Beta Breeding and Genetics at East Lansing, Michigan: Molecular Methods, Genetic Diversity, and Trait Elucidation

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ABSTRACT

Variety and germplasm development and release have been the main goals of the USDA-ARS sugarbeet (*Beta vulgaris* L.) breeding program at East Lansing, MI for over 70 years. Progress has been made in improving tolerance to *Aphanomyces* seedling disease, *Cercospora* leaf spot tolerance, *Rhizoctonia* crown and root rot tolerance, herbicide resistance, and low-tare (smooth root) root morphology. The genetic basis of these traits is not well understood, and the current program seeks to identify genes that influence the expression of disease, quality, and morphological traits, and locate them on beet chromosomes. The out-crossing nature of sugarbeet is not well suited for large-scale genetic analyses. A strategy has been adopted that should allow genetic dissection of a variety of traits through standard genetic analyses. Briefly, a genetic male sterile seed parent that also carries a dominant self-fertility gene is paired with germplasm of interest. The resulting self-fertile hybrid is self-pollinated to produce a segregating F_2 population, which is simultaneously observed for segregation of targeted traits and tested for linkage of these traits using molecular markers. Segregation of male-sterility and self-fertility genes in the F_2 gives a range of options for further characterization. In addition, this strategy allows relatively rapid introduction of traits from wild and unadapted germplasm while simultaneously

determining the genetic basis for novel traits introduced through such crosses. Using this system will allow systematic exploration of linkage relation-ships between agronomic genes from diverse germplasm sources and molecular markers.

Additional Key Words: breeding systems, germplasm, molecular markers.

Historical Perspective at East Lansing

The USDA has been involved with sugarbeet at East Lansing, Michigan for over 70 years. By 1935, J.G. Lill of the USDA Agricultural Testing Laboratory was providing sugar and purity analyses for the Farmers and Manufacturers Beet Sugar Association, of Saginaw, MI (Buschlen, 1938). About 1946, H.L. Kohls was hired by Michigan State College to breed and evaluate hybrids for Michigan (Kohls, 1950). Although Kohls' tenure in sugarbeet was relatively short, it is notable that he created Michigan Hybrid 18. V.F. Savitsky reported recovering two plants with monogerm seed from a 1.6 hectare seed production field of Michigan Hybrid 18 in Oregon (McFarlane, 1993; Savitsky, 1950; Oldemeyer, 1998).

Shortly after WWII, G.J. Hogaboam assumed agronomic testing and breeding responsibilities for the USDA at East Lansing, until he retired in 1984. Hogaboam collaborated in developing germplasm with tolerance to *Aphanomyces* seedling disease, *Rhizoctonia* crown and root rot, *Cercospora* leaf spot, as well as monogerm and CMS / O-type, resulting in the wide use of US H20 (Coe and Hogaboam, 1971) and other varieties in Michigan. Beginning in 1979, J.W. Saunders introduced a biotechnological perspective to the program, initially focusing on shoot culture for clonal propagation and shoot regeneration from callus (Saunders, 1998), which has expanded to include somatic embryogenesis (Saunders and Tsai, 1999), and for a time, somatic cell selection for herbicide resistance (Saunders et al., 1992). From 1982 to 1994, J.C. Theurer developed smooth-root, low tare genotypes in combination with improved agronomic performance and increased disease tolerance (Saunders et al., 1999). J.M. McGrath assumed duties as a USDA Research Geneticist for sugarbeet breeding and genetics in 1996, in part to continue prior activities but also to expand the focus of the program by applying molecular genetics to sugarbeet improvement.

Current Focus and Program Development

The mission of the USDA Agricultural Research Service is to provide practical solutions to agricultural problems of national scope. Such solutions often require long-term commitments and high-risk approaches.

Sugarbeet improvement is an area where the USDA-ARS has had a pivotal role. The East Lansing program has three main objectives: a) to continue the germplasm enhancement activities initiated in earlier years, b) to examine the genetics of agronomic and disease resistance traits, and c) to examine problems of emergence and stand establishment. Each of these is inter-related, and the focus in this paper is strategies being used to satisfy the second objective.

Beet has been eaten by humans for thousands of years, either as a leafy vegetable or fleshy root (Ford-Lloyd and Williams, 1975). Wild beet, and related species, are endemic throughout the Mediterranean region and the maritime regions of Western and Northern Europe. For sugar, beet has a history of about 200 years, and much of the increase in sucrose percentage from initial to current levels occurred within the first 100 years (Fischer, 1989; Cooke and Scott, 1993). Erosion of genetic diversity in sugarbeet has been a concern, since: a) sugarbeet has been selected from a narrow founding population, b) selection pressure for increased sucrose content has been intense, and c) a limited number of varieties formed the original germplasm base of breeding programs in the U.S. and elsewhere, originally introduced from Europe. Also, recessive genes for expression of monogerm seed and cytoplasmic male sterility are nearly universal in modern cultivars, and this has imposed an additional genetic bottleneck (Bosemark, 1993), as well as resulted in cytoplasmic uniformity in commercial hybrids worldwide.

Genetic bottlenecks during sugarbeet breeding have not necessarily impacted genetic diversity as whole, since breeding programs have generally maintained allelic diversity by virtue of population improvement approaches used during most of the crop's history. Selection over the past 80 years has sub-divided the genetic diversity into individual breeding populations, at least among germplasm releases in the U.S. (McGrath et al., 1999). Genetic diversity in sugarbeet is clearly much less than in its wild progenitors, and it is likely that useful characters (e.g. disease resistances, seedling vigor, nutrient efficiency, photosynthate partitioning, etc.) may be extracted from accessions available through national and international germplasm conservation organizations.

Determining the genetic basis of a wide range of agronomic traits and expanding the number and types of agronomic genes available to sugarbeet breeders is a daunting task. Discovering genes for agronomic traits could be combined with deployment of these genes in enhanced germplasm, and significant benefits may result in a modest timeframe. Shifting away from some of the traditional breeding methods is required to merge gene discovery and deployment. New approaches should be flexible enough to be systematically applied to a large number of trait and

germplasm sources. A major limitation for genetic analyses in beet is the presence of a complex self-incompatibility system (Lundqvist et al., 1973), and this generally limits generating sufficient seed of a single hybrid individual necessary to determine segregation patterns. To circumvent this limitation, the dominant self-fertility gene (Owen, 1942) is used as a central part of the East Lansing program. Using enforced selfing, a large number of segregating populations can be created from crosses of single individuals, one of which has the self-fertility gene, by placing pollen-proof bags on confirmed hybrids at flowering. Enforcing crossing between parents relies on a recessive nuclear male-sterility gene that ensures all seed produced on the mother plant are hybrid. Through this approach, over 50 hybrid combinations have been made and over 500 segregating populations generated in the past two years.

An advantage of the self-fertility / male-sterility (Sf / ms) approach is the range of genetic materials generated. Molecular marker analyses fit well with this scheme as each F_2 population is derived from a single F_1 hybrid plant. With a marker set covering the genome at a reasonable density, each population can be genotyped. The marker information is used to develop hypotheses on the inheritance of traits via QTL-type approaches (Tanksley and Nelson, 1996), and these hypotheses can be verified in F_3 or back-cross populations derived from selected F_2 individuals. A disadvantage of the Sf / ms approach is that segregation distortion will occur at and near the self-incompatibility locus (loci) if one of the parents is self-incompatible. This is because the gametophytic incompatibility system of beet will arrest growth of pollen tubes expressing the same self-incompatibility allele, such as would be expected during self-pollination. Culture of excised ovules from hybrids may allow recovery of self-sterile plants if they are desired for population improvement breeding approaches.

Ideally, molecular markers for a trait should be genes involved in the expression of that trait. Current knowledge is incomplete regarding the numbers, locations, and gene action for most sugarbeet characters. Until this information is available, expressed genes in general may be a good substitute. We have begun to sequence expressed genes, with the aim to develop Expressed Sequence Tags (ESTs) for germplasm evaluation and characterization. Sequenced cDNA clones are currently used as RFLP markers, with PCR-based strategies to be developed from cDNA nucleotide sequences and the genetic loci they detect. To date, we have sequenced over 500 randomly isolated cDNA clones (Table 1) from two libraries. These are searched for sequence similarity to previously characterized genes, and inspected for 'candidate' genes presumed to be involved in the expression of a trait. Sucrose metabolism genes from many organisms have been have been cloned, for example, and Schneider et al. (1999)

Table 1. Sugarbeet gene products identified through nucleotide sequencing and database searching.

Functional Class	Number of ESTs	Examples ($e < 10^{1}$)
Amino Acid Metabolism	36 (6.5%)	Glutamine synthetase; Aspartate aminotransferase; Phosphoserine aminotransferase; Alanine aminotransferase; S-Adenosylmethionine synthetase; S-Adenosyl homocysteinease; Ornithine aminotransferase
Carbohydrate Metabolism	15 (2.7%)	alpha-Amylase; beta-Amylase, 1-D-deoxyxyllulose 5-phosphate synthase, UDP-glucuronlyltransferase, cellulase, GDP-mannose pyrophosphorylase
Monosaccharide Metabolism	28 (5.1%)	Fructose 1,6-bisphosphatase; Malate dehydrogenase; Phosphofructokinase; Glyceraldehyde 3-phosphate dehydrogenase; Phosphogluconate dehydrogenase; Aldolase; Enolase; Sucrose-phosphate synthase
Lipid Metabolism	9 (1.6%)	N-Acetylglucosaminyl-phosphatidylinositol biosynthetic protein; N-myristoyltransferase; AcetylCoA carboxylase; Fatty acid hydroxylase
Electron Transport	5 (0.9%)	Cytochrome c; Ubiquinol-cytochrome c reductase; Ubiquinone Oxidoreductase
Photosynthesis	8 (1.5%)	RUBISCO; Monodehydroascorbate reductase; Photosystem II 10 kD protein

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Table 1 (continued). Sugarbeet gene products identified through nucleotide sequencing and database searching.

Functional Class	Number of ESTs	Examples ($e < 10^{1}$)
Membrane Transport	29 (5.3%)	Ca^{2+} transporting ATPase; H^{+} transporting ATPase; Cu^{2+} transporting ATPase; Potassium transport protein; ABC-type transport protein; Porin; Amino acid/peptide transporter; Na^{+} /H^{+} exchanger protein; Vacuolar H^{+} ATPase; Oxoglutarate/Malate translocator protein
Secondary Metabolism	14 (2.5%)	Sesquiterpene cyclase; Raucaffricine-0-beta-D-glucosidase; Pectinesterase; Caffeoyl-CoA O-methyltransferase
Replication	3 (0.1%)	DNA polymerase; DNA gyrase
Transcription Factor	15 (2.7%)	AP2 domain-containing protein; DREB2A; Myb-related proteins; bZIP transcription factor
Translation	26 (4.7%)	Elongation factors (EF1-alpha; Tu); Initiation factors (EIF2, EIF4A, EIF5A)
Histones	8 (1.5%)	H2B; H3; H4
Signal Transduction/ Protein Kinase	23 (4.2%)	Calmodulin; Guanylate nucleotide binding protein; Serine-Threonine protein kinase; Receptor protein kinase; Mitogen-activated protein kinase

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Table 1 (continued). Sugarbeet gene products identified through nucleotide sequencing and database searching.

Functional Class	Number of ESTs	Examples ($e < 10^{1}$)
Environmental Stress-Related	43 (7.8%)	Drought & salt inducible proteins (RAN1, HAL3A, Di19); Heat shock proteins (HSP70, HSP80. HSP90); Betaine-aldehyde dehydrogenase; Alcohol dehydrogenase; PR-protein (chitinase; PR3); Trehalose-6-phosphate synthase; Aluminum-induced protein
Ribosomal Protein	68 (12.3%)	40S, 50S, 60S
Miscellaneous	77 (14%)	Actin; Tubulin; Ubiquitin; Extensin; Elastin; Nucleolin; Ubiquitin conjugating enzyme; ATP Synthase; Proteasome assembly proteins; Phytase; Aminoacyl-tRNA synthetase; Histone deacetylase; RNAse; Maturase; Nucleoside triphosphatase; Inorganic pyrophosphatase; Glutathione S-transferase
Unknown	175 (31.8%)	
Total	**551**	

are using these as candidate genes involved in sucrose accumulation. Other strategies such as differential mRNA display and representational difference analysis (Liang and Pardee, 1992; Lisitsyn et al., 1995) may be useful in identifying differentially expressed genes, such as stress-induced genes during germination (de los Reyes and McGrath, unpublished).

Given an informative set of genetic markers, current technology would allow rapidly genotyping individuals in populations. Molecular marker profiles for each plant in a breeding population could be done prior to agronomic evaluation, if the current technologies were developed for beet. It is unlikely that any single population is going to detect all genes involved in trait expression. However, the combined power of multiple populations, sites, and years will identify most if not all genes or alleles that contribute to quantitative traits. Integrating this information will require a common set of markers that are uniformly distributed throughout the genic portion of the genome, and ones that are easily assayed with minimal cost. Analyzing the combined data sets will be challenging, and computational algorithms using artificial intelligence, fuzzy logic, and data mining approaches, for example, will need to be developed.

Marker tags alone are of little value to the grower or processor, so strategies to rapidly screen agronomic performance of segregating populations will need to be implemented. Scoring populations for agronomic traits is a significant undertaking that is often labor-intensive and subject to environmental variability. Imaging analysis and remote sensing are just beginning to be applied to agriculture and may show promise in scoring traits. Imaging can analyze each plant individually, and make repeated measures of single plants over time (e.g. non-destructive testing), if mounted on a mobile platform. An application would follow the course of field emergence over time, and perhaps pinpoint critical aspects amenable for a targeted breeding effort. Imaging can also show relative growth rates, leaf area indices, and canopy cover changes during the growing season. *Cercospora* leaf spot disease progression may be assessed with the same equipment. Other applications may be spectral analyses (hyper-spectral imaging) as early indicators of plant stress. Near-infrared reflectance spectroscopy may allow identification of specific molecules such as secondary metabolites and sucrose. Such capability would allow for high-throughput agronomic evaluation (e.g. screening large populations consistently and rapidly), at least as a first approximation, which would help to refine breeding and selection decisions. Such activities are beyond the current scope and expertise of the USDA at East Lansing, but collaborations to achieve these goals should be fostered.

In summary, the emphasis of USDA program at East Lansing is shifting towards integration of biotechnologies for sugarbeet improvement.

A strategy has been developed which will be tested over the coming years. Many opportunities for collaboration exist now and in the future (as they have in the past), and we appreciate the support of the sugar community in fostering past and future achievements.

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