North Central Soybean Research Program

Iron Deficiency Chlorosis: Getting to the Root of the Problem

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Soybean cultivars or breeding materials that combine complete tolerance to the IDC and acceptable yields are not available. The confounding factor is that the conditions necessary for screening are not found in all disease prone ("hot") fields each year. To address this concern, molecular markers have been identified that are being used to test breeding materials. The purpose of this continuing project is to address iron deficiency chlorosis in the upper Midwest using a combination of molecular genetics and gene cloning and manipulation. This is a coordinated project that combines the results of two distinct, but complementary approaches to solving this significant production constraint.

Project Objectives

- Screen advanced soybean breeding lines from the North Dakota, Minnesota, and Nebraska public breeding programs with current the collection of IDC associated markers
- Search for breeding program specific IDC markers using multiple mapping populations;
- Evaluate expression patterns of modern IDC tolerant and susceptible lines to discover genes responsive to iron stress that can be developed into effective markers

Reporting Period Accomplishments

Iron Deficiency Chlorosis (IDC) is a disease resulting from lack of useable iron and eventually results in yield loss at the end of the season. This is particularly important in the Midwestern part of the U.S because soil conditions favor the development of IDC. Even short-term iron deficiency can have a lasting effect on yield. Therefore, the project is designed to characterize soybean's short and long-term response to iron stress.

Objective 1. Advanced generation breeding lines, selected from the results of previous year's breeding efforts have been selected. These lines were selected based on their yield potential and response under IDC inducing conditions. These lines are currently being prepared for planting in the 2016 growing season in the three target states: Minnesota, Nebraska, and North Dakota. DNA is being extracted from these lines, and the material will be screened at NDSU for the suite of markers developed from previous NCSRP funded research.

These SNPs markers were discovered using using materials bred specifically for the North Dakota and Minnesota environments. They will be tested to determine if they are also useful for Nebraska.

Objective 2. The DNA described in objective 1 will also be used to discover a new SNP markers by using genotypye-sequencing technology coupled with a genome-wide association study (GWAS). The GWAS will be performed once the IDC response data is reported from this coming growing season.

During the 2015 field season, near isogenic lines (NIL) derived from the 'Fiskeby III' x 'Mandarin Ottawa' recombinant inbred line (RIL) mapping population were identified and planted in Danvers, Minnesota at our IDC nursery. 417 plants represented either the Fiskeby III or Mandarin Ottawa genotype at the chromosome Gm05 quantitative trait locus (QTL). These NILs were used as a validation population of the Gm05 QTL, and we found significant reduction ($p < 0.0001$) in mean IDC rating using a 1-9 scale with 1 being not affected by IDC and 9 being killed by IDC. The average IDC score for the Fiskeby III QTL was 4.10, while the average score for NIL lines carrying the Mandarin Ottawa genotype at the QTL was 4.88.

To further fine map the Gm05 QTL, 750 plants heterozygous at the QTL were planted and genotyped in St. Paul, MN in 2015. Plants were recovered that had novel recombination that will be useful for narrowing the QTL and mapping the gene responsible for this IDC tolerance. 92 NIL pairs (184 plants) have been selected from this group for planting in the Danvers, MN IDC nursery in May 2016. 1000 plants from this population with recombination events and that remain heterozygous over regions of the QTL will also be planted in St. Paul during the 2016 field season for further fine mapping.

Objective 3. Our research takes advantage of two near isogenic lines that are 98% genetically identical but differ in their iron response. Clark plants are iron efficient, while Isoclark plants are iron inefficient and develop symptoms of IDC under iron stress conditions. In a previous experiment, both Clark and Isoclark were grown in hydroponics in a greenhouse for a total of ten days. Plants were grown in either iron sufficient conditions, iron stress conditions for two days or iron stress conditions for 10 days. Plants were grown simultaneously to control for the age of the plants. RNA-seq was used to compare gene activity in Clark and Isoclark under the different treatments.

We are now characterizing the function of 19 genes that responded to iron stress in either Clark or Isoclark using Virus Induced Gene Silencing (VIGS). Using VIGS we can temporarily shut down the activity of specific genes and see how that change alters the ability of the plants to respond to iron stress. Ten genes were chosen because they responded the most to either short or long term iron stress, while another nine genes were selected because they responded to iron stress and showed sequence similarity to iron stress genes from other species. VIGS constructs have been constructed for all 19 genes and silenced plants have been preliminarily phenotyped in soil. We are now in the process of phenotyping under iron stress conditions.

In addition to understanding the immediate response to iron stress, we are also interested in understanding what happens to plants when they recover from iron stress. Clark was grown under iron sufficient conditions for seven days, exposed to iron deficiency stress for 24 hours, followed by 48 hours in iron sufficient conditions. Root and leaf tissues were harvested and used for RNA extraction and sequencing. This data is currently being analyzed to provide a base line of expression data and knowledge about plant physiological responses against which IDC improved lines identified by other members of the grant will be measured.