EUJAYL, IMAD A.[1]*, IVAN SIMKO[2] and CARL A. STRAUSBAUGH[1], [1]USDA-ARS, NWISRL, 3793 North 3600 East, Kimberly, ID 83341 and [2] USDA-ARS, Crop Improvement and Protection Unit, 1636 E. Alisal Street, Salinas, CA 93905. **Association Analysis of Beet Curly Top Disease Resistance Genes in Sugarbeet.**

ABSTRACT

Association analysis is an approach to overcome common bi-parental genetic linkage mapping limitations. This linkage-disequilibrium based approach was successfully applied in other crops and sugarbeet, thus allowing for detection of markers linked to genes of resistance. A pool of 168 diploid hybrids and germplasm were genotyped with 39 polymorphic SNP and 30 SSR markers. The same population was phenotyped for curly top reaction in the field and the greenhouse for two seasons. To compare performance of the two marker systems, the population structure analysis was performed on a subset of 62 genotypes that were analyzed with both SSR and SNP makers. The estimated number of subpopulations was affected by marker system, and varied from *K*=2 to 4. Analysis of population structure based on *K*=3 indicated clustering of varieties from Betaseed and American Crystal, while varieties from Hilleshog and Seedex formed a different cluster. Varieties from Holly Hybrids were distinctly separated from other clusters with a notable exception of accession HH06. Analysis of the complete population of 168 accessions, confirmed clustering detected on a subset of individuals. The USDA-ARS germplasm showed similarity to the Hilleshog – Seedex cluster, while a population from KWS clustered separately. The association analysis revealed several genomic regions associated with the disease reaction and putative alleles associated with SNP markers located on chromosomes 2, 5, 7, and 9.

Rationale and Objectives:

Developing bi-parental genetic mapping populations in sugarbeet is time-consuming and hindered by limited number of publically available inbred parental lines. Association analysis, an approach based on linkage disequilibrium in a diverse population proved an efficient and practical approach. This analysis allows for detection of recombination events across generations, rather than a single segregating population. Additionally, using older generations as well as elite germplasm will leverage the power to identify tightly linked loci.

Association analysis has been used to study bolting gene in sea beet and sugar content in sugarbeet. DNA markers with significant linkage disequilibrium with the *B* gene, sugar content and beet yield were identified.

The objectives of this study were to analyze the population structure (estimate *K*) of a large collection of experimental and commercial varieties from six seed companies, and elite germplasm from USDA-ARS for suitability for association analysis, and to use this population to identify markers associated with resistance to curly top.

Procedures:

A population of 132 pre-commercial and commercial hybrids were obtained from six sugarbeet seed companies; Betaseed Inc. , ACH Seeds Inc., Hilleshog, Seedex , and Holly Hybrids and KWS. Additionally, 36 elite germplasm from the USDA-ARS pre-breeding and

genetics programs were used in ths study. This population was phenotyped for curly top and rhizomania in 2006 and 2007. Curly top was scored based on a disease index of 0 to 9 (0=healthy and 9= dead). Rhizomania was scored in a naturally heavily infested field based on a 0 to 9 disease index.
DNA was isolated from leaves from a single plant per genotype using Qiagen DNeasy® Kit. Thirty polymorphic SSR markers, including 13 genomic SSRs and 17 EST-SSRS were used for genotyping. Out of the total number of SSRs, 19 markers with known chromosomal location and not linked to each other, while 11 EST-SSRs were not mapped. PCR amplification of all SSR were performed with M-13 tailed F-primers, labeled with FAM, PET, or NED, and electrophoresed using ABI 3100. SSR were scored for variation in fragment size (bp).
The population was genotyped with 39 polymorphic SNP markers using the SNPlex assay.
The data was analyzed using the statistical package program *Structure*. The population was used for subdivisions and. The program parameters were set to a Burn-in period and Reps of 100,000.
To evaluate the diversity of the population Neighbor joining clustering analysis was applied.

Conclusions:

Association analysis in sugarbeet provides a practical and efficient approach for identifying DNA markers associated with traits of interest to expedite germplasm enhancement. Additionally, it's suitable for studying multi-traits in contrast to bi-parental segregating populations designed for a trait-by-trait linkage analysis. Curly top resistance is a quantitative trait and the genes regulating the trait spread randomly across the genome. The genetic control of this trait renders it difficult to focus on a specific chromosomal genomic region. To improve the efficiency of association analysis genome-wide analysis will be used and will be coupled to conventional genetic linkage mapping and Bulked Segregant Analysis. Accordingly, we established a project to develop DArT markers for sugar beet. DArT markers are multilocus dominant markers that can be detected in any regions of the genome. A diverse population of 94 genotypes representing cultivated sugarbeet, sea beet and wild relatives has been used to develop DArT markers. Approximately 700 to 1000 markers will be used to genotype this population and analyzed to explore DNA markers associated with genes regulating curly top resistance in sugarbeet.