

**Breeding for genetics: Development of Recombinant Inbred Lines (RILs) for gene discovery and deployment.**

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**Abstract**

Genetic diversity underlies breeding advancement, and selection is the act of concentrating relevant genes (alleles) in populations for ultimate deployment to growers. Measures of genetic diversity indicate perhaps fewer than 25% of the alleles present in *Beta vulgaris* are present in sugarbeet, suggesting future genetic gains will be found within wild and unadapted germplasm. Germplasm collections exist, but attention to deploying their allelic diversity is stymied, with notable exceptions. Systematic efforts to deploy this germplasm held in public trust are needed, coupled with systematic efforts to identify, map, and catalog relevant agronomic genes. Both objectives can benefit from development of RILs, which represent 'immortal' segregating genetic populations. RILs have not been developed for sugarbeet, and their utility lies in the simple (if lengthy) construction process, their defined genetic relationships within populations, their homozygosity which limits phenotypic diversity to environmental variance components, and the ability a single molecular map to phenotypic values from many environments. RILs are derived via single seed decent (facilitated by self-fertility) from an initial segregating population, are highly inbred progeny derived from a single parent, and sample the genetic diversity of parental lines used to construct the hybrid. Our strategy has been to use a common male sterile (nuclear or cytoplasmic) self fertile seed parent (e.g. C869) to which one of 37 pollen donors has been mated to date, generally followed by selection for vigor and fecundity of the F<sub>1</sub> hybrid, then by single-seed descent for five generations. As of October 2004, 5,968 S<sub>2</sub>, 819 S<sub>3</sub>, 245 S<sub>4</sub>, and 102 S<sub>5</sub> plants are in process. Phenotypic, sucrose, and water content evaluations on one in-process population (C869 X Table beet) indicate the general utility of RILs for genetic analyses, and effectively couples the process of gene discovery with gene deployment in sugarbeet.

## Introduction

Genetic analyses in sugar beet are complicated by its breeding system, which is governed by self-incompatibility that prevents routine selfing, and highly transportable pollen (e.g. the slightest breeze) which increases risks of genetic contamination in controlled crosses. Few inbred lines have been constructed. Ones available have been based on a dominant self-fertility gene (or allele). Creating hybrids in Beta is problematic since flowers are slightly protandrous, each flower bears a single ovary, and mechanical disturbance (e.g. hand pollination) of the flower can result in premature abscission. Therefore, the seed parent for most of the RI populations to be developed has been male sterile in addition to carrying the self-fertility trait. Hybrids are created with ease by bagging a pollen donor with a male-sterile plant, the hybrids are self-fertile, and can be examined for agronomic performance in the field or greenhouse.

Construction of defined populations segregating for many traits has been ongoing at East Lansing over the past seven years. A strategy was taken initiated to capture genetic diversity of self-incompatible, open-pollinated USDA-ARS releases, wild species, and crop relatives [fodder and table (red) beets, and chard] in a self-fertile genetic background. Self-fertility enforces inbreeding, allowing unexploited opportunities to develop  $F_2$  (syn.  $S_2$ ) populations for genetic mapping and for progeny testing in  $F_3$  (syn.  $S_3$ ) populations for disease resistance and other agronomic evaluations. Many (>100) self-fertile hybrid populations have been made to date, yet remain to be characterized. Such early generation derivatives from an initial diverse hybrid are ephemeral, with their particular genetic combinations segregating in large part independently in later generations. Thus, multi-year, multi-location field trials are precluded, and estimates of heritability for any trait are less precise for  $F_2$  and  $F_3$  populations than they might be if populations were homozygous. We have continued selfing the progeny of  $F_2$  and  $F_3$  plants to determine whether agronomic trait genetics could be examined in advanced populations, and report some of the experiences here. The primary goal is to develop Recombinant Inbred Lines (RILs) from as many genetically divergent germplasm sources as practical to examine trait genetics. We use a rapid cycling strategy to attempt to advance 1.5 generations per year, with a goal to obtain  $F_6$  seed by single-seed-decent within four years.

A decision was made to create more populations with fewer individuals rather than a few populations with many individuals. This reflects a disadvantage at the start of whole scale inbreeding because recovering the positive donor trait alleles from heterozygous self-incompatible parental populations in a single fertile  $F_1$  hybrid as the primary source of one Recombinant Inbred I population may have a low probability, especially if parental sources were wild germplasm. More populations developed from the same cross would likely reveal more segregating loci related to trait genetics than would a narrower focus on a few larger populations, thus we have geared towards capturing the maximum amount of allelic diversity segregating in a slightly larger than practical number of segregating populations. However, any set of RI populations developed at these early stages is unlikely to capture all allelic diversity needed for formal genetic investigations in these proof-of-concept investigations. The intent here is to demonstrate that inbreeding can be accomplished and that inbreeding depression, segregation distortion, recessive lethality, and other Mendelian genetic masking phenomenon can be eliminated prior to the development of larger populations. The choice of parents continues to evolve as more and better information becomes available from on-going activities.

Hybrids have been grown under selection (e.g. disease nurseries) to identify the most highly developed phenotypes, and these individuals have been used to self-pollinate to generate F<sub>2</sub> populations. Beet improvement generally follows a population improvement approach, and most, if not all, disease resistance traits currently used in beet are expected to behave in a dominant fashion. It should be noted that heterozygosity introduces an additional source of experimental uncertainty in genetic analyses beyond that influenced by environment, and for this reason beet geneticists and breeders have sought to limit uncertainty associated with genetic heterozygosity through stably maintaining a genotype, either through anther-culture derived inbreds or by clonal propagation of elite genotypes. All of these methods have drawbacks, however the RIL population approach has not been attempted to date and it appears to offer an advantage of recovering a diverse array of adapted genotypes for further breeding and molecular analyses.

### **Materials and Methods**

Plant materials are listed in Table 1. For rapid cycling, plants were grown in an SC-10 super cells (e.g. tapered cone-shaped plastic pots) (Stuewe & Sons, Inc. Corvallis, OR), each 10 cubic inches (1.5 inch top diameter x 8.25 inch length). Each pot had a bottom drainage hole with four side drain holes on the tapered end, and held upright in polystyrene tray (Stuewe & Sons, Inc.). Pots were filled with high porosity commercial soil-less mix containing 70-80% horticultural sphagnum peat and perlite limed to 5.5 – 6.5 pH. Two to three seeds were planted per pot (for a total of 200 plants from each F<sub>2</sub> population). High-pressure, 400 watt, sodium lamp fixtures were placed 40" inches from the soil surface with one fixture for every 25 ft<sup>2</sup> of bench space. (Additional blue light produced by metal halide bulbs may be necessary to shorten the internodes of the plant during the first 7-week growth period). Plants were re-spaced in the trays at approximately three weeks of age to accommodate for the copious leaf growth, only the most vigorous plant in the pot was kept in cases of multiple seed emergence, and fertilization program was initiated with application once per week (Miracle Grow; 15-30-15, with 0.02% B, 0.07% Cu, 0.15% Fe, 0.05% Mn, 0.0005% Mo, and 0.06% Zn). Disease and insects were controlled on an as needed basis with both systemic and contact pesticides. Plants were grown for 7 weeks total in the greenhouse with average daytime temperature of 68 °F and average nighttime temperature of 64 °F. Vernalization for 14 weeks followed at a temperature of 40°F and illuminated by low-intensity fluorescent lamps with a photoperiod of 14 hours. Plants were not fertilized during this time, and watering was done on an as needed basis.

Plants were removed from vernalization at approximately 19 weeks of age and kept at 60 °F average day temperature and 55 °F average night temperature until most plants had bolted, usually with six weeks. A photoperiod of 17 hours was used, and a fertilization program identical as above resumed when plants began to bolt. Paper bags 4.5" x 7" in size were used to cover the inflorescence and tied at the bottom to control pollination. Wire stakes (20") were used to support the plant and pollination bags. Seed were harvested when their seedballs had dried, about six weeks after bolting, cleaned of leaf and stem debris, and stored at 40 °C with low humidity. Thus, from seed to seed, 30 – 40 weeks had elapsed (however the proportion of plants bolting in a population was variable and many plants needed re-vernalization) . The cycle was repeated for the next generation. Seed dormancy has not appeared to be a problem.

Agronomic trials were conducted at the Saginaw Valley Bean and Beet Farm (Saginaw, MI) in 2004. F<sub>5</sub> seed from the MSR population (Table 1 and below) was produced in 1

gallon pots in the greenhouse in the winter of 2004, and seed harvested from 157 green plants was planted as single 24' rows on June 4, 2004. Stand was variable, but most plots had >10 beets. Beets were lifted October 7, and five beets were individually weighed, sectioned longitudinally 2/3 of the beet's length from the crown, and cut was scanned with a handheld Near-Infra Red spectrometer. Statistical treatments were aided using the JMP package (SAS Institute).

## Results

*Bona fide* hybrids were made between self-fertile sugar beet and various *B. vulgaris* accessions representing the agronomic and morphological diversity of the species and ARS releases. These include, among others, hybrids with a common seed parent accession (C869, Curly Top virus resistance) with pollinators such as EL50 and US201 (both with *Cercospora* leaf spot resistance), USH20 (seedling vigor), EL51 (Rhizoctonia crown and root rot resistance), EL48 and SP6822 (Aphanomyces resistance), SR94 (smooth-root morphology), F1016 (root maggot resistance), EL0204 (rhizomania resistance), GW359 (root aphid resistance), SP85303 (oomycete resistance), L19 (high sucrose), KleinE (yield components), fodder beet (Mammoth Red and Wintergold), Table beet (W357B, Indian Table beet), Chard (leaf type), individuals from 100 Plant Introduction accessions that were selected for survival in a seedling disease nursery, and a dozen morphological mutants rescued from the East Lansing seed archives. Not all have yet made their way into RIL production.

The largest RIL populations are created to follow genetic segregation of specific East Lansing germplasm resistances (e.g. Aphanomyces, Rhizoctonia, Cercospora), sucrose accumulation, and smooth-root. The basic strategy for making F<sub>1</sub> hybrids has been to include a single C869 male sterile plant within a seed increase plot of the desired pollinator germplasm, such that the male sterile has the opportunity to acquire pollen from the donor population. For each RIL population, we target 94 plants by the F<sub>6</sub> generation as a successful outcome. This is because it is relatively easy to work with this number (plus one each of the parents) in a standard 96-well microtiter plate, and 94 individuals gives a reasonable approximation of segregation ratios for both molecular markers and simply inherited traits. We start with 200 plants and expect to lose one quarter of the plants in the F<sub>2</sub> due to segregation of the nuclear male sterile trait (i.e. the *A* locus). This is a problem if trait genes are linked to the *A* locus, so more recent F<sub>1</sub> hybrids are being made with the C869 CMS counterpart (however this may also segregate for *A*). An additional unknown proportion of F<sub>2</sub> plants may not yield seed either due to segregation of the self-fertility trait (symbolized as *S*, known to be a simply inherited trait but not known to be either an allele or an unlinked suppressor of self-incompatibility) or due to genetic load (e.g. segregation of lethal and sub-lethal alleles). The later problem might be expected with increasing heterozygosity of the pollen donor, particularly if the pollen donor is from a wild or feral population. To date, we have seen survival to be highly variable in the F<sub>2</sub>, ranging from 33 to 99% of individuals (Table 2). However, for most of the lines, many of the individuals in the population have either failed to bolt or otherwise have de-vernalized. For the three populations in Table 2 where >50 individuals have set seed, survival and seed set ranged from 62.4 to 91.0% of the population, consistent with expectations. Survival of F<sub>3</sub> plants has been excellent, and most of these and more advanced generations produce seed (data not shown), however segregation for male sterility and other genetic load traits is expected and observed.

Seed production in small pots is drastically reduced, with a range of 2 to 8 seeds (n=423; mean = 0.16 g; standard deviation = 0.12 g) produced per plant, which is sufficient for

single seed decent. Production of advanced generation populations in larger pots is markedly improved, with seed production in 1-gallon pots of a sugar x red F<sub>4</sub> population being sufficient to plant an un-replicated field trial (2 – 12 g seed/plant). Seed produced had good germination and vitality.

#### **Brief description of the specific populations:**

Populations in Table 1 fall into three broad categories encompassing the major breeding activities; crop type, pollinator, and O-type lineage. Within each category, populations have been chosen to represent one or more agronomic traits (described below). Generally, it is recognized that the initial donor of a particular trait, such as Cercospora resistance or restorer genes, comes from a single source. Thus, these trait genes are identical by decent, and their representation in multiple but distinct lineages differing by selection history, is a further means to increase the probability of capturing these alleles, but also allows an approximate segregation analysis of other potentially important traits in otherwise unrelated parents. Where indicated, the current status is of February 14, 2005.

#### **Crop Type:**

**7S x Red (SxR, see Table 1 for key):** This population was originally intended for a foundation genetic map where both parents were inbred and thus purged of deleterious alleles perhaps causing segregation distortion. In retrospect, the 7S parent contributed negative phenotypes of naked seed (e.g. little or no pericarp tissue) and resulting seed shatter. This population appears to segregate for vernalization / devernalization response since half the population consistently fails to bolt upon the first vernalization attempt. Current status: 113 F<sub>5</sub> plants and 145 F<sub>4</sub> plants vernalized and bolting in the greenhouse.

**C869 x Red beet W357B (MSR):** This population has been used extensively in our program for the past four years to examine the inheritance of sucrose, and is the population for the genetic map (unpublished). The red beet parent is a public germplasm release used widely in commercial red beet hybrids, and is self-fertile with ca. 8% sucrose content at field harvest. The female parent is ca. 15% sucrose at harvest. Little evidence of restricted recombination (e.g. clustering of markers) was obtained using AFLPs on the F<sub>2</sub> population. Current status: 75 unselected F<sub>4</sub> plants and 100 others represented as F<sub>5</sub> seed. This population was grown as F<sub>5</sub> plants in the field in 2004 (see below).

**C869 x PI540625 (AYI, APA, APB):** These populations were developed to examine two aspects of expanding the germplasm base of sugar beet. The first is to introgress a potentially novel genetic source of resistance to seedling damping-off caused by *Aphanomyces cochlioides*. The second is to examine the phenomenon of restricted recombination in sugar beets. The pollen parent is a wild *Beta vulgaris* spp. *maritima* collected from the north coast of France, with reported high levels of *Aphanomyces* resistance (via the GRIN system). Work over the past three years has confirmed at least two loci contributing a high level of resistance to *Aphanomyces* infection in seedlings two weeks of age. Field trials from 2002 – 2004 also showed a high level of resistance to the chronic disease phase. Recombination as assessed with AFLPs in the F<sub>2</sub> appears less restricted in this population than in other reported sugar beet molecular maps. Current status: 85 F<sub>4</sub>, 47 F<sub>3</sub>, and 216 F<sub>2</sub> plants in total vernalized and bolting.

**C869 x Indian Table beet (PI163182) (ITA, ITB):** This population is being developed as a potential source of field resistance to seedling diseases from a selection plot at the Bean and Beet Farm in Saginaw MI in 1999 where it performed comparably in disease and non-disease nurseries, and also as a source of allelic variation not present in sugar beet

germplasm. Current status: 166 F<sub>3</sub> and 113 F<sub>2</sub> plants in total vernalized and bolting.

C869 x Fodder (**MRF, WGF, SFA**): These populations are being developed to examine inheritance of animal fodder crop use type. Current status: 73 F<sub>3</sub> and 474 F<sub>2</sub> plants in total vernalized and bolting.

C869 x Chard (**RSC**): This population is being developed to examine inheritance of leaf crop use type. Current status: 1 F<sub>3</sub> and 97 F<sub>2</sub> plants in total vernalized and bolting. 102 plants in this population died. Additional populations from this and other chard accessions are available as F<sub>2</sub> seed.

C869 x Klein E (**KLA, KLB**): These populations are being developed from legacy germplasm. Klein E is likely to be the same or substantially similar to the first sugar beets grown in the U.S. Current status: 399 vernalized F<sub>3</sub> plants.

**Agronomic traits:**

C869 x SP6822 (**SPA, SPB, AYA, AYB**): These populations were developed to examine inheritance of agronomic traits and *Aphanomyces* resistance. SP6822 is the pollinator for USH20, a hybrid with high emergence potential that was widely grown for sucrose in Michigan from 1975 to 1985. Its seed parent is similar to EL45cms listed below. Current status: 3 F<sub>4</sub> plants, 339 F<sub>3</sub> plants, and 231 F<sub>2</sub> plants (total) vernalized and bolting.

C869 x EL50 (**CRA, CRB, CRC**): These populations are being developed to examine inheritance of resistance to leaf spot caused by *Cercospora beticola*. EL50 is among the most resistant germplasm available and is well adapted to Great Lakes growing conditions. Resistance has been described as variable and complex (5 – 8 QTLs) and is markedly influenced by environment, and availability of RI populations segregating of resistance will be invaluable. Current status: 13 F<sub>3</sub> and 363 F<sub>2</sub> plants total vernalized and bolting.

C869 x US201 (**CRD**): Similar to EL50 but using a progenitor *Cercospora* resistant pollen donor. Current status: 178 F<sub>2</sub> plants vernalized and bolting.

C869 x L19/2 (not currently being increased this cycle): This population is being developed to examine inheritance of high sucrose content from L19/2 (ca. 20%). This Z-type germplasm was reselected from L19 for adequate performance in Great lakes growing regions, however is extremely susceptible to the range of disease pressures in these areas. Current status: F<sub>3</sub> seed from 200 F<sub>2</sub> plants each, in each of 2 populations.

C869 x [SP6822-17 X Z430] (**SPC, SPD**): These populations derived from an initial hybrid of SP6822 with self-fertile, high sucrose Z-type germplasm, similar to L19. Current status: 200 F<sub>2</sub> plants vernalized and bolting.

C869 x C869 (**ORA**): This population is being developed as a control population for field comparisons with other RI populations. Current status: 6 F<sub>3</sub> and 146 F<sub>2</sub> plants vernalized and bolting.

C869 x SR94 (**SRA, SRB**): This population is being developed to examine inheritance of the smooth-root trait. SR94 also has near commercial levels of sucrose, reasonable yield potential, and good *Aphanomyces* and *Cercospora* resistance. Current status: 10 F<sub>3</sub> and 359 F<sub>2</sub> plants total vernalized and bolting.

C869 x EL51 (**RTA**): This population is being developed to examine resistance to *Rhizoctonia* crown and root rot caused by *Rhizoctonia solani*. EL51 is among the most *Rhizoctonia* resistant germplasm, with parentage from USDA-ARS Ft. Collins releases as well as independent USDA-ARS East Lansing selections. This and another population was screened in the greenhouse (J. Weiland, USDA-ARS, Fargo ND cooperating) and this RTA population had a greater number of resistant individuals. Current status: 10 F<sub>3</sub> and 121 F<sub>2</sub>

plants vernalized and bolting.

**O-type lineage:**

**C869 x EL48 (ELA, ELB):** These populations are being developed to examine the inheritance of elite 'traditional' East Lansing seed parent germplasm release materials. EL48 is monogerm, self-sterile, and predominantly O-type, with moderate sucrose concentrations (ca. 15%), and higher resistance to *Aphanomyces* as compared with the USDA-ARS Salt Lake City UT germplasm from which it is derived. It has low heterozygosity and a narrow germplasm base. Current status: 6 F<sub>3</sub> and 362 F<sub>2</sub> plants total vernalized and bolting.

**C869 x EL45 (ELC, ELD):** Similar to RILs from EL48. Current status: 388 F<sub>2</sub> plants total vernalized and bolting.

**C869 x SP657-0 (SPE, SPF):** Similar to RILs from EL45 except SP657 was developed from Eastern U.S. germplasm sources. Current status: 393 F<sub>2</sub> plants total vernalized and bolting.

**EL45cms x C869 (ELE):** reciprocal cross of C869 x EL45 to be used to examine maternal transmission of self-fertility. Current status: 200 F<sub>2</sub> plants vernalized and bolting.

**C869 x USH20 (UHA, UHB):** USH20 is the hybrid between EL45 (and EL44 CMS) with SP6822, used here as an alternate estimate for O-type segregation as well as seedling vigor exhibited by this hybrid. Current status: 400 F<sub>2</sub> plants vernalized and bolting.

F<sub>2</sub> seed in being obtained from 10 plants of each of the following: C869 x PI169025, PI169030, PI357360, and PI5990770 (four separate wild germplasm lines from seedling disease nursery selections); C869 x SP85303 (resistance to oomycete diseases); C869 x GW359 (original *Cercospora* resistance source, highly heterozygous); C869 x HiGerm Group (high emergence field selected populations from poorly stored seedlots); and others.

**Field performance of green segregates from F<sub>5</sub> field-selected Sugar x Red derivatives**

This test was done to estimate agronomic performance of inbred lines of beets, in this case derived from a cross between sugar beet and red beet, as a tool in genetic analyses. Inbreds often show reduced vigor relative to hybrids, and this was the case here, but not all inbreds performed poorly. It should be noted that, while this population was strictly derived by single seed decent, field selection had occurred in the F<sub>2</sub>, F<sub>3</sub> and F<sub>4</sub> generations, and thus the most poorly performing segregates would have been removed by natural and conscious selection, however care was exercised to prevent bias (e.g. root color). In these generations, up to five individuals per line were self-fertilized and the 157 individuals compared here as F<sub>5</sub> progeny were originally derived from ca. 50 F<sub>2</sub> individuals.

Compared to the commercial check variety C913 (which had three replicate plots), the average performance of the MSR population was 61% that of C913 with respect to beet yield (ranging from 0.66 to 5.5 lbs), 88% with respect to sucrose percentage (range 11.5 to 23.5 %), 102% with respect to water content (71.4 to 81.1%), 93% with respect to dry weight (18.9 to 28.6%), and 95% with respect to sucrose as a percentage of dry weight (59.8 to 78.1). The conclusion is that agronomic measures can be made on inbred beets, and that this information will be helpful in examining the magnitude of environmental variability for each trait of interest. In the present case, variability was highest in the beet weight and fresh weight sucrose percentage measures (CV = 45.5 and 10.7%, respectively) while Coefficients of Variation were 7% or less for all other measures. In particular, water content between beets within a plot (line) differed only slightly (<2%). It should be noted that differences between all lines considered were highly significant, and these reflect predominantly genetic differences.

## Discussion

Results from this activity are preliminary and selective, and are meant to be illustrative. Recombinant Inbreds are common in model plant systems and self-pollinated crops but have not been developed for beets. Initially, it was hoped a comprehensive public genetic map in beets would be available by the time  $F_2$  populations has designed and constructed for the purpose of segregation analyses of trait loci were ready. The ability to construct these populations soon exceeded the ability to analyze and map their respective genes, and we considered this an opportunity to develop RILs from these initial mapping populations. Thus, some of the earliest populations examined were simply selfed over successive generations as a proof of concept since beets are considered relatively intolerant of inbreeding. However, more precise data are being collected from the majority of lines being constructed, but these are still at an early stage of development.

A single hybrid has a single allele captured from each of its parents, in this case a common self-fertile, nuclear male sterile seed parent (progenitors of C869, a Salinas ARS release) and a variable pollen donor germplasm. C869 was constructed using inter-pollinated fertile and sterile plants, and thus is heterozygous. Pollen donors in most cases, with the exception of red beet W357B, are predominantly self-sterile and presumed highly heterozygous. Thus, any single hybrid generated will not have captured the allelic diversity of the pollen donor. For some traits, such as crop type (table, fodder, or leafy) this may not pose a challenge to discriminating genes that influence crop type since most or all individuals in these populations have been continuously selected for their respective defining characters, and these traits are 'fixed' in their populations. For other traits, such as disease resistance, such 'fixed' populations may not be available. This is because, in general, disease resistance and other agronomic traits are developed through population improvement approaches, and an improved population may simple be a change in relative allele frequencies for a few major genes controlling that trait.

The probability of 'capturing' an agronomic allele in a single hybrid needs to be considered in developing RILs, as does the probability of 'capturing' alleles of all genes when the trait is not simply inherited, and there is no *a priori* way to determine this. Balancing the number of populations desired to capture relevant diversity with the available resources is important, and one way to do this is to ensure reasonable agronomic performance of the hybrids. Typically,  $F_1$  hybrids are field evaluated in at least one 24' long plot (10 – 30 beets) and only the 5 to 10 most vigorous and disease free beets are accepted for the self-fertilization program. Each beet is self fertilized, and a second selection for seed quantity is made with those having the most seed produced taken to the next generation. Depending on the trait, one to four populations will enter into RIL production, however it is recognized that even having four RIL populations for complex traits such as *Cercospora* resistance may not be enough to fully capture the allelic diversity.

To capture all allelic diversity in a pollen donor population, one or a few male sterile, self fertile plants will be included in each seed increase we make at East Lansing. Typically, these seed increases are done from mother roots selected in a particular environment, either the agronomic tests or disease nurseries. Remnant seed from these hybrids are available for further RIL development if needed in the future, for expanding the allelic diversity captured or for marker assisted removal of deduced beneficial allelic contributions to discover additional genes and alleles in subsets of these initial pollen donors. Similarly, using  $F_1$  plants for RIL development that have the greatest seed yield affords the opportunity to



expand the RIL population in the future, if necessary. A pre-selection of potential pollen donors can be done in an environment that would concentrate the relevant alleles to be captured in the F<sub>1</sub>, such as growing the pollen donor population in a disease nursery initially, as we commonly do.

Plants in small pots are finicky, and de-vernalization has been a problem. The causes are not completely clear, however we currently keep vernalized plants cool and under high light intensity until the vernalization response (flowering) appears to be committed (bolting). In one year, aphid control was particularly vigorous, and plants seemed to have been stressed and remained vegetative. Transplanting to larger pots in that instance allowed a proportion of the plants to bolt, but the majority had to be re-vernalized. Our current thinking is that temperature control at the root is more difficult due to the small size of the pots, and they can warm up faster and hasten de-vernalization.

It should be noted that development of RIL populations is expected to be routine in the future, at least for the East Lansing location. The final requirements for the seed parent, in the case of East Lansing, was that it needed to be generally susceptible to the range of diseases prevalent in the Great Lakes region as well as have reasonable vigor and yield in Great Lakes growing areas in the absence of disease. With few exceptions, all RI populations have been developed using the USDA-ARS Salinas CA line C869 as the seed parent (Lewellen 2004), since it was the only germplasm available satisfying all criteria above. The two exceptions to date have been using an inbred sugar beet (7S) hand crossed with an inbred red beet (W357B) for purposes of developing an unbiased genetic map devoid of segregation distortion around the nuclear male-sterility locus, and a cross using male-fertile C869 segregant as the pollen parent on a traditional East Lansing CMS parent (EL45). It should also be noted that from the perspective of the Western germplasm pool, most RI populations will be developed from a single germplasm with >4,000 potential individuals at the end of the process.