2011 Soybean Breeder's Workshop – Abiotic Stress Research Summary

Prepared by James E. Specht

--- Iron-Deficiency Chlorosis (IDC) Tolerance ---

Silvia R. Cianzio, Professor, Iowa State University, Ames, IA - Phone: 515-294-1625 E-mail: scianzio@iastate.edu

Silvia reports that she and Randy Shoemaker (Research Geneticist, USDA-ARS, Ames) have collaborated with respect to IDC on population development with germplasm releases. Sources of IDC resistance include AR2 and AR3. Other sources are advanced experimental lines that were identified in 2010. Populations were developed from single crosses, with F3 and F4 derived lines classified by maturity evaluated on calcareous soils. Beginning this winter, we are also conducting nutrient solution test in the greenhouse. For promising lines (i.e., those with IDC scores < 2.5), molecular markers will be used if trace genomic segments to AR2 and AR3. Silvia provided some information on two available releases: (1) AR2 (ISURF Docket # 033810), MG: II, Trait: IDC/molecular marker, Line: A00-882060, Released: 1/8/2006, Parentage: A97-770012 x Pioneer 9254, and (2) AR3 (ISURF Docket # 03380), MG: I, Trait: IDC/molecular marker, Line: A00-882130, Released: 1/13/2006, Parentage: A97-770012 x Pioneer 9254. Silvia noted that some basic research is in progress to identify QTLs, but it is not far enough along for a report.

Ted Helms, Professor, North Dakota State University, Fargo, ND - Phone: 701-231-8136 E-mail: ted.helms@ndsu.edu

Ted reports that his recent research was aimed at determining whether there would be a yield advantage to planting two cultivars on fields where a portion of that field was prone to IDC. We used paired sites within the same field. One site was planted on the area of the field where IDC was present whereas the second paired site was planted where IDC was absent. The same 18 commercial soybean cultivars were planted on the paired sites. Results showed that visual scores for IDC were not sufficient to identify the highest-yielding cultivar for the portion of a field where IDC was present. The experimental data revealed that the average field yield was the greatest when two cultivars were planted, when one was more suited to the IDC-prone areas. Alternatively, a cultivar with partial IDC tolerance was also useful because yield was greater in the IDC areas of the field. **Citation: Helms, T.C., R.A. Scott, W.T. Schapaugh, R.J. Goos, D.W. Franzen, and A.J. Schlegel. 2010. Soybean iron-deficiency chlorosis tolerance and yield decrease on calcareous soils. Agron. J. 102: 492-498.**

James H. Orf, Professor, University of Minnesota, St. Paul, MN - Phone: 612-625-8275 E-mail: orfxx001@umn.edu

Jim reports that he uses breeding lines or varieties from our program and from other breeding programs that have good (low) iron deficiency scores for crossing. He has developed breeding material for both general purposes and special purposes that has good iron deficiency chlorosis tolerance. He has also crossed the best iron deficiency chlorosis tolerant lines with high yielding disease and insect resistant lines. He has screened our material in nurseries that have shown iron deficiency chlorosis in the past (usually high pH >7.5 and higher salts). Generally he waits until he has conducted preliminary yield tests so that he does not have so much material to test (as the areas for nurseries are small and ill-defined, i.e., vary from year to year. We have used a scale from 1 to 5 with 1 being completely green and 5 having necrotic leaves and dead growing points. Readings are taken at the second to third trifoliolate stage and then about two weeks later. Lines with very poor chlorosis scores (4 or 5) are dropped from the program and higher chlorosis scores get preference in deciding which lines to advance. He currently has an MS graduate student who is conducting a QTL study using different sources of iron deficiency chlorosis tolerance (sources not previously researched by others). The marker work is complete but we have had difficulty getting good phenotypic data from the field.

-- Salt Tolerance--

Pengyin Chen, Professor, University of Arkansas, Fayettevile, AR - Phone: 479-575-7564 E-mail: pchen@uark.edu

Pengyin reports that he is using 'S-100' and 'Lee 68' as salt-tolerant germplasm and 'Dare' and 'Williams' as salt-sensitive checks in his screening program. He also notes that he is trying to develop a more effective screening method for salt tolerance in soybean. At first, his group screened seven soybean genotypes with contrasting responses to salt stress in hydroponics. We found that foliar symptoms of excessive salt were obvious when plants were subjected to 120mM NaCl. Although the method was simple, it was not cost-effective to screen a large number of soybean genotypes in hydroponics. Thus, we tried to screen the differential genotypes for salt response in pots in the greenhouse. We compared four levels of NaCl concentrations (0, 80, 120, and 160mM), three growing media (commercial potting mix, soil, and sand), and four timings of salt treatment (VC, VE, V1, and V2 stages). It appeared that 120mM NaCl treatment at VE or V1 stages in sand or soil was the best means of detecting response differentials to salt between tolerant and sensitive lines. In the summer of 2010, we made crosses of S-100 x Williams and Dare x Lee 68 to begin the development of two salt QTL mapping populations. **Citation: Valencia, R., P. Chen, T. Ishibashi, and M. Conatser. 2008. A rapid and effective method for salt tolerance in soybean. Crop Sci. 48:1773-1779.**

--- **Aluminum Tolerance** --

David A. Lightfoot, Professor, Southern Illinois University, Carbondale, IL - Phone: 618-453-1797 E-mail: ga4082@siu.edu

David reports that his group is working on the genetic control of tolerance to aluminum toxicity in soybean. Recombinant inbred lines (RILs) derived from the cross of 'Essex' by 'Forrest' cultivars were tested in sand culture in green house. The major objective was to find genotypes of soybean tolerant to aluminum stress. Suitable genotypes can be detected easily and in a short duration using methodologies like sand culture and hydroponics. Plants were grown in green house and then transferred after three weeks in a growth chamber. Root lengths were measured before, and 72 hours after, an Al treatment. The results of the study were based on parameters like root tolerance index (RTI), and relative mean growth (RMG). RILs 85, 40 and 83 had tolerance to Al stress. Resistance was inferred to be related to elevated citrate excretion in roots. QTL were detected and confirmed in NILs. Two major and nine minor loci were detected. Breeding from these lines is in progress, and the lines are available to any group. RILs and markers may be used to provide increased tolerance to soybean against Al stress. **Citation: Sharma AD, H. Sharma, and DA Lightfoot. 2011. The genetic control of tolerance to aluminum toxicity in the 'Essex' by 'Forrest' recombinant inbred line population. Theor. Appl. Genet. 122:687-694 (1 March 2011).**

--- **Heat Tolerance** ---

J. Rusty Smith, Research Geneticist, USDA-ARS-CG, Stoneville, MS - Phone: 662-686-5499 E-mail: rusty.smith@ars.usda.gov

Rusty reported on research focusing on the development of soybean lines with tolerance to high-heat induced seed deterioration. High seed germinability is selected by growing material in the early soybean production system in MS across multiple years, using pedigree breeding for segregating generations, and then using delayed harvest of yield trials across years and locations for pure lines. Rusty noted that the yield testing of selected lines across locations in 2011. The following selected high-germinability PIs were crossed with high-yielding soybean lines: PIs 417154, 603784, 588016, 417185, 603756, 587982A, 423938, 594445, 393540, 594779, 594438, 594550, 603764B, 507029, 588026A, 417321, 603751A, 603602, 85009-1, 416875, 417017, 417050, 417328, 423932, 423940, 423941, 423945, 587576, 594692, 603670, 603719A, 603746, and 603723. The selected adapted lines include Glenn, Osage, R99-1613F, UA 4805, JTN 5503, JTN 5303, 5002T, 5601T, LG01-5087-5, LG04-4866, LG04-6863, LG04-6000, S99-11509, S99-11896, S01-9391, Jake, LD00-3309, Stoddard, K 1639-2, and DT98-9102. **Citation: Smith, J.R., A. Mengistu, R.L. Nelson, and R.L. Paris. 2008. Identification of Soybean Accessions with High Germinability in High-Temperature Environments. Crop Science 48:2279-2288.**

--- **Flood / Water-Logging Stress Tolerance** ---

Pengyin Chen, Professor, University of Arkansas, Fayettevile, AR - Phone: 479-575-7564 E-mail: pchen@uark.edu

Pengyin reports that the germplasm sources he is using for flood tolerance are 'Archer' and 'Peking'. Based on field flood screening work, he has identified two slow-wilting PIs (PI 471931 and PI 471938) and an old cultivar RA-452 as being flood tolerant. His group is using conventional breeding methods to improve yield potential of flood tolerant lines by crossing with high-yielding cultivars. Breeding populations, progeny rows, and preliminary tests are grown under irrigated conditions. Lines are selected based on adaptability and yield potential. Advanced lines are evaluated under irrigated and flooded conditions. The flood test is done in the field. Lines are grown in replicated 10' single-row plots, with flood stress imposed by surrounding each field block with raised soil levees. Lines are subjected to flooded condition at both the V4 and R1 stages for two weeks. A visual rating of a 0 to 9 scale, based on leaf chlorosis, stunting, and plant death, are taken two weeks after ceasing the flood conditions. The visual rating of "0" indicates no obvious flood damage while that of "9" means ≥ 90% plants damaged or dead. The same system is used to screen diverse germplasm for flood tolerance. Pengyin reports no material from this screening is ready for release just yet.

-- **Drought or Water Deficit Stress Tolerance** ---

H. Roger Boerma, Professor, University of Georgia, Athens, GA - Phone: 706-542-0927 Email: rboerma@uga.edu

Roger reported on QTLs governing fibrous roots – a trait believed to improve drought tolerance and productivity. PI 416937 was previously shown to possess fibrous roots and has been used as a parent in breeding programs to improve drought tolerance and productivity. To identify the genomic locations and genetic basis of this trait, a recombinant inbred line population (RIL) was derived from a cross between ' Benning' and PI 416937. Phenotypic data for rooting score were collected in the field for 2 years under rain-fed conditions at the Plant Sciences Farm near Athens GA. The selective genotyping approach was used to reduce the costs and work associated with conducting the QTL analysis. A total of five QTLs were identified on chromosomes Chr 1 (Satt383), Chr 3

(Satt339), Chr 4 (Sct_191), Chr 8 (Satt429), and Chr 20 (Sat_299), and together explained 51% of the variation in root score. Detected QTLs were co-localized with QTLs related to root morphology from previous studies, suggesting that fibrous root QTL may be associated with other morpho-physiological traits. **Citation: Hussein Abel-Haleem, Lee, GJ, and HR Boerma. 2010. Identification of QTL for increased fibrous roots in soybean. Theor. Appl. Genet. 17 Dec 17 2010 (DOI: 10.1007/s00122-010-1500-9)**

Roger also reported on QTLs for a trait known as slow-wilting under drought. PI 416937 was identified as slow wilting in North Carolina by Tommy Carter. A population of 150 RILs from Benning x PI 416937 was planted in various rain-fed locations in Arkansas, North Carolina, and Kansas. In a combined analysis across environments, the Multiple Interval Mapping model of QTL-Cartographer detected seven QTLs on different chromosomes (Chr 2, 4, 5, 12, 14, 17 and 19). Individually, these QTL explained from 4 to 26% of the phenotypic variation in wilting score. When placed in a multiple regression model the seven QTL explained ~ 70% of the phenotypic variation. The largest QTL (R2 = 26%) was located on Chr 12 (Lg-H) and the allele for slow wilting was inherited from PI 416937. This QTL was detected in all environments. QTL on Chr 1, 5, 13, and 17 were previously detected for slow wilting in other populations. A manuscript is in preparation by co-authors: A.-H. Hussein, R.Boerma, T. Carter, Jr., T. Sinclair, Larry Purcell, and A. King

Roger also reported on the development of NIL for yield under drought conditions. In a study to discover QTL for seed yield in the recombinant inbred line (RIL) population of Hutcheson x PI 471938 data were collected in 14 field environments (9 irrigated and 5 rain-fed environments in Georgia, Arkansas, Missouri and North Carolina). Three QTL on Chr 17 (Lg-D20, Chr 13 (Lg-F), and Chr 9 (Lg-K) were found for seed yield in the 5 rain-fed environments, but not in the irrigated environments. The alleles for increased seed yield were inherited from PI471938 for all three QTL. Additional research found these QTL were also associated with the slow wilting trait in two rain-fed environments in North Carolina. Using the heterogeneous inbred family approach, near-isogenic lines (NIL) for these QTL were produced by reselection within F4:8 lines of the Hutcheson x PI471938 population. The F8-derived NILs pairs are 97% identical except for the genomic regions near the QTL of interest (homozygous for either the PI 471938 or Hutcheson allele at the specific QTL). These slow wilt NILs were evaluated in 8 rain-fed environments in Georgia, Arkansas, and North Carolina from 2008 to 2010. The NILs with the PI 471938 allele(s) at one or more of the slow wilt QTL exceeded the yield of their sibs with Hutcheson allele(s) in rain-fed environments. For example, one NIL pair with the PI 471938 alleles at the Chr 17 (Lg-D2) QTL and Chr 13 (Lg-F) QTL out-yielded its NIL sib by 2 bu/ac, and another NIL pair with the PI 471938 allele at the Chr 9 (Lg-K) QTL out-yielded its NIL sib by 3.6 bu/ac. The collaborators on this project are A.-H. Hussein, R. Boerma, T. Carter, and L. Purcell. No manuscript just yet.

Pengyin Chen, Professor, University of Arkansas, Fayettevile, AR - Phone: 479-575-7564 E-mail: pchen@uark.edu

Pengyin reports that his research group is working on two drought tolerant traits. One is sustained nitrogen fixation under drought and the other is slow-wilting trait. Germplasm sources for the sustained nitrogen fixation trait are ‘Jackson’, PI 227557, and PI 506675. Sources of the slow-wilting trait are PI 471931, PI 471938, PI 416937, and NTCPR94-5157. In addition, we use ‘RA-452’ and ‘Stressland’ to develop drought tolerant lines. These two lines tend to sustain and yield well under drought conditions in Arkansas. Pengyin also noted that his group is taking both a basic approach and using conventional breeding methods. He is trying to enhance sustained nitrogen fixation / slow-wilting traits by combining each target trait from different genetic backgrounds and to combine these two traits. Crosses are also made between sustained N2-fix / slow-wilting trait and high-yielding cultivars to improve yield potential. Breeding populations, progeny rows, and preliminary yield tests are grown under irrigated conditions to select for adaptability and yield potential. Then, the advanced lines selected are evaluated under both irrigated and rainfed conditions. Again, we focus on yield potential and adaptability for genotype selection with particular interest in lines with less yield reduction under rainfed condition. *In general, the lines that perform well in irrigated condition also do well under rainfed condition.* Pengyin noted that the germplasm lines R01-416F and R01-581F had high yield potential and sustained nitrogen fixation under moderate drought condition and were released to breeders in 2007. **Citation: Chen, P., C.H. Sneller, L.C. Purcell, T.R. Sinclair, C.A. King, and T. Ishibashi. 2007. Registration of soybean germplasm lines R01-416F and R01-581F for improved yield and nitrogen fixation under drought stress. J. Plant Reg. 1(2):166-167.**

David A. Lightfoot, Professor, Southern Illinois University, Carbondale, IL - Phone: 618-453-1797 E-mail: ga4082@siu.edu

David reported that recombinant inbred lines (RILs) derived from the crosses of Essex by Flyer (and Essex x Hartwig) were tested in field +/- irrigation and in a rainout shelter using drip irrigation at different rates. The drip irrigation allows the experimenter to “dial in” a water delivery rate. **Citation: Mansour, HAG, MY, Tayel, DA Lightfoot, and AGM El-Gindy. 2010. Energy and water saving by using modified closed circuits of drip irrigation system. Agricultural Sciences 1(3): 154-177.**

David also reported that trigonelline content (a compatible solute for soy , instead of glycine-betain in cereals, seems to be a good biomarker of water deficit tolerance and that RILs and marker may be used in basic research and QTL detection to provide increased tolerance to water stress. **Citation: Cho, Y, EB Turnipseed, DA Lightfoot, and AJ Wood. 2008. Trigonelline in mature seeds and developing seedlings of *Glycine max*. Biologia Plantarum 52: 370-372.**

James H. Orf, University of Minnesota, St. Paul, MN - Phone: 612-625-8275 E-mail: orfxx001@umn.edu

Jim reports that for his drought tolerance research, he has been using materials identified by Dr. Tommy Carter as slow wilting, as well some as early maturity PI accessions (578425, 437152, 437161, 612711, 592960, plus Hendricks) that we have subsequently identified at our research test site at Becker, MN, which has very sandy soil that dries out relatively quickly. Wilting data is taken after plants enter the reproductive phase, using a 1 to 5 scale where 1 is no wilting and 5 is severe wilting with many dead leaves.

Dr. Carter has assisted by crossing our MG 0 lines with his MG V and VI breeding lines and the PI accessions that he has identified as slow wilting. We have selected slow wilting early maturing segregates derived from these matings and have crossed them with slow wilting types (PI's, breeding lines, varieties) of early maturity (MG 00, 0, I) as well as with high yielding general purpose and special purpose breeding material from our conventional breeding effort. We have generally screened F5 or later lines at Becker for slow wilting and then evaluated the best lines in irrigated and non-irrigated plots at Becker as well as other rain fed sites in MN before entering material in regional tests. We have also tested materials for slow wilting from other researchers at Becker including scientists from Nebraska, Arkansas and North Carolina. Currently we are in our third cycle of breeding and selection and have developed early maturity (MG 0 and I) high yielding germplasm that has the slow wilting trait. No releases/publications yet.

Shawn Conley, Associate Professor, University of Wisconsin, Madison, WI - Phone: 608-262-7975 E-mail: spconley@wisc.edu

Shawn reports that in 2009-2010, he conducted field studies aimed at characterizing the effect of plant stress (both abiotic and biotic) on soybean canopy reflectance. Five varieties from the WI Soybean Variety Testing program (Southern Region RR test) that exhibit differential response to plant stress (aphid resistance, water stress, BSR, and white mold) were identified and selected through data collected from our 2009 variety trials, personal experience, and company ratings. By imposing management regimes of irrigation, insect stress relief, and population, we hoped to induce a gradient of plant stress responses that range from no stress to severe stress. The experimental design was a randomized completed block split-split plot design with 4 replications. The whole plot was water management strategy, the sub-plot was to be insect management strategy (however, this was not used in 2009 or 2010, due to low insect density levels), and the sub-sub-plot was a factorial arrangement of population and variety. A Crop Circle ACS-430 active crop canopy sensor was used and it provides vegetation index data (NDVI, SRI, and other data) as well as basic reflectance information from plant canopies and soil. The ACS-430 provides reflectance data from the red edge, red, and near infrared wavelengths. Calculated indices can be used in conjunction with other agronomic references to index basic nutrient response, crop condition, yield potential, stress, and pest and disease impact in a quantitative objective manner. The unit can be used to monitor changing field (crop, plant) conditions during the growing season and/or the effects of different levels of an input compared to a local standard. Eleven crop canopy reflectance measurements were taken from 21-Jun to 30-Aug in 2010. The 2010 growing season provided ample rain, so water was not expected to be a limiting factor, but there was a significant difference in yield between the irrigated (65.5 bu/ac) and non-irrigated (61.1 bu/ac) treatments, though it did not show up in our reflectivity data. There was no measureable, insect, or disease stress to evaluate. The main differences we noticed in yield and how the yield differences were represented in the reflectance data was mostly the result of reflectivity differences among varieties. We were still able to use the reflectivity data to predict yield. We found that calculated NDRE (Normalized Difference Red-Edge) and Chl I (Chlorophyll Index-Red-Edge) indices from the reflectance data on 2-Aug had a significant relationship to grain yield. These indices are both commonly used and trusted as measures of biomass or stress. They were chosen because our data showed mostly a red-edge variation and these two indices are calculated using the red-edge data. This significant relationship can be further refined by adding more varieties to an experiment and normalizing all treatments to predict end of the season yield from an in-season reflectance measurement. Based on the data collected from this study and a non-related experiment that looked at planting date, we believe that better correlations between crop canopy reflectance and yield will occur later in the season. We intend to continue this expanded work in 2011. Though we experienced a lack of stress indices across all locations in 2010, we do not expect that to occur again in 2011, and therefore hope to continue to develop predictive indices from future stress events. Lastly, we are still evaluating the vast amount of reflectance data we collected in 2010 and hope to identify additional reflectance indicator regions that will assist growers in management and yield predictions.

James E. Specht, University of Nebraska, Lincoln, NE - Phone: 402-472-1536 E-mail: jspecht1@unl.edu

Jim reports that he has been evaluating the response to water scarcity (and water abundance) of F4- and F5-derived lines extracted from populations derived from matings of northern elite cultivars (like George Graef's semi-determinate NE3001) with Tommy Carter's southern breeding lines that have emanated Tommy's matings of southern elite cultivars with slow canopy wilting southern PI accessions. This has required heavy selection pressure on northern maturity in the large F2 generation populations and thereafter during generation advance. This year, for example, three selections from a population derived from the mating of Tommy's **N98-7165 x NE3001** were exceptional yield performers (relative to the yield and maturity checks in multiple 2010 Nebraska Tests of such lines) were submitted to the 2011 Preliminary Uniform Tests.

Several years ago, Jim obtained seed of about 1500 MG II & III PI accessions that had been collected from drought-prone regions of China. After conducting a seed increase and discarding accessions that were not agronomic (i.e., shattering, susceptible to diseases, lodged badly, etc.), the list was narrowed to about 350 accessions that, along with 42 high-yielding checks were grown in 2-row plot, 4-rep performance irrigated and rainfed performance trials in 2003-04. Fortuitously, a very severe drought occurred in 2003 which allowed the collection of yield data in very water-scarce year (2003) and a typical rainfed year (2004), along with irrigated yield data each year. Using this data, we selected about 20 MG II and 20 MG III accessions with the highest yields in the 2003 drought (that also turned out to have highest yields under irrigation) for two more years (2005-06) of confirmatory yield trials. We eventually narrowed the drought-tolerant list to the 13 MG II and 12 MG III (a total of 25) accessions shown below and mated these also to Dr. Graef's high-yielding cultivar NE3001. The progeny were generation-advanced to F4 stage in 2010. About 150-200 F4 plants were pulled to undergo an F4.5 progeny row seed increase in 2011 that will result in F4-derived RIL populations that can be yield-tested in 2012 in USA drought-prone areas (and in control irrigated trials in those same areas). In the past, it has been difficult to find progeny that are as high-yielding as the elite parent in both water-scarce and water-abundant production environments. Jim hopes that such progeny can be found in these 25 populations. Six of the below PI accessions were also mated to IA3023 as part of the NAM project that Dr. Brian Diers described to the Workshop participants yesterday.

Accession	Descriptor Notes	Accession	Descriptor Notes
PI 404.188A	II I WTBr SYBl	PI 089.009-2	III D WGBr DYBf
PI 437.169B	II I PGBr DYIb	PI 507.295	III D WGBr IYLbf (Na)
PI 437.341	II I PGBr DYIb	PI 398.881	III I PTBr DYBl
PI 437.863A	II I WGBr IYLbf	PI 561.370	III I PTBr IYBl
PI 438.164B	II I PGBr SYIb	PI 561.330B	III I WGBr IYBf
PI 438.258	II I PTBr DYBr	PI 091.089	III I WGBr SYBf
PI 476.352A	II I WTBr SYBl	PI 437.578	III I WGTn IYBf
PI 506.476	II I WGBr IYBf	PI 427.136	III I WTBr DYBl
PI 507.681B	II I WGBr IYY	PI 092.686	III I WTBr IYBl
PI 518.751	II I PGBr IYIb	PI 507.692A	III I WTBr IYBl
PI 538.400	II SD WGDbr IYBf	PI 507.708	III I WTBr IYBl
PI 567.170B	II D WGBr IYBf	PI 574.486	III I WTBr IYBl
PI 567.217C	II I WGBr IYY	NE3001	III SD WGBr DYBf