposed because of activities that have integrated pre-breeding with cultivar development. The project has focused on corn germplasm adaptation and improvement, inbred line development, extensive hybrid testing of NDSU lines and industry lines, and continuous training of applied corn breeders. Corn breeding and testing efforts have significantly increased. The program has conducted 65 replicated experiments in 2008 including 22,360 plots across 22 locations, an expansion from <5,000 plots across five locations before 1999. There were several experiments in each location and the size of location research sites, managed exclusively for corn breeding, ranged from 2 to 16 acres per location. Results were based on genetic and statistical unbiased principles with the most efficient choice of experimental designs, randomization, and replication. Millions of research data points (a range of one to 10 measurements per plot and from 10 to 35 traits per experiment) were generated and thousands of corn genotypes have been tested mainly on incomplete block experimental designs in order to increase accuracy. Randomized complete block designs, often used by scientists, have not been accurate designs for North Dakota (ND) soil and environmental conditions. Planting of all experiments was conducted from May 1 to May 31 (it is too risky to plant before May 1 even for research). Harvest has especially been challenging in 2008 due to October rainfall and low average seasonal temperature. As a consequence, harvest took longer than expected. It usually takes us 40-50 days with the number of experiments we manage in 2008 harvest was extended to December. This was a good year to wait for adequate moisture levels on early maturing corn. Most early maturing hybrids including ND lines were below 20%. Compared to top industry checks top NDSU hybrids had over 200% better drought and cold tolerance (through traditional breeding approaches and unique testing locations, e.g. high Montana elevations, western ND), over 150% improvement in grain moisture at harvest and fast dry down (especially in 2008), and over 110% on test weight, lodging resistance, extractable and fermentable starch, grain oil, grain protein, and over 105% for grain yield under dry land and, especially, under irrigated conditions. Most of these lines have been advanced for more multi-location, multi-trait, and multi-stage evaluation in 2009. Summer and winter nurseries including over 20,000 rows have generated diverse breeding techniques for observation, selection, and seed production. Winter nursery activities have focused on drought tolerance and seed production for hybrid testing. Summer nurseries included over 100,000 pollinations across 15 workers during July-August (we could use more). As a consequence, thousands of new crosses and lines were developed, produced, recombined, maintained, and distributed. Mating designs have produced thousands of progenies for basic and applied genetic and breeding studies. In addition to pre-breeding selection efforts, four reciprocal full-sib recurrent selection programs have been created to maximize genetic improvement of different heterotic groups. Breeding data, publications, breeding techniques, nursery details, and product releases has been disseminated through the web and numerous reports.

We have initiated and continued extensive partnerships with seed companies on testers, technology access (e.g. GMO, double-haploids, SNP marker technology, robotics), breeding approaches to drought tolerance, value added traits (e.g. starch utilization), test weight, and fast dry down with traditional and modern technologies. As a consequence of our trip to southern MN (Olivia, MN) breeding access to germplasm resulted to be very successful. Four graduate students, one research specialist, and several trainees were mentored in our breeding program during this period. National and international visitors were hosted and walked our nurseries, including representatives from major U.S. companies. All trainees were exposed to self- and cross-pollinating crop breeding techniques from pre-breeding to inbred line and hybrid corn development, release decisions, and intellectual property. Without exceptions, all graduate students (including former MS and Ph.D. students) were hired by public and private institutions. Industry cooperation has facilitated latest technology access to graduate students improving the efficiency of thesis projects (e.g. SNP marker technology reduces time and resources for NDSU, no need for academic labs that could become obsolete quickly). The breeding program, through its activities, has also directly served several graduate advanced teaching courses (e.g. genetics and plant improvement, quantitative genetics, crop breeding techniques, and advanced plant breeding).

Nine state unbiased performance trials were conducted to aid ND farmers in their objective hybrid selection. The decision of choosing new hybrids was not dependent on 'local' results. Hybrids were tested across locations within regions to aid producers select the most stable hybrid for their farms with minimum risk. We made sure results were not limited to one site. Top hybrids across locations were more stable, repeatable, and flexible to environmental change. Outreach has been extensive regionally, nationally, and internationally through continuous cooperation (e.g. industry), annual conferences, meetings, field days, annual visits from national and international scientists, and several invited national and international plenary lectures on early maturing corn breeding.

Without exceptions, early maturing hybrids including NDSU experimental lines showed better performance than industry hybrids in 2008 which shows the importance of the need to have reliable and stable early maturing hybrids developed exclusively for ND environmental conditions exclusively. ND challenging conditions were especially present in 2008. Data of all experiments are available upon request. Scientific peer-review publications, plenary lectures, and scientific oral presentations are also available upon request.

### **TEACHING AND ADVISING**

My teaching and advising goals are to work for the common good of our department and help NDSU increase a diverse graduate student enrollment with quality recruitment and instruction of two graduate courses (PLSC727 and PLSC781). A major challenge facing graduate education at NDSU though is how to recruit top quality students. For instance, the rapid expansion of the commercial sector and the lack of applied plant breeding graduates have generated a large demand for plant breeders. My position as Associate Professor/Corn Breeder has been essential to meet the future demands for plant breeders in the U.S. Our program has had a very high demand from potential students and employers. However, based on my experience advising over 15 MS and Ph.D. students in the past 10 years, there is a large variation in student quality. As a consequence, national large seed companies (e.g. Monsanto, DuPont/Pioneer, AgReliant Genetics) as well as international institutions have joined forces with the NDSU corn breeding program to attract top qualified individuals for graduate education and capacity building. An amount of \$395,000 has been secured by the NDSU corn breeding program on graduate student education fellowships with seed

companies in the past eight years. From those, over \$300,000 were from DuPont from which I received the DuPont Young Professor Award. Only in 2008, approximately \$100,000 dollars were awarded for graduate student education in corn breeding and genetics.

NDSU, represented by myself, has been recognized by the Senior Chief Director of the Plant Protection and Production Division from the Food and Agriculture Organization (FAO) of the United Nations in Rome, Italy (after an invited presentation) for being one of the foundations of the International Partnership Platform (IPP) of the Global Plant Breeding Task Forces for the International Plant Breeding Capacity Building Effort (GIPB) in April 2008. These efforts target high quality graduate student education. As a consequence of this advising initiative and cooperation, the Gates Foundation has made an award of \$1.2 Million dollars. Moreover, I was contacted by a Monsanto Global Manager to continue with these efforts through the Gates Foundation in December 2008. I was also contacted to provide direct advice to the Directors of the Plant Breeding Centers of Excellence at Universities of Illinois and North Carolina which have become very successful gathering funds and national leadership. These contacts, specifically the one by Monsanto, have encouraged me to apply for the current position of NDSU Assistant Dean for Graduate Studies. Also, I have provided the basic ideas for the Applied Plant Breeding Institute brochure and I have shared its goals nationally and internationally. I have disseminated information of it at annual meetings, poster and oral presentations, as well as in plenary invited lectures in 2008. I have also been actively involved in the national and international search for a new Department Head since July 2008. As a consequence, two of the five current candidates resulted from these unbiased efforts to find the top leading candidate.

My specific teaching goals were to continue, in cooperation with other faculty, to improve and update PLSC727 and PLSC781 based on student needs. Besides being trained by NDSU on 'Blackboard' and 'Campus Connection' and interacting with faculty with related courses, extensive industry cooperation was achieved with the purpose to link academic and industry experience. I have been able to access the latest technology (under confidentiality agreements) to avoid exposing students to materials and laboratory techniques that are currently obsolete. Both graduate classes were, in 2008, in the top three priorities for industry based on plant breeding training needs nationally (Monsanto, January 15, 2008). I have encouraged these classes to be required by all graduate students of plant breeding, genetics, and genomics students to the Curriculum Committee. I will also continue to use online resources for graduate students as it has been done in previous years for instruction management. All class contents are unique and have been written and updated by myself online based on corn breeding experience at NDSU in the past 10 years. Previous reports have stated that PLSC727 and PLSC781 syllabus, course materials, handouts, techniques have been made available on-line to anyone interested in crop breeding techniques and quantitative genetics. On-line videos and links with actual research experience applicable to courses were prepared and are under testing. Assessment data were generated for PLSC727 and PLSC781 and were submitted internally as requested. Both reports showed learning improvement (e.g. over 50% improvement based on student responses). However, it was not clear how the improvement was obtained since I felt that problem-based learning was not helpful particularly in PLSC781. A group of faculty including myself and representing several NDSU departments was involved with discussing these learning challenges and other problems of learning. Discussions on student challenging were very helpful and applied.

There has been demand to instruct these classes internationally. I was invited to instruct Quantitative Genetics (PLSC781) to professors and Ph.D. students representing different regions of Portugal (Lisboa, Coimbra, Oporto, and Braga) at the Instituto Politecnico and Escola Superior Agraria de Coimbra (Department of Plant Breeding), a teaching load of six hours per day in June 2008. As a consequence, NDSU Plant Sciences, ND Agricultural College, and NDSU Corn Breeding Units, as well as the USDA were formally recognized in a letter and a common award was obtained. Formal evaluations were conducted in the same scale as NDSU, they all were well above average, and are available upon request.

For innovation I have added as new initiative the availability of credits for undergraduate and graduate students on 'corn breeding experience'. Breeding techniques in a cross-pollinated crop such as corn are very diverse. Due to the need of developing not only inbred lines but also hybrids, both self and cross-pollinated breeding techniques are utilized. With this innovation, students are able to be exposed to both types of techniques and not just the ones for self-pollinated crops. This could be useful for even faculty learning the principles behind hybrid cultivar development which are much more complex (e.g. canola, sunflower).

I wrote preliminary exam questions (Oct 2008 and April 2008). I have also led the collaborative national and international edition effort to develop a book on Cereals (breeding) where most of my NDSU faculty colleagues were involved with. Springer was the Editorial.

I have served as advisor to the National Corn Germplasm Committee at the ASTA, and the United Tribal Council (UTC) representing numerous Native American reservations on a common goal to develop a federal common research project. As a consequence, a SARE grant was obtained for \$150,000. I have also served as advisor to the biofuel grant awarded to the NDSU Department of Agribusiness and Applied Economics.

My advising goal is to continue to train as many good graduate students as possible by emphasizing the recruitment of students with excellent potential. The NDSU corn breeding program had the uniqueness to train the next generation of breeders through not only germplasm adaptation and improvement but also on cultivar development and release decisions. I served as major advisor of two MS students (C. Eno from Cameroon, and S. Sharma from Nepal) and two Ph.D. students (J. Yang from China and T. Laude from Philippines), several interns, seminar presenters, and as a member of a graduate student committee (M. Ostlie). Retention of students was 100% and one professor from Bangladesh was accepted as Ph.D. student. Theses and dissertations focused on:

- 1) Adapting tropical and late temperate corn germplasm to increase the genetic diversity of North Dakota corn hybrids (S. Sharma)
- 2) Understanding the genetics of dry down and test weight in early-maturing industry x NDSU hybrids (J. Yang)
- 3) Evaluation of recurrent selection populations for grain quality and amino acid composition (T. Laude)

All of these research projects were directly involved with the breeding program goals of adaptation, genetic improvement for cold and drought tolerance among other traits, and the development of new and unique germplasm and lines.

Five manuscripts were published or accepted for publication from former graduate students (two from Eno, one from Sezegen, one from Osorno, one from Jumbo). M. Melani is the only former graduate student from the NDSU corn breeding program that has not published his research and it is, therefore, the exception. Student work by Eno and Yang has actively assisted in the release publication of four early maturing inbred lines and four early maturing populations.

I have served as member of the Curriculum and Graduate Studies (Experimental Design) Departmental Committees. I have participated on the creation of an undergraduate course on plant breeding and statistics, the horticultural program, and worked actively toward a better and unbiased Ph.D. prelim system for the benefit of future Ph.D. graduates representing NDSU and its reputation at other institutions.

## Description of the importance of the project

North Dakota corn hybrids are still late maturing, lack stress tolerance, and often, like in 2008, end up with poor quality. The demand for new early maturing corn hybrids in the state is going to increase due to the current costs associated with farming. The knowledge and products generated with the North Dakota State University (NDSU) corn breeding program have aided in the maintenance of corn production interest and the significant increase in ND corn acreage (2.6 million acres) and production (larger than wheat in number of bushels). The NDSU corn breeding program needs to keep strong as the most northern public breeding program in North America. NDSU lines have been recognized by industry as 'workhorse' females yielding over 100 Bu/ac for seed hybrid production for earlier maturing and faster drying hybrids.

Corn value decreases when harvested at high moisture levels. Identifying and developing new early maturing corn inbred lines in close cooperation with the seed industry are a long-term solution for maintaining profit statewide. This project allows extensive testing of hybrids across the state so that commercial corn hybrids available in the ND market become earlier and safer for ND producers. NDSU and industry complement each other growing trials across all locations so that hybrids are exposed to all environmental ND challenging conditions. In addition, the NDSU corn breeding program will increase the value added alternatives to ND farmers.

Extensive breeding efforts for early maturing corn have changed the conventional wisdom favoring longer maturing hybrids and have increased active cooperation to more than 30 public and private partners. Early maturing hybrids (80RM) have shown to outperform later maturing hybrids (85-90RM) after extensive testing. Additional data will be essential for ND corn producer decision making. Even across and within maturity groups, the faster a hybrid can dry down the better it can adapt to the challenging ND environmental conditions. NDSU corn breeding research leads the efforts toward understanding the genetics behind dry down and test weight, toward developing a new technique for measuring dry down (has been very challenging to measure this trait with accuracy), and toward identifying those hybrids with similar maturity but significantly better dry down capabilities genetically.

The lack of early maturing industry testers and the uniqueness of our program (e.g. only program with extensive testing in northern and western ND) have influenced industry to offer breeding rights access to NDSU recognizing the industry need to develop better early maturing hybrids and, thus, recognizing the NDSU corn breeding efforts and its capabilities to develop better products than the one available in the U.S. northern market. These and other industry partnerships have saved NDSU over \$500,000 in corn breeding research during 2008-2009.

Our breeding program has initiated the first long-term project to increase the genetic diversity of hybrids on farms in the U.S. northern Corn Belt. As a long time member of the USDA-GEM network, our breeding program has created a unique source of tropical and late temperate genetic materials adapted to ND through the EarlyGEM program, NDSU leads these efforts nationally. In fact, we have generated the first thesis project for the GEM national network. This had practical implications toward new ways of conducting corn breeding for very early maturing areas with highest potential for significant and fast genetic gain attracting national and international attention (e.g. invited plenary lectures in Western and Eastern Europe, similar corn growing latitudes and needs).

For ND producers, ethanol plants are a new market for their corn and an incentive. The development of new early-maturing hybrids is a unique opportunity to increase offer and demand for a desirable corn-ethanol relationship. These products allow corn to be planted earlier due to cold tolerance and to be harvested earlier due to a faster dry down, which is especially important for ND corn producers. The development of elite early-maturing inbred lines reduces the risks associated with late planting, drought, early frost, poor quality, and seed production costs.

This project addresses SBARE and ND Corn Council Utilization strategic goals at the same time by conducting research that is relevant to the state needs and by working toward enhancing the profitability of ND corn producers and ND corn industry, partnering with ND corn producers and industry, and generating new information.

## 2008 Publications

The number of corn breeding publications has achieved a new record high in 2008. The following peer-reviewed manuscripts were published:

- Hammond, J., and Carena, M.J. 2008. A breeding plan for molecular markers. ASA-CSSA-SSSA, Houston, TX.
- Hallauer, A.R., and Carena, M.J. 2008. Maize breeding. In: M.J. Carena (ed.). Cereal breeding. Springer, NY (in press).
- Carena, M.J. 2008. How many commercial maize heterotic patterns are available? ASA-CSSA-SSSA, Houston, TX.
- Carena, M.J., Pollak, L., Salhuana, W., and Denuc, M. 2008. Development of unique lines for early-maturing hybrids: Moving GEM germplasm northward and westward. *Euphytica* (in press).
- Bahadir, S., and Carena, M.J. 2008. Divergent recurrent selection for cold tolerance in two improved maize populations. *Euphytica* (in press).
- Eno, C., and Carena, M.J. 2008. Response to stratified mass selection for earliness in four elite maize populations non-adapted to North Dakota. *Maydica* (in press).
- Osorno, J., and Carena, M.J. 2008. Creating groups of maize genetic diversity for grain quality: implications for breeding. *Maydica* 53:131-141.
- Carena, M.J., and Wanner, D.W. 2008. Development of genetically broad-based inbred lines of maize for early maturing (70-80RM) hybrids. *J. Plant Reg.* 3:107-111.
- Carena, M.J. 2008. Increasing the genetic diversity of northern U.S. maize hybrids: Integrating pre-breeding with cultivar development. In *Conventional and molecular breeding of field and vegetable crops*. Novi Sad, Serbia.
- Yang, J., Carena, M.J. 2008. Identification of early maturing lines with high test weight and fast dry down. Maize Genetics Conference.
- Carena, M.J. 2008. Development of new and diverse lines for early-maturing hybrids: Traditional and modern maize breeding. In *Modern variety breeding for present and future needs*. J. Prohens and M.L. Badenes (Eds.). EUCARPIA, Valencia, Spain.
- Carena, M.J., Eno, C., and Wanner, D.W. 2008. Registration of NDBS11(FR-M)C3, NDBS1011, and NDBSK(HI-M)C3 maize germplasms. *J. Plant Reg.* 2:132-136.

Jumbo, M.B., and Carena, M.J. 2008. Combining ability, maternal, and reciprocal effects of elite early-maturing maize population hybrids. *Euphytica* 162:325-333.

#### **Major Synergistic Activities**

Elected ASTA Crop Germplasm Committee (2007-present)  
Public Breeding Member of Federal Multi-institutional U.S. Germplasm Enhancement Maize (GEM, 1999-present)  
Elected Secretary/Treasurer (2006-2011), NCR Corn Breeding Research Committee (NCCC-167, 2006-2011)  
Elected Chair, Early maturity subcommittee (NCCC-167, 2003-present)  
Executive Member, FAO Global Plant Breeding Initiative, 2005 – present)  
Executive Member, Elected Chair, Multi-state Plant Breeding Coordinating Committee (SCC-80, 2006-2007)  
Coordinating Editor, *Euphytica* (International Journal of Plant Breeding, 2001 – present)  
Editorial Board, *Maydica* (Journal of Maize and Allied Species, 2006 – present)  
Chair, Maize Registration Committee, Crop Science Society of America (2003 – present)  
Senior Editor, Springer (2006-present)  
Plenary Lecturer at Limagrain (2001), FAO (2007), EUCARPIA (2008), Serbia (2008)

#### **Graduate Students and Visiting Scientists**

Clarissa Barata (Brasil)	MS Graduate, currently at Monsanto Trait Integration
Alyson Hyrkas (USA)	MS Graduate, currently at USDA Illinois
McDonald Jumbo (Malawi)	MS Graduate, currently Ph.D. student at U. of Delaware
Marcelo Melani (Argentina)	MS/Ph.D. graduate, currently corn breeder at Dow/Mycogen
Juan Osorno (Colombia)	Ph.D. graduate, currently NDSU dry bean breeder
Bahadır Sezegen (Turkey)	MS graduate, currently Ph.D. student at NDSU
Colins Eno (Cameroon)	MS graduate, currently Ph.D. student OSU
Junyun Yang (China)	Ph.D. student
Santosh Sharma (Nepal)	MS student
Tonette Laude (Phillippines)	Ph.D. student
Fernanda B. da Silva (Brasil)	Visiting Scientist (2003-2004)
Xihua Zhang (China)	Visitng Scientist (2005-2006)
Duane Wanner	Research Specialist I (1971-present)
Gregory Lammers	Research Specialist II (2008-present)

**NCCC-167 Annual Report**  
**Ohio Agricultural Research and Development Center**  
**The Ohio State University**  
Rich Pratt, Professor  
Dept. of Horticulture and Crop Science  
Wooster, OH

**Mission and Program Description**

The OSU maize breeding and genetics program focuses on improvement of maize germplasm for resistance to infection by pathogenic agents causing foliar disease. Research is also conducted to examine compositional traits as they relate to grain quality characteristics suitable for food, feed, and industrial end-uses. Collaborative research is undertaken in projects involving molecular genetics and breeding for organic production systems. I advise graduate students, serve on graduate student advisory committees, teach two graduate level courses, and serve as Chair of the Graduate Studies Committee.

**Program Highlights, March 2008 to February 2009**

*IPM-CRSP Project:*

*Verification of Quantitative Trait Loci (QTLs) Conferring Resistance to Multiple Foliar Pathogens of Maize and their Utility for Marker-Assisted Selection:*

The original project was concluded and the former graduate advisee (Dr. Asea Godfrey) has become the Director of the Cereals Program (maize and rice) for the National Agricultural Research Organization in Uganda. The first publication from his dissertation research will be published in *Phytopathology* (please see below). Breeding lines with high levels of resistance were intercrossed to begin pyramiding resistance genes and crosses of these lines were made to QPM stocks and to Corn Belt testers. Further research and breeding will be continued as part of a new IPM CRSP project.

*Maize Breeding*

Grain samples from diverse populations were evaluated for compositional traits. A large range of phenotypic values were observed. A new Foss InfraXact is being used and the Tecator 1225 has been updated with new calibrations from ISU. Observed compositional values for breeding lines evaluated in 2008 (at 0% mois. basis) were as follows: protein 13.3 – 16.6%, oil 3.4 – 5.1%, and starch 64.0 to 67.7% (B73 values were 12.6%, 4.4, and 67.7%, respectively). Performance evaluations were conducted at two locations in Ohio and in the 700-800 maturity test in Texas, Georgia, and Ohio. Testcrosses between OSU breeding lines were made with Iowa Stiff-Stalk Synthetic (BSSS) and Corn Borer Synthetic testers. Selfing and test-cross production continued in other populations. Other specialty breeding programs are continuing. One testing site is now in transition to organic certification. High wind conditions following Hurricane Ike were experienced in 2008 field tests in Wooster and at South Charleston.

*Host Resistance Research*

Corn Redness Syndrome: Research on maize redness (causal agent is the stolbur phytoplasma) continued in cooperation with scientists at the Institute for Plant Protection and the Environment in Zemun, Serbia. The epidemiology of the disease has now been described and will be published in *Phytopathology* (please see below). Diagnostic procedures necessary to detect the pathogen are being established and research to examine the genetically variable genomic regions of maize bushy stunt and the Stolbur phytoplasmas is underway. Sequencing of the complete genomes of maize bushy stunt phytoplasma and stolbor phytoplasma have been initiated.

## **Significant Accomplishments and Impacts:**

### *IPM-CRSP Project*

Research has contributed to our understanding of host responses to infection, assisted the development of effective selection protocols, and increased our understanding of the number and action of genes for resistance. More efficient and expedient development of maize that can resist yield loss will result.

### *Host Resistance*

Research has established the causal agents, insect vector, and epidemiology of maize redness in Serbia. Management and breeding approaches to address disease losses can now be formulated.

## **Collaborators:**

Peter Balint-Kurti, USDA/ARS, NCSU  
Pat Carr, NDSU, ND  
Dave Christensen, Seed We Need, MT  
Asea Godfrey, NARO, Uganda  
Walter Goldstein, M.F.I, WI  
Saskia Hogenhout, John Innes Centre, U.K.  
Maury Johnson, Blue River Organics, IA  
S. Krnjajić, Institute for Plant Health and Environment, Serbia  
Suzanne Nelson, Native Seeds/SEARCH  
Peg Redinbaugh, USDA/ARS, OSU  
Joe Scheerens, OSU  
Margaret Smith, Cornell University, NY  
Ivo Toševski, CABI, Switzerland  
B. Vivek, CIMMYT, Zimbabwe

## **Current Personnel**

- |                  |                          |
|------------------|--------------------------|
| 1. Rich Pratt    | Project Leader           |
| 2. Mark Casey    | Research Assistant       |
| 3. Si Hwan Ryu   | Ph.D. Student            |
| 4. Valdir Correa | Ph.D. Student            |
| 5. Kalie Rock    | Student Assistant (2008) |
| 6. Cameron Exner | Student Intern (2008)    |

## **Publications:**

### **Journal Articles:**

- Asea, G., B. Vivek, G. Bigirwa, P.E. Lipps, and R.C. Pratt. 2009. Validation of consensus quantitative trait loci associated with resistance to multiple foliar pathogens of maize. *Phytopathology: (In Press)*
- Jović, J., T. Cvrković, S. Krnjajić, A. Petrović, M.G. Redinbaugh, R.C. Pratt, S.A. Hogenhout and I. Toševski. 2009. Maize redness transmitted by *Reptalus panzeri*: the disease cycle in Serbia. *Phytopathology: (In Press)*

## **Review Articles and Book Chapters:**

- Pratt, R.C., D.M. Francis, and L.S. Barrero-Meneses. 2008. Genomics of Tropical Solanaceous Species: Established and Emerging Crops. p. 453-467. *In*: R. Ming and P. Moore (eds.) Genomics of Tropical Crops. Springer/Verlag, New York.
- Poland, J., P. Balint-Kurti, R. Wisser, R. Pratt and R. Nelson. 2009. Quantitative disease resistance in plants. Trends in Plant Science. (In Press)
- Redinbaugh, M., and R.C. Pratt. 2009. Virus Resistance. p. 251-270. *In*: Handbook of Maize: Its Biology 2nd ed. (J. Bennetzen and S. Hake, eds.). Springer-Verlag. New York.

**Grants and Contracts:**

- 2006–2008, AgBioScience Innovations Grant, OARDC, Commercialization of multi-functional plant germplasm. Co-PI.
- 2007–2008, Organic Farming Research Foundation. Development of Corn Borer-Resistant Corn for Organic Farming Systems. Collaborator.
- 2007, International Agricultural Science and Technology Fellows Program. USDA/FAS. Co-PI.
- 2008–2010, Development of Genetic Variation for Anthocyanin and Carotenoid Pigment Content in Native American Maize. OARDC Research Enhancement Competitive Grant Program, Seed Grants. Co-PI.
- 2009–2011, Integrated Pest Management in Uganda. USAID IPM CRSP. (Pending) Co-PI.

**Goals for Next Year:**

Complete G. Asea research publication submission. Analyze data and prepare results of maize redness research on yield impacts for publication. Submit manuscript describing type three secretion system of *Pantoea stewartii*. Continue evaluation of germplasm for selected traits and conduct field evaluations of testcrosses. Continue characterization of genetically variable regions of maize bushy stunt phytoplasma.

## NCCC167 2008-9 ANNUAL REPORT FROM THE UNIVERSITY OF WISCONSIN

The overall goal of our research program is to develop germplasm and scientific information that support the use of corn for silage and as a feedstock for the biofuel industry.

### *Current Research Projects:*

1. **Corn silage breeding program:** The University of Wisconsin operates a corn silage breeding program focused on the development of corn varieties with enhanced compositional attributes and forage yield. One important source of germplasm of this breeding program is the Wisconsin Quality Synthetic (WQS), specifically designed to produce high-quality inbreds for use as parents for silage hybrids. WQS is continuously improved using a S2-testcross recurrent selection breeding method, and inbreds derived from succeeding cycles of improvement are developed to the S6 stage and released (See <http://cornbreeding.wisc.edu/>). The nutritional improved characteristic of WQS germplasm are low neutral detergent fiber (NDF), high *in vitro* true digestibility (IVTD), high NDF digestibility (NDFD), and low lignin concentration. Crosses derived from WQS C3 were evaluated in our 2008 summer trials, and additional S6 lines will be available for release Fall 2009. During summer 2007 the 20 best S2 families from WQS C3 were recombined to create WQS C4. A set of 200 S1s from WQS C4 were developed during the winter nursery of 2007-08 from this later cycle and visually evaluated during the 2008 summer nursery for *per se* agronomic performance. A selected subset of those lines was selfed during summer 2008 and testcrossed during winter nursery 2008-09 for evaluation during summer 2009.

In 2005, a new breeding population from a heterotic group complementary to WQS was initiated, and this population is designated the GEM Quality Synthetic. The breeding population GQS will be advanced in a manner similar to WQS. Selected families from Cycle 0 (GQS C0) of this population were recombined in summer 2007 to give rise to GQS C1. Approximately 200 S1 families were generated in summer 2008. Selected S2 families will be further selfed and crossed by appropriate testers for evaluation during summer 2009 for evaluation in 2010. Advanced lines from GQS C0 will be evaluated in summer 2009. Hybrids from the cross of inbred lines from GQS and WQS should have both excellent forage yield and quality because both sides of the hybrid pedigree have been under selection for these characteristics. This will increase the interest in and value of inbred germplasm from the UW silage breeding program. Additionally, inbred lines have also been developed from breeding populations provided by the USDA Germplasm Enhancement of Maize (GEM) project. Selection has been conducted for nutritional composition on both an inbred *per se* and hybrid topcross bases. Five promising lines have been identified and were released in Fall 2008 from these GEM breeding populations. More releases are expected to occur in Fall 2009, if evaluations warrant it.

### 2. Evaluation of S2-topcross selection for silage quality in the Wisconsin Quality Synthetic (WQS)

**corn population:** WQS was evaluated after the second cycle of selection by Frey et al. (2004 – Crop Sci 44: 1200-1208). While selection was successful for several silage quality traits, many of the changes were not statistically significant, and it was not clear if increases in silage quality could be attributed to increased digestibility of the stover or an increased contribution from the highly digestible ear. The primary objective of M.S. student Tim Gustafson research project was to evaluate the forage yield and quality attributes of the WQS population after the fourth cycle of selection on both a whole plant and stover level to further investigate the trends previously seen.

In summer 2007, cycles 0 through 3 of the WQS (WQS C0 and WQS C3, respectively) population *per se* as well as respective testcrosses to two stiff-stalk testers, LH244 and LH332, were grown in three replications at two locations (Madison and Arlington, WI) in a randomized complete block design. A brown midrib hybrid, a high lignin and low-digestibility check, two commercial grain hybrids as well as

two hybrids using the inbred parent W604S, a high quality silage inbred developed from WQS, crossed by inbred lines LH244 and LH332 were also included. The experiment was repeated in 2008 including WQS C4 *per se* as well as corresponding testcrosses by LH244 and LH332.

The entire experiment was done in triplicate: one trial (WQS WP) for whole plant silage analysis, a second trial (WQS ST) with ears stripped at silage stage for stover analysis, and a third trial (WQS BIOFEED) ear-stripped and harvested at physiological maturity. WQS BIOFEED was investigated for changes in stover composition at full maturity during the selection process for silage stage traits. Forage and grain yields, quality composition and other agronomic traits were evaluated. Results from that evaluation indicate that significant linear improvement was seen in whole-plant yield, stover yield, and whole-plant quality both in the population *per se* and in testcrosses. Stover quality was not improved through selection. Starch content has increased in whole-plant samples while ear percent has remained relatively constant suggesting an increase in the grain-to-cob ratio. Overall milk yield on a Mg of dry matter and on a hectare bases have increased with selection. Changes in silage dry matter yield have been greater on a percent basis than changes in silage quality suggesting that the current selection protocol of selecting S2 topcrosses first for yield then for quality may be more efficient at improving yield. A separate selection directly focused on stover quality may be necessary if more rapid improvement in stover composition is desired. An oral presentation describing these results was presented by Tim Gustafson at the NCCC167 annual meeting at the Allerton House Conference Center in March of 2009.

**3. Quantitative trait loci controlling yield and feedstock quality:** Corn stover has been identified as an important feedstock for production of cellulosic ethanol in North America. One component of improving the economics of this potential industry is to develop corn hybrids that have higher stover yield and quality, and therefore produce more fermentable carbohydrates per area of land. Because these quantitative traits are relatively novel and may be highly important to producers, fundamental questions regarding their inheritance and genetic architecture should be answered to most effectively accomplish that goal. In this study, Ph.D. student, Aaron Lorenz evaluated 206 recombinant inbred lines (RILs) of the intermated B73/Mo17 (IBM) population on a *per se* basis. A subset of the above RILs was testcrossed to a common inbred tester and 127 of those hybrids were also evaluated. Progenies were evaluated at multiple environments (inbred - four, hybrid - two) in a randomized complete block design with two replications. Traits assessed included stover yield, theoretical ethanol potential and other relevant stover constituents. Results from this research indicate that no undesirable correlations were observed between agronomic and cell wall traits. Polysaccharide content was either positively correlated to, or not correlated to NDFD, indicating that increasing both carbohydrate content and convertibility is feasible. QTL were identified for all traits evaluated, but few QTL were in common between inbred *per se* and testcross evaluations, despite moderate to high genotypic correlations between progeny types. This study provides information that will support the development of dual-purpose corn hybrids that are high in grain yield and also high in yield of digestible cellulosic feedstock. A publication defining genetic loci controlling plant biomass accumulation and cellulosic biomass characteristics will be submitted in April 2009.

**4. Diallel experiment evaluating dry tonnage contribution and quality of corn stover for biofuel:** An experiment was conducted to evaluate the compositional attributes of leaf blade, leaf sheath, stalk, cob, midrib and husk fractions of 23 hybrids. Twenty hybrids derived from a factorial mating design between a set of high-quality inbred lines (W601S, W602S, W603S, W604S and W605S) and four standard inbred testers (HC33, LH244, LH332 and TR725). A brown-midrib hybrid, a Leafy hybrid, and a commercial grain hybrid were also included as checks. Plants were harvested and

dissected into the aforementioned parts at four developmental stages corresponding to V3, V12, R3 and R6. Tissues

were dried and ground for compositional analysis, which included neutral (NDF) and acid detergent fiber (ADF) and *in vitro* ruminal digestibility. In addition, dry matter weights for each component were taken at the R6 stage. Evaluations were conducted at two locations on field plots arranged in a randomized complete block design with two replications. The objective of this experiment was to determine the relative contribution of individual plant parts to whole plant yield and composition as well as assess the relationship of plant part composition between different stages of development. Preliminary results for NDF and ADF indicate significant differences among hybrids for most plant parts. Stalk was the largest fraction of whole plant dry matter at R6 and stalk ADF was associated with whole plant ADF at R6. For stalk ADF, no association was found between early developmental stages (V3 and V12) and R6, suggesting that phenotyping for quality at early stages of development is not indicative of quality at R6. Furthermore, we found a significant entry-by-harvest time interaction for leaf blade ADF. Plant part-by-entry and plant part-by-location interactions were significant in R6 and indicate that the relative quality of hybrids could significantly depend on the plant part evaluated and the relative quality of plant parts could significantly depend on the growing environment. An oral presentation describing these results was presented by graduate student Candy Hansey at the NCCC167 annual meeting at the Allerton House Conference Center in March of 2009. A publication is expected to be completed in 2009.

**5. Evaluation of endogenous modifiers of expression of *grassy tillers1* mutant:** The ability to increase plant biomass per acre of land is an ever-growing concern as the biofuel industry continues to expand. Our hypothesis is that lateral branch (tiller) formation is one important method to increase biomass production in corn. *Grassy tillers1* (*gt1*) effects lateral branch development in corn. The penetrance of *gt1* is highly dependent on genetic background. E. G. Anderson's mutant *gt1* allele was backcrossed into W117Ht, W64A and W182BN to generate three sets of near isogenic lines used for studying *gt1*. The goal of this study is to characterize the phenotype and expression pattern of *gt1* in backgrounds with varying penetrance, to better understand how it can be used in breeding programs to increase biomass production per unit of area.

Two field trials were conducted to answer some more specific questions.

I. Effect of density on biomass yield of tillering and non-tillering morphologies - It is hypothesized that a tillering corn morphology will result in increased biomass production per unit of land. Twelve hybrids with varying degrees of tillering have been developed from the crosses of three non-tillering genotypes and three tillering genotypes to test this hypothesis. In addition, *leafy1*, brown midrib and a grain check were also included. Hybrids were evaluated at two different densities (70,000 and 20,000 plants ha<sup>-1</sup>) in a randomized complete block design restricted into a split plot layout with two replications at two locations in WI for two years. At physiological maturity ears were stripped from all plants in each plot. Ears were dried, shelled and cob biomass and grain yield were measured on a plot basis. The remaining above ground biomass (stover) was machine harvested, dried and weighed. Stover and cob weights were added to determine total biomass production per plot. In addition to that, morphological traits including tiller number and tiller width were also measured.

II. Effect of row-crop versus equivalent spacing on biomass production potential - It is hypothesized that a planting regime that provides equal distance among plants would result in increased biomass production per unit of land. The basis of this hypothesis is that equivalent spacing would allow maximum light interception by all plants and will thus maximize biomass production. To test this hypothesis two planting regimes were evaluated. The first planting regime was the traditional rowcrop planting. The second planting regime (equivalent spacing) had the same plant density ha<sup>-1</sup> however plants were planted with equal distance between plants within and between rows. This experiment also included three hybrids resulting from crosses among tillering lines and three hybrids produced by the

cross of the non-tillering isolines. In addition *leafy1*, brown midrib and other grain checks were also included under both planting treatments. Planting treatments were evaluated using a randomized complete block design restricted into a split plot layout with two replications at two locations in WI for two years. In both planting treatments planting densities were approximately 70,000 plants ha<sup>-1</sup>. Biomass yield and morphological traits were evaluated as described for field trial I. These two experiments will be repeated again in 2009 for additional environments of data. III. In addition to that transcriptional profiling of near isogenic lines with varying degrees of penetrance was conducted to determine which genes are uniquely differentially expressed in backgrounds with varying levels of penetrance as well as genes that are always differentially expressed will provide insight into the genetic mechanisms controlling *gt1*. Long oligonucleotide spotted arrays probed with RNA from meristematic tissue of different genotypes were used to evaluate differential gene expression 48 hours prior to initial elongation of the lower axillary meristem. Understanding how genes interact with other genes in a pathway can provide insight as to which gene combinations will be the most effective. A poster describing the preliminary results of the transcriptional profiling work was presented at the Maize Genetics Conference in Washington, DC in February of 2008. A poster describing these field trials preliminary results was presented at the Maize Genetics Conference in St Charles, IL in March of 2009.

**6. Great Lakes Bioenergy Research Center (GLBRC):** Two primary areas of research are being sponsored by the GLBRC for our group in collaboration with Shawn Kaeppeler and Heidi Kaeppeler.

I) Development of rapid-cycling, small statured corn varieties - Corn is an important model organism and crop species. Corn will be the largest near-term cropland source of lignocellulosic biomass with over 85 million acres grown in 2008. It is also the nearest model species relative of other high priority feedstocks including switchgrass and Miscanthus. The goal of this project is to develop rapid flowering, small-statured varieties of corn amenable to high-throughput genetic analysis in controlled environments and at locations without access to agronomic production fields.

II) Exploitation of the endogenous genetic variation of corn to identify genes related to important traits for the improvement in the utilization of biomass as a source of feedstock for the biofuel industry. The aim of this project is to identify the genetic locations of genes that explain the differences among diverse corn genotypes. This will be accomplished through the utilization of quantitative trait locus mapping analysis of populations such as the Intermated B73 X Mo17 (IBM) and the Nested Association Mapping (NAM). Potentially interesting regions will be further dissected by utilizing a diverse set of inbred lines chosen to represent a broad diversity of corn that will mature in Wisconsin. Preliminary phenotypic data gathering on this material took place in 2008 and will continue in 2009.

## The Future of Corn Breeding

In March of 2007, leaders in the maize genetics community met for a two-day retreat to discuss the strengths, challenges, and initiatives that define the future of maize genetic research in the post-genome era. The maize genetics community generated a report entitled “The Future of Maize Genetics: Planning for the Sequenced Genome Era” with the following short-term and long-term planning objectives:

### Short Term Planning (1-3 years):

1. Current sequencing/annotation will be completed and additional map-based sequencing efforts initiated.
2. Centralized databases with increased funding are needed now.
3. Indexed reverse genetic resources need to be finalized and will accelerate many areas of research. Current mutagenesis libraries should be indexed with new technologies.
4. Expression platforms and informatic tools should be selected and developed.
5. Transformation capacity should be enhanced in the public sector. Continuous support mechanisms for public transformation need to be resolved.

### Long Term Planning (Start now, with sustained efforts over the next decade):

1. Databases and stock center capacity will be enhanced, coordinated and supported.
2. Map-based sequences of other inbreds, races, and teosintes will be available.
3. A phenomics initiative will be underway, with large scale and multi-dimensional phenotyping capabilities for multiple environments available for the entire community.
4. The maize scientific community will be large, diverse, well-trained, and interactive.

The Maize Genetics Executive Committee adopted to these planning objectives, and has pursued funding to realize these objectives.

In March of 2009, the NCCC167 Corn Breeding group was asked to hold similar discussions about the future of corn breeding in the post-sequenced genome era. Many of the “Challenging unanswered questions in biology” presented in the 2007 maize genetics report are in line with those of breeders, although from a slightly different viewpoint:

- How is genomic diversity maintained, and how does it change *during selection*?
- What is the underlying molecular genetic basis for specific traits?
- Can natural variation provide information to develop novel breeding traits?
- How are *genetic effects* impacted by interaction with the environment (*i.e. GxE*)?
- What is the genetic, molecular and physiological basis of hybrid vigor (heterosis)?

With respect to the Maize Genetics Community short-term planning list, the Corn Breeding Community related most closely with objectives concerning databases. For the Corn Breeding Community to apply genetic and genomic techniques to breeding programs, the breeding community needs a database for integrating pedigrees, phenotypes, markers, QTL, and genes.

Of the currently available databases, Gramene is the closest to meeting our needs, except that phenotype and pedigree information would need to be incorporated.

The Maize Genetics Community long-term planning list is largely compatible with the expertise of the breeding community: germplasm, phenotypes, and integrative research.

- While the breeding community acknowledges the importance of B73 for the reference genome sequence, breeders, by their very nature, cannot be restricted to using a single inbred line or even a set of 100 inbred lines. Breeders constantly generate new materials by breeding and selection, often resulting in inbred lines that are used in genetic studies.
- Phenotypes are an area of specialty for corn breeders, although breeders tend to define a phenotype differently than geneticists (i.e. a phenotype is more than just the result of a single gene knockout). Specific needs in this area include a need to standardize phenotypes across the community (e.g. consistent disease rating scales) and high-throughput phenotyping tools.
- Breeding is integrative discipline, requiring knowledge in breeding methodology, statistics, quantitative genetics, plant pathology, entomology, physiology, etc. The number of institutions that have active corn breeding programs and/or rigorous quantitative genetics programs has diminished over the last decade, leaving fewer institutions to meet private sector demand for graduates with corn breeding experience. Thus the corn breeding community will need to be large, diverse, and well-trained. The breeding community must also strive for more collaboration. These collaborations will be between public and private corn breeding programs, and between breeding and genetics. A primary need in this area is a funding mechanism to support breeding-oriented collaborations.

Over the past decade, there have been increasing concerns about the role of public corn breeders, especially in light of the fact that the North Central Region Corn Breeding Committee has recently changed from a "research committee" to a "communications committee." An added challenge to public corn breeders is to "bridge the gap" between breeders and the larger community of maize geneticists, i.e. to take results of genetic studies from the lab to the field. Finally, public corn breeders must find a niche that is distinct from private corn breeders, yet foster collaborations that are beneficial to both private and public breeders.

While the Maize Genetics Community is following a clear advancement of research, breeders are experiencing a paradigm shift. It used to be that breeders were simply trying to advance a trait via selection; now breeders are being asked to use far more information than before. How are breeders going to get the traits to the field, integrating new tools with selection. The private sector has figured this out, but the public hasn't. If this is merely a capacity issue, perhaps the Corn Breeding Community need to ask for more money to increase our capacity? Perhaps the Corn Breeding Community should mimic the Maize Genetics Executive Committee, which has been very successful in their ability to prioritize the needs of the community as a whole and pursue funding to address those needs.

At the 2008 NCCC-167 business meeting, an ad hoc executive committee (Bill Beavis, Torbert Rocheford, Liz Lee, Wenwei Xu, Jim Hawk, and Bill Tracy) was formed to begin addressing some of the above issues. Goals included 1) define who might be interested in the breeding group; 2)

implement a survey of important topics; 3) decide on an interim meeting date and location; and 4) define the mission of the new group. A subset of the members met at the Plant Breeding (SCCC-80) meeting in June, but little progress was made on reaching these goals.

At the 2009 NCCC-167 meeting, a provisional Corn Breeding Executive Committee (CBEC) (Martin, Paul, Sherry, Travis Frey, and Tom Brutnell) was formed. The provisional committee was charged with the following responsibilities: 1) draft a mission statement for Corn Breeding Community; 2) draft by-laws for the CBEC; 3) expand the Corn Breeding Community by emailing various groups including the IL Corn Breeders School attendees, the 2006 Plant Breeding Survey responders, the Maize BioList, and the Maize Genetics Community ListServe, using self-definition as corn breeder as the primary qualification; 4) approve the by-laws by the newly defined group; 5) conduct formal elections for the Executive Committee; and 6) develop a new web page for the broader Corn Breeding Community, independent of the NCCC167 group.

To summarize, the Corn Breeding Community has identified several short term goals and/or needs: development of databases for integration of breeding information; establishment of a high throughput phenotyping capacity; increased collaboration between genetics and breeding research, and between public and private breeders; and a mechanism for funding breeding-oriented research projects. In order to address these needs, the Corn Breeding Community will need to expand its membership, and appoint an Executive Committee that will represent the Corn Breeding Community and pursue funding to support public corn breeding.

Attendance:

<u>Researchers/Faculty/Staff</u>		<u>Students</u>	
Tom Brutnell	Cornell Univ.	Anastasia Bodnar	Iowa State Univ.
Peter Peterson	Iowa State Univ.	Milly Kanobe	Iowa State Univ.
Walter Goldstein	Michael Fields Ag. Inst.	Andrew Burt	Univ. Guelph
Travis Frey	Monsanto	Gloria Iriarte	Univ. Guelph
David Mies	Syngenta	Talon Becker	Univ. Illinois
Richard Pratt	The Ohio State Univ.	Eunsoo Choe	Univ. Illinois
Martin Bohn	Univ. Illinois	Yang Fu	Univ. Illinois
Kevin Montgomery	Univ. Illinois	Andrew Hauck	Univ. Illinois
Natalia de Leon	Univ. Wisconsin	Cathy Kandianis	Univ. Illinois
Shawn Kaeppler	Univ. Wisconsin	Greg Mahone	Univ. Illinois
Bill Tracy	Univ. Wisconsin	Juan Marroquin	Univ. Illinois
Candice Gardner	ARS Ames, IA	Joana Novair	Univ. Illinois
Paul Scott	ARS Ames, IA	Sofia Silva	Univ. Illinois
Sherry Flint-Garcia	ARS Columbia, MO	Tim Gustafson	Univ. Wisconsin
Chris Grainger	Univ. Guelph	Candice Hansey	Univ. Wisconsin