McGRATH, J. MITCHELL* and LINDA E. HANSON, USDA-ARS, SBRU, 494 PSSB, Michigan State University, East Lansing, MI 48824-1325. **Overview of breeding and enhancement activities at East Lansing, Michigan.**

ABSTRACT

The ARS breeding and germplasm enhancement program at East Lansing, Michigan has been active for over 50 years, and was instrumental in breeding for resistance to Aphanomyces seedling disease, germplasm conversion for hybrid seed production, and developing smooth-root germplasm to reduce soil tare. Throughout this process, the focus has been on practical agronomic conversion to useful varieties and germplasm for the humid, rain-fed, sugar beet growing regions as typified by the Great Lakes region. For many reasons, our understanding of the genetic basis of these traits, and perhaps the majority of traits in sugar beet, has lagged behind our ability to recombine different disease resistances with sucrose yield, as well as application of technologies that would be useful to dissect the genetic basis of useful heritable variation for sugar growers. Three requisites to dissect the genetics of any trait are a (1) a population in which the trait(s) of interest segregates, (2) one or more measurable characteristics, e.g. traits, and (3) a context that allows clarification of the underlying genetic processes (e.g. markers and methods). Sugar beet suffers in the first instance by its complex self-incompatibility system, a trait that precludes self-fertilization for traditional, powerful, Mendelian genetic approaches to trait dissection. Over 10 years of deploying the self-fertility (S^f) allele has allowed developing a number of very interesting populations for genetic analyses. The process of inbreeding has not been as detrimental for fecundity as expected although it is unlikely inbreds would ever supplant hybrids for sucrose production. Traditional germplasm enhancement activities are still a strong component of the program, however stacking traits and resistances in such populations will eventually require marker-assisted approaches. The combination of population and marker development, ongoing, with existing expertise in measuring phenotypic variation is expected to facilitate introgression of novel alleles from wild germplasm as part of a more directed approach to sugar beet germplasm enhancement.

Plant breeding has two general goals, product enhancement, and crop protection. Product enhancement includes improving quality attributes such as yeild per acre and sucrose percentage, while crop protection encompasses any trait that would otherwise depress the aggregate yield of sucrose (or seed production, if hybrid seed is the end product). Over the past hundreds and thousands of years, selection has operated along these two paths, alternating between evaluation of the crop and selecting better performing plants or progeny for the next generation. This interplay between what plant breeders call the phenotype, which is the end product of the genetic potential of the variety or population minus the actual performance as it is affected by the specific environment, and the populations that contribute to the next generation of seed, is basically the operation of plant breeding.

Traditional methods of sugar beet breeding are followed at East Lansing, which has been in operation in one form or another since the early 1920's. Important contributions of the early East Lansing program have been contributions of Aphanomyces and Cercospora resistant varieties and germplasm as well as conversion of open-pollinated varieties that now allows the seed industry to make better and higher yielding hybrids for commercial growers. Some of these contributions have been reviewed, and an update of more recent work will appear within the next year in the Journal of Sugar Beet Research. Here, this expanded abstract simply serves to provide an update of the activities at the East Lansing germplasm enhancement program over the past 10 years, and provides a window to the goals and expectations for the next 10 years.

Currently, we have two broad areas of population development at East Lansing. The first follows traditional methods of mass and recurrent selection, where progeny from mother roots selected in one year and environment (e.g. disease nursery) are grown in nurseries in subsequent years. The best mother roots from these nurseries are selected and recombined (i.e. allowed to inter-pollinate) from which seed from these mother roots are evaluated in the same or different nurseries. The process by which new quality or protection traits are added to the best germplasm for humid, rainfed sugar beet growing areas is termed introgression. Introgression is desireable in many cases since none of our varieties or germplasm is perfect; there is always something desired, and the environment is rarely cooperative in allowing the full genetic potential to be realized. Plus, some traits are simply not available within the available populations. Adding traits via introgression can be a step backwards initially since often undesirable characters are also introduced, and need to be removed in subsequent rounds of selection and seed production. This process takes years. Slide 1 shows the current germplasm and goals of East Lansing traditional breeding. In a nutshell, enhancement of emergence, stand establishment, biomass production, and storability are quality enhancement issues; and improving persistence, Rhizoctonia, Fusarium, and nematode resistance are broadly crop protection issues. However, in a larger sense, these activities are more designed to widen the genetic base of East Lansing germplasm such that continued genetic gains are possible in the future.

Slide 1: Traditional sugar beet (self-sterile) population development for humid, rain-fed areas.

Re-adapted recurrent parent materials

=> Smooth-root, sugar yield, Aphanomyces, Cercospora, Rhizoctonia

using derivatives of SR97 / EL0204 / EL50 / EL51 / others

Introgression / selection materials

Seedling vigor / salt tolerant germination – Ames 3051 (poster)

Long-term seed viability (EL55)

Seedling disease resistance (SR98, Rhizoctonia & EL54, Aphanomyces)

Nematode resistance (RKN & SBCN) – from Salinas germplasm

CMS & O-type vigor selection – lower priority, not much inherent vigor

Low water / high biomass content – easy selection target

Projects in early stages

Storability…

Fusarium resistance…

There are some inherent problems with the traditional methods of sugar beet improvement (Slide 2), although it is emphasized that the process works and has provided inumerable benefits for everyone associated with the sugar industry, including consumers. However, one role of public breeding in the current commercially-dominated seed industry is to provide long term value to the industry by examining the process of traditional breeding and asking what and how we might overcome limitations in our knowledge and understanding of the processes which result in a profitable crop for growers. That is, to keep the industry viable and healthy, the job in part is to bring all tools to bear on understanding the genetics of sugar beet profitability *in toto*, and bring these to industry's table for application. It should be noted that in this very competitive environment, each seed company is following similar strategies as outlined

below. The major difference is in the outputs. Industry needs to remain profitable, and their activities are driven by and follow the balance sheet to some extent, while public breeders seek to understand and provide solutions for more fundamental processes that support long-term viability of the industry. One of these activities should be to understand the genes required for economic sustainability. It seems telling that something is not quite right when we do not fully understand the genetics of sucrose accumulation, the basic trait of the crop. This has as much to do with the biology of beets as with the lack of available technology to dissect such traits using traditional approaches available for sugar beet.

Slide 2: Limitations of current traditional breeding.

Problems with traditional breeding are:

Difficult to discern genetic contributions from environmental influences.

Slow progress to fixation of alleles in populations.

Pollen control to limit cross contamination.

Multiple alleles lead to complex gene interactions.

Gene discovery requires analyses of many individuals.

Problems are obviously surmountable (repeat, re-select, repeat).

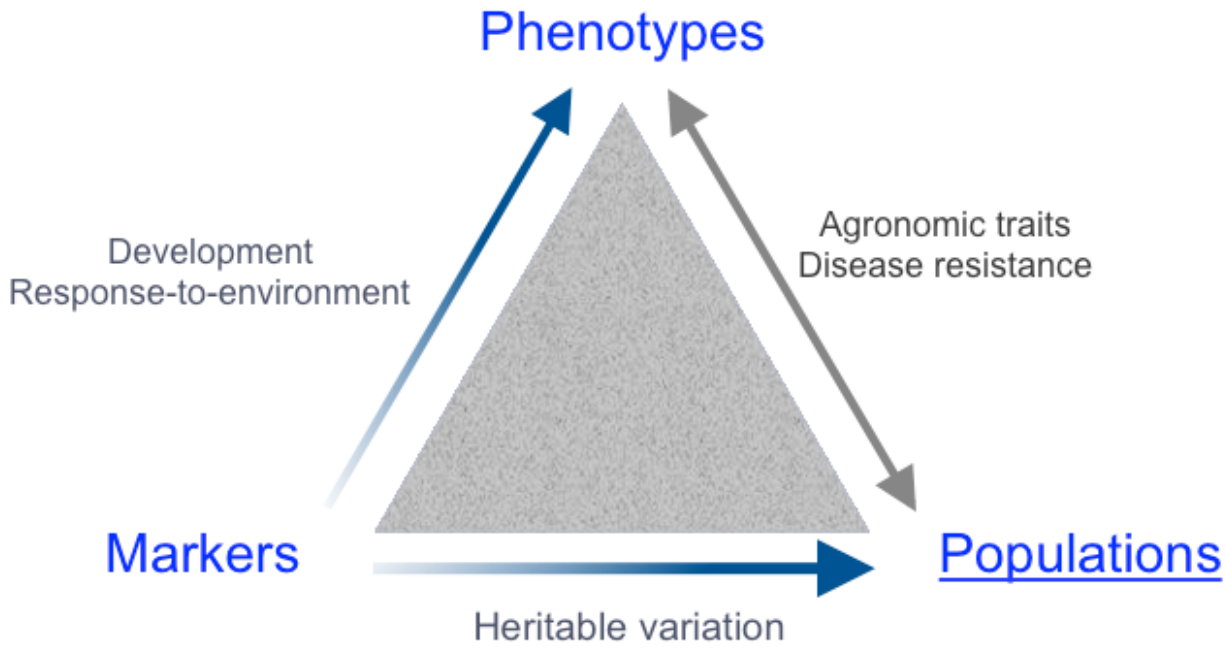
Is there another way that would meet germplasm needs,
and facilitate gene discovery and generate alternative perspectives?

In re-examining the role of traditional breeding, which provides enhanced germplasm to reduce input costs and improve quality, it is clear that a multitude of biochemical processes contribute to the final product delivered to the consumer, many of which are surmised but we suspect there are additional processes to be discovered with direct impact to growers and consumers. Rather than try to create a list of potential targets for improvement based on our current biochemical understanding, why not allow the beet to prompt us which process appear to be more important from its perspective? While this may seem a crazy notion, the tools are becoming available that this idea is not so dramatic to envision. There are limits to sugar beet, as we well know. One might imagine that these limits could be overcome, but how? The easy answer is to simplify the question, ask what tools are need to gain at least a preliminary understanding, and then leverage this understanding into a meaningful result.

The question most if not all sugar beet breeders, at one time or another, is to what extent what is observed and selected is the product of genetics (and heritable, where selection will be successful if all goes well) and how much of the phenotype is due to solely environment (and not heritable, thus selection would be a waste of time). The other question is, "And how much will it cost to know?" This is perhaps where the public breeding enterprise might help in the long run. That is, we should make the long-term investment in resources needed to address these questions in a cost efficient manner. So, what resources might be needed?

A picture of the resources needed in a general systematic approach to dissecting the genetic basis of agronomic traits might be considered in relationship to Slide 3. The relation between phenotypes (e.g. sucrose yield, rhizomania resistance) and populations discussed above are not the best for genetic dissection of traits. The new part of the approach considered is termed 'markers', but in reality this only means that we need a reproducible, independent measure of populations and phenotypes that allows the genetic contribution to be unambiguously determined. Most often what is envisioned are DNA-based markers since they are becoming cheaper, are extremely abundant (in theory), and the technologies to examine them are well developed. The genome sequence of beets fits this concept, and is actually ideal as a source of markers since it is comprehensive in scope, and will be available within the next five years. Markers in and of themselves are not useful *per se*, but they provide exceptional context and clarity. For example, the marker concept can be used to discover genes that are important for sucrose accumulation by contrasting gene expression young and old plants, if a developmental context were to be used. Alternatively, markers could be used to locate areas of the beet chromosomes that are more frequently inherited with high or low sucrose, for example. However, this report is not about markers, but of the requisite populations that will be needed, now that the marker approach is firmly accepted by science (we still need a sufficient but undetermined number of markers for sugar beet, and this is a current priority).

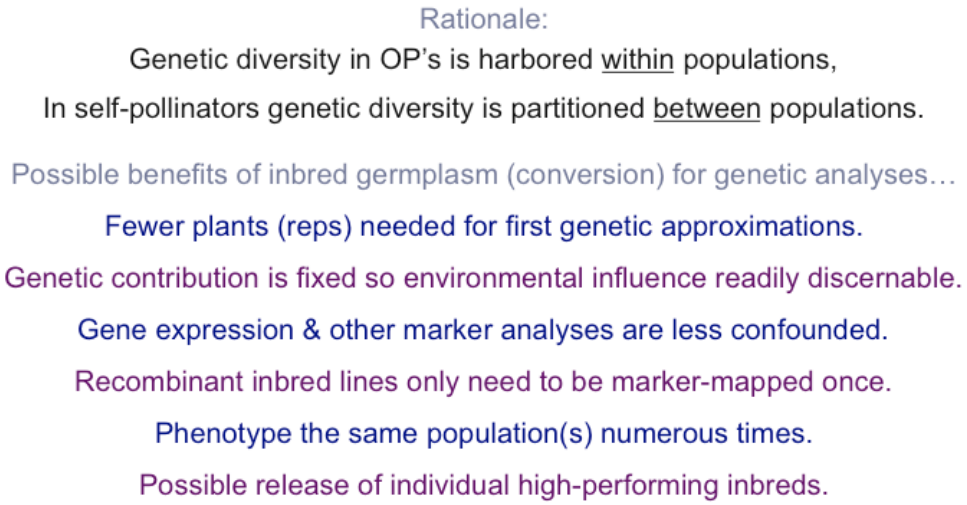
Slide 3: Broad concept of modern breeding, whereby markers can be used to provide context and clarity to agronomic processes in sugar beet.



The key to understanding the genetic components of agronomic performance are the populations themselves. The phenotype is known *a priori* as an agronomic trait of interest. The markers are useful, but only in context of the phenotype, which a property of a population in an environment. Thus, if the sugar beet population structure hinders genetic dissection of traits, what tools and tricks are available to overcome such limitations? This million dollar question has been addressed in many ways, including cloning a single plant, and developing doubled haploids which are presumed to be genetically uniform within a plant, and thus any differences in phenotype are presumed to be strictly a response to the specific environment. We have

approached the problem somewhat differently by inbreeding populations with the aid of the self-fertility gene (Slide 4). This has been a long-term endevour (since 2000), and is beginning to realize its promise. This is the second major breeding program ongoing at East Lansing.

Slide 4: Inbreeding beets may have significant advantages for genetic dissection of traits for sugar beet improvement.



The important points to remember are that beets are out-crossing by nature, and that any population of beets can have many different genes (or alleles; varients of one gene), and thus cloud any rationale genetic analysis. By strictly enforced inbreeding, each plant reduces its genetic variability by half in each generation such that after six generations less than 3% of the 30,000 or so genes in sugar beet are in the same state at each genetic locus. This simplifies analysis. If we start with a single diploid plant and self all its progeny, individually, that arise over these six generations, we have an inbred population where there are only two variants, maximum, at each genetic locus (due to the diploid nature of the intial individual), and we have captured most or all of the variants in one or more selfed individuals. This gives the benefit of knowing the amount of phenotypic variation for any trait, by looking at genetically identical (or nearly so) progeny within an inbred population, but also the genetic contribution when we measure the variation between populations of inbred individuals.

To date, we have developed such so-called 'Recombinant Inbred Lines' from three different traditional populations, and are beginning the time consuming process of their evaluation. Already, we are seeing the high environmental variation for root yield but less environmental variation for sugar content, in line with expectations. And we are continuing to develop additional populations. These populations will be an exceptional resource for beet genetics, and will dovetail nicely with discovery of genes and biochemical processes as we enter the genomic age of sugar beet. Characters such as Fusarium stalk blight resistance, Rhizoctonia damping-off and crown and root rot, and Cercospora leaf spot resistances have been shown to vary within these Recombinant Inbred Lines, and the next step will be to partition this genetic variablity on to individual chromosomes using markers as genetic loci. Knowing the genes and loci will be the start, from which we can find and then design, if needed, new or different genes and alleles in order to more precisely add value to the sugar beet germplasm.

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