UTILIZING GENETIC RESOURCES FOR PREBREEDING OF STRESS RESISTANT SUGAR BEET GERMPLASM: USING MOLECULAR TOOLS

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Abstract

In the last 30 years, disease pressure has compelled sugar beet breeders to utilize other cultivated beets and wild relatives as genetic resources, despite the reluctance of earlier breeders, who had avoided these sources due to the many undesirable traits introgressed with the resistance genes. In the U.S., public breeders do much of the sugar beet prebreeding. The development of molecular biology has impacted both the methodology and objectives of this germplasm enhancement effort. While introgressing genes; marker development, gene mapping, and gene discovery increasingly are becoming additional goals of the prebreeding effort. For example, the discovery of the vernalization gene in sugar beet, *BvFL1*, was part of a project to use molecular markers to understand the genetic variation present in populations of wild sea beet. Other wild beet populations, used as disease resistant donors, are providing one parent in mapping populations to indentify individual genes or QTLs responsible for that source of resistance. As genetic resources provide new sources of genetic variation in commercial variety development, breeders also are developing the population structure and information necessary to enable genomic and proteomic analyses of the genetic resources available to sugar beet breeders. The identification of major genes within other sections of *Beta*, (or other taxa) facilitates transgenic approaches to disease resistance. This prebreeding effort has increased the use of wild relatives as genetic resources and underscores the need for these populations in our genebank collections.

Key words: *Beta vulgaris*, disease resistance, *Beta vulgaris* ssp. maritima, genetic resources, sugarbeet

Introduction

By the middle of the 20th century, in response to the mechanization of agriculture, the cultivation and production of sugar beet (*Beta vulgaris* L.) had undergone tremendous changes (Francis, 2006). Seed companies had moved from open-pollinated and anisoploid varieties to true hybrids using the cytoplasmic male sterility and genetic fertility restoration system developed by V. F. Owen with monogerm O-type maintainer lines (Desprez & Desprez, 1993; Owen, 1945; Savitsky, 1952a; Savitsky, 1952b). As the area cultivated grew, so did the need for increased resistance to pests (Lewellen, 1992). However until the 1980s, the undesirable traits from wild and other domesticated beet groups – colored pigments in the root, multiple crowns, sprangled roots (difficult to harvest, soil tare), annual life cycle, and low sucrose concentration and extractability – created a reluctance to use this pool of genetic resources for disease resistance (Coons, 1975; Frese *et al*., 2001; Lewellen, 1992; Panella & Lewellen, 2005). The one notable exception was the success of Otavio Munerati, who used wild sea beet (*Beta vulgaris* subspecies *maritima* (L.) Arcang. – Bvm) germplasm in Italy as a source of resistance genes for protection against leaf spot (caused by *Cercospora beticola*, Sacc.) (Munerati *et al*., 1913).

A number of events in the 1980s paved the way for increased use of wild sea beet and other domesticated beet groups (referred to hereafter as 'exotic germplasm') as genetic resources in sugar beet improvement. In the U.S., the USDA-ARS Sugar Beet Crop Advisory Committee began an aggressive program of evaluation of exotic germplasm for resistance to important sugar beet pests and diseases (Doney, 1998; Panella & Lewellen, 2007). Concurrently, in Europe, N. O. Bosemark's (1989) theoretical breeding scheme was expanded and integrated into commercial and public programs in Europe and the U.S. (Frese *et al*., 2001; Panella & Lewellen, 2007). The World *Beta* Network, founded under the aegis of the IPGRI in 1987 (Frese, 1990), joined by the ECPGR *Beta* Working Group, begun in 1998 (ECPGR-BWG, 2008), have provided the frame-

work for international collaboration and cooperation. Then, between 1996 and 2002, European project partners (researchers and seed companies) collaborated in germplasm evaluation and data documentation in the GENRES CT95 42 project (Panella & Frese, 2003).

Along with the increasing introgression of genes from exotic sugarbeet genetic resources, the last fifteen years has seen a tremendous increase in the tools available for genetic analyses. Linkage mapping has grown from the use of isozymes (Abe & Tsuda, 1987) to full genome maps (Barzen *et al*., 1992; Grimmer *et al*., 2007b; McGrath *et al*., 2007b; Schneider *et al*., 2007); there are a plethora of molecular markers available and the possibility of full genome and proteome analyses is being realized (Catusse *et al*., 2008; Larson *et al*., 2007; Larson *et al*., 2008; Puthoff & Smigocki, 2007). There is no room here to adequately review this literature as is seems to be growing exponentially, and reviewed annually (Bosemark, 2006; McGrath *et al*., 2007a; McGrath & Skaracis, 2005; Zhang *et al*., 2008). Increasing access to these technologies is impacting the way we evaluate and use genetic resources.

How are Molecular Technologies Being Used in Prebreeding?

First, what is meant by prebreeding? If we look at the utilization of genetic resources as a pipeline from the genetic resource to the release of a cultivar to the farmer (figure 1), we see that the pre-breeding step is crucial, if the accessions in our genebanks are to be used by commercial seed companies. It is in this step that the un-adapted germplasm is evaluated, and bred into a genetic background that allows it to fit into a commercial breeding program, which operates under the constraint of meeting short term goals (Stander, 1993).

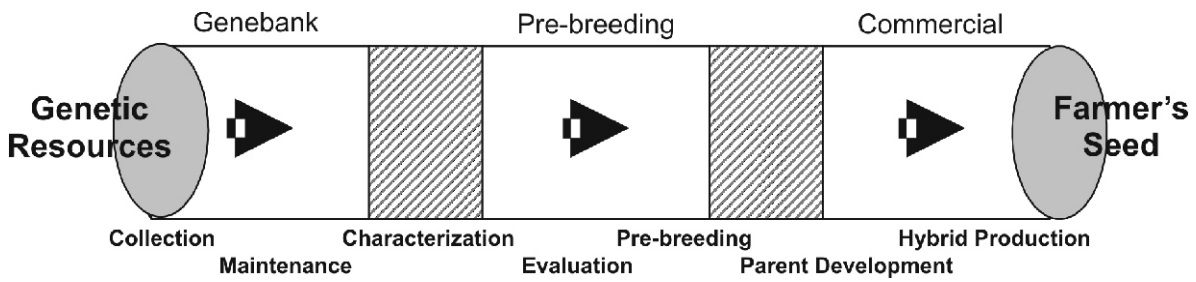

Figure 1. In the US, the utilization of sugar beet genetic resources flows from the genebanks through the USDA-ARS public breeder to the commercial seed company, which produces the varieties that reach the farmer. Utilization of genetic resources depends upon the prebreeding step to allow commercial breeders to access the genetic diversity available in our genebanks in a genetic background that they can use.

The prebreeding process is a long-term project, which, in sugar beet, may require 10 to 15 years of patient work; and, in the U.S., is accomplished by USDA-ARS public breeders (Doney, 1998; Panella & Lewellen, 2007). Much of this work has been done by crossing into long range populations using the recurrent selection method described by Bosemark (1989). In sugarbeet, prebreeding has been used to introgress disease (Campbell & Bugbee, 1993; Lewellen & Schrandt, 2001) and pest (Lange & De Bock, 1994) resistance, plant architecture traits (Theurer, 1993), and combining ability (Doney, 1993). However with new tools available, commercial breeders want as much information as possible to more quickly utilize the enhanced germplasm they are receiving from the public breeders. Information on inheritance always has been important and some new sources of resistance have been provided in a format to allow molecular (or traditional) genetic analyses – e.g., different sources of resistance backcrossed into a common genetic background (Lewellen, 1998).

Molecular markers of every kind are being used more commonly in breeding programs, and, with marker assisted selection, genes can be more efficiently introgressed. Public prebreeding programs utilize breeding schemes that facilitate germplasm enhancement concurrent to molecular marker discovery in the populations in which they are working. More marker discovery is taking place on the hybrid from the exotic source and sugar beet parent. (Friesen *et*

al., 2006; Grimmer et al., 2007a)Obviously if a new source of resistance can be provided to a commercial seed company with a marker in hand, or the population structure from which to quickly extract the marker, that germplasm is of more value.

Characterizing and Evaluating Genetic Resources

Evaluation of germplasm continues to be critical in identifying those accessions that have genes of interest. This is clearly illustrated in the screening for pest and disease resistance, mentioned in the introduction, and, over time work has been done on the development of disease screening techniques, in the field, greenhouse, and laboratory (e.g., (Azorova & Subikova, 1996; Büttner *et al*., 2004; Panella, 1998; Panella, 2000; Ruppel *et al*., 1979; Ruppel & Gaskill, 1971; Scholten *et al*., 2001; Scholten & Lange, 2000). Evaluation methodology is another piece of information that commercial seed companies want with enhanced germplasm – how to screen for the phenotype enhanced in that germplasm.

As we attempt to utilize exotic germplasm, especially wild relatives of sugar beet, it is important to characterize and understand the genetic diversity of those populations. Molecular markers provide a useful tool to supplement characterization by morphological traits and disease response. Although neutral markers may be used to quantify diversity, in looking at genetic diversity with molecular markers, it is critical to look at agriculturally important traits. In sugar beet, life history – annual or biennial – is such a trait. In a study of Bvm genetic diversity along the Atlantic coast of France, we were able to identify and characterize a key vernalization regulatory gene, *BvFL1*, mined from an EST database (Reeves *et al*., 2007). This discovery is not only useful in characterizing those populations but has tremendous research and commercial applications.

Gene discovery, be it in wild or domesticated populations, provides a new way to characterize genetic resources as well as providing the most useful marker – the gene itself. Single nucleotide polymorphisms (SNPs) within genes (and having a phenotypic effect) will become more common and more useful as we characterize them and understand their pleiotropic effects. They are a useful tool to describe alleles and measure allelic diversity. Already, important progress is being made in developing useful SNPs in sugar beet (Grimmer e*t al.,* 2007b; Schneider *et al*., 2007).

How will the changing technologies impact prebreeding?

As more markers (protein or DNA) are developed, and more genes influencing important traits are cloned and characterized, prebreeders will be introgressing characterized genes rather than empirical phenotypes. They also will be going out of sugar beet's primary genepool and into the secondary and tertiary gene pools (Harlan & de Wet, 1971) because transgenic approaches will allow insertion of genes directly into elite sugar beet parents (as well as into other crops). Evaluation will not be concentrated only on whether the trait is present but on the biochemistry and physiology of how a particular phenotype is manifested. Mapping genes is important, but more important may be the discovery and knowledge of allelic diversity and allelic interactions (at crucial loci) and their effects on phenotype.

Accurately screening germplasm will be important not only in determining the magnitude of the allelic effect at specific loci but also in understanding the pleiotropic effects of allelic interactions at different loci. Characterization and basic research will play a larger role in the prebreeding process because knowledge of the genetic composition of genetic resources will become more important in making breeding decisions. This knowledge is what the seed companies will need to decide which sources of a gene or genes to use developing their commercial hybrids.

These changes will also impact the way our genebanks and genetic resources databases are used. Currently, as mentioned above, *Beta* genetic resources are well characterized for a number of important morphological, agronomic and disease response descriptors (Panella & Frese, 2003). In the future we will need descriptor data from molecular analyses. It will be important to describe allelic diversity within an accession, not just among accessions. To describe gene and allele frequencies, it will be necessary to enter individual plant data into our genebank databases to describe the accessions. Fortunately our genebanks are moving in that direction (Volk & Rich-

ards, 2008). I think that the importance of prebreeding and the close working relationship between prebreeders and genebanks is summarized well by JR Stander (1993):

> "The broadening of the genetic base of crops requires a system of well maintained and appropriately described collections. Classification, characterization, and pre-breeding of material in collections will greatly stimulate their use by private plant breeders. In the absence of these activities private breeders will, in general, continue to ignore the collections."

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