ABSTRACT

TODD, STEVEN MICHAEL. Application of Near Infrared Spectroscopy to Study Inheritance of Sweetpotato Composition Traits. (Under the direction of Dr. G. Craig Yencho.)

As interest in new sweetpotato (*Ipomoea batatas* (L.) Lam.) markets such as starch feedstocks, anthocyanin production, chips and French fries, and other processed food products have increased, breeders have begun developing sweetpotatoes with unique compositions. This dissertation describes a three-pronged strategy to understand the genetic control of sweetpotato composition and modify it using near infrared spectroscopy (NIRS), quantitative inheritance studies, molecular markers, and exotic germplasm.

In our first experiment, a 5 x 5 NCII crossing block with 25 full-sib families was designed to study the inheritance of sweetpotato starch and anthocyanin content. Linear regression modeling was used to determine the effect of general combining ability (GCA) and specific combining ability (SCA) on dry matter, total monomeric anthocyanin (TMA) concentration, fresh yield, and total dry matter and anthocyanin yield. All five traits were moderately to highly heritable with significant general combining abilities. Yield and dry matter yield had significant specific combining abilities and significant differences among parents were discovered for all traits. Yield, dry matter, dry matter yield, and TMA yield were significantly impacted by spatial gradients within the field, but TMA concentration was not. Phenotypic and genotypic correlations among traits indicated that many traits of interest shared either genotypic and/or phenotypic correlations.

In our second experiment, a nested crossing block was used to estimate the heritability of sweetpotato yield and storage root composition traits in a population incorporating exotic germplasm obtained from the US sweetpotato germplasm repository and

a core set of elite US sweetpotato lines crossed in a modification of the NCI design. Yield traits were recorded in the field and biochemical composition was phenotyped using NIRS. Heritability was measured on a half-sib family basis and a full-sib family basis to allow comparison between the commonly used polycross nurseries and paired crossing blocks. Parent offspring regression, which has been commonly used by sweetpotato breeders, was also used to provide another heritability estimate. Starch and sugar contents had relatively high heritabilities on both a GCA (h^2>0.32) and SCA basis (h^2>0.77). Yield traits had low heritability on a GCA basis (h^2<0.16), but moderate heritability on an SCA basis (h^2= 0.21 – 0.51). Heritability trends suggested that polycross nurseries could be effective for modifying sweetpotato composition, while paired crosses would be more effective for the modification of sweetpotato yield. Based on the performance of a wide range of crosses between exotic and heirloom varieties, we hypothesize that the global sweetpotato germplasm base contains many useful alleles for continued sweetpotato improvement.

Our final study involved a previously described sweetpotato quantitative trait loci (QTL) mapping population developed from a Tanzania x Beauregard cross (Cervantes-Flores 2006). This population was phenotyped using NIRS to identify QTL for sugar and starch content. In Beauregard, six QTL were associated with decreased starch and dry matter content and eight QTL were associated with increased sugar content. One QTL in Beauregard was associated with decreased yield. In Tanzania, two QTL were associated with increased starch and two QTL were associated with decreased starch; there were also two QTL associated with decreased sugars and one associated with increased sugar content; one QTL was associated with decreased culls. In most cases, newly identified QTL co-locate with those previously described.

Collectively, this research represents a significant effort in sweetpotato to merge molecular markers with NIRS phenotyping, and it has opened the doorway to further developments that merge these two new technologies for sweetpotato improvement.

Application of Near-Infrared Spectroscopy to Study Inheritance of Sweetpotato Composition Traits

by
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BIOGRAPHY

Steven Michael Todd was born on May 19, 1981 in Springfield, IL. He is the middle child of James and Jean Todd. When he was four, his family moved to the small town of Auburn, IL. There he attended school, ran track and cross-country, and participated in Boy Scouts. It was in this town, surrounded by corn and soybean fields, that he first learned his love of science and saw agriculture in action.

After graduation from Auburn High School in 1999, Steven went on to Bradley University, majoring in Biochemistry. At Bradley, he got his first job in agricultural research, working at the USDA-ARS's National Center for Agricultural Utilization Research facility just a few blocks from campus. Here he learned he could turn his interests in science and agriculture into a career.

From Bradley University, he moved on to Texas A&M University for a master's degree under Dr. David Stelly. Here, while working on cotton genomics, he first learned about plant breeding. After earning a master's degree, he went to work for the Monsanto cotton breeding program in Leland, MS.

After working for a time in MS, Steven made a decision that would lead him to this day: he would take a major next step in his career and earn a PhD in plant breeding. After applying to multiple graduate schools, he made the decision to attend NC State University due to its desirable location in Raleigh and world-renowned plant breeding faculty. He spent the next few years learning about plant breeding, breeding sweetpotatoes and potatoes, and making professional contacts. As is traditional with graduate school work, the compilation of these activities is now recorded in this dissertation. I hope that the results of this work

may result in better sweetpotato varieties which can provide a livelihood to NC farmers and foodstuffs to an evergrowing world and that the skills I learned here can make a positive contribution to the world through agricultural development.

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No piece of scientific work or large work project of any kind is accomplished without contributions from numerous people, and this one is no exception. These people certainly deserve credit for their contributions to this work and/or my grad school experience in general.

Members of the NCSU sweetpotato research team who contributed include my advisor: Craig Yencho, and other committee members Ken Pecota, Van-Den Truong, Bryon Sosinski, and Mari Chinn have all shared their extensive knowledge of sweetpotatoes during my time here. I would also like to thank committee member Jim Holland, who was always willing to share his understanding of quantitative genetics, which provided critical support during the data analysis phase of the project. Many other members of the NCSU faculty have provided important pieces of advice throughout my graduate work here including John Williamson, Jonathan Schultheis, Mark Clough, Brian Jackson, and others too numerous to list. Extension agents Billy Little and Alan Thornton helped me obtain excellent experience interfacing breeding research with growers' needs.

I would like to thank Ken and Ana Pecota, Jarred and Liz Driscoll, and Nic George for their friendship, advice, and encouragement throughout my graduate school experience. I also appreciate the friendship, encouragement, and professional networking opportunities I have shared at NCSU with the other plant breeding and horticultural science grad students.

Many more people have contributed technical support to various aspects of the project. Meri Reeber, Ben Winslow, and the greenhouse staff in the NCSU Department of Horticultural Science took excellent care of my plants in the greenhouse for many months.

The station crews at the Horticultural Crops Research Station in Clinton, NC, and at the Cunningham Research Station, Kinston, NC, provided excellent support planting, maintaining, and harvesting my experiments. Alejandro Merchan, Summer Wilkinson, and Shelby Curro provided great technical assistance in the lab and greenhouse. Dhana Savithri, Debbie Clare, and Cari Furiness provided important support using the near-infrared spectrometer.

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CHAPTER 1: INTRODUCTION

I. Importance of crop composition

In recent years, the impact of crop composition for both nutritional value and industrial use has become an issue of increasing importance in the agricultural industry. This interest has resulted in numerous agricultural research teams giving increased attention to genetic modification of crop composition. The most notable of these efforts has been that of the Consultative Group on International Agricultural Research's (CGIAR) HarvestPlus initiative to increase the nutritional value of crops grown by farmers in underdeveloped countries as a measure to alleviate malnutrition (Pfeiffer and McLafferty 2007). Other groups have also begun to focus on modification of crop composition to improve human health or processed products. Soybean breeders have developed soybeans with modified fatty acid content that increases shelf-life, reduces saturated fat content, or eliminates *trans*-fats in vegetable oil (Fehr 2007). Multiple crops have drawn attention for their potential as a cellulosic biofuel source, and breeders of these crops have begun efforts to improve processing efficiency by lowering lignin content (Dhugga 2007). Sugarcane and sugarbeet breeders have long focused on increasing sugar content in new varieties (Jackson 2005, Biancardi et al. 2010).

Modification of crop composition can also affect important industrial feedstocks such as starch. Different starch physical and chemical characteristics can have significant impacts on the quality and final application of starch. Many starch characteristics including amylose content, crystal size and shape, amylopectin branch patterns, and lipid content vary across

species, cultivars of species, and environments. These characteristics affect physical properties of starch such as gelatinization temperature, retrogradation, taste, and color, which in turn impact the quality of the final product. While starch properties are often chemically modified after harvest to improve quality for specific applications, genetic improvement (via both traditional breeding and transgenics) may allow development of improved starches *in planta*, thus reducing the need for post-harvest modification (Bertolini 2010).

Studies have shown a direct, significant effect of feedstock composition on enzyme digestibility, biofuel yields, and processing input requirements from a given feedstock. While overall starch content is clearly a major factor in determining biofuel yield, starch feedstock composition can cause significant changes in final ethanol yield and therefore, economic viability. It has been shown that susceptibility of sweetpotato (*Ipomoea batatas*) starch to α-amylase digestion is proportional to amylose content (Zhang and Oates 1999). Since the sweetpotato starch conversion process is heavily reliant on starch digestion by α-amylase (Duvernay 2008), increasing the amylose content of the feedstock is likely to improve ethanol yields, thereby improve the economics of the conversion process. In common biofuel feedstocks such as maize and grain sorghum, changes in amylose content have resulted in significant changes in total ethanol yields (Wang et al. 2008, Sharma et al. 2006, Wu et al. 2006). Starch modification in sweetpotatoes has been shown to reduce energy requirements for ethanol production. Japanese researchers have demonstrated that by using sweetpotato cultivars with low gelatinization temperatures, input requirements for ethanol production can be significantly reduced (Srichuwong et al. 2012). While these case studies demonstrate the potential influence of feedstock composition on final bioproducts yield, the

differences between grain and root starches also demonstrate the need to avoid a one size fits all approach to feedstock modification.

II. Application of NIRS to breeding programs

Improvement of crop composition through breeding requires high-throughput, highly-reproducible technologies to determine crop composition. Near infrared reflectance spectroscopy (NIRS) has been successfully used to analyze starch quantity and composition in multiple crops including sweetpotato (Katayama et al. 1996, Ishiguro and Yamakawa 1998, Lu et al. 2006, Zum Felde etl al. 2009, Lebot et al. 2011). NIRS has been widely used to determine amylose content in rice (*Oryza sativa*) in which starch structure plays a major role in cooking quality (Wu and Shi 2004). NIRS systems have also been developed to determine a wide range of constituents in potato (*Solanum tuberosum*) including starch content, starch composition, sugar content, and protein content (Büning-Pfaue et al. 1998, Hartmann and Büning-Pfaue 1998, Haase 2006). A NIRS system has been developed at the International Potato Center (CIP) that is used to analyze starch content, β-carotene content, anthocyanin content, and mineral content in sweetpotatoes. Furthermore, continued development of the system by CIP researchers has resulted in incorporation of NIRS data into the breeding program (CIP 2011, Zum Felde et al. 2009, Tumwegamire et al. 2011). More recent proof-of-concept work has produced sweetpotato NIRS models for several traits using a system comparable to the one described herein (Lebot et al. 2011).

In many crops, NIRS has begun to play a valuable role in helping breeders meet important objectives for modification of crop composition. Wheat breeders have developed a

system to use NIRS to identify seeds subject to early germination, thus allowing selection against this critical trait (Smail et al. 2006). The oil quality traits of rapeseed cultivars have been successfully measured with NIRS (Font et al. 2006) and sugar content has been measured in tomato using NIRS (Peiris et al. 1998).

III. Sweetpotato: diversity of uses

In the United States, sweetpotatoes have traditionally been used primarily as a holiday side-dish, but around the world, they are utilized in a wider variety of ways. Many areas of the world utilize sweetpotato as a staple crop, and farmers grow starchy high-dry matter sweetpotatoes. In other areas, especially China, which grows ~75% of the world's sweetpotatoes (FAO-STAT 2008), the crop is commonly used as an animal feedstock (Woolfe 1992). The viability of sweetpotatoes as an ethanol source has been demonstrated in China, and Japan. Chinese sweetpotato breeders have released varieties of high-starch sweetpotato such as YanShu22 (Lin et al. 2008) and Quanshu9 (Yu et al. 2008) for fuel ethanol production. China has significantly different sweetpotato production systems than the US, but Japan, which has a similar production system to the US, has successfully grown sweetpotatoes for industrial use. Leading Japanese varieties such as 'Konahomare' and 'Koganesengan' have been grown for starch production for decades, providing a source of sugar syrups in Japan, similar to high-fructose corn syrup in the US (Woolfe 1992, Kumagai et al. 2002).

There is an increasing need in the US to develop expanded resources for use in industrial starch, biofuels, and other bio-products (Hughes and Qureshi 2010). Driven by an

increasing world population, improved standards of living in many regions, and environmental and national security concerns about fossil fuels, US and global demand for fuel and industrial starch has increased, leading to record prices for oil and agricultural commodities. To meet this increasing demand, bio-renewables are becoming a more important part of the energy and industrial products market meaning new feedstock supplies must be developed (Henry 2010). To date, most of the US biofuel and industrial starch supply has relied on maize grain. While the US is the world's top producer of maize, with 39% of global production (FAO-STAT 2006-2010), increasing demand, and competition with the animal and human food needs has made the limitations on maize production for biofuels apparent. Further, sufficiently high levels of maize production required for biofuel economic feasibility are only achieved in limited areas of the US, primarily the Midwestern Corn Belt, and the high input requirements for maize result in a low net energy gain when used as a biofuel (Shapouri and McAloon 2004).

Alternative biofuel feedstock sources may be viable in certain regions of the United States (Shapouri and Salassi 2006). Because sweetpotatoes provide a high starch yield per acre, and they can grow on poor soils and are commonly grown in the Southeastern US, sweetpotatoes may provide a starchy feedstock that can be easily incorporated into existing biofuel infrastructure. (Woolfe 1992, Ziska et al. 2009). Most sweetpotato production in the US occurs in the Southeast, a corn deficit region well outside of the Midwestern Corn Belt. The largest sweetpotato producing states are North Carolina, California, Mississippi, and Louisiana (NASS 2007-2011). Sweetpotatoes perform well in the warm southern environments, where high summertime temperatures may negatively impact maize yields,

and in relatively low fertility soils (Woolfe 1992). In addition to biofuels, many chemicals (such as polymers and solvents) in everyday use are derived from petroleum, however as the era of cheap petroleum comes to an end, the rise of biobased chemicals and fuels will play an important role in the future of energy and chemical products. Starch can provide an important source of six-carbon sugars, which can be broken into smaller organic molecules and formed into a range of polymers that either replace traditional petrochemicals or compose completely new classes of chemicals (van Haveren et al. 2008). These can be used to make bioplastics, industrial solvents, and fuel additives (Drumright et al. 2000, Werpy and Petersen 2004).

Starch is also a critical ingredient in many foods, such as baked goods, batters, artificial flavorings, and thickeners for various food products, sauces, and dressings. With such a broad range of needs for texture, stability, and processing, no individual starch can meet all the needs of various industries. To adapt starch to different applications, processors use physical, chemical, and biochemical modifications to improve starch for specific applications. Such chemical modifications alter starch gelatinization temperatures, freeze-thaw stability, gelling properties, and film strength (Bertolini 2010). By breeding sweetpotatoes with different starch structures, we can develop better starches for particular applications with less chemical modification. Furthermore, starch can be hydrolyzed into sugars, which can be used either directly or to develop a variety of chemicals. Development of sugar syrups for artificial sweeteners is a leading use of sugars derived from starches (Bertolini 2010).

In addition to sweetpotatoes as a potential source of starch, purple-fleshed

sweetpotatoes can be developed as a potential source of anthocyanins. Anthocyanins are phenolic compounds that have been shown to have both antioxidant properties and applications as natural colorants. Purple-fleshed sweetpotatoes provide a potential source of the anthocyanins, cyanidin and peonidin (Suda et al. 2003). In Japan, a successful industry has arisen based on using purple-fleshed sweetpotatoes as sources of anthocyanins as natural food colorants. This industry has been supported by the efforts of Japanese sweetpotato breeding teams to develop purple-fleshed sweetpotatoes with high concentrations of anthocyanins such as 'Ayamurasaki' (Yoshinaga 1995), 'Murasakimasari' (Kumagai et al. 2002), and 'Akemurasaki' (Sakai et al. 2009). Anthocyanins are often used as natural colorants in drinks and foods, and the ability of anthocyanins to change color according to pH allows them to be applied to food products that require various colors (Suda et al. 2003).

While use as an industrial feedstock provides potential new uses for sweetpotatoes, new developments in the food market have even greater potential to affect the sweetpotato industry. In recent years, consumers have become more concerned about the quality and nutritional content of food (Grunert 2013), and the industry has successfully marketed the health aspects of sweetpotatoes (Loebenstein 2009). Advances in food technology have facilitated the rise of new sweetpotato processing companies such as Yamco (http://www.yamco.net) and new investments in sweetpotato by established food companies such as ConAgra LambWeston, McCain Foods, and Simplot Foods (Yencho, personal communications). These new markets have led to a recent expansion of US sweetpotato acreage as growers endeavor to meet expanding demand for sweetpotatoes. However, processing of sweetpotatoes involves challenges related to final product quality that may be

partially met through modification of sweetpotato composition.

IV. Increasing farm diversity

The global agricultural industry is changing rapidly and farmers need new options for product diversity that can allow them to better meet the needs of customers and respond to changing market conditions. Farmers employ many strategies for farm diversification including crop diversification, market diversification, and agritourism. Diversification brings potential benefits to farmers including risk reduction associated with potential market fluctuations during the course of the season and additional income streams to improve farm economic viability (Harwood et al. 1999, Paul and Nehring 2005).

Farm diversification is not a monolithic action, but the result of individual decisions by many farmers. Many factors influence an individual farmer's decision to diversify including risk reduction, emergence of new markets, improvement of revenue streams, and personal motivation (Barbieri and Mahoney 2009). Potential roadblocks to diversification include lack of suitable crops, additional requirements for capital equipment, the need for new production knowledge, and minimal market availability (Harwood et al. 1999). A grower's individual decision to diversify is made after considering the impact of these and many other factors.

The large sweetpotato industry already in North Carolina indicates that the needed cultural knowledge and capital equipment is already available in growing regions of the state. By developing new sweetpotato types adapted to North Carolina, our breeding team is working to provide suitable crops. We are also working with private companies to encourage

development of new markets for sweetpotatoes. While by no means a panacea, further development of sweetpotato potential may meet the needs of a subset of growers seeking options for diversification, allowing them to make the "diversification leap" into new crops and markets.

V. Industrial sweetpotato breeding in the U.S.

Earlier sweetpotato breeding efforts in the US have focused on development of high-starch sweetpotato lines for use in domestic energy production resulting in the release of named cultivars such as 'HiDry' (Hamilton et al. 1985) and 'Sumor' (Dukes et al. 1987). However, such breeding efforts have not been consistently maintained, being developed primarily in response to national energy crises such as World War II or the 1970's oil embargo. Since achievement of plant breeding goals requires a continued focus of breeding teams and consistent funding support, this intermittent support is one reason no viable sweetpotato starch and biofuel industry exists in the US.

The North Carolina State University (NCSU) Sweetpotato Breeding and Genetics program has developed several high starch sweetpotato lines that have potential to contribute to new markets. Many sweetpotato varieties grown around the world contain much higher starch than the traditional orange-fleshed varieties commonly grown in the United States. While the leading orange-fleshed US cultivars Covington and Beauregard each have 18% dry matter, experimental white- and cream-fleshed lines developed by the breeding program contain 30-34% dry matter (Yencho, unpublished data). Because starch content is closely correlated with dry-matter content, sweetpotato breeders often use dry matter as a substitute

measurement for starch content. About 70% of staple-type sweetpotato dry matter is starch, with the rest consisting of sugars, proteins, ash, and other minor components (Woolfe 1992). In recent years, our team has focused on development of high-yielding, high-starch sweetpotato lines. We have developed experimental varieties that have produced higher starch yields than 'HiDry' or the high dry matter Korean sweetpotato line 'Suwon 122' (Yencho, unpublished data). By maintaining a focus on development of high-dry matter sweetpotato clones, we propose to develop an alternative starch feedstock supply for use in the Southeast.

VI. Exotic Germplasm and Heritability

Genetic diversity and heritability form the basis of global breeding efforts in every crop. As our program begins efforts to modify sweetpotato composition to meet various industrial and nutritional needs, the need to determine heritability and diversity for important new traits is paramount. A critical component of this dissertation research is to determine both the available genetic diversity and heritability for amylose content. While previous efforts to increase starch content in US sweetpotato cultivars have been successful, we are unaware of efforts to modify more detailed components of sweetpotato such as sugar or amylose content in US sweetpotato germplasm. The extent of variation in traits such as amylose and sugar content in US germplasm in unknown, but is likely to be fairly narrow within the elite germplasm pool due to a significant founder effect and highly directional selection for β -carotene content and taste. However, studies overseas have shown a broad range of composition traits in broader germplasm pools (Collado et al. 1999). More recent

breeding efforts in Japan have resulted in the development of 'Quicksweet', a sweetpotato with short branched starch and low gelatinization temperature (Katayama et al. 2002, Katayama et al. 2006). These results clearly demonstrate that breeding efforts can modify sweetpotato starch to have desirable properties. Such breadth suggests that it will be possible to modify sweetpotato composition by incorporating exotic germplasm into varieties adapted to US cultivation.

In addition, Japanese breeding programs have studied novel breeding and production methods such as inbred-hybrid sweetpotato breeding, introgression of exotic germplasm, cut seed piece planting, and true seed planting. By lowering production costs and/or raising productivity, these technologies may prove useful in a changing industry. The Japanese approach shows that useful sweetpotato traits can be found in ex-US germplasm and that non-traditional market development can be successful if research programs and industry work together (Iwama et al. 1990, Komaki et al. 1998, Kumagai et al. 2002, Taniguchi 2004).

VII. Proposed research

The studies in this dissertation were designed to determine the potential to modify sweetpotato composition through breeding. Our overarching hypothesis was that we could use the breeding techniques described below to better understand the range of traits that exist in sweetpotato germplasm, determine their relative heritabilities, and begin to systematically improve these traits for the newly emerging processing industries in sweetpotato. We developed a 3-pronged approach to achieve this goal. First, we determined the combining ability for anthocyanin and dry matter content using an NCII mating experiment. Second, we

implemented an NCI experiment to test study the heritability of sweetpotato composition traits and the potential of exotic germplasm to modify sweetpotato composition. Third, we used newly developed NIRS models of key traits to phenotype a genome mapping population to identify QTL for sugar, reducing sugar, starch, and dry matter.

Analysis of anthocyanin and dry-matter content using an NCII design

In our first experiment (Chapter 2), our team developed an experimental sweetpotato population using an NCII crossing pattern with parents of varying flesh colors and dry matter contents. Preliminary results indicated high levels of segregation for numerous important sweetpotato traits including anthocyanin, dry-matter, and β-carotene content, and yield (Teow et al. 2007, Yencho unpublished data). We used linear modeling and standard quantitative genetic statistical procedures available in JMP (SAS Institute, Cary, NC) and SAS (SAS Institute, Cary, NC) to determine the combining abilities for the 10 lines included in the experiment. These analyses enabled us to determine the importance of general and specific combining ability for dry-matter content, yield, and anthocyanin content. The results of this research are enabling us to develop improved methods for developing high-anthocyanin purple-fleshed sweetpotato clones to support a developing natural colorants industry in NC.

NCI experiment to estimate trait heritability

In our second experiment (Chapter 3), we conducted a large-scale NCI breeding study on the inheritance of yield, starch, and sugar content related traits in sweetpotato. This

experiment allowed our team to estimate heritability of new composition traits, study the effects of exotic germplasm, and compare the benefits of paired crosses and polycross nurseries. This experiment also describes our team's first application of NIRS into our genetic studies.

As the work described in Chapter 2 demonstrated, numerous incompatibilities, sterility issues, and flowering difficulties often inhibit crossing of sweetpotato genotypes and reduce the size of breeding populations. By using a nested design with re-randomization, we were able to reduce the impact of incompatibilities, allowing us to develop a final mating population of 105 parents.

Since most US sweetpotato varieties trace their ancestry to a handful of ancestral lines, they likely have a narrow genetic base, which may limit our ability to modify sweetpotato quality in new directions (Jarrett 1987, Pecota personal comments). This population allowed us to estimate heritability for new traits and study the effects of exotic and heirloom germplasm on US sweetpotato breeding. To help overcome this challenge, the population described in Chapter 3 not only contains leading US tablestock orange-fleshed, but also high dry-matter white, yellow, and cream-fleshed lines from the US as well as overseas sweetpotato varieties from Asia, the South Pacific, Latin America, and Africa. Such a broad array of germplasm is more likely to contain alleles that may benefit emerging sweetpotato markets than the relatively narrow base of North American tablestock germplasm.

While incorporation of new germplasm is one critical part of developing sweetpotato for new markets, development of phenotyping tools for new traits is its indispensable

counterpart. By further developing and applying previously described NIRS tools (George, personal communication), we have developed a system that allows identification of the genetic basis of new traits such as sugar and starch content and determine the best crossing approaches to improve our germplasm base.

Identification of sweetpotato QTLs using NIRS

Our third and final experiment (Chapter 4) took advantage of a genome map developed from a cross between a white East African landrace 'Tanzania' and an orange-fleshed American cultivar 'Beauregard'. An AFLP based map was developed and QTL for starch, dry matter, beta-carotene, and nematode resistance have been previously identified using this genetic resource (Cervantes-Flores 2006, Cervantes-Flores et al. 2008a, Cervantes-Flores et al. 2008b, Cervantes-Flores et al. 2011). By maintaining the TB (Tanzania x Beauregard) mapping population, our team has enabled continued study of sweetpotato genomics. By merging previously described linkage mapping data with the NIRS tools described in this dissertation, we have confirmed the location of previously described QTL for starch and dry matter content and identified new QTL for sugar and reducing sugar content. The research in this chapter advances our understanding of basic sweetpotato genomic architecture in the United States and serves as another step toward marker assisted selection in sweetpotato.

Conclusion

The research presented in this dissertation demonstrates several complementary steps forward for sweetpotato breeding. It demonstrates that sweetpotato composition can be readily modified using traditional cross-breeding and has resulted in development of multiple new technologies that are being incorporated into the program's overall breeding effort. The NIRS technologies described are allowing our team to move into a new generation of sweetpotato phenotyping and the broad germplasm base developed as part of chapter 3 provides new genetic resources, allowing us to meet the needs of both growers and downstream users of sweetpotatoes.

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CHAPTER 2: COMBINING ABILITY OF SWEETPOTATO GERMPLASM FOR YIELD, STARCH AND ANTHOCYANIN PRODUCTION

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Abbreviations: TMA, total monomeric anthocyanins; NCII, North Carolina Design II; GCA, general combining ability; SCA, specific combining ability; DM, dry matter content; CIE, International Commission on Illumination.

Key Words: anthocyanins, general combining ability, heritability, NCII design, specific combining ability, starch, sweetpotato, *Ipomoea batatas*

Abstract

Interest in the potential of sweetpotatoes for the production of industrial products is increasing. As part of our efforts to develop sweetpotatoes (*Ipomoea batatas* (L.) Lam.) for starch and anthocyanin production in the Southeast US, we developed a 5 x 5 NCII mating design resulting in 25 full-sib families consisting of 20-25 offspring each to estimate the relative importance of general and specific combining abilities for dry matter, total monomeric anthocyanin (TMA) concentration, fresh yield, total dry matter and anthocyanin yields. All five traits had significant general combining abilities. Yield and dry matter yield had significant specific combining abilities. Significant differences among parents were discovered for all traits. Yield, dry matter, dry matter yield, and TMA yield were significantly impacted by spatial gradients within the field, but TMA concentration was not. Many trait pairs of interest had either genotypic and/or phenotypic correlations. Genotypic and phenotypic correlations among yield, dry matter, and dry matter yield; as well as among yield, TMA, and TMA yield suggested that improving one trait will not negatively impact other traits of importance.

Introduction

Increasing demand for bioproducts and rising interest in the development of alternative crops to promote farm diversification have stimulated research on alternative feedstocks for production of starch, sugars, and other natural plant-derived products (Harwood et al. 1999, Henry 2010). By developing new feedstock crops, breeders can simultaneously help achieve these goals. Sweetpotatoes with higher starch content may allow farmers to produce sweetpotatoes for industrial starch production, which can be used directly in the food and paper industries or to produce a number of biobased chemicals including biofuels and bioplastic precursor molecules (Ellis et al. 1998, Werpy and Petersen 2004), thus opening new markets. In addition to potential markets for sweetpotato starch, sweetpotatoes may also have potential as a source of anthocyanins for use as a natural food colorant. Anthocyanins can be used as a functional food ingredient due to their recognized antioxidant and anti-cancer properties (Philpott et al. 2004, Teow et al. 2007). In some regions of the world, purple-fleshed sweetpotatoes are commonly consumed as tablestock sweetpotatoes, while in others, notably Japan and China, purple-fleshed varieties are also used as a source of industrial anthocyanins (Suda et al. 2003).

Sweetpotatoes may be suitable as an industrial bioproduct feedstock because they can be grown on marginal soils with low inputs of fertilizer and pesticides, thus reducing competition with food and feed crops and reducing the need for chemical inputs which require fossil fuels in their production (Woolfe 1992). Increased farm diversity reduces risk over specialized farms because at times when one commodity has lower prices, another may have higher prices. However, a grower's decision to diversify is impacted by additional

factors including management knowledge, required capital investments, and availability of suitable crops and/or livestock (Harwood et al., 1999). As interest in bioproducts and biofuels increases, plant breeders have diversified their efforts to include development of new crops for bioproduct development (White et al. 1994). Breeders' ability to develop new suitable crops is likely to meet the needs of a portion of growers, allowing them to diversify, reduce risk, and meet rising demand for bioproducts and biofuels.

Development of regionally-adapted, high-starch and/or purple-fleshed sweetpotatoes may allow development of new sweetpotato-based industries in North Carolina and the Southeast US. In response to the rising needs for feedstocks and crop diversification, our program has begun intensive breeding efforts to increase biomass and anthocyanin production from sweetpotatoes. To date, most sweetpotato breeding efforts in the US have focused on development of orange-fleshed, low dry-matter, tablestock varieties. As a result, little information is available for critical bioproduct production traits such as dry-matter and starch content, total fresh yield, and anthocyanin levels. To further understand the genetic basis of these critical traits for bioproduct production, an NCII design (Comstock and Robinson 1948) experiment was undertaken to identify combining abilities and genetic variances underlying these traits.

In sweetpotatoes, most heritability studies reported to date have relied on parent-offspring regression (Jones et al. 1969, Zhang 1994), with relatively few using intra-class designs (Qiwei et al. 1988, Mwanga et al. 2002). Furthermore, previous studies have focused on traits of importance for tablestock varieties, which may have little relevance for industrial sweetpotato production (Jones et al. 1969, Zhang 1994). NCII designs have been widely

used to estimate heritability components in diploid crops (Yu et al. 1991, HaoChuan et al. 2009). They allow partitioning of variance components for a given trait into additive and dominance effects. Identification of additive and dominance components is important for breeding because it allows crossing and selection procedures to be better optimized for a trait of interest. The autohexaploid, clonally propagated nature of sweetpotatoes changes the interpretation and application of heritability information from that of the traditional sexually propagated diploid crops; however, it is still critical for development of appropriate breeding schemes. Because each parent passes on three copies of each allele to its offspring, half-sibs in autohexaploids share a dominance variance component (Gallais 2003). The clonally propagated nature of sweetpotatoes allows breeders to maintain dominance and epistatic variance in ways that breeders of sexually propagated crops cannot.

In this paper, we report on an NCII experiment to identify combining abilities of potential sweetpotato parents for dry matter and anthocyanin production. These two components may allow development of new sweetpotato processing industries in the Southeastern US. Dry matter is closely correlated with starch content (Hall and Smittle 1983). The relative ease of measuring dry matter content has allowed breeders to use it as an estimate of relative starch content. While many purple-fleshed varieties have low anthocyanin concentrations and/or are unadapted to North Carolina, our efforts are showing that adapted varieties with high anthocyanin concentrations can be developed through breeding. We report on this progress herein.

Materials and Methods

Crossing Block Development

In 2003, a diallel mating design was carried out to test compatibility of sweetpotato lines to aid in selection of parents for further study. Parents were selected to represent high dry matter clones and purple fleshed clones that could be used in a breeding program to increase dry matter content, starch, and anthocyanin yield. Twenty-two parents were crossed in all possible combinations to determine compatibility. From this crossing block, five female and five male parents were found to be compatible in all needed combinations and used to develop the NCII mating block described in this paper (Table 1).

Field Trials

Twenty-five offspring of each full-sib family were evaluated in the field at the Horticultural Crops Research Station (HCRS) in Clinton, NC during the 2004 growing season. Each seed was planted and sprouted in the greenhouse in 6 x 12 cell seedling trays and the seedling was transplanted to the field. Asexual propagation allowed five vegetative cuttings to be obtained from each entry, which were then transplanted into the field in a modified randomized complete block experimental design (RCBD) with five-plants per plot. The plants were spaced 30 cm in the row and rows were on 1.06 m centers. Inorganic fertilizer (N-P-K) was applied per NCSU's sweetpotato crop production recommendations (Wilson et al. 1989). The trial was cultivated twice and irrigation applied once as needed. For the modified RCBD, the five blocks (i.e. replications) in the experiment consisted of 5 offspring from each full-sib family. Therefore, the 25 siblings were planted out as five plants

per plot, with the first five siblings in replicate one, siblings 6-10 in replicate two, siblings 11-15 in replicate three, etc. The crop was planted on June 29, 2004 and harvested on November 1, 2004, 125 days after planting (DAP).

Data Collection

Sweetpotatoes were harvested with a one-row chain digger and weighed in the field to determine total fresh yield. Storage root samples were collected and brought to the laboratory to determine their percent dry matter (DM) and total monomeric anthocyanins (TMA) content. Samples between 125 and 250g were processed and lyophilized to remove moisture and then weighed to determine dry weights. DM was calculated as freeze-dried weight/fresh weight. TMA, expressed as mg cyanidin-3-glucoside /100 g FW, was determined as previously described (Teow et al. 2007). The color of the freeze-dried powders was measured using a tristimulus colorimeter (Model D25/DP9000, Hunter Associate Laboratories, Inc., Reston, VA, USA) and expressed as L*, a*, b* values. The instrument (45°/0°geometry, D25 optical sensor) was calibrated against a standard white tile (L* = 92.75, a* = –0.76, b* = –0.07). Freeze-dried samples were placed into a 35 mm petri dish and covered,. L*, a*, and b* values were obtained by averaging three readings per sample. Hue angle in degree (H*) and chroma (C*) were calculated as arctan (b*/a*) and $\sqrt{(a^*)^2 + (b^*)^2}$, respectively (Hutchings, 1994).

Data Analysis

Data were analyzed using JMP v.9.0 (SAS Institute, Cary NC). Tests were carried out for general combining ability (GCA) and specific combining ability (SCA), for each of 5 traits: fresh yield, DM, TMA, dry matter yield (=fresh yield x DM), and TMA yield (=fresh yield x TMA). The linear model $Y = \mu + \beta_1 F + \beta_2 M + \beta_3 (F*M) + \beta_4 B + \varepsilon$. In this model, Y represents the phenotypic trait, μ represents the intercept (population mean), β_1 represents the coefficient of regression for females and F represents the female, β_2 represents the coefficient of regression for males and M represents the male, β_3(F*M) represents female*male interaction and its coefficient, β_4B is the in-field block effect and its coefficient, and ε is the error term. All factors were considered fixed effects. GCA was considered significant for a trait if the female and/or male parents had a statistically significant effect. SCA was considered significant if the parents demonstrated a statistically significant female*male interaction for the trait. Trait correlations were analyzed using the multivariate option in JMP v.9.0 (SAS Institute, Cary, NC). Phenotypic correlations were calculated on the raw data from individual plots. Family mean correlations were calculated based on the LSMean of each full-sib family.

Results

Family Diversity

Twenty-five full-sib families with 20-25 members each were generated in the crossing block. The families showed extensive diversity for storage root shape, color, and size. Offspring flesh colors included white, cream, orange, white with purple, purple and

purple with orange. Intensity of orange and purple flesh color varied widely even among full-sibs. Most families contained offspring with colors and/or coloring patterns that were not present in either parent. Such diversity is likely due to the highly heterozygous, autohexaploid nature of sweetpotatoes. L96-117 x NC415, a cross of an orange parent by a purple, generated one of the most diverse full sib families in the trial, with offspring bearing orange, purple, and cream flesh (Figure 1a). NCDM01-192 x NC415 a cross between the two darkest purple parents in the population, produced offspring ranging from dark purple to cream, with diverse color patterns (Figure 1b). Xushu18 x NC415 shows that dark purple offspring can still be obtained in a white x purple cross (Figure 1c). A cross of 2 white sweetpotatoes (Xushu18 x NCFTA94) shows that while white sweetpotatoes produce predominantly white offspring, traces of purple can occasionally be found in some white x white crosses (Figure 1d).

Fresh Yield

The model for total fresh yield included females, males, interaction, and block effects. Male and female parents both had a significant effect on offspring yield, indicating a significant general combining ability. The significant female*male interaction indicated that specific combining ability significantly influenced offspring yield. The significance of the replication effect indicated that within field variation had a significant impact on fresh yield of sweetpotatoes.

The R^2 value of the model was 0.38. Comparing the mean squares of each component in the model suggests the relative importance of each component in the model.

In-field blocks showed the largest mean square, indicating it had the largest impact on fresh yield in this experiment. This was followed closely by females, with males and SCA having much smaller effects (Table 2).

Both male and female parents showed significant differences in the yield of their offspring. Among females, the Chinese cultivar, Xushu18, produced offspring with the highest fresh yield weight. These were significantly higher yielding than the offspring of any other parent. Following Xushu18 were the North American lines NCDM01-192 and L96-117 with no significant differences between them. Okinawa, a landrace from the Pacific, and NC1650-8N produced the lowest yielding offspring, each with less than half the yield of Xushu18 (Table 3).

The fresh yield range for paternal parents was generally narrower than for female parents, in agreement with the larger mean square for yield associated with females. The highest yielding male parents were the North American lines NCFTA94 and O'Henry, followed by the landrace Camote Morado (PI 531093) and the purple-fleshed line NC 415. The male parent with the lowest yielding offspring was NC1554, which produced offspring with significantly lower yields than either NCFTA94 or O'Henry, but not significantly lower than Camote Morado or NC415 (Table 3).

Since yield was significantly impacted by specific combining ability, full-sib families were analyzed to identify those that exhibited significant SCA. Six families were found to have significant SCAs, as their yields were different than expected based on the GCAs of the two parents. Of these, four families had yields lower than expected from the parental GCAs, while two families had yields higher than expected (Table 4).

Dry matter content

Significant specific combining ability effects for DM content were not observed in this study however, it was influenced by both male and female general combining abilities and block effects. The R^2 for the model was 0.57, indicating that parental combining ability together with in-field variation was a good indicator of offspring DM in this set of genetic materials. The mean squares of female, male, and replication indicated that their impact on dry matter content was approximately equal, although males appeared to have a slightly larger effect than females (Table 2).

Both female and male parents were ranked by their ability to produce high-dry matter offspring. Among female parents, NCDM01-192 and Okinawa produced offspring with the highest dry matter content with 38.7% and 38.2% dry matter respectively. These were followed by NC1650-8N, Xushu18, and L96-117 respectively; with L96-117 being significantly lower than any other female parents at 33.3% dry matter (Table 3).

Among male parents, NC1554 produced the offspring with the highest dry matter content at 39.2%. This was followed by C. Morado and NCFTA94 at 38.2% and 37.8%, respectively. The male parents that produced the lowest dry matter were NC415 and O'Henry (Table 3).

Dry matter yield

Dry matter yield from each plot was calculated by multiplying fresh yield by DM. Dry matter yield was influenced by general combining ability, specific combining ability, and block variation. The mean square suggests that females had the largest impact on dry

matter yield, followed by block variation, specific combining ability, and male parents (Table 1). The R^2 value for this model was 0.35, suggesting that, with this set of materials, parental combining ability plus block effect was a moderate predictor of offspring dry matter yield.

Both males and females were ranked by their ability to produce offspring with high dry matter yield. Among females Xushu18 and NCDM01-192 produced offspring with the highest dry matter yield, but they were not significantly different from each other. L96-117 and Okinawa offspring produced the third and fourth highest dry matter yields, without a significant difference between them, while NC1650-04 produced offspring with the lowest dry matter yields (Table 3).

The difference among dry matter yields for male parents was much narrower than for female parents. NCFTA94 and Camote Morado produced offspring with the highest dry matter yields, with no significant difference between them. However, NCFTA94 produced progeny with significantly higher dry matter yields than all parents except Camote Morado. O'Henry, NC415, and NC1554 produced offspring with the third, fourth, and fifth highest dry matter yields, respectively. There were no significant differences among any of the four lowest dry matter yielding parents (Table 3).

Analysis of female*male interactions revealed several families with significant specific combining abilities for dry matter yield. L96-117 x C. Morado produced significantly higher dry matter yields than predicted by GCAs of the two parents. NC1650-8N x NC415, L96-117 x O'Henry, Okinawa x C. Morado, and Xushu18 x NCFTA94 all produced offspring with significantly lower dry matter yields than would be expected from parental GCAs (Table 5).

Anthocyanin content

As part of our breeding efforts to develop purple-fleshed sweetpotatoes for both tablestock use and industrial anthocyanin production, we also studied the inheritance of TMA concentration in the populations. Our model included the impact of females, males, female x male interactions (SCA) and block variation and resulted in an R^2 of 0.21, suggesting low to intermediate heritability. Both males and females had significant effects on anthocyanin concentration, but neither specific combining ability nor block variation had a significant impact on anthocyanin concentration (Table 2).

Both males and females were ranked by the anthocyanin contents of their offspring. Among the female parents, NCDM01-192, Okinawa, NC1650-8N, and L96-117 produced the offspring with the highest anthocyanin contents respectively, with no significant differences between them. Among males, NC415 produced the offspring with the highest anthocyanin contents at 96 mg anthocyanins/100g flesh. This was followed by Camote Morado and NC1554. O'Henry and NCFTA94, two white fleshed parents, produced the offspring with the lowest anthocyanin concentrations (Table 3).

TMA Yield

Because the total amount of anthocyanins is affected by both the total fresh yield and the anthocyanin concentration, we also undertook an analysis to determine the effect of general and specific combining ability of TMA yield. The R^2 value for a model including females, males, females*males, and block variation was 0.18, indicating that, in this set of genetic materials, TMA yield was not highly heritable. Females, males, and blocks had

significant effects on TMA yield. Mean squares analyses indicated that TMA yield was primarily influenced by GCA (Table 2).

Both male and female parents were ranked by their ability to produce offspring with high TMA yield. Among females, NCDM01-192 produced offspring with the highest TMA yield. Its TMA yield was more than double any other female parent and significantly higher than every other female parent. Female parents L96-117, Xushu18, Okinawa, and NC1650-8N ranked 2-5, with no significant differences found between any of the four parents. Among male parents, NC415 produced offspring with the highest TMA yield, although not significantly higher than Camote Morado or NC1554, which ranked 2 and 3 for TMA yield, respectively. The male parents with the lowest TMA yield were O'Henry and NCFTA94 (Table 3).

Trait Correlations

Phenotypic and family mean correlations among traits described in this article revealed several correlations relevant to sweetpotato breeding. Both phenotypic and family mean correlations provide information relevant to improvement of our key traits of interest: dry matter yield and TMA yield.

Phenotypic and family mean correlations among yield, dry matter, and dry matter yield indicate that improving fresh yield would likely have a positive impact on dry matter yield. The family mean correlation between yield and dry matter yield was strongly positive (0.96). Yield had a negative phenotypic correlation with storage root dry matter content (-0.21) but there were no statistically significant correlations between dry matter content and

dry matter yield. Strong positive correlations were found between yield and dry matter yield.

TMA concentration and TMA yield possessed significant family mean correlations with dry matter (Table 6), but their phenotypic correlations with dry matter were much weaker. TMA and TMA yield both had weak family mean correlation with yield, and a weak phenotypic correlation (0.12) was found between TMA and dry matter content. TMA yield had a strong phenotypic correlation with TMA (0.80) and a positive correlation with yield (0.25).

To evaluate the potential of colorimetry for evaluation of anthocyanin content, phenotypic relationships between CIE colorimetric values and TMA concentration were calculuated. TMA correlations with CIE color values showed negative correlations with L* and b* and a positive correlation with a* (Table 7). TMA also had a strong logarithmic relationship with hue (r^2= 0.87).

Discussion

We studied combining abilities for five traits of importance to sweetpotato breeding. Total fresh yield was influenced by both general and specific combining abilities. Maternal parents had the greatest impact on fresh yield of their offspring, followed by males and specific combining abilities, respectively. The R^2 for the combining ability model of fresh yield was 0.38, suggesting that fresh yield is somewhat difficult to predict via parental combining abilities. The relative difficulty in predicting total fresh yield agrees with past experience of our program as we have found that unselected (first year) full and half sib families vary widely for total fresh yield. Because all traits have significant GCAs, it is

likely that all traits can be improved via polycross nurseries, which are used by many sweetpotato breeding programs today. Polycross nurseries have the advantage of producing large numbers of seed that are difficult to develop with paired crosses by hand because of the breeding behavior of sweetpotato.

The different effects of females and males on yield could be the result of two potential reasons. There may indeed be a significant maternal effect on total yield, or due to the small sample size, there is a possibility that greater diversity among the female parents causes them to have a larger variance component. While previous studies on maternal effects have been undertaken in sweetpotato, results have been mixed. Lin et al. (2007) demonstrated significant maternal effects on yield in one population, but not in another. In other clonally propagated crops, significant maternal effects on yield have been found in potato (*Solanum tuberosum*) (Sanford and Hanneman Jr. 1980).

The significant SCA for fresh yield suggests that our program may need to adjust our polycross nursery breeding approach. Polycross nurseries have been common in sweetpotato breeding since they were first proposed by in the 1960's (Jones 1965). Polycross nurseries have provided breeders with the ability to generate large numbers of seed with minimal amounts of labor. However, because they allow only direct control of the female in a given cross, their ability to take advantage of SCA for traits of importance is reduced. While within family selection followed by asexual propagation can take advantage of SCA, the number of seed produced by well-performing full-sib families is unknown and likely reduced in a polycross nursery as compared to paired crosses. This limitation has led to the suggestion that sweetpotato breeding programs should consider using more paired crosses

which are more efficient in terms of genetic gain (Gruneberg et al. 2009), but in breeding programs that have limited technical resources (i.e. labor for manual crossing), the limited seed that can be obtained from paired crosses means that the primary means of generating genetic variability for these programs will continue to be polycross nurseries. However, it may be possible to combine the attributes of paired crosses and polycrosses using a "modified polycross nursery". Typically, parents for polycross nurseries are selected based on the phenotypic qualities of the parents and then randomly ordered within a nursery. To improve traits with significant SCA such as yield, it may be advantageous to first identify high SCA crosses through smaller scale paired crossing experiments. Then, to generate larger numbers of seedlings, parents could be selected based on the performance of their offspring and located in polycross nurseries such that parents with favorable SCA were closer to each other in the nursery than may happen through standard randomization. This "modified polycross format" may increase the likelihood of combining desirable parents and could take advantage of the strengths and alleviate the weaknesses of polycross nurseries (i.e., large seed numbers, but uncertain parentage).

Dry matter content is often used in sweetpotatoes as a substitute for starch content due to the correlation between the two traits (Hall and Smittle 1983) and the relative ease of measuring dry matter. Our model, which included males, females, female*male interaction and blocking by rep explained 57% of the variation in dry matter. We found no significant SCA for dry matter content. Previous studies have found inbreeding to be an effective method of increasing dry matter (Komaki et al. 1998), a discovery which suggests dry matter is primarily controlled by additive effects, as a significant dominance effect would result in

inbreeding depression. The high GCA and non-significant SCA for dry matter content in this experiment also support the hypothesis that dry matter content is primarily influenced by additive effects. As such, dry matter content could likely be effectively improved using polycross nurseries.

For development of industrial sweetpotatoes, fresh yield and dry matter content should both be considered as components of dry matter yield. To further study this primary trait, dry matter yield was calculated for each plot by multiplying fresh yield by DM. Since dry matter and fresh yield are negatively correlated, it was necessary to calculate combining abilities for dry matter yield independently. Maternal parents had the largest impact on dry matter yield of their offspring, followed by paternal parents and then specific combining abilities. It is likely that females had such a large impact on dry matter yield primarily through their impact on fresh yield.

Interest in sweetpotato anthocyanins for industrial food colorants and anti-oxidant additives is rising as demand for natural products and health foods increase (Shahidi 2000). Purple-fleshed sweetpotatoes have significant untapped potential as a source of cyanidin and peonidin (Odake et al. 1992). The anthocyanins from purple-fleshed sweetpotatoes have shown significant anti-oxidant activity, which is associated with anti-cancer and other health promoting properties (Teow et al. 2007). To develop this potential market in the US, we are developing purple-fleshed varieties adapted to local growing conditions. Since few purple-fleshed varieties are native to the US, we are developing varieties by crossing purple-fleshed lines from other parts of the world and selecting for adaptation to our region and for high concentrations of anthocyanins. To better understand inheritance of TMA, we included

several purple-fleshed varieties from different sources in our crossing block and measured TMA in the offspring. Our model of TMA included female parents, male parents, female*male interactions, and block variation, but explained only 21% of TMA variation. This relatively low R^2 value is likely because even purple x purple crosses often produce significant numbers of white offspring (Figure 1b). The discovery that white-fleshed by white-fleshed crosses can occasionally yield purple offspring (Figure 1d) provides further explanation as to the low R^2 value for this trait. We found that O'Henry produced white offspring at a higher frequency than FTA94, but O'Henry had several offspring with much higher TMA concentration than those of FTA94. Thus O'Henry would be a better parent for producing offspring with high TMA concentration even though the frequency of purple offspring would likely be lower, the frequency of offspring with higher anthocyanin content would likely be higher. Based on these observations, we hypothesize that the frequency of purple offspring a parent produces is not necessarily related to the TMA concentration of the purple offspring produced.

Continued breeding experience by our team has shown these two situations (i.e. presence of purple offspring in families from white parents and presence of white offspring in families from purple parents) are the general rule rather than the exception (data not shown). Biosynthesis of anthocyanins is determined by the presence of all necessary steps in the biochemical pathway (Holton and Cornish 1995). Due to the highly heterozygous, autohexaploid breeding nature of sweetpotatoes (Cervantes et al. 2008), clones may have a complete anthocyanin pathway even if they have multiple copies of null alleles for a particular step in the pathway. Independent assortment and reduction of ploidy during

meiosis can result in gametes that do not contain active alleles for particular steps in the pathway. When these gametes are combined during fertilization, two purple-fleshed parents can produce offspring with no anthocyanins. On the other hand, genetic recombination can produce offspring with a complete anthocyanin pathway even in cases in which neither or just one parent has a complete anthocyanin pathway (Figure 1d) (Ishiguro et al. 2001, Mano et al. 2007). In this population, males had a much larger impact on TMA than females. This was likely due to the small population size. In particular, NC415 is known to have very high TMA and its use as 20% of the males may have resulted in an outsized effect. While other clones in the experiment range from white to purple, no clone in this population had TMA levels as high as NC415.

Similar to dry matter yield, fresh yield and TMA should both be considered as component traits for TMA yield in industrial sweetpotatoes. We calculated TMA yield by multiplying TMA by fresh yield to estimate the total anthocyanins that could be produced per plot. For this population, anthocyanin yield is more heavily influenced by anthocyanin content than fresh yield. This trend is likely affected by the diverse nature of the population, which contained several white and purple parents. Since non-purple by non-purple crosses produced several families with almost no TMA, no amount of fresh yield in these families would overcome the complete lack of TMA in their offspring. Similar to TMA, males had a much larger effect than females for TMA yield, while females had a much larger effect on total fresh yield, but this was not the case in TMA yield. TMA yield also had a low R^2 value, more closely resembling that for TMA than for fresh yield. Trait correlations also showed that TMA yield was much more closely correlated with TMA than with fresh yield. While

this conclusion is likely to hold for crossing blocks similar to this one, its potential impact on our purple breeding program is currently unknown as we will not attempt to develop purple varieties by crossing only white sweetpotatoes since these families are unlikely to be productive in a concentrated effort to develop high TMA yielding cultivars.

One significant discovery from this experiment that will impact our breeding efforts is that crossing two purple sweetpotatoes generally results in offspring with significantly higher TMA than crossing a white by a purple sweetpotato. In recent years we have significantly expanded our collection of purple sweetpotato germplasm beyond that described in this experiment. In order to improve adaptation, we will be crossing less adapted purple-fleshed lines with highly adapted white and orange-fleshed varieties. During the first generation of these crossing efforts, we may expect lower average TMA concentrations in purple offspring from purple x non-purple crosses than purple x purple crosses. This suggests that recurrent selection will be required to develop adapted varieties with high TMA, as even the purple offspring from purple by white crosses will likely have lower TMA than would be obtained from purple-fleshed by purple-fleshed crosses.

The trait correlations we found will also have significant impacts on our breeding program (Table 6). We have found that dry matter yield is much more significantly impacted by total yield than by dry matter content. This suggests that our efforts will be better spent improving fresh yield in relatively high dry matter lines than attempting to increase their dry matter further. We have also found the TMA yield of sweetpotatoes is more closely correlated with the TMA content than with fresh yield in this population. However, since this population contains several high-yielding parents with no anthocyanin content, this

correlation may change in another population or crossing block. Another interesting discovery from this experiment was that TMA concentration is closely correlated with the L*, a*, and b* values on the CIE color scale, furthermore, TMA concentration of purple clones had a logarithmic relationship to hue (R^2=0.87) (Table 7). These results suggest colorimetric quantification of TMA may be a valid high-throughput means of selecting for higher anthocyanin content, although the presence or absence of beta-carotene in purple-fleshed clones may impact colorimetric readings in unpredictable ways.

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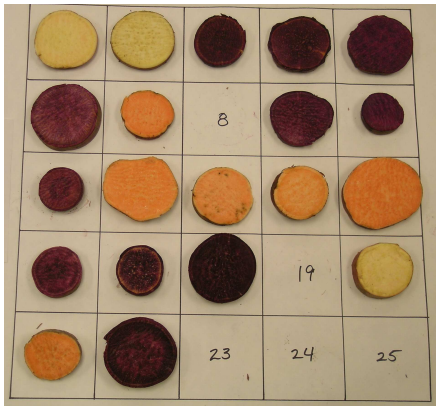
a.



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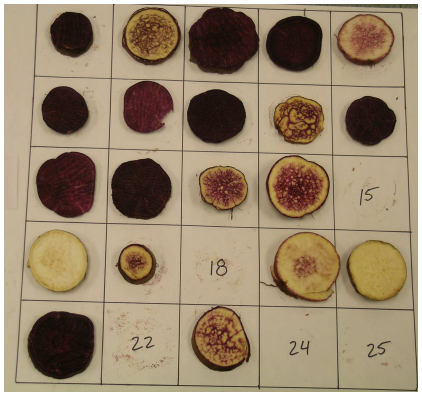

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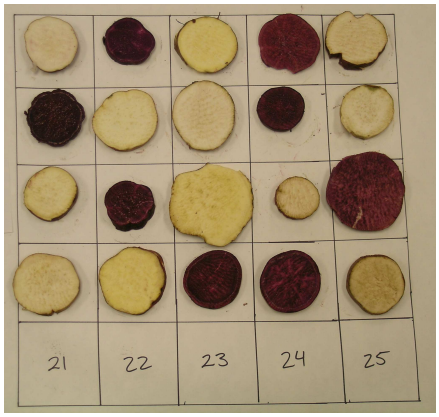

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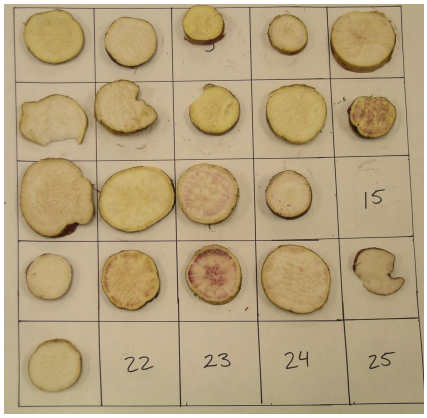

Figure 1: An example of the digital images taken of each segregating family. Each storage root slice represents a single member of a full-sib family consisting of 20-25 siblings **a.** L96-117 x NC415, an orange parent crossed by a purple. **b.** NCDM01-192 x NC415, the two darkest purple parents in the population. **c.** Xushu18 (white parent) x NC415 (purple parent). **d.** A cross of 2 white sweetpotatoes (Xushu18 x NCFTA94).

Table 1: Female and male parents. Parents represent a population that could be used to increase dry matter and anthocyanin content in North American cultivars. Parents were crossed in a 5x5 factorial crossing block to estimate combining abilities for yield, anthocyanins, and dry matter related traits. Each parental clone is named and its dry matter content, flesh color and origin are listed.

Female	**Dry Matter**	**Flesh color**	**Origin**
NC1650-8N	22	cream with purple	USA
NCDM01-192	30	cream with purple	USA
L96-117	19	orange	USA
Okinawa	29	purple with cream	Japan
Xushu 18	26	cream	China
Male			
NC1554	30	medium purple	USA
C. Morado PI 531093	30	medium purple	South America
NC415	26	dark purple	unknown
NCFTA94	32	white	USA
O'Henry	20	cream	USA

Table 2: ANOVAs for traits of interest. Fresh yield is measured in kg / plot, DM represents dry matter content (% fresh weight), DM Yield represents dry matter yield (kg / plot), TMA represents total monomeric anthocyanin content (mg / 100 g fresh weight), and TMA yield represents anthocyanins per plot (mg / plot). The mean square of each parameter is shown along with its degrees of freedom. The effect of female parents, male parents, SCA, and field variation is included in the full model. *, **, and *** indicate significance at the 0.05, 0.01, and 0.001 levels respectively.

ANOVAs for Traits of Interest

	Fresh Yield		**DM**		**DM Yield**		**TMA**		**TMA Yield**	
	df	**MS**	**df**	**MS**	**df**	**MS**	**df**	**MS**	**df**	**MS**
Full Model	**28**	**58*****	**28**	**263*****	**28**	**5.7*****	**28**	**25629*****	**28**	**54964040*****
Female	4	159***	4	457***	4	18.7***	4	20459*	4	93194170***
Male	4	26***	4	662***	4	2.6**	4	127368**	4	176243624***
F x M	16	14***	16	17	16	1.6***	16	6137	16	14731196
Block	4	185***	4	635***	4	13.8***	4	2589	4	46154930*
Error	485	5	485	12	485	0.6	434	6142	434	16611643

Table 3: LSMean values of half-sib families for each trait of interest. DM represents dry matter content (% fresh weight), DM Yield represents dry matter yield (kg / plot), TMA represents total monomeric anthocyanin content (mg / 100 g fresh weight), and TMA yield represents anthocyanins per plot (mg / plot). *, **, and *** indicate family is significantly different from the population mean with a significance of 0.05, 0.01, and 0.001 respectively. Parents with the same letter indicator are not significantly different from each other for the trait specified.

Half-sib family LSMeans for Traits of interest

	Fresh yield (kg / plot)	DM (% fresh wt)	DM Yield (kg / plot)	TMA (mg / 100 g FW)	TMA Yield (mg / plot)
Females					
NC1650-8N	1.5***,c	37.1b	0.6***,c	40.71a,b	303**,b
NCDM01-192	3.8**,b	38.7***,a	1.4***,a	72.24***,a	2992***,a
L96-117	3.4b	33.3***,c	1.1b	40.50a,b	1353ab
Okinawa	2.3***,c	38.2***,a,b	0.9***,b	48.09a,b	753b
Xushu18	4.7***,a	36.9b	1.7***,a	33.69b	1292b
Males					
NC1554	2.4***,b	39.2***,a	1.0*,b	57.27b	1705a
C. Morado PI 531093	3.1a,b	38.2***,a,b	1.2a,b	62.89*,b	2183*,a
NC415	3.0a,b	36.4c	1.1b	96.24***,a	2999***,a
NCFTA94	3.7**,a	37.8**,b	1.4***,a	8.63***,c	-203***,b
O'Henry	3.5a	32.6***,d	1.1b	10.20***,c	10***,b

Table 4: Specific combining abilities for families with statistically significant combining abilities for yield. Estimates are the differences from the expected value based on the GCA of each parent. Positive estimates indicate a specific combination that yields higher than would be expected from the combined GCA of the two parents while negative estimates indicate a lower than expected yield. Combinations not listed did not have a statistically significant SCA. *, **, and *** indicate significance at the 0.05, 0.01, and 0.001 levels respectively.

Specific Combination	Estimate
NC1650-8N*NC415	-0.8*
L96-117*C. Morado	1.2**
L96-117*NCFTA94	0.9*
L96-117*O-Henry	-1.8***
Okinawa*C. Morado	-0.9*
Xushu18*NCFTA94	-1.4**

Table 5: Specific combining abilities for full-sib families with statistically significant combining abilities for dry matter yield. Combinations not listed did not have a statistically significant SCA. Positive estimates indicate a specific combination with higher dry matter yield than would be expected from the combined GCA of the two parents while negative estimates indicate a lower than expected dry matter yield. Combinations not listed did not have a statistically significant SCA. *, **, and *** indicate significance at the .05, .01, and .001 levels respectively.

Specific Combination	**Estimate**
NC1650-8N*NC415	-0.3*
L96-117*C. Morado	0.4**
L96-117*O'Henry	-0.6***
Okinawa*C. morado	-0.3*
Xushu18*NCFTA94	-0.4**

Table 6: Phenotypic (top) and family mean (bottom) trait correlations. *, **, and *** indicate significance at the .05, .01, and .001 levels, respectively. TMA represents total monomeric anthocyanins (mg/ 100g fresh weight) and TMA Yield represents anthocyanins per plot (mg/ plot). Phenotypic correlations were calculated based on individual plot data. Family mean correlations were calculated based on the LSMean values of full-sib families.

	Dry Matter	**Dry Matter Yield**	**TMA**	**TMA Yield**
Yield	-0.21***	0.97***	-0.08	0.25***
	-0.14	0.96***	-0.18	0.19
Dry Matter		0.00	0.12**	0.04
		0.13	0.37	0.33
Dry Matter Yield			-0.06	0.27***
			-0.07	0.29
TMA				0.80***
				0.89***

Table 7: Linear correlations of CIE colorimetric values with anthocyanin concentration (TMA) in sweetpotato storage roots. The strong correlations between TMA and the L*, a*, and b* values suggest the possibility of using colorimetry to screen for sweetpotatoes with increased anthocyanin levels. *, **, and *** indicate a p-value of >0.05, 0.01, and >0.001, respectively. While not shown in the table, TMA of purple clones had a logarithmic relationship with hue ($r^2 = 0.87$).

	L*	**a***	**b***	**b*/a***	**Hue**	**Chroma**
TMA	-0.81***	0.74***	-0.75***	0.02	0.01	0.19***

CHAPTER 3: USE OF AN NCI DESIGN TO ESTIMATE EFFECTS OF EXOTIC GERMPLASM ON A NORTH AMERICAN SWEETPOTATO POPULATION

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Abbreviations: NCI, North Carolina Design I; DM, dry matter content; NIRS, near-infrared spectroscopy; TY, total yield; DW, dry weight basis; FW, fresh weight basis.

Key Words: exotic germplasm, heritability, NCI design, paired crosses, polycross, reducing sugar, starch, sugar, sweetpotato, *Ipomoea batatas*

Abstract

A nested mating design was used to estimate the heritability of yield and chemical composition traits in sweetpotato. A population incorporating exotic germplasm obtained from the US sweetpotato germplasm repository and a core set of elite US sweetpotato lines was crossed using a modified NCI mating experiment. Parents and offspring were planted in 2010 and 2011 in Kinston, NC. Yield traits were measured in the field and biochemical composition was phenotyped using near infrared spectroscopy. Heritability was calculated on both a half-sib and full-sib family basis, enabling comparisons of heritability for the polycross and paired cross breeding strategies commonly used in sweetpotato. Starch and sugar contents (glucose, fructose, sucrose, and total sugars) had relatively high heritabilities on both a GCA (h^2>0.32) and SCA basis (h^2>0.77). Yield traits exhibited low heritability on a half-sib basis (h^2<0.16), but moderate heritability on a full-sib basis (h^2= 0.21 - 0.51). Heritability was also calculated using parent-offspring regressions to compare with sibling covariance methods and we observed that composition traits had high heritabilities and resulted in similar estimates with sibling analysis as with parent-offspring regression. However, yield traits produced heritability estimates with smaller standard errors using sib-analysis than using parent-offspring regression. Percent culls had a highly skewed distribution and more accurate heritability estimates were obtained using parent-offspring regression. Heritability estimates suggest that polycross nurseries made of parents with desirable phenotypes can be effective for modifying sweetpotato composition while the use of exotic germplasm to improve yield will likely require paired crosses with progeny testing.

Introduction

Sweetpotato breeding programs have been active in the US since the early 20th century (Hernandez et al. 1959). Due to the need to develop varieties that would set storage roots in the temperate zones of the US, early breeding programs relied heavily on a small number of varieties that were able to develop storage roots and flowers to allow genetic recombination under such conditions. This resulted in significant genetic bottlenecking. Since that time, highly directional selection for lower-dry matter (ca. 18 -20%) orange-fleshed varieties and the loss of breeding programs across the US have narrowed the germplasm base even further (Jarrett 1987, Pecota personal communications). The effect of these factors on yield, disease resistance and biochemical diversity in US sweetpotato germplasm is unknown, but has likely resulted in relatively narrow ranges among elite US cultivars for most of these traits.

The emergence of new value-added processing opportunities in the sweetpotato chip, French fry and natural colorant market sectors will require development of varieties with unique biochemical compositions. While dry matter content has long been measured in sweetpotato breeding programs (Jones 1986), this trait provides only one component of sweetpotato composition, and it is insufficient to meet the needs of emerging markets. To modify sweetpotato biochemical composition, a more precise, high-throughput method of screening sweetpotato biochemical composition is needed. Near-infrared spectroscopy (NIRS) screening has been widely applied to crop breeding in recent years as breeders in many crops have begun efforts to modify crop composition (Osborne 2006, Lee et al. 2011). While sweetpotato breeding technology lags behind many other crops, breeders are

beginning to incorporate NIRS into their selection programs (Katayama et al. 1996, Lu et al. 2006a, Lu et al. 2006b, Tumwegamire et al. 2011). To advance sweetpotato breeding to the next-generation and meet the needs of new markets, such as processing for chips and fries and industrial uses such as starch and anthocyanins, the NCSU sweetpotato breeding and genetics program has begun incorporating NIRS technologies to screen experimental lines for storage root composition.

In addition to phenotyping, estimating the heritability of key processing traits is also important because this information can be incorporated into sweetpotato crossing decisions. While there are several different methods of defining heritability (Holland et al. 2003), it can be broadly defined as the ability to modify a given trait through breeding. Many methods of measuring heritability have been developed including parent offspring regression, diallels, and the North Carolina (NC) designs (Comstock and Robinson 1948, Griffing 1956, for review see: Hallauer and Miranda 1988). The NCI design has been a staple of plant quantitative genetics for decades and for sweetpotato breeding it can serve two complementary purposes. First, it enables sweetpotato breeders to avoid sexual incompatibility barriers that commonly occur in structured mating designs of sweetpotato. Second, it enables breeders to theoretically compare the rate of genetic gain that would be expected for traits using polycross nurseries and/or paired crosses for sweetpotato genetic improvement.

Most published studies of sweetpotato heritability have relied on parent-offspring regression (Jones et al. 1969, Jones 1986), but some have also used half-sib families (Thompson et al. 1994, Kim et al. 1996). While NCII designs have been implemented in

sweetpotatoes before (Qiwei et al. 1988), previous experience with them in our laboratory showed that incompatibilities can create significant challenges for factorial crossing blocks (Chapter 2). Due to low seed set and high incompatibility rates, paired crosses with sweetpotatoes require significant investment of resources. To overcome these difficulties, polycross nurseries have been commonly used since the late 1960's (Jones 1965). These have allowed screening increased numbers of genotypes, but resulted in selection on half-sib families rather than specific combinations of parental lines. As a result, it has been suggested that sweetpotato breeders would improve breeding efficiency and progress if they used paired-crossing mating strategies (Grüneberg et al. 2009). While each method has potential advantages, the best approach depends on breeding goals and available resources. Since paired cross nurseries require far more resources than polycross nurseries, programs with limited resources must balance the benefits from paired crossing with the need to apply resources to other stages in the breeding process. The best approach may also depend on project goals and traits of importance, since traits which are heavily influenced by additive effects can be efficiently modified with polycross nurseries, while those with significant dominance and epistatic effects should be modified through paired crosses.

While use of an NCI mating design allowed comparison of a large number of families to better estimate heritability, screening large numbers of genotypes while controlling for field variation is a significant challenge in sweetpotato, as it is a bulky commodity that produces a crop of storage roots that are relatively hard to harvest, handle and grade. To allow phenotyping of the large number of genotypes obtained through the mating design, an augmented design was adopted for the first time by our breeding program. The augmented

design was proposed in the 1950s (Federer 1960, Federer and Raghavarao 1975) but it has received limited application in sweetpotato breeding programs (Marchese et al. 2010). Augmented designs use replicated check varieties to estimate variance across a set of incomplete blocks, while unreplicated experimental lines are spread throughout the set of incomplete blocks (Arellano 2007). This design allows screening of larger numbers of genotypes with fewer resources than would be possible with more traditional designs. Augmented designs have the potential to increase throughput of genotype screening in breeding programs, particularly in early generation trials where breeders have must screen large numbers of genotypes with wide genotypic variation. They are likely to be especially useful in efforts to incorporate exotic germplasm into elite breeding populations, as genetic variation will generally be greater than with adapted populations and a higher percentage of the population will be poorly adapted, necessitating screening larger numbers of progeny.

In this experiment, development of NIRS provided a critical ability to screen experimental varieties while expansion of the germplasm base maximized genetic variation to improve the ability to produce genotypes with unique compositions. By merging high throughput NIRS phenotyping with an augmented design, it was possible to screen large numbers of new genotypes. The merger of these technologies created an improved system that facilitates the breeding of sweetpotatoes to meet the needs of new markets.

Materials and Methods

Germplasm

A diverse set of germplasm, consisting of 141 clones, was selected for the experiment

(Table 1). The germplasm contained a core set of US germplasm including experimental lines from the NCSU breeding program, current major and historic US cultivars, and germplasm from around the world contained within the US sweetpotato germplasm repository. Lines from NCSU included orange and yellow fleshed tablestock varieties, high dry matter white varieties, and purple-fleshed lines for industrial anthocyanin production. Additional genotypes were selected from major regions of the world including Asia, Africa, Latin America, and the South Pacific to maximize the parental variation sampled. Genotypes with white, cream, yellow, orange, and purple flesh were included. While information on many genotypes was limited, efforts to maximize genetic variation were based on information available at the outset of the experiment.

Crossing block

During the winter of 2009-2010, a nested crossing block modeled after the traditional NCI design was established in the greenhouses at NCSU. Parents were randomized using Microsoft Excel and families were developed using a cyclical mating pattern with 4 females per male. The only initial adjustment to the order was to move “Covington”, which was known to be female sterile, to become the male in its respective half-sib family.

Previous experience in sweetpotato crossing has shown that numerous incompatibilities are likely to be found in any sweetpotato crossing block (Chapter 2). During crossing, incompatibilities were discovered, and females from incompatible crosses were grouped together and randomly assigned to new half-sib families. This process was repeated multiple times during the crossing season, with the total number of compatible

crosses increasing after each re-randomization. In this way, the number of crosses in the experiment was maximized with minimal impact on the expectation of random mating (Appendix A). Crossing continued until 15 true seed were obtained from each family or until changing environmental conditions in the spring rendered further crossing efforts ineffective, whichever came first.

Field Trials

In spring 2010, true seeds from the crossing block were planted in 72 cell flats at the NCSU greenhouses in Raleigh, NC and then transported to the greenhouses at the Horticultural Crops Research Station (HCRS), Clinton, NC. Parents of the crossing block, including parents in the original crossing block from which offspring could not be obtained, were produced in the greenhouses in Clinton, NC. After an initial growing period in the greenhouse, seedlings were transplanted in the field in Clinton, NC, and five cuttings per clone were obtained after several weeks of growth and transplanted into the experimental field.

The offspring and parents of the crossing block were grown in an augmented design with check lines (which consisted of the parents of the NCI crossing block plus 36 additional lines) replicated in three incomplete blocks to estimate within-field variation and a single replication for each offspring at the Lower Coastal Research Station, Kinston, NC (35°22’31” N, 77°33’28” W) during the 2010 and 2011 growing seasons. Each clone was planted into a five-plant plot with 30 cm spacing between plant and 1.2 m break between plots on rows 1.06 meters apart. Variation in seedling development required two plantings

with an early planting on July 8, 2010 and a late planting on July 19, 2010. Both plantings were harvested November 8-9, 2010. Prior to harvest the sweetpotato vines were removed using a flail mower. The plots were then dug with a two-row chain digger and the storage roots harvested by hand. Plots were sorted into total yield (TY) and culls for rot and/or cracking and weights were taken for each class. Fresh Biomass was calculated as TY + cull weight and Dry Biomass was calculated as Fresh Biomass x % Dry Matter.

During harvest of the 2010 trial a three-root sample of each clone was collected and saved for seedling production for the 2011 season in addition to a sample for NIRS analysis described below. In 2011, all test entries (both parent and offspring) were bedded in the greenhouses at HCRS, Clinton, NC. Five plant cuttings were taken from each offspring entry and 15 cuttings were taken from each parental entry. In 2011, an early planting was carried out on May 18, 2011 and a late planting was done on June 28, 2011. Both early and late plantings were harvested October 25-26, 2011.

Sample Collection and Preparation

During harvest in both 2010 and 2011, a sample of three US #1 size roots (roots ~4.5 - 8.8 cm diameter and ~7.6 – 22.8 cm long) was collected from each plot for later NIRS analysis. Samples were cured at ~29 °C and 85% RH for 7 days in storage rooms at the HCRS, Clinton, NC. On January 9, 2011 (samples from 2010) and January 11-12, 2012 (samples from 2011), samples were washed, chopped coarsely using a food-processor, and weighed to obtain fresh sample weights at HCRS then stored at -4°C until freeze drying. Samples were freeze dried until warm to the touch (between 1 and 13% moisture), weighed

to obtain dry sample weights, and milled on a Cyclotec 1093 sample mill (FOSS Hillerød, Denmark) with a 1mm screen.

NIRS Phenotyping

Milled samples were scanned using a FOSS XDS Rapid Content Analyzer (FOSS NIRSystems, Inc, Laurel, MD) near-infrared spectrometer with ISIScan v4.0 software (Infrasoft International LLC, State College, PA). Milled samples (~5 mL) were placed in a quartz bottom cup (part number IH-0386, FOSS NIRSystems, Hillerød, Denmark) and scanned at wavelengths from 400 nm to 2500 nm using 2 nm increments (Infrasoft International 2008, Drapcho, personal communications).

After scanning, a subset of 10% of each population was selected for analysis by wet chemistry using the "Expand Product Library" function in WinISI software (Infrasoft International LLC, State College PA). This function identified samples with spectra that complemented samples previously scanned (George, personal communications) to develop a more complete NIRS model and ensure that the subset of spectra selected for wet chemistry was representative of the total population of spectra (Infrasoft International 2005, Drapcho, personal communications).

Wet chemistry

Amylose content analysis was conducted using an iodine assay similar to that described by Jarvis and Walker (1993). Samples were washed with ethanol, dissolved in NaOH, pH adjusted with citric acid and reacted with a 1% Lugol's solution. The absorbance

of the blue product was measured on a Perkin-Elmer Lambda Bio20 UV-Vis spec at wavelengths of 504, 548, 580, 630, 700, 720, and 800 nm. A standard curve was developed using purified potato amylose from Sigma-Aldrich (A0512) (Sigma-Aldrich, St Louis, MO) and purified potato amylopectin from Sigma-Aldrich (10118) (Sigma-Aldrich, St Louis, MO). Absorbance standard curves for both amylose and amylopectin showed peaks in comparable ranges to those identified by earlier researchers (Jarvis and Walker 1993).

Sugar analysis was conducted with the Megazyme Sucrose, D-Fructose, D-Glucose kit (Megazyme, Wicklow, Ireland). Total starch analysis was done with the Megazyme Total Starch Kit (Megazyme, Wicklow, Ireland). For each trait, protocols provided by Megazyme were followed. Megazyme kits were adopted for the analysis because they provide a simple affordable method that can be performed by personnel with minimal training.

Residual moisture was measure by weighing ~0.5 g into a microcentrifuge tube, allowing the tube to oven dry at 37 °C for 3-5 days, and taking the oven dried weight. Residual moisture was calculated as: (1- (oven-dried weight / freeze dried weight). Dry matter of the sweetpotato was calculated as: ((freeze dried weight *(1- residual moisture)) / fresh weight)).

Data analysis

Following wet chemistry, dry-matter based statistical models for prediction based on NIRS were developed for each trait of interest using the "Develop Equation with Full Spectrum" option in WinISI v4.0 (Infrasoft International LLC, State College, PA) (Infrasoft International 2005, Drapcho, personal communications). Partial least squares regression was

used to develop models for each individual trait. Optimal equations were selected by comparing all available combinations of scatter corrections and derivative treatments. Two outlier passes were used with the default critical T values set at 2.5, critical H values set at 12.0, and critical F values set at 8.0. The entire spectrum from 400-2500 nm was used to develop the model. After the best model was identified using the all available function, the complete dataset was divided into a calibration set and a validation set (Table 2). An equation was developed from the calibration set using the same mathematical treatments as for the complete data set. This calibration equation was then used to estimate phenotypic values for each component in the validation set to provide an estimate of the accuracy of the model (Lebot et al. 2011). The dry-matter based values of all samples were then predicted with the best model using the "Compare Predicted and Reference Values" function in WinISI. Percent amylose was calculated by dividing the amylose content of the sample by the starch content of the sample (Williams et al. 1958). Fresh weight basis traits were calculated by multiplying the dry weight basis values by the dry matter content.

Heritability analysis was conducted using Proc Mixed in SAS v9.2 for Linux (SAS Institute, Cary, NC). The linear model used was:

$$Y = \mu + \beta_0 G + \beta_1 M(G) + \beta_2 F(M*G) + \beta_3 C(F*M*G) + \beta_4 Y + \beta_5 P(Y) + \beta_6 R(Y) + \beta_7 Y*M(G) + \beta_8 Y*F(M*G) + \beta_9 Y*C(F*M*G) + \varepsilon$$

Y represents the phenotypic trait of interest, μ represents the intercept, $\beta_0 G$ represents the generation (parents or offspring) and its coefficient, $\beta_1 M(G)$ represents the effect of the male parent nested within generation and its coefficient, $\beta_2 F(M*G)$ represents the effect of female parent nested with male parent, $\beta_3 C(F*M*G)$ represents the effect of clone nested

within full-sib family and its coefficient, β_4Y represents the effect of year and its coefficient, β_5P(Y) represents the effect of planting time within year, β_6R(Y) represents the effect of rep within year, and ε represents the error. β_7Y*M(G) represents the interaction of years and males within generations, β_8Y*F(M*G) represents the interaction of years and females within males and generations, and β_9Y*C(F*M*G) represents the interaction of years with clones within females, males, and generations. All effects except generation were considered random and the 'group' option in the random statement was used to separate the genetic effects and GxE effects of parents and offspring. Due to half-sib basis heritability estimates for some traits that were not significantly greater than zero, a likelihood ratio test was performed for dry biomass, fresh biomass, and yield to determine the significance of the male effect in the above model by eliminating the male main effect in the model above and comparing the models using the -2 log likelihood statistic provided by SAS.

Proc IML (Interactive Matrix Language) was used to convert the covariances into heritability estimates. Heritability of each trait was calculated on a family means basis for both independent half-sib families and non-independent full-sib families (Holland et al. 2003). Estimators of heritability are shown below:

Half-sib families:

$$h^2 = \frac{\sigma^2_M}{\sigma^2_{M+} \sigma^2_{F(M)}/ f + \sigma^2_{MY} / y + \sigma^2_{F(M)Y} / yf + \sigma^2_{err} / yfr}$$

Full-sib families:

$$h^2 = \frac{\sigma^2_{F(M)} + ((f(m\text{-}1))/(mf\text{-}1))\ \sigma^2_M}{((f(m\text{-}1))/(mf\text{-}1))\ \sigma^2_{M+} ((f(m\text{-}1))/(mf\text{-}1))\ \sigma^2_{MY} / y + \sigma^2_{F(M)} + \sigma^2_{F(M)Y}/y + \sigma^2_{err} / yr}$$

Heritability is represented by h^2; σ^2 represents the variance attributable to a factor listed in its respective subscript. F, M, and Y represent females within males, males, and years, respectively; while *f*, *m*, and *y* represent the harmonic mean of females within males, males, and years. Sample SAS code used for calculating heritability of fresh weight basis biomass is contained in Appendix A. Phenotypes of check clones, half-sib families, and full-sib families were calculated using best linear unbiased predictors (BLUPS). Parent-offspring regressions were calculated using LSMeans obtained through Proc Mixed in SAS (Stadler and Saxton 2004) and regressing the LSMeans of the full-sib or half-sib offspring within each year on the parents of the other (Thompson et al. 1994, Kim et al. 1996), this corrected for GxY but since the experiment was grown at the same location each year, it did not eliminate potential bias caused by GxL effects. Correction factors for environmental effect on phenotypic variance were included by calculating the standard deviation of the 36 non-parental check clones each year then dividing the standard error of one year by that of the other. This produced two correction factors: $\sigma_{2011}/\ \sigma_{2010}$ was multiplied by the regression slope for the 2010 offspring on the 2011 parents and $\sigma_{2010}/\ \sigma_{2011}$ was multiplied by the regression slope of the 2011 offspring on the 2010 parents (Falconer 1989, Nyquist and Baker 1991). Heritability estimates are presented using h^2_{HS} (half-sib families) representing the half-sib heritability obtained when a sweetpotato is crossed with an unselected population; h^2_{SP} (selected polycross) representing twice the half-sib heritability and would be obtained when crossing in a polycross nursery in which the breeder has selected all parents within the nursery; and h^2_{FS} (full-sib families) representing the heritability of full-sib families as would be obtained with paired crosses. Because three traits: dry biomass, fresh biomass,

and yield did not have heritabilities significantly greater than zero, maximum likelihood ratio tests were performed to test the significance of the male effect by eliminating it from the full model and comparing the log-likelihoods of the full and reduced models using a chi-square test.

Results

NIRS

Development of the NIRS standard curves allowed simultaneous estimation of 7 traits of interest for sweetpotato breeding (Table 2): dry matter (% fresh weight), fructose (g/100g dry weight), glucose (g/100g dry weight), sucrose (g/100g dry weight), total sugar content (g/100g dry weight), starch content (% dry weight), and amylose (% dry weight). The NIRS standard curves were adjusted for the residual moisture present in the samples. This was done because small amounts of residual moisture were either present in the sample after freeze-drying or absorbed from the atmosphere during milling and processing. This differential moisture accumulation was likely due to variation in handling of samples and the different dry matter contents observed in each genotype, and the adjustment enabled more accurate final dry matter calculations as a quality control measure. The NIRS models predicted glucose (R^2 = 0.96, relative percent difference (RPD) = 3.28) most accurately. Starch was also predicted with high accuracy (R^2 = 0.96, RPD = 2.90) although this prediction was based on a relatively low sample number (N = 132). The NIRS predictions for fructose (R^2 = 0.93, RPD = 2.59), sucrose (R^2 = 0.87, RPD = 1.89), total sugar content (R^2 = 0.90, RPD = 2.23), dry matter (R^2 = 0.82, RPD = 1.72) and amylose content (R^2 = 0.89, RPD = 2.14) were

also highly significant though not as accurate. All NIRS estimates of the above traits were adjusted for residual moisture content (R^2 = 0.92, RPD = 2.46).

Trait Correlations

Significant correlations (r) were observed for many of the traits studied (Table 3). Traits related to fresh yield included dry biomass, fresh biomass, TY, cull weight, percent culls, and dry matter. Comparisons among these traits demonstrated that both culls and percent culls were negatively correlated with yield (r= -0.22 and -0.54) while culls and percent culls were positively correlated with each other (r= 0.73). Both dry biomass and fresh biomass were positively correlated with both yield and cull weight but negatively correlated with percent culls. No correlation was found between dry matter and TY or fresh biomass.

Correlations related to dry matter and storage root sugars and starch represented another important group of correlations (Table 3). Significant negative correlations were found between dry matter and dry weight based (DW) glucose (r= -0.75), DW sucrose (r= -0.43), DW fructose (r= -0.75), and DW total sugars (r= -0.82). Dry matter content was positively correlated with both DW starch content (r= 0.83), and sample amylose content (r= 0.85). Dry matter content was also negatively correlated with fresh weight basis (FW) glucose content (r= -0.62), FW fructose content (r= -0.64) and FW total sugar content (r= -0.54). However, dry matter content showed no correlation with FW sucrose content (r= 0.022). Dry matter content also showed significant positive correlations with FW starch content (r= 0.96) and starch amylose content (r= 0.32).

Among the sugars tested, DW sucrose content showed no correlation with DW fructose content or DW glucose content. While DW fructose and DW glucose content were closely correlated with each other (r= 0.98). DW glucose, DW sucrose, and DW fructose content all showed significant positive correlations with total sugar content (r= 0.76, 0.64, and 0.74). On a fresh weight basis, both glucose and fructose showed significant negative correlations with sucrose (r= -0.42 and -0.44). FW glucose and FW fructose were closely correlated with each other (r= 0.97) and on a fresh weight basis, glucose, sucrose, and fructose were all significantly correlated with FW total sugar content (r= 0.50, 0.51, and 0.47). DW and FW content of individual sugars were closely correlated for fructose, sucrose, glucose, and total sugars (r= 0.94, 0.87, 0.94, and 0.87).

Among correlations related to amylose content, sample amylose content was most closely correlated to starch content (r= 0.91). Amylose content of the sample and starch percent amylose also showed a highly significant correlation (r= 0.53). Dry weight starch content and percent amylose in the starch were significantly, but weakly, correlated (r= 0.15).

Heritability Estimates

Yield traits (dry biomass, fresh biomass, TY, culls, and percent culls) tended to have very low heritabilities on a GCA basis with estimates ranging from 0.08 to 0.16 using sib analysis (Table 4) and from 0.02 to 1.05 using parent-offspring regression (Table 5). Most h^2_{GCA} and h^2_{SP} values were not significantly different from zero, except for those related to culls. Yield traits had moderate heritabilities on an SCA basis, ranging from 0.39 to 0.51 with sib analysis (Table 4) and 0.16 to 0.77 using parent-offspring regression (Table 5).

Genetic variances of yield traits were also generally not significantly greater than zero (Table 6), furthermore, likelihood ratio tests indicated that dry biomass, fresh biomass, and yield did not have half-sib genetic effects significantly greater than zero (Table 7).

Dry matter content, dry weight starch content, fresh weight starch content, amylose content, and percent amylose content were another group of important traits. Heritabilities for dry matter, starch, and amylose were high on both a GCA and SCA basis (Table 4, Table 5). Percent amylose had a lower GCA heritability (h^2=0.13) but moderate SCA heritability (h^2=0.39). Heritability estimates were similar for these traits using either sib analysis or parent-offspring regression.

Reducing sugar traits included fructose and glucose on both a fresh weight and dry weight basis and have an impact on quality of processed products. These traits had high heritabilities on both a GCA (h^2=0.51-0.54) and an SCA basis (h^2=0.84-0.87). Estimates for h^2_{SCA} were slightly higher using parent-offspring regression than using sibling analysis while estimates for h^2_{SP} were similar for the two methods (Table 4, Table 5).

Sucrose and total sugar content are an important group of traits because of their impact on taste and sweetness of sweetpotatoes. While not identical, these traits were related as sucrose is one component of total sugar content. These traits had significant heritability on both a GCA and SCA basis. Total sugar content (h^2= 0.45-0.53) was slightly more heritable than sucrose content (h^2=0.32-0.37). Estimates for sugar heritability were similar using parent offspring-regression as using sib analysis (Table 4, Table 5).

Discussion

The emergence of new processing markets in the sweetpotato industry requires the development of varieties with new traits. This experiment provides initial insight into the potential opportunities and impediments to be expected as breeders seek to incorporate new germplasm into North American breeding populations. It also improves our understanding of the potential trade-offs between polycross nurseries and paired crosses in sweetpotato breeding. NIRS screening protocols allowed high-throughput screening of important processing and culinary quality traits including sugar and starch content. The incorporation of germplasm from around the world ensured high genetic diversity for the traits of interest and use of an augmented design allowed field screening of more genotypes than would otherwise be possible.

The NCI design used for these experiments has several advantages and provides a valid estimate of heritability, but it is also limited by certain assumptions. The traditional analysis and interpretation of an NCI design requires random selection of parental lines from a population. However, this is ineffective to study integration of exotic and heirloom germplasm for improvement of US sweetpotato breeding populations. Also, the traditional NCI analysis is based on assumptions of diploid inheritance, no environmental correlation among relatives, no maternal effects, no linkage disequilibrium, a non-inbred population, random sampling of a reference population, and random mating within the selected population sample. These assumptions allow statistically valid analysis of quantitative inheritance and heritability estimates; however, in actual breeding populations, none of these conditions are likely to occur (except for diploid inheritance, which is dependent on the crop

species of interest). For example, rather than selecting and mating plants at random, breeders select parents with particular desirable traits to help meet program goals and mate them in desirable combinations. Breeders then interpret the results of experiments in a limited manner that accounts for various degrees of violation of these assumptions. The resources required to develop cross-pollinating sweetpotato nurseries prevent many breeding programs, which are focused on cultivar development, from developing and maintaining large, random-mating, unselected populations for genetic studies. Furthermore, the germplasm in the US sweetpotato germplasm repository is not a random population of sweetpotato clones, and random selection of parents from it would almost certainly exclude many lines, such as developed cultivars from Asia, that would be included in an effort to expand US sweetpotato germplasm. Such an effort is likely to rely on using a main base of elite US germplasm and supplementing it with exotic lines, especially those that may have desirable traits despite poor adaptation (Steinhauer 1948), and would not be well modeled by random selection of clones available within North American breeding populations and/or the sweetpotato germplasm repository.

The NCI population described in this paper is representative of a sweetpotato population that incorporates exotic germplasm into US sweetpotato germplasm. It includes a core of elite US germplasm crossed randomly with ex-US varieties from around the world. During selection of ex-US germplasm, efforts were made to maximize genetic diversity and weight the population toward clones considered most likely to contain traits of interest for North America. However, information on many lines was limited, reducing the ability to identify the lines most likely to have beneficial traits.

The quantification of glucose, fructose, starch, and sugars and by NIRS enabled reasonably accurate, high throughput screening capability (Table 2) across a broad range of sweetpotato phenotypes (Table 1). This made it suitable for screening of populations such as the NCI design described here, since genetic variation for several traits was high and genotypes could be quantified for each trait. This level of screening allowed identification of genotypes that may produce offspring with desirable compositions, as was illustrated by the relatively high heritability for most composition traits, values which could not have been obtained with our conventional phenotyping methods. The NIRS model developed with this work is comparable to other recently developed sweetpotato NIRS models. The International Potato Center has developed a more comprehensive system and has obtained more accurate measures for traits than our system (Zum Felde et al. 2009). Our model estimates were similar to those developed by Lebot and colleagues using similar protocols (Lebot et al. 2011).

Phenotype distributions (Appendix B) demonstrated that for several traits, the total range for North American germplasm was similar to that for the ex-US lines. However, notable differences were found in the distributions of many traits. The North American germplasm tended to have higher biomass than ex-US lines, although some ex-US lines had very high biomass. North American cultivars also showed a bifurcated distribution for dry matter, likely caused by historical selection effects. Most selection in North America has tended toward low dry matter, however, during the 1970s and 1980s there were efforts to develop biofuel types and in recent years, the NCSU sweetpotato breeding program has made concerted efforts to develop higher dry matter lines, many of which were included in this

experiment. North American lines also showed greater sugar content and greater reducing sugar content, which were also likely due to the effect of historical selection for sweet, low dry matter, orange lines in North America, and more recent selection for high dry matter industrial type sweetpotatoes.

Rankings of full-sib families suggested that genetic bottlenecks, drift, and/or inbreeding depression could be decreasing yield improvement potential in North American breeding populations. TY heritability via half-sib families was not significantly greater than zero, but was moderate on a full-sib basis (Table 4, Table 5). This data agreed with previous studies showing that SCA for yield is much greater than GCA (Jones 1969, Martin and Jones 1986, Komaki et al. 1998). Some of the highest yielding (fresh weight basis) full-sib families were developed from crosses of adapted x exotic parents including: “TIS 3017” x “Covington” (rank = 1), “CN1345-8” x “DM04-197” (rank =3), and “KalmeghS-30” x “W-250” (rank = 9). We noted that this did not reflect yield of US #1 roots; but rather TY, the total root weight less culls for gross misshapes, rot, and severe growth cracks. Increasing root biomass through exotic germplasm may be correlated with lower pack-out in early generation crosses. This possibility was further illustrated through analysis of cull weight and cull percent. Rankings of check clones for cull weight showed that adapted and unadapted clones were spread throughout the ranking of 139 clones. While unadapted clones might have been expected to produce higher cull weights, the results showed clones with a low cull weight were often so unadapted that they had almost no yield at all, neither TY nor culls, while high yielding clones produced both more TY and more cull mass. Percent culls complemented the cull weight for measuring clone productivity. Clones with low percent culls were either so

poorly adapted they produced almost no storage roots at all, or were the most highly adapted North American cultivars. Families with the lowest percent culls were often those that had at least one highly adapted parent. However, percent culls did have much higher heritability estimates using parent-offspring regression, perhaps because the phenotypic distribution of the population was highly skewed to the left for this trait.

The low relationship of yield with half-sib parental phenotype was likely partially due to the random nature of the crossing block, which resulted in families developed from adapted by adapted parents, adapted by unadapted parents, and unadapted by unadapted parents. These results suggested that addition of select exotic lines into the population via paired crossing may have the long-term benefit of increasing yield through increasing genetic diversity and heterosis (Iwanaga 1988). The yield heritability estimates obtained here agree with those previously estimated of 0.21-0.60 (Jones 1969), and 0.25-0.57 (Jones 1986, Martin and Jones 1986).

Sugar and reducing sugar heritability in the population was very high with $h^2 > 0.77$ for SCA heritability and $h^2 > 0.32$ for GCA heritability. These high heritabilities are in agreement with high sugar heritabilities observed in other crops such as apricot (Bassi et al. 1996), sugarcane (Cox et al. 1994), and soybean (Openshaw and Hadley 1981). The rankings of check clones, half-sib families, and full-sib families showed that several families on the extremes (both low and high values for an individual trait) were produced from at least one exotic parent, indicating that exotic germplasm likely contains genes that can modify sweetpotato composition beyond that present in the elite North American germplasm, although not always in the desired direction (Figure 1). Rankings of full-sib and half-sib

families showed that while efforts to increase sucrose content would be unlikely to benefit from incorporation of overseas germplasm (notable exception “KalmeghS-30”), several heirloom varieties and non-NC lines produced offspring with very high sucrose contents. These lines included “Oklamar”, “W-250” and “W-392”. Efforts to decrease reducing sugar content would not be likely to benefit as much from incorporation of exotic germplasm or heirloom varieties due to strong negative correlation of reducing sugar content with high dry matter. In this experiment, several families with low reducing sugar contents also had very high dry matter content, including “Tinto” x “DM02-180” and “Kyushu100” x “DM02-180”. These staple-type sweetpotatoes are desirable in some regions, such as Africa and parts of Asia, but their dry matter content may be unacceptably high for the processing industry in North America. However, if efforts to incorporate heirloom varieties were undertaken to increase sucrose content in processing varieties, the resultant population is likely to be different enough from the population described here as to render predictions of reducing sugar and dry matter in the offspring unreliable.

While North American clones did show a broad range of starch contents, the rankings of full- and half-sib families indicated that further efforts to increase starch content could benefit from inclusion of high-starch exotic lines such as “Kyukei-97” and “Yukimusume”. Families produced from these clones tended to have very high starch content. The performance of these parents reflects the preference overseas for staple-type sweetpotatoes. In particular, Japanese sweetpotato breeders have focused heavily on developing high starch lines since World War II (Komaki et al. 1998).

Trait correlations suggested that most traits of interest did not have correlations that

would inhibit breeding. Fresh yield traits were not highly correlated with any composition trait, suggesting the possibility of breeding high yielding clones with any desired composition. Very strong correlations were found between starch and dry matter content, in agreement with earlier studies (Hall and Smittle 1983). Close correlations were also found between glucose and fructose, matching biochemical models showing the two reducing sugars are produced by the breakdown of sucrose in carbon sinks. Sugar traits were negatively correlated with starch content, in agreement with earlier biochemical studies showing that once carbon is transported to a sink in the form of sucrose, subsequent biochemical pathways can result in production of either starch or sugar (Fernie et al. 2002). On a fresh weight basis, reducing sugars were negatively correlated with sucrose content, suggesting opportunities for breeding the low reducing sugar, high sucrose lines desired by the processing industry. Development of processing varieties for the chip and fry industries calls for varieties with low reducing sugar, high sucrose content, and moderate dry matter content. These results showed that some varieties, such as “Oklamar”, and some families, including “Oklamar” x “W-392”, had compositions that would likely meet these requirements, indicating that such varieties could be developed through a concentrated selection program.

Comparisons of family and parental performance uncovered a few families in which offspring outperformed the high parent (for reducing sugars offspring were compared to the low parent rather than high parent because lower reducing sugars are more desirable) on a family mean basis, but individual offspring that outperformed either parent were more common (Figures 1a-d). Improved performance of offspring family means usually occurred

for families developed from parents with average or below average performance. While these families often exhibited improved performance as compared to their parents, they were generally not the best performing families. This indicates that even poorly performing parents can contain alleles for improved performance. Of greater relevance to sweetpotato breeding was the ability to develop individual offspring that outperformed even well-performing parents for individual traits. The asexually propagated nature of sweetpotatoes enables breeders to capture and fix additive, dominance, and epistatic effects at the F1 and perpetuate them through clonal propagation. The development of offspring that outperform well-performing parents indicates that sweetpotato breeding has not reached a plateau. While for low heritability traits, the application of this information to a breeding program is much more difficult, each trait investigated showed the potential selecting improved clones, and the trend was especially apparent for improvement of sucrose and reducing sugars. Dry biomass was a relatively low heritability trait, with few clear trends of well-performing families, but individual clones did show improved performance. Comparisons of parents and offspring indicated that even parents with high dry matter such as “DM02-180” could produce offspring with even higher dry matter through crossing with other high dry matter lines. For increasing sucrose content, “NC03-007” x “W-250” and “KalmeghS-30” x “W-250” both produced individual offspring that outperformed “W-250”, the male with the highest sucrose content and the high parent in both crosses. While several clones with low reducing sugar were able to produce offspring with even lower reducing sugar.

Application of the NCI design in combination with parent-offspring regression allowed comparison of parent-offspring regression with sib analysis for sweetpotatoes.

Sweetpotato breeders have often used parent offspring regression to study heritability (Jones et al. 1969, Jones 1986) and at times have applied half-sib analysis (Thompson et al. 1994, Kim et al. 1996). To our knowledge, this is the first time that an NCI design has been used in sweetpotatoes. In this experiment, high heritability traits, including sugars and starch content, could be estimated equally well using either method (Table 4, Table 5). However, for low heritability traits, such as yield, the sib analysis method was more effective, producing estimates with much lower standard errors. In this experiment, traits with low heritability often did not have half-sib genetic covariance components that were significantly greater than zero (Table 6). However for one trait, percent culls, parent-offspring regression outperformed sib analysis. This may have been due to the highly skewed distribution of the trait as discussed above.

Crossbreeding of sweetpotatoes has historically been difficult due to the complex nature of the genome, large number of incompatibilities, and low seed set. To overcome these hurdles, sweetpotato breeders have deployed innovative technologies such as grafted plants to induce flowering (Hernandez et al. 1959) and polycross nurseries (Jones 1965). While these technologies have often been necessary for sweetpotato breeding, they have not come without costs. In particular, the widespread use of polycross nurseries in sweetpotato breeding has resulted in limited knowledge of the pedigrees of most modern cultivars and reduced the ability to take advantage of specific combining ability and the possible exploitation of heterotic effects gained from genetically diverse crosses. Incorporation of exotic germplasm and heterosis have played an important role in improving potato; another autopolyploid, asexually propagated, highly heterozygous crop that also underwent a severe

genetic bottleneck upon transport to temperate zones (Mendoza and Haynes 1974, Cubillos and Plaisted 1976, Luthra et al. 2005). As such, questions have arisen within the sweetpotato breeding community regarding the trade-offs between paired crosses and polycross nurseries (Grüneberg et al. 2009). As a nested design, this experiment offered an opportunity to compare half-sib families, similar to those developed using polycross nurseries, with full-sib families, as would be obtained with paired crosses. These results indicated high heritabilities for all composition traits investigated can be obtained using polycross nurseries (Table 4), indicating that they would be satisfactory to modify sweetpotato composition. However, yield traits had relatively low heritabilities on a half-sib family basis, even when accounting for control of both parents as could be obtained in a polycross nursery in which the breeder has direct control of all parental lines (Table 4). These traits had much higher heritability on a full-sib family basis, indicating significant SCA for the traits. This suggested that yield could be more efficiently improved using paired crosses than polycrosses. Given these results, an inbred-hybrid system such as that described by Komaki and colleagues (1998) could be worthy of investigation. In such a system, polycross nurseries could be used to develop populations with desirable compositions, paired crosses could then be used to increase yield in the selected offspring.

This article describes the first step in efforts to broaden sweetpotato germplasm to meet the emerging needs of the sweetpotato processing industry and showed that many traits of interest for improving North American lines could be found in exotic or heirloom germplasm sources. The high heritability estimates for sugar and starch traits indicated that polycross nurseries consisting of parents with desirable phenotypes would be satisfactory for

improving these traits. Yield traits showed low GCA heritability and moderate SCA heritability, suggesting that efficient incorporation of yield genes from exotic sources would likely require use of paired crosses with progeny testing. Use of exotic and/or heirloom germplasm to develop populations with novel compositions may be accomplished by crossing the exotic/heirloom germplasm in a polycross nursery. Early generation selection pressure should focus on overall yield and biochemical composition, while pressure for shape and uniformity should increase in later generations.

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Table 1: Parental clones selected for inclusion in the NCI design. While initial information about many clones was limited (note "-" indicating unknown origin), efforts were made to maximize diversity by including clones from many regions of the world. Clones written in italics were selected for initial inclusion in the experiment but could not successfully produce offspring so were used only as checks, two clones used initially as parents were unable to produce storage roots in the field (note "-" for % DM in table).

Clone	Origin	%DM	flesh color
Taihaku Saitama No.1	Japan	24	cream-yellow
6618-001	Africa	24	cream
6625-001	Africa	26	cream
Beauregard	LSU	15	orange
Batata Blanca	Venezuela	22	cream
Benihayato	Japan	21	orange
BM85-42	NC	28	white-cream
BP1SP2	USDA	23	white-cream
Centennial	LSU	22	orange
CN1345-8	AVRDC	29	orange
CN1489-43	AVRDC	28	white-cream
Cordner	TX & OK	18	orange
Covington	NC	19	orange
Darby	LSU	15	orange
NCDM01-158	NC	29	white
NCDM02-105	NC	27	cream
NCDM02-180	NC	30	white
NCDM03-035	NC	25	cream
NCDM03-092	NC	27	cream
NCDM04-001	NC	29	yellow
NCDM04-051	NC	28	white
NCDM04-146	NC	27	cream
NCDM04-197	NC	26	orange
NCDM04-226	NC	28	cream
NCDM05-090	NC	23	white
Evangeline	LSU	19	orange

Table 1 continued.

Clone	Origin	%DM	flesh color
Excel	USDA	21	orange
Francia	Puerto Rico	23	cream
FT4-89	NC	26	white
FTA94	NC	27	white
GA90-16	UGA	21	cream
Georgia Jet	UGA	17	orange
Guangshu7	China	21	white
Guangshu70-9	China	20	yellow
Hatteras	NC	14	orange
Heart-O-Gold	LSU	19	orange
Hernandez	LSU	17	orange
HiDry	USDA	28	white
HM-145	MS	27	cream
IB.05	Samoa	22	cream
Jewel	NC	18	orange
Jishu5	China	26	cream
Julian	LSU	24	orange
KalmeghS-30	India	23	cream-orange
Kemb10	Africa	26	cream
Koganesengan	Japan	27	cream
Kotopuki	Japan	24	cream
Kyukei-63	Japan	29	cream
Kyukei-97	Japan	27	white-cream
Kyushu100	Japan	28	yellow
Bonita	LSU	20	cream
L-258	PNG	23	white
L-329	PNG	25	white
Liaoshu40	China	23	cream
Liberty	USDA	21	cream
Ma’alau	PNG	27	cream
Macana	Puerto Rico	22	cream
MD320	MD	23	cream
MD810	MD	20	orange
MD822	MD	16	orange

Table 1 continued.

Clone	Origin	%DM	flesh color
MDP217-84	MD	25	cream
Merenge	Africa	25	white
Minamiyutaka	Japan	30	white
Mojave	Puerto Rico	27	white
Morada Sombica	Venezuela	26	white
Mugande	Africa	29	white
Murasaki-29	LSU	28	cream
Nancy Hall	Florida	27	cream
NC03-007	NC	22	cream
NC03-030	NC	22	cream
NC03-089	NC	17	orange
NC03-302	NC	23	cream
NC03-395	NC	18	orange
NC04-097	NC	24	cream
NC04-412	NC	20	orange
NC04-531	NC	20	orange
NC06-185	NC	22	orange
NC1880	NC	24	yellow-orange
NC413	NC	27	purple
NC93-17	NC	16	orange
NonDaHong	Asia	19	cream
Norin#2	Japan	24	cream
Norin#4	Japan	20	white
O. 4	China	20	orange
Okinawa100	Japan	-	-
Oklamar	OSU	22	orange
OK-P-10	OSU	27	orange
Papota	Puerto Rico	23	white
Patriot	USDA	19	orange
PDM P4	NC	26	white
Pelican Processor	USDA	28	cream
Perla	Puerto Rico	23	white
PI324887	-	28	cream
PI531113	-	26	cream
PI564112	-	29	cream
PI564114	-	22	cream

Table 1 continued.

Clone	Origin	%DM	flesh color
Porto Rico	PR	23	orange
NCPur01-192	NC	26	purple
NCPur04-118	NC	30	purple
NCPur05-028	NC	26	purple
NCPur05-055	NC	27	purple
NCPur05-087	NC	-	purple
NCPur06-014	NC	29	purple
Regal	USDA & TX	20	orange
Resisto	USDA	22	orange
Ruddy	USDA	17	orange
Seon-mi	South Korea	25	white
Sumor	USDA	24	cream
Suwon122	Korea	25	yellow
Suwon147	Korea	24	cream
Tainung65	Taiwan	23	cream
Tanzania	Africa	24	cream
TIB10	Africa	22	cream
TIB11	Africa	17	orange
TIB4	Africa	25	orange
Tinian	Tinian	24	cream
Tinto	Mexico	29	white-purple
TIS2498	Nigeria	28	cream
TIS2525	Nigeria	23	cream
TIS2532	Nigeria	22	cream
TIS3017	Nigeria	23	cream
TIS3290	Nigeria	25	cream
TIS9232	Nigeria	27	white-purple
Travis	LSU	27	orange
UGA204	UGA	15	orange
Vardaman	MS	27	cream
Viola	Puerto Rico	20	orange
W-241	USDA	18	orange
W-245	USDA	18	orange
W-250	USDA	19	orange

Table 1 continued.

Clone	Origin	%DM	flesh color
W-392	SC	21	orange
Wagabolige	Africa	27	white
Wanmun Large	PNG	25	cream
White Bunch	USDA	27	cream
Whitestar	USDA	28	white
Woksaken	PNG	28	white
Won-mi	Korea	21	white-purple
Xiangnonhuangpi	China	24	cream
Xushu18	China	24	white
YanShu1	China	23	white
Yukimusume	Japan	27	cream

Table 2: Statistical summary of NIRS prediction models developed using WinISI. The complete model includes all samples for each trait for which both wet chemistry data and spectra were obtained. The calibration equation and validation set were developed from different subsets of the complete data set to evaluate equation quality. Identifiers are: *N*: number of samples used to make the calibration equation. *Mean*: mean of samples. *SD*: standard deviation of samples. *Min.*: estimated minimum. *Max*: estimated maximum. *SEC*: standard error of calibration. R^2: R^2 value of equation. *Bias*: prediction bias of equation vs. lab data. *SEP(C)*: standard error of prediction corrected for bias. *RPD*: SD / SEP(C). *#PLS*: number of terms in partial least squares regression *PreTreat*: scatter correction applied to equation. IMSC: inverse multiplicative scatter correction. SNV & DET: scales spectrum to standard deviation of 1.0 and removes linear and curvature. ScLin: remove scale and linear. *Deriv*: derivative treatment applied to equation. Pretreat and derivative treatments were made using options available in WinISI v.4 software. In the derivative treatment, the first number is the derivative, the second is the gap, and the third and fourth numbers are smoothing functions.

Table 2 continued.

Complete Model													
Constituent	**N**	**Mean**	**SD**	**Min**	**Max**	**SEC**	**R^2**	**Bias**	**SEP (C)**	**RPD**	**#PLS**	**PreTreat**	**Deriv**
Dry Matter	340	24.34	5.13	8.9	39.7	2.15	0.82	-1.38	2.99	1.72	10	IMSC	1441
Moisture	352	5.02	2.46	0.0	12.4	0.69	0.92	-0.46	1.00	2.46	11	SNV & DET	3441
Glucose	347	2.85	2.91	0.0	11.6	0.61	0.96	-0.41	0.89	3.28	15	ScLin	1441
Sucrose	355	9.39	5.39	0.0	25.6	1.95	0.87	-1.31	2.85	1.89	15	IMSC	2441
Fructose	346	2.05	2.01	0.0	8.1	0.54	0.93	-0.36	0.77	2.59	15	None	2441
Sugar	353	14.82	8.28	0.0	39.7	2.58	0.90	-1.72	3.72	2.23	11	None	4441
Starch	132	44.50	15.19	0.0	90.1	3.11	0.96	-2.42	5.24	2.90	9	MMSC	4441
Amylose	227	16.46	5.17	1.0	32.0	1.69	0.89	-1.12	2.42	2.14	11	MSC	1441

Calibration Equation										Validation set		
Constituent	**N**	**Mean**	**SD**	**Est. Min**	**Est. Max**	**SEC**	**R^2**	**SECV**	**1-VR**	**R^2**	**SEP(C)**	**N**
Dry Matter	289	24.2	4.9	9.4	39.1	2.54	0.74	2.68	0.71	0.79	2.60	50
Moisture	298	5.0	2.4	0	12.1	0.77	0.89	0.80	0.89	0.79	1.20	50
Glucose	300	2.8	2.7	0	11.0	1.21	0.80	1.27	0.79	0.77	1.40	49
Sucrose	299	9.1	5.1	0	24.3	2.80	0.69	3.02	0.64	0.56	4.00	49
Fructose	300	2.1	2.0	0	8.2	0.94	0.79	1.05	0.74	0.74	1.03	49
Sugar	301	14.8	8.3	0	39.7	2.99	0.87	3.20	0.85	0.83	3.61	48
Starch	102	45.4	14.3	2.5	88.3	4.22	0.91	4.68	0.89	0.88	6.35	30
Amylose	194	16.6	5.1	1.2	31.9	1.96	0.85	2.14	0.82	0.84	1.90	37

Table 3: Phenotypic correlations among traits in the NCI population grown at Kinston, NC in 2010 and 2011. *, **, and *** indicate statistical significance at p-values of 0.05, 0.01, and 0.001 respectively. Trait abbreviations are: *TY*: total yield, *DWStarch*: dry weight basis starch, *FWStarch*: fresh weight basis starch, *DWFruc*: dry weight basis fructose, *FWFruc*: fresh weight basis fructose, *DWGluc*: dry weight basis glucose, *FWGluc*: fresh weight basis glucose, *DWSuc*: dry weight basis sucrose, *FWSuc*: fresh weight basis sucrose, *DWSug*: dry weight basis total sugars, *FWSug*: fresh weight basis total sugars.

	Dry Biomass	**Fresh Biomass**	**TY**	**Culls**	**%culls**	**DM**	**DWGluc**	**DWSuc**	**DWFruc**	**DWSug**
Fresh Biomass	0.92***									
TY	0.79***	0.84***								
Culls	0.29***	0.33***	-0.22***							
%culls	-0.12***	-0.12***	-0.54***	0.73***						
DM	0.32***	-0.03	0.02	-0.09***	-0.13***					
DWGluc	-0.13***	0.16***	0.11***	0.10***	0.06*	-0.75***				
DWSuc	-0.08***	0.07***	0.07***	0.01	0.00	-0.43***	0.03			
DWFruc	-0.13***	0.16***	0.10***	0.12***	0.07***	-0.75***	0.98***	0.00		
DWSug	-0.15***	0.16***	0.11***	0.09***	0.05**	-0.82***	0.76***	0.64***	0.74***	
DWStarch	0.28**	-0.02	0.00	-0.05*	-0.07***	0.83***	-0.68***	-0.61***	-0.65***	-0.91***
Am	0.41***	0.12***	0.16***	-0.05**	-0.14***	0.85***	-0.59***	-0.62***	-0.58***	-0.84***
FWGluc	-0.08***	0.17***	0.11***	0.10***	0.05*	-0.62***	0.94***	-0.09***	0.90***	0.63***
FWSuc	0.07***	0.06***	0.08***	-0.01	-0.05*	0.02	-0.35***	0.87***	-0.37***	0.27***
FWFruc	-0.06	0.17***	0.11***	0.12***	0.07**	-0.64***	0.93***	-0.11***	0.94***	0.62***
FWSug	-0.01	0.20***	0.15***	0.10***	0.02	-0.54***	0.51***	0.70***	0.48***	0.87***
FWStarch	0.33***	-0.01	0.03	-0.07***	-0.11***	0.96***	-0.72***	-0.54***	-0.70***	-0.87***
PerAm	0.39***	0.33***	0.36***	-0.02	-0.18***	0.32***	0.03	-0.25***	-0.07***	-0.18***

Table 3 continued.

	DWStarch	Am	FWGluc	FWSuc	FWFruc	FWSug	FWStarch
Fresh Biomass							
Yield							
Culls							
%culls							
DM							
DWGluc							
DWSuc							
DWFruc							
DWSug							
DWStarch							
Am	0.91***						
FWGluc	-0.55***	-0.47***					
FWSuc	-0.22	-0.25***	-0.42***				
FWFruc	-0.52***	-0.46***	0.97***	-0.44***			
FWSug	-0.74***	-0.66***	0.50***	0.51***	0.47***		
FWStarch	0.94***	0.92***	-0.61***	-0.12***	-0.60***	-0.67***	
PerAm	0.15***	0.53***	0.004	-0.15***	-0.055	-0.13***	0.29***

Table 4: Heritabilities with standard errors of 18 traits measured in a sweetpotato population representing expansion of the North American sweetpotato germplasm pool. h^2_{GCA} (general combining ability) is the heritability on a half-sib family basis if cross-pollinated with an uncontrolled population. h^2_{SP} is twice the GCA heritability and represents the heritability in a polycross nursery in which all parents have been selected with consideration for their effect on the trait of interest. h^2_{SCA} (specific combining ability) represents heritability on a full-sib family basis, comparable to paired cross nurseries. Trait abbreviations are: *DWStarch*: dry weight basis starch, *FWStarch*: fresh weight basis starch, *DWFruc*: dry weight basis fructose, *FWFruc*: fresh weight basis fructose, *DWGluc*: dry weight basis glucose, *FWGluc*: fresh weight basis glucose, *DWSuc*: dry weight basis sucrose, *FWSuc*: fresh weight basis sucrose, *DWSug*: dry weight basis total sugars, *FWSug*: fresh weight basis total sugars.

	h^2_{GCA}	h^2_{SP}	h^2_{SCA}
Dry Biomass	0.04 ±0.05	0.08 ±0.10	0.21 ±0.12
Fresh Biomass	0.11 ±0.07	0.22 ±0.14	0.39 ±0.11
Yield	0.08 ±0.06	0.16 ±0.12	0.40 ±0.09
Culls	0.16 ±0.06	0.32 ±0.12	0.48 ±0.08
% Culls	0.15 ±0.07	0.30 ±0.14	0.51 ±0.08
Dry Matter	0.46 ±0.10	0.92 ±0.20	0.83 ±0.04
DWStarch	0.41 ±0.10	0.82 ±0.20	0.77 ±0.05
FWStarch	0.43 ±0.10	0.86 ±0.20	0.79 ±0.05
Amylose	0.44 ±0.10	0.88 ±0.20	0.80 ±0.04
% Amylose	0.13 ±0.06	0.26 ±0.12	0.39 ±0.10
DWFruc	0.54 ±0.09	1.08 ±0.18	0.86 ±0.03
FWFruc	0.52 ±0.09	1.04 ±0.18	0.85 ±0.03
DWGluc	0.54 ±0.09	1.08 ±0.18	0.87 ±0.03
FWGluc	0.51 ±0.09	1.02 ±0.18	0.84 ±0.03
DWSuc	0.37 ±0.10	0.74 ±0.20	0.79 ±0.04
FWSuc	0.32 ±0.09	0.64 ±0.18	0.77 ±0.04
DWSug	0.53 ±0.09	1.06 ±0.18	0.86 ±0.03
FWSug	0.45 ±0.09	0.90 ±0.18	0.78 ±0.05

Table 5: Heritabilities obtained by parent offspring regressions with standard errors. h^2_{HS} is twice the regression slope of a half-sib parent-offspring regression in which half-sib family mean values are regressed on the male parent. h^2_{FS} is the slope of offspring family means regressed on the midparent values and represents heritability on a full-sib family basis, comparable to paired cross nurseries. Both parents and offspring were grown in 2010 and 2011 and offspring from one year were regressed onto parental values from the other. See Table 4 for comparison to heritability estimates obtained via variance-covariance of half-sib and full-sib families.

	h^2_{HS}		h^2_{FS}	
Offspring Grown In:	2010	2011	2010	2011
Dry Biomass	0.02 ±0.18	0.28 ±0.14	0.16 ±0.10	0.32 ±0.08
Fresh Biomass	0.16 ±0.24	0.35 ±0.16	0.26 ±0.12	0.36 ± 0.08
Yield	0.29 ± 0.23	0.33 ±0.16	0.38 ±0.10	0.33 ±0.08
Culls	0.64 ±0.26	0.28 ±0.11	0.44 ±0.11	0.32 ±0.05
% Culls	1.05 ±0.22	0.46 ±0.14	0.77 ±0.10	0.46 ±0.06
Dry Matter	1.03 ±0.24	1.03 ±0.16	0.71 ±0.09	0.64 ±0.07
DWStarch	0.93 ±0.19	0.76 ±0.15	0.59 ±0.09	0.57 ±0.06
FWStarch	0.91 ±0.20	0.93 ±0.17	0.63 ±0.09	0.65 ±0.07
Amylose	0.96 ±0.21	0.75 ±0.19	0.66 ±0.09	0.66 ±0.07
% Amylose	0.42 ±0.21	0.34 ±0.29	0.37 ±0.10	0.31 ±0.12
DWFruc	0.89 ±0.13	1.08 +0.14	0.6 ± 0.07	0.72 ±0.07
FWFruc	0.77 ±0.15	1.10 ±0.13	0.63 ±0.08	0.77 ±0.06
DWGluc	0.89 ±0.12	1.07 ±0.15	0.59 ±0.07	0.7 ±0.07
FWGluc	0.72 ±0.13	1.04 ±0.12	0.57 ±0.07	0.77 ±0.06
DWSuc	0.80 ±0.18	0.51 ±0.16	0.57 ±0.07	0.65 ±0.06
FWSuc	0.69 ±0.14	0.5 ±0.16	0.52 ±0.06	0.61 ±0.06
DWSug	1.10 ±0.17	0.99 ±0.14	0.63 ±0.08	0.70 ±0.06
FWSug	0.86 ±0.14	0.87 ±0.15	0.49 ±0.07	0.73 ±0.06

Table 6: Genetic covariances with standard errors. Trait abbreviations are: *DWStarch*: dry weight basis starch, *FWStarch*: fresh weight basis starch, *DWFruc*: dry weight basis fructose, *FWFruc*: fresh weight basis fructose, *DWGluc*: dry weight basis glucose, *FWGluc*: fresh weight basis glucose, *DWSuc*: dry weight basis sucrose, *FWSuc*: fresh weight basis sucrose, *DWSug*: dry weight basis total sugars, *FWSug*: fresh weight basis total sugars.

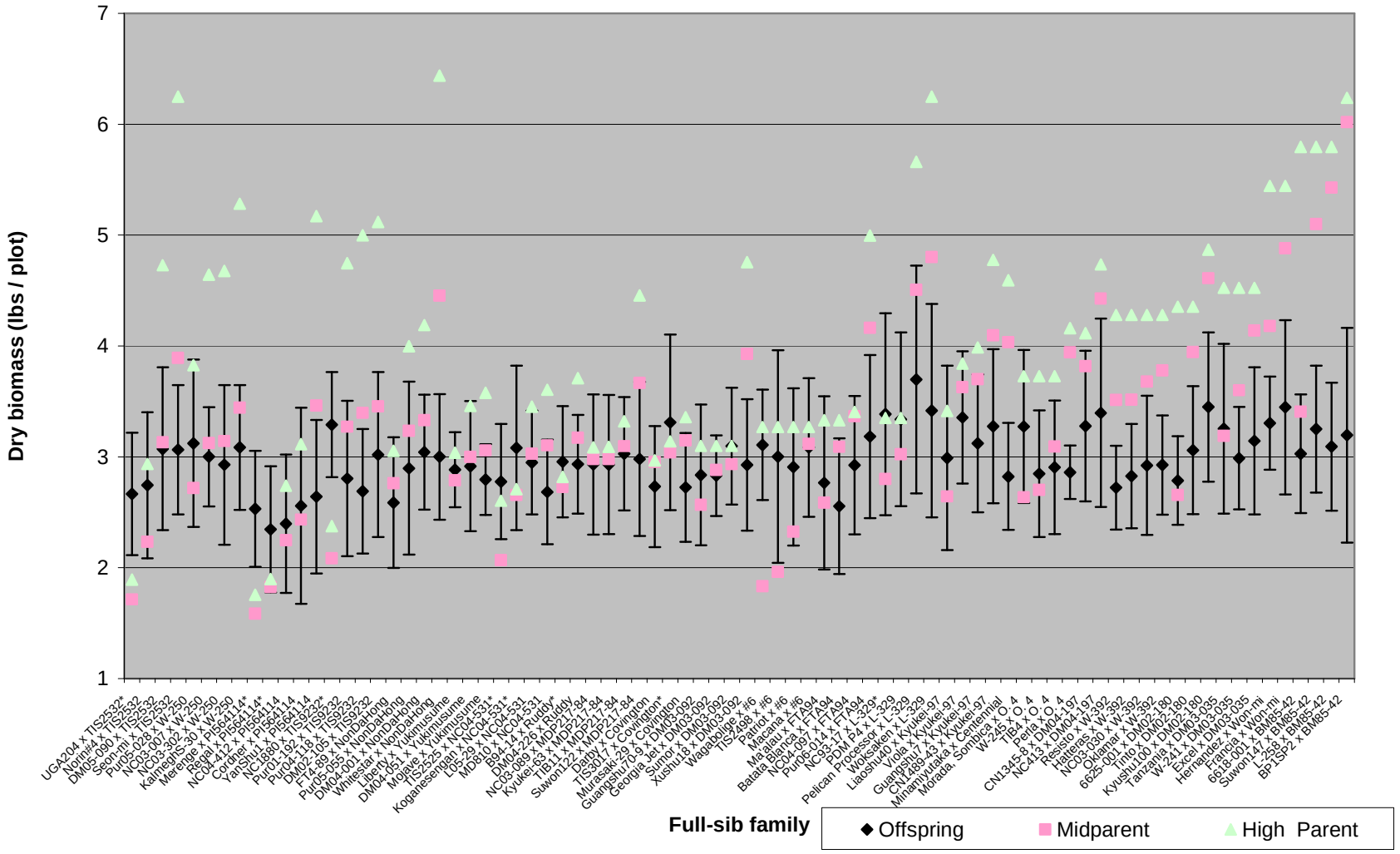
	Covariance	
	GCA	**SCA**
Dry Biomass	0.09 ±0.13	0.22 ±0.17
Fresh Biomass	4.01 ±2.87	7.33 ±3.36
TY	2.79 ±2.34	7.04 ±2.92
Culls	2.42 ±1.03	3.51 ±1.16
% Culls	63.04 ±34.88	124.44 ±41.40
Dry Matter	5.73 ±2.27	9.13 ±2.40
DWStarch	25.01 ±9.86	37.22 ±10.36
FWStarch	5.12 ±2.03	7.74 ±2.13
Amylose	3.76 ±1.44	5.52 ±1.51
% Amylose	0.55 ±0.29	0.77 ±0.32
DWFruc	1.66 ±0.60	2.30 ±0.62
FWFruc	0.04 ±0.01	0.05 ±0.01
DWGluc	2.61 ±0.95	3.73 ±0.98
FWGluc	0.06 ±0.02	0.08 ±0.02
DWSuc	3.36 ±1.367	6.01 ±1.51
FWSuc	0.10 ±0.04	0.19 ±0.05
DWSug	16.69 ±6.06	24.09 ±6.27
FWSug	0.26 ±0.09	0.33 ±0.09

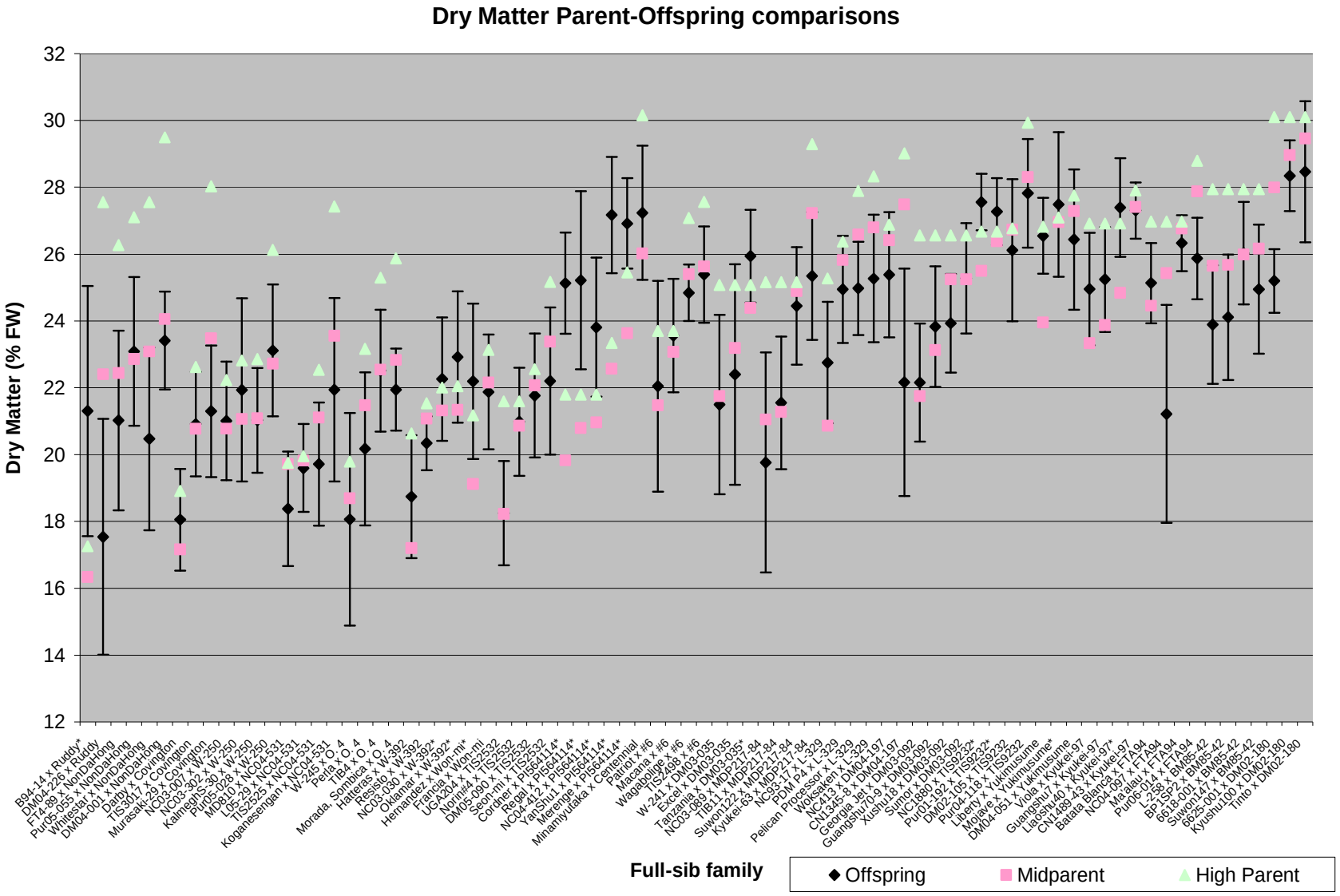
Table 7: Likelihood ratio tests for select traits. Heritability estimates for dry biomass, fresh biomass and yield were not significantly greater than zero on a half-sib family basis. To estimate the significance of half-sib effects, a likelihood ratio test was performed by eliminating the main effect of males from the full model to develop a reduced model. Results indicate that heritability on a half-sib basis for these traits was not significant. The -2 ln likelihood value of both the reduced model and full model were obtained from SAS Proc Mixed. The difference between the two models is shown and the p-value was calculated based on a chi-square curve with 1 degree of freedom.

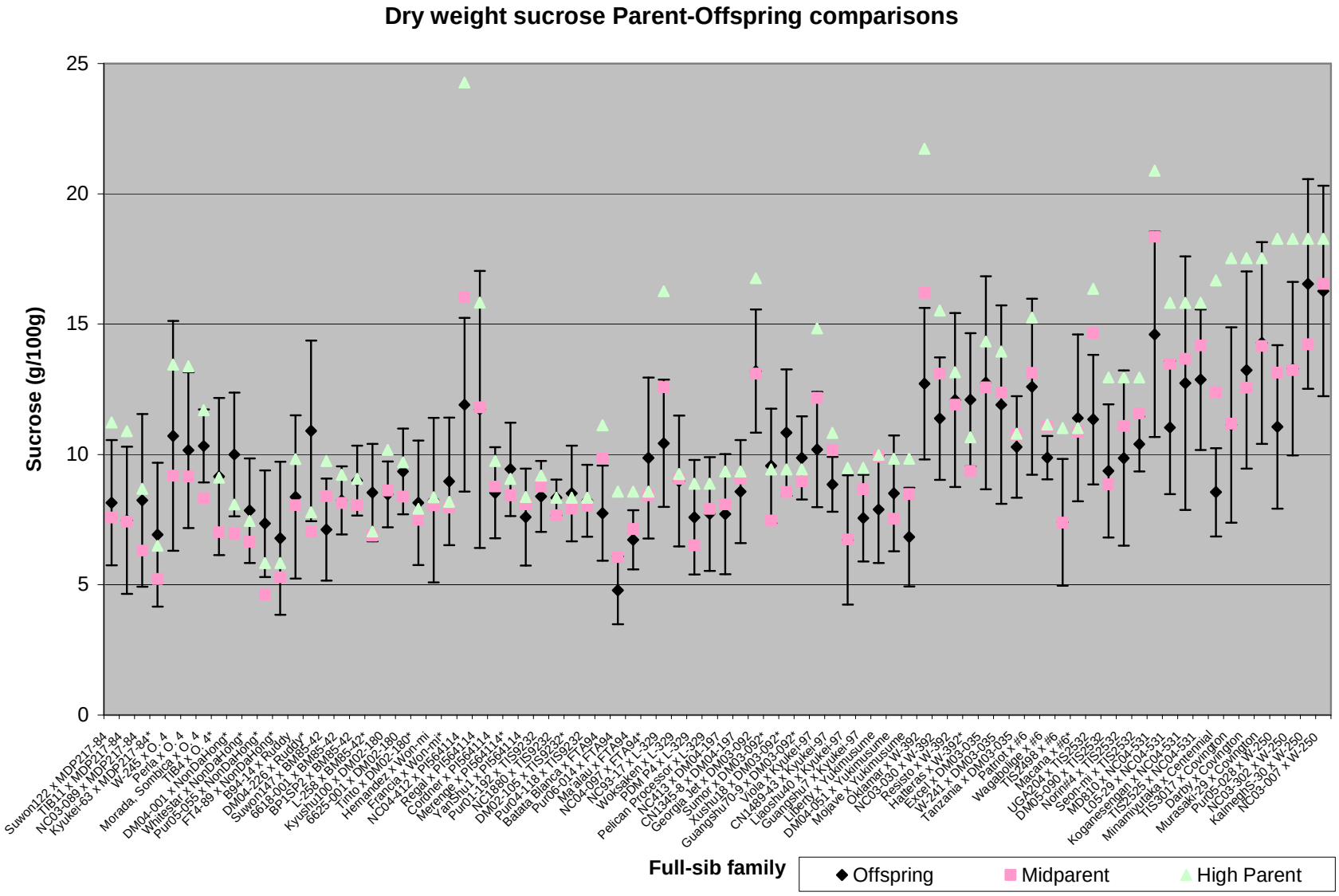
Trait	Reduced model	Full model	Difference	P-value
Dry Biomass	10878	10877.4	0.6	0.44
Fresh Biomass	18927	18924.2	2.8	0.09
Yield	19152.9	19150.9	2	0.16

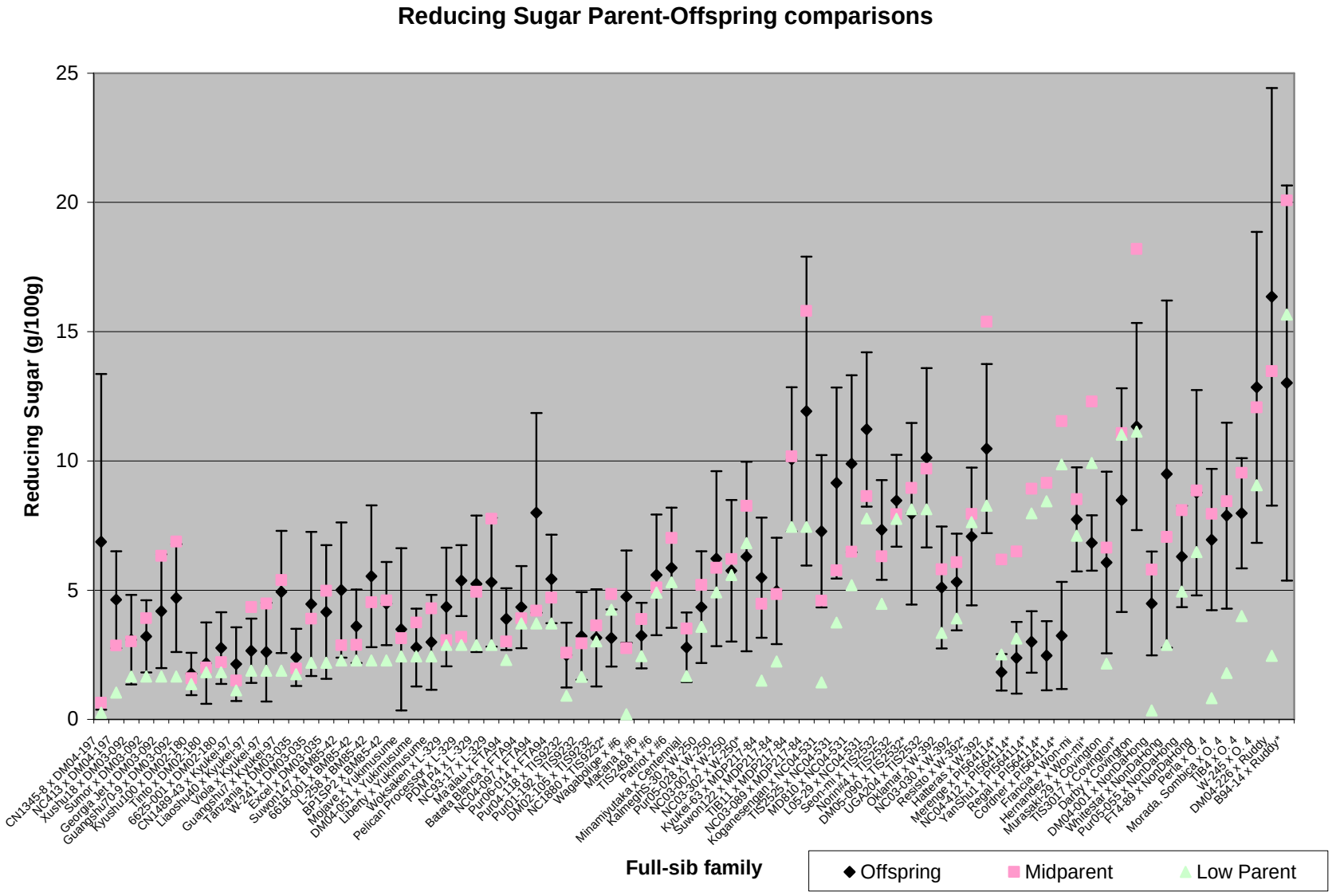
Figure 1: Graphs showing parents and offspring for each of 80 full-sib families in which both parents and offspring were grown at Lower Coastal Research Station, Kinston, NC in 2010 and 2011. Genotypic values (BLUPs) are shown for high parent (for reducing sugar, the low parent value is shown rather than the high parent because clones with lower reducing sugar are preferred), midparent, and offspring family mean for each of four traits: dry biomass, dry matter, dry weight sucrose content, and dry weight reducing sugar content. Error bars indicate one standard deviation above and below the mean of the full-sib family and are included to provide an estimate of the range of variation for a trait that can be expected from a particular cross. Families in which the mean value of the offspring is greater than the high parent are marked with an asterisk.

Dry biomass Parent-Offspring comparisons




Dry Matter Parent-Offspring comparisons
Dry Matter (% FW)
32
30
28
26
24
22
20
18
16
14
12
B94-14 x Ruddy*
DM04-226 x Ruddy
FTA-88 x NonDaHong
Pur05-053 x NonDaHong
Whitestar x NonDaHong
DM04-001 x NonDaHong
Darby x Covington
TIS3017 x Covington
Murasaki-29 x Covington
NC03-007 x W-250
NC03-302 x W-250
KalmeghS-39 x W-250
Pur05-028 x W-250
MD810 x W-250
L05-29 x NC04-531
TIS2525 x NC04-531
Koganesengan x NC04-531
W-245 x O. 4
Perla x O. 4
TIB4 x O. 4
Morada, Sombica x O. 4
Hatteras x W-392
Resisto x W-392
NC03-030 x W-392
Okamar x W-392*
Hernandez x W-392*
Franqui x Won-mi*
UGA204 x Won-mi
Norin#4 x TIS2532
DM05-090 x TIS2532
Seon-mi x TIS2532
Cordner x TIS2532
Regal x PI564114*
NC04-412 x PI564114*
YanShu1 x PI564114*
Merenge x PI564114*
Minamiyutaka x PI564114*
Centennial
Patriot x #6
Macana x #6
Wagabadige x #6
TIS2488 x #6
W-241 x DM03-035
Excel x DM03-035
Tanzania x DM03-035*
NC03-089 x MDP217-84
TIB11 x MDP217-84
Suwon122 x MDP217-84
Kyuket-63 x MDP217-84
NC93-177 x L-329
PDM P4 x L-329
Pelican Processor x L-329
Woksaken x L-329
NC4213 x DM04-197
CN1345-8 x DM04-197
Georgia Jet x DM03-092
GuangShu70-9 x DM03-092
Xushu18 x DM03-092
Sumor x DM03-092
NC1880 x DM03-092
Pur01-192 x TIS9232*
DM02-105 x TIS9232*
Pur04-118 x TIS9232
Liberty x Yukimusume
Mojave x Yukimusume*
DM04-051 x Yukimusume*
Viola x Kyuket-97
GuangShu7 x Kyuket-97
Liaoshu40 x Kyuket-97
CN1489-43 x Kyuket-97*
Batata Blanca x Kyuket-97
NC04-097 x FTA94
Ma'alau x FTA94
Pur06-014 x FTA94
L-258 x FTA94
BP-15P2 x BM85-42
6618-001 x BM85-42
Suwon141 x BM85-42
6625-001 x BM85-42
Kyushu100 x DM02-180
Tinto x DM02-180
Full-sib family
Offspring
Midparent
High Parent


Dry weight sucrose Parent-Offspring comparisons
Sucrose (g/100g)
25
20
15
10
5
0
Full-sib family
Offspring
Midparent
High Parent


Reducing Sugar Parent-Offspring comparisons
Reducing Sugar (g/100g)
25
20
15
10
5
0
Full-sib family
Offspring
Midparent
Low Parent

CHAPTER 4: IDENTIFICATION OF SWEETPOTATO QTL USING NEAR INFRARED SPECTROSCOPY PHENOTYPING

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Abbreviations: QTL, quantitative trait loci; DM, dry matter content; NIRS, near-infrared spectroscopy; DW, dry weight basis; FW, fresh weight basis; CIM, composite interval mapping; MIM, multiple interval mapping.

Key Words: exotic germplasm, heritability, NCI design, paired crosses, polycross, reducing sugar, starch, sugar, sweetpotato, *Ipomoea batatas*

Abstract

A previously described sweetpotato quantitative trait loci (QTL) mapping population developed from a cross between Tanzania, an African landrace and Beauregard, a major USA cultivar, was phenotyped using near infared spectroscopy (NIRS) to identify QTL for storage root glucose, fructose, sucrose and starch content and yield. Transgressive segregation was observed for all traits. In Beauregard, six QTL were identified that decreased starch and dry matter content, explaining 37.6% of variation. Eight QTL were associated with increased sugar content, explaining 27.8% of sugar variation. One QTL in Beauregard was associated with decreased yield, explaining 4.8% of yield variation. In Tanzania, two QTL were associated with increased starch and two QTL were associated with decreased starch, explaining 16.6% of variation. Two QTL in Tanzania were associated with decreased sugars and one was associated with increased sugar content, explaining 19.3% of sugar variation. One QTL was associated with decreased culls, explaining 4.6% of cull variation. In most cases, newly identified QTL co-locate with those previously described. QTL for increased sugar content frequently-colocated with those for decreased starch and dry matter content, and vice versa. This research represents the first effort in sweetpotato to merge molecular markers with NIRS phenotyping.

Introduction

The proliferation of molecular genetics technologies for plant breeding has opened the door to faster crop improvement, but it also presents new challenges to relate genes to important phenotypic traits. As DNA sequencing and analysis capabilities have improved, breeders in many crops have begun to use quantitative trait loci (QTL) mapping to identify genes linked to traits of importance. Identification of QTL for key traits provides the first stage toward marker-assisted selection (Collard et al. 2005, Shendure and Ji 2008), and marker-assisted breeding is now being implemented in many major crops (Boopathi 2013)

Significant growth in the sweetpotato processing sector has resulted in many changes in the sweetpotato industry, and breeders are now seeking to modify the starch and sugar content of sweetpotato storage roots to meet new industry needs. Previous genome mapping efforts in several crops have identified QTL for various composition traits, including amylose and sugar content. Amylose content has long been an important trait in rice breeding due to its effect on cooking quality. As such, multiple studies have been undertaken in rice and multiple loci for amylose content have been found (Bao et al. 2002, Aluko et al. 2004). Similarly, QTL for sugar content have also been identified in several crops including sugarcane (Ming et al. 2001, Aitken et al. 2006), sweet sorghum (Murray et al. 2008, Ritter et al. 2008), sugarbeet (Weber et al. 2000, Schneider et al. 2002), tomato (Eshed and Zamir 1995, Fridman et al. 2002), and potato (Menendez et al. 2002).

Sweetpotato research teams around the world have developed QTL mapping populations and begun identification of sweetpotato QTL for traits of importance. African researchers have developed populations from regional varieties to map QTL for resistance to

important sweetpotato diseases (Mwanga et al. 2002, Mcharo et al. 2005). Studies have also identified QTL for root-knot nematode resistance (Nakayama et al. 2012). Taiwanese sweetpotato breeders have developed a QTL map for yield traits in populations of Nancy Hall x Tainung 27 (Chang et al. 2009). Earlier work by the NCSU sweetpotato breeding program produced a QTL mapping population from a Tanzania x Beauregard cross, resulting in a sweetpotato linkage map with QTL identified for yield, dry matter, beta-carotene, starch, and root-knot nematode resistance (Cervantes-Flores 2006, Cervantes-Flores et al. 2008a, Cervantes-Flores et al. 2008b, Cervantes-Flores et al. 2011).

Turning the advances in DNA sequencing into improved crops calls for merging the new DNA sequencing technologies with high-throughput phenotyping tools. As a step toward this goal, scientists have begun to integrate near-infrared spectroscopy (NIRS) phenotyping and QTL mapping to facilitate the identification of important loci for composition traits in various crops. NIRS phenotyping has been used to help identify QTL controlling for endosperm color in wheat (Pozniak et al. 2007) and for grain texture in barley (Beecher et al. 2002), and it has become important to selection procedures in many crops including soybean, maize, and wheat (Osborne 2006, Lee et al. 2011).

While NIRS has been used as a breeding tool in many agronomic crops for years, its application in sweetpotatoes is relatively new. To date, research on NIRS phenotyping in sweetpotatoes has focused on measuring starch content and quality traits, nutritional components, and sugar contents. These studies, largely driven by the International Potato Center's Quality and Nutrition Laboratory (Yencho, personal communication; http://www.cipotato.org/qnlab), have produced initial standard curves that are now being

used as phenotyping tools during sweetpotato selection (Katayama et al. 1996, Lu et al. 2006a, Tumwegamire et al. 2011).

As part of the efforts to incorporate next-generation technologies into our sweetpotato breeding program, NIRS phenotyping work to place additional quality traits on the previous sweetpotato QTL map was undertaken (Cervantes-Flores 2006, Cervantes-Flores et al. 2008a, Cervantes-Flores et al. 2008b, Cervantes-Flores et al. 2011). This chapter describes the next phase of QTL mapping efforts in which the TB population was phenotyped using NIRS and the resulting composition data were analyzed to detect QTL associated with these traits.

Materials and Methods

Plant Material

The population used for this study was a full-sib family developed from a cross of Tanzania x Beauregard (mericlone B94-14). These parents were selected due to their diversity in a number of traits (Table 1) with Tanzania being a white-fleshed high dry matter African landrace and Beauregard being an orange-fleshed low dry matter North American cultivar. Two hundred thirty-eight full-sibs were phenotyped for this study (Cervantes-Flores 2006, Cervantes-Flores et al. 2008a, Cervantes-Flores et al. 2008b, Cervantes-Flores 2011). The population was maintained through vegetative propagation in virus-free greenhouses on the NCSU campus in Raleigh, NC since its development.

Field Seasons

During the 2004 growing season, the TB population was grown at the Horticultural Crops Research Station in Clinton, NC (35°01'30" N, 78°16'35" W) and at the Lower Coastal Research Station (35°22'31" N, 77°33'28" W) at Kinston, NC as described previously (Cervantes- Flores 2006). During the 2010 growing season, the population was planted at the Lower Coastal Research Station in Kinston in five plant plots with 30 cm between plants, 1.06 m between rows, and 1.22 meter alleys between plots. Vegetative tip cuttings were transplanted on July 1, 2010.

Harvest, Sample Collection and Processing

Storage root sample collection for composition analysis from the 2004 season was previously described (Cervantes-Flores 2006). The samples were freeze-dried and stored in a freezer at -4°C since processing.

The trial in Kinston 2010 was harvested on October 28-29, 2010 using a two-row chain digger. Storage roots from each plot were picked up by hand, graded into total useable yield and culls (rot, growth cracks, and/or gross mishapes), and weighed in the field. A sample of three US #1 size roots (roots ~4.5 - 8.8 cm diameter and ~7.6 – 22.8 cm long) was collected from each plot for later NIRS analysis. Samples were cured at ~29 °C and 85% RH for one week then stored at ~13 °C and 85% RH in storage rooms at the Horticultural Crops Research Station (HCRS), Clinton, NC. On December 1, 2010, samples were washed and food-processed at HCRS and then stored at -4°C until freeze-drying. Samples were freeze-dried to between 1 and 13% moisture and milled on a Cyclotec 1093 sample mill (FOSS

Hillerød, Denmark) with a 1mm screen.

NIRS Phenotyping

Milled samples were scanned using a FOSS XDS Rapid Content Analyzer (FOSS NIRSystems, Inc, Laurel, MD) NIRS with ISIScan software (Infrasoft International LLC, State College, PA), as described in Chapter 3. Samples (~5 mL) were placed in a quartz bottom cup (part number IH-0386, FOSS NIRSystems, Hillerød, Denmark) and scanned at wavelengths from 400 nm to 2500 nm using 2 nm increments (Infrasoft International 2008, Drapcho, personal communications).

After NIRS scanning, a subset of 10% of each population was selected for analysis by wet chemistry. Using the "Select Samples from Spectra File" function in WinISI software (Infrasoft International LLC, State College PA). This function identified samples with spectra that represented the total range of spectra from the population (Infrasoft International 2005, Drapcho, personal communications).

Chemical Analyses

Amylose content analysis was conducted using an iodine assay similar to that described by Jarvis and Walker (1993). Samples were washed with ethanol, dissolved in NaOH, pH adjusted with citric acid and reacted with Lugol's iodine solution. The absorbance of the blue product was measured on a Perkin-Elmer Lambda Bio20 UV-Vis spec at wavelengths 504, 548, 580, 630, 700, 720, and 800 nm. A standard curve was developed using purified potato amylose from Sigma-Aldrich (A0512) (Sigma-Aldrich, St Louis, MO)

and purified potato amylopectin from Sigma-Aldrich (10118) (Sigma-Aldrich, St Louis, MO). The standard curve obtained using purified potato amylose and amylopectin showed a peak extinction coefficient for amylose of 16.74 at 630 nm and a peak of amylopectin absorbance of 3.07 at 548 nm (Fig 1). Absorbance standard curves for both amylose and amylopectin showed peaks in comparable ranges to those identified by earlier researchers (Jarvis and Walker 1993).

Sugar analysis was conducted with the Megazyme Sucrose, D-Fructose, D-Glucose kit (Megazyme, Wicklow, Ireland). Total starch analysis was done with the Megazyme Total Starch Kit (Megazyme, Wicklow, Ireland). For each trait, protocols provided by Megazyme were followed.

NIRS Model Development

Following wet chemistry, statistical models were developed for each trait of interest using WinISI (Infrasoft International LLC, State College, PA), as described in Chapter 3 (Infrasoft International 2005, Drapcho, personal communications). All models were identical to those described in Chapter 3 except for amylose, for which a new formula was developed due to variation in amylose standards (Sigma-Aldrich product A0512). Protocols for amylose model development using WinISI were as described in Chapter 3 (Table 2). A dry-matter based model was developed using the "Develop Equation with Full Spectrum" option in WinISI. Optimal equations were selected by comparing all combinations of scatter corrections and derivative treatments. The dry-matter based values of all samples were then predicted with the best model using the "Compare Predicted and Reference Values" function

in WinISI.

QTL Identification

Analysis of variance was performed for each trait in JMP 9.0 (SAS Institute, Cary, NC) using the model:

$$Y = \mu + \beta_0 G + \beta_1 G*E + \varepsilon$$

In which Y is the trait of interest, μ is the population mean, $\beta_0 G$ is the effect of genotype, β_1 G*E is the genotype by environment interaction, and ε is the error.

QTL mapping was carried out using QTLCartographer v 2.5_011 (Wang et al. 2012). Genotyping and linkage mapping data was described previously (Cervantes-Flores 2006, Cervantes-Flores et al. 2008a, Cervantes-Flores et al. 2008b, Cervantes-Flores et al. 2011). AFLP markers were used to develop individual linkage maps for Beauregard and Tanzania. The Beauregard map consisted of 726 single-dose AFLPs grouped into 90 linkage groups and the Tanzania map contained 947 single-dose AFLPs joined into 86 linkage groups.

QTL for dry matter, glucose, fructose, sucrose, starch, and amylose were identified using the Composite Interval Mapping (CIM) and Multiple Interval Mapping (MIM) functions in WinQTL Cartographer v2.5_011 (Wang et al. 2012). Correlations within individual traits across environments (0.12 to 0.69) were considered too low for reliable QTL mapping via averaging data across environments (Cervantes-Flores 2006). As such, QTL analysis was performed on traits within individual environments. Initial analysis with CIM was used to screen for possible QTL. Due to the highly-heterozygous polyploid nature of the genome and the use of single dose markers for QTL analysis, the cross was analyzed as a B2

cross (ie. a backcross to parent 2). Model 6 in QTL Cartographer was used with the 'Forward and Backward Regression' option. The default setting of five background markers and a window size of 10 cM were used. Initial CIM threshold levels were set using the permutation test function with 500 permutations. Following CIM analysis, MIM was used to further investigate all traits. Initial MIM models were created using peaks from CIM analysis ≥ 2.0 LOD in instances where the peak was not located on the end of the linkage group and ≥ 2.5 LOD where the peak was on the end of the linkage group. Initial models were optimized using the "Optimize model" option and initial QTL were tested for significance. Criteria were set for a minimum distance of 5.0 cM between QTL and a score statistic equivalent to $p<0.05$ (Robertson-Hoyt et al. 2006, Silva et al. 2012). The "Refine Model" option was used to detect further main QTL, identify interactions, and perform 1-dimensional genome scans to identify any additional QTL with epistatic interactions with identified QTL, and test the significance of QTL using the score test option. Addition of QTL was continued until no more QTL or interactions could be added which met the above criteria.

QTL map images were created using MapChart v2.2 (Voorips 2002). To simplify trait interpretation, traits were coded as in the following example:

FWStarch10K

FW (fresh weight basis vs. dry weight basis)
Starch (biochemical component or phenotypic trait)
10K (environment: Kinston NC 2010 (10K), Kinston NC 2004 (04K), Clinton NC 2004 (04C))

Results

NIRS model

As described in Chapter 3, most variables were accurately predicted using NIRS, but each prediction model required unique scatter correction and derivative treatments for model optimization (Table 2). R^2 value, which is the percent variation explained by the model, and relative percent difference, which is the ratio of the measurement error to the samples standard deviation, were used to determine accuracy of the NIRS models. Amylose was predicted with $R^2 = 0.82$ and RPD = 1.62.

Correlations

Pearson correlation coefficients (r) were calculated for all combinations of traits using individual plot data (Table 3). A comparison of fresh weight and dry weight basis values for individual traits indicated strong positive correlations in all traits (r >0.86). Among sugar traits, glucose and fructose showed strong positive correlations with each other on both a fresh weight (r =0.97) and dry weight basis (r =0.98). Sucrose was negatively correlated with the reducing sugars glucose and fructose. Dry weight basis sucrose had weak negative correlations with the reducing sugars (r =-0.14 and r =-0.11), while fresh weight basis sucrose showed significant negative correlations with the reducing sugars (r =-0.46 and r =-0.51). Individual traits exhibited significant positive correlations across environments in all cases (data not shown). On a dry weight basis, starch content was negatively correlated with all sugar content traits: fructose (r=-0.53), glucose (r=-0.56), sucrose (r=-0.44), and total sugars (r=-0.80). Dry matter content showed positive correlations with both fresh weight

basis and dry weight basis starch content, although correlations with fresh weight basis starch content (r =0.83) were higher than those with dry weight basis starch content (r =0.61). Amylose showed positive correlations with both percent amylose (r =0.58) and starch (r =0.75 and r =0.88).

Phenotypic distributions

Phenotypic distributions were calculated for all traits (Figure 2) using LSMeans for clones. Distributions for individual traits ranged from normally distributed to highly skewed. Yield, dry matter and starch traits tended to have normal to nearly normal distributions, although starch was slightly skewed to the right. Reducing sugars (glucose and fructose) tended to be highly skewed distributions with long tails, indicating that most samples had very little to no reducing sugars and a few had high concentrations of reducing sugars. Sucrose and total sugars tended to have nearly normal distributions, although slightly skewed to the left. Amylose and percent amylose tended to be nearly normal distributions but slightly skewed toward higher concentrations. All traits exhibited transgressive segregation. Individual traits on a fresh weight and dry weight basis tended to have similar distributions.

QTL Identification

QTL were identified for glucose, sucrose, fructose, total sugars, and starch on both a dry weight and wet weight basis as well as for amylose (dry weight basis) and percent amylose (amylose / starch) in each of three environments. QTL for total marketable yield (TMY) (defined as roots that did not show rot, severe growth cracks, or gross misshapes that

would interfere with industrial processing), total yield, cull weight, and percent culls (cull weight / total yield) were identified from the population grown at LCRS, Kinston, NC in 2010. ANOVAs for composition traits (Table 4) showed starch and dry matter traits had significant genetic and genotype by environment effects, while sucrose had only a significant GxE effect, and reducing sugars had no significant GxE component. 149 QTL spread across 44 linkage groups were identified for individual traits within individual environments in the Beauregard genome map (Table 5, Appendix C) while 108 QTL across 44 linkage groups were found for individual traits in individual environments in the Tanzania map (Table 6, Appendix C). Furthermore, 30 significant additive x additive interactions were found between loci in the Beauregard map and 16 significant interactions were found in the Tanzania map (Table 7a, 7b).

While numerous QTL were found for individual traits within individual environments (Table 5, Table 6, Appendix C), they were considered reliable (Table 8a, Table 8b) if QTL for an individual trait, or for multiple closely related traits, were co-localized across multiple environments. QTL which appeared across multiple environments were merged into an integrated genome map to reduce spurious QTL and produce a more comprehensible picture of sweetpotato genetic architecture (Figure 3, Figure 4).

In the Beauregard map, 14 QTL were identified for starch, reducing sugar, and sugar content, and 1 QTL was identified for yield (Figure 3, Table 8a). Linkage group (LG) B01.03 contained QTL that lowered starch content and increased reducing sugar content. LGs B04.23, B05.25, B11.62, B12.70, and B13.75 all contained QTL that lowered starch content and increased sugar content. Linkage groups B11.61 and B11.64 contained QTL that

increased reducing sugars, while LG B13.73 contained a QTL that decreased yield. Together these QTL explained 27.8% of total sugar variation, 37.6 % of total variation in starch and dry matter, and 4.8% of yield variation. No QTL interactions appeared across multiple environments (Table 7a).

In the Tanzania map, 8 QTL were identified for starch and sugar content and 1 was identified for cull yield (Figure 4, Table 8b). Linkage groups T01.05 and T01.06 contained QTL that increased starch content and decreased sugar content, LG T03.15 contained a QTL that increased sugar content, and LG T07.40 contained a QTL that decreased starch content and increased reducing sugar content. In comparison, LG T72 contained a QTL that decreased sugar content, while LG T07.41 contained a QTL that decreased culls. Together these QTL explain 19.3% of sugar variation, 16.6% of total starch and dry matter variation, and 4.6% of cull variation. No significant QTL interactions were found across environments (Table 7b).

Discussion

In recent years, researchers around the world have made efforts to identify QTL for traits of importance in sweetpotato (Mwanga et al. 2002, Mcharo et al. 2005, Cervantes-Flores et al. 2008a, 2008b, 2011, Chang et al. 2009, Nakayama et al. 2012) and begun efforts to use NIRS for screening of quantitative traits in sweetpotato (Katayama et al. 1996, Lu et al. 2006a, Lu et al. 2006b). This work further develops previous work (Cervantes-Flores 2006, Cervantes-Flores et al. 2008a, Cervantes-Flores et al. 2008b, Cervantes-Flores et al. 2011) and to the best of our knowledge, this is the first publication to apply NIRS

phenotyping to identify sweetpotato QTL and also the first work to identify QTL associated with sugar content in sweetpotato.

The TB mapping population was grown in 3 environments (Clinton, NC, 2004; Kinston, NC, 2004; and Kinston, NC, 2010) and samples from each environment were phenotyped for yield and composition traits. Phenotyping and QTL analysis of yield, starch, dry matter and β-carotene for the 2004 season were previously described by Cervantes-Flores (2006) and Cervantes-Flores, et al. (2011); however, they are discussed here for purposes of comparison with the new data.

Significant positive correlations were found between starch and dry matter content, in agreement with previous research (Hall and Smittle 1983, Cervantes-Flores et al. 2011). This correlation is likely because starch forms one of the largest components of sweetpotato dry matter content. Close correlations were also found between glucose and fructose, which is in agreement with previous knowledge that these reducing sugars are produced by the breakdown of sucrose in carbon sinks (Fernie et al. 2002). A negative correlation was found between sucrose and the two reducing sugars, this correlation is also in agreement with the biochemical breakdown of sucrose resulting in the formation if glucose and fructose and it suggests opportunities to breed for lower reducing sugars and higher sucrose content simultaneously. All sugar traits were negatively correlated with starch content, in agreement with earlier biochemical studies showing that once carbon is transported to a sink in the form of sucrose, subsequent biochemical pathways can result in production of either starch or sugar (Fernie et al. 2002).

In our earlier QTL studies, dry matter was determined via oven drying and starch

content was determined by the Megazyme Total Starch Kit for all samples (Megazyme International, Wicklow, Ireland) (Cervantes-Flores 2006). During our NIRS studies, these traits were analyzed again on the same populations using our NIRS standard curves. The correlations between the two analyses of these four traits (Dry matter Clinton 2004, Dry matter Kinston 2004, Starch content Clinton 2004, and Starch content Clinton 2004) showed significant positive correlations of between 0.65 and 0.76, with the population from Kinston showing slightly higher correlations for both traits. Considering differences in methodologies and the potential for sample degradation, we consider this to be a close correlation. However, the high GxE influence on some traits (Table 4) may have been caused by sample degradation.

The QTL identified in this chapter often colocalized with those identified previously. In "Beauregard", QTL for starch and dry matter on LGs B01.03, B04.23, B11.62, and B12.70 discovered with NIRS correlated with those found previously (Figure 3). QTL that lower starch content frequently co-located with those that increased sugar content (LGs B01.03, B04.23, B11.62, B12.70, and B13.75). Furthermore, on LGs B05.25 and B11.61, QTL that affected either sugar content or reducing sugar content appeared to be close to those affecting starch content (Cervantes-Flores 2006, Cervantes-Flores et al. 2011). Combining these results with previous knowledge of the biochemical pathways associated with carbon sinks suggests that these QTL may be enzymes in the cytoplasmic pathways that determine whether photosynthate is transferred to the amyloplast for conversion into starch or remains in the cytoplasm as sugars (Fernie et al. 2002).

On the Tanzania map, QTL correlations were also found relating sugars and starch to

each other and to yield (Figure 4). The linkage group T01.05 was shown to have a QTL that increased starch and decreased sugar content, which coincided with those found by Cervantes-Flores (2006) that increased dry matter and decreased yield. QTL that decreased sugar content colocalized with those that decreased yield (Cervantes-Flores 2006) on LGs T01.05 and T72, while on T07.40, QTL that increased reducing sugars and yield (Cervantes-Flores 2006) colocalized. On T07.41, a newly discovered QTL for decreased culls appeared near a previously discovered QTL for increased yield (Cervantes-Flores 2006).

Our sweetpotato genetic map research has located numerous QTL derived from both Tanzania and Beauregard. In many cases, QTL that affect various, seemingly unrelated traits overlap. Lower sugar content is often associated with higher dry matter (especially in Beauregard), and higher sugar content is sometimes associated with higher yield (especially in Tanzania). There are multiple possible explanations for these QTL correlations on the Beauregard and Tanzania maps. QTL associated with sugar content from Beauregard may represent genes associated with the processing of sugar into starch in the carbon sink (Fernie et al. 2002). Due to our reliance on single copy markers for QTL identification, markers and associated QTL, which may be present in multiple copies within a parent, will not appear in our map. Tanzania may have multiple copies of sucrose-to-starch genes and the single-copy QTL associated with sugar content in Tanzania, and specific alleles may be responsible for converting the products of photosynthesis into yield in North Carolina. The correlations between yield and storage root sugar content may also be caused by overall genotype adaptation due to the fact that Beauregard was selected in North America to meet the local needs of high yield and sweet taste while Tanzania was selected for low sweetness and

adaptation to a much different environment. Alternatively, they may be caused by physiology since photosynthate in the storage root may be funneled to either starch, thereby increasing dry matter, or cell division, thereby increasing yield. While we do not have definitive evidence of the mechanisms underlying these correlated QTL, we propose that they form parts of pathways associated with sugar related enzymes that allocate photosynthate along various possible pathways within carbon sinks. Photosynthate (in the form of sucrose) is transported to carbon sinks such as sweetpotato roots, from there it can remain as sugars in the cytoplasm, be transported to amyloplasts to serve as starch, or be used in cell division and storage root growth (Frommer and Sonnewald 1994).

This research demonstrates both the advantages and limitations of our laboratory's current stage of development with NIRS phenotyping tools. Development of NIRS phenotyping tools in our program to date has consisted of developing initial standard curves and prediction equations for several traits of importance to sweetpotato breeding in the US. Our standard curves for all the traits in this research project have acceptable R^2, and standard errors: SEP (standard error of prediction), SEC (standard error of calibration), and SECV (standard error of cross-validation) values to play a role in selection in our breeding program. Other sweetpotato breeding teams around the world have developed NIRS standard curves with somewhat better scores due to their more comprehensive systems and higher numbers of scanned samples (Katayama et al. 1996, Lu et al. 2006a, 2006b, Zum Felde et al. 2009). However, the RPD (standard deviation / SEP) values for several traits are relatively low for the fine scale phenotyping needed for QTL mapping. With RPD values ranging from 1.62 – 3.28, our NIRS phenotyping capabilities at this stage have only a rough screening capability

(Hurburgh and Igne 2010). The low resolution power of the NIRS system combined with the possibility of sample degradation over time limits our ability to identify QTL for many traits, especially those that may have only a small effect on phenotype or for traits that may have minimal differences between the two parents (Table 1). As our NIRS models improve through scanning and wet chemistry of more samples and improved protocols, we expect these results to improve, allowing NIRS to play an increased role in both our applied breeding and basic research. Considering the nature of the two parents: highly diverse with one parent, Tanzania, poorly adapted to North Carolina; the use of AFLPs, which do not readily transfer between populations; and the complexity of the sweetpotato genome; it is unlikely that MAS will play a significant role in our applied breeding in the near future. However, the valuable role MAS is playing in other crops suggests that basic research toward developing a viable MAS system for sweetpotatoes may be a worthwhile endeavor.

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Table 1: LSMeans for phenotypic values of each parent: Beauregard and Tanzania. Yield trait data was collected at Kinston, NC in 2010. Composition traits show ranges obtained from environments at Clinton NC 2004, Kinston NC 2004, and Kinston NC 2004. Composition traits are shown on both a fresh weight (FW) basis and a dry weight (DW) basis.

Trait	**Beauregard**	**Tanzania**
Total Yield (lbs/ plot)	22.45	3.86
Total Marketable Yield (lbs/ plot)	20.87	3.13
Culls (lbs/ plot)	1.57	0.73
% Culls (% Total Yield)	6.48	17.02
DM (NIRS estimate) (% FW)	15.38 - 21.28	23.86 - 28.65
DW Fructose (g/ 100g DW)	1.64 - 4.7	0.0 - 0.06
DW Glucose (g/ 100g DW)	1.81 - 5.82	0.0 - 0.09
DW Sucrose (g/ 100g DW)	4.77 - 13.89	5.06 - 12.45
DW Sugar (g/ 100g DW)	9.64 - 25.56	4.5 - 11.6
DW Starch (% DW)	36.25 - 57.89	52.05 - 66.89
FW Fructose (g/ 100g FW)	0.34 - 0.72	0.0 - 0.01
FW Glucose (g/ 100g FW)	0.37 - 0.89	0.0 - 0.02
FW Sucrose (g/ 100g FW)	1.02 - 2.14	1.42 - 2.96
FW Sugar (g/ 100g DW)	1.98 - 3.92	1.26 - 2.76
FW Starch (% FW)	5.59 - 12.38	12.43 - 18.36
Amylose Content (% DW)	9.8 - 14.45	14.09 - 18.73
% Amylose (% Starch)	23.48 - 27.09	27.07 - 28.65

Table 2: Statistical summary of NIRS prediction model for amylose developed using WinISI. For all other traits refer to Table 2, Chapter 3. Identifiers are: *N*: number of samples used to make the calibration equation. *Mean*: mean of samples. *SD*: standard deviation of samples. *Min.*: estimated minimum. *Max*: estimated maximum. *SEC*: standard error of calibration. R^2: R^2 value of equation. *SECV*: standard error of cross-validation. *1-VR*: coefficient of determination for cross-validation. *Wave*: number of wavelengths used to make calibration equation. *Bias*: prediction bias of equation vs. lab data. *SEP(C)*: standard error of prediction corrected for bias. *RPD*: SD / SEP(C). *PreTreat*: scatter correction applied to equation. *Deriv*: derivative treatment applied to equation. IMSC: inverse multiplicative scatter correction. Scatter and derivative treatments were made using options available in WinISI v.4 software as described in Chapter 3.

Amylose Complete Model														
N	**Mean**	**SD**	**Min**	**Max**	**SEC**	**R^2**	**SECV**	**1-VR**	**wave**	**bias**	**SEP(C)**	**RPD**	**PreTreat**	**Deriv.**
227	14.3	4.5	0.7	28.0	1.94	0.82	2.15	0.77	256	-1.29	2.80	1.62	IMSC	2441

Amylose Calibration Equation									**Amylose Validation Set**		
N	**Mean**	**SD**	**Est. Min**	**Est. Max**	**SEC**	**R^2**	**SECV**	**1-VR**	**RSQ:**	**SEP(C):**	**N**
192	14.1	4.8	0	28.4	2.1	0.80	2.28	0.77	0.57	2.49	36

Table 3: Pearson correlation coefficients for LSMeans of traits calculated on genotypic values across three environments (Clinton 2004, Kinston 2004, and Kinston 2010). DM = dry matter (% fresh weight). DWGluc = dry weight basis glucose (g/100g dry weight). DWFruc = dry weight basis fructose (g/100g dry weight). DWSuc = dry weight basis sucrose (g/100g dry weight). DWSug = dry weight basis total sugars (glucose + fructose + sucrose). DWStarch = dry weight basis starch (% dry weight). FWGluc = fresh weight basis glucose (DWGluc / (100-DM)). FWFruc = fresh weight basis fructose (DWFruc / (100-DM)). FWSuc = fresh weight basis sucrose (DWSuc / (100-DM)). FWSug = fresh weight basis sugars (DWSug / (100-DM)). FWStarch = fresh weight basis starch (DWStarch / (100-DM)). Am = amylose (% dry sweetpotato sample). PerAm = percent amylose (Am / DWStarch).

	Yield	**DM**	**DWGluc**	**DWFruc**	**DWSuc**	**DWSug**	**DWStarch**
DM	-0.18**						
DWGluc	0.25***	-0.61***					
DWFruc	0.24***	-0.63***	0.98***				
DWSuc	0.18**	-0.22***	-0.11	-0.14*			
DWSug	0.29***	-0.66***	0.71***	0.69***	0.56***		
DWStarch	-0.02	0.61***	-0.56***	-0.53***	-0.44***	-0.80***	
FWGluc	0.25***	-0.55***	0.98***	0.96***	-0.16**	0.67***	-0.51***
FWFruc	0.24***	-0.58***	0.96***	0.98***	-0.19**	0.65***	-0.48***
FWSuc	0.09	0.19**	-0.46***	-0.50***	0.86***	0.18**	-0.08
FWSug	0.27***	-0.40***	0.51***	0.47***	0.62***	0.90***	-0.66***
FWStarch	-0.09	0.83***	-0.67***	-0.67***	-0.35***	-0.81***	0.88***
Am	-0.12*	0.72***	-0.60***	-0.61***	-0.33***	-0.71***	0.75***
PerAm	-0.14*	0.47***	-0.27***	-0.31***	-0.03	-0.20***	0.05

Table 3 continued.

	FWGluc	FWFruc	FWSuc	FWSug	FWStarch	Am
DM						
DWGluc						
DWFruc						
DWSuc						
DWSug						
DWStarch						
FWGluc						
FWFruc	0.97***					
FWSuc	-0.46***	-0.51***				
FWSug	0.52***	0.48***	0.42***			
FWStarch	-0.61***	-0.62***	0.10	-0.57***		
Am	-0.55***	-0.55***	0.08	-0.48***	0.88***	
PerAm	-0.24***	-0.29***	0.17**	-0.02	0.31***	0.58***

Table 4: Analysis of variance tables for composition traits estimated using NIRS for the TB population. The model includes clone effect and clone* environment effect.

DM

Source	DF	MS	F	P-value
Full Model	661	13.7313	8.7546	<.0001
Clone	158	12.8609	8.1996	<.0001
Clone * Env	404	10.3187	6.5788	<.0001
Error	496	1.5685		

DWFruc

Source	DF	MS	F	P-value
Full Model	661	1.527	1.4524	<.0001
Clone	157	1.6264	1.547	0.0002
Clone * Env	404	0.7943	0.7555	0.9983
Error	496	1.0514		

DWGluc

Source	DF	MS	F	P-value
Full Model	661	2.5044	1.5191	<.0001
Clone	157	2.7166	1.6478	<.0001
Clone * Env	404	1.3637	0.8272	0.9767
Error	496	1.6486		

DWStarch

Source	DF	MS	F	P-value
Full Model	661	93.3879	5.0474	<.0001
Clone	157	31.3822	1.6961	<.0001
Clone * Env	404	119.8149	6.4758	<.0001
Error	496	18.5021		

DWSuc

Source	DF	MS	F	P-value
Full Model	661	24.408	3.5037	<.0001
Clone	157	7.2508	1.0408	0.3698
Clone * Env	404	31.6361	4.5413	<.0001
Error	496	6.9663		

DWSug

Source	DF	MS	F	P-value
Full Model	661	36.0934	2.7483	<.0001
Clone	157	17.3062	1.3178	0.0139
Clone * Env	404	39.5135	3.0087	<.0001
Error	496	13.133		

Table 4 continued.

FWFruc

Source	DF	MS	F	P-value
Full Model	661	0.0537	1.5116	<.0001
Clone	157	0.0671	1.8868	<.0001
Clone * Env	404	0.0311	0.8752	0.9191
Error	496	0.0356		

FWGluc

Source	DF	MS	F	P-value
Full Model	661	0.0891	1.5845	<.0001
Clone	157	0.1137	2.0234	<.0001
Clone * Env	404	0.053	0.9428	0.7314
Error	496	0.0562		

FWStarch

Source	DF	MS	F	P-value
Full Model	661	14.8354	4.753	<.0001
Clone	157	7.709	2.4698	<.0001
Clone * Env	404	17.6913	5.668	<.0001
Error	496	3.1212		

FWSuc

Source	DF	MS	F	P-value
Full Model	661	0.996	2.911	<.0001
Clone	157	0.3389	0.9904	0.5208
Clone * Env	404	1.2419	3.6296	<.0001
Error	496	0.3422		

Am

Source	DF	MS	F	P-value
Full Model	661	9.3366	4.2261	<.0001
Clone	157	4.8533	2.1968	<.0001
Clone * Env	404	11.2899	5.1103	<.0001
Error	496	2.2093		

FWSug

Source	DF	MS	F	P-value
Full Model	661	1.1129	2.7749	<.0001
Clone	157	0.5225	1.3027	0.0175
Clone * Env	404	1.3392	3.3392	<.0001
Error	496	0.4011		

Table 4 continued.

		PerAm		
Source	DF	MS	F	P-value
Full Model	661	7.1175	2.8646	<.0001
Clone	158	7.7877	3.1344	<.0001
Clone * Env	404	5.2141	2.0985	<.0001
Error	496	2.4846		

Table 5: QTL identified in Beauregard genome map using multiple interval mapping in QTL Cartographer. QTL were identified with specific environments (Clinton NC 2004, Kinston NC 2004, and Kinston NC 2010). The trait column shows the phenotypic trait within an individual environment. LG and position identify the location of the QTL within the genome. Linkage groups are the same as those described earlier (Cervantes-Flores et al. 2006, Cervantes-Flores et al. 2008b). Effect shows if the QTL increased or decreased the value of the trait. P-Value and % Variation explained show the likelihood of the QTL and the percent phenotypic variation it explains for the trait. QTL are grouped by the trait they affect.

Trait	LG	Position (cM)	Effect	P-Value	% Variation Explained
Amylose Content					
Am04C	B03.15	19.3	+	0.009	3.62
Am04C	B04.23	0	-	<0.0001	7.89
Am04C	B11.62	3.7	-	<0.0001	5.16
Am04K	B04.23	0	-	0.01	4.67
Am04K	B09.54	0	+	<0.0001	8.86
Am04K	B11.62	0	-	0.003	7.5
Am04K	B12.70	47.4	-	<0.0001	8.12
Am10K	B01.03	18.3	-	0.012	4.21
Am10K	B12.70	36.5	-	0.014	6.4
Percent Amylose					
PerAm04C	B03.13	7.6	+	<0.0001	4.27
PerAm04C	B03.15	15.3	+	<0.0001	7.55
PerAm04C	B04.23	0	-	<0.0001	7.35
PerAm04C	B05.29	5.7	-	0.007	4.92
PerAm04C	B11.62	3.7	-	<0.0001	5.41
PerAm04K	B02.09	4.8	+	0.017	6.54
PerAm04K	B05.25	39.5	-	<0.0001	8.71
PerAm04K	B05.25	86.9	+	0.012	6.17
PerAm04K	B12.71	6.9	+	<0.0001	6.75
PerAm10K	B08.43	38.5	-	0.024	2.9
PerAm10K	B09.49	34.2	-	<0.0001	6.88
PerAm10K	B14.81	8	-	0.041	4.47

Table 5 continued.

Fructose					
DWFruc04C	B01.03	17.1	+	<0.0001	3.73
DWFruc04C	B11.61	42.1	+	<0.0001	3.66
DWFruc04C	B11.64	49.2	+	<0.0001	0.99
DWFruc04C	B12.67	35.1	+	<0.0001	3.44
DWFruc04C	B13.76	0.1	+	<0.0001	2.54
DWFruc04K	B03.15	8.1	-	<0.0001	4.81
DWFruc04K	B03.16	12.1	+	0.25	-0.18
DWFruc04K	B03.16	51.2	+	0.011	1.97
DWFruc04K	B11.61	42.1	+	<0.0001	1.44
DWFruc04K	B12.70	40.1	+	<0.0001	3.82
DWFruc04K	B15.87	58.1	-	0.013	7.44
DWFruc10K	B11.61	42.1	+	0.001	3.57
DWFruc10K	B11.64	49.2	+	0.001	2.49
DWFruc10K	B13.75	10.1	+	0.001	4.27
FWFruc04C	B01.03	17.1	+	0.026	3.74
FWFruc04C	B11.61	42.1	+	<0.0001	3.98
FWFruc04C	B11.64	49.2	+	<0.0001	1.09
FWFruc04C	B12.67	35.1	+	0.002	3.71
FWFruc04C	B13.76	0.1	+	<0.0001	2.77
FWFruc04K	B03.15	8.1	-	<0.0001	6.07
FWFruc04K	B04.22	27.1	+	0.025	0.02
FWFruc04K	B08.45	7.2	-	<0.0001	1.01
FWFruc04K	B11.61	42.1	+	<0.0001	2.3
FWFruc04K	B13.73	5.2	-	<0.0001	2.68
FWFruc04K	B15.87	58.1	-	0.003	6.9
FWFruc10K	B05.27	33.2	+	0.005	5.88
FWFruc10K	B11.61	42.1	+	0.002	4.37
FWFruc10K	B11.64	49.2	+	<0.0001	3.27
FWFruc10K	B12.69	12.1	+	0.013	2.56

Table 5 continued.

Glucose					
DWGluc04C	B01.03	17.1	+	<0.0001	3.76
DWGluc04C	B11.61	42.1	+	<0.0001	4.27
DWGluc04C	B11.64	49.2	+	0.022	0.73
DWGluc04C	B12.67	35.1	+	0.001	4.39
DWGluc04K	B03.15	8.1	-	<0.0001	4.46
DWGluc04K	B11.61	42.1	+	0.01	2.76
DWGluc04K	B12.70	14.1	+	0.001	0.76
DWGluc04K	B13.75	0.1	+	0.009	3.06
DWGluc10K	B11.61	59.4	+	0.009	10.98
DWGluc10K	B11.64	61.1	+	<0.0001	8.42
DWGluc10K	B11.66	23.6	-	<0.0001	5
DWGluc10K	B12.69	12	+	0.047	3.74
DWGluc10K	B13.75	9.4	+	<0.0001	4.48
FWGluc04C	B01.03	17.1	+	<0.0001	3.74
FWGluc04C	B11.61	42.1	+	<0.0001	4.39
FWGluc04C	B11.64	49.2	+	0.02	0.73
FWGluc04C	B12.67	32.1	+	0.002	4.12
FWGluc04K	B03.15	8.1	-	<0.0001	4.62
FWGluc04K	B12.70	14.1	+	<0.0001	0.72
FWGluc04K	B13.75	0.1	+	<0.0001	2.87
FWGluc04K	B15.87	51.1	-	<0.0001	0.79
FWGluc10K	B05.27	33.2	+	0.016	5.24
FWGluc10K	B11.61	42.1	+	0.004	3.14
FWGluc10K	B11.64	49.2	+	0.018	2.51
FWGluc10K	B11.66	14.1	-	<0.0001	3.96
Dry matter					
NIRDM04C	B04.23	2	-	<0.0001	8.34
NIRDM04C	B06.36	31	+	0.002	3.38
NIRDM04C	B07.39	5.6	+	<0.0001	5.8
NIRDM04K	B02.07	60.1	-	0.007	6.81
NIRDM04K	B04.23	0	-	<0.0001	4.82
NIRDM04K	B05.25	14	-	0.007	14.68
NIRDM04K	B11.62	0	-	<0.0001	12.31
DM10K	B05.26	30.3	+	0.001	3.95
DM10K	B10.59	10.7	-	0.005	8.1
DM10K	B12.70	47.4	-	0.013	4.89
DM10K	B13.75	2	-	0.001	5.81
DM10K	B13.76	0.1	-	0.016	3.03

Table 5 continued.

Starch					
DWStarch04C	B01.02	23	+	0.019	6.12
DWStarch04C	B01.06	0	+	0.038	3.61
DWStarch04C	B13.74	2	-	0.003	4.44
DWStarch04K	B11.62	2.1	-	0.001	3.66
DWStarch04K	B12.70	40.1	-	0.002	7.21
DWStarch10K	B01.03	17.1	-	0.019	4.4
DWStarch10K	B05.25	26.1	-	0.897	2.37
DWStarch10K	B11.62	2.1	-	0.135	4.17
DWStarch10K	B13.75	0.1	-	0.119	4.84
FWStarch04C	B03.16	54.6	+	0.019	3.09
FWStarch04C	B04.23	4	-	0.001	5.62
FWStarch04C	B12.70	47.4	-	0.002	4.29
FWStarch04K	B04.21	13.2	+	0.006	4.17
FWStarch04K	B05.25	30.3	-	<0.0001	8.74
FWStarch04K	B11.62	3.7	-	<0.0001	11.87
FWStarch04K	B12.70	45.4	-	<0.0001	9.33
FWStarch04K	B89	4.1	+	0.047	2.56
FWStarch10K	B10.59	8.7	-	0.029	5.3
FWStarch10K	B12.70	45.4	-	0.001	4.93
FWStarch10K	B13.75	0	-	<0.0001	5.91
Sucrose					
DWSuc04C	B05.29	9.7	-	0.013	5.83
DWSuc04C	B12.67	35	-	0.005	5.75
DWSuc04K	B05.25	71.3	+	<0.0001	5.87
DWSuc10K	B05.25	31.1	+	<0.0001	3.71
DWSuc10K	B11.61	42.1	-	0.001	4.74
DWSuc10K	B11.62	2.1	+	<0.0001	6.06
DWSuc10K	B12.70	0.1	+	0.253	0.16
DWSuc10K	B13.73	13.1	-	0.112	2.29
FWSuc04C	B02.07	49.6	+	0.007	4.63
FWSuc04C	B12.67	35	-	0.003	5.27
FWSuc04K	B05.25	84.9	+	0.032	5.82
FWSuc04K	B11.61	12.4	+	0.006	3.9
FWSuc10K	B05.25	56.5	+	0.015	3.47
FWSuc10K	B06.33	37.2	+	0.015	5.14
FWSuc10K	B11.61	57.4	-	<0.0001	8.11
FWSuc10K	B11.65	55.6	+	0.003	7.35

Table 5 continued.

Total Sugar					
DWSug04C	B05.25	32.3	+	<0.0001	5.41
DWSug04C	B15.86	50.9	+	0.01	4.44
DWSug04K	B04.23	0.1	+	0.008	5.02
DWSug04K	B05.25	60.1	+	0.022	3.76
DWSug04K	B06.36	31.1	-	<0.0001	4.37
DWSug04K	B07.37	0.1	+	0.003	4.47
DWSug04K	B11.62	0.1	+	0.001	6.03
DWSug04K	B15.88	5.1	-	0.034	-0.06
DWSug10K	B04.23	0.1	+	0.268	1.22
DWSug10K	B05.25	31.1	+	0.019	2.27
DWSug10K	B11.62	0.1	+	0.047	4.93
DWSug10K	B13.73	13.1	-	0.039	4.34
DWSug10K	B13.75	10.1	+	0.145	3.96
FWSug04C	B05.25	44.1	+	0.049	3.88
FWSug04C	B15.86	50.9	+	0.005	4.39
FWSug10K	B05.25	53.1	+	0.155	0.77
FWSug10K	B05.28	10.1	-	0.062	4.07
FWSug10K	B11.62	0.1	+	0.003	4.55
FWSug10K	B13.73	13.1	-	0.016	3.77
Yield					
TMY10K	B15.86	52.9	-	0.004	5.33
TMY10K	B89	4.1	-	0.004	3.44
TY10K	B13.73	14.7	-	0.002	5.2
TY10K	B15.88	15.9	+	0.002	4.67
Culls10K	B04.21	53.3	-	<0.0001	2.92
PerCulls10K	B08.46	50.7	+	0.013	2.9
TMY10K	B13.73	14.7	-	0.002	4.3

Table 6: QTL identified in Tanzania genome map using multiple interval mapping in QTL Cartographer. QTL were identified with specific environments (Clinton NC 2004, Kinston NC 2004, and Kinston NC 2010). The trait column shows the phenotypic trait within an individual environment. LG and position identify the location of the QTL within the genome. Linkage groups are the same as those described earlier (Cervantes-Flores et al. 2006, Cervantes-Flores et al. 2008b). Effect shows if the QTL increased or decreased the value of the trait. P-Value and % Variation explained show the likelihood of the QTL and the percent phenotypic variation it explains for the trait. QTL are grouped by the trait they affect.

Trait	LG	Position (cM)	Effect	P-Value	% Variation Explained
Amylose Content					
Am04C	T05.28	22.1	+	0.005	3.63
Am04C	T06.32	65.4	+	0.027	2.2
Am04K	T02.07	72	-	0.035	4.76
Am04K	T04.20	35.1	-	0.009	5.76
Am04K	T07.40	45	-	0.001	7.01
Am10K	T01.05	21.1	+	0.001	2.9
Percent Amylose					
PerAm04C	T05.28	22.1	+	<0.0001	5
PerAm10K	T07.40	75.4	-	<0.0001	4.56
PerAm10K	T07.42	39.6	+	0.013	5.55
PerAm04C	T12.67	61.6	-	0.014	2.42
PerAm04K	T13.76	58.2	+	0.008	5.67
PerAm04K	T77	26	+	0.008	4.34

Table 6 continued.

Fructose					
DWFruc04C	T01.05	24.8	-	0.008	4
DWFruc04C	T05.25	14.1	-	<0.0001	4.24
DWFruc04K	T03.15	39.1	+	<0.0001	8.34
DWFruc04K	T03.18	12.2	-	<0.0001	1.9
DWFruc10K	T01.05	28.1	-	<0.0001	4.76
DWFruc10K	T02.10	35.1	+	<0.0001	4.14
DWFruc10K	T07.40	48.1	+	<0.0001	1.26
FWFruc04C	T01.05	21	-	0.008	4.1
FWFruc04K	T03.15	39.1	+	<0.0001	10.45
FWFruc04K	T03.18	74.1	+	0.05	2.73
FWFruc04K	T12.70	0.1	-	<0.0001	2.37
FWFruc10K	T01.05	28.1	-	<0.0001	4.19
FWFruc10K	T02.10	35.1	+	<0.0001	4.78
FWFruc10K	T07.40	54.2	+	0.006	2.71
Glucose					
DWGluc04K	T01.03	70.2	+	0.001	3.9
FWGluc04C	T01.05	24.9	-	0.007	4.97
DWGluc10K	T01.05	28.1	-	<0.0001	5.37
FWGluc10K	T01.05	31.2	-	<0.0001	3.93
DWGluc04K	T02.07	16.1	+	<0.0001	3.44
DWGluc10K	T02.10	35.1	+	<0.0001	4.59
FWGluc10K	T02.10	35.1	+	<0.0001	5.14
DWGluc04K	T03.15	36.1	+	0.001	4.36
FWGluc04K	T03.15	40.7	+	0.001	6.49
DWGluc04C	T06.36	40.8	-	0.002	3.68
DWGluc10K	T07.40	48.1	+	<0.0001	1.37
FWGluc10K	T07.40	48.1	+	0.001	1.07
FWGluc04C	T08.47	58.6	+	0.007	3.35
DWGluc04C	T72	17.1	-	<0.0001	4.21
FWGluc04C	T72	17.1	-	0.011	4.25
FWGluc04C	T83	26	-	<0.0001	8.1
Dry matter					
NIRDM04K	T01.05	18.1	+	<0.0001	4.6
NIRDM04K	T02.07	49.1	-	<0.0001	3.79
NIRDM04C	T03.13	44.6	+	0.032	2.75
NIRDM04C	T04.20	47.8	-	0.005	4.38
NIRDM04K	T07.40	48.1	-	<0.0001	7.5
NIRDM04K	T11.61	49.1	+	<0.0001	3.54
DM10K	T03.17	12.1	+	<0.0001	1.89
DM10K	T07.39	55.1	-	<0.0001	5.36

Table 6 continued.

Starch					
DWStarch04C	T03.13	76	+	0.021	4.62
DWStarch04K	T11.64	0	+	0.006	4.5
DWStarch10K	T01.05	24.8	+	<0.0001	7.39
DWStarch10K	T01.06	77.7	+	0.002	4.29
FWStarch04C	T03.13	44.6	+	0.023	3.58
FWStarch04C	T04.20	47.8	-	0.003	5.12
FWStarch04K	T02.07	49.1	-	0.004	4.11
FWStarch04K	T06.33	0.1	-	0.028	1.99
FWStarch04K	T07.40	48.1	-	0.003	6.57
FWStarch10K	T01.05	24.9	+	<0.0001	5.81
FWStarch10K	T01.06	77.7	+	<0.0001	4.48
FWStarch10K	T07.39	53.1	-	0.002	4.76
Sucrose					
DWSuc04C	T01.01	31.3	-	0.04	3.5
DWSuc04C	T01.06	46.3	-	0.007	3.38
DWSuc04C	T71	56.6	+	<0.0001	6.1
DWSuc04K	T06.31	13.2	+	<0.0001	9.39
DWSuc04K	T06.31	55.2	-	0.008	0.47
DWSuc04K	T08.45	69.1	+	0.001	4.07
DWSuc04K	T14.81	65.1	-	<0.0001	5.33
DWSuc10K	T60	5.1	-	0.006	3.85
DWSuc10K	T72	31.1	-	0.002	2.94
FWSuc04C	T01.01	31.3	-	0.014	4.12
FWSuc04C	T01.06	46.3	-	0.011	3.31
FWSuc04C	T71	56.6	+	<0.0001	5.78
FWSuc04K	T04.19	61.5	-	0.013	3.32
FWSuc04K	T06.31	14.5	+	<0.0001	7.25
FWSuc04K	T08.45	70.9	+	0.004	5.04
FWSuc04K	T09.49	19	-	0.004	4.66
FWSuc04K	T14.81	63	-	0.001	6.02
FWSuc10K	T05.29	63.9	+	<0.0001	5.35

Table 6 continued.

Total Sugar					
DWSug04C	T04.22	57.1	+	<0.0001	3.12
DWSug04C	T72	5.2	-	0.011	0.82
DWSug04K	T02.07	29.7	+	<0.0001	5.33
DWSug10K	T01.05	21.1	-	<0.0001	7.24
DWSug10K	T01.06	77.7	-	0.01	3.02
DWSug10K	T03.15	38.3	+	0.002	3.11
DWSug10K	T07.39	70.1	+	0.034	3.93
DWSug10K	T72	34.8	-	0.003	3.73
FWSug04C	T01.02	58.5	+	0.005	5.39
FWSug04C	T04.22	60.3	+	<0.0001	4.44
FWSug04C	T72	23.9	-	0.041	3.02
FWSug04K	T02.08	74.2	-	<0.0001	4.36
FWSug04K	T06.35	24.2	-	0.002	3.65
FWSug04K	T07.42	26.1	+	0.001	1.57
FWSug04K	T14.81	45.1	-	<0.0001	2.97
FWSug10K	T01.05	18.1	-	<0.0001	4.41
FWSug10K	T72	31.1	-	0.002	3.06
FWSug10K	T83	0.1	-	0.398	0.29
Yield					
TMY10K	T02.09	19.1	+	0.001	6.47
TMY10K	T03.18	47.3	-	0.012	2.92
TY10K	T08.43	2	-	<0.0001	3.61
TY10K	T14.81	52.2	+	<0.0001	6.39
TY10K	T02.10	31.8	+	<0.0001	4.57
Culls10K	T02.10	40.1	+	0.107	3
Culls10K	T05.25	14.2	-	<0.0001	2.19
Culls10K	T07.41	2.1	-	<0.0001	3.21
Culls10K	T09.52	58.1	+	0.139	5.01
PerCulls10K	T07.41	9.7	-	0.001	5.93

Table 7: Loci interactions identified using multiple interval mapping in QTL cartographer. Trait shows the trait within the environment the interaction is associated with. LG1 and LG2 identify the locations of the QTL within the interaction. Effect indicates if the interaction increases or decreases the phenotypic value of the trait. P-value indicates the likelihood of the interaction and % Variation indicates the percent phenotypic variation explained by the interaction. a. Loci interactions in the Beauregard genome map. b. Loci interactions in the Tanzania genome map. LG refers to the linkage group in which the QTL for the listed trait can be found. Refer to tables 4 and 5 for more information about individual QTL.

a.

Trait	LG1	LG2	Effect	P-Value	% Variation
DWFruc04C	B01.03	B12.67	+	<0.0001	0.01
DWFruc04C	B01.03	B13.76	+	<0.0001	0.11
DWFruc04C	B11.61	B11.64	+	<0.0001	0.01
DWFruc04K	B03.16	B15.87	-	<0.0001	5.61
DWFruc10K	B11.61	B11.64	+	0.001	0.11
DWGluc04C	B01.03	B11.61	+	<0.0001	0.49
DWGluc04K	B03.15	B12.70	-	<0.0001	0.42
DWStarch04K	B11.62	B12.70	-	0.006	0.1
DWStarch10K	B05.25	B11.62	-	0.01	1.03
DWStarch10K	B05.25	B13.75	-	<0.0001	2.05
DWStarch10K	B11.62	B13.75	-	0.002	0.05
DWSuc10K	B05.25	B13.73	-	0.004	3.37
DWSuc10K	B11.61	B12.70	-	<0.0001	0.68
DWSug04K	B06.36	B15.88	+	0.003	2.25
DWSug10K	B04.23	B11.62	+	<0.0001	2.61
DWSug10K	B04.23	B13.73	-	0.013	1.74
DWSug10K	B05.25	B13.73	-	0.013	0.2
DWSug10K	B05.25	B13.75	+	0.028	1.37
FWFruc04C	B01.03	B11.61	+	<0.0001	0.02
FWFruc04C	B01.03	B12.67	+	<0.0001	0.02
FWFruc04C	B01.03	B13.76	+	<0.0001	0.08
FWFruc04C	B11.61	B11.64	+	<0.0001	0.01

Table 7 continued.

FWFruc04K	B03.15	B11.61	-	0.002	0.2
FWFruc04K	B04.22	B11.61	+	0.032	0.35
FWFruc10K	B11.61	B12.69	+	0.001	1.85
FWGluc04C	B01.03	B11.61	+	<0.0001	0.29
FWGluc04K	B03.15	B12.70	-	<0.0001	0.43
FWGluc04K	B13.75	B15.87	-	0.006	0.71
FWGluc10K	B11.64	B11.66	-	0.012	0.16
FWSug10K	B05.25	B05.28	-	<0.0001	3.48

b.

Trait	**LG1**	**LG2**	**Effect**	**P-Value**	**% Variation**
Culls10K	T02.10	T09.52	+	0.003	2.08
Culls10K	T05.25	T07.41	+	<0.0001	1.26
DWFruc04K	T03.15	T03.18	-	<0.0001	0.33
DWGluc04K	T01.03	T03.15	+	0.002	0.24
DWSuc04K	T06.31	T14.81	+	0.001	3.28
DWSuc10K	T06.31	T14.81	+	0.05	0.96
DWSug04C	T04.22	T72	-	0.01	0.55
FWFruc04K	T03.15	T12.70	-	<0.0001	0.69
FWFruc10K	T01.05	T02.10	-	0.005	0.14
FWGluc04C	T08.47	T83	-	0.005	0.82
FWGluc10K	T01.05	T02.10	-	0.001	0.58
FWStarch04K	T02.07	T06.33	-	0.001	0.88
FWSug04K	T02.08	T14.81	+	<0.0001	1.41
FWSug10K	T01.05	T83	+	0.002	1.23
NIRDM04K	T01.05	T02.07	+	<0.0001	1.78
NIRDM04K	T02.07	T07.40	-	0.014	0.08

Table 8: QTL appearing across multiple environments which affect the same or closely related traits were merged into an integrated QTL map. Appearance of a QTL across multiple environments is considered as increased evidence of it being real. The trait affected is shown in the Trait column. Component traits are the number of individual traits identified in Table 4 and Table 5 that were combined to create the integrated QTL across environments. LG and position identify the location of the QTL and effect identifies if the QTL increases or decreases the phenotypic value of the trait. % Variation is the average of the percent phenotypic variation explained by the component traits. Refer to tables 4 and 5 for more information about individual QTL. 7a. Summary of traits appearing on the Beauregard map across multiple environments. 7b. Summary of traits appearing on the Tanzania map across multiple environments.

a.

Trait	Component Traits	LG	Position	Effect	% Variation
Reducing Sugars	4	B01.03	26.6	+	3.7
Starch and Amylose	2	B01.03	26.6 - 27.8	-	4.3
Sugars	2	B04.23	0.1	+	3.1
Starch, Dry matter, Amylose	5	B04.23	0 - 4	-	6.3
Sugars	9	B05.25	31.1 - 84.9	+	3.9
Starch and Dry matter	3	B05.25	14 - 30.3	-	8.6
Reducing Sugars	11	B11.61	42.1 - 59.4	+	4.1
Sugars	4	B11.62	23.3 - 25.3	+	5.4
Starch, Dry matter, Amylose	6	B11.62	23.2 - 26.9	-	6.5
Reducing Sugars	8	B11.64	49.2 – 61.1	+	2.5
Sugars	4	B12.70	0.1 - 40.1	+	1.4
Starch, Dry matter, Amylose	7	B12.70	36.5 - 47.4	-	6.5
Yield	2	B13.73	14.7	-	4.8
Sugars	5	B13.75	0.1 - 10.1	+	3.7
Starch, Dry matter	3	13.75	0 - 2	-	5.5

Table 8 continued.

b.

Trait	Component Traits	LG	Position	Effect	% Variation
Sugars	9	T01.05	37.1 - 50.2	-	4.8
Starch & Dry Matter	4	T01.05	37.1 - 43.9	+	5.2
Sugars	3	T01.06	46.3 - 77.7	-	3.2
Starch	2	T01.06	77.7	+	4.4
Sugars	5	T03.15	44.3 - 48.9	+	6.6
Reducing Sugars	4	T07.40	48.1 - 54.2	+	1.6
Starch, Dry matter, Amylose	4	T07.40	45 - 48.1	-	7.0
Culls	2	T07.41	13 - 20.6	-	4.6
Sugars	7	T72	5.2 - 34.8	-	3.1

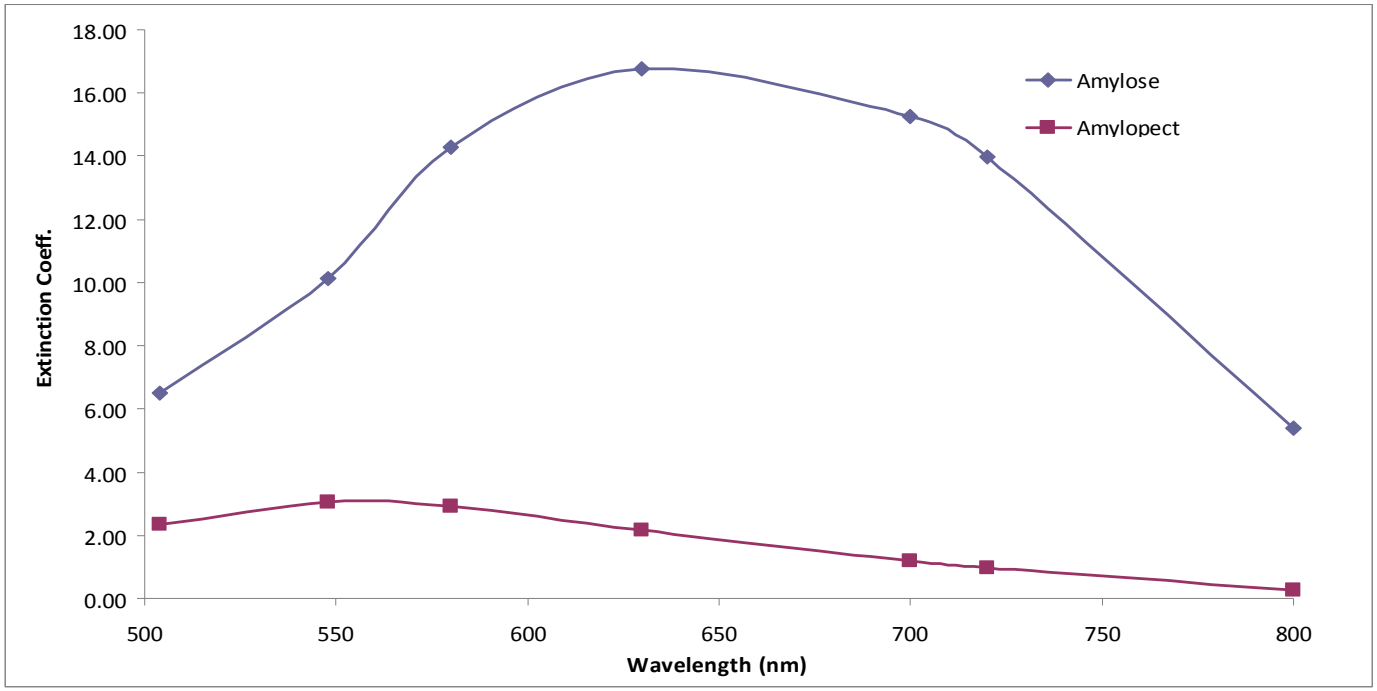
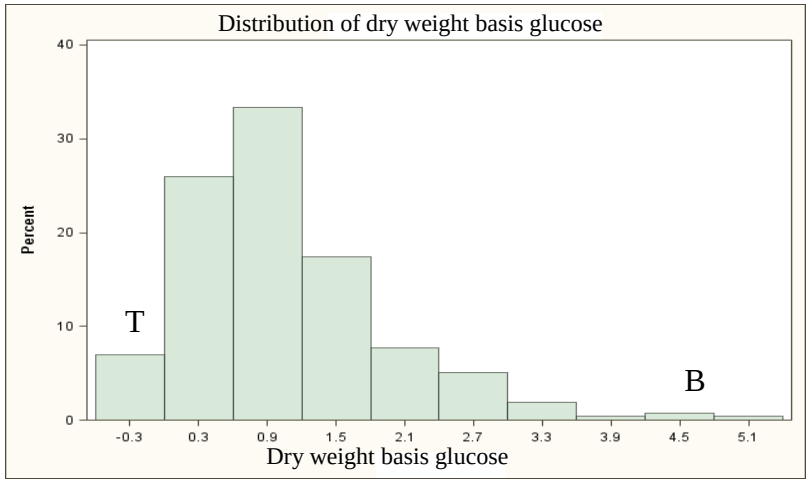
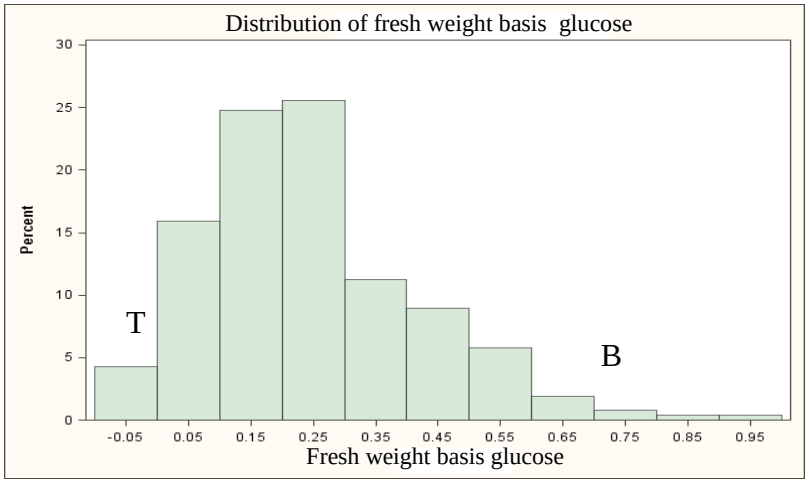
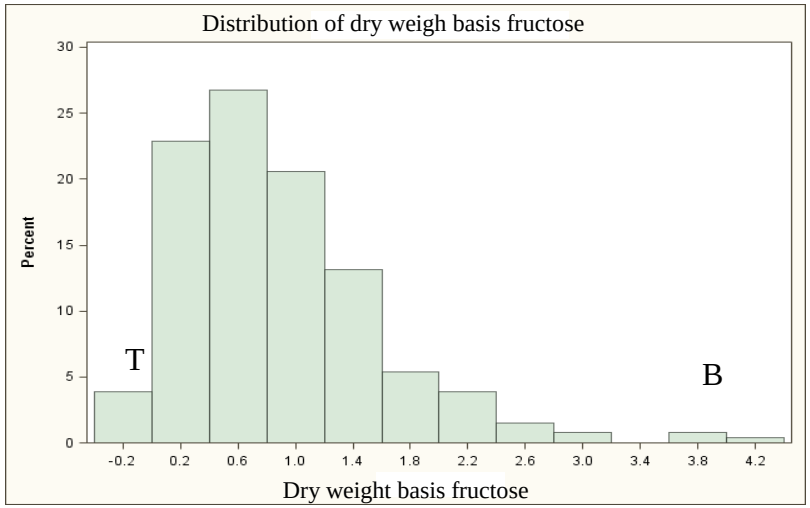


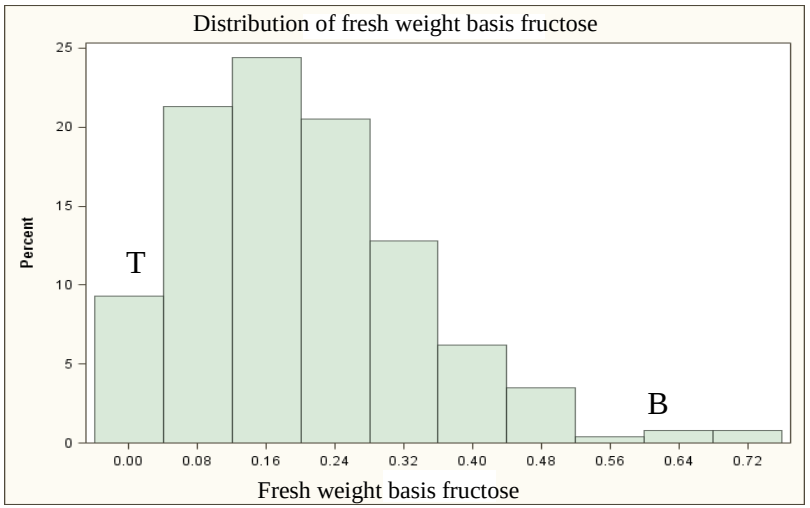
Figure 1: Iodine Standard developed from purified potato amylose from Sigma (A0512) and purified amylopectin from potatoes from Sigma (10118). Absorbance was measured at 504, 548, 580, 630, 700, 720, and 800 nm. Peak absorbance for amylose was at 630 nm while peak absorbance for amylopectin was at 548 nm.

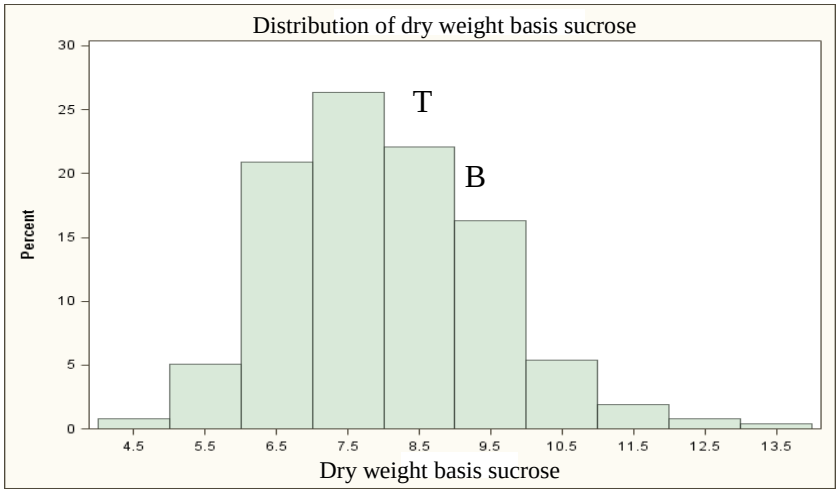
Figure 2: Histograms of clone LSMean values for traits in the TB population. Traits include dry weight basis glucose (g/100g), dry weight basis fructose (g/100g), fresh weight basis glucose (g/100g), fresh weight basis fructose (g/100g), dry weight basis dry weight basis sucrose (g/100g), dry weight basis total sugar content (g/100g), fresh weight basis sucrose (g/100g), fresh weight basis total sugar content (g/100g), dry weight basis starch content (g/100g), fresh weight basis starch content (g/100g), sample amylose content (% dried sweetpotato), and starch amylose content (% starch), yield (lbs/plot), and dry matter (% fresh weight). The letters 'T' and 'B in each histogram identify the phenotypes of the parents "Tanzania" and "Beauregard".

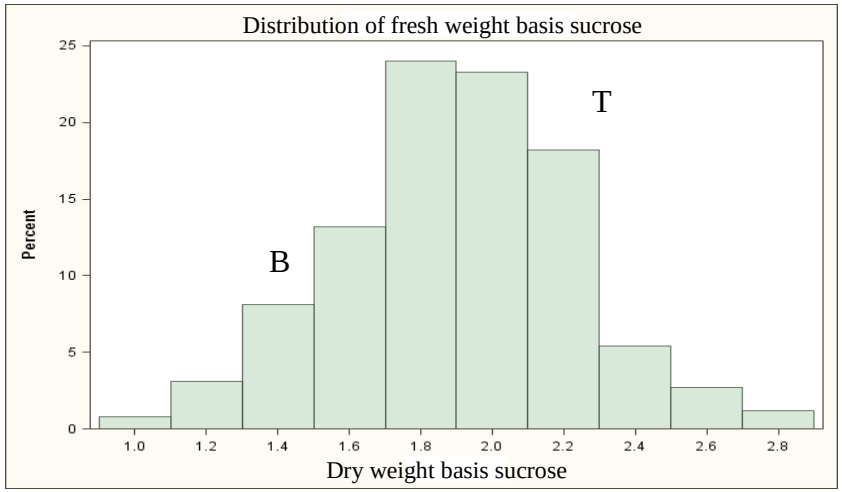

Distribution of dry weight basis glucose
Percent
40
30
20
10
0
T
B
-0.3
0.3
0.9
1.5
2.1
2.7
3.3
3.9
4.5
5.1
Dry weight basis glucose

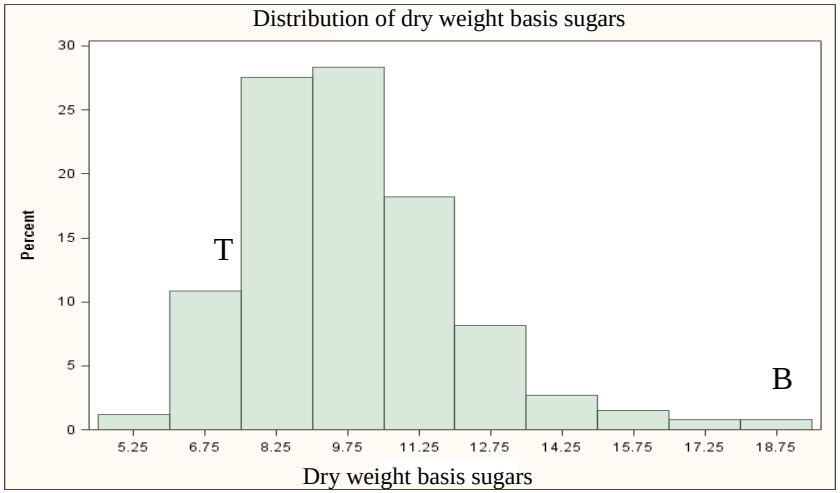

Distribution of fresh weight basis glucose
Percent
30
25
20
15
10
5
0
T
B
-0.05
0.05
0.15
0.25
0.35
0.45
0.55
0.65
0.75
0.85
0.95
Fresh weight basis glucose

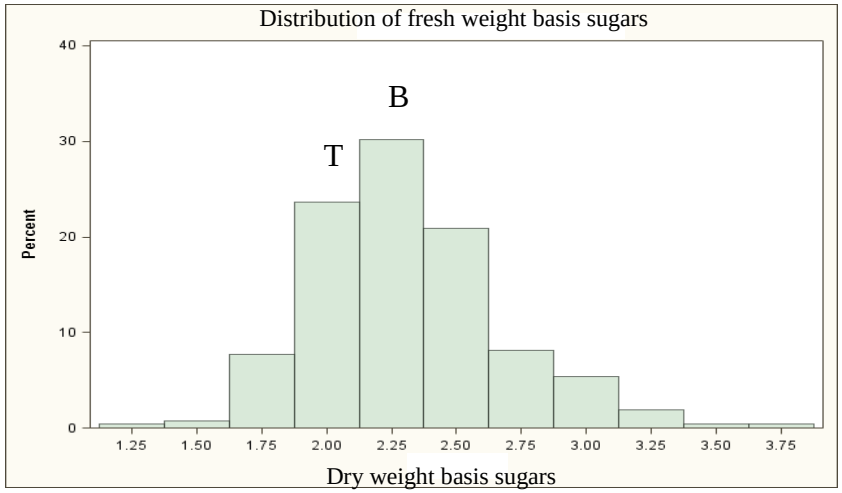

Distribution of dry weigh basis fructose
Percent
30
25
20
15
10
5
0
T
B
-0.2
0.2
0.6
1.0
1.4
1.8
2.2
2.6
3.0
3.4
3.8
4.2
Dry weight basis fructose

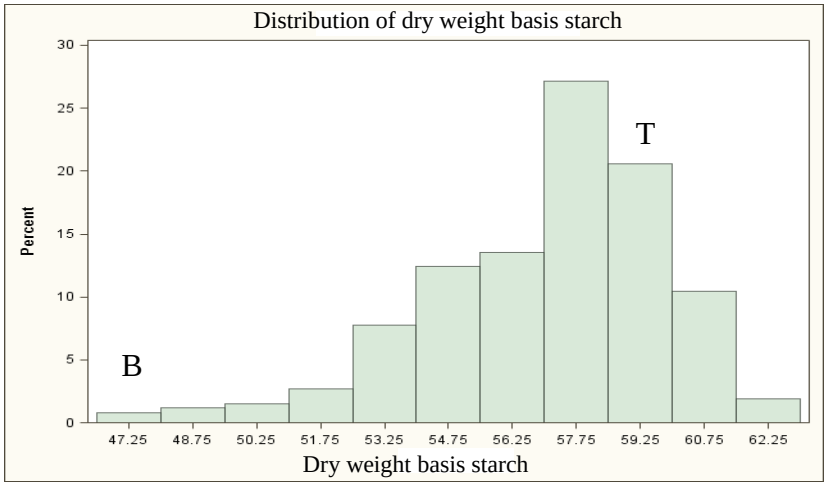

Distribution of fresh weight basis fructose
Percent
25
20
15
10
5
0
T
B
0.00
0.08
0.16
0.24
0.32
0.40
0.48
0.56
0.64
0.72
Fresh weight basis fructose

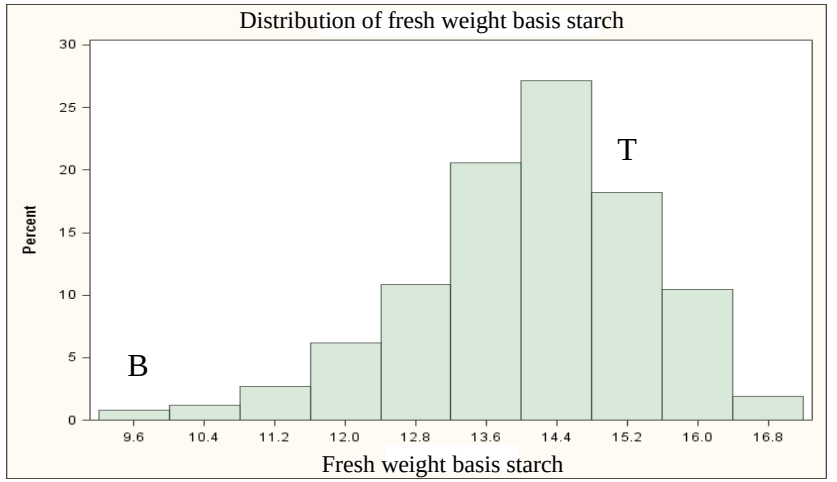

Distribution of dry weight basis sucrose
Percent
30
25
20
15
10
5
0
T
B
4.5
5.5
6.5
7.5
8.5
9.5
10.5
11.5
12.5
13.5
Dry weight basis sucrose

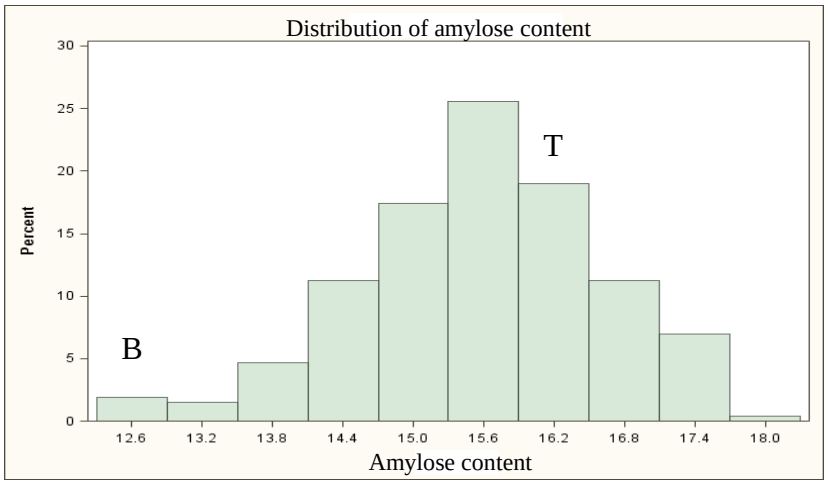

Distribution of fresh weight basis sucrose
Percent
25
20
15
10
5
0
T
B
1.0
1.2
1.4
1.6
1.8
2.0
2.2
2.4
2.6
2.8
Dry weight basis sucrose

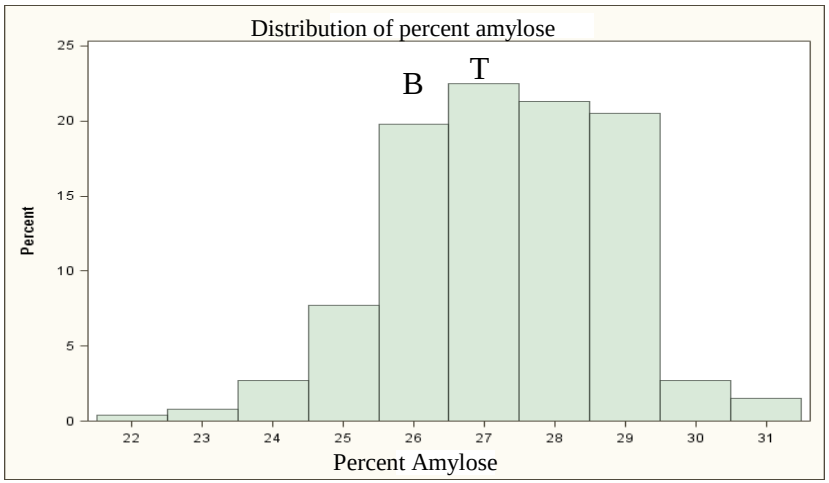

Distribution of dry weight basis sugars
Percent
30
25
20
15
10
5
0
T
B
5.25
6.75
8.25
9.75
11.25
12.75
14.25
15.75
17.25
18.75
Dry weight basis sugars

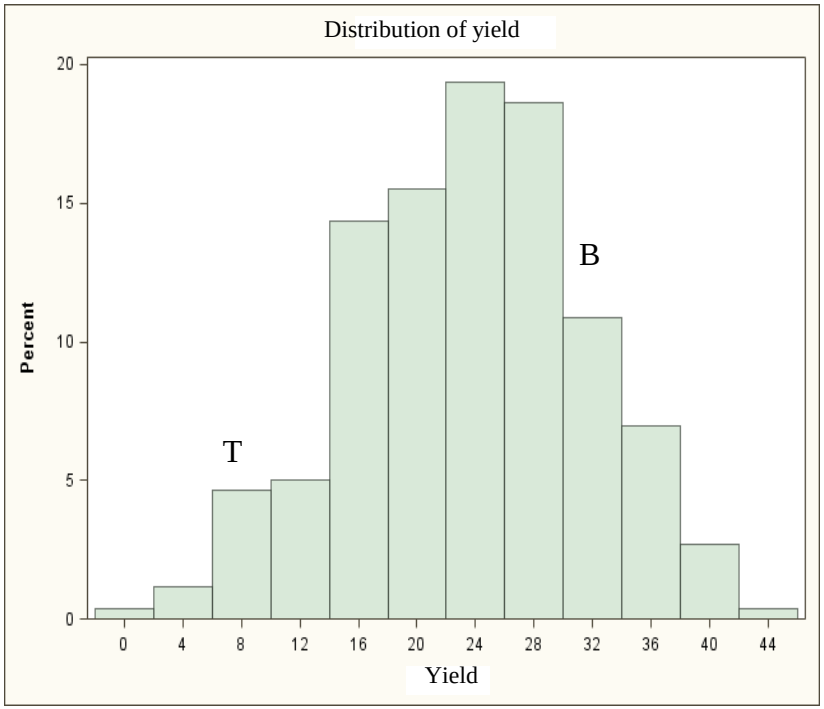

Distribution of fresh weight basis sugars
Percent
40
30
20
10
0
B
T
1.25
1.50
1.75
2.00
2.25
2.50
2.75
3.00
3.25
3.50
3.75
Dry weight basis sugars


Distribution of dry weight basis starch
Percent
30
25
20
15
10
5
0
B
T
47.25
48.75
50.25
51.75
53.25
54.75
56.25
57.75
59.25
60.75
62.25
Dry weight basis starch


Distribution of fresh weight basis starch
Percent
30
25
20
15
10
5
0
B
T
9.6
10.4
11.2
12.0
12.8
13.6
14.4
15.2
16.0
16.8
Fresh weight basis starch


Distribution of amylose content
Percent
30
25
20
15
10
5
0
B
T
12.6
13.2
13.8
14.4
15.0
15.6
16.2
16.8
17.4
18.0
Amylose content


Distribution of percent amylose
Percent
25
20
15
10
5
0
B
T
22
23
24
25
26
27
28
29
30
31
Percent Amylose


Distribution of yield
Percent
20
15
10
5
0
T
B
0
4
8
12
16
20
24
28
32
36
40
44
Yield

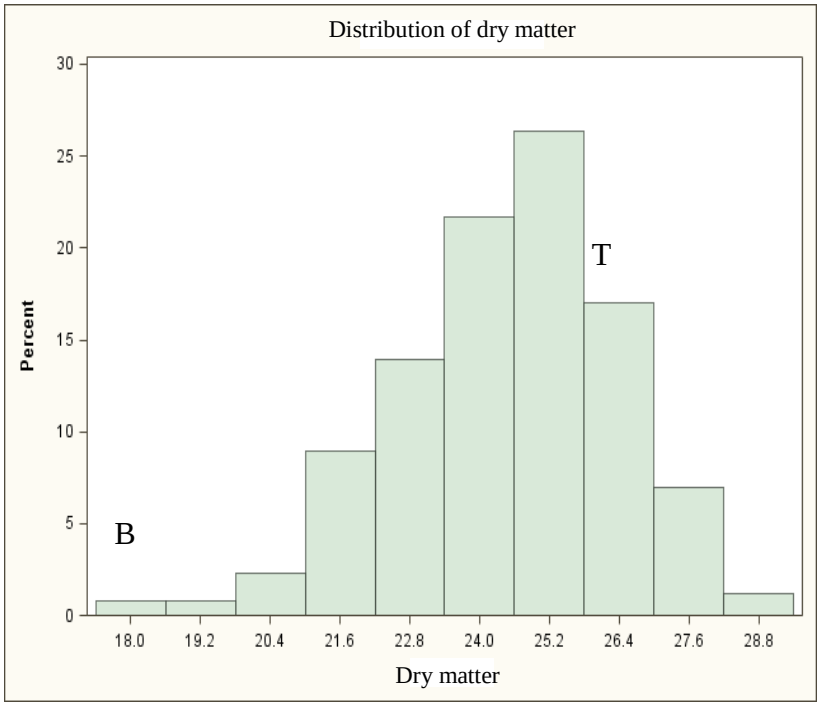
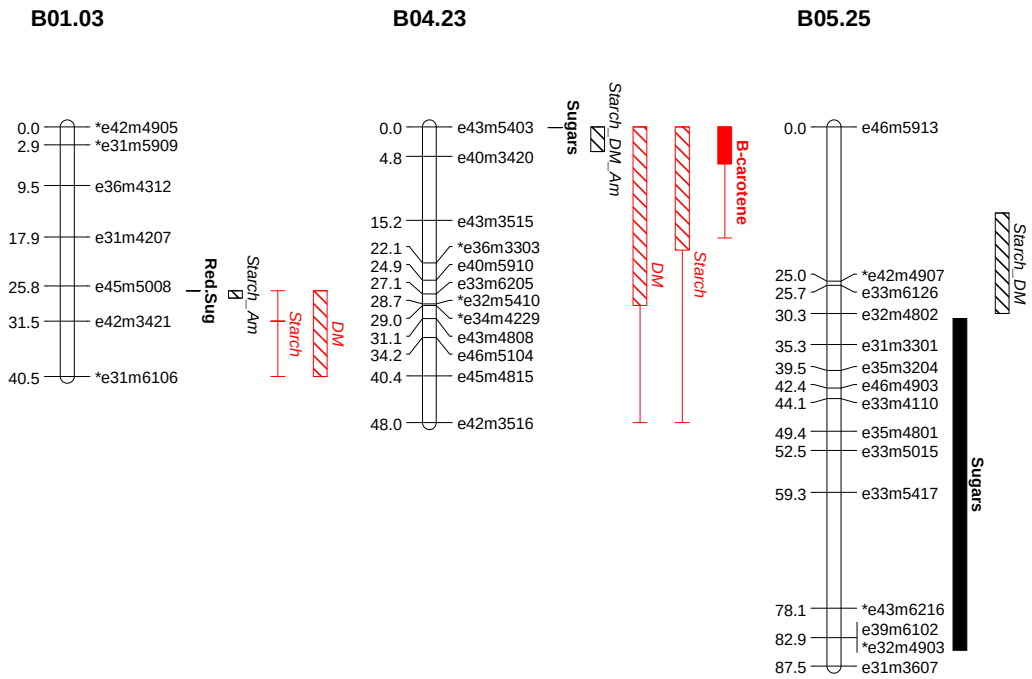

Distribution of dry matter
Percent
30
25
20
15
10
5
0
B
T
18.0
19.2
20.4
21.6
22.8
24.0
25.2
26.4
27.6
28.8
Dry matter

Figure 3: Beauregard composite map of QTL that appeared across multiple environments as determined by NIRS phenotyping. QTL that had a positive effect on a particular trait are shown with solid bars and bold type. QTL that had a negative effect on a particular trait are shown with bars with diagonal lines and italic print. For comparison purposes, QTL on these linkage groups identified previously (Cervantes-Flores et al. 2006) are shown in red.


B01.03
0.0 *e42m4905
2.9 *e31m5909
9.5 e36m4312
17.9 e31m4207
25.8 e45m5008
31.5 e42m3421
40.5 *e31m6106
Red.Sug
Starch_Am
Starch
DM
B04.23
0.0 e43m5403
4.8 e40m3420
15.2 e43m3515
22.1 *e36m3303
24.9 e40m5910
27.1 e33m6205
28.7 *e32m5410
29.0 *e34m4229
31.1 e43m4808
34.2 e46m5104
40.4 e45m4815
48.0 e42m3516
Sugars
Starch_DM_Am
DM
Starch
B-carotene
B05.25
0.0 e46m5913
25.0 *e42m4907
25.7 e33m6126
30.3 e32m4802
35.3 e31m3301
39.5 e35m3204
42.4 e46m4903
44.1 e33m4110
49.4 e35m4801
52.5 e33m5015
59.3 e33m5417
78.1 *e43m6216
82.9 e39m6102
*e32m4903
87.5 e31m3607
Starch_DM
Sugars
B11.61
0.0 e32m3817
9.4 *e34m4106
10.4 e40m4010
15.3 e35m4805
17.0 e33m4308
20.3 e42m3107
26.7 e41m4411
34.6 *e41m4902
41.4 e43m3901
48.9 *e44m4506
61.0 e41m3221
DM
Starch
Red.Sug
B11.62
0.0 *e46m3806
2.6 *e41m3502
4.6 *e37m3302
10.0 *e37m4102
11.5 *e44m6109
12.5 *e42m5408
15.8 *e43m3714
18.0 *e44m4907
18.7 *e37m3110
*e35m4207
*e31m6012
20.8 *e46m6015
*e32m3724
*e42m3812
*e40m3718
23.2 e32m4102
24.9 e41m4905
27.8 e36m5103
*e39m5115
36.6 *e31m3602
Sugars
Starch_DM_Am
DM
Starch
B-carotene
B11.64
0.0 e31m4414
13.2 e43m3312
14.0 e44m3701
16.9 e32m6108
18.6 e36m4010
*e43m6005
31.6 *e41m3205
32.3 *e44m4506
35.3 e39m4109
36.5 *e44m6007
42.2 e42m4808
49.1 e44m4204
62.3 e38m5402
Red.Sugars

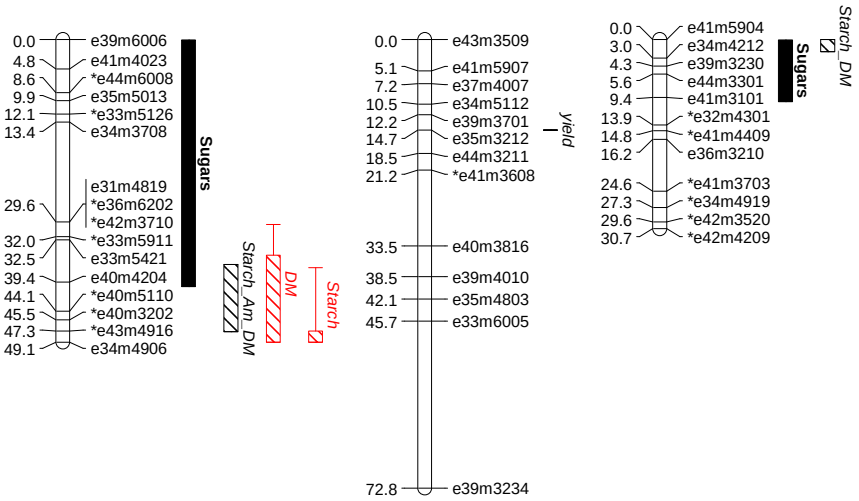
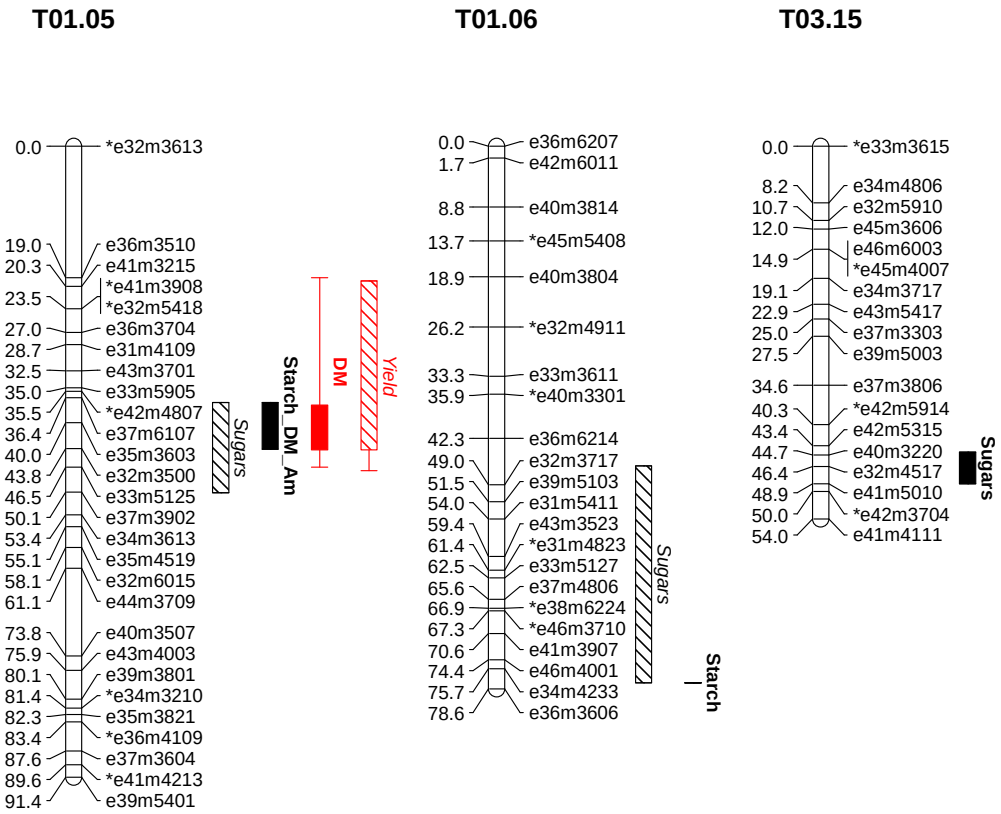
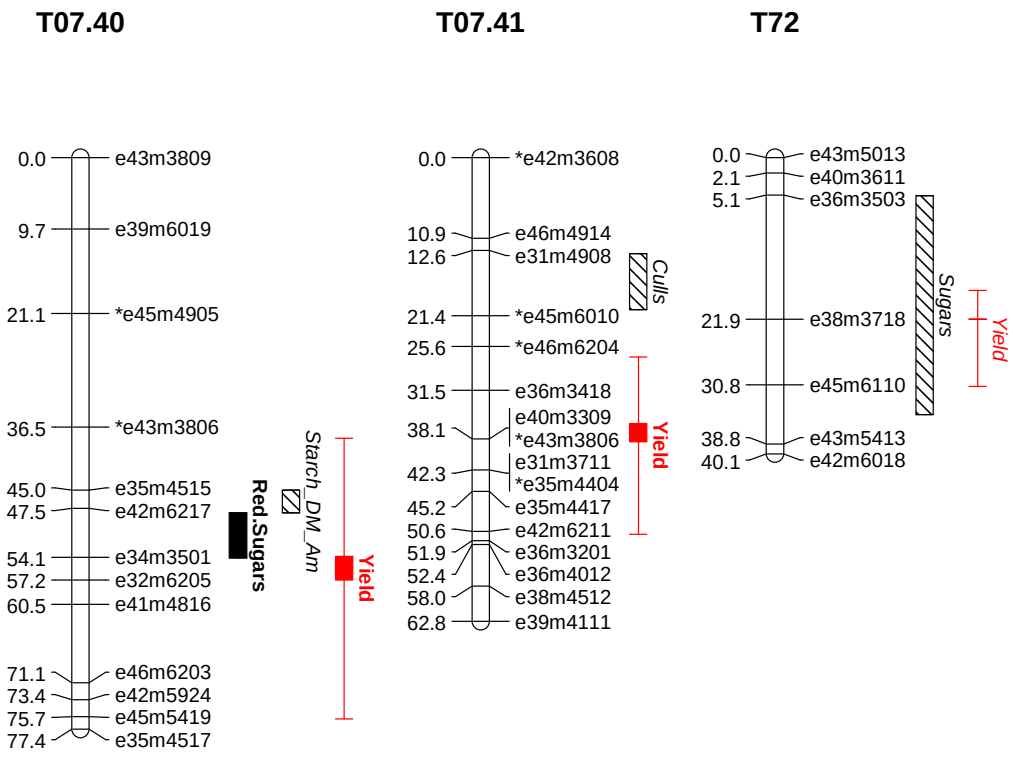

B12.70
B13.73
B13.75
0.0 e39m6006
4.8 e41m4023
8.6 *e44m6008
9.9 e35m5013
12.1 *e33m5126
13.4 e34m3708
29.6 e31m4819
*e36m6202
*e42m3710
32.0 *e33m5911
32.5 e33m5421
39.4 e40m4204
44.1 *e40m5110
45.5 *e40m3202
47.3 *e43m4916
49.1 e34m4906
Sugars
Starch_Am_DM
DM
Starch
0.0 e43m3509
5.1 e41m5907
7.2 e37m4007
10.5 e34m5112
12.2 e39m3701
14.7 e35m3212
18.5 e44m3211
21.2 *e41m3608
yield
33.5 e40m3816
38.5 e39m4010
42.1 e35m4803
45.7 e33m6005
72.8 e39m3234
0.0 e41m5904
3.0 e34m4212
4.3 e39m3230
5.6 e44m3301
9.4 e41m3101
13.9 *e32m4301
14.8 *e41m4409
16.2 e36m3210
24.6 *e41m3703
27.3 *e34m4919
29.6 *e42m3520
30.7 *e42m4209
Sugars
Starch_DM

Figure 4: Tanzania composite map of QTL which appear across environments as determined by NIRS phenotyping. QTL which had a positive effect on a particular trait are shown with solid bars and bold type. QTL which had a negative effect on a particular trait are shown with bars with diagonal lines and italic print. For comparison purposes, QTL on these linkage groups identified previously (Cervantes-Flores et al. 2006) are shown in red.


T01.05
T01.06
T03.15
0.0 *e32m3613
19.0 e36m3510
20.3 e41m3215
*e41m3908
23.5 *e32m5418
27.0 e36m3704
28.7 e31m4109
32.5 e43m3701
35.0 e33m5905
35.5 *e42m4807
36.4 e37m6107
40.0 e35m3603
43.8 e32m3500
46.5 e33m5125
50.1 e37m3902
53.4 e34m3613
55.1 e35m4519
58.1 e32m6015
61.1 e44m3709
73.8 e40m3507
75.9 e43m4003
80.1 e39m3801
81.4 *e34m3210
82.3 e35m3821
83.4 *e36m4109
87.6 e37m3604
89.6 *e41m4213
91.4 e39m5401
Sugars
Starch_DM_Am
DM
Yield
0.0 e36m6207
1.7 e42m6011
8.8 e40m3814
13.7 *e45m5408
18.9 e40m3804
26.2 *e32m4911
33.3 e33m3611
35.9 *e40m3301
42.3 e36m6214
49.0 e32m3717
51.5 e39m5103
54.0 e31m5411
59.4 e43m3523
61.4 *e31m4823
62.5 e33m5127
65.6 e37m4806
66.9 *e38m6224
67.3 *e46m3710
70.6 e41m3907
74.4 e46m4001
75.7 e34m4233
78.6 e36m3606
Sugars
Starch
0.0 *e33m3615
8.2 e34m4806
10.7 e32m5910
12.0 e45m3606
14.9 e46m6003
*e45m4007
19.1 e34m3717
22.9 e43m5417
25.0 e37m3303
27.5 e39m5003
34.6 e37m3806
40.3 *e42m5914
43.4 e42m5315
44.7 e40m3220
46.4 e32m4517
48.9 e41m5010
50.0 *e42m3704
54.0 e41m4111
Sugars


T07.40
T07.41
T72
0.0 e43m3809
9.7 e39m6019
21.1 *e45m4905
36.5 *e43m3806
45.0 e35m4515
47.5 e42m6217
54.1 e34m3501
57.2 e32m6205
60.5 e41m4816
71.1 e46m6203
73.4 e42m5924
75.7 e45m5419
77.4 e35m4517
Red.Sugars
Starch_DM_Am
Yield
0.0 *e42m3608
10.9 e46m4914
12.6 e31m4908
21.4 *e45m6010
25.6 *e46m6204
31.5 e36m3418
38.1 e40m3309
*e43m3806
42.3 e31m3711
*e35m4404
45.2 e35m4417
50.6 e42m6211
51.9 e36m3201
52.4 e36m4012
58.0 e38m4512
62.8 e39m4111
Culls
Yield
0.0 e43m5013
2.1 e40m3611
5.1 e36m3503
21.9 e38m3718
30.8 e45m6110
38.8 e43m5413
40.1 e42m6018
Sugars
Yield

CHAPTER 5: SWEETPOTATO BREEDING STEPS INTO THE NEW CENTURY

This dissertation helped chart a path to a new generational paradigm for sweetpotato breeding. The combination of new phenotyping technologies, germplasm, molecular markers, augmented designs, and quantitative inheritance studies have provided information that can be used to design new crossing and selection methods. As new sweetpotato markets emerge, such tools will be critical for development of new sweetpotato varieties. While many of the technologies described in this dissertation have existed individually for decades (Federer and Raghavaro 1975, Hallauer and Miranda 1988, Batten 1998, Asins 2002), and some have been applied to sweetpotato previously (Jones 1986, Cervantes-Flores 2006, Lu et al. 2006, Marchese et al. 2010, Tumwegamire et al. 2011), their merger opens new possibilities to advance both basic and applied sweetpotato research.

In recent years, the emergence of new markets for processed sweetpotato products, such as chips and fries, and industrial applications, such as anthocyanins, have created opportunities and new risks for North Carolina sweetpotato growers. The technologies described herein represent a portion of the NCSU sweetpotato team's response to these new developments.

The NCII design described in this dissertation provided a transition step to allow the NCSU sweetpotato breeding and genetics program to estimate combining ability for dry matter and anthocyanin content. It allowed the program to gain experience with quantitative inheritance studies while using established technologies for measuring composition. The NCII design showed that anthocyanin and dry matter content had significant general

combining abilities and that yield had a significant specific combining ability. The NCII design also revealed the impact of incompatibilities on quantitative genetics studies in sweetpotato. The small size of the NCII experiment (5 females and 5 males) was primarily due to the effect of incompatibilities in a factorial crossing block. This small size limited the conclusions that could be drawn from the experiment, as possible factors such as maternal effects cannot be identified through such a small population.

Near-infrared spectroscopy (NIRS) was applied in this dissertation on a large scale for the first time by the NCSU sweetpotato breeding program. Measurement of dry matter has long been a standard practice in sweetpotato breeding, however, it gives only a rough picture of crop composition. New customers, such as food and industrial processors, need sweetpotatoes with specific compositions to satisfy processing requirements. To evaluate potential sweetpotato cultivars for these traits, NIRS, a technology that allowed rapid screening of composition, was implemented. Proof-of-concept work was developed to allow estimation of dry matter, fructose, glucose, sucrose, total sugar, starch, and amylose content of sweetpotato samples (N. George personal communications). The application of NIRS in the two experiments described herein demonstrated both the benefits and limitations of the current stage of NIRS development. The NIRS standard curves were able to predict several sugar and starch traits with sufficient accuracy for rough screening, which allowed experiments with broad diversity, such as the NCI design, to be screened. However, development of the QTL map using NIRS demonstrated that the technology was not fully capable of the fine scale phenotyping needed to distinguish QTL, which tend to explain relatively little phenotypic variation.

The NCI design described here developed a method of studying quantitative inheritance in sweetpotatoes that overcame the incompatibilities that have plagued many such studies, and also showed the potential and limitations of polycross nurseries and paired crosses in sweetpotato breeding. The experiment demonstrated that polycross nurseries would be satisfactory for modification of composition traits, but that improvement of yield traits might be better approached through paired crosses. All composition traits studied in this dissertation, except percent amylose, showed high heritabilities through half-sib families, especially when accounting for selection on both parents as would happen in a polycross nursery. However, yield traits showed low heritabilities on a half-sib family basis, but moderate heritabilities on a full-sib family basis.

Finally, this research included an effort to merge NIRS phenotyping tools with molecular markers to step into a new generation of plant breeding. In recent decades, molecular markers have made significant impacts on plant breeding by allowing direct identification of genes underlying traits and selection for genes even in the absence of direct phenotyping for traits. As DNA sequencing and molecular markers prices have dropped exponentially in recent decades, the ability to gather molecular data on plants of interest has increased accordingly. However, to take advantage of these new molecular tools, improved phenotyping tools must be developed and applied to identify DNA sequences of importance to agricultural production (Furbank and Tester 2011). The research described in Chapter 4 merged molecular markers with new NIRS phenotyping tools that increased phenotyping throughput and allowed identification of QTL associated with new traits. Markers associated with sugar content, reducing sugar content, and starch content were identified. This work

confirmed and supplemented earlier work (Cervantes-Flores 2006, Cervantes-Flores et al. 2008a, Cervantes-Flores et al. 2008b, Cervantes-Flores et al. 2011) and provided a more complete understanding of sweetpotato genomics.

Together, the technologies described in these chapters provide an important step toward a new generation of sweetpotato breeding. Previous generational developments in sweetpotato breeding have included the development of grafting techniques to induce flowering (Hernandez et al. 1959) and use of polycross nurseries to increase seed numbers (Jones 1965). The lessons learned here open the door to the merger of NIRS phenotyping, new germplasm, new crossing methods, and molecular markers to develop a new generation of sweetpotato breeding. Merger of these new tools can allow development of new markets such as those for anthocyanins and chips and fries to the benefit of North Carolina sweetpotato growers.

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APPENDICES

Appendix A: Compatible and Incompatible Crosses in NCI mating design

Compatible Crosses		Incompatible Crosses
Macana x #6	L05-29 x NC04-531	Batata Blanca x Norin#4
Patriot x #6	MD810 x NC04-531	Cordner x PI564114
TIS2498 x #6	TIS2525 x NC04-531	DM01-158 x Covington
Wagabolige x #6	DM04-001 x NonDaHong	DM01-158 x DM03-035
6618-001 x BM85-42	FT4-89 x NonDaHong	DM01-158 x Won-mi
BP1SP2 x BM85-42	Pur05-055 x NonDaHong	DM02-180 x Mugande
L-258 x BM85-42	Whitestar x NonDaHong	DM02-291 x W-250
Suwon147 x BM85-42	Morada, Sombica x O. 4	DM04-146 x Won-mi
Minamiyutaka x Centennial	Perla x O. 4	Francia x L-329
Darby x Covington	TIB4 x O. 4	GA90-10 x Centennial
Murasaki-29 x Covington	W-245 x O. 4	Georgia Jet x Yukimusume
TIS3017 x Covington	Cordner x PI564114	Hayman x Centennial
6625-001 x DM02-180	Merenge x PI564114	Heart-O-Gold x TIS2532
Kyushu100 x DM02-180	NC04-412 x PI564114	HiDry x DM04-197
Tinto x DM02-180	Regal x PI564114	HM-145 x Centennial
Excel x DM03-035	YanShu1 x PI564114	Jishu5 x Centennial
Tanzania x DM03-035	B94-14 x Ruddy	Jishu5 x Kyukei-97
W-241 x DM03-035	DM04-226 x Ruddy	Julian x W-250
Georgia Jet x DM03-092	Okinawa100 x Ruddy	Kotopuki x DM03-092
Guangshu70-9 x DM03-092	DM05-090 x TIS2532	Kyukei-63 x FTA94
Sumor x DM03-092	Norin#4 x TIS2532	MD320 x DM03-035
Xushu18 x DM03-092	Seon-mi x TIS2532	MD807 x Covington
CN1345-8 x DM04-197	UGA204 x TIS2532	MD807 x Yukimusume
NC413 x DM04-197	DM02-105 x TIS9232	MD822 x Covington
Batata Blanca x FTA94	NC1880 x TIS9232	MD822 x PI 324887
Ma'alau x FTA94	Pur01-192 x TIS9232	Minamiyutaka x Centennial
NC04-097 x FTA94	Pur04-118 x TIS9232	Nancy Hall x O. 4
Pur06-014 x FTA94	KalmeghS-30 x W-250	NC06-185 x PI 324887
CN1489-43 x Kyukei-97	NC03-007 x W-250	Pelican Processor x Won-mi
Guangshu7 x Kyukei-97	NC03-302 x W-250	PI531113 x Covington
Liaoshu40 x Kyukei-97	Pur05-028 x W-250	PI564112 x Yukimusume
Viola x Kyukei-97	Hatteras x W-392	Picadito x Yukimusume
NC93-17 x L-329	NC03-030 x W-392	Ruddy x MDP217-84
PDM P4 x L-329	Oklamar x W-392	Suwon 147 x PI 324887
Pelican Processor x L-329	Resisto x W-392	TIB4 x Yukimusume
Woksaken x L-329	Francia x Won-mi	Tinian x Yukimusume
Kyukei-63 x MDP217-84	Hernandez x Won-mi	TIS 3290 x Centennial
NC03-089 x MDP217-84	Pur05-087 x Won-mi	TIS3017 x Won-mi
Suwon122 x MDP217-84	DM04-051 x Yukimusume	Vardaman x BM85-42
TIB11 x MDP217-84	Liberty x Yukimusume	Viola x Norin4
Koganesengan x NC04-531	Mojave x Yukimusume	Wanmun Large x PI564114
		Xiangnonhuangpi x W-392

Appendix B: SAS code for analysis of heritability for NCI design

```
options ps=60 ls=80;

PROC FORMAT;
VALUE c_sign
   LOW - < -3.5  =  "CXB95DC1"
  -3.5 - < -2.5  =  "CX7F87E0"
  -2.5 - < -1.5  =  "CXC1BAD3"
  -1.5 - < 0     =  "CXE0CCDF"
     0 - < 1.5   =  "CXE0CCDF"
   1.5 - < 2.5   =  "CXC1BAD3"
   2.5 - < 3.5   =  "CX7F87E0"
   3.5 -  HIGH   =  "CXB95DC1";

VALUE cf_col
   LOW - < -3.5  =  "CXF2D010"
  -3.5 - < -2.5  =  "CXF2D010"
  -2.5 - < -1.5  =  "CX2436F4"
  -1.5 - < 0     =  "CX663031"
     0 - < 1.5   =  "CX663031"
   1.5 - < 2.5   =  "CX2436F4"
   2.5 - < 3.5   =  "CXF2D010"
   3.5 -  HIGH   =  "CXF2D010";

VALUE FW_ratio
LOW  - <  -3.5 = "BOLD"
  -3.5 - < 3.5 = "LIGHT"
    3.5 - HIGH = "BOLD";

VALUE FS_ratio
  LOW  - < -3.5 = "ITALIC"
   -3.5 - < 3.5 = "ROMAN"
     3.5 - HIGH = "ITALIC" ;
  run;

PROC IMPORT OUT= WORK.a
            DATAFILE=  "NC1_hpc_Biomass"
            DBMS=xls  REPLACE;
         DATAROW=2;
      GUESSINGROWS=1200;
RUN;

proc contents data=a;
run;

title "Plots per clone per year";
proc freq data=a noprint;
where Biomass ne .;/*QC step*/
tables  year*male*female*clone /list missing out=outfq1  nopercent nocum;
run;
```

```
title "Checking Parents";
proc print data=outfq1;  /*QC step, tells how many offspring are in each
full-sib family*/
      where male eq "" and female eq "";
      run;

title"Offspring with extra plots/year ";
proc print data=outfq1; /*QC step. Is there more than one entry per year
listed for any offspring?*/
      where count gt 1 and male ne "";
      run;

title "Total plots per full-sib family";
proc freq data=outfq1; /*QC step.  Tells how many total plots (2010 +
2011) in each full-sib family*/
tables  male * female /list missing out=outfq2  nopercent nocum;
run;

title "Full-sib families per half-sib family ";
title2 "For harmonic means";
proc freq data=outfq2; /*QC step.  Tells how many full-sib families are in
each half-sib family*/
tables  male   /list missing out=outfq3  nopercent nocum;
run;

title "Plots per clone across environments";
title2 "For harmonic means";
proc freq data=a noprint;
where Biomass ne .;/*QC step*/
tables  male*female*clone /list missing out=outfq4  nopercent nocum;
run;

title "Offspring per full-sib family";
title2 "For harmonic means";
proc freq data=outfq4 noprint; /*# of offspring per full-sib family*/
tables  female /list missing out=outfq5  nopercent nocum;
run;

  data b; /*adds variable to tell if a particular entry is a parent or an
offspring*/
  length var_p $9 clone $25 male $25 female $25;
   set a;
   if female=" " and male=" " then do; female="P";  male="P";  VAR_P=
"Parents"; end;
      else VAR_P="Offspring";
run;

title "Plots per half-sib family";
proc freq data=b; /*QC step.  Number of offspring in half-sib family*/
tables  var_p*male   /list missing nopercent nocum;
run;
```

```
title "Plots per full-sib family";
proc freq data=b;/*QC step.  Number of offspring in full-sib family*/
where Biomass ne .;
tables  VAR_P *male*female /list missing out=outfq6  nopercent nocum;
run;

title "Full-sib families per half-sib family";
proc freq data=outfq6;/*QC step.  Number of half-sib families in full-sib
family*/
tables  VAR_P *male/list missing out=outfq8  nopercent nocum;
run;

data see ; /*how close is Satterthwaite to sum of all df's*/
 set outfq8;
 df=count-1;
 run;

title "Verifying Satterthwaite DF";
proc means  data=see sum;/*how close is Satterthwaite to sum of all df's*/
      var df;
      run;

title"Entries with missing data";
proc print data=b; where Biomass=. or female='';*(obs=20);/*QC step.
Entries with missing data*/
run;

title "Heritability matrices with solutions";
title2 "Random effects ";
proc mixed data=b asycov;
  class  year clone rep male female planting var_p;
  model  Biomass  = var_p / outp=outmx3  residual htype=3 solution;
  random   rep(year) planting(year) year;
  random year*male(var_p) year*female(male*var_p)/ group=var_p;
  random male(var_P)/ group = var_p solution;
  random female(male*var_P)/ group = var_p solution;
  random clone(male*female*var_p)/ group=var_p solution;
  random clone*year(male*female*var_p) / group=var_p;
  parms 0.1918          53.4688           10.1146     3.8819      0
      5.2364      0     4.0134      0.000013    3.4912      0.000013
      19.1000     38.7850     11.5508     22.9546           22.8312;
  ods output asycov=Biomass_asycov covparms = Biomass_covparm
SolutionR=BLUPs SolutionF=BLUEs;
  run;

title "Outliers";
proc print data=outmx3;
  where abs(StudentResid) gt 3.5;
  var var_P clone male female Year Rep Planting Tier Col Biomass Pred
      StdErrPred DF Alpha Lower Upper Resid StudentResid;
run;
```

```
 ************************************************************************;
Title "Heritabilities";
proc iml;
start seh(V, C, LG, LPg, LS, LPs, Hg, SEg, Hs, SEs);
Vpg = LPg`*V;
Vgg = LG`*V;
Vps = LPs`*V;
Vgs = LS`*V;
Hg = Vgg/Vpg;
dg = (1/Vpg)*(LG - (LPg*Hg));
VHg = dg`*C*dg;
SEg = sqrt(VHg);
Hs = Vgs/Vps;
ds = (1/Vps)*(LS - (LPs*Hs));
VHs = ds`*C*ds;
SEs = sqrt(VHs);
finish seh;

use Biomass_covparm; read all into v; use Biomass_asycov; read all into c;
* Note that SAS introduces an extra first column into the matrix which
must be removed;
C = C(|1:nrow(C), 2:ncol(C)|);
/*there were 23 males in the experiment.
Calculate harmonic means of other components:  # environments, #
females/male, and #entries/full-sib family.*/

/*23 = # of males;*/
/*fsh = harmonic mean of # of full-sib families per half sib family*/
use outfq3 where (Male ^="");
read all var ("COUNT")into fullsib;
print fullsib;
fsh = harmean (fullsib);
print fsh;

/*eh = harmonic mean of # of environments;*/
use outfq4 where (Male ^="");
read all var ("COUNT")into enviro;
print enviro;
eh = harmean (enviro);
print eh;

/*ch = harmonic mean of # of clones / full-sib family;*/
use outfq5 where (Female ^="");
read all var ("COUNT")into clonefreq;
print clonefreq;
ch = harmean (clonefreq);
print ch;

*this is genetic variance among HS families (GCA variance);
LG = {0, 0, 0, 0, 0, 0, 0, 1, 0, 0, 0, 0, 0, 0, 0, 0};
*Ls is genetic variance among FS families within HS families = GCAf + SCA;
```

```
LS = 0// 0// 0// 0// 0// 0// 0// ((fsh*(22))/((fsh*23)-1))// 0// 1// 0//
0// 0// 0// 0// 0// 0;
*LPs is PHENOTYPIC variance among FS families within HS families
*we should have clone * environment interaction specified and in this
denominator;
LPs = 0// 0// 0// ((fsh*22)/(((fsh*23)-1)*eh))// 0// (1/eh)// 0//
((fsh*22)/((fsh*23)-1))// 0// 1// 0// (1/ch)// 0// (1/ch*eh)// 0//
(1/ch*eh);
*Then we also need a term for LPg for HS family pheno variance:
Vg + Vsca/f + Vge/e + Vsca/fe + Vclone/c + Vclone*E/ce + Verr/fecr
c = mean number of clones per full sib family
r = 1 since this is an augmented design and all offspring have just 1 rep;
LPg = 0// 0// 0// (1/eh)// 0// (1/eh*fsh)// 0// 1// 0// (1/fsh)// 0//
(1/ch*fsh)// 0// (1/ch*eh*fsh)// 0// (1/ch*fsh*eh);

call seh (V, C, LG, LPg, LS, LPs, Hg, SEg, Hs, SEs);

title "Biomass Heritability on a Family Mean Basis";
print "Heritability: Polycross (HS Family Mean-Basis)", Hg, SEg;
print "Heritability: Paired Cross (FS family Mean-Basis)", Hs, SEs;
quit;

data randeff1;
set BLUPs;
if effect= 'Rep(Year)' then delete;
if effect= 'Planting(Year)' then delete;
if effect= 'Year' then  delete;
if effect= 'Year*male(var_p)' then delete;
if effect= 'Year*fema(male*var_)' then delete;
if effect= 'Yea*clo(mal*fem*var)' then delete;
if effect= 'male(var_p)' and var_p= 'Parents' then delete;
if effect= 'female(male*var_p)' and var_p= 'Parents' then delete;
if effect= 'clon(male*fema*var_)' and var_p= 'Offspring' then delete;
if effect= 'clon(male*fema*var_)' and var_p= 'Parents' and male^= 'P' then
delete;
if var_p= 'Offspring' and Male= 'P' then delete;
drop Rep Planting Year DF tValue Probt;
run;

data randeffparents;
set randeff1;
if var_p= 'Parents';
run;

data randhalfsib;
set randeff1;
if effect= 'male(var_p)';
rename Estimate= MaleEffect;
rename StdErrPred= MaleStderr;
run;

data randfullsib;
```

```
set randeff1;
if effect= 'female(male*var_p)';
rename Estimate= FemaleEffect;
rename StdErrPred= FemaleStderr;
run;

proc sort data=randfullsib;
by Male;
run;

data families;
merge randhalfsib randfullsib;
by male;
run;

data families2;
set families;
length fam_effect 6;
length fam_stderr 8;
fam_effect= MaleEffect + FemaleEffect;
fam_stderr= sqrt(MaleStderr**2 + FemaleStderr**2);
run;

data SolutionF2;
set BLUEs;
if Effect='Intercept' then Effect= 'Parents';
if var_p= 'Offspring' then Effect= 'Offspring';
drop DF tValue Probt;
run;

proc transpose data= work.SOLUTIONF2 out=SolutionF3;
run;

data SolutionF4;
set SolutionF3;
rename Col1= Parentals;
rename Col2= Children;
length Offspring_Effect 8;
run;

data SolutionF5;
set SolutionF4;
Offspring_effect= Parentals + Children;
run;

Proc transpose data= SolutionF5 out=SolutionF6;
run;

data fixPars;
set SolutionF6;
if _NAME_ = 'Parentals';
run;
```

```
data fixOffs;
set SolutionF6;
if _NAME_ = 'Offspring_Effect';
run;

data fixPars2;
set fixPars;
rename _NAME_= var_p;
rename Estimate= FixedEst;
rename StdErr= FixedStdErr;
run;

data fixPars3;
set fixPars2;
if var_p= 'Parentals' then var_p= 'Parents';
run;

data Parentranks;
merge fixPars3 RANDEFFPARENTS;
by var_p;
run;

data Parentranks2;
set Parentranks;
length totalest 8;
length totalstderr 8;
run;

data Parentranks3;
set Parentranks2;
totalest= FixedEst + Estimate;
totalstderr= StdErrPred;
run;

proc sort data=Parentranks3;
by descending totalest;
run;

title 'Rankings of check clones';
proc print data=parentranks3 label;
var Clone totalest totalstderr;
label totalest='Biomass' totalstderr='Std Err';
run;

data fixoffs2;
set fixoffs;
rename _NAME_= var_p;
run;

data fixoff3;
set fixoffs2;
```

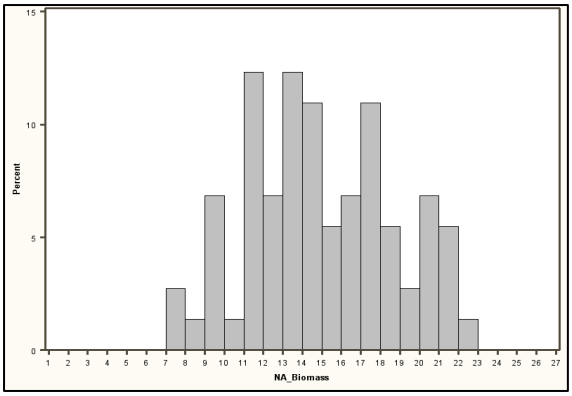
```
if var_p= 'Offspring_Effect' then var_p= 'Offspring';
run;

data halfsibscomb;
merge fixoff3 randhalfsib;
by var_p;
run;

data halfsibcomb2;
set halfsibscomb;
length halfsibeff 8;
length halfsibstderr 8;
run;

data halfsibcomb3;
set halfsibcomb2;
halfsibeff= Estimate + MaleEffect;
halfsibstderr= MaleStdErr;
run;

proc sort data= halfsibcomb3;
by descending halfsibeff;
run;

title 'Rankings of half-sib families';
proc print data=halfsibcomb3 label;
var Male halfsibeff halfsibstderr;
label halfsibeff='Biomass' halfsibstderr='Std Err';
run;

data fullsibcomb;
merge fixoff3 families2;
by var_p;
run;

data fullsibcomb2;
set fullsibcomb;
length fam_eff2 8;
length fam_stderr2 8;
run;

data fullsibcomb3;
set fullsibcomb2;
fam_eff2= Estimate + fam_effect;
fam_stderr2= fam_stderr;
run;

proc sort data=fullsibcomb3;
by descending fam_eff2;
run;

title 'Rankings of full-sib families';
```

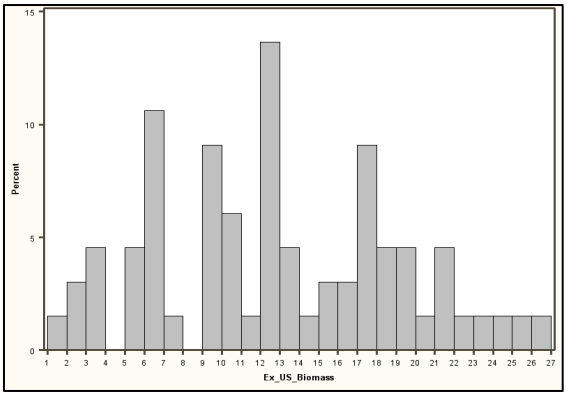
```
proc print data=fullsibcomb3 label;
var Male Female fam_eff2 fam_stderr2;
label fam_eff2='Biomass' fam_stderr2='Std Err';
run;
```

Appendix C: Histograms showing distribution of US and ex-US sweetpotato check clones for select traits.

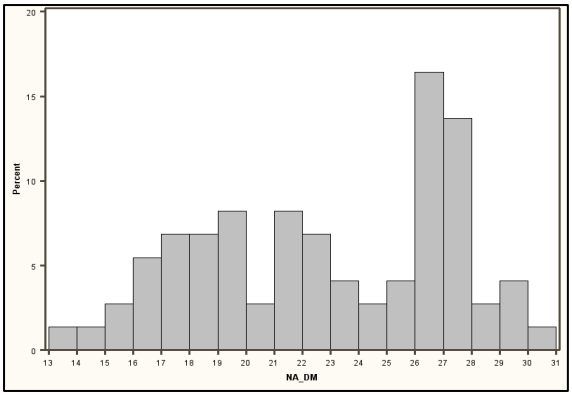
US Germplasm ex-US Germplasm

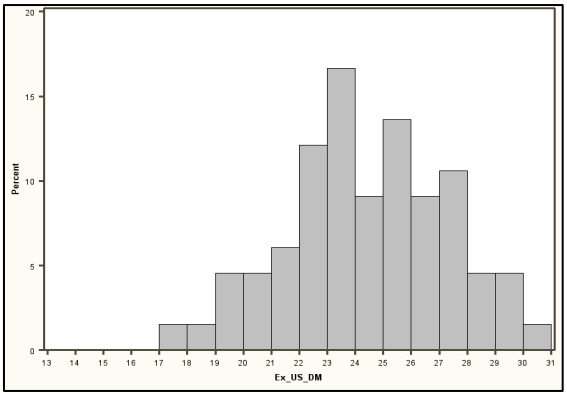
Biomass



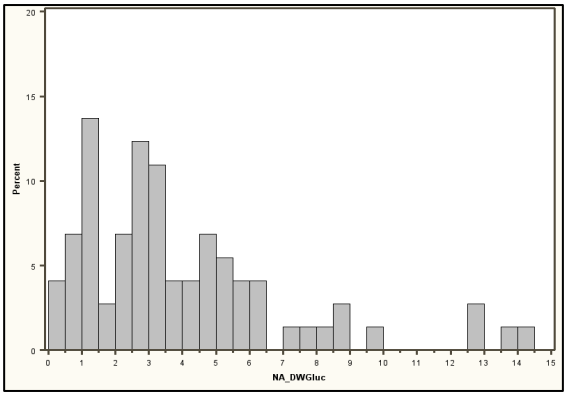


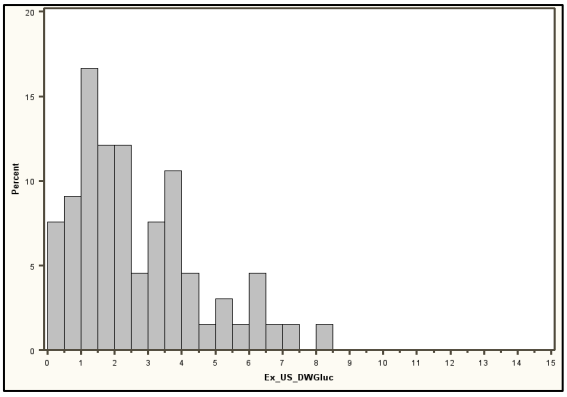
Dry Matter





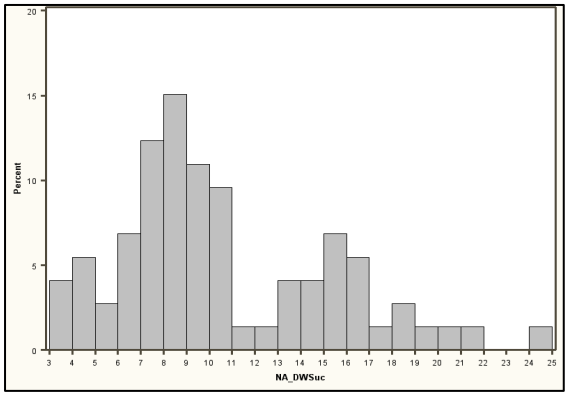
Dry Weight Basis Glucose

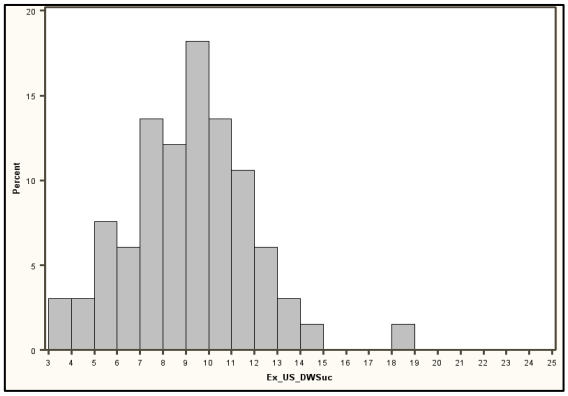




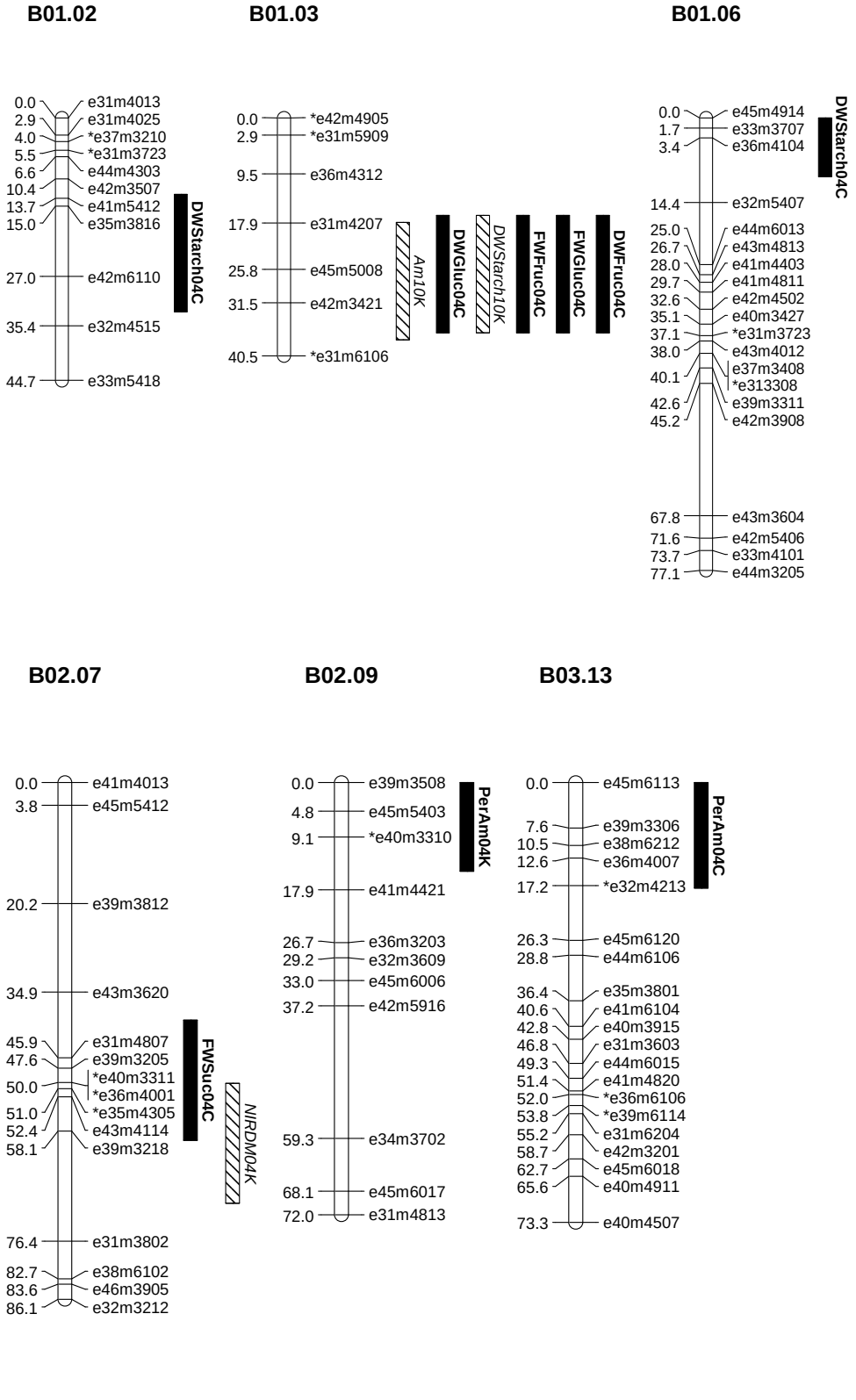
US Germplasm ex-US Germplasm

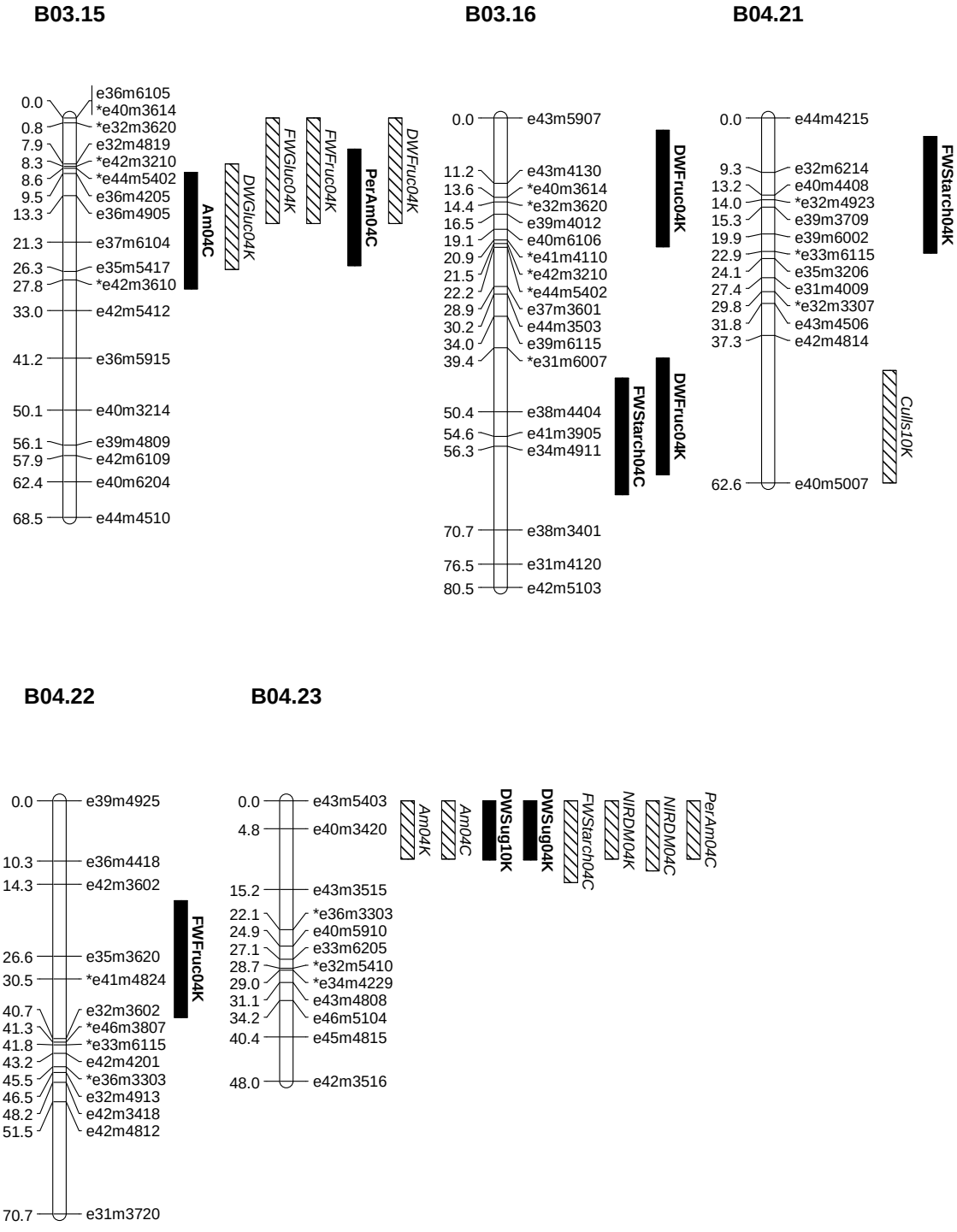
Dry Weight Basis Sucrose

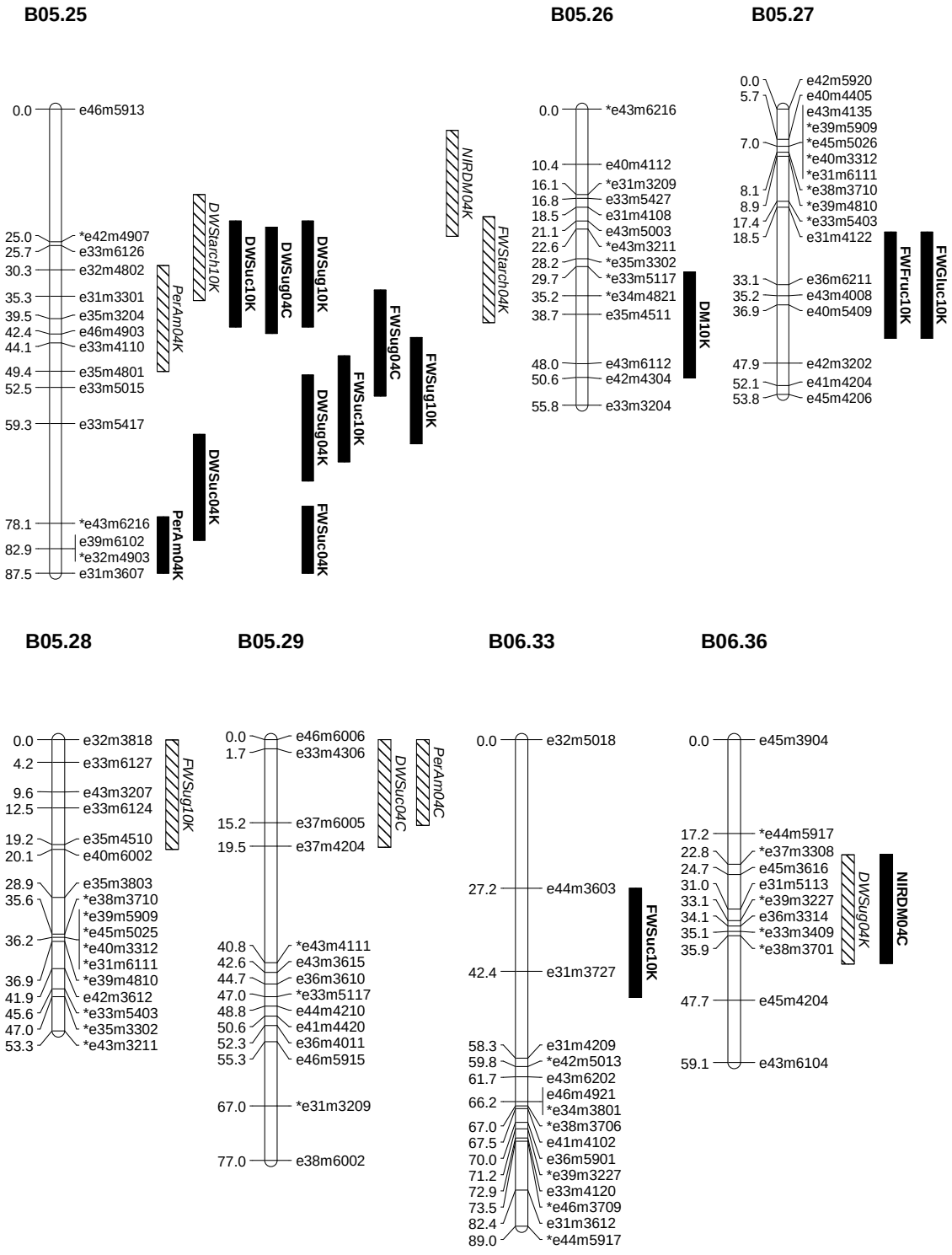


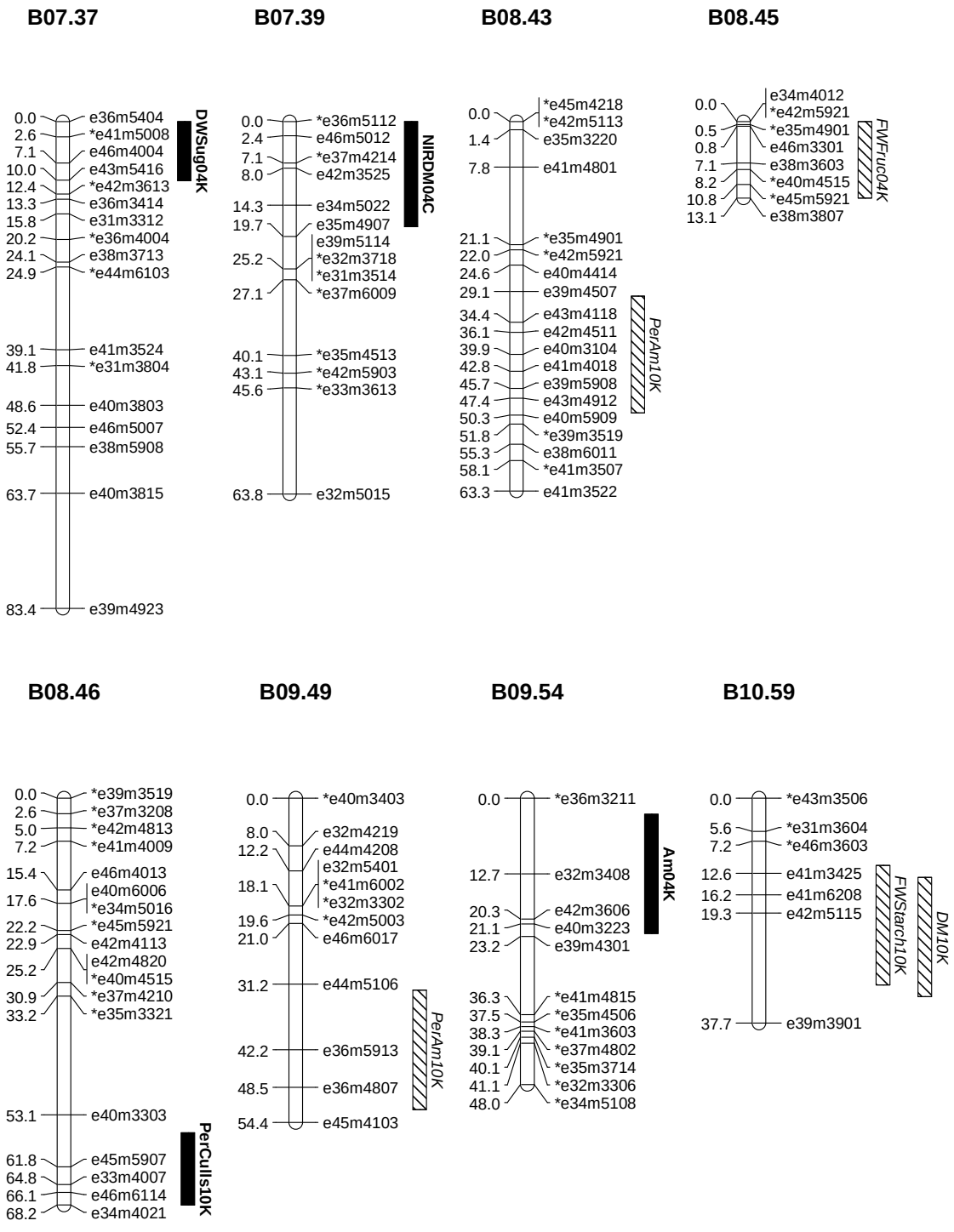


Appendix D: Comprehensive QTL maps for Beauregard and Tanzania




B03.15
0.0 e36m6105
*e40m3614
0.8 *e32m3620
7.9 e32m4819
8.3 *e42m3210
8.6 *e44m5402
9.5 e36m4205
13.3 e36m4905
21.3 e37m6104
26.3 e35m5417
27.8 *e42m3610
33.0 e42m5412
41.2 e36m5915
50.1 e40m3214
56.1 e39m4809
57.9 e42m6109
62.4 e40m6204
68.5 e44m4510
Am04C
DWGluc04K
FWGluc04K
FWFruc04K
PerAm04C
DWFruc04K
B03.16
0.0 e43m5907
11.2 e43m4130
13.6 *e40m3614
14.4 *e32m3620
16.5 e39m4012
19.1 e40m6106
20.9 *e41m4110
21.5 *e42m3210
22.2 *e44m5402
28.9 e37m3601
30.2 e44m3503
34.0 e39m6115
39.4 *e31m6007
50.4 e38m4404
54.6 e41m3905
56.3 e34m4911
70.7 e38m3401
76.5 e31m4120
80.5 e42m5103
DWFruc04K
FWStarch04C
DWFruc04K
B04.21
0.0 e44m4215
9.3 e32m6214
13.2 e40m4408
14.0 *e32m4923
15.3 e39m3709
19.9 e39m6002
22.9 *e33m6115
24.1 e35m3206
27.4 e31m4009
29.8 *e32m3307
31.8 e43m4506
37.3 e42m4814
62.6 e40m5007
FWStarch04K
Culls10K
B04.22
0.0 e39m4925
10.3 e36m4418
14.3 e42m3602
26.6 e35m3620
30.5 *e41m4824
40.7 e32m3602
41.3 *e46m3807
41.8 *e33m6115
43.2 e42m4201
45.5 *e36m3303
46.5 e32m4913
48.2 e42m3418
51.5 e42m4812
70.7 e31m3720
FWFruc04K
B04.23
0.0 e43m5403
4.8 e40m3420
15.2 e43m3515
22.1 *e36m3303
24.9 e40m5910
27.1 e33m6205
28.7 *e32m5410
29.0 *e34m4229
31.1 e43m4808
34.2 e46m5104
40.4 e45m4815
48.0 e42m3516
Am04K
Am04C
DWSug10K
DWSug04K
FWStarch04C
NIRDM04K
NIRDM04C
PerAm04C


B05.25
0.0 e46m5913
25.0 *e42m4907
25.7 e33m6126
30.3 e32m4802
35.3 e31m3301
39.5 e35m3204
42.4 e46m4903
44.1 e33m4110
49.4 e35m4801
52.5 e33m5015
59.3 e33m5417
78.1 *e43m6216
82.9 e39m6102
*e32m4903
87.5 e31m3607
DWStarch10K
PerAm04K
DWSuc10K
DWSuc04K
DWSug04C
DWSug10K
DWSug04K
FWSuc10K
FWSug04C
FWSug10K
FWSuc04K
B05.26
0.0 *e43m6216
10.4 e40m4112
16.1 *e31m3209
16.8 e33m5427
18.5 e31m4108
21.1 e43m5003
22.6 *e43m3211
28.2 *e35m3302
29.7 *e33m5117
35.2 *e34m4821
38.7 e35m4511
48.0 e43m6112
50.6 e42m4304
55.8 e33m3204
NIRDM04K
FWStarch04K
DM10K
B05.27
0.0 e42m5920
5.7 e40m4405
e43m4135
*e39m5909
7.0 *e45m5026
*e40m3312
*e31m6111
8.1 *e38m3710
8.9 *e39m4810
17.4 *e33m5403
18.5 e31m4122
33.1 e36m6211
35.2 e43m4008
36.9 e40m5409
47.9 e42m3202
52.1 e41m4204
53.8 e45m4206
FWGluc10K
FWFruc10K
B05.28
0.0 e32m3818
4.2 e33m6127
9.6 e43m3207
12.5 e33m6124
19.2 e35m4510
20.1 e40m6002
28.9 e35m3803
35.6 *e38m3710
*e39m5909
*e45m5025
36.2 *e40m3312
*e31m6111
36.9 *e39m4810
41.9 e42m3612
45.6 *e33m5403
47.0 *e35m3302
53.3 *e43m3211
FWSug10K
B05.29
0.0 e46m6006
1.7 e33m4306
15.2 e37m6005
19.5 e37m4204
40.8 *e43m4111
42.6 e43m3615
44.7 e36m3610
47.0 *e33m5117
48.8 e44m4210
50.6 e41m4420
52.3 e36m4011
55.3 e46m5915
67.0 *e31m3209
77.0 e38m6002
PerAm04C
DWSuc04C
B06.33
0.0 e32m5018
27.2 e44m3603
42.4 e31m3727
58.3 e31m4209
59.8 *e42m5013
61.7 e43m6202
e46m4921
66.2 *e34m3801
67.0 *e38m3706
67.5 e41m4102
70.0 e36m5901
71.2 *e39m3227
72.9 e33m4120
73.5 *e46m3709
82.4 e31m3612
89.0 *e44m5917
FWSuc10K
B06.36
0.0 e45m3904
17.2 *e44m5917
22.8 *e37m3308
24.7 e45m3616
31.0 e31m5113
33.1 *e39m3227
34.1 e36m3314
35.1 *e33m3409
35.9 *e38m3701
47.7 e45m4204
59.1 e43m6104
DWSug04K
NIRDM04C


B07.37
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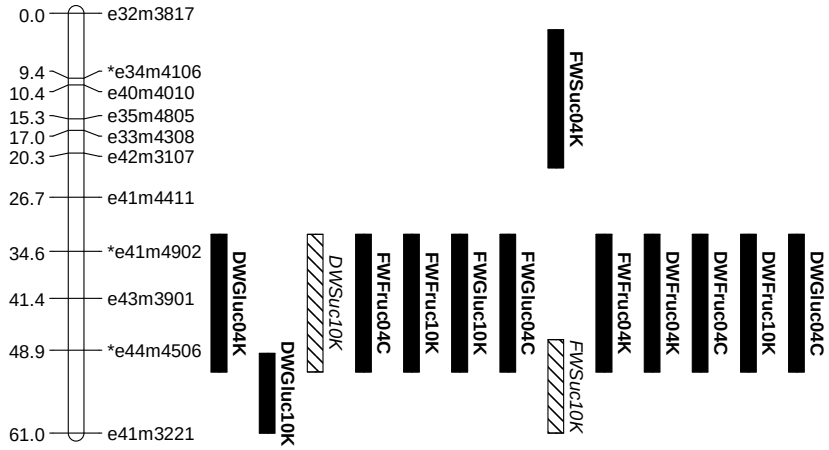
2.6 *e41m5008
7.1 e46m4004
10.0 e43m5416
12.4 *e42m3613
13.3 e36m3414
15.8 e31m3312
20.2 *e36m4004
24.1 e38m3713
24.9 *e44m6103
39.1 e41m3524
41.8 *e31m3804
48.6 e40m3803
52.4 e46m5007
55.7 e38m5908
63.7 e40m3815
83.4 e39m4923
DWSug04K
B07.39
0.0 *e36m5112
2.4 e46m5012
7.1 *e37m4214
8.0 e42m3525
14.3 e34m5022
19.7 e35m4907
e39m5114
25.2 *e32m3718
*e31m3514
27.1 *e37m6009
40.1 *e35m4513
43.1 *e42m5903
45.6 *e33m3613
63.8 e32m5015
NIRDM04C
B08.43
0.0 *e45m4218
*e42m5113
1.4 e35m3220
7.8 e41m4801
21.1 *e35m4901
22.0 *e42m5921
24.6 e40m4414
29.1 e39m4507
34.4 e43m4118
36.1 e42m4511
39.9 e40m3104
42.8 e41m4018
45.7 e39m5908
47.4 e43m4912
50.3 e40m5909
51.8 *e39m3519
55.3 e38m6011
58.1 *e41m3507
63.3 e41m3522
PerAm10K
B08.45
0.0 e34m4012
*e42m5921
0.5 *e35m4901
0.8 e46m3301
7.1 e38m3603
8.2 *e40m4515
10.8 *e45m5921
13.1 e38m3807
FWFruc04K
B08.46
0.0 *e39m3519
2.6 *e37m3208
5.0 *e42m4813
7.2 *e41m4009
15.4 e46m4013
e40m6006
17.6 *e34m5016
22.2 *e45m5921
22.9 e42m4113
e42m4820
25.2 *e40m4515
30.9 *e37m4210
33.2 *e35m3321
53.1 e40m3303
61.8 e45m5907
64.8 e33m4007
66.1 e46m6114
68.2 e34m4021
PerCulls10K
B09.49
0.0 *e40m3403
8.0 e32m4219
12.2 e44m4208
e32m5401
18.1 *e41m6002
*e32m3302
19.6 *e42m5003
21.0 e46m6017
31.2 e44m5106
42.2 e36m5913
48.5 e36m4807
54.4 e45m4103
PerAm10K
B09.54
0.0 *e36m3211
12.7 e32m3408
20.3 e42m3606
21.1 e40m3223
23.2 e39m4301
36.3 *e41m4815
37.5 *e35m4506
38.3 *e41m3603
39.1 *e37m4802
40.1 *e35m3714
41.1 *e32m3306
48.0 *e34m5108
Am04K
B10.59
0.0 *e43m3506
5.6 *e31m3604
7.2 *e46m3603
12.6 e41m3425
16.2 e41m6208
19.3 e42m5115
37.7 e39m3901
FWStarch10K
DM10K

B11.61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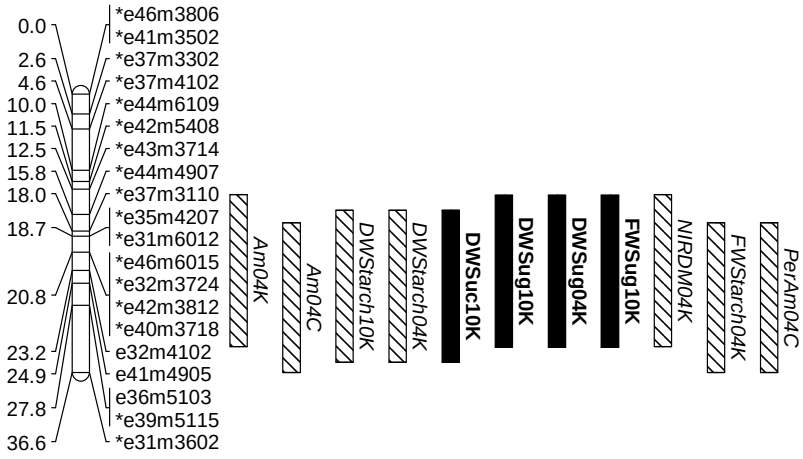
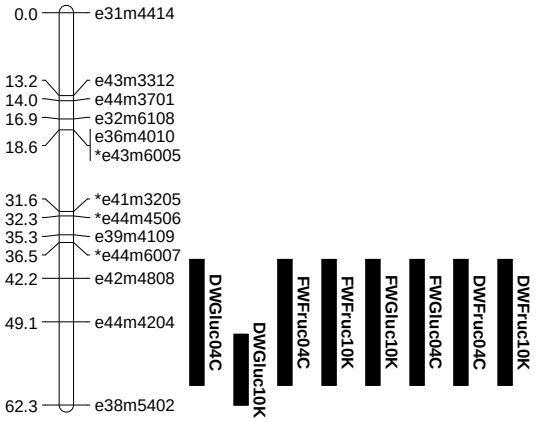
9.4
10.4
15.3
17.0
20.3
26.7
34.6
41.4
48.9
61.0
e32m3817
*e34m4106
e40m4010
e35m4805
e33m4308
e42m3107
e41m4411
*e41m4902
e43m3901
*e44m4506
e41m3221
DWGluc04K
DWGluc10K
DWSuc10K
FWFruc04C
FWFruc10K
FWGluc10K
FWGluc04C
FWSuc10K
FWSuc04K
FWFruc04K
DWFruc04K
DWFruc04C
DWFruc10K
DWGluc04C

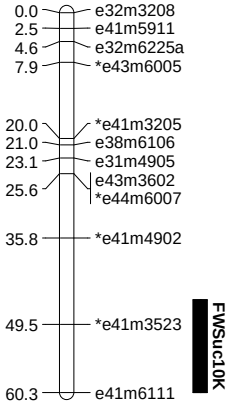
B11.62


0.0
2.6
4.6
10.0
11.5
12.5
15.8
18.0
18.7
20.8
23.2
24.9
27.8
36.6
*e46m3806
*e41m3502
*e37m3302
*e37m4102
*e44m6109
*e42m5408
*e43m3714
*e44m4907
*e37m3110
*e35m4207
*e31m6012
*e46m6015
*e32m3724
*e42m3812
*e40m3718
e32m4102
e41m4905
e36m5103
*e39m5115
*e31m3602
Am04K
Am04C
DWStarch10K
DWStarch04K
DWSuc10K
DWSug10K
DWSug04K
FWSug10K
NIRDM04K
FWStarch04K
PerAm04C

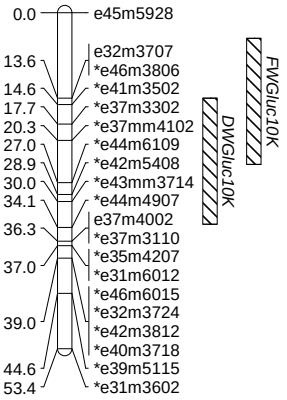
B11.64



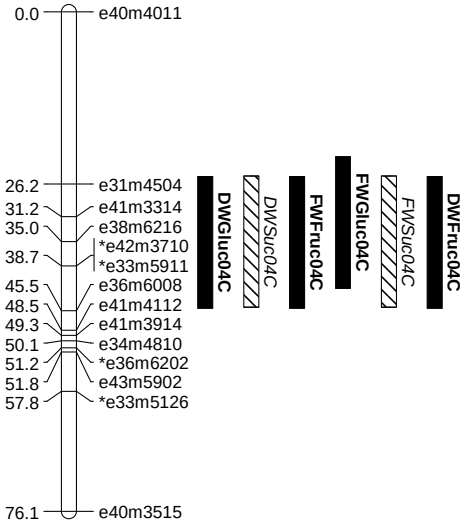
B11.65



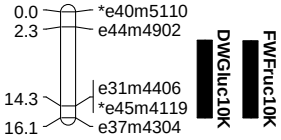
B11.66



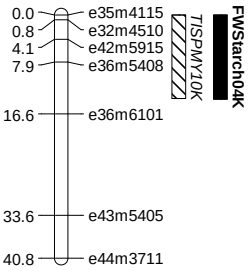
B12.67



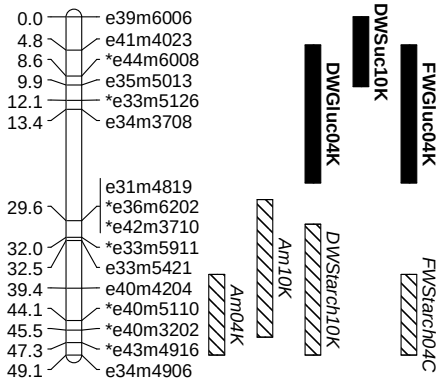
B12.69



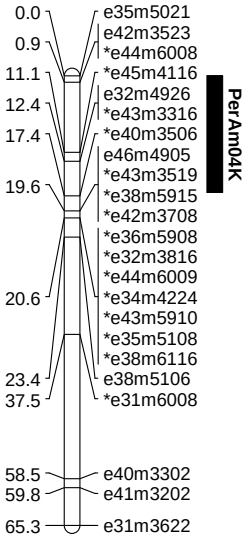
B89



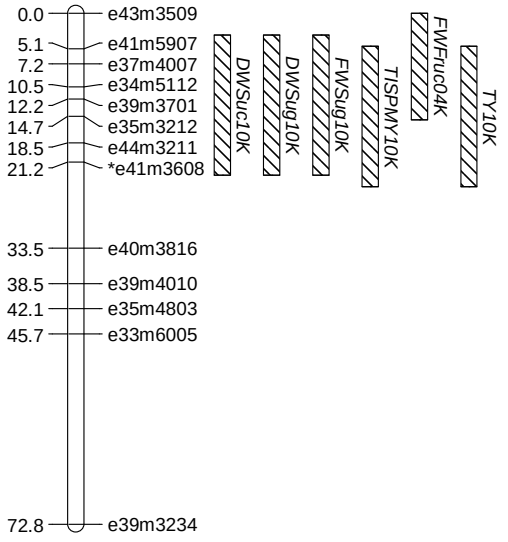
B12.70



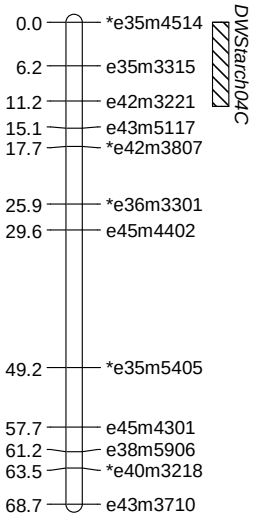
B12.71



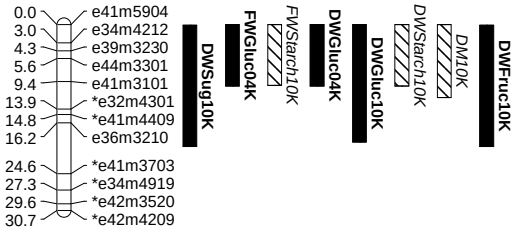
B13.73



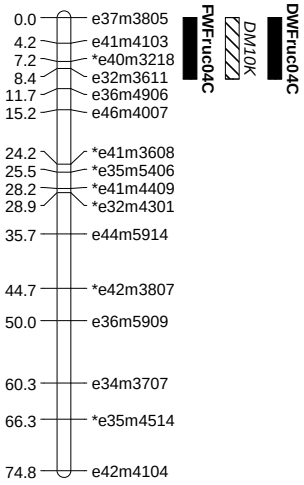
B13.74



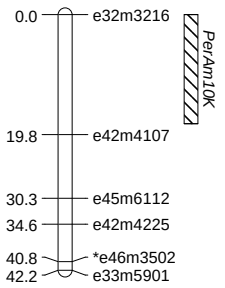
B13.75



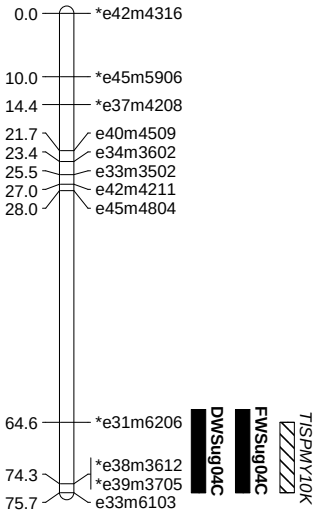
B13.76



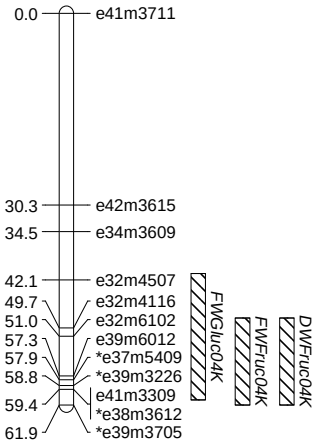
B14.81



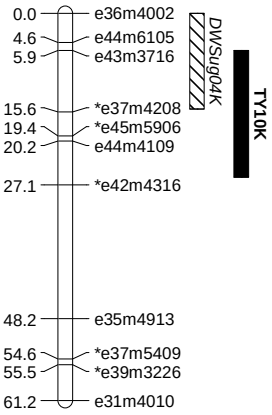
B15.86

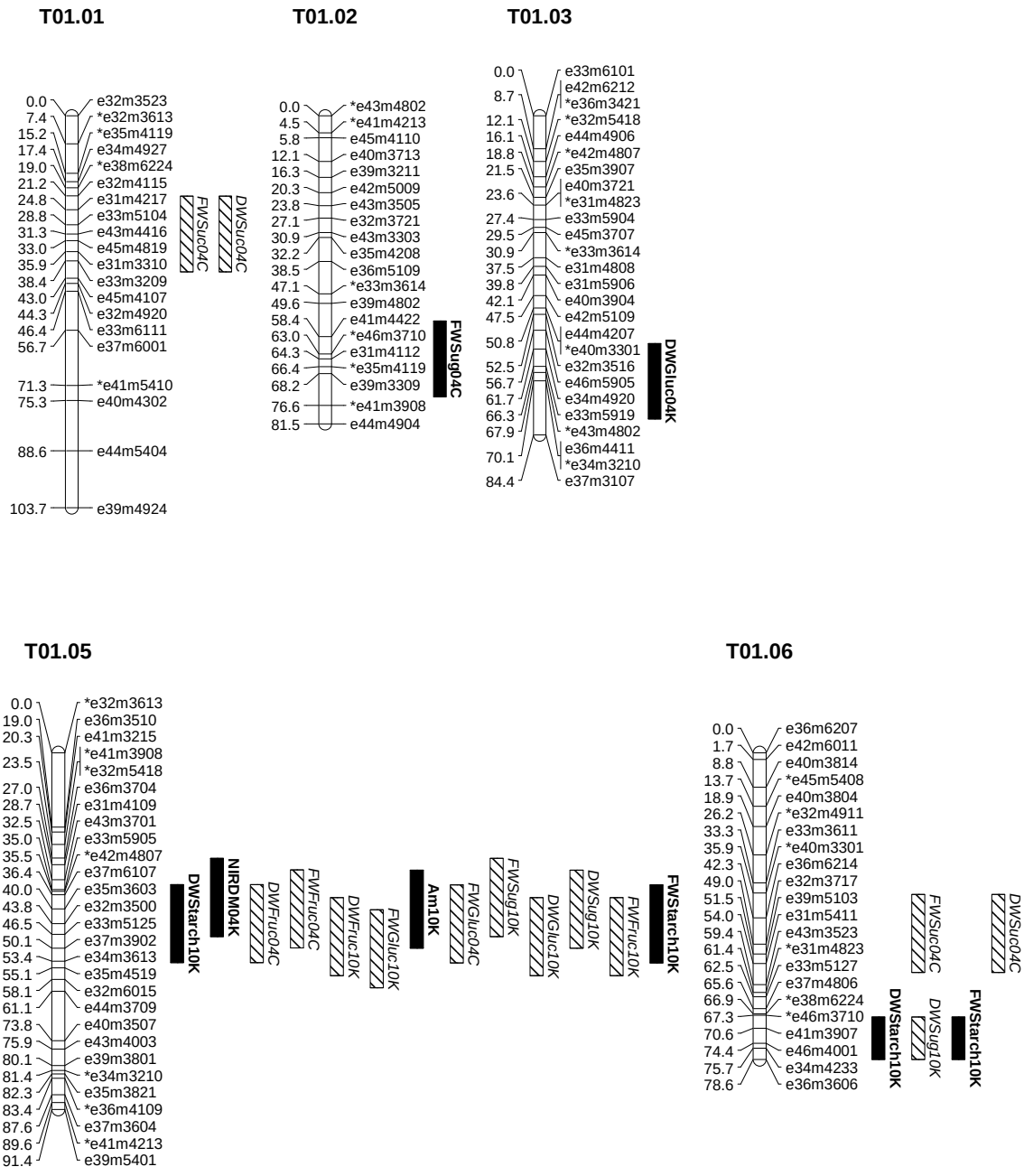


B15.87



B15.88

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7.4 *e32m3613
15.2 *e35m4119
17.4 e34m4927
19.0 *e38m6224
21.2 e32m4115
24.8 e31m4217
28.8 e33m5104
31.3 e43m4416
33.0 e45m4819
35.9 e31m3310
38.4 e33m3209
43.0 e45m4107
44.3 e32m4920
46.4 e33m6111
56.7 e37m6001
71.3 *e41m5410
75.3 e40m4302
88.6 e44m5404
103.7 e39m4924
FWSuc04C
DWSuc04C
T01.02
0.0 *e43m4802
4.5 *e41m4213
5.8 e45m4110
12.1 e40m3713
16.3 e39m3211
20.3 e42m5009
23.8 e43m3505
27.1 e32m3721
30.9 e43m3303
32.2 e35m4208
38.5 e36m5109
47.1 *e33m3614
49.6 e39m4802
58.4 e41m4422
63.0 *e46m3710
64.3 e31m4112
66.4 *e35m4119
68.2 e39m3309
76.6 *e41m3908
81.5 e44m4904
FWSug04C
T01.03
0.0 e33m6101
8.7 e42m6212
12.1 *e36m3421
16.1 *e32m5418
18.8 e44m4906
21.5 *e42m4807
23.6 e35m3907
27.4 e40m3721
29.5 *e31m4823
30.9 e33m5904
37.5 e45m3707
39.8 *e33m3614
42.1 e31m4808
47.5 e31m5906
50.8 e40m3904
52.5 e42m5109
56.7 e44m4207
61.7 *e40m3301
66.3 e32m3516
67.9 e46m5905
70.1 e34m4920
84.4 e33m5919
*e43m4802
e36m4411
*e34m3210
e37m3107
DWGluc04K
T01.05
0.0 *e32m3613
19.0 e36m3510
20.3 e41m3215
23.5 *e41m3908
*e32m5418
27.0 e36m3704
28.7 e31m4109
32.5 e43m3701
35.0 e33m5905
35.5 *e42m4807
36.4 e37m6107
40.0 e35m3603
43.8 e32m3500
46.5 e33m5125
50.1 e37m3902
53.4 e34m3613
55.1 e35m4519
58.1 e32m6015
61.1 e44m3709
73.8 e40m3507
75.9 e43m4003
80.1 e39m3801
81.4 *e34m3210
82.3 e35m3821
83.4 *e36m4109
87.6 e37m3604
89.6 *e41m4213
91.4 e39m5401
DWStarch10K
NIRDM04K
DWFruc04C
FWFruc04C
DWFruc10K
FWGluc10K
Am10K
FWGluc04C
FWSug10K
DWGluc10K
DWSug10K
FWFruc10K
FWStarch10K
T01.06
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1.7 e42m6011
8.8 e40m3814
13.7 *e45m5408
18.9 e40m3804
26.2 *e32m4911
33.3 e33m3611
35.9 *e40m3301
42.3 e36m6214
49.0 e32m3717
51.5 e39m5103
54.0 e31m5411
59.4 e43m3523
61.4 *e31m4823
62.5 e33m5127
65.6 e37m4806
66.9 *e38m6224
67.3 *e46m3710
70.6 e41m3907
74.4 e46m4001
75.7 e34m4233
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DWStarch10K
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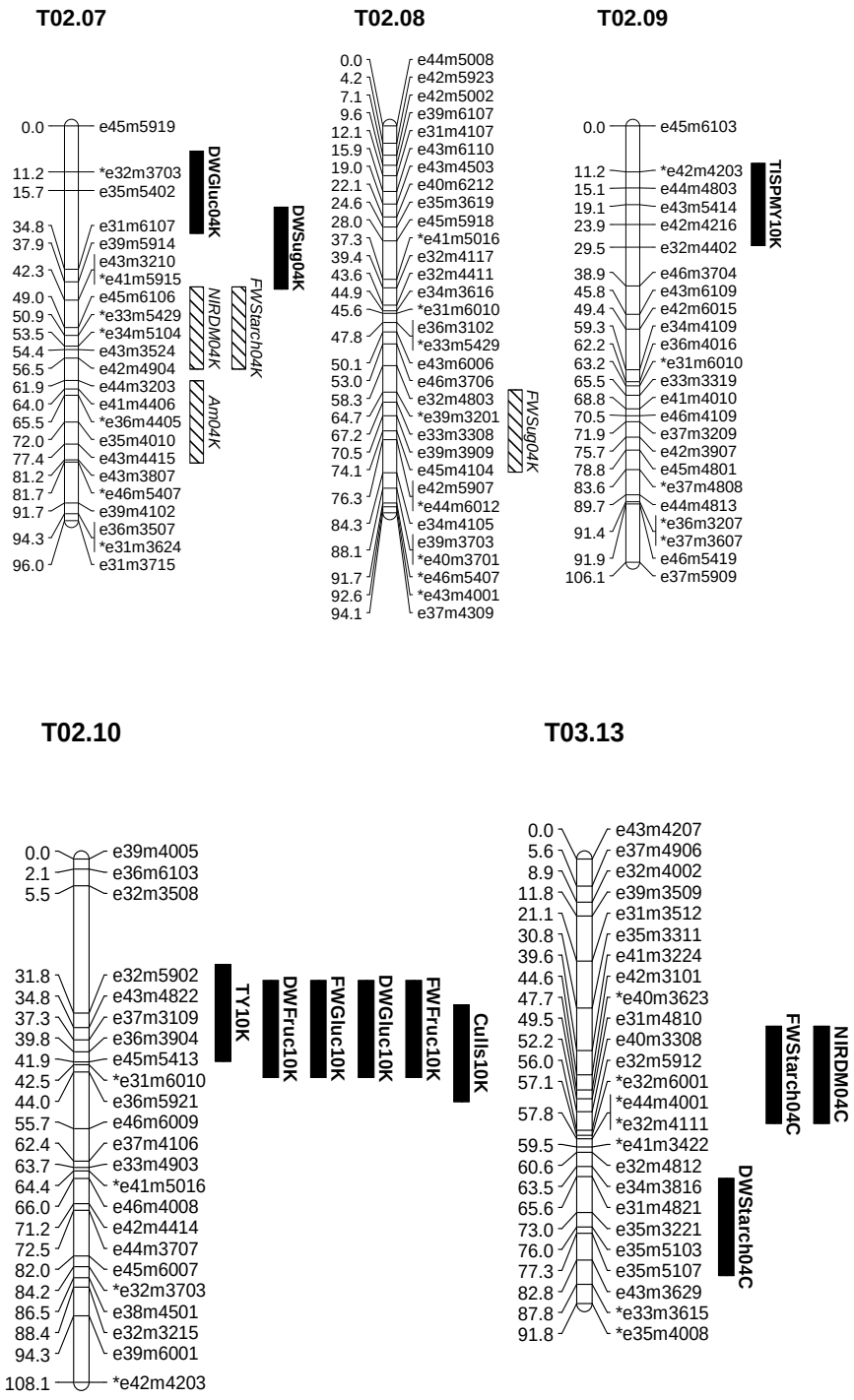
FWStarch10K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34.8 e31m6107
37.9 e39m5914
42.3 e43m3210
*e41m5915
49.0 e45m6106
50.9 *e33m5429
53.5 *e34m5104
54.4 e43m3524
56.5 e42m4904
61.9 e44m3203
64.0 e41m4406
65.5 *e36m4405
72.0 e35m4010
77.4 e43m4415
81.2 e43m3807
81.7 *e46m5407
91.7 e39m4102
94.3 e36m3507
*e31m3624
96.0 e31m3715
DWGluc04K
DWSug04K
FWStarch04K
NIRDM04K
Am04K
T02.08
0.0 e44m5008
4.2 e42m5923
7.1 e42m5002
9.6 e39m6107
12.1 e31m4107
15.9 e43m6110
19.0 e43m4503
22.1 e40m6212
24.6 e35m3619
28.0 e45m5918
37.3 *e41m5016
39.4 e32m4117
43.6 e32m4411
44.9 e34m3616
45.6 *e31m6010
47.8 e36m3102
*e33m5429
50.1 e43m6006
53.0 e46m3706
58.3 e32m4803
64.7 *e39m3201
67.2 e33m3308
70.5 e39m3909
74.1 e45m4104
76.3 e42m5907
*e44m6012
84.3 e34m4105
88.1 e39m3703
*e40m3701
91.7 *e46m5407
92.6 *e43m4001
94.1 e37m4309
FWSug04K
T02.09
0.0 e45m6103
11.2 *e42m4203
15.1 e44m4803
19.1 e43m5414
23.9 e42m4216
29.5 e32m4402
38.9 e46m3704
45.8 e43m6109
49.4 e42m6015
59.3 e34m4109
62.2 e36m4016
63.2 *e31m6010
65.5 e33m3319
68.8 e41m4010
70.5 e46m4109
71.9 e37m3209
75.7 e42m3907
78.8 e45m4801
83.6 *e37m4808
89.7 e44m4813
91.4 *e36m3207
*e37m3607
91.9 e46m5419
106.1 e37m5909
TISPMY10K
T02.10
0.0 e39m4005
2.1 e36m6103
5.5 e32m3508
31.8 e32m5902
34.8 e43m4822
37.3 e37m3109
39.8 e36m3904
41.9 e45m5413
42.5 *e31m6010
44.0 e36m5921
55.7 e46m6009
62.4 e37m4106
63.7 e33m4903
64.4 *e41m5016
66.0 e46m4008
71.2 e42m4414
72.5 e44m3707
82.0 e45m6007
84.2 *e32m3703
86.5 e38m4501
88.4 e32m3215
94.3 e39m6001
108.1 *e42m4203
TY10K
DWFruc10K
FWGluc10K
DWGluc10K
FWFruc10K
Culls10K
T03.13
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5.6 e37m4906
8.9 e32m4002
11.8 e39m3509
21.1 e31m3512
30.8 e35m3311
39.6 e41m3224
44.6 e42m3101
47.7 *e40m3623
49.5 e31m4810
52.2 e40m3308
56.0 e32m5912
57.1 *e32m6001
*e44m4001
57.8 *e32m4111
59.5 *e41m3422
60.6 e32m4812
63.5 e34m3816
65.6 e31m4821
73.0 e35m3221
76.0 e35m5103
77.3 e35m5107
82.8 e43m3629
87.8 *e33m3615
91.8 *e35m4008
FWStarch04C
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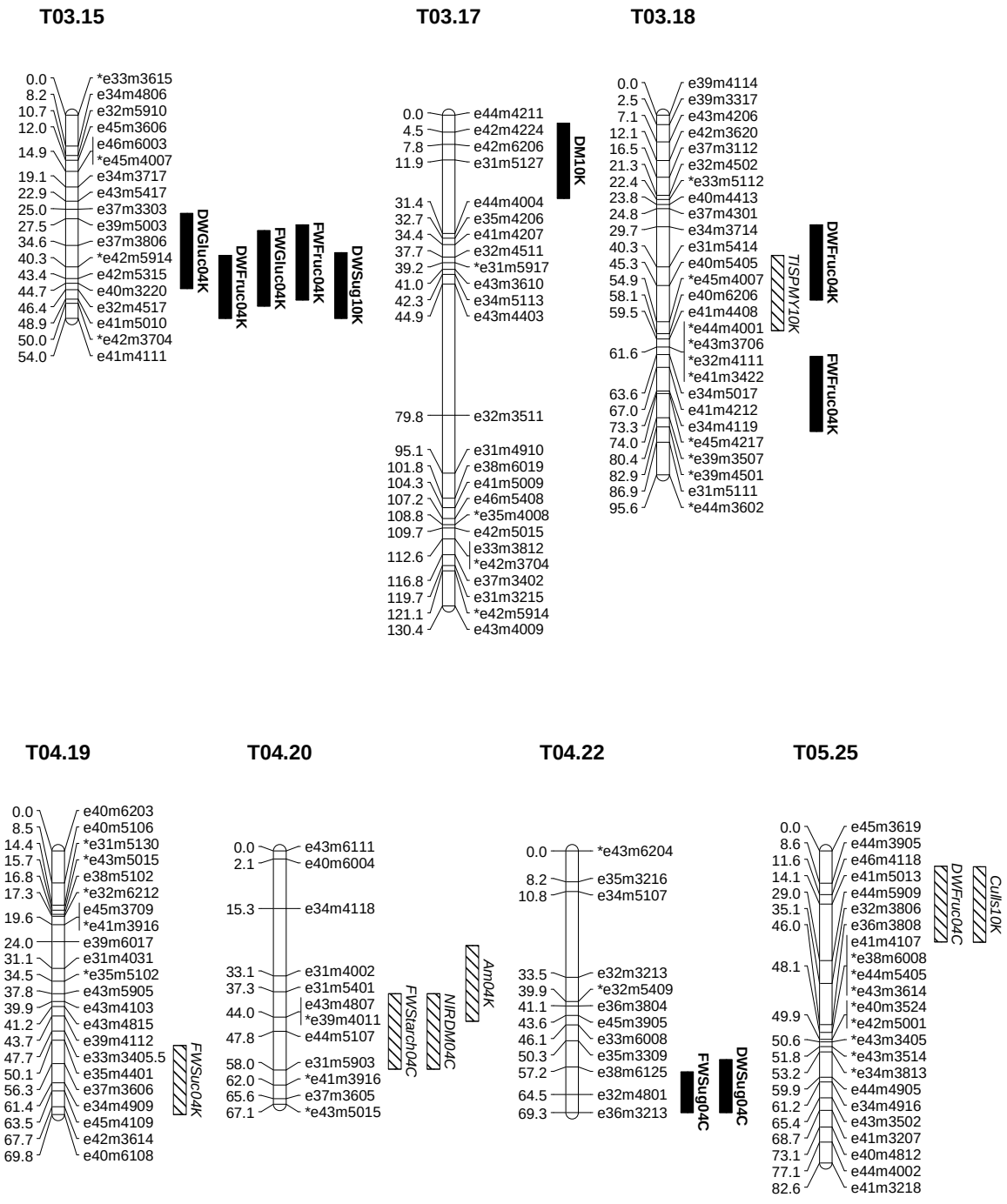
DWStarch04C


T03.15
T03.17
T03.18
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8.2 e34m4806
10.7 e32m5910
12.0 e45m3606
14.9 e46m6003
*e45m4007
19.1 e34m3717
22.9 e43m5417
25.0 e37m3303
27.5 e39m5003
34.6 e37m3806
40.3 *e42m5914
43.4 e42m5315
44.7 e40m3220
46.4 e32m4517
48.9 e41m5010
50.0 *e42m3704
54.0 e41m4111
DWGluc04K
DWFruc04K
FWGluc04K
FWFruc04K
DWSug10K
0.0 e44m4211
4.5 e42m4224
7.8 e42m6206
11.9 e31m5127
31.4 e44m4004
32.7 e35m4206
34.4 e41m4207
37.7 e32m4511
39.2 *e31m5917
41.0 e43m3610
42.3 e34m5113
44.9 e43m4403
79.8 e32m3511
95.1 e31m4910
101.8 e38m6019
104.3 e41m5009
107.2 e46m5408
108.8 *e35m4008
109.7 e42m5015
112.6 e33m3812
*e42m3704
116.8 e37m3402
119.7 e31m3215
121.1 *e42m5914
130.4 e43m4009
DM10K
0.0 e39m4114
2.5 e39m3317
7.1 e43m4206
12.1 e42m3620
16.5 e37m3112
21.3 e32m4502
22.4 *e33m5112
23.8 e40m4413
24.8 e37m4301
29.7 e34m3714
40.3 e31m5414
45.3 e40m5405
54.9 *e45m4007
58.1 e40m6206
59.5 e41m4408
*e44m4001
61.6 *e43m3706
*e32m4111
*e41m3422
63.6 e34m5017
67.0 e41m4212
73.3 e34m4119
74.0 *e45m4217
80.4 *e39m3507
82.9 *e39m4501
86.9 e31m5111
95.6 *e44m3602
TISPMY10K
DWFruc04K
FWFruc04K
T04.19
T04.20
T04.22
T05.25
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8.5 e40m5106
14.4 *e31m5130
15.7 *e43m5015
16.8 e38m5102
17.3 *e32m6212
19.6 e45m3709
*e41m3916
24.0 e39m6017
31.1 e31m4031
34.5 *e35m5102
37.8 e43m5905
39.9 e43m4103
41.2 e43m4815
43.7 e39m4112
47.7 e33m3405.5
50.1 e35m4401
56.3 e37m3606
61.4 e34m4909
63.5 e45m4109
67.7 e42m3614
69.8 e40m6108
FWSuc04K
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2.1 e40m6004
15.3 e34m4118
33.1 e31m4002
37.3 e31m5401
44.0 e43m4807
*e39m4011
47.8 e44m5107
58.0 e31m5903
62.0 *e41m3916
65.6 e37m3605
67.1 *e43m5015
Am04K
NIRDM04C
FWStarch04C
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8.2 e35m3216
10.8 e34m5107
33.5 e32m3213
39.9 *e32m5409
41.1 e36m3804
43.6 e45m3905
46.1 e33m6008
50.3 e35m3309
57.2 e38m6125
64.5 e32m4801
69.3 e36m3213
FWSug04C
DWSug04C
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8.6 e44m3905
11.6 e46m4118
14.1 e41m5013
29.0 e44m5909
35.1 e32m3806
46.0 e36m3808
e41m4107
48.1 *e38m6008
*e44m5405
*e43m3614
*e40m3524
49.9 *e42m5001
50.6 *e43m3405
51.8 *e43m3514
53.2 *e34m3813
59.9 e44m4905
61.2 e34m4916
65.4 e43m3502
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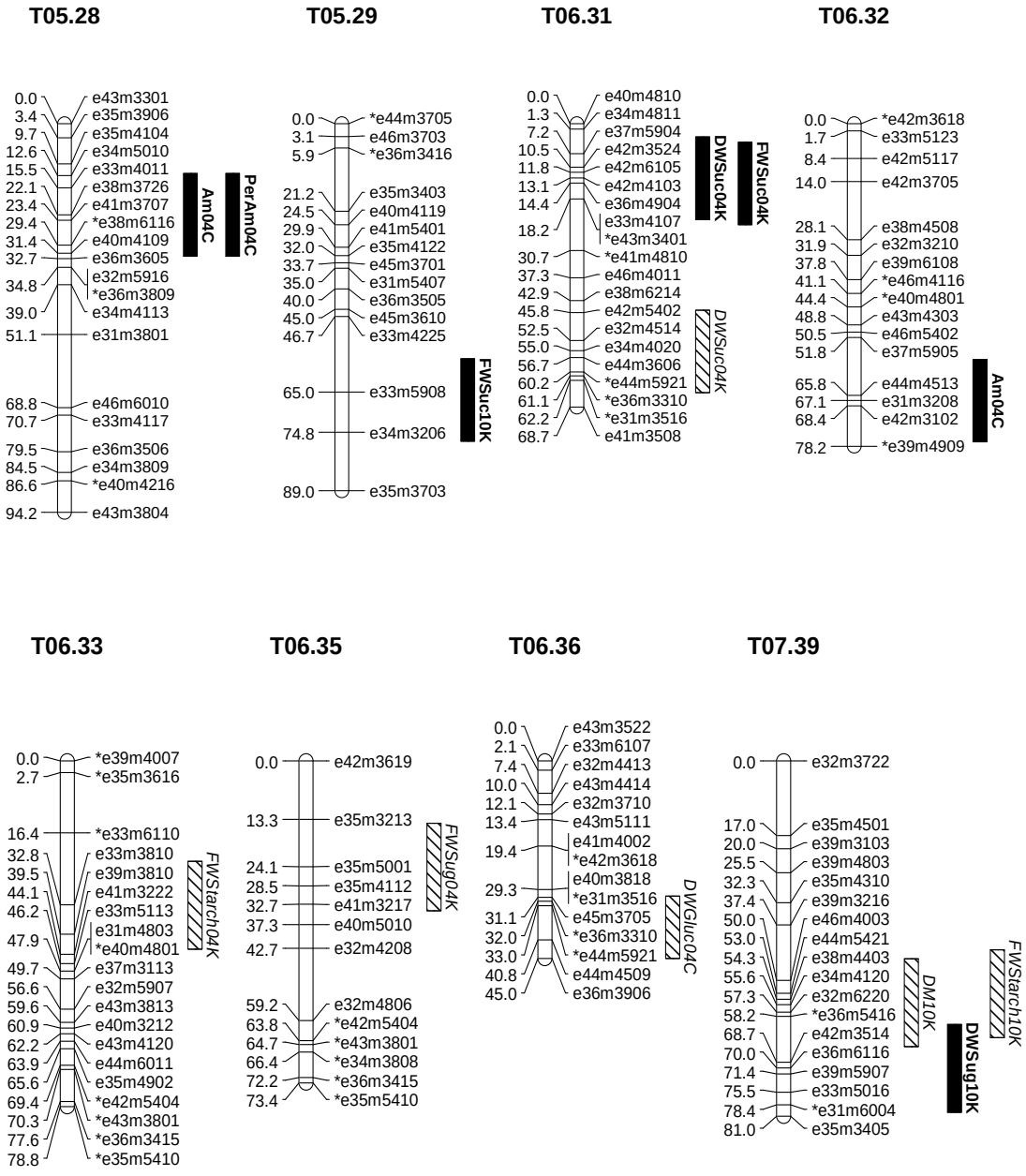
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15.5 e33m4011
22.1 e38m3726
23.4 e41m3707
29.4 *e38m6116
31.4 e40m4109
32.7 e36m3605
34.8 e32m5916
*e36m3809
39.0 e34m4113
51.1 e31m3801
68.8 e46m6010
70.7 e33m4117
79.5 e36m3506
84.5 e34m3809
86.6 *e40m4216
94.2 e43m3804
Am04C
PerAm04C
T05.29
0.0 *e44m3705
3.1 e46m3703
5.9 *e36m3416
21.2 e35m3403
24.5 e40m4119
29.9 e41m5401
32.0 e35m4122
33.7 e45m3701
35.0 e31m5407
40.0 e36m3505
45.0 e45m3610
46.7 e33m4225
65.0 e33m5908
74.8 e34m3206
89.0 e35m3703
FWSuc10K
T06.31
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1.3 e34m4811
7.2 e37m5904
10.5 e42m3524
11.8 e42m6105
13.1 e42m4103
14.4 e36m4904
18.2 e33m4107
*e43m3401
30.7 *e41m4810
37.3 e46m4011
42.9 e38m6214
45.8 e42m5402
52.5 e32m4514
55.0 e34m4020
56.7 e44m3606
60.2 *e44m5921
61.1 *e36m3310
62.2 *e31m3516
68.7 e41m3508
DWSuc04K
FWSuc04K
T06.32
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1.7 e33m5123
8.4 e42m5117
14.0 e42m3705
28.1 e38m4508
31.9 e32m3210
37.8 e39m6108
41.1 *e46m4116
44.4 *e40m4801
48.8 e43m4303
50.5 e46m5402
51.8 e37m5905
65.8 e44m4513
67.1 e31m3208
68.4 e42m3102
78.2 *e39m4909
Am04C
T06.33
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16.4 *e33m6110
32.8 e33m3810
39.5 e39m3810
44.1 e41m3222
46.2 e33m5113
47.9 e31m4803
*e40m4801
49.7 e37m3113
56.6 e32m5907
59.6 e43m3813
60.9 e40m3212
62.2 e43m4120
63.9 e44m6011
65.6 e35m4902
69.4 *e42m5404
70.3 *e43m3801
77.6 *e36m3415
78.8 *e35m5410
FWStarch04K
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24.1 e35m5001
28.5 e35m4112
32.7 e41m3217
37.3 e40m5010
42.7 e32m4208
59.2 e32m4806
63.8 *e42m5404
64.7 *e43m3801
66.4 *e34m3808
72.2 *e36m3415
73.4 *e35m5410
FWSug04K
T06.36
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2.1 e33m6107
7.4 e32m4413
10.0 e43m4414
12.1 e32m3710
13.4 e43m5111
19.4 e41m4002
*e42m3618
29.3 e40m3818
*e31m3516
31.1 e45m3705
32.0 *e36m3310
33.0 *e44m5921
40.8 e44m4509
45.0 e36m3906
DWGluc04C
T07.39
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17.0 e35m4501
20.0 e39m3103
25.5 e39m4803
32.3 e35m4310
37.4 e39m3216
50.0 e46m4003
53.0 e44m5421
54.3 e38m4403
55.6 e34m4120
57.3 e32m6220
58.2 *e36m5416
68.7 e42m3514
70.0 e36m6116
71.4 e39m5907
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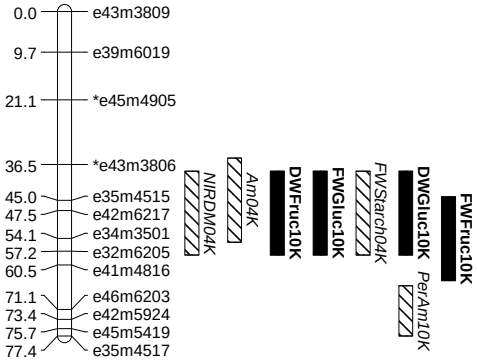
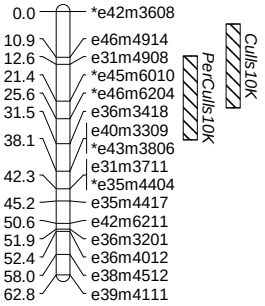
DM10K
FWStarch10K
DWSug10K

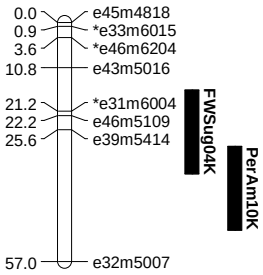
T07.40



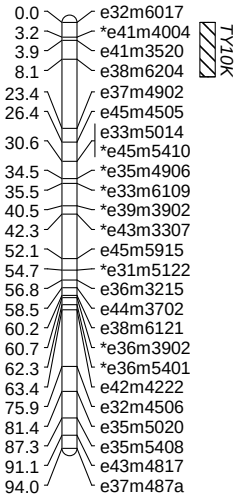
T07.41



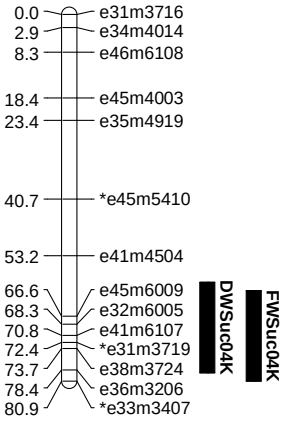
T07.42



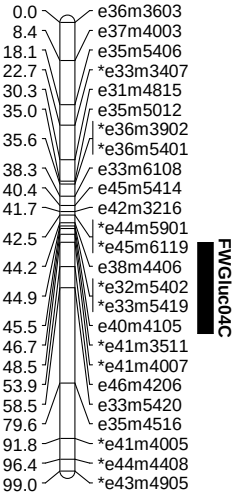
T08.43



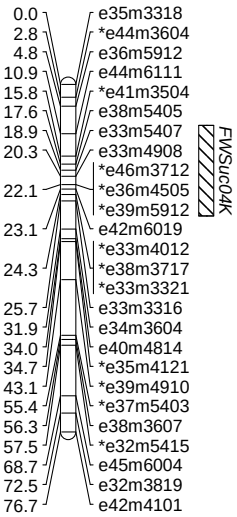
T08.45

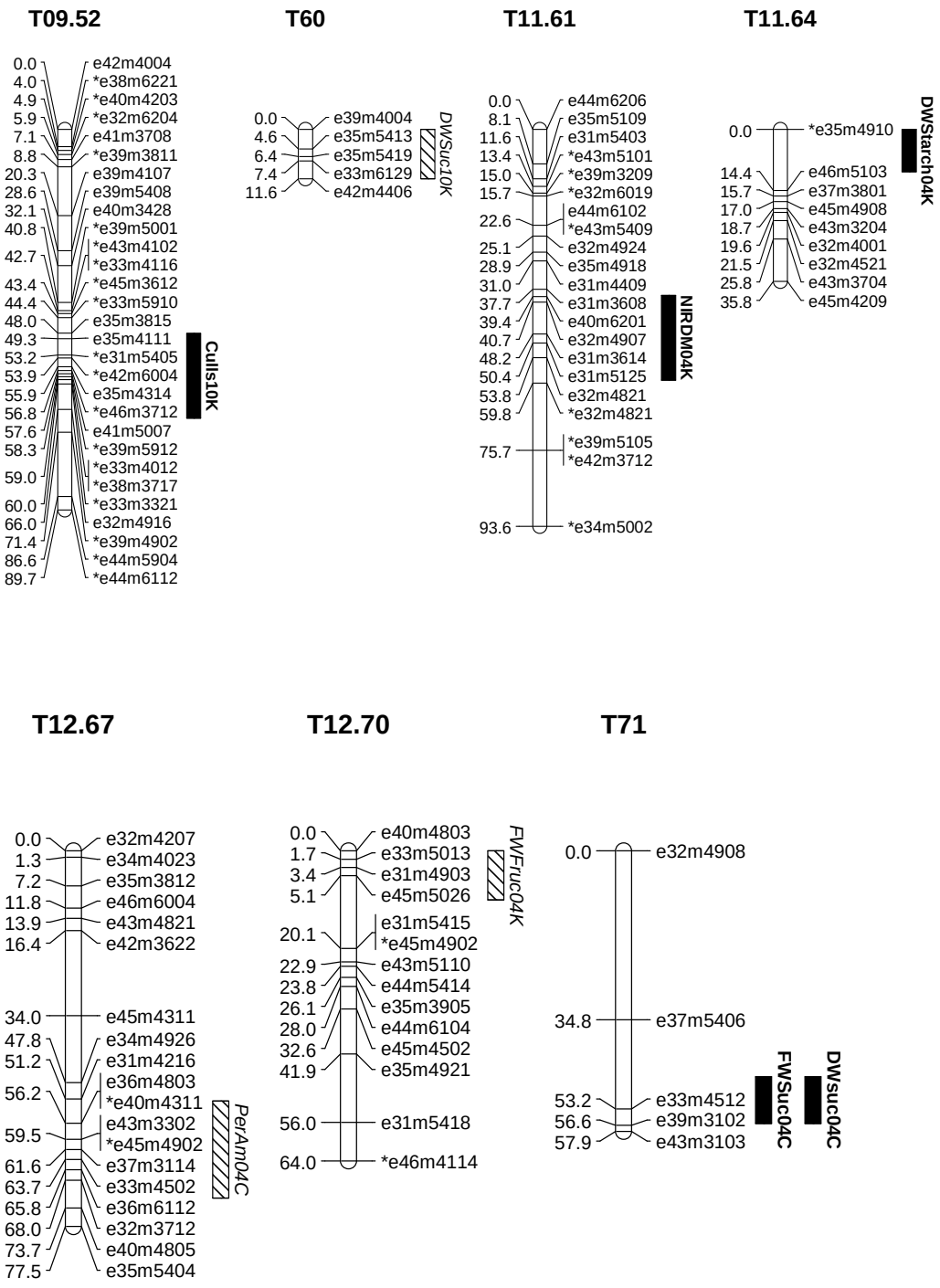


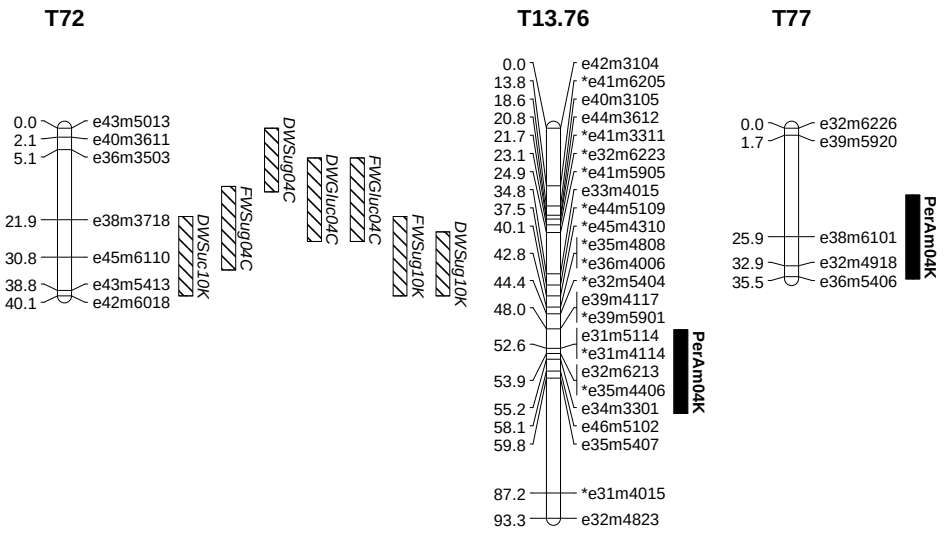
T08.47

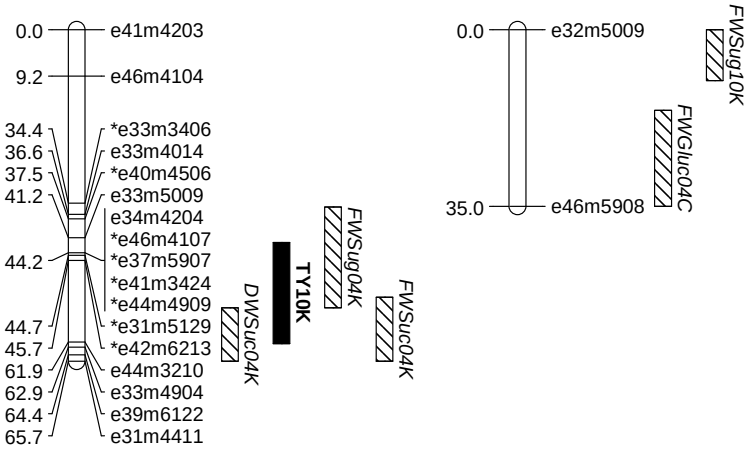


T09.49




T09.52
0.0 e42m4004
4.0 *e38m6221
4.9 *e40m4203
5.9 *e32m6204
7.1 e41m3708
8.8 *e39m3811
20.3 e39m4107
28.6 e39m5408
32.1 e40m3428
40.8 *e39m5001
42.7 *e43m4102
*e33m4116
43.4 *e45m3612
44.4 *e33m5910
48.0 e35m3815
49.3 e35m4111
53.2 *e31m5405
53.9 *e42m6004
55.9 e35m4314
56.8 *e46m3712
57.6 e41m5007
58.3 *e39m5912
59.0 *e33m4012
*e38m3717
60.0 *e33m3321
66.0 e32m4916
71.4 *e39m4902
86.6 *e44m5904
89.7 *e44m6112
Culls10K
T60
0.0 e39m4004
4.6 e35m5413
6.4 e35m5419
7.4 e33m6129
11.6 e42m4406
DWSuc10K
T11.61
0.0 e44m6206
8.1 e35m5109
11.6 e31m5403
13.4 *e43m5101
15.0 *e39m3209
15.7 *e32m6019
22.6 e44m6102
*e43m5409
25.1 e32m4924
28.9 e35m4918
31.0 e31m4409
37.7 e31m3608
39.4 e40m6201
40.7 e32m4907
48.2 e31m3614
50.4 e31m5125
53.8 e32m4821
59.8 *e32m4821
75.7 *e39m5105
*e42m3712
93.6 *e34m5002
NIRDM04K
T11.64
0.0 *e35m4910
14.4 e46m5103
15.7 e37m3801
17.0 e45m4908
18.7 e43m3204
19.6 e32m4001
21.5 e32m4521
25.8 e43m3704
35.8 e45m4209
DWStarch04K
T12.67
0.0 e32m4207
1.3 e34m4023
7.2 e35m3812
11.8 e46m6004
13.9 e43m4821
16.4 e42m3622
34.0 e45m4311
47.8 e34m4926
51.2 e31m4216
56.2 e36m4803
*e40m4311
59.5 e43m3302
*e45m4902
61.6 e37m3114
63.7 e33m4502
65.8 e36m6112
68.0 e32m3712
73.7 e40m4805
77.5 e35m5404
PerAm04C
T12.70
0.0 e40m4803
1.7 e33m5013
3.4 e31m4903
5.1 e45m5026
20.1 e31m5415
*e45m4902
22.9 e43m5110
23.8 e44m5414
26.1 e35m3905
28.0 e44m6104
32.6 e45m4502
41.9 e35m4921
56.0 e31m5418
64.0 *e46m4114
FWFruc04K
T71
0.0 e32m4908
34.8 e37m5406
53.2 e33m4512
56.6 e39m3102
57.9 e43m3103
FWSuc04C
DWsuc04C


T72
0.0 e43m5013
2.1 e40m3611
5.1 e36m3503
21.9 e38m3718
30.8 e45m6110
38.8 e43m5413
40.1 e42m6018
DWSug04C
FWGluc04C
DWGluc04C
FWSug10K
DWSug10K
FWSug04C
DWSuc10K
T13.76
0.0 e42m3104
13.8 *e41m6205
18.6 e40m3105
20.8 e44m3612
21.7 *e41m3311
23.1 *e32m6223
24.9 *e41m5905
34.8 e33m4015
37.5 *e44m5109
40.1 *e45m4310
42.8 *e35m4808
*e36m4006
44.4 *e32m5404
e39m4117
48.0 *e39m5901
52.6 e31m5114
*e31m4114
53.9 e32m6213
*e35m4406
55.2 e34m3301
58.1 e46m5102
59.8 e35m5407
87.2 *e31m4015
93.3 e32m4823
PerAm04K
T77
0.0 e32m6226
1.7 e39m5920
25.9 e38m6101
32.9 e32m4918
35.5 e36m5406
PerAm04K


T14.81
0.0 e41m4203
9.2 e46m4104
34.4 *e33m3406
36.6 e33m4014
37.5 *e40m4506
41.2 e33m5009
e34m4204
*e46m4107
44.2 *e37m5907
*e41m3424
*e44m4909
44.7 *e31m5129
45.7 *e42m6213
61.9 e44m3210
62.9 e33m4904
64.4 e39m6122
65.7 e31m4411
TY10K
FWSug04K
FWSuc04K
DWSuc04K
T83
0.0 e32m5009
35.0 e46m5908
FWSug10K
FWGluc04C