BREEDING PERSPECTIVES AND PROGRAMS AT EAST LANSING

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Abstract:

USDA-ARS sugar beet breeding activities for both *Aphanomyces* resistance and CMS / O-type conversion at East Lansing reach back to the 1940's, with variety testing activities at Michigan State University reaching back to circa 1911. Many of those contributions are well known in the sugar beet breeding community, and this contribution serves to update this community on the current breeding activities at East Lansing. The overall goal is to produce germplasm enhanced in one or more traits for release to the seed industry for ultimate incorporation into modern hybrids. Along this trajectory, germplasm is being developed for genetic analyses of traits important to the Great Lakes and Eastern U.S. growing regions. Two broad breeding methods are being deployed, each with multiple components. Open pollinated methods are being used to recombine existing germplasm releases to effect genetic progress through selection in the Eastern agro-environment. Selfing is being employed to create inbreds for genetic analyses. Both approaches result in populations that form one of the three essential legs of modern sugar beet breeding. The other two legs, phenotyping and markers, are also being developed. The relative efforts applied to these three legs needs to be balanced against available resources.

Introduction:

Recent germplasm releases at East Lansing have focused on developing and improving sucrose content in a shallow root-groove character that sheds soil harvested with the beets and thus minimizes the spread of disease from improperly disposed tare dirt (e.g. SR96 & SR97; McGrath 2003). Disposal of tare dirt is costly. This 'smooth-root' trait reduces adhered soil on beets by ca. 50%, promising a savings of $2 million to Michigan factories alone. Changing the root architecture was initiated in the 1940's, however agronomic and disease resistance limitations delayed its adoption. Currently, this germplasm is reasonably well adapted to the Eastern U.S. and is currently being deployed by industry. Its remaining limitation is low tolerance to damping-off and crown and root rot caused by *Rhizoctonia solani* AG2-2. The rhizomania resistance gene *Rz*1 has been incorporated into this background (e.g. EL0204; McGrath & Lewellen 2004). The smooth-root germplasm stream is serving, and will continue to serve, as our recurrent parent for introgression of new characters, such as sugarbeet cyst nematode resistance and salt-tolerant germination, as well as introgressing *Rhizoctonia* resistance from existing improved germplasm (see below). These populations are derived from traditional open-pollinated methods of sugar beet selection, including mother root selection in disease and agronomic nurseries, seed production in the winter greenhouses, and progeny testing in both the lab and the field.

The inability to sufficiently discriminate between phenotypic variance due to genotype or environment in open-pollinated populations of sugar beet is well known, and numerous methods have been suggested to alleviate this concern, including doubled haploids and clonal propagation, however little substantial progress has been made in separating these variance

components. To surmount these limitations, we have began an unprecedented deployment of the S^f allele (Owen 1942), a dominant Mendelian character conferring self-fertility to the normally self-incompatible sugar beet, in creating Recombinant Inbred Lines (RILs) that fix the genetic contributions to traits and thus allow an estimation of the environmental variance components. Such deployment has not been without its problems, and some of these are discussed below.

Results and Discussion:

Release of EL54: EL54 (PI 654357) is a sugarbeet germplasm derived from wild beet (*Beta vulgaris* ssp. *maritima*) accession WB879 (PI 540625), released in the interest of broadening the genetic base of sugar beet. The parent accession WB879, collected in 1989 from Port-de-Houet, France (3 m elevation), was initially identified as *Aphanomyces* resistant in the Sugarbeet Germplasm Committee's coordinated field trials in 1994 and reported as one of four accessions with very high resistance to *Aphanomyces* as judged by the lack of discoloration of hypocotyls. EL54 has shown excellent *Aphanomyces* resistance in field trials in Michigan, Minnesota, and North Dakota. EL54 also has shown a high degree of male sterility, and may be useful as an alternative source for deployment of cytoplasmic male sterility (CMS) for hybrid seed production. This male sterile phenotype was apparent only in the crosses leading to EL54 and not with any other sugar beet germplasm tested with this material.

The pedigree of EL54 started with a pair-cross of one plant from a population of twenty WB879 plants and SP6822 (PI 615525). SP6822 is a parent of the widely grown legacy hybrid USH20 (PI 631354). Seed was harvested from the WB879 parent, and this seed was planted at the Saginaw Valley Bean and Beet Farm (Saginaw, MI) under conditions conducive for Aphanomyces seedling damping-off (i.e. late planting into warmer soils at the end of April 1998, with a weekly irrigation schedule). Emergence and seedling vigor were judged excellent in visual comparisons with similar and other breeding materials in this test, and 28 roots were selected at the end of the season (October 1998) based on the absence of lateral rootlets (i.e. sprangles) and freedom from disease. After vernalization, only seven plants bolted and flowered, and all seven were male sterile in that their anthers were plump and yellow but failed to dehisce. One of these plants, 98B004-2, was backcrossed with a single SP6822 male fertile plant designated 98B15-1, and seed from this cross, designated the Y11-33 population, was tested for seedling *Aphanomyces* reaction in the laboratory. The laboratory seedling assay followed a protocol of soaking seeds in 0.3% hydrogen peroxide for 24 hours, germinating seedlings in water for 4 days with care taken not to submerge the cotyledons, inoculating the seedlings with a zoospore suspension (100 zoospores/ml) for 3 hours followed by a water rinse, and scoring the proportion of surviving seedlings at 4-days post inoculation. Y11-33 showed an intermediate disease reaction (22% seedling survival) between WB879 (13% seedling survival) and SP6822 (29% survival, note that these results are influenced by the frequency of seedling resistance traits in each population tested). The unselected Y11-33 population derived from remnant seed was grown in the greenhouse, vernalized, and the unusual result was that all 111 flowering plants of this population were male sterile. Ten vigorous Y11-33 plants were inter-pollinated with 10 male-fertile C869 plants, and seed was harvested separately from individual Y11-33 plants. EL54 is derived from plant Y11-33-110 that showed excellent seed set and non-shattering seed, unlike its nine sibling Y11-33 plants. Y11-33-110 was tested at the Saginaw Valley Bean and Beet Farm seedling disease nursery (Saginaw, MI) in 2003. 36 mother roots from the Saginaw nursery were selected based on good stand establishment, vigorous growth during the season, and freedom from disease and sprangles at harvest. Of these 36 roots, 28 were further selected

after washing the roots and selecting those roots with little or no surface checking or discoloration at and near the lenticels, one symptom of Aphanomyces root rot. After vernalization, these 28 plants showed a slight improvement in male fertility, and due to this reduced male fertility, these plants were again paired with 10 plants of SP6822 selected from the same nursery in Saginaw. Again, seed was harvested from Y11-33-110 mother plants. This seed was designated 03B081 (EL-A014205), and was increased in a 0.04 hectare plot in Oregon and harvested in four fractions (designated with a separate WC number); monogerm-male sterile (WC050864), monogerm-male fertile (WC050862), multigerm-male sterile (WC050191), and multigerm-male fertile (WC050860). This seed showed contamination (ca. 6%) with an unknown red chard-like pollinator. EL54 is a mixture of equal weights from each fraction. Seed of each individual fraction is available on request. EL54 and its progenitors have been evaluated in the Betaseed Aphanomyces nursery (Shakopee, MN) for five seasons. EL54 has consistently shown excellent *Aphanomyces* resistance (mean score = 2.4, sd. = 0.6 compared with means of resistant checks = 2.8, sd. = 1.1; scored on a scale of 1 to 8 where 1 is healthy and 8 is severely rotted at the end of the season). EL54's parentage includes SP6822 whose *Aphanomyces* tolerance is well known, and the degree that WB879 contributes to *Aphanomyces* resistance to EL54 is as yet uncertain. The mean nursery rating of EL54 as contrasted with SP6822 in the two years of direct comparison was 2.5 (sd. = 0.7) versus 2.4 (sd. = 2.0), respectively. The lower level of variability in EL54 scores over years suggests the trait is more consistent than currently deployed *Aphanomyces* resistance. Over two years of agronomic trials, EL54 has shown an average sucrose percentage of 14% (sd = 2.2) and a yield of 25.1 tons / acre (sd = 6.9). This compares at 82% of the sucrose percentage and 93% of the yield of the commercial check Syngenta Hilleshög E17 in the same nurseries.

Release of EL55: EL55 (PI 655304) is a sugarbeet germplasm derived from seed held in sub-optimal storage conditions at East Lansing for 20+ years, and has no smooth-root genetics in its background. EL55 was released in the interest of improving seed quality and performance in sugar beet. From over 4,000 legacy seedlots produced between 1961 and 1989 stored in ambient (high humidity) conditions, only 523 emerged under field conditions at the Saginaw Valley Bean and Beet Farm in Saginaw, MI in 2000. Only 71 of these stored seedlots gave commercially adequate stands (ca. 50% of planted seed), however each of these good seedlots was produced after 1988. Roots from the 12 oldest seedlots (i.e. those stored the longest) were collected as mother roots, and seed was produced. Using an accelerated seed aging protocol, this seed demonstrated slightly improved seed longevity. The majority of this germplasm is derived from remnant seed generated during development of seed parents in the germplasm conversion to hybrid seed production conducted by George Hogaboam. Thus, this material is also expected to be useful for developing cytoplasmic male sterility (CMS) parents for hybrid seed production. Reasonable to high levels of Aphanomyces blackleg and Cercospora leaf spot resistances can be expected in this material, as is customary of traditional East Lansing ARS germplasm.

The parentage of EL55 is complex, having been derived from 12 legacy seedlots. The 12 seedlots dated from 1961 through 1982 and were selected on the basis of their ability to germinate and grow healthy roots in the 2000 field nursery in Saginaw, Michigan. The 12 seedlots were 61G1X03, 77B2-01, 77B18X01, 78B32-01, 78B32X02, 79B15X70, 79B29-1, 79B31X04, 80B11-016, 80B14-23, 82B66X02, and 82B66-S3, where the first two digits of each seedlot designate the year of seed production. Each seedlot yielded a single root, with the exception of 78B32-01 (5 roots), and all of these were used as mother roots and intercrossed in the 2001 greenhouse. This seed was designated 00B041 (EL-A007070) and increased in Oregon

in 2003 as WC030246 (EL-A013698), with selection against colored roots. This seed was used for all testing. EL55 has been evaluated for agronomic performance, and disease reaction to leaf spot caused by *Cercospora beticola*, crown and root rot caused by *Rhizoctonia solani*, and damping-off and root rot caused by *Aphanomyces cochlioides*.

Germplasm contributing to EL55 generally represents intermediate generations from a cross section of germplasm enhancement activities important during the development of modern hybrids. Nine of the 12 breeding lines were targeted towards the development of seed parents (monogerm, cytoplasmic male sterile and their maintainer lines), while the remaining three breeding lines were used for developing pollinators. The pollinator lines were 77B2-01, 79B29-1, and 80B11-016. 77B2-01 was annotated as yielding high Recoverable White Sugar per Acre, good Cercospora leaf spot resistance, and good Aphanomyces blackleg resistance, slightly better than the legacy hybrid USH20. 79B29-1 is derived from 77B2, and is annotated as a red beet selection, however the red beet parentage is not clear. 80B11-016 is the result of a broad based selection among multigerm breeding lines, with its most recent progenitor being seed harvested from a single plant designated 78B24-21. Since seed production per plant was highly variable during these years of operation, selection for seed production was commonly practiced. Each of these breeding lines is multigerm, pollen fertile, and 100% red hypocotyls. The remaining nine breeding lines contributing to EL55 are 61G1X03, 77B18X01, 78B32-01, 78B32X02, 79B15X70, 79B31X04, 80B14-23, 82B66X02, and 82B66-S3, and all but two of these were intermediate populations in the development of CMS and O-type seed parent germplasm. The exceptions are 78B32-01 and 78B32X02, which are seed increases of EL36 (W6 17110 = O-type and W6 17111 = CMS, respectively). The oldest seedlot, 61G1X03, stems from a 1957 single root selection designated as the “09” clone. The same population gave rise to the “02” clone that was eventually released as EL40 (PI 590719). After three generations of selection for seed set, a single derivative plant of the multigerm “09” clone was pair crossed with C361HO, a monogerm sterile cytoplasm release from USDA-ARS Salinas, California. Seed was harvested from the C361HO parent, seed was further increased from 90 plants, and this seed was designated as 61G1X03. 77B18X01 is F1 seed from an unknown male sterile, monogerm sugar beet breeding line crossed with multigerm cv. ‘Winterkeeper’ red beet (NSL 6320). It is likely that the red and yellow roots segregating in EL55 are ultimately derived from ‘Winterkeeper’. 79B15X70 is a second backcross generation of a multigerm breeding line annotated as having good yield selected under conditions promoting Rhizoctonia crown and root rot development crossed with an monogerm O-type breeding line designated G318-11 as the recurrent parent. 79B31X04 is similar to 79B15X70 except that the CMS breeding line designated as I444-7 was used as the recurrent parent. 80B14-23 is also derived from monogerm selections performed under conditions promoting Rhizoctonia crown and root rot development, and is pollen fertile. 82B66X02, and 82B66-S3 are each derived from monogerm selections performed under conditions promoting Rhizoctonia crown and root rot, this progeny was indexed for O-type, and backcrossed to the O-type breeding line designated I436-3. 82B66X02 is BC5 seed produced in a seed increase plot, and 82B66-S3 is seed harvested from the I436-3 recurrent parent. EL55 was tested for agronomic performance in 2004, 2005, and 2007 at the Saginaw Valley Bean and Beet Farm in Saginaw, Michigan. Compared with the standard check variety Syngenta E17, EL55 showed 93% of the yield (24.4 versus 22.8 tons/acre for E17 and EL55, respectively), 80% of the sucrose percentage (E17 = 18.0 ± 0.5 % sucrose, EL55 = 14.5 ± 1.8 % sucrose), 105% of the % water (E17 = 75.4 ± 1.8 % water, EL55 = 78.9 ± 1.7 % water), and 121% of the 30-day stand count (2004 and 2005, only).

Release of SR98: SR98 (PI 655951) is a sugarbeet germplasm with smooth, low soil tare root and high levels of resistance to damping-off and crown and root rot caused by *Rhizoctonia solani* Kühn (AG2-2). Previous smooth–root releases have been highly susceptible to diseases caused by *R. solani*, and the SR98 has incorporated *Rhizoctonia*-resistant germplasm released from the USDA-ARS Ft. Collins, CO and East Lansing, MI *Rhizoctonia*-resistance breeding programs. SR98 has shown moderate yield potential in agronomic trials, and has shown reasonable resistance to Aphanomyces blackleg and Cercospora leaf spot as is customary of traditional East Lansing ARS germplasm. SR98 is diploid, self-sterile, multigerm, and biennial. SR98 was developed from a wide range of germplasm as part of a population enhancement effort to increase genetic diversity and foster recombination among advanced public sugar beet breeding materials. In the case of SR98, the bulk (62.5%) of germplasm contributing to the release derives from *Rhizoctonia*-resistant materials. These *Rhizoctonia*-resistant materials include EL51 (35%, 14 mother roots) and a mix of Ft. Collins derivatives from released materials (27.5%, 11 mother roots). Specifically, the Ft. Collins releases were selected under conditions favoring *Rhizoctonia* disease development in the 2001 East Lansing *Rhizoctonia* nursery, and seed emanating from healthy roots we designated the 'FC mix' (02B095, EL-A013703) were intercrossed with the bulk of other germplasm contributing to SR98 in the 2002 East Lansing greenhouse. 02B095 is composed of seed generated from two mother roots of FC750/1, two roots of FC709, four roots of FC709-2, one root of FC712, and two roots of FC714-1. SR98 also included five other germplasm lines; 17.5% (seven mother roots) contributed by EL0204 and its progenitor 96RM14-01 contributing the smooth-root trait and rhizomania (*Rz*1) resistance, 12.5% (5 mother roots) traditional smooth-root germplasms, previously unreleased, 96N7 and 95HS3, and 5% (two roots) of an advanced population of traditional East Lansing sugar beet (e.g. SP6822) crossed with fodder beet (e.g. Mescan's Round and Ovana) initially constructed as a source population for the introgression of the smooth-root trait from such fodder types, and one extra root (2.5%) of FC712. Each of these mother roots was selected for low disease expression of crown and root rot symptoms in the 2001 East Lansing Rhizoctonia crown and root rot nursery. All plants were interpollinated, and seed was harvested by mother root accession and subsequently tested individually. 'FCmix' was tested in the 2007 and 2008 East Lansing Rhizoctonia seedling disease nurseries, and seed was harvested from mother roots selected in this nursery in 2007. This seed, designated 07B154 (EL-A023047), is SR98. Thus, SR98 is expected to be segregating for many characters, however a majority of individuals are expected to be resistant to seedling or crown and root rot diseases, or both. SR98 is being released at this time because of the high priority for seedling disease resistance identified as needed in the Great Lakes growing region and the current lack of available improved germplasm. In the 2007 seedling *Rhizoctonia* nursery in East Lansing, SR98 had a September stand count of 30.0 plants (std. dev = 0, n=2 plots), compared with the resistant EL51 (mean = 25.5, std. dev. = 5.3, n = 4) and a susceptible unrelated smooth-root breeding line SR IC-2 (mean = 2.0, std. dev. = 1.4, n = 2). In the 2007 Ft. Collins, CO Rhizoctonia crown and root rot nursery, SR98 had a Disease Index (DI; 0 = no disease, 7 = dead) of 1.7, relative to the highly resistant, resistant, and susceptible checks of 1.5, 2.8, and 3.5, respectively. In 2008 at Saginaw, MI, 30-day emergence was 130.8% of eight commercial checks, yield was 85.3% of the same checks (19.6 T/A vs. 23.0 T/A), percent sucrose fresh weight was 90.7% of checks (16.8 vs. 18.6%), and percent dry matter was 88.8% of checks (21.2% vs. 23.9%). Sucrose percent expressed as a fraction of dry matter was 101.9% of checks (79.4% vs. 77.9%; range of commercial values = 75.0 to 80.3).

Recombinant Inbred Lines: Self-pollinated crops partition genetic diversity between populations, and genetic analyses of such populations is simplified relative to open pollinated crops such as sugar beet. To simplify the genetic analyses of beet traits we have begun a process of inbreeding using the S^f gene (or allele, the actual biochemical basis for this trait is as yet unclear). We have not attempted other inbreeding strategies such as doubled haploids, since the effort required for this exceeds our capacity and relatively few such experimental materials are available in the public sector. This may be a consequence due to segregation of lethal and sub-lethal alleles in inbred populations derived from open pollinated parents, a problem that also affects inbreeding via normal sexual means.

Hybrids were created using a nuclear or CMS seed parent that is homozygous for the dominant self-fertility gene. Typically, in our program, C869 (Lewellen 2004) has been used as the source seed parent. Pollen parents have been from at least 500 different crosses, representing all of the important crop types and much of the historical genetic legacy available in sugar beet. Currently, a male sterile, self-fertile plant is included in each open pollinated increase to capture the available genetic diversity in our traditional breeding stream. From these F_1's, more than 800 have been selfed (by bagging) to give F_2 seed, generally from roots of field-grown F_1 plants used as mother roots and selected for plant vigor and size. Such a selection likely eliminates dominant lethal and sub-lethal alleles and gene interactions that might be segregating in the original populations. From the 800 F_2's, >4800 F_3 families have been derived from >35 different F_2 populations (ca. >100 individuals per family) representing crop type and agronomic trait donors from populations segregating for traits important for the Great Lakes growing area. Additional inbreeding has resulted in the more advanced populations listed in Table 1. Deriving populations via single seed decent (SSD) from F_2 populations has resulted in more loss of vigor in advanced generations than by beginning the SSD process in the F_3.

Table 1: Advanced inbred populations at East Lansing.

Population	Parents	Primary Trait	Seed Generation	# of Families
SxR	"7S" x W357B	sugar content	F5	174
MSR	(C)869 x W357B	sugar, yield, reference map	F6	190
RTA	(C)869 x EL51	Rhizoctonia resistance	F5	126
CRB	(C)869 x EL50	Cercospora	F4	117
Y20	(C)869 x SP7622	Aphanomyces	F4	101
AYA	(C)869 x SP7622	Aphanomyces	F3:F6	91

These populations will be subject to genetic analyses at East Lansing, and their populations will be mapped with molecular markers, at least to a low-density map. The populations and the map will be released as germplasm (genetic stocks) such that additional phenotyping can be performed in other areas where disease and agronomic conditions may be different.

References:

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