Improvement of Sweet and Vegetable Corn Quality for Organic Production Systems

By

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A dissertation submitted in partial fulfillment of

the requirements for the degree of

Doctor of Philosophy

(Plant Breeding & Plant Genetics)

at the

UNIVERSITY OF WISCONSIN – MADISON

2023

Date of final oral examination: 11/30/2023

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Acknowledgements

I would like to thank Dr. Bill Tracy for his dedication to the craft of plant breeding. It was an honor to be a part of the program and to witness the passion you brought forth every day, even during long, grueling days in the field. I am inspired by you, Bill, to bring the same zeal and curiosity to my career. I would like to thank Pat Flannery for teaching me many practical skills, like how to measure and lay out a field, and for his attention to detail, organization, and record keeping, which not only helped me tremendously in my research but also taught me how critical these elements are in successful plant breeding. I would like to thank the members of my committee, Drs. Natalia de Leon, Julie Dawson, and Phil Simon, for their guidance and contribution to my development as a researcher.

Thank you to the other members of Team Sweet for the camaraderie, especially during pollination season. Many graduate and undergraduate students contributed to the research in this dissertation and without their collaboration this work would not have been possible. I would like to especially thank Cathleen McCluskey for her support and insight and Carl Branch for his patience and willingness to talk statistics. I want to also thank all the professors who taught Plant Breeding and Plant Genetics core classes while also leading research programs of their own. I am grateful for your commitment to teaching. I will continue to reference the knowledge you shared in the classroom, thank you.

Lastly, I want to thank my foundation, my family, without whom I would not be who or where I am. Thank you Mom, Dad, Sabrina, and Garrett, for your unwavering encouragement, enthusiasm, and love. To my husband, Kevin, I love you, thank you for your humor, good cooking, and for championing me through all the seasons.

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Abstract

Organic growers are interested in open pollinated sweet corn varieties due to their ability to be further bred and adapted to specific environments and management systems. Yet organic growers report that certain characteristics of open pollinated varieties, particularly a lack of uniformity for certain traits, hinders adoption and marketability. Additionally, growers at large seek new products to differentiate themselves in the marketplace and local chefs and restaurateurs seek new raw products to drive innovation in the kitchen. In particular, chefs report a need for different types of fresh eating corn, namely 'vegetable' types of corn that are less sweet, starchier, and better suited to cooking. The goal of this work was to determine best methods for the characterization and improvement of open pollinated sweet corn varieties and vegetable corn populations for organic agroecosystems.

Chapter one reviews the relevant literature. Chapters two and three determined the utility of total soluble solids content for sweet and vegetable corn quality improvement. Chapter two used a half diallel cross of lines near-isogenic for four commonly used endosperm types in sweet corn breeding, wild type, *sugary1, shrunken2,* and *waxy1,* to determine the combining ability for total soluble solids content and the relationship of this trait to carbohydrate traits over three harvest dates. Variation existed for carbohydrate traits and total soluble solids across endosperm types and for hybrids within an endosperm type. Total soluble solids differed between 19 and 22 or 25 days after pollination for *sugary1, shrunken2,* and *waxy1* endosperm. However, total soluble solids content correlated with soluble carbohydrates only when assessed across all endosperm types.

Chapter three used recurrent selection on total soluble solids content in a vegetable corn population to increase the length of the fresh harvest window under organic management. The

length of the fresh harvest window was not increased by selection on this trait and realized heritability was low for total soluble solids in this population, ranging from -0.24 to 0.27. Indirect responses to selection for tenderness, chalkiness, ear width, and ear length were negatively linear, an undesirable direction. There was a moderate positive correlation between perceived starchiness (chalkiness) and total sugar content ($r = 0.56$) as well as a strong correlation between kernel moisture content and total sugar content ($r = 0.73$), indicating that future work to improve the harvest window could use selection on sensory analysis or kernel moisture.

Chapter four evaluated a trial of experimental and commercially available open pollinated sweet corn varieties under organic management for uniformity of flowering time and a suite of traits of relevance to growers and consumers. Three open pollinated varieties bred in the Wisconsin Sweet Corn Breeding Program, ‘Who Gets Kissed’, ‘Who Gets Kissed Too’, and ‘Quick Kiss’ were as uniform as the open pollinated check variety for silk emergence. However, selection for earlier and more uniform flowering time in ‘Who Gets Kissed Too’ relative to ‘Who Gets Kissed’ has not significantly changed these traits, future selection work must use experimental designs that better control environmental variance to improve efficiency and make gains. Most open pollinated varieties in the trial performed as well as the hybrid check variety for stand counts, husk traits, tip fill and percent marketable ears and many varieties outperformed the open pollinated check for number of kernel rows and row configuration. Notably, experimental varieties ‘Who Gets Kissed Too’ and ‘Olympic Sweet’, and a variety released in 2022, ‘Honey Badger’, had a significantly higher ratings for sweetness at both harvest dates compared to the bottom three varieties in the trial. Within each harvest date, all *se1* varieties, except for Candy Mountain, performed just as well as the hybrid check for sweetness.

Additionally, all varieties, except for Who Gets Kissed, performed as well as the hybrid check for holding tenderness across both harvest dates. In general, methods to improve the uniformity of traits like flowering time and eating quality of open pollinated varieties could be improved by first quantifying the variability inherent in the variety via measuring a large sample of ears in multiple environments, information which could then be used to inform selection to better serve the needs of growers.

1 Chapter One: Literature Review

1.1 Maize Domestication

Maize (*Zea mays* ssp. *mays*) was domesticated from its wild progenitor, teosinte (*Zea mays* ssp. *parviglumis*), about 9,000 years ago in the Balsas River Valley in Southwest Mexico (Doebley, 2004; Hake & Ross-Ibarra, 2015; Yang et al., 2019). Indigenous peoples selected teosinte to fit their needs and desires, actively practicing what we call plant breeding today. Their efforts changed the plant dramatically, from a highly branched plant with many small tassels, ears, and kernels surrounded by a hard fruitcase, to the maize we see today with apical dominance, a few large ears, and exposed kernels (Stitzer & Ross-Ibarra, 2018).

This history has been intensely studied by the scientific community. Evidence for the current working theory of domestication includes fertility and cross compatibility between maize and teosinte ssp. *parviglumis*, gradual changes in ear morphologies in the archeological record indicating low selection pressure causing incremental change over time, as well as reductions in genetic variance in modern maize compared to teosinte for traits under selection, such as ear morphology (Yang et al., 2019). Population genetics analyses have revealed that after a single domestication event and resultant genetic bottleneck, there was likely subsequent gene flow from teosinte, including from teosinte ssp. *mexicana* in the highlands of Mexico (Matsuoka et al., 2002). This gene flow continues today in areas where teosintes grow wild near maize fields or through intentional crossing. Archeological and phylogenetic evidence suggests that ancestral maize was diversified in the highlands of southwest Mexico around 6,500 years ago and then people carried maize to other regions throughout the hemisphere (Barnes et al., 2022; Matsuoka

et al., 2002). Enough genetic diversity remained, and remains today, to allow maize to spread and adapt to vastly diverse environments worldwide and to continue to be selected by plant breeders.

1.2 Sweet Corn Biology

1.2.1 Kernel Development & Structure

The maize kernel is composed of three main tissues, the endosperm, the embryo, and the pericarp (Kiesselbach, 1949). The endosperm is triploid and is formed through double fertilization, a hallmark of angiosperms. The mature maize pollen grain (male gametophyte) contains three haploid cells, two sperm cells and a vegetative cell. The mature embryo sac (female gametophyte) contains eight haploid cells, the egg cell with two synergid cells, two polar nuclei, and three antipodal cells. When a pollen grain lands on a silk and germinates, the vegetative nucleus forms the pollen tube which grows down the silk and allows the sperm cells to enter the embryo sac. One of the sperm cells fertilizes the egg cell, forming the diploid zygote. The other sperm cell fuses with two polar nuclei to form the triploid endosperm (Kiesselbach, 1949). The pericarp, the outer most layer of the maize kernel, is the ovary wall of the maternal plant.

Each maize kernel is a caryopsis, a one seeded fruit commonly called a grain. In the case of sweet corn, it is the immature caryopses that we consume as a fresh “vegetable”. (The designation of vegetable is botanically inaccurate but is how the United States Department of Agriculture (USDA) categorizes sweet corn. In this dissertation “sweet corn” will be used to describe maize eaten as a fresh vegetable.) As such, the biochemical composition and texture of the kernel tissues are important traits for breeding. The endosperm is the site of carbohydrate

synthesis and storage, a process discussed in detail below. The pericarp protects the seed from damage and pathogens and is the first tissue we encounter when biting into an ear of sweet corn. Tenderness in sweet corn is defined by how easily the pericarp breaks apart when sweet corn is bitten. Sweet corn pericarp is only a few cell layers thick, generally between 5 to 15 cell layers, and is measured in microns, with thinner pericarp perceived as more tender and preferred for fresh consumption (Tracy & Galinat, 1987).

Briefly, the development of the maize kernel from pollination to fresh eating stage is as follows. Within hours after pollination, the endosperm starts to divide mitotically while the embryo sac grows and forms a central vacuole. Cell walls form around the nuclei produced by the mitotic divisions of the endosperm within days, filling up what was hollow space in the embryo sac. This process continues until about 10 days after pollination (DAP), when cell division and differentiation occurs at the outer edges of the kernel, away from the embryo (Figure 1.1) (Kiesselbach, 1949).

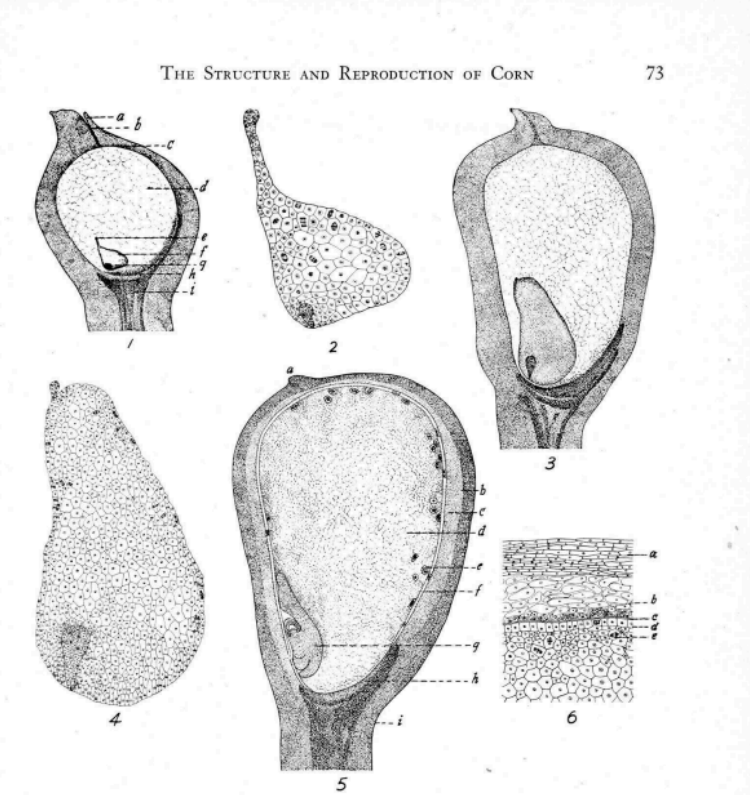

FIG. 58.—Series of longitudinal sectional drawings of developing kernel after fertilization to show region of cell division in endosperm.

1, pistil, five days after fertilization. a, silk scar; b, stylar canal; c, carpels which will form pericarp; d, nucellus; e, antipodals; f, endosperm; g, embryo; h, placental-hilar-funicular region; i, vascular tissue of carpels. X 10.

2, endosperm, embryo and antipodals from "1." Cell division as indicated by mitosis, generally scattered throughout endosperm up to this stage. X 55.

3, kernel about 10 days after pollination. X 10.

4, endosperm, embryo, and antipodals from "3." Cell division has become limited to outer region of endosperm, away from the embryo. No cell division was ever found in basal part of endosperm except in the youngest stages. X 30.

5, kernel about 20 days after pollination. a, silk scar; b, pericarp; c, inner part of carpels breaking down; d, endosperm; e, location of mitosis in endosperm; f, aleurone; g, embryo; h, hilar region; i, pedicel. X 10.

6, section through endosperm and pericarp of "5" to show detail. a, pericarp; b, inner part of carpels breaking down; c, nucellar membrane; d, aleurone; e, endosperm showing mitosis. X 30.

Figure 1.1. From (Kiesselbach, 1949) page 73. Stage 5 shows the locations of the endosperm (d.), pericarp (b.), and embryo (g.) in the developing maize kernel as well as the sites of mitosis in the endosperm at 20 days after pollination.

1.2.2 Sweet Corn Carbohydrates

The carbohydrates from grasses in the *Poaceae* family have been important for the flourishing of humankind for millennia and remain vital for nutritional needs today, and as such are a target for breeding in many cereals (Tetlow & Emes, 2017). Field corn, or wild type, is generally assumed to have a dominant functional allele at all the major endosperm starch

synthesis loci in the starch synthesis pathway. With functional alleles in the starch synthesis pathway, starch in maize endosperm is approximately 75% amylopectin and 25% amylose (Tracy et al., 2019).

1.2.3 Starch Synthesis Pathway

By weight, more than 70% of the wild type maize kernel is composed of polysaccharides (Tracy et al., 2019). Starch synthesis and storage occurs in the maize endosperm, with synthesis active from about 10 to 30 DAP. After 30 DAP, the kernel begins to dehydrate and starch is stored in the amyloplast stroma in the endosperm as a future food source for the embryo (Finegan et al., 2022; Hannah & Boehlein, 2017). While much has been elucidated about the genetics of starch synthesis in maize by using recessive mutations to determine enzymes and their functions, much remains unknown, such as the regulation of genes contributing to starch synthesis, or which enzymatic step is most rate limiting (Hannah & Boehlein, 2017).

The biochemical pathway understood today is as follows, condensed for clarity (Figure 1.2). The synthesis of starch begins with sucrose. Sucrose is produced through photosynthesis and transported through the phloem to the developing kernel, entering via the pedicel (Felker & Shannon, 1980). Sucrose enters the cytosol of the endosperm through the basal endosperm transfer layer (BETL) where it is cleaved into fructose and UDP-glucose by sucrose synthase. The gene *Shrunken1* encodes for sucrose synthase (Hannah & Boehlein, 2017). UDP-glucose is converted to glucose-1-phosphate by uridine-diphosphate glucose (UGPase) and then to ADP-glucose and pyrophosphate. The latter step is catalyzed by ADP-glucose pyrophosphate (AGPase) with the addition of ATP. AGPase has four subunits, two small and two large. The gene *Shrunken2* encodes for the two large subunits and *Brittle2* encodes for the two small

subunits of this enzyme (Tracy et al., 2019). ADP-glucose is moved into the amyloplast via a membrane bound adenylate transporter encoded by *Brittle1*.

In the amyloplast, ADP-glucose is converted into crystalline starch granules via a series of processes catalyzed by three main types of enzymes: starch synthases, starch branching enzymes, and starch debranching enzymes. Starch synthases form $\alpha-(1\longrightarrow 4)$-linked glucan chains through catalyzing the transfer of glucose from ADP-Glucose onto the C4 end of the glucan chain and the ADP is exported from the amyloplast (Tetlow & Emes, 2017). Starch branching enzymes initiate $\alpha-(1\longrightarrow 6)$ branch linkages in the $\alpha-(1\longrightarrow 4)$-linked glucan chains of amylose, amylopectin, and water soluble polysaccharides (WSP) via hydrolytic cleavage of bonds. The position and prevalence of these branches confer structural properties of starch, such as the formation of the parallel double helices that provide water insolubility in the case of amylose and amylopectin. Maize, in common with most higher plants, has two classes of starch branching enzymes, starch branching enzyme I (SBEI) and starch branching enzyme II (SBEII). SBEII has two isoforms in cereals, SBEIIa and SBEIIb, which display tissue specificity. SBEIIb is the most common form in maize and is the most abundant protein in the amyloplast stroma (Tetlow & Emes, 2017). The mutation *amylose extender* is the loss of function of SBEIIb, which dampens starch synthesis by 20%. Finally, starch debranching enzymes hydrolyze $\alpha-(1\longrightarrow 6)$ glucose linkages. Maize can have both a pullulanase and isoamylase type of debranching enzyme and the isoamylase type has three isoforms in angiosperms, including maize: Isoamylase1 *(*encoded by *Su1), Isoamylase2 (Isa2),* and *Isoamylase3 (Isa3)* (Tracy et al., 2019).

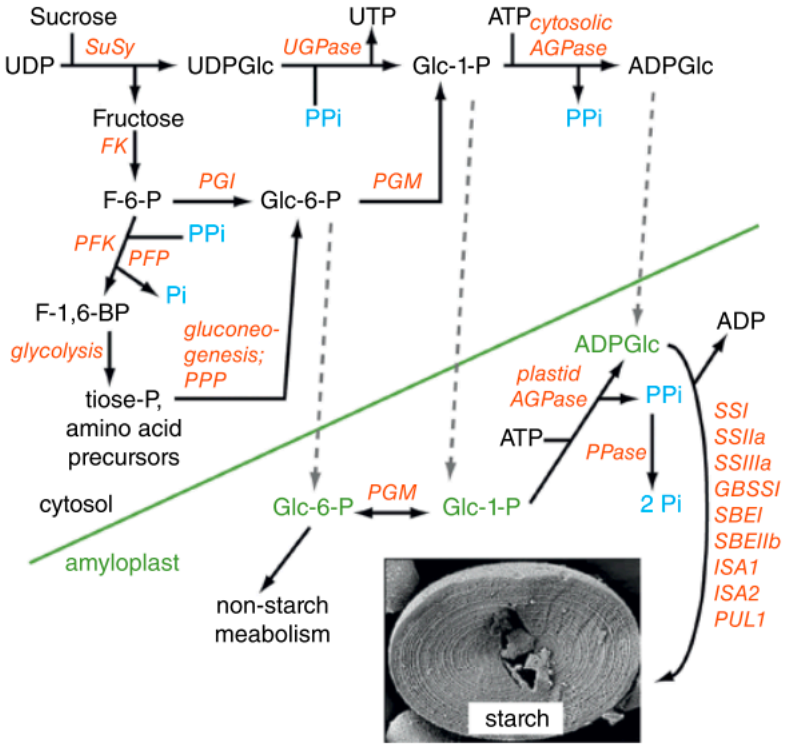

Figure 1.2. The starch synthesis pathway in developing corn endosperm based on recent literature on higher plant biochemistry. Cytosolic substrates are black, amyloplast substrates are green, and enzymes are red. Model proposed by Drs. Alan Myers and Karen Koch. ADP, adenosine diphosphate; ADPGlc, adenosine diphosphate glucose; AGPase, ADP-glucose pyrophosphorylase; ATP, adenosine triphosphate; FK, fructose kinase; F-6-P, fructose-6-phosphate; GBSS1, glucose bound starch synthase; Glc-1-P, glucose-1-phosphate; Glc-6-P, glucose-6-phosphate; ISA, isoamylase; PFK, phosphofructokinase-1; PFP, diphosphate-fructose-6-phosphate 1-phosphotransferase; PGM, phosphoglucomutase; PPP, pentose phosphate pathway; Pi, inorganic phosphate; PGI, phosphoglucose isomerase; Ppase, plastidial soluble inorganic pyrophosphatase; Ppi, pyrophosphate; PUL, pullulanase; SBE, starch branching enzyme; SS, starch synthase; SuSy, sucrose synthase; UDP, uridine diphosphate; UDPGlc, uridine-diphosphate glucose; UGPase, UDP-glucose pyrophosphorylase; UTP, uridine triphosphate (Hennen-Bierwagen & Myers, 2013).

1.3 Important Sweet Corn Alleles for Breeding

1.3.1 Overview

Sweet corn (*Zea mays* L.) breeding uses recessive loss of function alleles that affect the starch synthesis pathway, resulting in changes to sugar and starch accumulation in the endosperm

(Boyer & Shannon, 1983; Brewbaker & Martin, 2015). These alleles impact the shelf life and eating quality of sweet corn, conferring increased sweetness and variability in texture. At least eight recessive alleles have been utilized in sweet corn breeding (Table 1.1) (Tracy et al., 2019). The insoluble starch fraction of sweet corn consists of polysaccharides amylopectin and amylose. Amylopectin is a branched glucose polymer while amylose is a linear glucose polymer (Brewbaker & Martin, 2015; Fergason, 1994). Sweet corn also contains water soluble polysaccharides (WSP), which is a highly branched molecule. A high ratio of WSP to insoluble starch confers a desirable creamy mouthfeel to sweet corn (Culpepper & Magoon, 1924; Marshall & Tracy, 2003). The sugar fraction of sweet corn consists of mono- and di-saccharides, primarily sucrose, glucose, and fructose, with sucrose as the most abundant sugar by weight at the time of fresh harvest and thus conferring the majority of the sweet flavor we perceive (Pollak, 2010). While fructose tastes sweeter than sucrose, sweet corn has relatively small fractions of fructose and glucose.

Table 1.1 Wild type genes encoding enzymes that are involved in the starch synthesis pathway in maize endosperm. Adapted from (Tracy et al., 2019).

Chromosome	**Gene**	**Enzyme**	**Used in Sweet Corn**	**References**
5	*Amylose-extender1 (Ae1)*	Starch branching enzyme 2a	Yes	Fisher et al. 1996
5	*Brittle1 (Bt1)*	Adenylate transporter	Yes	Sullivan et al. 1991
4	*Brittle2 (Bt2)*	AGPase small subunit	Yes	Hannah and Nelson 1976
10	*Dull1 (Du1)*	Starch Synthase3	Yes	Gao et al. 1998
6	*Isoamylase2 (Isa2)*	Isoamylase2	No	Kubo et al. 2010
9	*Shrunken1 (Sh1)*	Sucrose synthase	No	Chourey and Nelson 1976
3	*Shrunken2 (Sh2)*	AGPase large subunit	Yes	Hannah and Nelson 1976
5	*Starch branching enzyme Ia (SbeIa)*	Starch branching enzyme	No	Yao et al. 2004

8	*Starch branching enzyme IIa (SbeIIa)*	Starch branching enzyme	No	Blauth et al. 2002
4	*Sugary1 (Su1)*	Isoamylase1	Yes	James et al. 2005
6	*Sugary2 (Su2)*	Starch Synthase2a	No	Zhang et al. 2004
2	*Sugary Enhancer1 (Se1)*	Unknown	Yes	Von Mogel et al. 2014
9	*Waxy1 (Wx1)*	Granule-bound starch synthase	Yes	Nelson and Rines 1962
2	*Zeapullulanase1 (Zpu1)*	Pullulanase	No	Dinges et al. 2003

1.3.2 Sugary1 locus

Historically, the first known mutation to affect the starch synthesis pathway resulting in sweet corn is a mutation at the *sugary1* locus (*su1* or *su1-ref),* located on the short arm of chromosome four. The *su1* mutation occurred naturally and independently at least five times in several locations in North, Central, and South America (*su1-ne (su1-ref), su1-sw, su1-nc, su1-cm, su1-p)* (Fonseca et al., 2015; Trimble et al., 2016; Viesselmann et al., 2014).

The wild type *Su1* allele encodes a starch debranching isoamylase enzyme (ISA1), which is required for the formation of amylopectin (Shuler et al., 2017). The *su1-ne (su1-ref), su1-sw, su1-nc* alleles produce a non-catalytic protein. Others such as *su1-cm* and *su1-4582* produce no protein at all. While still others make an active protein, but with reduced activity (Kubo et al., 2010; Trimble et al., 2016).

Early in kernel development, those alleles with no protein activity cause an accumulation of sugars and WSP in the endosperm at the expense of amylopectin (Marshall & Tracy, 2003). While *Su1* corn at 20 days after pollination (DAP) contains 5.9% total sugar, 2.8% WSP, and 66.2% starch, *su1* corn at the same stage contains 15.6% total sugar, 22.8% WSP, and 28% starch (Creech, 1965). The different *su1* alleles result in varying concentrations of WSP and

starch (Shuler et al., 2017). These ratios change as the corn matures, with most of the sugars in *su1* corn converting to WSP and starch over time. This conversion is rapid in *su1* corn and quality quickly deteriorates post-harvest. While sweet corn with the *su1* allele, or "sugary" sweet corn, was the only commercial type of sweet corn until the 1960s, the very short shelf life and harvest window are the reasons why, today, *su1* sweet corns are seldom grown commercially for fresh consumption (Tracy et al., 2019). The *su1* allele is still used today in combination with other alleles impacting the starch synthesis pathway. The *su1* mutation confers a distinct wrinkled and glassy kernel phenotype in dry seed.

1.3.3 Shrunken2 locus

Estimates are that nearly 100% of the sweet corn grown in the U.S. commercially for the fresh market and about 75% of the sweet corn for the processing market today contain the recessive mutation at the *shrunken2* locus (*sh2* or *sh2-ref*) (Hu et al., 2021; Tracy et al., 2019). These sweet corn types are commonly called "supersweet". The *sh2* locus was characterized by John Laughnan in the 1950s at the University of Illinois from a stock given to him by a colleague at the University of Michigan, E.B. Mains. Laughnan released the first supersweet cultivar in 1961 by backcrossing the *sh2* allele into sugary inbreds and producing supersweet inbreds and hybrids in a sugary background (Tracy, 1997). The *sh2* locus is on chromosome three and the loss of function allele was caused by a complex chromosome rearrangement (Kramer et al., 2015). The functional allele encodes the large subunit of the enzyme adenosine diphosphate glucose pyrophosphorylase (AGPase) whereas the mutant *sh2* allele causes decreased AGPase activity and, as a result, sugars build up because they are not converted to starch. (Hannah & Nelson, 1976).

At 20 DAP, *sh2* corn contains two to four times the total sugars of *su1* and very little WSP. Creech (1968) reported 34.8% total sugar, 4.4% WSP, and 18.4% starch. The double recessive mutant, *su1su1sh2sh2,* results in even more sugar and less starch, because the *su1* loss of function acts after the *sh2* loss of function in the biochemical pathway (Tracy, 1997). Most importantly, supersweets convert sugars to WSP and starch more slowly than sugary corns, allowing the corn to be harvested over longer periods of time, shipped greater distances, and stored longer without a greatly reduced decline in quality (Tracy, 1997). Poor post-harvest handling can accelerate the conversion in either mutant. The *sh2* mutation confers a distinct highly shriveled, opaque phenotype in dry seed.

1.3.4 Sugary enhancer1 locus

The *sugary enhancer1* (*se1*) mutation is used in combination with *su1* to produce *su1su1se1se1* cultivars. The mechanism of *se1* is unknown but it functions as a recessive modifier of *su1.* The sequence of *Se1* is on chromosome two, and the *se1* phenotype is observed when this gene is deleted in some genetic backgrounds (Zhang et al., 2019). The double recessive mutant *su1su1se1se1* doubles the levels of sugars compared to *su1* alone, including an increase in maltose, and maintains WSP at the expense of starch (Zhang et al., 2019). The rate of conversion of sugars to WSP and starch is comparable to *su1.* The *su1* mutation confers a distinct wrinkled and glassy kernel phenotype in dry seed. The dry seed of *su1su1se1se1* cultivars have a similar wrinkled, glassy appearance as *su1su1* that is often lighter in color, but the phenotypic expression is genetic background dependent (Tracy et al., 2019).

1.3.5 Waxy1 locus

"Sticky" or "glutinous" corn types contain the *waxy1 (wx1)* allele and are a common vegetable corn type in East Asia. In tropical climates, corn with *wx1* endosperm performs better than supersweet corns, which succumb to insect, disease, and abiotic pressures (Brewbaker & Martin, 2015). In the U.S. the *wx1* allele was first identified in 1909 from corn brought from China, where *wx1* was likely isolated shortly after the introduction of corn in the 1600s (Boyer & Shannon, 1983; Brewbaker & Martin, 2015). There are at least nine alleles of *waxy1*, (*wx-D7, wx-D10, wx-Cin4, wx-124, wx-Reina, wx-Xuanwei, wx-PIF/Harbinger, wx-hAT, wx-Elote2)*, which are mutations in the coding or promotor regions of the Waxy gene located on chromosome nine (Wu et al., 2022). The *wx1* mutation results in endosperm starch that is 100% amylopectin, while wild type maize with functional alleles in the starch synthesis pathway is approximately 75% amylopectin and 25% amylose (Brewbaker & Martin, 2015; Fergason, 1994). The composition of the starch fraction affects the mouthfeel of the corn, most noticeably after cooking. Waxy corn is a very popular vegetable in East Asia, but in the U.S., waxy corn is grown commercially for the processed starch and as animal feed but is rarely utilized as a vegetable. The *wx1* mutation confers a phenotype that is full and dull in appearance in dry seed and the presence of the mutation can be confirmed via iodine staining of the kernel or the pollen (Fergason, 1994).

1.4 Sweet Corn Production in the United States

According to the USDA, in 2021, sweet corn was harvested on 356,700 total acres in the United States with certified organic sweet corn occupying 11,887 of those harvested acres. In Wisconsin in 2021, sweet corn was harvested on 53,200 total acres with certified organic sweet corn occupying 1,907 of those harvested acres. Wisconsin ranked third for certified organic

sweet corn acreage (1,907 acres) behind Washington (4,013 acres) and Minnesota (2,079 acres) in 2021, however Wisconsin ranked second in the nation for the number of certified organic farms producing sweet corn, 61, behind California with 86 farms. Certified organic sweet corn generated $34,346,754 in sales nationwide in 2021, with $3,594,562 generated in Wisconsin. While nationwide in 2021, fresh market sweet corn generated more revenue than processing sweet corn, the trend is the opposite in Wisconsin. In Wisconsin, the sweet corn processing market generated $36,513,000 in production value overall with $3,448,360 in certified organic sales. The sweet corn fresh market generated $16,454,000 in production value overall with $146,202 in certified organic sales in 2021 (USDA-NASS, 2023).

1.5 Breeding Sweet Corn for Organic Environments

1.5.1 Genotype x Environment Interactions

In essence, plant breeding seeks to produce crop varieties that perform well in a target population of environments (TPE). Organic farming environments often differ from their conventional counterparts in fundamental ways. For example, availability of plant nutrients from organic sources of fertilizer often differs from that of inorganic sources, particularly nitrogen availability where mineralization of organic matter can be slow in cold soils. Pest, disease, and weed management strategies in organic systems are also quite different from conventional management because pesticide type and use differ, for instance (Burger et al., 2008; Lammerts van Bueren et al., 2002). Organic growers often rely upon crop rotation to break pest and disease cycles. Inherent in all farming systems is diversity, diversity of plant and soil communities across space and time, but conventional management has more ability to homogenize environments through inputs that act quickly. As such, organic environments are often more variable in comparison and often show higher genotype x environment (GxE) interactions (Lammerts van

Bueren & Myers, 2012). Some trials with conventional and organic environments show rank change genotype x environment (management) interactions in cultivar performance (Lammerts van Bueren et al., 2011; Lammerts van Bueren et al., 2002; Murphy et al., 2007; Wolfe et al., 2008). However, developing varieties with broad adaptation that perform well in both types of management systems is possible. One study in maize reported several hybrids performed well in both organic and conventional environments with relatively high and consistent phenotypic and genotypic correlations for dry matter across the two management systems, but inconsistent and low correlations across the two management systems for yield (Burger et al., 2008). This demonstrates that GxE is trait specific, therefore determining the degree to which a trait is impacted by the environment is important when developing breeding strategy. In instances of traits with high GxE across management systems, organic growers would be best served by breeding in the environment of intended use (Fess et al., 2011). Whereas if a trait exhibits low GxE and if the genetic correlation between the performance in the breeding environment and the TPE is sufficiently high, then indirect selection could be the most effective strategy.

Carbohydrate composition of sweet corn is generally reported as highly heritable due to the large effect of recessive alleles (ie. *su1* or *sh2*) that confer mutations in the starch synthesis pathway and largely impact carbohydrate traits (Tracy, 1997). One study reported heritability estimates of 0.84 for total sugar and 0.71 for total polysaccharides (Dagla et al., 2015). Heritability is relative to the environments in which a trait is measured and the genotypes on which a trait is measured, and as such can be artificially inflated or deflated depending on the variability of the included environments and genotypes. A study with two highly contrasting environments, one with silt loam and the other with a sandy soil type, reported more variability due to GxE than to genotype or environment main effects for sweet corn quality traits, with the

percentage of variability explained for total sugar content as 24% for genotype, 6.4% for environment, and 65.3% for GxE (Wong et al., 1994). Environmental variables like high temperatures and high rainfall have also been found to be associated with lower sugar content, illustrating the importance of testing cultivar performance across locations and years (Culpepper & Magoon, 1927). The differences among the environments in the TPE need to be considered when deciding how to allocate resources and meet breeding goals.

1.5.2 Organic Sweet Corn Production

Almost all the sweet corn grown commercially for the fresh and processing markets in the U.S. today are supersweet (*sh2*) hybrids. Yet supersweets have inherent agronomic deficiencies, including poor germination and poor early vigor in comparison to *su1* sweet corn (Churchill & Andrew, 1984; Guzman et al., 1983; Styer & Cantliffe, 1984; Wilson Jr. et al., 1994). The poor germination of supersweets is due to many factors, among them low seed weight, leaking of sugars from the kernel attracting pathogens, high levels of sugars, and low levels of polysaccharides in the kernel endosperm (Tracy, 1997). Seed weight and polysaccharides are positively correlated with germination (Viesselmann et al., 2014). Fungicide seed treatments are commonly used to aid in germination, yet commonly used seed treatments are disallowed in organic production. Further, poor germination can result in uneven stands, and together with poor seedling vigor can put a heavy weed control burden on organic producers. Mechanical or cultural weed control methods organic producers rely upon can be costly. In a half-diallel cross of seven sweet corn inbreds, Zystro et al. (2012) found that early season plant height and early season leaf area were negatively correlated with early- and late-season weed mass, respectively (Zystro et al., 2012).

Consequently, organic sweet corn producers could benefit from breeding that prioritizes traits like germination, early vigor, and plant and leaf morphologies that close the canopy quickly to shade out weeds. Variation exists for seed weight and germination among supersweet lines (Adetimirin et al, 2006). Recurrent selection for improved field emergence in supersweet corn found linear responses to direct selection on field emergence and kernel weight as well as indirect linear increase in total starch content (Juvik et al., 1993). Organic growers could benefit from market classes of corn with higher levels of starch than modern supersweet varieties to benefit from the improved germination. Stakeholders participating in the University of Wisconsin – Madison Seed to Kitchen trials reported interest in corn varieties that were "less sweet, more starchy", and suited to "cooking or polenta" (Dawson & Healy, 2018, p. 242) These needs could be met with field corn varieties for grain with high culinary value or new market classes of fresh eating corn that are less sweet, such as the "vegetable corn" types explored in part of this research. In other parts of the world, for example in South America, fresh eating corns are much less sweet than the cultivars grown commercially in the U.S.. The market for vegetable corn types is increasing in the U.S., especially as more food system stakeholders, like chefs, become involved in the breeding process.

1.5.3 Hybrid Maize

Modern maize breeding is dominated by the inbred – hybrid model. A hybrid is the first filial generation from the mating of two genetically distinct inbred individuals, resulting in a population where the individuals are heterozygous, but the group is genetically homogenous. Reasons for the proliferation of the inbred-hybrid model are many, among them are that the genetic and phenotypic uniformity of hybrids allow many aspects of industrialized farming to function well, such as the use of mechanization, that maize shows severe inbreeding depression

and thus strong heterosis, and that hybrid seed is a profitable business model for seed companies whereby the hybrid needs to be purchased by farmers every year. The first inbreds and hybrids were developed out of open pollinated varieties. The genetic base for 87% of the hybrid field corn grown in the United States, at the time of publication in 2004, was just five open pollinated populations (Troyer, 2004). These five open pollinated populations exhibited wide adaptation to the temperate climate of the U.S. and are 'Reid Yellow Dent', 'Minnesota 13', 'Lancaster Sure Crop', 'Northwestern Dent', and 'Leaming Corn', populations developed by farmer breeders in the mid to late 1800s (Troyer, 2009). The first hybrid corn varieties were double cross hybrids, made by crossing two single cross hybrids. The first double cross hybrid was made by Donald F. Jones in 1917 in Mt. Carmel, Connecticut (Troyer, 2009). Once more vigorous inbreds were developed, double cross hybrids were replaced by single cross hybrids beginning in the 1970s, and single crosses are the type grown today (Troyer, 2009). Hybrid seed coupled with advances in agronomy, increased inputs, improved pest management, irrigation, and many other factors have been a boon for field corn yields in the U.S. (Tracy et al., 2004). Since the 1930s, corn yields have been steadily rising (Troyer, 2009). In 2001, the highest tonnage crop in the world was corn, surpassing rice and wheat (Troyer, 2009).

1.5.4 Open Pollinated Varieties

Open-pollinated varieties (OPs or OPVs) remain an alternative to hybrids. OPVs have advantages comparatively, in that they can continue to be bred on farm allowing for continued adaptation to specific environmental, management, and climactic conditions. An open pollinated cultivar is propagated by saving seed from the open intermating of individuals in a naturally cross-pollinating species such that the population is both genetically heterozygous and heterogeneous. In a survey conducted by Lyon et al. (2015) of Wisconsin organic farmers, a

majority of respondents stated that they preferred to use OPVs over hybrids. A majority also responded that they believed the development of OPVs should be a priority for organic agriculture. However, responses also detailed downsides to OPVs, such as a lack of vigor compared to hybrids, a lack of uniformity resulting in poorer marketability compared to hybrids, or the potential for contamination in saved seed (Lyon et al., 2015). These are salient points, as the inherent diversity within OPVs is what allows for further selection, adaptation, and buffering capacity, but "too much" diversity, particularly for traits that growers and consumers value uniformity within, can put growers of OPVs at a disadvantage compared to those growing more uniform cultivars. Retailers demand uniform and unblemished produce (Collart et al., 2022). Consumers often judge produce quality by appearance and often expect a degree of uniformity and consistency for traits related to appearance and quality attributes (Collart et al., 2022; Yiridoe et al. 2005; Wuest et al., 2021). Farmers also value uniformity in traits that support their systems, such as disease resistance or plant architecture (National Research Council, 1993). Therefore, there is a balance in variability and uniformity that must be struck within OPVs.

The development of varieties, like OPVs, or mixtures in the case of self-pollinating species, in partnership with growers is often touted as the best way to ensure that relevant traits are incorporated and that growers will adopt the variety once it is released. This methodology is called Participatory Plant Breeding (PPB). PPB was first formally described in 1996, but practice dates earlier, and involves establishing breeding goals and conducting selection and evaluation collaboratively on farm with participation from farmers, breeders, and other stakeholders (Rhoades & Booth, 1982; Shelton & Tracy, 2015; Witcombe et al., 1996). PPB was first used to better serve smallholder farmers in low income countries operating on marginal land whose needs were not being met by the formal seed system (Shelton & Tracy, 2015). The idea behind

PPB is that the expertise of the breeder and farmer are combined to form a collaborative knowledge base of what is needed in a variety and how to achieve the goals, as well as to take advantage of any GxE for specific adaptation to the farm(s) where the selection takes place. PPB activity is gaining traction in the U.S., a recent review article flagged 47 PPB projects in the global north (Colley et al., 2021). Colley et al. (2021) highlighted that these projects were mostly in response to the needs of organic growers for adapted varieties and/or for a seed development process that aligns with the four principles of the organic movement as designated by the International Federation of Organic Agriculture Movements (IFOAM): health, ecology, fairness, and care (Colley et al., 2021; Lammerts van Bueren & Myers, 2012).

A sweet corn PPB project in the U.S. led to the release of an OPV, 'Who Gets Kissed,' in 2015 (Shelton & Tracy, 2015). This project was a collaboration between Minnesotan farmer Martin Diffley, the Organic Seed Alliance in Port Townsend, WA, breeder John Navazio, and Drs. Adrienne Shelton, William Tracy, and Jared Zystro at University of Wisconsin – Madison. Two OP sweet corn varieties were improved in tandem, an early and a late variety, with the late variety ultimately released. Martin identified quality as one of the most important traits, and the PPB methodology led to a linear response to selection for improvement in flavor and in tenderness in the early and late populations, respectively, across the four cycles of selection, in addition to improvement in other traits. Who Gets Kissed continues to undergo selection in the Wisconsin breeding program and in others, including in Western Oregon and Washington States. Several new OPVs recently released or soon to be released, 'Sweet Kisses', 'Olympic Sweet', and 'Quick Kiss', are related to Who Gets Kissed, or the early population developed concurrently, highlighting how OPVs can continue to be shaped into new forms and adapted to new environments by farmers and breeders.

1.6 Total Soluble Solids

Total soluble solid (TSS) content is the percentage of total solid constituents dissolved in solution. The solid soluble fraction of fruits and vegetables can include sugars, amino acids, other acids like ascorbic or citrate, pectins, minerals, and phenols dissolved in the water-based juice (Beckles, 2012). Plant components such as cellulose, lignin, and fat are not soluble and therefore not part of TSS. TSS is commonly measured with a refractometer and reported in the units of degrees Brix (°Brix), where 10 °Brix equates to 10% solids in solution.

In many fruit breeding programs, such as tomato, watermelon, grape, strawberry, apricot, and winter squash, TSS is used as an approximate measure of percent sugars and high TSS is desirable (Baccichet et al., 2023; Beckles, 2012; Breksa et al., 2015; Campbell et al., 2021; Huang et al., 2022; Hultengren et al., 2016). TSS provides an inexpensive and quick measurement and correlates sufficiently with total sugars in some crops, but it is not very precise. Factors like maturity, post-harvest storage conditions and time, fruit size, management practices, and time of day might all affect a TSS measurement to various degrees.

The ratio of TSS to titratable acidity (TA) is also often used as a selection benchmark for balanced flavor in some fruits. For example, a TSS:TA of 12.5 is a common minimum threshold used for breeding fresh-eating tomatoes and higher TSS:TA ratios in strawberries are associated with increased consumer desirability (Beckles, 2012; Schwieterman et al., 2014; Whitaker et al., 2013). Dry matter content is a measure of all constituents other than water in a fruit or vegetable. Therefore, TSS is related to dry matter content, the extent to which these two traits correlate varies among crops, but both typically have an inverse relationship with the size of fruit. For example, a study in tomato reported a correlation coefficient of 0.84 between TSS and fruit dry matter content, another in summer squash reported a correlation coefficient of 0.33, and TSS and

dry matter were highly correlated in a study of peach, nectarine, and plum with a correlation coefficient of 0.91 across 13 cultivars (Itoh et al., 2020; Martínez-Valdivieso et al., 2015; Scalisi & O'Connell, 2021).

In sweet corn, however, TSS is likely not a reliable proxy for total sugars. TSS measurements would include both total sugars and WSP, if present, in addition to other soluble constituents. Both sugars and WSP are desirable constituents, but sugars confer sweetness while WSP confers a creamy mouthfeel, and it is impossible to know with a TSS measurement alone what proportion is due to sugars versus WSP. A study using a single sweet corn cultivar, Silver Queen, a sugary type, reported that as TSS increased (+5 °Brix) texture ratings shifted from "moderately delicate" to "slightly rigid" (Collins & Taylor, 1976). This change in texture could reflect a change in the quantity of WSP or a change in dry matter, among other possibilities, however neither was quantified in this study. Other studies found that *sh2* hybrids had lower TSS than both *se1* and *su1* hybrids, and TSS increased over consecutive harvest dates for *su1* and *se1* hybrids while remaining constant in *sh2* hybrids (Hale et al., 2005; Lee et al., 1999; Zhu et al., 1992). Correlations reported in the literature vary considerably but are often negative between TSS and total sugars. Hale et al. (2005) reported a coefficient of -0.51, while Zhu et al. (1992) reported insignificant but contrasting correlations, -0.79 for a *sh2* cultivar and 0.68 for a *se1* cultivar, while Lee et al. (1999) did not report any significant correlations. These are in opposition to the strong positive correlations between TSS and total sugars in other crops (Breksa et al., 2015; Hale et al., 2005; Lee et al., 1999; Zhu et al., 1992). The mixed results and often negative correlations are evidence that TSS is not a reliable proxy for total sugars in sweet corn. None of these studies quantified the change in WSP, so to our knowledge it is unknown if

TSS correlates with total sugars plus WSP, how that relationship behaves over time as these ratios change, among endosperm mutants, or among cultivars.

1.7 Sensory Analysis in Breeding

Perception of flavor is a complex phenomenon involving our physiology as well as complex and poorly understood mediation of stimuli by our brains, where environment, culture, memory, and emotion all play a role. The idea that, "flavor is not in the food; it is created by the brain," is a foundational principle of neurogastronomy and communicates this complexity and inherent variability (Shepherd, 2015, p. 1). Our mouths have five receptor types that allow us to perceive five distinct flavors, sweet, sour, bitter, salty, and umami. Our olfactory system, on the other hand, has at least 320 distinct receptors, illustrating that much of the nuance of our experience of flavor stems from aroma (Kaeppler & Mueller, 2013).

Breeders of horticultural crops often consider flavor as an important trait, but evaluation is difficult due both to subjectivity as well as to other constraints, such as the large number of breeding lines in a typical breeding program. Quality assessment techniques often used in food science contexts, such as conventional descriptive analysis where trained panelists develop repeatable descriptions of product attributes, are difficult in a breeding context due to time and budget constraints. The training for panelists doing this type of analysis can take up to 120 hours before the descriptive analysis itself even takes place (Dawson & Healy, 2018).

Historically, individual breeders taste breeding lines and make decisions about acceptable or unacceptable flavor and textural attributes (Hampson et al., 2000; Bowen et al., 2019). Breeders are often considered well versed in the market demands for quality of their crop, the range of variability for quality attributes present within a crop, as well as what is generally considered acceptable in the marketplace. But given the variability of flavor perception it begs

the question of whether there is a more equitable method of making decisions about quality that might better capture the spectrum of people's experiences who eat the crop. More recently, groups such as the Seed to Kitchen Collaborative (SKC) at University of Wisconsin – Madison and the Culinary Breeding Network (CBN) at Oregon State University have pursued alternative methods to evaluate quality that are often quick and tap a wider audience, including experts. Some of these rapid sensory analysis methods include survey techniques like 'check all that apply', intensity scales (rate on 1-5 scale), or hedonic rating (like/dislike) as well as non-survey-based techniques, such as 'projective mapping', where individuals place samples in physical space based on perceived similarity or difference (Dawson & Healy, 2018). Experts can include culinary professionals such as chefs, sommeliers, coffee roasters, distillers, or brewers, who are experienced with discerning and describing flavor. These alternative methods are typically, by design, more rapid than traditional methods, and use experts, semi-trained, or untrained panelists to quickly differentiate samples based on specific attributes, to identify consumer preference among samples, or to develop a sensory profile of samples, among many other potential objectives.

In sweet corn breeding, main flavor attributes and correlated quantitative measurements include sweetness (total sugars, with sucrose as primary driver), tenderness (pericarp thinness), creaminess (WSP content), and aroma (sulfur containing volatile compounds in combination with other volatiles) (Azanza et al., 1996; Bailey & Bailey, 1938; Flora & Wiley, 1974; Winter et al., 1955). Care must be taken not to equate a quantitative measure with a sensory experience, but rather these measurements can be used as baselines or to exclude breeding lines that might fall outside of an acceptable range (Chen, 2020). All of the above quantitative measures require considerable time and expertise, and most are destructive. At present, in sweet corn breeding

programs, there is a lack of quantitative measurements that are correlated with flavor attributes *and* are rapid and cheap enough to screen material early in the breeding pipeline. At early stages in the breeding pipeline when there are thousands of genotypes to be evaluated, laborious quantitative measures or sensory evaluation of many segregating breeding lines is not feasible.

1.8 Chapter One References

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2 Chapter Two: Genetic Variation for Endosperm Carbohydrates and Total Soluble Solids Content in *shrunken2*, *sugary1*, *waxy1*, and Wild Type Near Isogenic Corn Lines across Three Harvest Dates

2.1 Abstract

Sweet corn cultivars must meet stringent quality standards to be accepted in the marketplace. Breeding for eating quality traits like sweetness typically involve taste ratings by breeders or quantification of carbohydrate content. Total soluble solids content is used as a proxy for sweetness in many fruit crops. Using a diallel cross of near isogenic corn lines for endosperm types *sugary1, shrunken2, waxy1* and wild type, a combining ability analysis for carbohydrate traits and total soluble solids content determined the relationship of these traits over three harvest dates. Variation existed for total sugar, sucrose, glucose, fructose, total polysaccharides, and starch content within and across endosperm types and harvest dates, but strong correlations with total soluble solids content were present only when assessed across all endosperm types. Strong similarities existed among wild type, waxy1, and sugary1 near isogenic lines for general combining ability for carbohydrate traits, while shrunken2 near isogenic lines had different desirable combiners. Line C40 was a desirable general combiner for carbohydrate traits among wild type, waxy1, and sugary1 endosperm types, while Ia5125, P39, and Ia453 were desirable general combiners for shrunken2 endosperm. This experiment also determined that total soluble solids content is not a useful trait in sweet corn breeding for quality traits.

2.2 Introduction

Endosperm carbohydrates from grasses (*Poaceae*) have been important for the flourishing of humankind for millennia and remain vital for nutritional needs today (Tetlow & Emes, 2017). The wild type corn (*Zea mays* L.) kernel contains 60 to 70% starch by weight, the majority located in the endosperm (De Vries et al., 2016). Starch is the most abundant source of calories in diets worldwide, as such, carbohydrate traits are an important breeding target (Svihus & Hervik, 2016). Sweet and waxy corns are important vegetable crops worldwide (Ketthaisong et al., 2014). Sweet corn was planted on 355,100 acres in the United States in 2022. Fresh market production of sweet corn was valued at $539.43 million, and production of sweet corn for processing generated $269.37 million, in 2022 (USDA-NASS, 2023).

As fresh vegetables, eating quality is one of the most important traits. Sweet corn breeding uses mutations in the starch synthesis pathway that alter the composition of the carbohydrates in the endosperm, conferring variation in quality traits like sweetness and texture. Specifically, sweetness is determined by the amount of total sugar in the endosperm, with sucrose as the most abundant and therefore most important sugar (Reyes et al., 1982). Texture, typically defined as creamy, watery, crispy, or gritty, is determined by the ratio of water soluble polysaccharides (WSP) to insoluble polysaccharides (starch) within the endosperm (Culpepper & Magoon, 1927).

Like sweet corn, waxy corn eating quality is also impacted by the composition of carbohydrates in the endosperm, with sweetness and glutinosity as two attributes that drive consumer liking. A high level of glutinosity or stickiness is preferred, which is determined by the quantity and physical structure of amylopectin in the endosperm (Dermail et al., 2022; Gong & Chen, 2013; Ketthaisong et al., 2014). Sweetness is also determined by the total sugar content

with higher levels of sugar desirable (Dermail et al., 2022). Hybrids between sweet and waxy corn, or synergistic cultivars, aim to increase the sugar content while maintaining the desirable glutinosity (Fuengtee et al., 2020; Lertrat & Thongnarin, 2008). Unlike sweet corn, waxy corn has lower moisture content at fresh harvest stage, between 40-45% compared to 75-85% in sweet corn, though both types are commonly consumed as cooked fresh green ears (Gong & Chen, 2013; Kachhadiya et al., 2018; Ketthaisong et al., 2014; Szymanek et al., 2020; Tracy, 1997).

Within an endosperm type, selections in breeding pipelines for quality traits are typically made by tasting experimental material, often by an individual breeder (Zystro et al., 2021). The perception of taste is biologically complex and an inherently subjective experience, which complicates this process (Klee & Tieman, 2018). Other methods to assess eating quality, such as using trained sensory panels or laboratory methods to quantitatively measure flavor components, are costly and time consuming, and therefore often limited to evaluating late-stage breeding material (Dawson & Healy, 2018; Hagenguth et al., 2022). Given the tradeoff between high eating quality and agronomic traits like germination and vigor, it is likely that lines with favorable alleles for eating quality are discarded early in the breeding process (Harakotr et al., 2022; Rowe & Garwood, 1978; Tracy et al., 2019). A rapid and inexpensive method of quantifying carbohydrate content would serve to speed up the breeding process and allow for material to be evaluated earlier in the pipeline for quality traits.

Total soluble solids (TSS) content is the percentage of total solid constituents dissolved in solution. The soluble solid fraction of fruits and vegetables can include sugars, soluble polysaccharides, amino acids, other acids like ascorbic or citrate, pectins, minerals, and phenols dissolved in the water-based juice (Beckles, 2012). TSS is commonly measured with a refractometer and reported in the units of degrees Brix (°Brix), where 10 °Brix equates to 10%

solids in solution. In many fruit breeding programs, such as tomato, watermelon, grape, strawberry, and winter squash, TSS is used as an approximate measure of percent total sugars and high TSS is desirable (Beckles, 2012; Breksa et al., 2015; Campbell et al., 2021; Huang et al., 2022; Hultengren et al., 2016). Given the effectiveness and affordability of the TSS phenotype in fruit breeding programs, it is worthwhile to investigate the combining ability of TSS among commonly used endosperm types to determine if the same technology could be utilized in sweet corn breeding.

Historically, *sugary1* (*su1* or *su1-ref),* located on the short arm of chromosome four, is the first known allele to alter the starch synthesis pathway. Numerous mutations are known at the *Su1* locus (Dinges et al., 2001; James et al., 1995). At least five mutations occurred independently in several locations in North, Central, and South America (*su1-ne (su1-ref), su1-sw, su1-nc, su1-cm, su1-p)* and were maintained and used by indigenous people (Fonseca et al., 2015; Tracy et al., 2006; Trimble et al., 2016; Viesselmann et al., 2014).

The wild type *Su1* allele encodes isoamylase1 (ISA1), a starch debranching enzyme, which is required for the formation of amylopectin, while the mutant alleles either produce a non-catalytic protein, no protein, or an active protein with reduced activity (Kubo et al., 2010; Shuler et al., 2017; Trimble et al., 2016). Early in kernel development, those alleles with no protein activity cause an accumulation of sugars and WSP in the endosperm at the expense of amylopectin (Marshall & Tracy, 2003). Wild type (WT), endosperm at 20 days after pollination (DAP) contains 5.9% total sugar, 2.8% WSP, and 66.2% starch, *su1-ref* endosperm at the same developmental stage contains 15.6% total sugar, 22.8% WSP, and 28% starch (Creech, 1965). Different *su1* alleles result in varying concentrations of WSP and starch (Shuler et al., 2017).

These ratios change as the endosperm matures, with most of the sugars in *su1* converting to WSP and starch over time.

Estimates are that nearly 100% of the sweet corn grown in the U.S. commercially for the fresh market and about 75% of the sweet corn for the processing market today contain the recessive mutation, *shrunken2* (*sh2* or *sh2-ref*) (Hu et al., 2021; Tracy et al., 2019). These sweet corn types are commonly called "supersweet". The *sh2* locus is on chromosome three and *sh2-ref* is a loss of function allele caused by a complex chromosome rearrangement (Kramer et al., 2015). The functional allele encodes the large subunit of the enzyme adenosine diphosphate glucose pyrophosphorylase (AGPase) whereas the mutant *sh2* allele causes decreased AGPase activity, resulting increased sugars and decreased polysaccharides (Hannah & Nelson, 1976). At 20 DAP, *sh2* endosperm contains two to four times the total sugars of *su1* and very little WSP. Creech (1968) reported 34.8% total sugar, 4.4% WSP, and 18.4% starch.

"Sticky," "glutinous," or waxy corn types contain the *waxy1 (wx1)* allele. There are at least nine alleles of *waxy1*, (*wx-D7, wx-D10, wx-Cin4, wx-124, wx-Reina, wx-Xuanwei, wx-PIF/Harbinger, wx-hAT, wx-Elote2)*, which are mutations in the coding or promotor regions of the *Waxy1* gene located on chromosome nine (Wu et al., 2022). The *wx1* mutation results in endosperm starch that is 100% amylopectin, while wild type corn with functional alleles in the starch synthesis pathway is approximately 75% amylopectin and 25% amylose (Brewbaker & Martin, 2015; Fergason, 1994).

The combining ability for TSS content in corn is unknown. Using near isogenic lines (NILs) for commonly used endosperm mutations, *sh2, su1,* and *wx1,* as well as WT, the objectives of this study were to determine the genetic variability for TSS among hybrids within

commonly used endosperm types, as well as to determine how TSS relates to carbohydrate composition across harvest dates.

2.3 Materials and Methods

2.3.1 Germplasm

Experimental entries included four six-line half diallel crosses with fifteen hybrids each, or sixty total hybrids, generated by crossing four sets of six inbreds in a half diallel mating design without parents per Griffing's Method Four, Model One (Griffing, 1956). The sets of inbreds include four near-isogenic lines (NILs) in six inbred genetic backgrounds, generated in the method described by Finegan et al. (2022). The six inbreds were Connecticut 40 (C40), Connecticut 68 (C68), Iowa 453 (Ia453), Iowa 5125 (Ia5125), Illinois 101t (Il101t), and Purdue 39 (P39). Each NIL is homozygous for one of three recessive alleles conferring major endosperm mutations (the mutation at *wx1*, *su1*, or *sh2*), or is wild type (WT, which contains dominant alleles at all known loci in the starch synthesis pathway). The inbreds were chosen because they represent diverse genetic backgrounds in sweet corn ancestry and have been used extensively in sweet corn breeding and development (Hu et al., 2021; Shelton & Tracy, 2013; Zystro et al., 2012).

2.3.2 Experimental Design

To generate hybrids, NILs were crossed in winter 2019 at Tuniche Seed Services, a winter nursery in Rancagua, Chile (34°06'S, 70°44'W). Due to a lack of sufficient seed for eight hybrids, a subset of the NILs were crossed again in the summer of 2020 at West Madison Agricultural Research Station (43°04'N, 89°32'W) to generate enough seed to plant all hybrids among all four diallel crosses in 2021.

The experiment was grown at West Madison Agricultural Research Station which has a Plano silt loam (fine-silty, mixed mesic Typic Argiudoll) soil type. The experimental design was a randomized complete block design (RCBD) used in two years, 2020 and 2021, with two planting dates per year and two replications per planting date. Plots were single rows 3.5 m long, with 0.76 m between rows, and an alley of 0.91 m between plots. Plots were direct seeded with twenty-five seeds per row then subsequently thinned to twelve plants per row at the V5 growth stage.

A complete *su1* and *wx1* diallel cross was grown in each experimental year, while only a partial diallel cross of *sh2* and WT was grown in 2020, due to seed shortages in 2020. Complete diallel crosses of all four groups were grown in 2021. The specific *sh2* hybrids grown in 2020 were C40 x P39, C68 x C40, C68 x Il101t, Ia453 x C40, Ia5125 x C68, Ia5125 x Ia453, Ia5125 x P39, Il101t x Ia5125, P39 x C68, and P39 x Il101t. The specific WT hybrids grown in 2020 were C40 x Ia5125, C40 x P39, C68 x C40, C68 x Il101t, Ia453 x C68, Ia5125 x C68, Ia5125 x Ia453, Ia5125 x P39, Il101t x C40, Il101t x Ia5125, P39 x Ia453, and P39 x Il101t. The letters in the inbred naming system refer to the state in which the inbred was developed, with Connecticut, Indiana, Louisiana, or Minnesota (C), Indiana or Michigan (P), Iowa (Ia), and Illinois (Il) (Gerdes et al., 1993). TSS and carbohydrate content was collected on all diallel crosses across all planting dates and years. Combining ability analysis was conducted on the WT, *su1,* and *wx1* diallel crosses across both years and on the *sh2* diallel cross in 2021, see 2.4 Combining Ability Analysis.

Seeds were treated with Maxim XL® fungicidal seed treatment prior to planting. In 2020, hybrids were planted on May 22 and June 5. In 2021, hybrids were planted on May 18 and June 3. After planting and pre-emergence, an herbicide mix of Callisto® (0.36 L ha^{-1}), Dual II

Magnum® (1.75 L ha^{-1}), Princep® (0.56 kg ha^{-1}), and Glyphosate® (2.33 L ha^{-1}) was applied, which kept plots weed free.

2.3.3 Field Data

A minimum of six ears per plot were self-pollinated on the same day within individual plots. Plots were pollinated on different days depending on when six ears were ready, and pollination dates ranged from six to seven days depending on planting date. Plots were harvested on three dates, 19, 22, and 25 days after pollination (DAP). Two ears were harvested on each date. On each ear the TSS content (%) was measured using an Atago Pal-1 digital refractometer (Atago USA Inc., Bellevue, WA) using a protocol modified from Hale et al. (2005). Specifically, the refractometer was calibrated and zeroed per the manufacturer's instructions. A 3 x 3 cm section of immature kernels were cut from the center of the ear, placed in a conventional kitchen garlic press, and the liquid extract was squeezed onto the reader well of the refractometer. Care was taken to ensure no solids were in the extract. All instruments were washed in water and thoroughly dried between samples.

After the TSS were measured, the ears were frozen with liquid nitrogen. A sample of kernels from the center of each ear was shelled, bulked, and placed into 50 mL polypropylene tubes. Shelling equipment was wiped with absorbent towels to dry and remove any kernel residue in between samples. The kernels were stored at -80 C until processed through a Labconco FreeZone 4.5L 77500/77510 series freeze dryer to remove all moisture (Labconco Corporation, Kansas City, MO). Each sample was freeze dried for five consecutive days while kept at -50 C. After freeze drying, samples were ground using an Udy cyclone mill, sifted through a 0.5mm screen, and stored at room temperature prior to carbohydrate content analysis. The mill was cleaned using a vacuum in between samples.

2.3.4 Near Infrared Spectroscopy

The experimental design yielded 620 samples in 2020 and 719 in 2021. Each freeze dried and ground sample were scanned into a Foss ds2500 Near Infrared Spectroscopy (NIRS) instrument (FOSS, Hillerød, Denmark). The Foss ds2500 measures the reflectance of light at wavelengths of 400 to 2500 nm in 0.5 nm increments. The NIRS instrument was calibrated with standards prior to scanning in experimental samples and the sample holder was cleaned with compressed air and Kimwipes® in between samples. After reflectance data was generated for each sample from each year, WinISI software was used to center the spectra and check for outliers (FOSS, Hillerød, Denmark). Any detected outliers were rescanned. If the rescanned sample remained in outlier range, it was removed. Five samples were identified as outliers and removed in the 2020 dataset and eleven in the 2021 dataset. Planting dates and replications were pooled within years such that a prediction equation for carbohydrate traits was generated for each year. A calibration set was selected using an H statistic of 1 (Au et al., 2020). This statistic yielded a calibration set of 109 samples in 2020 and 117 samples in 2021, which is 17.6% and 16.3% of the overall data set respectively. The calibration sets contained roughly equal representation of all endosperm types, planting dates, and harvest dates.

2.3.5 Laboratory Data

Reference values for seven carbohydrate traits were generated for the calibration sets using wet chemistry in the laboratory. Two Neogen Megazyme assay kits were used (Megazyme, Bray, Ireland): The first (K-SUFRG) for quantifying the concentrations of sucrose, D-fructose (fructose), and D-glucose (glucose), and total sugars, and the second (K-TSTS-100A) for quantifying the concentration of total polysaccharides, starch, and water soluble polysaccharides (WSP). The calibration samples were run in triplicate lab replications following the methods

described by De Vries et al., (2016). The three replications were averaged to generate a reference value for each calibration sample for each of the seven carbohydrate traits.

2.3.6 Statistical Analysis: Prediction Model Validation

Two prediction models for each experimental year for the seven carbohydrate traits were developed using partial least squares regression. The predictions were validated using external validation, whereby a set of samples that were not a part of the calibration set were analyzed in the lab for carbohydrate content and those reference values compared to the predicted values. The validation samples were chosen randomly from the samples within a year, excluding the samples used for calibration, while ensuring that the set included representation from the four endosperm types, two planting dates, and three harvest dates. For validating the sugar traits (sucrose, glucose, fructose, total sugar) twenty-four samples were used and for validating the polysaccharide traits (total polysaccharides, starch, WSP) twenty-two samples were used. Like the calibration set, validation samples were run in triplicate lab replications and averaged.

Prediction accuracy was assessed using regression of the predicted values on the reference values for the validation set and assessing the coefficient of determination (R^2) for each trait. Root mean squared error of prediction (RMSEP) values were also investigated to assess prediction accuracy, calculated using the formula listed in Egesel & Kahriman (2012).

While sweet corn carbohydrate traits are generally considered highly heritable due to the large effect of recessive endosperm mutations on these traits, carbohydrate traits are also highly impacted by timing of harvest (Ledenčan et al., 2022; Szymanek, 2009). It is of interest in a breeding program to determine if a prediction model for carbohydrate traits calibrated with one year of data can be used to accurately predict a second year. To assess prediction accuracy

between years, the models calibrated within each year were used to predict the other, unknown year (Lane et al., 2020; Teh et al., 2020).

2.3.7 Statistical Analysis: Combining Ability

All analyses were run with R Statistical Software (R Core Team, 2021). A type III analysis of variance (ANOVA) for all traits was conducted, with F-tests on the mean squares used to determine significant sources of variation after model assumptions of normality and equal variance of the residuals were verified. Due to the large effect of endosperm mutations on carbohydrates, the assumption of homoscedasticity of the residuals was violated when all four sets of diallel crosses were analyzed together. Therefore, the experiment was analyzed as four separate diallel crosses. Within each diallel cross, the hybrids, harvest dates, planting dates, replications nested in planting dates, and interactions were considered fixed effects. The model used was:

$$Y_{ijklm} = \mu + hybrid_{ij} + planting\ date_k + replication(planting\ date)_{kl} + harvest\ date_m + (hybrid\ x\ planting\ date)_{ijk} + e_{ijklm} \quad (1)$$

Where Y_{ijklm} is the phenotypic value measured for hybrid *ij* in planting date *k* and replication *l* on harvest date *m,* μ is the grand mean, $hybrid_{ij}$ is the effect of hybrid *ij,* $planting\ date_k$ is the effect of planting date *k* (*k* = early 2020, late 2020, early 2021, late 2021), $replication(planting\ date)_{kl}$ is the effect of replication *l* nested in planting date *k,* $harvest\ date_m$ is the effect of harvest date *m,* $(hybrid\ x\ planting\ date)_{ijk}$ is the effect of the interaction between hybrid *ij* and planting date *k,* and e_{ijklm} is the random error term. Stepwise model selection was conducted and the model with the lowest AIC score used. Outliers were identified using the rosnerTest() command from the EnvStats package and removed (Millard, 2013). Post hoc multiple comparisons tests were conducted using the emmeans() command form

the Emmeans package with planting dates treated as random effects using Tukey's Honest Significant Difference (HSD) with an alpha level of 0.05 (Lenth, 2022).

The statistical analysis of the general (GCA) and specific combining abilities (SCA) was conducted in R using the lm.diallel() command in the lmDiallel package using Griffing's Method Four, Model 1 (Griffing, 1956; Onofri et al., 2021). The model considered the hybrid effect from model 1 as:

$$hybrid_{ij} = GCA_i + GCA_j + SCA_{ij} \quad (2)$$

Where $hybrid_{ij}$ is the value of the GCA effect of the *i*th inbred parent plus the GCA effect of the *j*th inbred parent plus the SCA effect of the cross between the *i*th and *j*th inbred parents. When estimating the significance of GCA and SCA effects, the p-value was adjusted by the false discovery rate to control for multiple tests (Benjamini & Hochberg, 1995). Only GCA and SCA effects that were significant ($p \leq 0.05$) are reported. When a significant (p-value ≤ 0.05) hybrid x planting date, GCA x planting date, or SCA x planting date interactions were found for a trait, Spearman's coefficient of determination, rho, was calculated to determine if planting dates could be pooled. For all other traits planting dates were pooled.

A complete diallel cross for the *sh2* hybrids was only possible in planting dates grown in 2021. Due to seed shortages in 2020, five of the fifteen *sh2* hybrids were missing, including crosses with all six parents, which precluded the construction of a design matrix. Therefore, the *sh2* diallel cross is only analyzed in the two planting dates grown in 2021. A complete diallel cross for WT hybrids was also not possible in 2020 due to seed shortages, with three of the fifteen WT hybrids missing. However, one of the six parents, Ia5125, was not missing any crosses and therefore a design matrix was constructed per Wu & Matheson (2000), to account for the missing crosses in the calculation of GCA and SCA in 2020. Combining ability analysis was

therefore conducted on WT, *su1,* and *wx1* diallel crosses grown in both years and among the *sh2* diallel cross grown in 2021.

2.4 Results and Discussion

2.4.1 Prediction Models

The models for predicting glucose and fructose were found to have high prediction accuracy for these traits based on the coefficient of determination of external validation (Table 2.1; Appendix Figures A1-1 and A1-2). The regression of predicted vs. reference values for sucrose and total sugars in both years were found to have discontinuous variation due to large differences among endosperm types for these traits, and therefore inflated coefficients of determination of external validation when all endosperms were analyzed together (Table 2.1). This effect was observed among both the calibration set and the validation set (Appendix Figures A1-1, A1-2, A1-3, and A1-4). Therefore, the validation set was subset by endosperm types with low (*su1, wx1,* WT) and high (*sh2*) trait values for sucrose and total sugar to control for this variability and fairly assess prediction accuracy. The *su1, wx1,* WT endosperms had less than 20% sucrose and total sugar on a dry weight basis, while *sh2* endosperm had greater than 20% sucrose and greater than 30% total sugar on a dry weight basis. Prediction accuracy for the low and high trait value groups was generally high and consistent across the models built for each year for sucrose and total sugar (Table 2.1; Appendix Figures A1-5, A1-6, A1-7, and A1-8).

Like the regression for sucrose and total sugar, the regression for total polysaccharides, starch, and WSP in both years were found to have discontinuous variation due to large differences among endosperm types for these traits, and therefore inflated coefficients of determination when all endosperms were analyzed together (Table 2.1). This effect was observed among both the calibration set and the validation set (Appendix Figures A1-9, A1-10, A1-11,

and A1-12). Therefore, the validation set was subset into low and high trait value groups to control for this variability to fairly assess prediction accuracy.

The coefficients of determination for predicting *su1, wx1,* and WT endosperms with high quantities of total polysaccharides (>30% on a dry weight basis), and for predicting *wx1* and WT endosperms with high quantities of starch (>30% on a dry weight basis), were generally high in the models built for each year (Table 2.1; Appendix Figures A1-13, A1-14, A1-15, and A1-16). The coefficients of determination of models built in 2020 versus 2021 for endosperms with high levels of starch differed, but the RMSEP was similar in each year, 2.82 in 2020 and 3.19 in 2021 (Table 2.1). The coefficients of determination for predicting *su1* endosperms with high quantities of WSP (>15% on a dry weight basis) were moderate for the models built in each experimental year and the RMSEP for WSP for *su1* endosperms was 2.81 in 2020 and 2.21 in 2021(Table 2.1; Appendix Figures A1-17 and A1-18).

The coefficients of determination for predicting *sh2* endosperms with low quantities of total polysaccharides (<20% on a dry weight basis) differed between the models built for each year (Table 2.1; Appendix Figures A1-19 and A1-20). Hybrids with *shrunken2* endosperm have little variability for total polysaccharides, with reference values ranging from 11% to 14.4% on a kernel dry weight basis among the hybrids and harvest dates included in the 2020 validation set and from 14.5% to 17.8% in the 2021 validation set (Appendix Figures A1-19 and A1-20). This lack of variability could explain the low R^2 in 2020 (Table 2.1) (Blakeney & Flinn, 2005). Given that carbohydrate traits are highly heritable in sweet corn, it is unlikely that the year effects would affect prediction accuracy to a large degree (Tracy, 1997). The RMSEP was similar among years, 2.88 in 2020 and 2.74 in 2021. Lastly, the coefficients of determination for predicting *sh2, wx1,* and WT endosperms with low quantities of WSP (<6% on a dry weight

basis) were very low (Table 2.1; Appendix Figures A1-23 and A1-24). Previous research has shown that hybrids with *sh2, wx1,* and WT endosperm have low quantities of WSP and low variation for WSP among hybrids and among harvest dates (Creech, 1965). The lack of quantity coupled with the lack of variability for this trait could explain the low prediction accuracy. WSP ranged from -2.44% to 5.82% and -2.52% to 5.99% in the validation reference values for *sh2, wx1,* and WT endosperms in 2020 and 2021, respectively, and from 1.32% to 6.76% and -2.26% to 6.08% in the predicted values in 2020 and 2021, respectively, on a kernel dry weight basis (Appendix Figures A1-23 and A1-24). Negative WSP values can be generated for reference values because WSP content is determined by subtracting starch content from total polysaccharide content, which are quantified in two separate assays. Both the reference and the predicted values fall within the biological range reported by Creech, (1965). WSP ranged from 14.90% to 27.78% and 22.35% to 29.68% in the validation reference values for *su1* endosperms in 2020 and 2021, respectively, and from 17.15% to 24.90% and 21.14% to 27.07% in the predicted values in 2020 and 2021, respectively, on a kernel dry weight basis (Appendix Figures A1-17 and A1-18). This demonstrates that the models' predictions differentiate among endosperm types with low and high quantities of WSP.

The poor prediction accuracy for WSP could also be affected by the error associated with the reference values. Interlaboratory error for starch quantification using enzymatic assays is between 1.9 and 5% (McCleary et al., 1997; McCleary et al., 2019). The RMSEPs fell into a similar range as the error associated with the reference method, from 0.30 to 4.83 for prediction models for all traits. The predicted WSP values fall into a range established by prior research, yet the models had poor prediction accuracy when endosperms with high and low trait values were analyzed separately, resulting in especially poor prediction of WSP for *sh2, wx1,* and WT

endosperm types. Therefore, in analysis, the predicted WSP values were only used for *su1* endosperms.

Prediction models trained with one year and used to predict unknown hybrids within the same year generally had higher, albeit similar, prediction accuracy than between year models (Table 2.1). Other authors report similar results for between year versus within year prediction models for maize yield and for apple dry matter using NIRS data (Lane et al., 2020; Teh et al., 2020). Between year models had higher prediction accuracy for some traits, for example for glucose or when the 2020 model was used to predict sucrose content of hybrids with *sh2* endosperm (high trait value) in 2021 (Table 2.1). Due to higher prediction accuracy for most traits, within year models were used to predict all carbohydrate traits in analysis.

Table 2.1. Prediction accuracy metrics for partial least square regression prediction models for carbohydrate traits. Models trained on calibration set from each experimental year, 2020 and 2021 ("Training Year") and used to predict unknown hybrids in each experimental year, 2020 and 2021 ("Predicted Year"). Reported values are coefficients of determination (R^2) of regression of external validation reference values on model predicted values for the overall model with all hybrids and endosperm types, a reduced model predicting hybrids with endosperm types with high levels of a carbohydrate (high trait value), and a reduced model predicting hybrids with endosperm types with low levels of a carbohydrate (low trait value). NA = not applicable.

Trait	**Overall Model R^2**				**Reduced Model R^2 (high trait value)**				**Reduced Model R^2 (low trait value)**			
Training Year	*2020*	*2021*	*2020*	*2021*	*2020*	*2021*	*2020*	*2021*	*2020*	*2021*	*2020*	*2021*
Predicted Year	*2020*	*2021*	*2021*	*2020*	*2020*	*2021*	*2021*	*2020*	*2020*	*2021*	*2021*	*2020*
Total Sugar	0.98	0.99	0.98	0.99	0.93	0.96	0.84	0.91	0.87	0.86	0.84	0.90
Sucrose	0.96	0.97	0.97	0.94	0.79	0.71	0.90	0.51	0.58	0.64	0.58	0.35
Glucose	0.61	0.87	0.90	0.64	NA	NA	NA	NA	NA	NA	NA	NA
Fructose	0.74	0.89	0.89	0.71	NA	NA	NA	NA	NA	NA	NA	NA
Total Polysac-charides	0.97	0.97	0.96	0.97	0.78	0.70	0.54	0.78	0.07	0.84	0.69	0.02
Starch	0.98	0.97	0.97	0.98	0.82	0.39	0.69	0.69	0.83	0.61	0.56	0.55
WSP	0.92	0.94	0.92	0.94	0.56	0.42	0.27	0.69	0.01	0.04	0.08	0.07

2.4.2 Analysis of Variance

In the ANOVA, hybrid, harvest date, and planting date were all significant sources of variation for all traits among the four diallel crosses, with the exception that harvest date was not a significant source of variation for TSS among WT hybrids and planting date was not a significant source of variation for total polysaccharides among *sh2* hybrids (Appendix Tables A2-1, A2-2, A2-3, A2-4).

Spearman's rho indicated that planting dates could be pooled within years but not across years for all traits in the *wx1* diallel cross (Appendix Table A3-1). Spearman's rho indicated that planting dates and years could be pooled for all traits in the wild type diallel cross (Appendix Table A3-2). Spearman's rho indicated that planting dates and years could be pooled for all traits except starch in the *su1* diallel cross (Appendix Table A3-3). Spearman's rho indicated that planting dates could be pooled within 2021 for total polysaccharides and starch but planting dates could not be pooled within 2020 for these traits in the *sh2* diallel cross (Appendix Table A3-4). In instances of GCA x planting date and SCA x planting date interactions, planting dates were pooled when Spearman's rho was greater than 0.75 (Appendix Tables A3-5, A3-6, A3-7, and A3-8).

Combining ability analysis found that GCA was a significant source of variation for all traits in all four diallel crosses measured in all planting dates among the WT, *su1,* and *wx1* diallel crosses and among the *sh2* diallel cross measured in planting dates in 2021 (Appendix Tables A2-1, A2-2, A2-3, A2-4). SCA was a significant source of variation for many traits in ANOVA, but significant SCA effects were rare and are therefore not reported. Harvest date was a significant source of variation, but high correlations were found for GCA among the three harvest dates, and therefore combining ability analysis is reported averaged over harvest dates

(Appendix Tables A4-1, A4-2, A4-3, A4-4, and A4-5). Predictability is the ratio of two times the mean square for GCA to two times the mean square for GCA plus the mean square for SCA and is therefore a measure of the relative contribution of GCA and SCA to hybrid performance. Predictabilities ranged from 0.58 to 0.99 for all traits, indicating that GCA is generally a better predictor of hybrid trait performance than SCA (Table 2.2).

Table 2.2. Predictability, $(2 * MS_{GCA}) : (2 * MS_{GCA} + MS_{SCA})$, with MS = mean square, GCA = general combining ability, SCA = specific combining ability, for carbohydrate traits measured on 15 hybrids within each endosperm type from six-line half diallel crosses grown in Madison, WI in four planting dates in 2020 and 2021. Due to interactions, the *wx1* diallel cross could not be pooled over years and is therefore presented for each year. WSP = water soluble polysaccharides. NA = not applicable. † = measured in two planting dates in 2021.

Trait	**WT diallel cross**	***sh2* diallel cross†**	***su1* diallel cross**	***wx1* diallel cross 2020**	***wx1* diallel cross 2021**
Total Soluble Solids (%)	0.98	0.98	0.94	0.88	0.92
Total Sugar (mg/g)	0.99	0.98	0.97	0.58	0.93
Sucrose (mg/g)	0.97	0.99	0.95	0.75	0.91
Glucose (mg/g)	0.97	0.99	0.99	0.67	0.94
Fructose (mg/g)	0.98	0.99	0.99	0.66	0.95
Total Polysaccharides (mg/g)	0.98	0.97	0.99	0.76	0.90
Starch (mg/g)	0.98	0.99	0.94	0.74	0.83
WSP (mg/g)	NA	NA	0.98	NA	NA

2.4.3 Carbohydrate Content

Averaged over hybrids, harvest dates, and four planting dates, the *sh2* mutation resulted in a significant increase of 245.0 mg/g in total sugar, 200.0 mg/g in sucrose, 23.6 mg/g in glucose, and 20.0 mg/g in fructose compared to WT (Appendix Table A5-1). The *sh2* mutation resulted in a significant decrease of 290.0 mg/g in total polysaccharides, 262.0 mg/g in starch, and 45.1 mg/g in total carbohydrate content compared to WT (Appendix Table A5-1).

Comparatively, the *su1* mutation did not have as large of an effect on sugars, conferring a significant increase of 32.0 mg/g in total sugar, 31.1 mg/g in sucrose, and 1.5 mg/g in glucose, compared to WT (Appendix Table A5-1). The *su1* mutation was not different from WT in fructose content but contributed to a significant decrease of 43.0 mg/g in total polysaccharides, 220.0 mg/g in starch, and 11.4 mg/g in total carbohydrate content, compared to WT (Appendix Table A5-1). The *wx1* mutation had an even smaller effect on sugars, conferring a significant increase of 18.0 mg/g in total sugar, 8.1 mg/g in sucrose, 4.3 mg/g in glucose, and 3.9 mg/g in fructose (Appendix Table A5-1). The *wx1* mutation contributed to a significant increase in starch of 7.0 mg/g, but a decrease in total polysaccharides of 15.0 mg/g compared to WT and was not different from WT for total carbohydrate content (Appendix Table A5-1). The carbohydrate contents in this study align with the ranges reported in prior research (Creech, 1965).

Among hybrids within an endosperm type, carbohydrate content varied, and averaging over hybrids within an endosperm type did not reveal the full spectrum of variation. For example, averaged over harvest dates, four *su1* hybrids had a significantly lower quantity of total sugar than C40 x Ia5125 *wx1*, despite the *su1* mutation conferring an increase in total sugar compared to *wx1* on average (Appendix Table A5-2). Many hybrids with C40 as a parent had high amounts of total sugar across all four endosperm types. For example, C40 x Ia5125 with WT, *wx1, su1,* or *sh2* endosperm had significantly higher total sugar than 10 of the 15 WT, *wx1, su1,* or *sh2* hybrids, respectively, when averaged over harvest dates (Appendix Table A5-2).

Harvest date also had a large effect on carbohydrate content. Averaged over *su1* hybrids, total sugars decreased 49.1 mg/g between 19 DAP and 25 DAP (Figure 2.1). Similarly, total sugars decreased 52.0 mg/g averaged over WT hybrids and 56.5 mg/g averaged over *wx1* hybrids between 19 DAP and 25 DAP (Figure 2.1). In contrast, total sugar content of *sh2* hybrids had a

less severe decrease over harvest dates, with 17.7 mg/g between 19 DAP and 25 DAP (Figure 2.1). A similar but opposite trend was observed in total polysaccharide content over harvest dates (Figure 2.1). Pairwise comparisons among harvest dates within endosperm types showed that total sugar and total polysaccharide content were significantly different at each harvest date, except for total sugar among *sh2* hybrids, which remained stable from 19 to 22 DAP before decreasing at 25 DAP (Figure 2.1).

The linear trends observed among total sugar and total polysaccharide content over consecutive harvest dates were not reflected in TSS content. Due to the presence of WSP, *su1* endosperm had much higher TSS compared to the other three endosperm types, with 21.43% TSS averaged over harvest dates compared to 14.54% for *sh2,* 8.94% for *wx1,* 8.13% for WT endosperm (Figure 2.1). WT TSS did not vary over harvest dates, while *su1, sh2,* and *wx1* had significantly lower TSS at 19 DAP compared to 22 DAP and 25 DAP (Figure 2.1). *su1* also had the largest increase in TSS from 19 to 25 DAP, increasing by almost 3% (Figure 2.1). There was variation for TSS among hybrids within endosperm types. TSS ranged from 5.36% to 10.26% among WT hybrids, from 6.55% to 12.25% among *wx1* hybrids, and from 12.36% to 16.43% among *sh2* hybrids, over three harvest dates (Appendix Tables A5-3, A5-4, A5-5, A5-6). *su1* hybrids had a greater range, comparatively, from 16.18% to 26.53% over three harvest dates (Appendix Table A5-7).

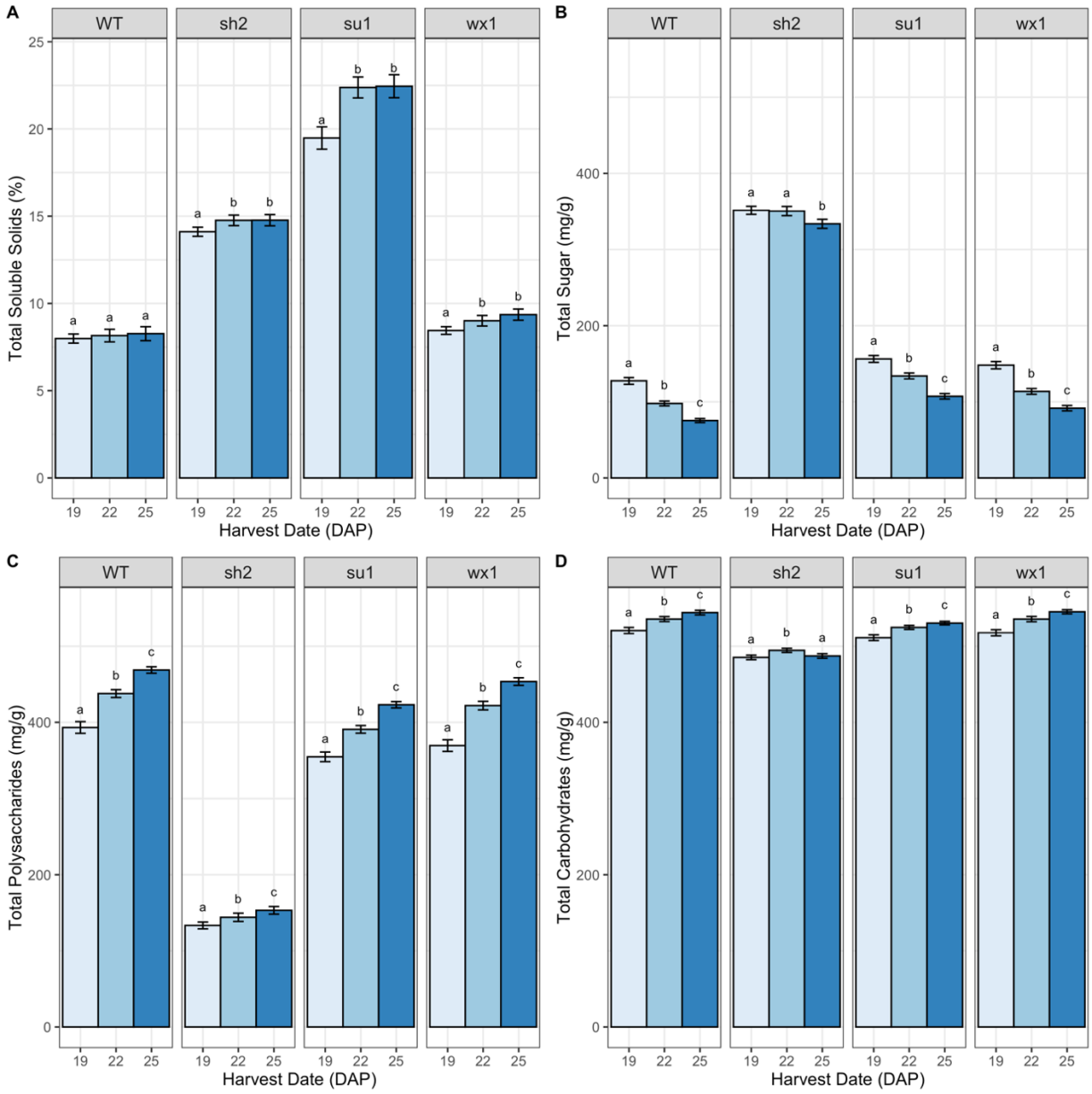

Figure 2.1. Mean ± standard error of total soluble solids (%) (A), total sugar content (mg/g) (B), total polysaccharides (mg/g) (C), total carbohydrates (mg/g) (D) of four endosperm types, (*waxy1* (*wx1*), Wild Type (WT), *sugary1* (*su1*), *shrunken2* (*sh2*)) across three harvest dates (19, 22, 25 days after pollination (DAP)) averaged over hybrids generated from four six-line half diallel crosses measured in four planting dates across two years, 2020 and 2021, at West Madison Agricultural Research Station. Within an endosperm type, harvest dates that share a lowercase letter are not significantly different at alpha = 0.05 from Tukey's Honest Significant Difference pairwise comparison tests.

2.4.4 General Combining Ability: Sugar Traits among Near Isogenic Lines

The general combining abilities for all traits are reported over four planting dates for WT, *su1,* and *wx1* diallel crosses. The *wx1* diallel cross is reported for each experimental year due to rank change interactions. The *sh2* diallel cross is reported over two planting dates in 2021 due to seed shortages. *sh2* NILs Ia5125 and P39 had positive GCA for total sugar (Table 2.3). In the *su1, wx1,* and WT diallel crosses, these two NILs did not have significant GCA for total sugar (Tables 2.4 – 2.7). Instead, C40 had a positive GCA for total sugar and C68 had a negative GCA for total sugar in the *su1, wx1* in 2021*,* and WT diallel crosses (Tables 2.4, 2.6, 2.7). Additionally, C40 had a positive GCA for sucrose, glucose, and fructose in the *su1* and WT diallel crosses as well as a positive GCA for sucrose in the *wx1* diallel cross in 2021 (Tables 2.4, 2.6, 2.7). C68 had a negative GCA for sucrose, glucose, and fructose in *su1* and *wx1* in 2021 diallel cross, and a negative GCA for glucose and fructose in the *sh2* and WT diallel cross (Tables 2.3, 2.4, 2.6, 2.7).

Averaged over hybrids at 19 DAP, having C40 as a parent led to significantly higher total sugar content than when C68 was a parent in *su1, wx1, sh2,* and WT diallel crosses (Appendix Table A5-8). The same effect was observed at 22 DAP and at 25 DAP (AppendixTable A5-8). This finding agrees with Finegan et al. (2022), who used the same set of NILs and reported C40 per se had higher total sugar levels at 21 DAP in *su1* and WT NILs compared to C68. However, while Finegan et al. (2022) found no difference in total sugar between *sh2* C40 and C68 NILs per se at 21 DAP, we observed a significant difference in total sugar content between these two *sh2* NILs when used in hybrid combination at three harvest dates, 19, 22, and 25 DAP.

These results suggest that C40 may contribute alleles that positively affect total sugar and sucrose content when paired with mutations at the *su1*, *wx1,* or *sh2* locus, or with functional

alleles in the starch synthesis pathway (WT), while C68 may carry alleles that negatively affect these traits in these endosperm types.

P39 had a positive GCA for TSS across all four diallel crosses while Ia5125 had a negative GCA for TSS across all four diallel crosses except for TSS measured in 2020 in the WT diallel cross (Tables 2.3 – 2.7). P39 had a positive GCA for total sugar and sucrose in the *sh2* diallel cross but did not have a significant positive GCA for any other sugar traits across the four diallel crosses (Tables 2.3 – 2.7). Ia5125 had a negative GCA for TSS across all four diallel crosses and a negative GCA for sucrose across the *su1, wx1* in 2020, and WT diallel crosses, but a positive GCA for glucose and fructose across the *sh2, su1,* and WT diallel crosses and for total sugar in the *sh2* diallel cross (Tables 2.3 – 2.7). Therefore, there was not a consistent pattern between the GCA for TSS and GCA for other sugar traits in any of the diallel crosses.

Table 2.3. General combining abilities for *shrunken2* NILs from a six-line half diallel cross averaged over three harvest dates measured in two planting dates in 2021. GCA = general combining ability, NIL = near isogenic line, ns = not significant. *,**,*** significant at 0.05, 0.01, and 0.001 probability levels, respectively. ∴ = measured in early planting date in 2021. ⋮ = measured in late planting date in 2021.

	GCA Values					
***shrunken2* NIL**	**Total Soluble Solids (%) ∴**	**Total Soluble Solids (%) ⋮**	**Total Sugar (mg/g)**	**Sucrose (mg/g)**	**Glucose (mg/g)**	**Fructose (mg/g)**
C68	ns	-0.53*	ns	20.55***	-6.92***	-6.80***
Ia5125	ns	-0.71**	14.80***	ns	9.79***	10.17***
Ia453	ns	ns	ns	14.52***	-4.45**	-3.39*
P39	1.66***	1.39***	11.05**	11.83**	ns	ns
C40	ns	ns	ns	-8.39*	5.51***	4.89**
Il101t	-1.17***	-0.92***	-34.41***	-31.51***	-3.19*	-3.85*

Table 2.4. General combining abilities for *sugary1* NILs from a six-line half diallel cross averaged over three harvest dates measured in four planting dates in 2020 and 2021. GCA = general combining ability, NIL = near isogenic line, ns = not significant. *,**,*** significant at 0.05, 0.01, and 0.001 probability levels, respectively.

	GCA Values

***sugary1* NIL**	**Total Soluble Solids (%)**	**Total Sugar (mg/g)**	**Sucrose (mg/g)**	**Glucose (mg/g)**	**Fructose (mg/g)**
C68	ns	-18.97***	-7.50***	-4.58***	-5.00***
Ia5125	-2.09***	ns	-4.69**	5.01***	5.75***
Ia453	2.24***	ns	ns	-3.28**	-3.78**
P39	1.27**	ns	ns	-3.02**	-3.16***
C40	-0.82*	22.29***	12.47***	5.12***	5.01***
Il101t	-1.01*	ns	-3.47*	ns	ns

Table 2.5. General combining abilities for wax*y1* NILs from a six line half diallel cross averaged over three harvest dates measured in two planting dates in 2020. GCA = general combining ability, NIL = near isogenic line, ns = not significant. *,**,*** significant at 0.05, 0.01, and 0.001 probability levels, respectively. ∮ = measured in early planting date in 2020. ∯ = measured in late planting date in 2020.

	GCA Values							
***waxy1* NIL**	**Total Soluble Solids (%)**	**Total Sugar (mg/g)**	**Sucrose (mg/g) ∮**	**Sucrose (mg/g) ∯**	**Glucose (mg/g) ∮**	**Glucose (mg/g) ∯**	**Fructose (mg/g) ∮**	**Fructose (mg/g) ∯**
C68	ns	ns	ns	ns	ns	ns	ns	ns
Ia5125	-1.38***	ns	-8.28*	ns	ns	ns	ns	ns
Ia453	ns	ns	ns	ns	ns	ns	ns	ns
P39	0.85***	ns	ns	ns	ns	ns	ns	ns
C40	ns	ns	ns	ns	ns	ns	ns	ns
Il101t	0.65**	ns	ns	ns	ns	ns	ns	ns

Table 2.6. General combining abilities for wax*y1* NILs from a six line half diallel cross averaged over three harvest dates measured in two planting dates in 2021. GCA = general combining ability, NIL = near isogenic line, ns = not significant. *,**,*** significant at 0.05, 0.01, and 0.001 probability levels, respectively.

	GCA Values				
***waxy1* NIL**	**Total Soluble Solids (%)**	**Total Sugar (mg/g)**	**Sucrose (mg/g)**	**Glucose (mg/g)**	**Fructose (mg/g)**
C68	-0.52*	-20.17***	-8.33***	-6.44**	-5.91**
Ia5125	-0.88***	ns	ns	ns	ns
Ia453	0.57**	ns	ns	ns	ns
P39	0.45*	ns	ns	ns	ns
C40	0.63**	12.21*	7.74***	ns	ns
Il101t	ns	ns	-5.46**	ns	ns

Table 2.7. General combining abilities for wild type NILs from a six-line half diallel cross averaged over three harvest dates measured in four planting dates in 2020 and 2021. GCA = general combining ability, NIL = near isogenic line, ns = not significant. *,**,*** significant at 0.05, 0.01, and 0.001 probability levels, respectively. ∫ = measured in two planting dates in 2020. ∬ = measured in two planting dates in 2021.

	GCA Values					
Wild type NIL	**Total Soluble Solids (%)∫**	**Total Soluble Solids (%)∬**	**Total Sugar (mg/g)**	**Sucrose (mg/g)**	**Glucose (mg/g)**	**Fructose (mg/g)**
C68	ns	ns	-12.32***	ns	-5.11***	-4.94***
Ia5125	-2.53***	-1.74***	ns	-5.66***	5.17***	5.48***
Ia453	0.70**	ns	-8.19*	ns	-3.35*	-3.33*
P39	ns	0.82***	ns	ns	-2.92*	-2.94*
C40	1.13***	1.26***	18.11***	8.99***	5.48***	5.20***
Il101t	0.60**	ns	ns	ns	ns	ns

2.4.5 General Combining Ability: Polysaccharide Traits among Near Isogenic Lines

NILs with positive GCA for total sugar had negative GCA for total polysaccharides and vice versa. Specifically, among *sh2* NILs, C68, P39, and Ia5125 had negative GCA for total polysaccharides (Table 2.3). Across *su1, wx1,* and WT NILs, C40 had a negative GCA for total polysaccharides and C68 a positive GCA (Tables 2.9 – 2.12). For the *sh2* NILs, Ia5125 had a negative GCA for total polysaccharides and a positive GCA for starch. Ia453, had a positive GCA just for starch, whereas P39 had a negative GCA for both traits (Table 2.8). Ia453 had a positive GCA for total polysaccharides among WT, *su1,* and *wx1* in 2020 NILs (Tables 2.9, 2.10, 2.12). Ia453 had a negative GCA for starch in three of the four planting dates among *su1* NILs, a positive GCA for starch among *wx1* NILs in 2020, but did not have a significant effect on starch among WT NILs (Tables 2.9, 2.10, 2.12). Ia453 had the largest, positive GCA for WSP among *su1* NILs in all four planting dates, demonstrating that Ia453 contributes polysaccharide content that is primarily WSP, not starch (Table 2.9).

Il101t had the largest GCA for total polysaccharides among *sh2* NILs, 31.96 mg/g (Table 2.8), which corresponds to a large negative GCA for total sugar, -34.41 mg/g (Table 2.3). Among *su1* NILs, Il101t had a positive GCA for starch in planting date 1, 2, and 3, similar to the GCA found for starch among *sh2* Il101t (Tables 2.8 and 2.9). These findings suggest that Il101t may contribute favorable alleles for starch production in both *su1* and *sh2* endosperm types, while Ia453 may contribute favorable alleles for total polysaccharide among *su1* and WT endosperm types, for WSP with *su1* endosperm, and for starch with *sh2* endosperm.

Table 2.8. General combining abilities for *shrunken2* NILs from a six-line half diallel cross averaged over three harvest dates measured in two planting dates in 2021. GCA = general combining ability, NIL = near isogenic line, ns = not significant. *,**,*** significant at 0.05, 0.01, and 0.001 probability levels, respectively.

GCA Values		
***shrunken2* NIL**	**Total Polysaccharides (mg/g)**	**Starch (mg/g)**
C68	-9.01**	ns
Ia5125	-7.80*	11.73**
Ia453	ns	13.20***
P39	-11.68***	-15.36***
C40	ns	ns
Il101t	31.96***	23.74***

Table 2.9. General combining abilities for *sugary1* NILs from a six-line half diallel cross averaged over three harvest dates measured in four planting dates in 2020 and 2021. GCA = general combining ability, NIL = near isogenic line, WSP = water soluble polysaccharides, ns = not significant. *,**,*** significant at 0.05, 0.01, and 0.001 probability levels, respectively. ⊕ = measured in early planting date in 2020. ⊖ = measured in late planting date in 2020. ⊗ = measured in early planting date 2021. ⊙ = measured in late planting date 2021. ∅ = measured in both planting dates in 2021.

GCA Values								
***sugary1* NIL**	**Total Polysaccharide-s (mg/g)**	**Starch (mg/g) ⊕**	**Starch (mg/g) ⊖**	**Starch (mg/g) ⊗**	**Starch (mg/g) ⊙**	**WSP (mg/g) ⊕**	**WSP (mg/g) ⊖**	**WSP (mg/g) ∅**
C68	17.71***	ns	18.77**	ns	ns	17.34***	ns	9.37*
Ia5125	-13.26**	ns	-14.29*	ns	ns	-14.36***	-11.75*	ns

Ia453	15.21***	-13.94**	-15.97*	-21.90**	ns	18.22***	29.41***	28.48***
P39	9.81*	ns	ns	ns	ns	9.13*	ns	ns
C40	-28.59***	-9.46*	-14.17*	ns	ns	-15.17***	-12.58**	-18.42***
Il101t	ns	12.93**	17.70**	24.90***	ns	-15.16***	-19.20***	-25.14***

Table 2.10. General combining abilities for wax*y1* NILs from a six line half diallel cross averaged over three harvest dates measured in two planting dates in 2020. GCA = general combining ability, NIL = near isogenic line, ns = not significant. *,**,*** significant at 0.05, 0.01, and 0.001 probability levels, respectively.

GCA Values		
***waxy1* NIL**	**Total Polysaccharides (mg/g)**	**Starch (mg/g)**
C68	ns	ns
Ia5125	ns	ns
Ia453	23.64**	20.98*
P39	ns	ns
C40	-25.30**	-18.13*
Il101t	ns	ns

Table 2.11. General combining abilities for wax*y1* NILs from a six line half diallel cross averaged over three harvest dates measured in two planting dates in 2021. GCA = general combining ability, NIL = near isogenic line, ns = not significant. *,**,*** significant at 0.05, 0.01, and 0.001 probability levels, respectively.

GCA Values		
***waxy1* NIL**	**Total Polysaccharides (mg/g)**	**Starch (mg/g)**
C68	20.41**	21.47*
Ia5125	ns	ns
Ia453	ns	ns
P39	ns	ns
C40	-19.76**	-19.48*
Il101t	ns	ns

Table 2.12. General combining abilities for wild type NILs from a six-line half diallel cross averaged over three harvest dates measured in four planting dates in 2020 and 2021. GCA = general combining ability, NIL = near isogenic line, ns = not significant. *,**,*** significant at 0.05, 0.01, and 0.001 probability levels, respectively. $\oplus$ = measured in early planting date in 2020. $\ominus$ = measured in late planting date in 2020. $\otimes$ = measured in early planting date 2021. $\odot$ = measured in late planting date 2021.

GCA Values

Wild type NIL	Total Polysaccharides (mg/g)	Starch (mg/g) ⊕	Starch (mg/g) ⊖	Starch (mg/g) ⊗	Starch (mg/g) ⊙
C68	11.43*	ns	ns	ns	ns
Ia5125	-16.58***	ns	ns	ns	ns
Ia453	20.44***	ns	ns	ns	ns
P39	11.63*	ns	ns	ns	ns
C40	-26.45***	-25.49**	ns	ns	-33.34***
Il101t	ns	ns	ns	ns	ns

2.4.6 Correlations

In agreement with prior research, among all endosperm types as well as among hybrids within a single endosperm type, total sugar was strongly inversely correlated with total polysaccharides (Figure 2.2, A) (Creech, 1965). Likewise, sucrose was strongly correlated with total sugar (Figure 2.2, A). Similarly, glucose and fructose were strongly correlated with total sugar (Figure 2.2, A). Among all endosperm types, WSP and soluble carbohydrates (WSP plus Total Sugar) were highly correlated with TSS, yet total sugar and sucrose were weakly correlated with TSS (Figure 2.2, A). Among endosperm types with low WSP content (*sh2, wx1,* WT), total sugar and sucrose were highly correlated with TSS (Figure 2.2, C). The correlations among carbohydrate traits and TSS are stronger than those reported by Hale et al. (2005), who found that TSS was moderately negatively correlated with total sugars and sucrose, among nine cultivars harvested at three dates with *su1, sh2,* and *sugaryenhancer1* endosperm types. The range and relative value of TSS content determined in this experiment among hybrids and endosperm types agreed with the values reported by Hale et al. (2005). However, the total sugar and sucrose content reported by Hale et al. (2005) for *su1* and *sh2* hybrids was much lower than the content found in this experiment and others (Creech, 1965; Finegan et al., 2022).

Correlations with TSS are much lower within a single endosperm type. For example, among hybrids with *su1* endosperm, WSP was weakly correlated with TSS and soluble

carbohydrates did not significantly correlate with TSS (Figure 2.2, B). Total sugar was weakly negatively correlated with TSS in *su1* endosperm (Figure 2.2, B). Among hybrids with *sh2* endosperm, total sugar was weakly positively correlated with TSS (Figure 2.2, D). Among hybrids with *wx1* or WT endosperm, total sugar was not correlated with TSS (Appendix Figure A6). The correlations indicate that TSS content reflects the major differences among WSP (all endosperms) or total sugar content (*sh2, wx1,* WT endosperms) among endosperm types but is not sensitive to differences among hybrids within an endosperm type for carbohydrate traits. Therefore, TSS content is of little use in sweet corn breeding for carbohydrate traits when the aim is to select on heritable differences within endosperm types, not across endosperm types.

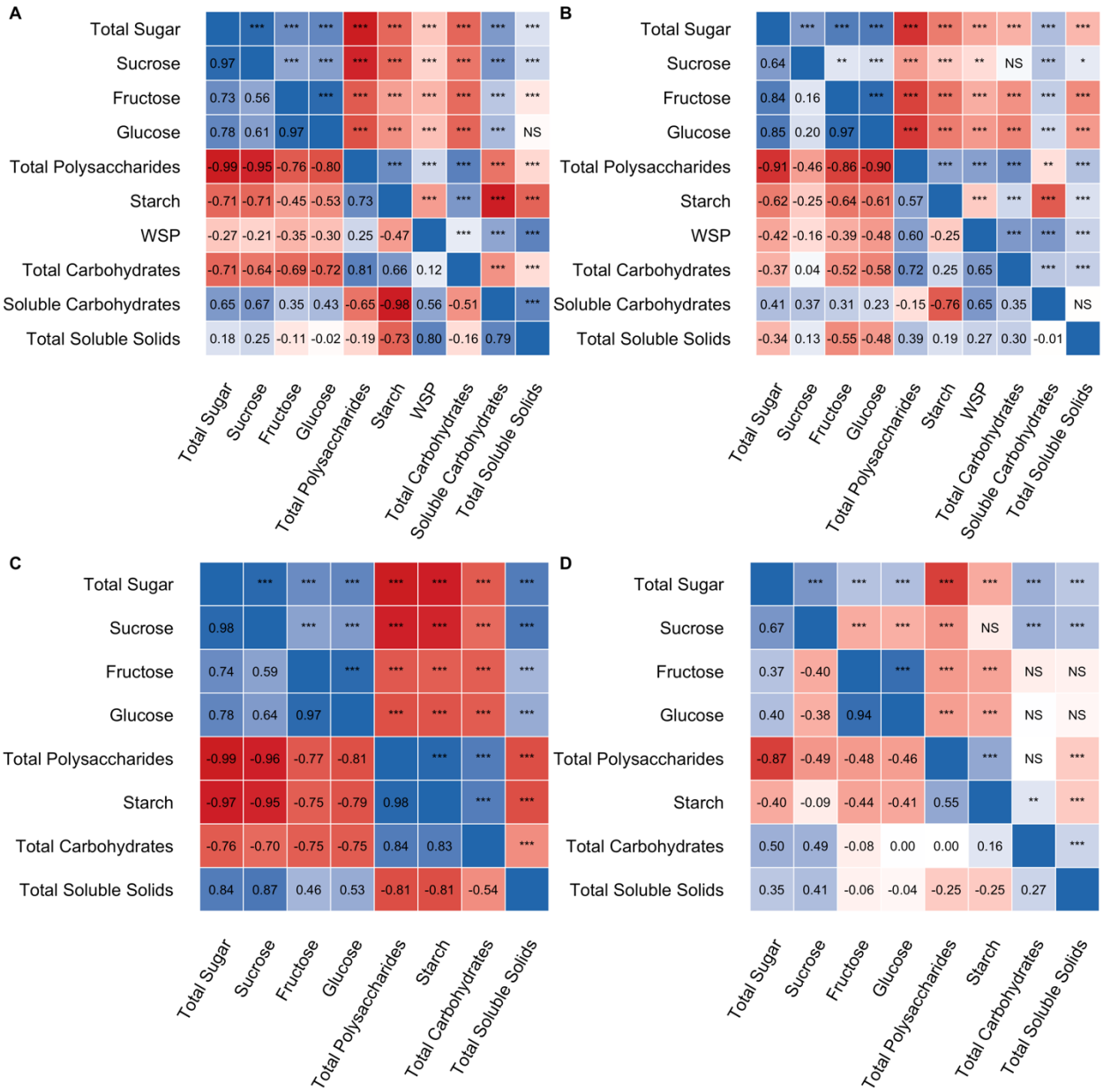

Figure 2.2. Pearson correlation coefficients (lower diagonals) and significance (upper diagonals, *,**,*** correspond to 0.05, 0.01, 0.001 probability levels, respectively) among traits measured in four planting dates at West Madison Agricultural Research Station in 2020 and 2021 averaged over three harvest dates. Averaged over all endosperm types (A), among *sugary1* endosperm (B), averaged over endosperm types with low levels of WSP (C), among *shrunken2* endosperm (D). NS = not significant. Cells are colored white for correlation coefficients of 0, colored blue for positive correlation coefficients, with increasing saturation as coefficient approaches 1, and colored red for negative correlation coefficients, with increasing saturation as coefficient approaches -1. NS = not significant.

2.5 Conclusion

Total soluble solids content is not a useful trait for sweet corn breeding, supporting the conclusions of Hale et al. (2005). Sweet corn breeding aims to select upon differences in eating quality traits within endosperm types. Using a half diallel mating design of NILs, analysis revealed that while TSS varies among hybrids and is significantly lower at 19 DAP compared to 22 or 25 DAP for *su1, wx1,* and *sh2* endosperm, TSS does not strongly correlate with total sugar within an endosperm type nor does TSS behave similarly across endosperm types. Future research on selection methodology for eating quality could focus on determining the repeatability among taste tasters or evaluating rapid sensory methods to determine which best function in the context of breeding sweet corn.

For all other carbohydrate traits, similar NILs were desirable combiners in the *su1, wx1,* and WT diallel crosses. Different NILs were desirable combiners in the *sh2* diallel cross. The main effects of the *su1* and *wx1* mutations are slight reductions in dry seed weight but differences among polysaccharide ratios. Such differences are due to changes late in the starch synthesis pathway, affecting granule bound starch synthase (*wx1*) and isoamylase1 (*su1*), respectively (Shuler et al., 2017; Tracy et al., 2019). In contrast, the *sh2* mutation causes a large decrease in the amount of total carbohydrate (total sugars plus total polysaccharides) compared to *su1, wx1,* and WT hybrids (Tracy, 1997). Among the hybrids in this experiment, those with WT endosperm produced 533.7 mg/g which was no different from *wx1* endosperm, 533.0 mg/g and *su1* endosperm produced slightly, though significantly, less with 522.3 mg/g. Those with *sh2* endosperm produced significantly less total carbohydrate than the other three endosperms, 488.6 mg/g on average (Appendix Table A5-1). Furthermore, the *sh2* mutation is severe, knocking out AGPase activity early in the pathway causing a marked increase in the expression of genes

associated with starch and protein synthesis, an effect which can be replicated by increasing the content of sucrose in developing wild type kernels (Giroux et al., 1994). Finegan et al. (2022) reported that the *su1* mutation caused few changes in the endosperm transcriptome relative to WT, and changes occurred later in kernel development, at 28 DAP. Conversely, *sh2* caused notable changes in the endosperm transcriptome (Finegan et al., 2022). Differences in kernel total carbohydrate, severity of mutation and temporal effects on the starch synthesis pathway, and endosperm transcriptome between *sh2* and WT, *wx1*, or *su1* may explain the differences observed between desirable combiners. Specifically, inbred C40 was a desirable combiner for carbohydrate traits in *su1, wx1,* and WT endosperm types. Inbreds Ia5125, P39, and Ia453 were desirable combiners for carbohydrate traits in the *sh2* endosperm.

The desirable combiners identified in this research could be used in future breeding efforts for quality traits or as testers for new inbreds. This experiment also determined that TSS content is not a reliable tool for selection of sweet corn quality within these major endosperm types.

2.6 Chapter Two References

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2.7 Chapter Two Appendix

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A5-7. Total soluble solids among *sugary1* hybrids over three harvest dates
A5-8. Total sugar among hybrids with C68 or C40 as a parent

VI. Appendix Figure A6: Pearson Correlations

A6. Correlations among traits measured on hybrids with *waxy1* or wild type endosperm

I. Appendix Figures A1: Prediction Models

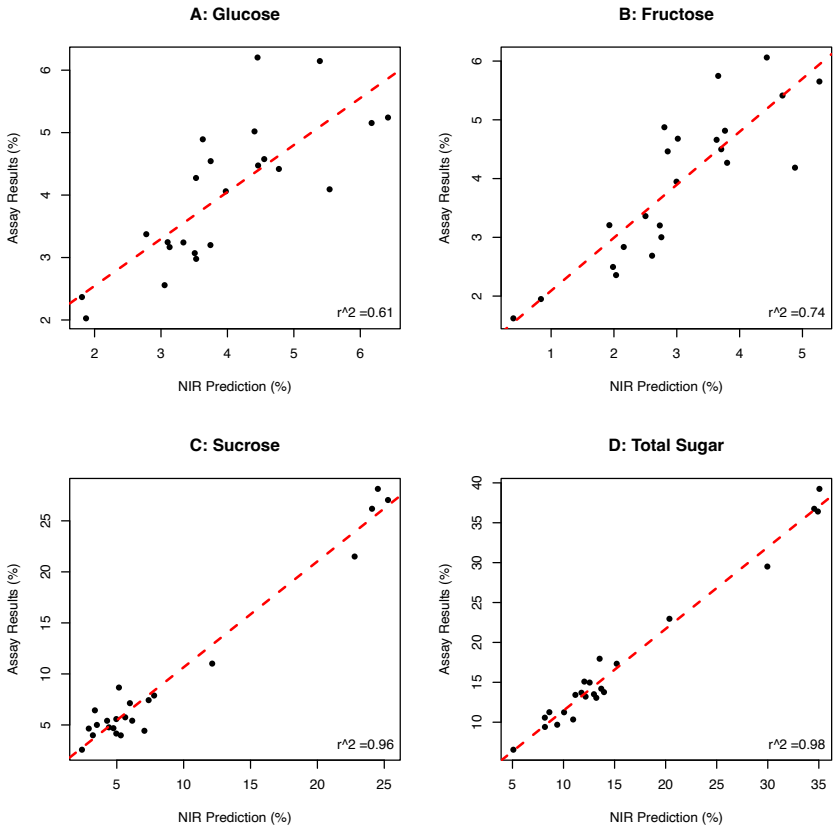

Figure A1-1. Regression of predicted values (NIR Prediction %) on reference wet lab values (Assay Results %) for the validation set selected in 2020 for Glucose (A), Fructose (B), Sucrose (C), and Total Sugar (D). The coefficient of determination of the model is reported in the lower right corner of each graph.

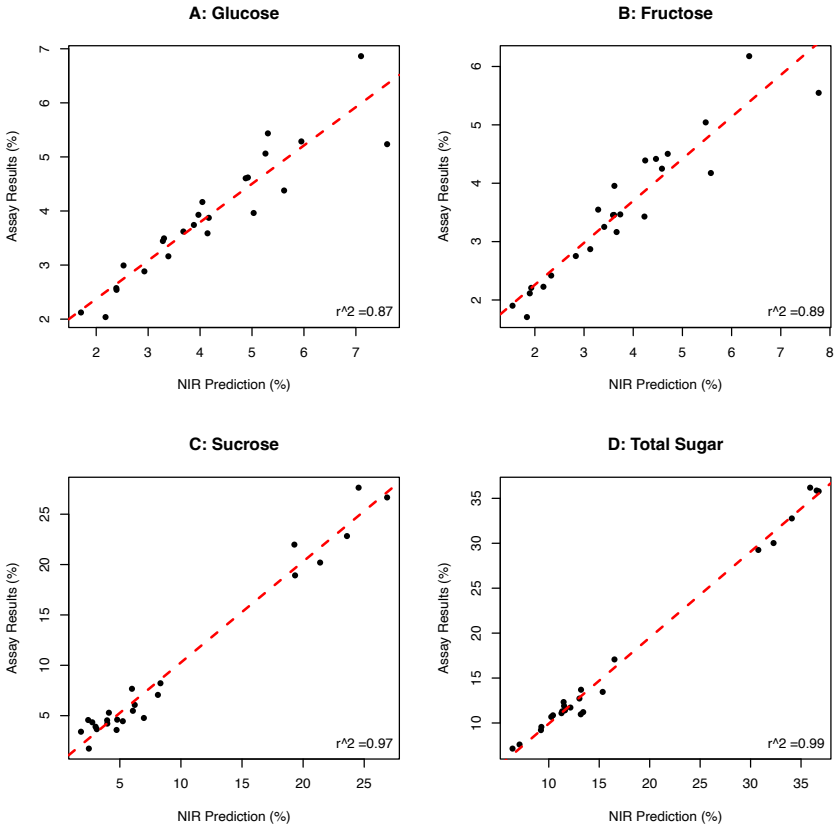

Figure A1-2. Regression of predicted values (NIR Prediction %) on reference wet lab values (Assay Results %) for the validation set selected in 2021 for Glucose (A), Fructose (B), Sucrose (C), and Total Sugar (D). The coefficient of determination of the model is reported in the lower right corner of each graph.

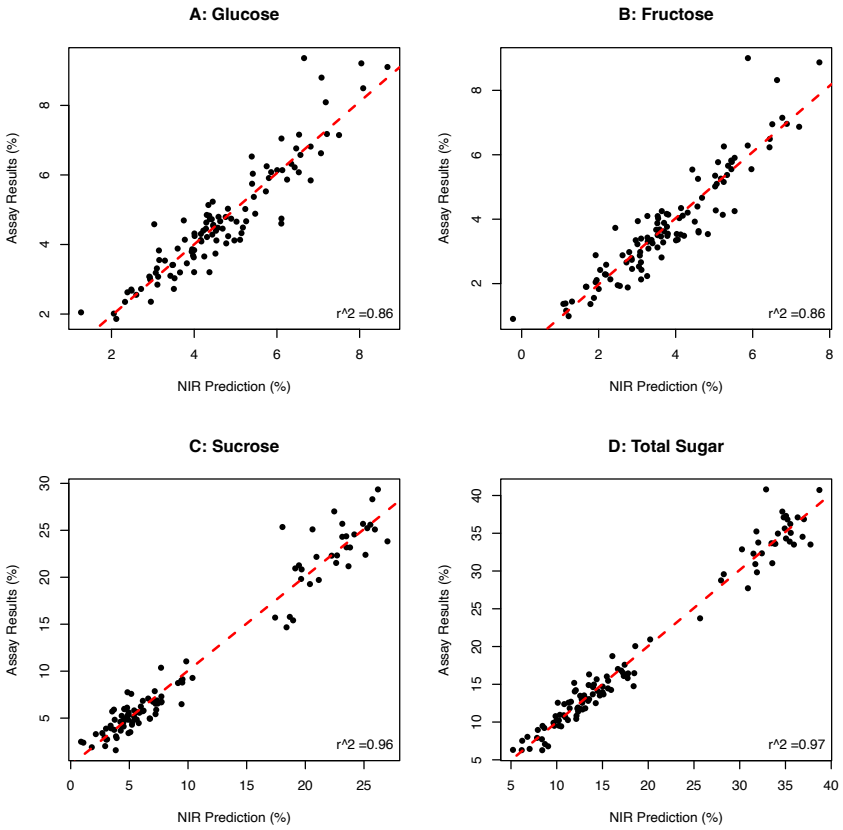

Figure A1-3. Regression of predicted values (NIR Prediction %) on reference wet lab values (Assay Results %) for the calibration set selected in 2020 for Glucose (A), Fructose (B), Sucrose (C), and Total Sugar (D). The coefficient of determination of the model is reported in the lower right corner of each graph.

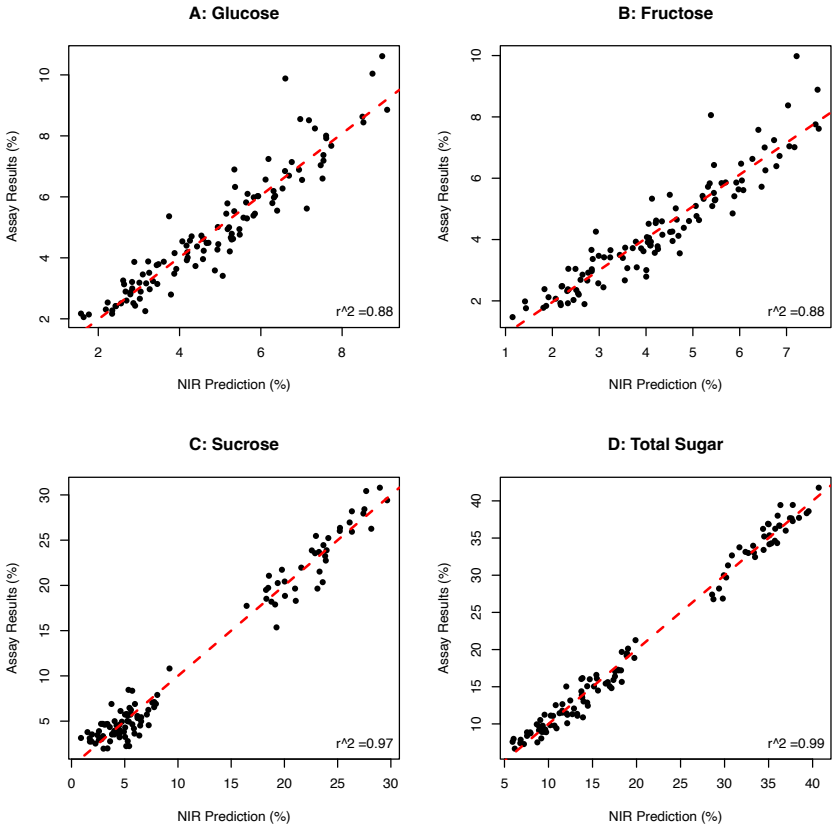

Figure A1-4. Regression of predicted values (NIR Prediction %) on reference wet lab values (Assay Results %) for the calibration set selected in 2021 for Glucose (A), Fructose (B), Sucrose (C), and Total Sugar (D). The coefficient of determination of the model is reported in the lower right corner of each graph.

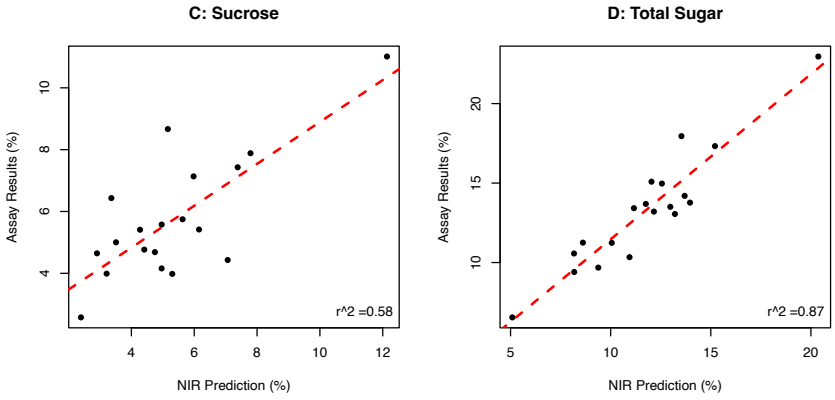

Figure A1-5. Regression of predicted values (NIR Prediction %) on reference wet lab values (Assay Results %) for the *sugary1, waxy1,* and wild type hybrids in the validation set selected in 2020 for Sucrose (C) and Total Sugar (D). The coefficient of determination of the model is reported in the lower right corner of each graph.

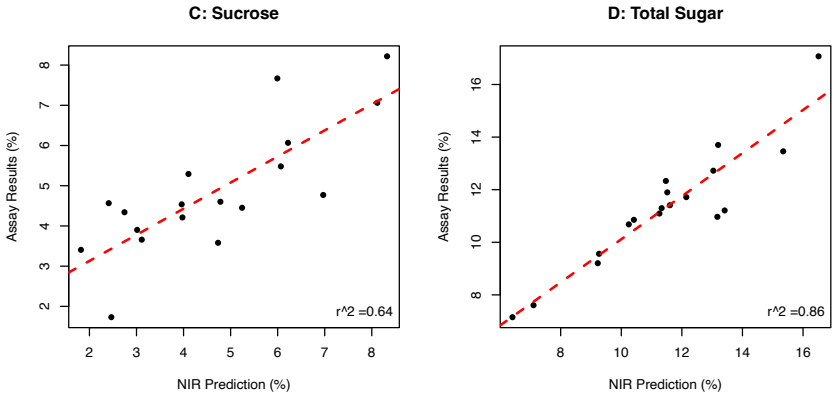

Figure A1-6. Regression of predicted values (NIR Prediction %) on reference wet lab values (Assay Results %) for the *sugary1, waxy1,* and wild type hybrids in the validation set selected in 2021 for Sucrose (C) and Total Sugar (D). The coefficient of determination of the model is reported in the lower right corner of each graph.

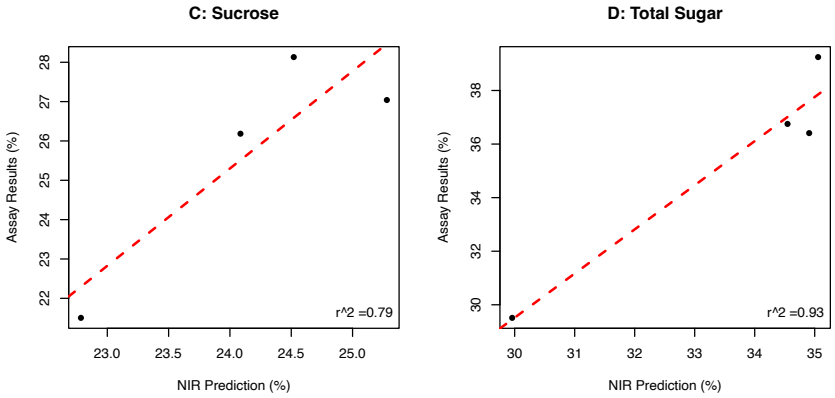

Figure A1-7. Regression of predicted values (NIR Prediction %) on reference wet lab values (Assay Results %) for the *shrunken2* hybrids in the validation set selected in 2020 for Sucrose (C) and Total Sugar (D). The coefficient of determination of the model is reported in the lower right corner of each graph.

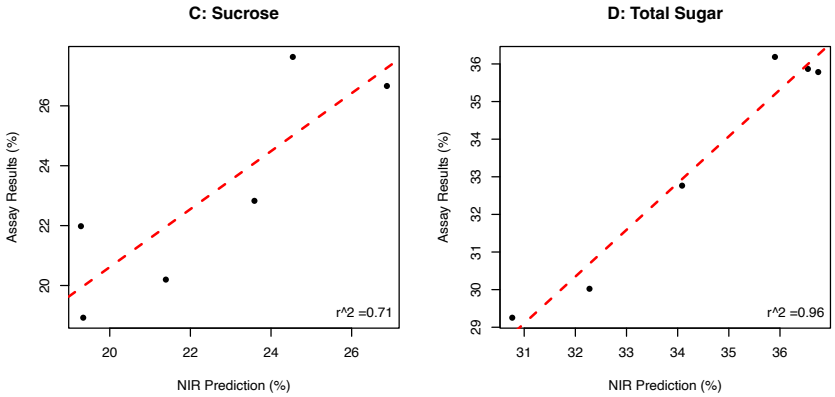

Figure A1-8. Regression of predicted values (NIR Prediction %) on reference wet lab values (Assay Results %) for the *shrunken2* hybrids in the validation set selected in 2021 for Sucrose (C) and Total Sugar (D). The coefficient of determination of the model is reported in the lower right corner of each graph.

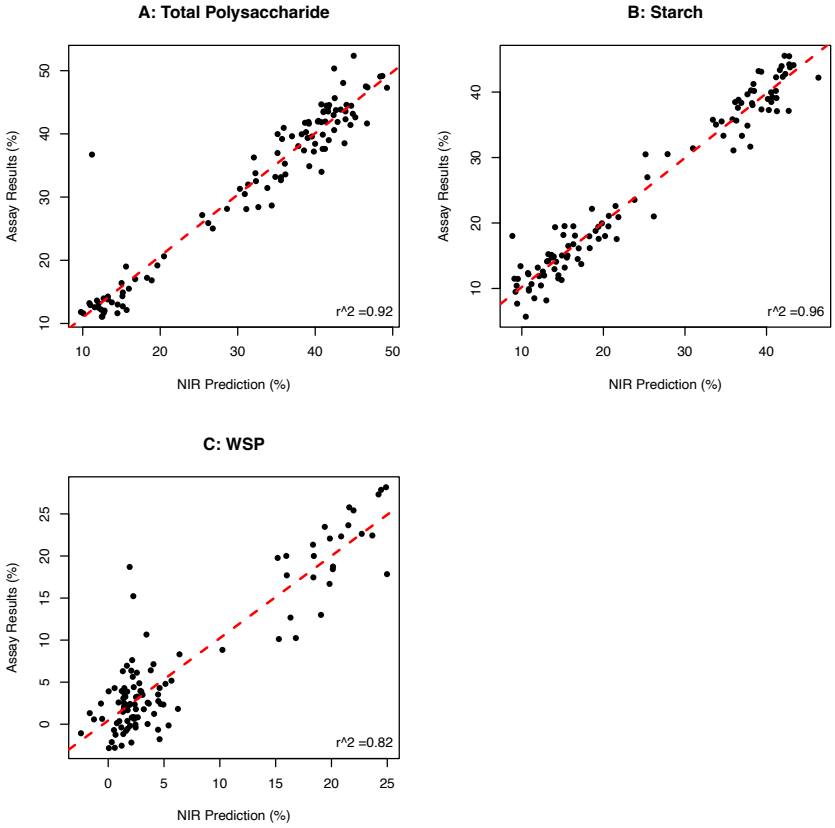

Figure A1-9. Regression of predicted values (NIR Prediction %) on reference wet lab values (Assay Results %) for the calibration set selected in 2020 for Total Polysaccharides (A), Starch (B), and water soluble polysaccharides (C). The coefficient of determination of the model is reported in the lower right corner of each graph.

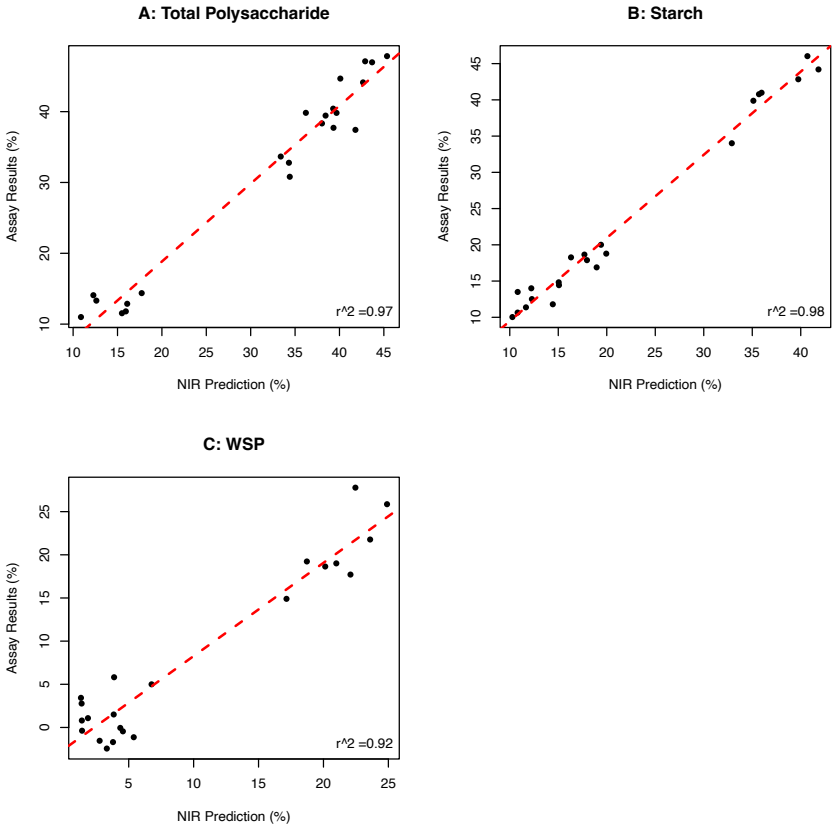

Figure A1-10. Regression of predicted values (NIR Prediction %) on reference wet lab values (Assay Results %) for the validation set selected in 2020 for Total Polysaccharides (A), Starch (B), and water soluble polysaccharides (C). The coefficient of determination of the model is reported in the lower right corner of each graph.

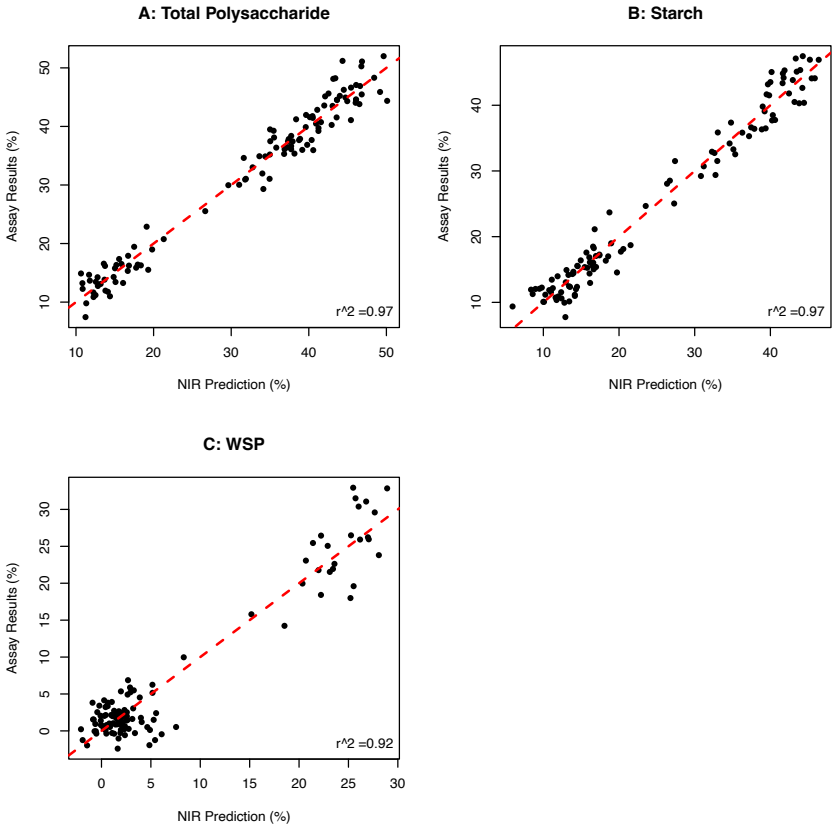

Figure A1-11. Regression of predicted values (NIR Prediction %) on reference wet lab values (Assay Results %) for the calibration set selected in 2021 for Total Polysaccharides (A), Starch (B), and water soluble polysaccharides (C). The coefficient of determination of the model is reported in the lower right corner of each graph.

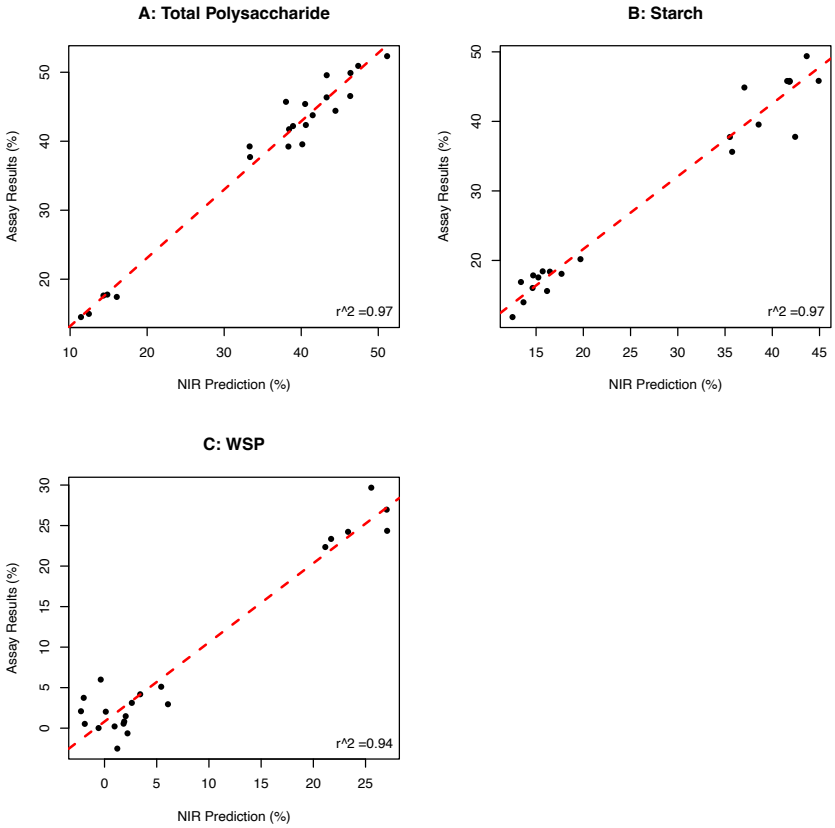

Figure A1-12. Regression of predicted values (NIR Prediction %) on reference wet lab values (Assay Results %) for the validation set selected in 2021 for Total Polysaccharides (A), Starch (B), and water soluble polysaccharides (C). The coefficient of determination of the model is reported in the lower right corner of each graph.

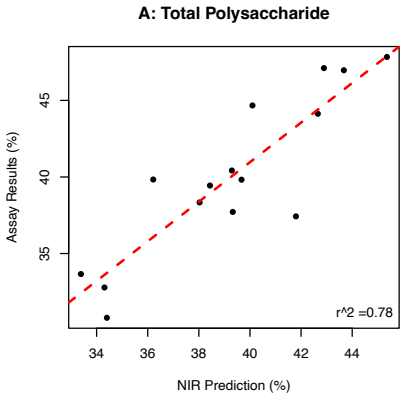

Figure A1-13. Regression of predicted values (NIR Prediction %) on reference wet lab values (Assay Results %) for *sugary1, waxy1,* and wild type hybrids the validation set selected in 2020 for Total Polysaccharides. The coefficient of determination of the model is reported in the lower right corner.

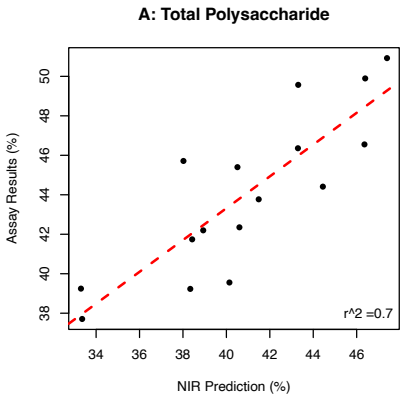

Figure A1-14. Regression of predicted values (NIR Prediction %) on reference wet lab values (Assay Results %) for *sugary1, waxy1,* and wild type hybrids the validation set selected in 2021 for Total Polysaccharides. The coefficient of determination of the model is reported in the lower right corner.

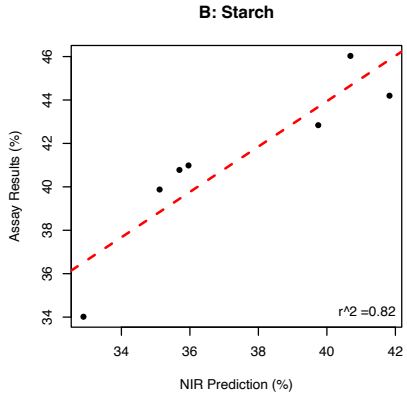

Figure A1-15. Regression of predicted values (NIR Prediction %) on reference wet lab values (Assay Results %) for *waxy1* and wild type hybrids the validation set selected in 2020 for Starch. The coefficient of determination of the model is reported in the lower right corner.

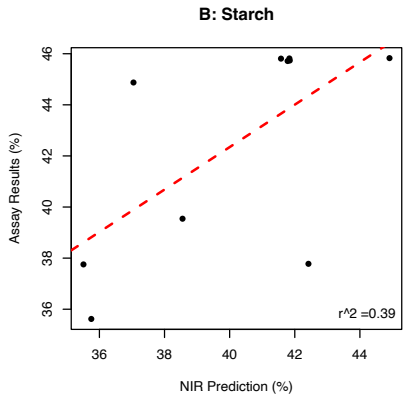

Figure A1-16. Regression of predicted values (NIR Prediction %) on reference wet lab values (Assay Results %) for *waxy1* and wild type hybrids the validation set selected in 2021 for Starch. The coefficient of determination of the model is reported in the lower right corner.

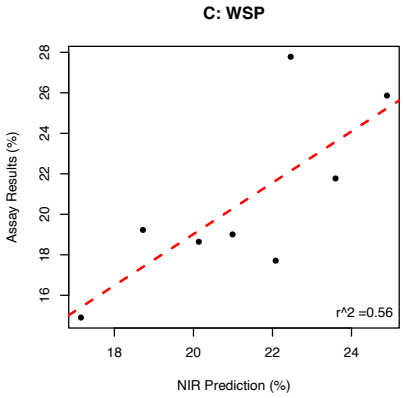

Figure A1-17. Regression of predicted values (NIR Prediction %) on reference wet lab values (Assay Results %) for *sugary1* hybrids the validation set selected in 2020 for water soluble polysaccharides. The coefficient of determination of the model is reported in the lower right corner.

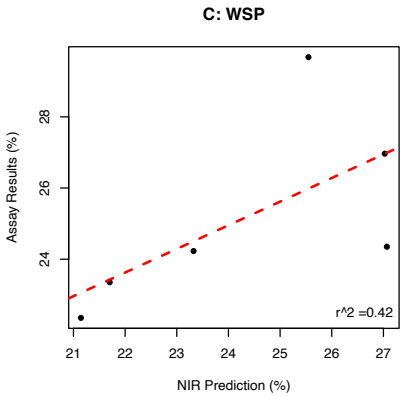

Figure A1-18. Regression of predicted values (NIR Prediction %) on reference wet lab values (Assay Results %) for *sugary1* hybrids the validation set selected in 2021 for water soluble polysaccharides. The coefficient of determination of the model is reported in the lower right corner.

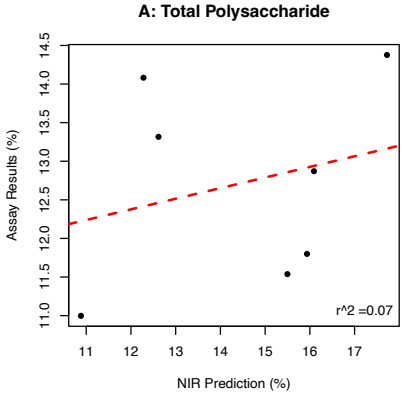

Figure A1-19. Regression of predicted values (NIR Prediction %) on reference wet lab values (Assay Results %) for *shrunken2* hybrids the validation set selected in 2020 for Total Polysaccharides. The coefficient of determination of the model is reported in the lower right corner.

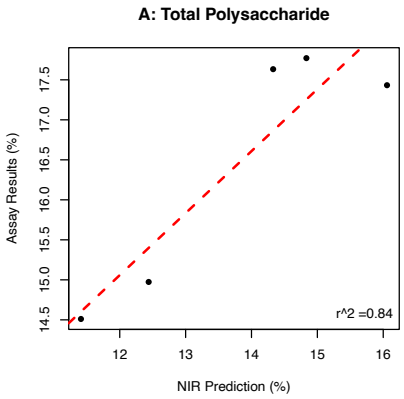

Figure A1-20. Regression of predicted values (NIR Prediction %) on reference wet lab values (Assay Results %) for *shrunken2* hybrids the validation set selected in 2021 for Total Polysaccharides. The coefficient of determination of the model is reported in the lower right corner.

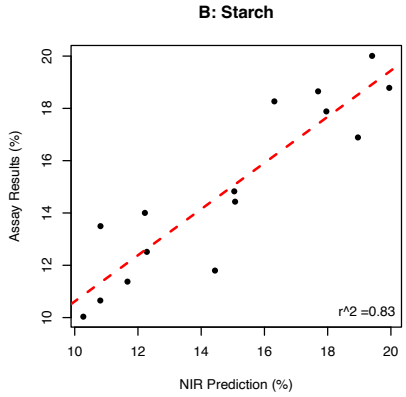

Figure A1-21. Regression of predicted values (NIR Prediction %) on reference wet lab values (Assay Results %) for *sugary1* and *shrunken2* hybrids the validation set selected in 2020 for Starch. The coefficient of determination of the model is reported in the lower right corner.

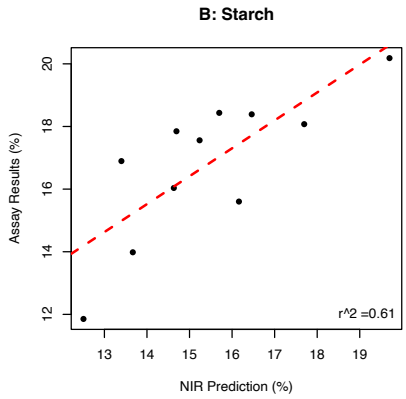

Figure A1-22. Regression of predicted values (NIR Prediction %) on reference wet lab values (Assay Results %) for *sugary1* and *shrunken2* hybrids the validation set selected in 2021 for Starch. The coefficient of determination of the model is reported in the lower right corner.

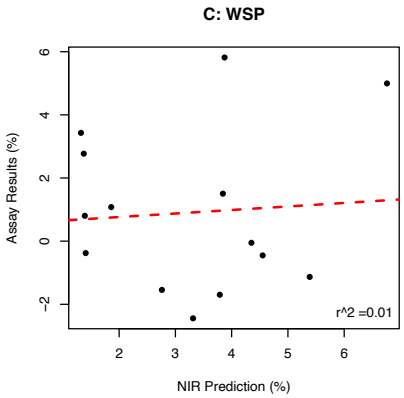

Figure A1-23. Regression of predicted values (NIR Prediction %) on reference wet lab values (Assay Results %) for *shrunken2, waxy1,* and wild type hybrids the validation set selected in 2020 for water soluble polysaccharides. The coefficient of determination of the model is reported in the lower right corner.

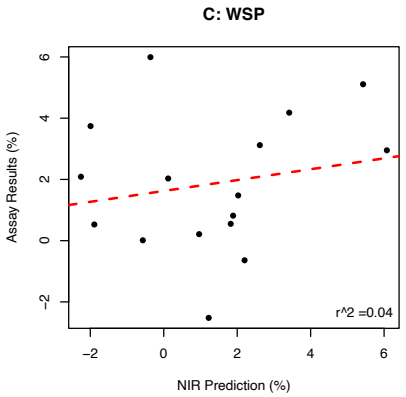

Figure A1-24. Regression of predicted values (NIR Prediction %) on reference wet lab values (Assay Results %) for *shrunken2, waxy1,* and wild type hybrids the validation set selected in 2021 for water soluble polysaccharides. The coefficient of determination of the model is reported in the lower right corner.

II. Appendix Tables A2: Analysis of Variance

Table A2-1. Significance of F-tests on mean squares from an analysis of variance for total soluble solids and carbohydrate traits of 15 *shrunken2* (*sh2*) hybrids from a six-line half-diallel cross measured at West Madison Agricultural Research Station in four planting dates in 2020 and 2021. *,**,*** significant at 0.05, 0.01, and 0.001 probability levels, respectively. ns = not significant, PD = planting date, Rep = replication, HD = harvest date, GCA = general combining ability, SCA = Specific combining ability. † = measured in the two planting dates in 2021.

	Source of Variation								
Trait	PD	Rep(PD)	HD	*sh2* Hybrid	GCA†	SCA†	Hybrid x PD	GCA x PD†	SCA x PD†
Total soluble solids	**	ns	***	***	***	ns	ns	*	ns
Total sugar	*	ns	***	***	***	ns	ns	ns	ns
Sucrose	**	ns	***	***	***	ns	ns	ns	ns
Glucose	**	***	***	***	***	ns	ns	ns	ns
Fructose	**	*	***	***	***	ns	ns	ns	ns
Total polysaccharides	ns	ns	***	***	***	**	***	ns	ns
Starch	*	**	***	***	***	ns	**	ns	ns

Table A2-2. Significance of F-tests on mean squares from an analysis of variance for total soluble solids and carbohydrate traits of 15 *sugary1* (*su1*) hybrids from a six-line half-diallel cross measured at West Madison Agricultural Research Station in four planting dates in 2020 and 2021. *,**,*** Significant at 0.05, 0.01, and 0.001 probability levels, respectively. ns = not significant, PD = planting date, Rep = replication, HD = harvest date, GCA = general combining ability, SCA = Specific combining ability.

	Source of Variation								
Trait	PD	Rep(PD)	HD	*su1* Hybrid	GCA	SCA	Hybrid x PD	GCA x PD	SCA x PD
Total soluble solids	***	ns	***	***	***	***	ns	ns	ns
Total sugar	***	ns	***	***	***	***	*	**	ns
Sucrose	**	ns	***	***	***	***	ns	ns	ns
Glucose	**	ns	***	***	***	ns	**	***	ns
Fructose	**	**	***	***	***	ns	**	***	ns
Total polysaccharides	***	*	***	***	***	**	**	***	ns
Starch	***	***	***	***	***	***	***	***	ns
WSP	***	*	***	***	***	***	***	***	ns

Table A2-3. Significance of F-tests on mean squares from an analysis of variance for total soluble solids and carbohydrate traits of 15 *waxy1* (*wx1*) hybrids from a six-line half-diallel cross measured at West Madison Agricultural Research Station in four planting dates in 2020 and 2021. *,**,*** Significant at 0.05, 0.01, and 0.001 probability levels, respectively. ns = not significant, PD = planting date, Rep = replication, HD = harvest date, GCA = general combining ability, SCA = Specific combining ability.

	Source of Variation								
Trait	PD	Rep(PD)	HD	*wx1* Hybrid	GCA	SCA	Hybrid x PD	GCA x PD	SCA x PD
Total soluble solids	***	ns	***	***	***	***	**	***	*
Total sugar	**	ns	***	***	***	***	***	***	***
Sucrose	***	ns	***	***	***	***	***	***	**
Glucose	***	ns	***	***	***	***	***	***	***
Fructose	**	ns	***	***	***	***	***	***	***
Total polysaccharides	***	**	***	***	***	***	***	***	***
Starch	***	ns	***	***	***	***	***	***	***

Table A2-4. Significance of F-tests on mean squares from an analysis of variance for total soluble solids and carbohydrate traits of 15 wild type (WT) hybrids from a six-line half-diallel cross measured at West Madison Agricultural Research Station in four planting dates in 2020 and 2021. *,**,*** Significant at 0.05, 0.01, and 0.001 probability levels, respectively. ns = not significant, PD = planting date, Rep = replication, HD = harvest date, GCA = general combining ability, SCA = Specific combining ability.

	Source of Variation								
Trait	PD	Rep(PD)	HD	WT Hybrid	GCA	SCA	Hybrid x PD	GCA x PD	SCA x PD
Total soluble solids	***	ns	ns	***	***	***	***	***	**
Total sugar	***	*	***	***	***	ns	ns	ns	ns
Sucrose	*	**	***	***	***	ns	ns	ns	ns
Glucose	***	ns	***	***	***	**	*	**	ns
Fructose	***	ns	***	***	***	***	**	***	ns

Total polysaccharides	***	***	***	***	***	**	***	***	*
Starch	***	*	***	***	***	*	***	***	*

III. Appendix Tables A3: Spearman Correlations among Planting Dates

Table A3-1. Nonparametric correlations (Spearman's rho) among planting dates for *waxy1* endosperm. 1 = early planting date 2020, 2 = late planting date 2020, 3 = early planting date 2021, 4 = late planting date 2021.

waxy1 Diallel Cross		
Trait	**Planting Date**	**Spearman's rho**
Total Soluble Solids	1 (2020) – 2 (2020)	0.83***
Total Soluble Solids	1 (2020) – 3 (2021)	0.69***
Total Soluble Solids	1 (2020) – 4 (2021)	0.65***
Total Soluble Solids	2 (2020) – 3 (2021)	0.52***
Total Soluble Solids	2 (2020) – 4 (2021)	0.43
Total Soluble Solids	3 (2021) – 4 (2021)	0.74***
Fructose	1 (2020) – 2 (2020)	0.80***
Fructose	1 (2020) – 3 (2021)	0.26
Fructose	1 (2020) – 4 (2021)	0.21
Fructose	2 (2020) – 3 (2021)	0.24
Fructose	2 (2020) – 4 (2021)	0.36
Fructose	3 (2021) – 4 (2021)	0.81***
Glucose	1 (2020) – 2 (2020)	0.75***
Glucose	1 (2020) – 3 (2021)	0.17
Glucose	1 (2020) – 4 (2021)	0.18
Glucose	2 (2020) – 3 (2021)	0.32
Glucose	2 (2020) – 4 (2021)	0.43
Glucose	3 (2021) – 4 (2021)	0.79***
Starch	1 (2020) – 2 (2020)	0.81***
Starch	1 (2020) – 3 (2021)	0.52*
Starch	1 (2020) – 4 (2021)	0.30
Starch	2 (2020) – 3 (2021)	0.69***
Starch	2 (2020) – 4 (2021)	0.46
Starch	3 (2021) – 4 (2021)	0.81***
Sucrose	1 (2020) – 2 (2020)	0.52*
Sucrose	1 (2020) – 3 (2021)	-0.11
Sucrose	1 (2020) – 4 (2021)	-0.09
Sucrose	2 (2020) – 3 (2021)	-0.05
Sucrose	2 (2020) – 4 (2021)	0.16
Sucrose	3 (2021) – 4 (2021)	0.67**
Total Polysaccharides	1 (2020) – 2 (2020)	0.94***
Total Polysaccharides	1 (2020) – 3 (2021)	0.31
Total Polysaccharides	1 (2020) – 4 (2021)	0.36
Total Polysaccharides	2 (2020) – 3 (2021)	0.36

Total Polysaccharides	2 (2020) – 4 (2021)	0.41
Total Polysaccharides	3 (2021) – 4 (2021)	0.89***
Total Sugar	1 (2020) – 2 (2020)	0.84***
Total Sugar	1 (2020) – 3 (2021)	0.075
Total Sugar	1 (2020) – 4 (2021)	-0.09
Total Sugar	2 (2020) – 3 (2021)	0.175
Total Sugar	2 (2020) – 4 (2021)	0.12
Total Sugar	3 (2021) – 4 (2021)	0.88***
*,**,*** Significant at 0.05, 0.01, 0.001 probability levels, respectively		

Table A3-2. Nonparametric correlations (Spearman's rho) among planting dates for wild type endosperm. 1 = early planting date 2020, 2 = late planting date 2020, 3 = early planting date 2021, 4 = late planting date 2021.

Wild type Diallel Cross		
Trait	**Planting Date**	**Spearman's rho**
Total Soluble Solids	1 (2020) – 2 (2020)	0.89***
Total Soluble Solids	1 (2020) – 3 (2021)	0.86***
Total Soluble Solids	1 (2020) – 4 (2021)	0.84***
Total Soluble Solids	2 (2020) – 3 (2021)	0.80**
Total Soluble Solids	2 (2020) – 4 (2021)	0.80**
Total Soluble Solids	3 (2021) – 4 (2021)	0.74**
Fructose	1 (2020) – 2 (2020)	0.87***
Fructose	1 (2020) – 3 (2021)	0.85***
Fructose	1 (2020) – 4 (2021)	0.95***
Fructose	2 (2020) – 3 (2021)	0.69*
Fructose	2 (2020) – 4 (2021)	0.86***
Fructose	3 (2021) – 4 (2021)	0.79***
Glucose	1 (2020) – 2 (2020)	0.80**
Glucose	1 (2020) – 3 (2021)	0.89***
Glucose	1 (2020) – 4 (2021)	0.90***
Glucose	2 (2020) – 3 (2021)	0.61*
Glucose	2 (2020) – 4 (2021)	0.80**
Glucose	3 (2021) – 4 (2021)	0.79***
Starch	1 (2020) – 2 (2020)	0.59*
Starch	1 (2020) – 3 (2021)	0.69*
Starch	1 (2020) – 4 (2021)	0.78**
Starch	2 (2020) – 3 (2021)	0.62*
Starch	2 (2020) – 4 (2021)	0.77**
Starch	3 (2021) – 4 (2021)	0.65**
Total Polysaccharides	1 (2020) – 2 (2020)	0.77**
Total Polysaccharides	1 (2020) – 3 (2021)	0.79**
Total Polysaccharides	1 (2020) – 4 (2021)	0.88***
Total Polysaccharides	2 (2020) – 3 (2021)	0.67*
Total Polysaccharides	2 (2020) – 4 (2021)	0.94***
Total Polysaccharides	3 (2021) – 4 (2021)	0.82***

*,**,*** Significant at 0.05, 0.01, 0.001 probability levels, respectively

Table A3-3. Nonparametric correlations (Spearman's rho) among planting dates for *sugary1* endosperm. 1 = early planting date 2020, 2 = late planting date 2020, 3 = early planting date 2021, 4 = late planting date 2021.

sugary1 Diallel Cross		
Trait	**Planting Date**	**Spearman's rho**
Total Sugar	1 (2020) – 2 (2020)	0.84***
Total Sugar	1 (2020) – 3 (2021)	0.88***
Total Sugar	1 (2020) – 4 (2021)	0.78***
Total Sugar	2 (2020) – 3 (2021)	0.91***
Total Sugar	2 (2020) – 4 (2021)	0.88***
Total Sugar	3 (2021) – 4 (2021)	0.87***
Fructose	1 (2020) – 2 (2020)	0.91***
Fructose	1 (2020) – 3 (2021)	0.91***
Fructose	1 (2020) – 4 (2021)	0.88***
Fructose	2 (2020) – 3 (2021)	0.85***
Fructose	2 (2020) – 4 (2021)	0.84***
Fructose	3 (2021) – 4 (2021)	0.88***
Glucose	1 (2020) – 2 (2020)	0.87***
Glucose	1 (2020) – 3 (2021)	0.86***
Glucose	1 (2020) – 4 (2021)	0.89***
Glucose	2 (2020) – 3 (2021)	0.82***
Glucose	2 (2020) – 4 (2021)	0.89***
Glucose	3 (2021) – 4 (2021)	0.83***
Starch	1 (2020) – 2 (2020)	0.49
Starch	1 (2020) – 3 (2021)	0.67**
Starch	1 (2020) – 4 (2021)	0.27
Starch	2 (2020) – 3 (2021)	0.70**
Starch	2 (2020) – 4 (2021)	0.61*
Starch	3 (2021) – 4 (2021)	0.35
Total Polysaccharides	1 (2020) – 2 (2020)	0.94***
Total Polysaccharides	1 (2020) – 3 (2021)	0.96***
Total Polysaccharides	1 (2020) – 4 (2021)	0.93***
Total Polysaccharides	2 (2020) – 3 (2021)	0.85***
Total Polysaccharides	2 (2020) – 4 (2021)	0.86***
Total Polysaccharides	3 (2021) – 4 (2021)	0.93***
WSP	1 (2020) – 2 (2020)	0.82***
WSP	1 (2020) – 3 (2021)	0.79***
WSP	1 (2020) – 4 (2021)	0.82***
WSP	2 (2020) – 3 (2021)	0.96***
WSP	2 (2020) – 4 (2021)	0.83***
WSP	3 (2021) – 4 (2021)	0.84***
*,**,*** Significant at 0.05, 0.01, 0.001 probability levels, respectively		

Table A3-4. Nonparametric correlations (Spearman's rho) among planting dates for *shrunken2* endosperm. 1 = early planting date 2020, 2 = late planting date 2020, 3 = early planting date 2021, 4 = late planting date 2021.

shrunken2 Diallel Cross		
Trait	**Planting Date**	**Spearman's rho**
Total Polysaccharides	1 (2020) – 2 (2020)	0.44
Total Polysaccharides	1 (2020) – 3 (2021)	0.35
Total Polysaccharides	1 (2020) – 4 (2021)	0.79
Total Polysaccharides	2 (2020) – 3 (2021)	0.78
Total Polysaccharides	2 (2020) – 4 (2021)	0.75
Total Polysaccharides	3 (2021) – 4 (2021)	0.97***
Starch	1 (2020) – 2 (2020)	0.69
Starch	1 (2020) – 3 (2021)	0.49
Starch	1 (2020) – 4 (2021)	0.81*
Starch	2 (2020) – 3 (2021)	0.71
Starch	2 (2020) – 4 (2021)	0.72
Starch	3 (2021) – 4 (2021)	0.92***
*,**,*** Significant at 0.05, 0.01, 0.001 probability levels, respectively		

Table A3-5. Nonparametric correlations (Spearman's rho) among planting dates for GCA of *waxy1* endosperm. 1 = early planting date 2020, 2 = late planting date 2020, 3 = early planting date 2021, 4 = late planting date 2021.

waxy1 Diallel Cross		
Trait	**Planting Date**	**Spearman's rho**
GCA for Total Soluble Solids averaged over harvest dates	1 (2020) – 2 (2020)	0.94**
GCA for Total Sugar averaged over harvest dates	1 (2020) – 2 (2020)	0.83*
GCA for Sucrose averaged over harvest dates	1 (2020) – 2 (2020)	0.71
GCA for Glucose averaged over harvest dates	1 (2020) – 2 (2020)	0.49
GCA for Fructose averaged over harvest dates	1 (2020) – 2 (2020)	0.60
GCA for Total Polysaccharides averaged over harvest dates	1 (2020) – 2 (2020)	0.83*
GCA for Starch averaged over harvest dates	1 (2020) – 2 (2020)	0.83*
GCA for Total Soluble Solids averaged over harvest dates	3 (2021) – 4 (2021)	0.77
GCA for Total Sugar averaged over harvest dates	3 (2021) – 4 (2021)	0.94**
GCA for Sucrose averaged over harvest dates	3 (2021) – 4 (2021)	0.77

GCA for Glucose averaged over harvest dates	3 (2021) – 4 (2021)	0.94**
GCA for Fructose averaged over harvest dates	3 (2021) – 4 (2021)	1***
GCA for Total Polysaccharides averaged over harvest dates	3 (2021) – 4 (2021)	0.94**
GCA for Starch averaged over harvest dates	3 (2021) – 4 (2021)	0.89*
*,**,*** Significant at 0.05, 0.01, 0.001 probability levels, respectively		

Table A3-6. Nonparametric correlations (Spearman's rho) among planting dates for GCA of wild type endosperm. 1 = early planting date 2020, 2 = late planting date 2020, 3 = early planting date 2021, 4 = late planting date 2021.

Wild type Diallel Cross		
Trait	**Planting Date**	**Spearman's rho**
GCA for Total Soluble Solids averaged over harvest dates	1 (2020) – 2 (2020)	0.83*
GCA for Total Soluble Solids averaged over harvest dates	1 (2020) – 3 (2021)	0.77
GCA for Total Soluble Solids averaged over harvest dates	1 (2020) – 4 (2021)	0.83*
GCA for Total Soluble Solids averaged over harvest dates	2 (2020) – 3 (2021)	0.71
GCA for Total Soluble Solids averaged over harvest dates	2 (2020) – 4 (2021)	0.6
GCA for Total Soluble Solids averaged over harvest dates	3 (2021) – 4 (2021)	0.94**
GCA for Glucose averaged over harvest dates	1 (2020) – 2 (2020)	0.77
GCA for Glucose averaged over harvest dates	1 (2020) – 3 (2021)	0.94**
GCA for Glucose averaged over harvest dates	1 (2020) – 4 (2021)	0.94**
GCA for Glucose averaged over harvest dates	2 (2020) – 3 (2021)	0.89*
GCA for Glucose averaged over harvest dates	2 (2020) – 4 (2021)	0.89*
GCA for Glucose averaged over harvest dates	3 (2021) – 4 (2021)	1***
GCA for Fructose averaged over harvest dates	1 (2020) – 2 (2020)	0.94**
GCA for Fructose averaged over harvest dates	1 (2020) – 3 (2021)	0.89*
GCA for Fructose averaged over harvest dates	1 (2020) – 4 (2021)	0.94**

GCA for Fructose averaged over harvest dates	2 (2020) – 3 (2021)	0.94**
GCA for Fructose averaged over harvest dates	2 (2020) – 4 (2021)	0.89*
GCA for Fructose averaged over harvest dates	3 (2021) – 4 (2021)	0.94**
GCA for Total Polysaccharides averaged over harvest dates	1 (2020) – 2 (2020)	0.89*
GCA for Total Polysaccharides averaged over harvest dates	1 (2020) – 3 (2021)	0.83*
GCA for Total Polysaccharides averaged over harvest dates	1 (2020) – 4 (2021)	0.94**
GCA for Total Polysaccharides averaged over harvest dates	2 (2020) – 3 (2021)	0.89*
GCA for Total Polysaccharides averaged over harvest dates	2 (2020) – 4 (2021)	0.94**
GCA for Total Polysaccharides averaged over harvest dates	3 (2021) – 4 (2021)	0.94**
GCA for Starch averaged over harvest dates	1 (2020) – 2 (2020)	0.54
GCA for Starch averaged over harvest dates	1 (2020) – 3 (2021)	0.66
GCA for Starch averaged over harvest dates	1 (2020) – 4 (2021)	0.6
GCA for Starch averaged over harvest dates	2 (2020) – 3 (2021)	0.66
GCA for Starch averaged over harvest dates	2 (2020) – 4 (2021)	0.94**
GCA for Starch averaged over harvest dates	3 (2021) – 4 (2021)	0.71
*,**,*** Significant at 0.05, 0.01, 0.001 probability levels, respectively		

Table A3-7. Nonparametric correlations (Spearman's rho) among planting dates for GCA of *sugary1* endosperm. 1 = early planting date 2020, 2 = late planting date 2020, 3 = early planting date 2021, 4 = late planting date 2021.

sugary1 Diallel Cross		
Trait	**Planting Date**	**Spearman's rho**
GCA for Total Sugar averaged over harvest dates	1 (2020) – 2 (2020)	0.77

GCA for Total Sugar averaged over harvest dates	1 (2020) – 3 (2021)	0.83*
GCA for Total Sugar averaged over harvest dates	1 (2020) – 4 (2021)	0.77
GCA for Total Sugar averaged over harvest dates	2 (2020) – 3 (2021)	0.94**
GCA for Total Sugar averaged over harvest dates	2 (2020) – 4 (2021)	1***
GCA for Glucose averaged over harvest dates	1 (2020) – 2 (2020)	0.94**
GCA for Glucose averaged over harvest dates	1 (2020) – 3 (2021)	1***
GCA for Glucose averaged over harvest dates	1 (2020) – 4 (2021)	0.94**
GCA for Glucose averaged over harvest dates	2 (2020) – 3 (2021)	0.94**
GCA for Glucose averaged over harvest dates	2 (2020) – 4 (2021)	0.89*
GCA for Fructose averaged over harvest dates	1 (2020) – 2 (2020)	0.94**
GCA for Fructose averaged over harvest dates	1 (2020) – 3 (2021)	1***
GCA for Fructose averaged over harvest dates	1 (2020) – 4 (2021)	0.89*
GCA for Fructose averaged over harvest dates	2 (2020) – 3 (2021)	0.94**
GCA for Fructose averaged over harvest dates	2 (2020) – 4 (2021)	0.94**
GCA for Total Polysaccharides averaged over harvest dates	1 (2020) – 2 (2020)	1***
GCA for Total Polysaccharides averaged over harvest dates	1 (2020) – 3 (2021)	1***
GCA for Total Polysaccharides averaged over harvest dates	1 (2020) – 4 (2021)	1***
GCA for Total Polysaccharides averaged over harvest dates	2 (2020) – 3 (2021)	1***
GCA for Total Polysaccharides averaged over harvest dates	2 (2020) – 4 (2021)	1***
GCA for Starch averaged over harvest dates	1 (2020) – 2 (2020)	0.43

GCA for Starch averaged over harvest dates	1 (2020) – 3 (2021)	0.77
GCA for Starch averaged over harvest dates	1 (2020) – 4 (2021)	0.20
GCA for Starch averaged over harvest dates	2 (2020) – 3 (2021)	0.89*
GCA for Starch averaged over harvest dates	2 (2020) – 4 (2021)	0.71
GCA for Starch averaged over all harvest dates	3 (2021) – 4 (2021)	0.66
GCA for WSP averaged over harvest dates	1 (2020) – 2 (2020)	-0.26
GCA for WSP averaged over harvest dates	1 (2020) – 3 (2021)	0.89*
GCA for WSP averaged over harvest dates	1 (2020) – 4 (2021)	0.94**
GCA for WSP averaged over harvest dates	2 (2020) – 3 (2021)	0.94**
GCA for WSP averaged over harvest dates	2 (2020) – 4 (2021)	1***
GCA for WSP averaged over all harvest dates	3 (2021) – 4 (2021)	0.94**
*,**,*** Significant at 0.05, 0.01, 0.001 probability levels, respectively		

Table A3-8. Nonparametric correlations (Spearman's rho) among planting dates for GCA of *shrunken2* endosperm. 3 = early planting date 2021, 4 = late planting date 2021.

shrunken2 Diallel Cross		
Trait	**Planting Date**	**Spearman's rho**
GCA for Total Soluble Solids	3 (2021) – 4 (2021)	0.60
*,**,*** Significant at 0.05, 0.01, 0.001 probability levels, respectively		

IV. Appendix Tables A4: Spearman Correlations among Harvest Dates

Table A4-1. Nonparametric correlations (Spearman's rho) among harvest dates (19, 22, 25 DAP) for GCA of *waxy1* endosperm in 2020 planting dates.

waxy1 Diallel Cross		
Trait	**Harvest Date**	**Spearman's rho**
Total Soluble Solids	19 - 22	0.77
Total Soluble Solids	19 - 25	0.83*
Total Soluble Solids	22 - 25	0.94**
Total Sugar	19 - 22	0.37
Total Sugar	19 - 25	0.83*
Total Sugar	22 - 25	0.77
Sucrose	19 - 22	0.20

Sucrose	19 - 25	0.37
Sucrose	22 - 25	0.89*
Glucose	19 - 22	0.94**
Glucose	19 - 25	0.83*
Glucose	22 - 25	0.77
Fructose	19 - 22	0.94**
Fructose	19 - 25	0.71
Fructose	22 - 25	0.60
Total Polysaccharides	19 - 22	1***
Total Polysaccharides	19 - 25	0.83*
Total Polysaccharides	22 - 25	0.83*
Starch	19 - 22	0.94**
Starch	19 - 25	0.94**
Starch	22 - 25	0.83*
*,**,*** Significant at 0.05, 0.01, 0.001 probability levels, respectively		

Table A4-2. Nonparametric correlations (Spearman's rho) among harvest dates (19, 22, 25 DAP) for GCA of *waxy1* endosperm in 2021 planting dates.

waxy1 Diallel Cross		
Trait	**Harvest Date**	**Spearman's rho**
Total Soluble Solids	19 - 22	0.94**
Total Soluble Solids	19 - 25	0.77
Total Soluble Solids	22 - 25	0.89*
Total Sugar	19 - 22	0.83*
Total Sugar	19 - 25	1***
Total Sugar	22 - 25	0.83*
Sucrose	19 - 22	0.83*
Sucrose	19 - 25	0.94*
Sucrose	22 - 25	0.77
Glucose	19 - 22	0.66
Glucose	19 - 25	0.71
Glucose	22 - 25	0.37
Fructose	19 - 22	0.77
Fructose	19 - 25	0.83*
Fructose	22 - 25	0.77
Total Polysaccharides	19 - 22	0.94**
Total Polysaccharides	19 - 25	0.94**
Total Polysaccharides	22 - 25	0.89*
Starch	19 - 22	0.83*
Starch	19 - 25	1***
Starch	22 - 25	0.83*
*,**,*** Significant at 0.05, 0.01, 0.001 probability levels, respectively		

Table A4-3. Nonparametric correlations (Spearman's rho) among harvest dates (19, 22, 25 DAP) for GCA of *sugary1* endosperm averaged over planting dates in years 2020 and 2021.

sugary1 Diallel Cross		
Trait	**Harvest Date**	**Spearman's rho**
Total Soluble Solids	19 - 22	0.94**
Total Soluble Solids	19 - 25	0.94**
Total Soluble Solids	22 - 25	1***
Total Sugar	19 - 22	0.83*
Total Sugar	19 - 25	1***
Total Sugar	22 - 25	0.83*
Sucrose	19 - 22	0.94**
Sucrose	19 - 25	1***
Sucrose	22 - 25	0.94**
Glucose	19 - 22	0.89*
Glucose	19 - 25	0.89*
Glucose	22 - 25	0.83*
Fructose	19 - 22	0.94**
Fructose	19 - 25	0.94**
Fructose	22 - 25	0.83*
Total Polysaccharides	19 - 22	1***
Total Polysaccharides	19 - 25	0.94**
Total Polysaccharides	22 - 25	0.94**
Starch	19 - 22	0.77
Starch	19 - 25	0.66
Starch	22 - 25	0.89*
WSP	19 - 22	1***
WSP	19 - 25	0.89*
WSP	22 - 25	0.89*
*,**,*** Significant at 0.05, 0.01, 0.001 probability levels, respectively		

Table A4-4. Nonparametric correlations (Spearman's rho) among harvest dates (19, 22, 25 DAP) for GCA of *shrunken2* endosperm in 2021 planting dates.

shrunken2 Diallel Cross		
Trait	**Harvest Date**	**Spearman's rho**
Total Soluble Solids	19 - 22	0.71
Total Soluble Solids	19 - 25	0.14
Total Soluble Solids	22 - 25	0.71
Total Sugar	19 - 22	0.89*
Total Sugar	19 - 25	0.31
Total Sugar	22 - 25	0.60
Sucrose	19 - 22	0.94**
Sucrose	19 - 25	0.71
Sucrose	22 - 25	0.54
Glucose	19 - 22	1***
Glucose	19 - 25	1***
Glucose	22 - 25	1***
Fructose	19 - 22	1***

Fructose	19 - 25	0.94**
Fructose	22 - 25	0.94**
Total Polysaccharides	19 - 22	1***
Total Polysaccharides	19 - 25	0.43
Total Polysaccharides	22 - 25	0.43
Starch	19 - 22	0.89*
Starch	19 - 25	0.83*
Starch	22 - 25	0.83*
*,**,*** Significant at 0.05, 0.01, 0.001 probability levels, respectively		

Table A4-5. Nonparametric correlations (Spearman's rho) among harvest dates (19, 22, 25 DAP) for GCA of wild type endosperm averaged over planting datesin years 2020 and 2021.

Wild type Diallel Cross		
Trait	**Harvest Date**	**Spearman's rho**
Total Soluble Solids	19 - 22	0.94**
Total Soluble Solids	19 - 25	0.83*
Total Soluble Solids	22 - 25	0.77
Total Sugar	19 - 22	1***
Total Sugar	19 - 25	0.77
Total Sugar	22 - 25	0.77
Sucrose	19 - 22	0.71
Sucrose	19 - 25	0.37
Sucrose	22 - 25	0.77
Glucose	19 - 22	1***
Glucose	19 - 25	1***
Glucose	22 - 25	1***
Fructose	19 - 22	0.94**
Fructose	19 - 25	0.94**
Fructose	22 - 25	0.89*
Total Polysaccharides	19 - 22	0.94**
Total Polysaccharides	19 - 25	1***
Total Polysaccharides	22 - 25	0.94**
Starch	19 - 22	0.89*
Starch	19 - 25	0.66
Starch	22 - 25	0.77
*,**,*** Significant at 0.05, 0.01, 0.001 probability levels, respectively		

V. Appendix Tables A5: Hybrid Trait Means

Table A5-1. Estimated marginal means for carbohydrate traits of endosperm types (WT = wild type, *sh2 = shrunken2, su1 = sugary1, wx1 = waxy1*) averaged over hybrids from four six-line diallel crosses grown at West Madison Agricultural Research Station in two planting dates with two replications per planting date in 2020 and 2021. Total Carbohydrate is the addition of total sugar and total polysaccharides. Within a trait, means that share the same lower-case letter are

not statistically different at alpha = 0.05 from Tukey's Honest Significant Difference pairwise comparison tests.

Endosperm Type	Total Soluble Solids (%)	Total Sugar (mg/g)	Sucrose (mg/g)	Glucose (mg/g)	Fructose (mg/g)	Total Polysacc-harides (mg/g)	Starch (mg/g)	Total Carbohy-drate (mg/g)
WT	8.34 a	100.0 a	36.0 a	34.7 a	28.9 a	433.0 d	393.0 c	533.7 c
sh2	14.77 c	345.0 d	236.0 d	58.3 d	48.9 c	143.0 a	131.0 a	488.6 a
su1	21.43 d	132.0 c	67.1 c	36.2 b	28.2 a	390.0 b	173.0 b	522.3 b
wx1	8.94 b	118.0 b	44.1 b	39.0 c	32.8 b	415.0 c	400.0 d	533.0 c

Table A5-2. Estimated marginal means for total sugar (mg/g) of hybrids near-isogenic for endosperm type (WT = wild type, *sh2 = shrunken2, su1 = sugary1, wx1 = waxy1*), averaged over harvest dates, from four six-line diallel crosses grown at West Madison Agricultural Research Station in two planting dates per year with two replications per planting date in 2020 and 2021. Means that share the same letter or number are not statistically different at alpha = 0.05 from Tukey's Honest Significant Difference pairwise comparison tests.

Hybrid	Endosperm Type	Total Sugar (mg/g)	Pairwise Comparison
C68 x Il101t	WT	84.8	1
Ia453 x C68	WT	84.8	1
P39 x Ia453	WT	88.8	12
Il101t x Ia453	WT	89.6	123
Ia5125 x C68	WT	92.3	1234
P39 x Il101t	WT	94.7	1234
Il101t x Ia5125	WT	100.3	2345
C68 x C40	WT	100.9	23456
C68 x Il101t	wx1	102.1	23456
Ia453 x C68	wx1	102.2	23456
P39 x C68	WT	103.7	345678
Ia5125 x Ia453	WT	104.1	4567
P39 x Ia453	wx1	106.2	4567890
Il101t x Ia453	wx1	107.0	456789 A
Ia5125 x P39	WT	108.1	567890AB
Ia5125 x C68	wx1	109.6	567890AB
C40 x P39	WT	110.8	567890ABC
Il101t x C40	WT	111.1	567890ABCD
P39 x Il101t	wx1	112.0	567890ABC
Ia453 x C40	WT	113.3	67890ABCDE
C68 x Il101t	su1	116.8	7890ABCDEFG
Ia453 x C68	su1	116.9	7890ABCDEFG
Il101t x Ia5125	wx1	117.7	7890ABCDEF
C68 x C40	wx1	118.3	890ABCDEFG
C40 x Ia5125	WT	120.2	90ABCDEFGH
P39 x Ia453	su1	120.8	ABCDEFGH

P39 x C68	wx1	121.1	ABCDEFGH
Ia5125 x Ia453	wx1	121.5	BCDEFGHI
Il101t x Ia453	su1	121.6	0 BCDEFGHI
Ia5125 x C68	su1	124.3	CDEFGHIJ
Ia5125 x P39	wx1	125.5	DEFGHIJ
P39 x Il101t	su1	126.7	EFGHIJ
C40 x P39	wx1	128.2	FGHIJK
Il101t x C40	wx1	128.5	FGHIJK
Ia453 x C40	wx1	130.7	GHIJKL
Il101t x Ia5125	su1	132.4	HIJKL
C68 x C40	su1	132.9	HIJKLM
P39 x C68	su1	135.8	IJKLM
Ia5125 x Ia453	su1	136.1	JKLM
C40 x Ia5125	wx1	137.5	JKLM
Ia5125 x P39	su1	140.2	KLMN
C40 x P39	su1	142.8	LMN
Il101t x C40	su1	143.1	LMN
Ia453 x C40	su1	145.3	MN
C40 x Ia5125	su1	152.2	N
C68 x Il101t	sh2	329.0	O
Ia453 x C68	sh2	329.1	O
P39 x Ia453	sh2	333.1	OP
Il101t x Ia453	sh2	333.9	OPQ
Ia5125 x C68	sh2	336.5	OPQR
P39 x Il101t	sh2	339.0	OPQR
Il101t x Ia5125	sh2	344.6	PQRS
C68 x C40	sh2	345.2	PQRST
P39 x C68	sh2	348.0	QRST
Ia5125 x Ia453	sh2	348.4	RST
Ia5125 x P39	sh2	352.4	STU
C40 x P39	sh2	355.1	STU
Il101t x C40	sh2	355.4	STU
Ia453 x C40	sh2	357.6	TU
C40 x Ia5125	sh2	364.4	U

Table A5-3. Estimated marginal means and standard error for total soluble solids (%) of wild type (WT) hybrids measured at three harvest dates (19, 22, and 25 days after pollination (DAP)) from a six-line diallel cross grown at West Madison Agricultural Research Station in two planting dates per year with two replications per planting date in 2020 and 2021.

WT Hybrid	Harvest Date (DAP)	Total Soluble Solids (%)	Standard Error
(Ia5125 x C68)	19	5.36	0.26
(Ia5125 x C68)	22	5.53	0.26
(Ia5125 x C68)	25	5.64	0.26
(Il101t x Ia5125)	19	6.00	0.26

(Il101t x Ia5125)	22	6.17	0.26
(Ia5125 x Ia453)	19	6.18	0.26
(Il101t x Ia5125)	25	6.28	0.26
(Ia5125 x Ia453)	22	6.35	0.26
(Ia5125 x Ia453)	25	6.46	0.26
(Ia5125 x P39)	19	6.93	0.26
(Ia5125 x P39)	22	7.10	0.26
(Ia5125 x P39)	25	7.21	0.26
(Il101t x Ia453)	19	7.62	0.35
(C40 x Ia5125)	19	7.70	0.26
(Il101t x Ia453)	22	7.79	0.35
(C40 x Ia5125)	22	7.87	0.26
(Il101t x Ia453)	25	7.90	0.35
(C40 x Ia5125)	25	7.98	0.26
(C68 x Il101t)	19	8.31	0.26
(Ia453 x C68)	19	8.35	0.26
(P39 x C68)	19	8.44	0.37
(C68 x Il101t)	22	8.47	0.26
(Ia453 x C68)	22	8.52	0.26
(C68 x Il101t)	25	8.58	0.26
(P39 x C68)	22	8.61	0.37
(Ia453 x C68)	25	8.63	0.26
(Ia453 x C40)	19	8.64	0.35
(P39 x C68)	25	8.72	0.37
(Ia453 x C40)	22	8.81	0.35
(Ia453 x C40)	25	8.92	0.35
(C68 x C40)	19	8.96	0.26
(P39 x Il101t)	19	8.96	0.26
(C68 x C40)	22	9.13	0.26
(P39 x Il101t)	22	9.13	0.26
(C68 x C40)	25	9.24	0.26
(P39 x Il101t)	25	9.24	0.26
(C40 x P39)	19	9.39	0.26
(P39 x Ia453)	19	9.40	0.26
(C40 x P39)	22	9.56	0.26
(P39 x Ia453)	22	9.57	0.26
(C40 x P39)	25	9.67	0.26
(P39 x Ia453)	25	9.68	0.26
(Il101t x C40)	19	9.98	0.26
(Il101t x C40)	22	10.15	0.26
(Il101t x C40)	25	10.26	0.26

Table A5-4. Estimated marginal means and standard error for total soluble solids (%) of *shrunken2 (sh2)* hybrids measured at three harvest dates (19, 22, and 25 days after pollination

(DAP)) from a six-line diallel cross grown at West Madison Agricultural Research Station in two planting dates per year with two replications per planting date in 2020 and 2021.

sh2 Hybrid	Harvest Date (DAP)	Total Soluble Solids (%)	Standard Error
(C68 x Il101t)	19	12.36	0.22
(Il101t x Ia453)	19	12.61	0.30
(Il101t x Ia5125)	19	12.74	0.23
(Ia5125 x C68)	19	12.88	0.22
(C68 x Il101t)	25	13.01	0.22
(C68 x Il101t)	22	13.02	0.22
(Il101t x Ia453)	25	13.25	0.30
(Il101t x Ia453)	22	13.26	0.30
(Il101t x Ia5125)	25	13.39	0.23
(Il101t x Ia5125)	22	13.40	0.23
(Il101t x C40)	19	13.51	0.30
(Ia5125 x C68)	25	13.53	0.22
(Ia5125 x C68)	22	13.54	0.22
(Ia453 x C68)	19	13.84	0.30
(Ia5125 x Ia453)	19	13.92	0.22
(C68 x C40)	19	14.05	0.23
(Il101t x C40)	25	14.16	0.30
(Il101t x C40)	22	14.17	0.30
(C40 x Ia5125)	19	14.27	0.30
(Ia453 x C40)	19	14.30	0.22
(Ia453 x C68)	25	14.49	0.30
(Ia453 x C68)	22	14.50	0.30
(Ia5125 x Ia453)	25	14.57	0.22
(Ia5125 x Ia453)	22	14.58	0.22
(C68 x C40)	25	14.70	0.23
(C68 x C40)	22	14.71	0.23
(C40 x Ia5125)	25	14.92	0.30
(C40 x Ia5125)	22	14.93	0.30
(Ia453 x C40)	25	14.95	0.22
(Ia453 x C40)	22	14.96	0.22
(P39 x C68)	19	15.00	0.23
(Ia5125 x P39)	19	15.00	0.22
(P39 x Il101t)	19	15.47	0.22
(C40 x P39)	19	15.61	0.22
(P39 x C68)	25	15.64	0.23
(Ia5125 x P39)	25	15.65	0.22
(P39 x C68)	22	15.65	0.23
(Ia5125 x P39)	22	15.66	0.22
(P39 x Ia453)	19	15.77	0.30
(P39 x Il101t)	25	16.12	0.22
(P39 x Il101t)	22	16.13	0.22

(C40 x P39)	25	16.26	0.22
(C40 x P39)	22	16.27	0.22
(P39 x Ia453)	25	16.42	0.30
(P39 x Ia453)	22	16.43	0.30

Table A5-5. Estimated marginal means and standard error for total soluble solids (%) of *waxy1 (wx1)* hybrids measured at three harvest dates (19, 22, and 25 days after pollination (DAP)) from a six-line diallel cross grown at West Madison Agricultural Research Station in two planting dates per year with two replications per planting date in 2020.

wx1 Hybrid	Harvest Date (DAP)	Total Soluble Solids (%) in 2020	Standard Error
(Ia453 x C68)	25	6.55	0.64
(Ia5125 x C68)	25	6.85	0.64
(Ia5125 x C68)	19	7.35	0.64
(Ia5125 x C68)	22	7.45	0.64
(Ia5125 x Ia453)	19	7.73	0.64
(C40 x Ia5125)	19	7.78	0.64
(Il101t x Ia5125)	19	7.85	0.64
(C40 x Ia5125)	22	8.00	0.64
(C40 x P39)	19	8.08	0.64
(Ia5125 x Ia453)	25	8.10	0.64
(Ia453 x C68)	22	8.15	0.64
(Ia453 x C68)	19	8.38	0.64
(Il101t x Ia5125)	22	8.45	0.64
(Ia5125 x P39)	22	8.50	0.64
(Ia5125 x Ia453)	22	8.60	0.64
(Ia5125 x P39)	19	8.60	0.64
(Il101t x Ia5125)	25	8.80	0.64
(C40 x Ia5125)	25	8.88	0.64
(P39 x Il101t)	19	8.95	0.75
(Il101t x Ia453)	19	8.98	0.64
(C68 x Il101t)	19	9.05	0.64
(C68 x C40)	19	9.05	0.64
(C40 x P39)	22	9.23	0.64
(Ia5125 x P39)	25	9.23	0.64
(Il101t x Ia453)	22	9.28	0.64
(P39 x Ia453)	19	9.28	0.64
(Ia453 x C40)	19	9.30	0.64
(C68 x C40)	25	9.36	0.75
(Il101t x C40)	19	9.53	0.64
(Il101t x C40)	22	9.53	0.64
(C40 x P39)	25	9.62	0.64
(C68 x C40)	22	10.08	0.64
(P39 x C68)	19	10.13	0.64
(P39 x Il101t)	25	10.29	0.75

(Il101t x C40)	25	10.30	0.64
(Ia453 x C40)	22	10.43	0.64
(P39 x C68)	22	10.53	0.64
(Il101t x Ia453)	25	10.65	0.64
(C68 x Il101t)	25	10.85	0.64
(C68 x Il101t)	22	10.88	0.64
(P39 x Ia453)	22	10.90	0.64
(P39 x Ia453)	25	10.98	0.64
(Ia453 x C40)	25	11.00	0.64
(P39 x C68)	25	11.53	0.64
(P39 x Il101t)	22	12.25	0.64

Table A5-6. Estimated marginal means and standard error for total soluble solids (%) of *waxy1 (wx1)* hybrids measured at three harvest dates (19, 22, and 25 days after pollination (DAP)) from a six-line diallel cross grown at West Madison Agricultural Research Station in two planting dates per year with two replications per planting date in 2021.

wx1 Hybrid	Harvest Date (DAP)	Total Soluble Solids (%) in 2021	Standard Error
(Il101t x Ia5125)	25	6.72	0.64
(Ia5125 x C68)	19	6.98	0.54
(Il101t x Ia5125)	19	7.20	0.54
(Il101t x Ia5125)	22	7.28	0.54
(Ia5125 x C68)	22	7.48	0.54
(C68 x C40)	19	7.55	0.54
(Ia5125 x Ia453)	19	7.55	0.54
(C68 x Il101t)	22	7.60	0.54
(C68 x Il101t)	19	7.60	0.54
(Ia5125 x P39)	19	7.78	0.54
(Ia5125 x C68)	25	7.78	0.54
(P39 x C68)	19	7.93	0.54
(Ia5125 x Ia453)	22	7.93	0.54
(Il101t x C40)	19	7.95	0.54
(C40 x Ia5125)	19	8.05	0.54
(Ia5125 x Ia453)	25	8.13	0.54
(Ia453 x C68)	22	8.13	0.54
(C40 x Ia5125)	22	8.18	0.54
(Ia453 x C68)	19	8.25	0.54
(P39 x C68)	22	8.50	0.54
(C68 x Il101t)	25	8.55	0.54
(C40 x Ia5125)	25	8.58	0.54
(Il101t x Ia453)	19	8.68	0.54
(P39 x Il101t)	22	8.73	0.54
(C40 x P39)	22	8.78	0.54
(P39 x Ia453)	22	8.88	0.54
(C68 x C40)	22	8.90	0.54

(C40 x P39)	19	8.95	0.54
(Ia5125 x P39)	22	9.00	0.54
(P39 x Ia453)	19	9.03	0.54
(P39 x Il101t)	19	9.08	0.54
(Ia453 x C68)	25	9.10	0.54
(Il101t x C40)	22	9.15	0.54
(P39 x C68)	25	9.15	0.54
(C40 x P39)	25	9.28	0.54
(Il101t x Ia453)	22	9.33	0.54
(Il101t x Ia453)	25	9.35	0.54
(P39 x Il101t)	25	9.40	0.54
(Il101t x C40)	25	9.93	0.54
(C68 x C40)	25	9.95	0.54
(Ia453 x C40)	22	10.18	0.54
(P39 x Ia453)	25	10.23	0.54
(Ia5125 x P39)	25	10.48	0.54
(Ia453 x C40)	25	10.87	0.64
(Ia453 x C40)	19	10.93	0.54

Table A5-7. Estimated marginal means and standard error for total soluble solids (%) of *sugary1 (su1)* hybrids measured at three harvest dates (19, 22, and 25 days after pollination (DAP)) from a six-line diallel cross grown at West Madison Agricultural Research Station in two planting dates per year with two replications per planting date in 2020 and 2021.

su1 Hybrid	Harvest Date (DAP)	Total Soluble Solids (%)	Standard Error
(Ia5125 x C68)	19	16.18	0.49
(C40 x Ia5125)	19	17.08	0.49
(Il101t x Ia5125)	19	17.09	0.49
(C40 x P39)	19	18.34	0.49
(C68 x Il101t)	19	18.43	0.49
(Il101t x C40)	19	18.58	0.49
(Ia5125 x P39)	19	18.88	0.49
(P39 x Il101t)	19	18.91	0.49
(Ia5125 x C68)	22	19.08	0.49
(Ia5125 x C68)	25	19.15	0.49
(C68 x C40)	19	19.54	0.49
(Ia5125 x Ia453)	19	19.78	0.49
(C40 x Ia5125)	22	19.98	0.49
(Il101t x Ia5125)	22	19.99	0.49
(C40 x Ia5125)	25	20.06	0.49
(Il101t x Ia5125)	25	20.07	0.49
(Il101t x Ia453)	19	20.33	0.49
(Ia453 x C40)	19	20.58	0.49
(C40 x P39)	22	21.24	0.49
(C40 x P39)	25	21.31	0.49

(C68 x Il101t)	22	21.32	0.49
(C68 x Il101t)	25	21.40	0.49
(Il101t x C40)	22	21.47	0.49
(Il101t x C40)	25	21.55	0.49
(Ia5125 x P39)	22	21.77	0.49
(P39 x Il101t)	22	21.81	0.49
(Ia5125 x P39)	25	21.85	0.49
(P39 x Il101t)	25	21.89	0.49
(Ia453 x C68)	19	22.10	0.49
(C68 x C40)	22	22.44	0.49
(C68 x C40)	25	22.52	0.49
(Ia5125 x Ia453)	22	22.68	0.49
(Ia5125 x Ia453)	25	22.76	0.49
(P39 x C68)	19	22.78	0.49
(Il101t x Ia453)	22	23.23	0.49
(Il101t x Ia453)	25	23.30	0.49
(Ia453 x C40)	22	23.48	0.49
(Ia453 x C40)	25	23.55	0.49
(P39 x Ia453)	19	23.56	0.49
(Ia453 x C68)	22	25.00	0.49
(Ia453 x C68)	25	25.07	0.49
(P39 x C68)	22	25.68	0.49
(P39 x C68)	25	25.75	0.49
(P39 x Ia453)	22	26.46	0.49
(P39 x Ia453)	25	26.53	0.49

Table A5-8. Estimated marginal means for total sugar (mg/g) of hybrids with either C40 or C68 as a parent, near-isogenic for endosperm type (WT = wild type, *sh2 = shrunken2, su1 = sugary1, wx1 = waxy1*), from four six-line diallel crosses grown at West Madison Agricultural Research Station in two planting dates per year with two replications per planting date in 2020 and 2021. Within a harvest date, means that share the same letter are not statistically different at alpha = 0.05 from Tukey's Honest Significant Difference pairwise comparison tests.

Parental NIL	**Endosperm Type**	**Harvest Date**	**Total Sugar (mg/g)**	**Pairwise Comparison**
C68	WT	19	119.2	a
C68	wx1	19	139.4	b
C40	WT	19	143.6	b
C68	su1	19	144.4	b
C40	wx1	19	163.9	c
C40	su1	19	168.8	c
C68	sh2	19	338.9	d
C40	sh2	19	363.4	f
//				
C68	WT	22	88.9	a
C68	wx1	22	104.6	b

C40	WT	22	111.3	bc
C68	su1	22	124.8	cd
C40	wx1	22	127.0	d
C40	su1	22	147.2	e
C68	sh2	22	337.9	f
C40	sh2	22	360.3	g
//				
C68	WT	25	67.6	a
C68	wx1	25	84.3	b
C40	WT	25	89.0	bc
C68	su1	25	100.0	cd
C40	wx1	25	105.8	d
C40	su1	25	121.4	e
C68	sh2	25	320.5	f
C40	sh2	25	341.9	g

VI. Appendix Figure A6: Pearson Correlations

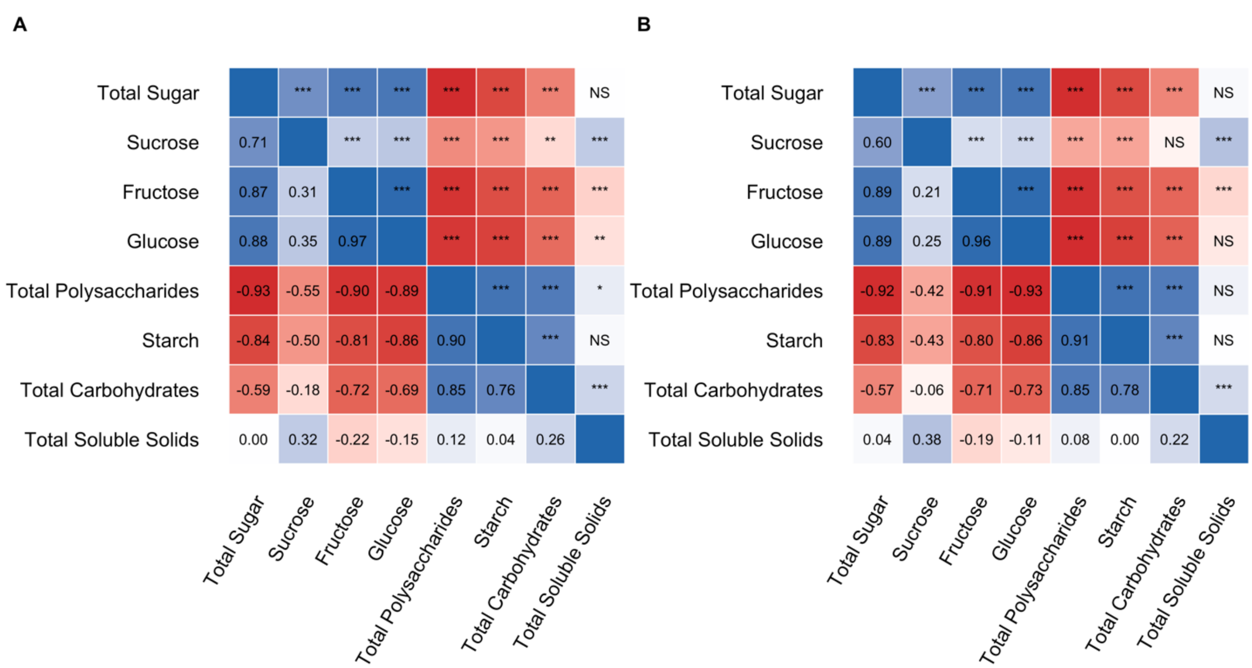

Figure A6. Pearson correlation coefficients (lower diagonals) and significance (upper diagonals, *,**,*** correspond to 0.05, 0.01, 0.001 probability levels, respectively) among traits measured on hybrids in four planting dates at West Madison Agricultural Research Station in 2020 and 2021 averaged over three harvest dates. NS = not significant. Among *waxy1* endosperm (A). Among wild type endosperm (B). Cells are colored blue for positive correlation coefficients,

with increasing saturation as coefficient approaches 1, and colored red for negative correlation coefficients, with increasing saturation as coefficient approaches -1.

3 Chapter Three: Evaluation of Gain from Three Cycles of Recurrent Selection for Total Soluble Solids Content in a Sweet x Field Corn 'Vegetable' Population under Organic Management

3.1 Abstract

There is interest in developing corn varieties for fresh harvest that are starchier, less sweet, and better suited to cooking applications than modern supersweet (*shrunken2)* corn varieties. Relative to field corn, modern sweet corn varieties have elevated sugar content, moisture content, as well as thin pericarps conferring elevated tenderness; all traits that confer an extended fresh harvest window and allow for use as a fresh vegetable. Methods to select for a higher starch ideotype while maintaining an acceptable fresh harvest window are unknown and unexplored. This experiment determined the gain from three cycles of recurrent selection on total soluble solids content in a sweet x field, or 'vegetable', corn population under organic conditions. Selection was not effective in changing the TSS content, total sugar, sucrose, glucose, fructose content, or moisture content, or shifting ratings of eating quality in a desirable direction, over cycles of selection. Significant negative indirect responses to selection were observed for tenderness, chalkiness, ear width, and ear length. TSS content was found to have very low to negligible heritability at two harvest dates in the population, with the variance in the measurement primarily due to error. Realized heritability was low across cycles, with a negative realized heritability in the first two cycles of selection (-0.24) and a positive, but low, realized heritability in the third cycle of selection (0.27). A moderate positive correlation between perceived chalkiness (starchiness) and total sugar content (0.56) as well as a strong positive

correlation between kernel moisture content and total sugar content (0.73) indicate that future work for improving the harvest window in this population could focus on selection via sensory analysis or kernel moisture content.

3.2 Introduction

Recurrent selection is the cyclical improvement of a population for traits of interest (Bernardo, 2020). This process results in the accumulation of desirable alleles for the traits of interest within a population, while also maintaining genetic variation for continued gain. Improved populations can be used as is or as source material for generating new inbreds. When generating new inbreds from populations improved via recurrent selection, the chance of fixing alleles for traits of interest are increased relative to an unimproved population, because the frequency of desirable alleles has been increased (Bernardo, 2020).

While progress can be achieved rapidly, within a few cycles, long term gains are also possible. The Illinois long-term selection experiment, initiated in 1896 and with over 100 cycles of divergent selection, demonstrates that gain can continue over many cycles for quantitatively controlled traits (Dudley, 2007). The population means of the high oil and high protein populations in this experiment were still increasing after 100 cycles of selection, illustrating that the limit had still not been reached for these traits in these populations. Theory demonstrates that these traits are likely controlled by 50 or more loci that were at a low frequency in the initial population (Bernardo, 2020).

For recurrent selection to be effective, certain criteria need to be considered. The breeder's equation illustrates the components that influence the response to selection (R):

$$R = i\, h^2\, \sigma_p / t$$

Where,

R = the response to selection,

i = the selection intensity,

h^2 = the narrow sense heritability,

σ_p = the phenotypic standard deviation,

t = the generation interval

The selection intensity, i, refers to the proportion of families that are selected to advance to the next cycle of selection. Selection intensity is increased by either selecting fewer families or by evaluating more families. In cross pollinated species like corn, increasing the selection intensity too much by selecting too few families for advancement, increases the coefficient of inbreeding and risks inbreeding depression. Increasing the selection intensity by selecting fewer families also decreases the genetic variability within the population, decreasing the potential for future gain. Therefore, it is generally better to increase the selection intensity by evaluating more families. However, with a finite budget there is a tradeoff. Increasing the number of families evaluated often means reducing the replication of families, which reduces the precision of the evaluation (Zystro et al., 2019).

The narrow sense heritability, h^2, is the ratio of additive genetic variance to phenotypic variance. Phenotypic variance includes variance due to the genotype and due to the environment. The additive genetic variance is the variation in breeding values and is therefore what drives the response to selection (Falconer & Mackay, 2009). If there is not variation in breeding values for the trait of interest in the population, no gains can be made. If heritability is low, the tradeoff between reducing environmental variation via replication and increasing selection intensity is greater than if heritability is high and the impact of the environment is low. The type of progeny

that are evaluated and recombined, for example selfed families or half-sib families, impacts the coefficient of additive variance and thus the response to selection as well.

Lastly, the generation interval, t, in the denominator of the breeder's equation, divides the response to selection into units of time. The number of generations that can be grown in a specific unit of time, for example a calendar year, is dependent upon the biology of the crop and available resources. Often breeding programs can achieve two or more generations per year by using off season winter nurseries or with crops, methodology, or management that can achieve a seed-to-seed cycle quickly. Response to selection per year, then, can be increased by increasing the number of selection cycles per year.

The motivation for this recurrent selection experiment began with a request from stakeholders for fresh eating corn that was less sweet, more starchy, and suited to cooking (Dawson & Healy, 2018). The Wisconsin Sweet Corn Breeding Program approached this request by crossing a *sugaryenhancer1 (se1)* sweet corn inbred with a starchy *Su1* field corn population, self-pollinating the F1, then intermating the progeny to generate a new population. The resultant population had increased starch relative to modern sweet corn varieties, but the fresh eating harvest window of the population was very narrow, and improvement was necessary for the population to be a viable variety for growers.

Harvest window is the length of time that a sweet corn ear remains sweet and tender in the field and can therefore be harvested over a period of time while remaining high quality. Harvest date is typically determined by the number of days after pollination (DAP). A desirable harvest window is characterized by a lack of a precipitous decline in total sugar levels, tenderness, and moisture as the DAP increases. A narrow harvest window is undesirable because

it increases the risk of crop loss or lower market price if the quality is below market threshold, as well as burdens the grower with less flexibility in harvest timing (Wong et al., 1994).

In modern sweet corn varieties with the *sh2* allele, sugars and moisture content remain elevated from about 20 days after pollination (DAP) to 27 DAP, remaining succulent, tender, and allowing high quality harvest throughout this period (Tracy, 1997; Wong et al., 1994). Supersweet *(sh2)* sweet corn at peak fresh harvest stage, around 23 DAP, generally contain 75% to 80% moisture and have >300 mg/g total sugar in the endosperm (Dodson-Swenson & Tracy, 2015; Soberalske & Andrew, 1978). While in field corn varieties, moisture is already dropping at this time point, with 70% moisture at 24 DAP, and the sugar content is much lower than sweet corn, with about 100 mg/g total sugar in the kernel endosperm (Nielsen, 2021). The pericarp is the outer most layer of the kernel, which protects the seed from damage, pathogens, and from splitting due to osmotic pressure during development, and is also the first tissue we encounter when biting into an ear of sweet corn (Tracy & Galinat, 1987). Tenderness in sweet corn is defined by how easily the pericarp breaks apart when sweet corn is bitten (Bailey & Bailey, 1938). Sweet corn pericarp is only a few cell layers thick, generally between 5 to 15 cell layers, and is measured in microns, with thinner pericarp perceived as more tender and preferred for fresh consumption (Tracy & Galinat, 1987). The length of the fresh harvest window in sweet corn is therefore impacted by several processes that occur during kernel development, including the quantity of and ratios between carbohydrates accumulated in the endosperm, the tenderness of the pericarp, and the moisture content of the kernel.

After pollination, kernel development can be divided into three phases, the lag phase, grain filling phase, and maturation drying phase (Maiorano et al., 2014). During the lag phase, from pollination until about 14 DAP, the kernel accumulates water rapidly, but has not yet

accumulated carbohydrates. During grain filling, from 14 DAP to about 45 DAP, the kernel stores polysaccharides (starches) in the case of field corn, or mono- and disaccharides (sugars) and polysaccharides in the case of sweet corn, in the endosperm. At 45 DAP, about 90% of the total dry matter is accumulated in the kernel. After the midpoint in grain filling, water loss occurs as more carbohydrates are stored in the kernel at the expense of water. During the final phase, the maturation drying phase, more water loss occurs and dry matter accumulates until physiological maturity (Zhang et al., 2023). Physiological maturity is the point at which the kernel has accumulated maximum dry matter and the 'black' or 'abscission' layer, composed of collapsed cells, forms at the base of the kernel, preventing the exchange of water and carbohydrates between the kernel and the rest of the plant (Maiorano et al., 2014). At physiological maturity, field corn moisture is around 35% and the endosperm contains 85% of the total dry matter of the kernel due to carbohydrates (Li et al., 2022; Li et al., 2021). As the kernel develops, the pericarp thickens and builds hemicellulose and lignin in the outer cell layers and finally compresses as it reaches physiological maturity, transforming from a soft tissue that can be bitten through or punctured with a fingernail to a hard protective layer that cannot be punctured with a fingernail at physiological maturity (Kiesselbach, 1949; Zhang et al., 2022).

Kernel development is impacted by genetics, management, and the environment, and is particularly sensitive to heat and drought stress during reproductive stages and early grain filling (Cirilo et al., 2011; Martínez et al., 2022; Zhang et al., 2023). Kernel moisture and sugar content both decrease with maturity but the content and rate of loss is impacted by genotype, environment, and management (Ledenčan et al., 2022). For example, Soberalske and Andrew (1978) reported that in isogenic series for endosperm type, the *Su1 sh2* endosperm lost moisture and sugar content much slower than any other endosperm type over successive harvest dates, and

that genotypes within endosperm type exhibited different rates of moisture and sugar loss (Soberalske & Andrew, 1978).

Sweet corn carbohydrate content includes water soluble polysaccharides (WSP), insoluble polysaccharides (starch), sucrose, glucose, and fructose. Of these carbohydrates, the sugars (sucrose, glucose, and fructose) and the WSP are water soluble. The perception of sweetness is highly correlated with consumer liking and the sugar content, particularly sucrose content, in the endosperm (Evensen & Boyer, 1986; Ledenčan et al., 2022). Therefore, sweet corn breeding targets elevated sugar (sucrose) content. Sweet corn carbohydrate content is typically quantified using enzymatic assays in the laboratory (De Vries et al., 2016). In other crops, however, more rapid methods, including total soluble solids (TSS) content, are used to estimate total sugar. TSS is a measure of the soluble constituents dissolved in solution. The solid soluble fraction of fruits and vegetables can include sugars, amino acids, other acids like ascorbic or citrate, pectins, minerals, and phenols, which are dissolved in the water-based juice (Beckles, 2012). TSS is measured with a refractometer and reported in the units of degrees Brix (°Brix), where 10 °Brix equates to 10% solids in solution. TSS is often used in fruit breeding, such as in tomato, watermelon, grape, strawberry, apricot, and winter squash, where TSS is an approximate measure of percent sugars (Baccichet et al., 2023; Beckles, 2012; Breksa et al., 2015; Campbell et al., 2021; Huang et al., 2022; Hultengren et al., 2016). High TSS is associated with high ratings of sweetness by taste panelists in these crops (Baccichet et al., 2023; Beckles, 2012; Schwieterman et al., 2014; Whitaker et al., 2013).

Dry matter content is a measure of all constituents other than water in a fruit or vegetable. Therefore, TSS is related to dry matter content, the extent to which these two traits correlate varies among crops, but both typically have an inverse relationship with the size of fruit. For

example, a study in tomato reported a correlation coefficient of 0.84 between TSS and fruit dry matter content, another in summer squash reported a correlation coefficient of 0.33, and TSS and dry matter were highly correlated among stone fruits with a correlation coefficient of 0.91 across 13 cultivars (Itoh et al., 2020; Martínez-Valdivieso et al., 2015; Scalisi & O'Connell, 2021). Moisture content of sweet corn kernels, the inverse of dry matter content of the kernels, exhibited strong negative correlations with TSS in the literature, often at or above -0.90 across cultivars (Campbell & McKerlie, 1967; Drake & Nelson, 1979)

TSS provides an inexpensive and quick measurement, but it is not very precise. For example, a refractometer, such as the Atago PAL-1, costs $350 USD, can be recalibrated for use over many years and thousands of samples, and the measurement itself takes about a minute or less (Atago USA, Inc., Bellevue, Washington, USA). However, factors like maturity, post-harvest storage conditions and time, fruit size, management practices, time of day, and operator error might all affect a TSS measurement to various degrees (Nookaraju et al., 2010). Enzymatic assays in the laboratory are precise, with repeatability within 2%, but expensive (*Megazyme Knowledge Base FAQ*, 2021). For example, the assay used in the Wisconsin Sweet Corn Breeding program to quantify total sugar, glucose, fructose, and sucrose (K-SUFRG) costs $2.38 per sample for the kit, samples are typically replicated in triplicate in the lab for a single genotype, and the assay requires other materials, such as disposable cuvettes, and lab equipment, such as a spectrophotometer, to process (Megazyme, Bray, Ireland). The enzymatic assays are also time consuming to conduct, with the K-SUFRG assay requiring four hours to quantify the sugar content of twenty-four samples. In a breeding program with thousands of genotypes replicated over years and environments, the number of samples can accumulate rapidly.

In sweet corn, studies using TSS to approximate total sugars report mixed results. TSS is likely not a reliable proxy for total sugars in sweet corn varieties that contain WSP, such as *su1* and *se1* endosperms. While both sugars and WSP are desirable constituents, sugars confer sweetness while WSP confers a creamy mouthfeel, and because both are water soluble it is impossible to know with a TSS measurement alone what proportion is due to sugars versus WSP. A study using a single sweet corn cultivar, 'Silver Queen', an *su1* type, reported that as TSS increased (+5 °Brix) texture ratings shifted from "moderately delicate" to "slightly rigid" (Collins & Taylor, 1976). Other studies found that *sh2* hybrids had lower TSS than both *se1* and *su1* hybrids, and TSS increased over consecutive harvest dates for *su1* and *se1* hybrids while remaining constant in *sh2* hybrids (Hale et al., 2005; Lee et al., 1999; Zhu et al., 1992). Correlations reported in the literature vary considerably but are often negative between TSS and total sugars. Hale et al. (2005) reported a coefficient of -0.51, while Zhu et al. (1992) reported contrasting correlations, -0.79 for a *sh2* cultivar and 0.68 for a *se1* cultivar. No studies could be found that used TSS as selection criteria in sweet corn, to our knowledge it is unknown if selection on TSS would result in changes to sugar content, harvest window, or perception of quality traits.

While enzymatic assays provide a quantitative measurement of carbohydrate content, the Wisconsin Sweet Corn Breeding Program also uses "bite tests", taste tests to qualitatively assess the eating quality of varieties in development in the program. Bite tests rate varieties on a 1 to 5 scale for sweetness and tenderness, among other attributes, with 1 as poor or low and 5 as high or excellent. Sweet corn carbohydrates are influenced by major genes of large effect, namely the recessive alleles, like *sh2*, conferring mutations in the starch synthesis pathway that largely

impact carbohydrate content relative to wild type, as well as many quantitative trait loci (QTL) of smaller effect size (Azanza et al., 1996; Hislop, 2022).

Considering the changes in carbohydrates that occur during kernel development and their relationship to the fresh eating harvest window, this experiment determined if the fresh eating harvest window could be lengthened by three cycles of recurrent selection on total soluble solids content at 24 DAP. The hypothesis was that selection on TSS at 24 DAP would increase the total sugar content at 24 DAP in cycle 0 relative to cycle 3 and therefore lengthen the harvest window as measured by bite tests in cycle 0 relative to cycle 3.

3.3 Materials and Methods

3.3.1 Germplasm Development

Two populations were derived from a cross between We11413, a Wisconsin *se1* inbred that was very tender, and a *Su1* Pozolero Morado population, provided by Dr. Jose Ron Parra from the University of Guadalajara. Pozolero Morado was an improved population bred by Dr. Ron Parra from samples of Elotes Occidentales collected from the Mexican states of Jalisco, Zacatecas, Michoacán, and Nayarit (M. Willcox, personal communication, March 1, 2021). Elotes Occidentales are considered multipurpose and used both as fresh ears for elotes and as dry grain, typically nixtamalized for pozole, ground for atole due to the slight sweetness of the grain, for pinole, or chicales (M. Willcox, personal communication, March 1, 2021). Dr. Ron Parra used half sib recurrent selection to select for yield, 8-rows, and grain type (M. Willcox, personal communication, March 1, 2021).

The cross between We11413 and Pozolero Morado was made in the summer of 2014 at West Madison Agricultural Research Station (WMARS). In the summer of 2015, the F1 was self-pollinated. The resulting progeny went through three cycles of sib mating. In the winter of

2019 at a winter nursery in Tuniche, Chile, the population was split into two groups based on dry kernel phenotype, *su1* and *Su1*, and each population was separately sib pollinated to form cycle 0 (C0). The original objective of this experiment was to conduct recurrent selection on each of these populations, the *su1* and the *Su1*. However, the *su1* population was discontinued after two cycles of selection due to extremely poor germination in 2022, resulting in too few plants to evaluate and advance. The remainder of this paper will refer to the population that was self-pollinated (@), sib mated (#), and selected for the *Su1* phenotype, or (We11413 x Pozolero Morado)@###*Su1*#, as "the population".

3.3.2 Experimental Design: Years 2020 - 2022

In the summer of 2020, 25 rows of C0, and in the summer of 2021, 25 rows of cycle 1 (C1) were planted at WMARS with rows thinned to 12 plants per row. In the summer of 2022, 40 rows of the cycle 2 (C2) population were planted at WMARS with rows thinned to 12 plants per row. Due to labor constraints during the global pandemic, in 2020, the population was planted in the conventional field, but in subsequent summers the population was planted in the organic field at WMARS. In each of the summers in years 2020-2022 as many plants as possible were self-pollinated on the same day. The objective was to self-pollinate at least 100 plants. In 2020, or C0, 108 plants were pollinated. In 2021, or C1, 156 plants were pollinated. In 2022, or C2, 244 plants were pollinated. Each self-pollinated plant was labelled with a unique number identifier that remained with the ear on that plant through harvest.

3.3.3 Trait Evaluation: Years 2020 - 2022

At 19 and again at 24 days after pollination (DAP), the TSS were measured on each ear using an Atato Pal-1 digital refractometer (Atago USA Inc., Bellevue, WA) and a method

modified from Hale et al. (2005). Specifically, the refractometer was calibrated and zeroed per the manufacturer's instructions. A 3x3 cm section of kernels from the longitudinal center of the ear, with the ear still attached to the plant, were cut off with a knife, placed in a conventional kitchen garlic press, and the liquid extract squeezed onto the reader well of the refractometer. Care was taken to ensure that no solids were in the extract. All instruments were washed in water and thoroughly dried between samples. Only plants with successful pollination that had at least 50% pollinated kernels on the ear were measured to ensure enough kernels were present to be used as seed. In 2020, or C0, 92 plants were measured. In 2021, or C1, 125 plants were measured. In 2022, or C2, 173 plants were measured. At maturity, all the ears from the plants were individually harvested with the unique identifier and dried in a seed dryer at WMARS for five days.

3.3.4 Cycle Formation: Years 2020 - 2022

In each cycle (2020 (C0), 2021 (C1), 2022 (C2)), ~10% of the plants with the highest TSS at 24 DAP were selected for advancement. Only plants where the TSS at 24 DAP was higher than the TSS at 19 DAP were selected. In 2020, or C0, seed from 10 plants were advanced. In 2021, or C1, seed from 12 plants were advanced. In 2022, or C2, seed from 17 plants were advanced. Seed from the selected plants were then sent to a winter nursery where the resulting plants were crossed on a plant-to-plant basis, creating full sib ears. The crossing design was such that sib pollinations from plants derived from the same ear were not possible. This was achieved by a paired row crossing scheme where the first row had seed from plant one and the second row contained a balanced bulk of seed from all the other ears except for plant one, and rows one and two were crossed in both directions, and this pattern repeated for all selected plants. The seed from the full sib ears were combined into balanced bulks, whereby each ear

contributed an equal number of seeds to the next cycle. In the winter of 2022, seed from the balanced bulks of each cycle were sent to the winter nursery and sib mated to generate enough seed, from the same seed environment, to be planted in the summer of 2023 to evaluate gain from selection. In summary, C0 was planted in the summer of 2020 and selected plants formed C1 in the winter of 2020, in the summer of 2021 C1 was planted and selected plants formed C2 in the winter of 2021, and in the summer of 2022 C2 was planted and selected plants formed cycle 3 (C3) in the winter of 2022, and all four cycles were also grown in the winter of 2022 to increase seed stocks.

3.3.5 Experimental Design: Year 2023

In the summer of 2023, the four cycles, C0, C1, C2, and C3, were planted in a RCBD in two organic environments with three replications per environment at WMARS to evaluate the gain from selection. The populations were planted in six row plots and thinned to twelve plants per row. At least forty plants per plot were self-pollinated all on the same day to collect TSS and taste test data. Each self-pollinated ear was labelled with a unique number identifier that remained with the ear throughout data collection. At least ten plants per plot were left to open pollinate to collect data on ear and husk traits.

3.3.6 Trait Evaluation: Year 2023

Evaluated traits included stand counts, plant, and ear heights. Plant and ear heights were measured after pollination but prior to harvest. Plant height was measured as the height from the ground to the ligule of the leaf subtending the tassel. Ear height was measured as the height from the ground to the ear branch node at the base of the upper most ear. Eight randomly chosen

plants per row were measured for both plant and ear height, excluding the first and last plants per row. Stand counts were taken when plants reached the V5 stage and prior to thinning.

At 19 and 24 DAP, TSS content was measured on ten self-pollinated plants per plot. The TSS content was measured on the two dates on the same ear. At 19 DAP, the TSS was measured in the field with the ear still connected to the plant and at 24 DAP the ears were harvested and the TSS content measured before the ear was flash frozen with liquid nitrogen. The kernels were then removed from the ear and stored at -80 degrees Celsius until processed through a Labconco FreeZone 4.5L 77500/77510 series freeze dryer to remove all moisture (Labconco Corporation, Kansas City, MO). The kernels from the ten ears from plots in one environment were weighed pre- and post- freeze drying and the percent kernel moisture was calculated based on these weights. The freeze-dried kernels ground using an Udy cyclone mill, sifted through a 0.5mm screen, and stored at room temperature until lab assays to quantify the sugar content were conducted. To prepare the kernels for the lab assays, the freeze-dried kernels from the ten ears from each plot were combined in equal proportion to form a bulk sample for each plot. Therefore, across the four cycles grown in two environments with three replicates, there were twenty-four samples. Quantification of total sugar, sucrose, glucose, and fructose content was conducted in triplicate laboratory replicates for each of the twenty-four samples using Neogen Megazyme assay kit K-SUFRG (Megazyme, Bray, Ireland).

At 19 and again at 24 DAP, taste tests were conducted by two tasters. On the two harvest dates up to ten ears were harvested from the self-pollinated ears. Only ears that had a successful pollination were selected for tasting, therefore the number of tasted ears varied from three to ten ears per plot per harvest date. These ears were broken in half and two tasters rated the sweetness, tenderness, and chalkiness on a 1 – 5 scale for each ear. Sweetness was defined as 1 with no

perceptible sweetness and 5 as very sweet. Tenderness was defined as how easily your teeth shear through the kernels upon biting, with 1 defined as extremely tough and 5 defined as a pleasant pop upon biting with little resistance. Chalkiness was chosen as a measure of how starchy the kernels tasted when chewed, with 1 defined as very chalky and 5 defined as no perceptible chalkiness. In summary, up to 10 ears per plot were rated by two tasters at two harvest dates, for a total of up 40 ratings per plot. Prior to tasting, tasters were calibrated by tasting ears from C0 and C3 that were planted in another field at an earlier planting date than the trial. Tasters sampled twenty ears from each of the cycles and discussed the variation that existed within these populations for the three quality attributes and agreed on the upper and lower bounds of the rating for each attribute.

At approximately 25 DAP, ten open pollinated ears per plot were harvested to evaluate husk appearance, husk protection, tip fill, row configuration, number of kernel rows, ear length, and ear width. The ten ears were laid side by side on a table. Husk appearance and husk protection are rated on a 1-5 scale, with 1 as poor and 5 as excellent. Husk protection evaluates how far the husks extend past the cob and how tightly the husks were wrapped around the silks to deter insect predation. Husk appearance rates the visual appeal of the husks considering disease presence, the number and size of the flag leaves, with more and larger leaves as desirable, and husk color, with a dark green as desirable. Next, the ears were husked. Tip fill was rated on 1-5 scale with 1 as poor and 5 as excellent. Tip fill rates the extent of how developed kernels were at the tip of the ear. A row configuration score was given on a 1-5 scale with 1 as poor (no definable rows or large gaps between rows) and 5 as excellent (straight rows, no gaps). Next, the ears were laid on a measuring board and the ear length and width were measured in centimeters for each ear.

3.3.7 Statistical Analysis: Year 2023

A linear model was built using the lm() command from the R Stats package in R via backward model selection (R Core Team, 2021). Models were evaluated using the Akaike information criterion (AIC) and adjusted R^2 as criteria to choose the best fit model. The model treated environment, replication nested in environment, cycle, harvest date (DAP), where applicable, and taster, where applicable, as fixed effects. The model was:

$$Y_{ijk} = \mu + cycle_i + environment_j + (cycle\ x\ environment)_{ij} + replication(environment)_{jk} + e_{ijk} \quad (1)$$

Where Y_{ijk} is the phenotypic value measured for cycle *i* in environment *j* and replication *k,* μ is the grand mean, $cycle_i$ is the effect of cycle *i,* $environment_j$ is the effect of environment *j,* $(cycle\ x\ environment)_{ij}$ is the effect of the interaction between cycle *i* and environment *j,* $replication(environment)_{jk}$ is the effect of replication *k* nested in environment *j,* and e_{ijk} is the random error term. Model 1 was used for response variables not collected over levels of harvest date or taster, specifically: total sugar, sucrose, glucose, fructose, plant and ear heights, stand, ear length and width, husk appearance and protection, number of kernel rows, rowing, tip fill, and moisture content. Model 2 included the terms from model 1 but added the main effect of harvest date. The model was:

$$Y_{ijkl} = \mu + cycle_i + environment_j + harvest\ date_k + (cycle\ x\ environment)_{ij} + replication(environment)_{jl} + e_{ijkl} \quad (2)$$

Where Y_{ijkl} is the phenotypic value measured for cycle *i* in environment *j* on harvest date *k* in replication *l,* μ is the grand mean, $cycle_i$ is the effect of cycle *i,* $environment_j$ is the effect of environment *j,* $harvest\ date_k$ is the effect of harvest date *k,* $(cycle\ x\ environment)_{ij}$ is the

effect of the interaction between cycle *i* and environment *j,* $replication(environment)_{jl}$ is the effect of replication *l* nested in environment *j,* and e_{ijkl} is the random error term. Model 2 was used for response variables collected over levels of harvest date, but not taster, specifically: TSS. Finally, model 3 included the terms from model 2 but added the main effect of taster as well as the interaction among taster and harvest date. The model was:

$$Y_{ijklm} = \mu + cycle_i + environment_j + harvest\ date_k + taster_l + (cycle\ x\ environment)_{ij} + (taster\ x\ harvest\ date)_{kl} + replication(environment)_{jm} + e_{ijklm} \quad (3)$$

Where Y_{ijklm} is the phenotypic value measured for cycle *i* in environment *j* on harvest date *k* rated by taster *l* in replication *m,* μ is the grand mean, $cycle_i$ is the effect of cycle *i,* $environment_j$ is the effect of environment *j,* $harvest\ date_k$ is the effect of harvest date *k,* $taster_l$ is the effect of taster *l,* $(cycle\ x\ environment)_{ij}$ is the effect of the interaction between cycle *i* and environment *j,* $(taster\ x\ harvest\ date)_{kl}$ is the effect of the interaction between taster *l* on harvest date *k,* $replication(environment)_{jm}$ is the effect of replication *m* nested in environment *j,* and e_{ijklm} is the random error term. Model 3 was used for the three quality traits, sweetness, tenderness, and chalkiness.

Outliers were checked using the rosnerTest() command from the EnvStats package in R (Millard, 2013). Model assumptions, including normality and equal variance of the residuals, were verified graphically. Analysis of variance (ANOVA) was conducted on plot means for all traits, with F-tests on the mean squares used to determine significant sources of variation. In instances of cycle x environment interactions, Spearman's rank correlations were calculated to determine if the interaction was due to a change in magnitude or a change in rank among cycles across environments. Post hoc pairwise tests were conducted using the emmeans() command

from the Emmeans package in R with p-values adjusted using Tukey's Honest Significant Difference and the confidence intervals adjusted by the Dunn-Sidak correction for multiple comparisons with an alpha level of significance of 0.05 (Lenth, 2022). Traits with significant differences in cycle means were fit to linear and quadratic models via orthogonal polynomial contrasts with a single intercept (Eberhart, 1964). Variance components were estimated using the VarCorr function in the lme4 package to calculate entry mean heritability, with cycle, environment, cycle x environment, harvest date (where applicable), and replication nested within environment treated as random variables (Bates et al., 2015). Realized heritability, or the response to selection divided by the selected differential, was calculated based on the cycle population means and means of the selected plants within cycle (Bernardo, 2014).

Equations used to calculate the coefficient of inbreeding are defined below. In this experiment S_1 families were recombined, which have an F_p of 0, therefore the effective population size is equal to two times the number of S_1 families recombined (Hallauer & Miranda, 1988). Wright's coefficient of inbreeding was calculated using the equation, $F = 1/(2N_e) + [1 - 1/(2N_e)]F'$. Where, F is the coefficient of inbreeding, N_e is the effective population size, and F' is the coefficient of inbreeding from the previous generation. The F' was assumed to be 0 for cycle 0. The effective population size is calculated using the equation, $N_e = 2N/(1 + F_p)$. Where, N_e is the effective population size, N is the number of lines recombined, and F_p is the coefficient of inbreeding for the parental plants of the lines that are recombined.

3.4 Results and Discussion

3.4.1 Analysis of Variance

The traits with significant variation due to cycle were tenderness, chalkiness, ear length and ear width (Tables 3.1 and 3.2). The environment was a significant source of variation for total sugar, glucose, fructose, ear length, husk protection, and sweetness (Tables 3.1 and 3.2). There was a significant cycle x environment interaction for TSS, ear width, sweetness, and tenderness (Table 3.1 and 3.2). Among the eating quality traits (sweetness, tenderness, chalkiness), harvest date was a significant source of variation for all three traits, taster was a significant source of variation for tenderness and chalkiness, and there was a taster x harvest date interaction for all three traits (Table 3.2). Replication nested in environment was not a significant source of variation for any trait (Tables 3.1 and 3.2). The Spearman correlations among cycle ranks between environments were 0.96 (p-value ≤ 0.001) for ear width, 0.89 (p-value ≤ 0.001) for TSS, 0.92 (p-value ≤ 0.001) for sweetness, and 0.96 (p-value ≤ 0.001) for tenderness. The Spearman correlations among taster ranks between harvest dates were 0.99 (p-value ≤ 0.001) for both tenderness and chalkiness. Results will be presented averaged over environments and tasters for all traits.

Table 3.1. P-values from F-tests on mean squares in analysis of variance of four cycles grown in two organic environments with three replications per environment at West Madison Agricultural Research Station in 2023. *,**,*** correspond to significant at .05, .01, .001 probability levels, respectively. ns = not significant, NA = not applicable, Rep = replication, Env = environment. † = the terms in the model for this trait were cycle and replication because this trait was only collected in one environment.

	Source of Variation				
Trait	*Cycle*	*Environment*	*Harvest Date*	*Cycle x Environment*	*Rep(Env)*
Total Soluble Solids	ns	ns	ns	*	ns
Total Sugar	ns	***	NA	ns	ns
Sucrose	ns	ns	NA	ns	ns
Glucose	ns	***	NA	ns	ns

Fructose	ns	**	NA	ns	ns
Ear Height	ns	ns	NA	ns	ns
Plant Height	ns	ns	NA	ns	ns
Stand	ns	ns	NA	ns	ns
Ear Length	**	*	NA	ns	ns
Ear Width	*	ns	NA	*	ns
Husk Appearance	ns	ns	NA	ns	ns
Husk Protection	ns	*	NA	ns	ns
Number of Kernel Rows	ns	ns	NA	ns	ns
Rowing	ns	ns	NA	ns	ns
Tip Fill	ns	ns	NA	ns	ns
Moisture†	ns	NA	NA	NA	ns

Table 3.2. P-values from F-tests on mean squares in analysis of variance of four cycles grown in two organic environments with three replications per environment at West Madison Agricultural Research Station in 2023. Two tasters rated the three traits on each harvest date. *,**,*** correspond to significant at .05, .01, .001 probability levels, respectively. ns = not significant, Rep = replication, Env = environment.

	Source of Variation						
Trait	*Cycle*	*Environment*	*Taster*	*Harvest Date*	*Cycle x Environment*	*Taster x Harvest Date*	*Rep(Env)*
Sweetness	ns	***	ns	***	*	*	ns
Tenderness	***	ns	***	***	*	***	ns
Chalkiness	*	ns	***	***	ns	***	ns

3.4.2 Quality Traits

The three quality traits -- sweetness, tenderness, and chalkiness -- were all significantly lower at the later harvest date compared to the early harvest date when averaged over cycles of selection (Figure 3.1).

Figure 3.1. Ratings of sweetness, tenderness, and chalkiness (1-5 scale) at two harvest dates, 19 and 24 days after pollination, averaged over four cycles of selection grown in two organic environments with three replications per environment at West Madison Agricultural Research Station in 2023. Within each trait, means that share the same letter are not significantly different by a Tukey's Honest Significant Difference test at a significance level of 0.05.

Among cycles, averaged over harvest date, C0, C2, and C3 were all significantly different from one another for tenderness and C0 was significantly different from C3 for chalkiness (Table 3.3). Tenderness was significantly different between C0 and C2 and between C0 and C3 at the 19 DAP harvest date but only different between C0 and C3 at 24 DAP (Table 3.4). Chalkiness was not significantly different among cycles at 19 DAP but C0 was significantly different from C3 at 24 DAP (Table 3.4). There were no significant differences among cycles for

sweetness (Table 3.3). A linear coefficient was significant when fit to tenderness and chalkiness among cycles of selection, but a quadratic coefficient was not (Table 3.5). The coefficients were negative, indicating that there was a negative linear indirect response to selection for tenderness and chalkiness when selecting on TSS content.

Table 3.3. Estimated marginal mean ratings of sweetness, tenderness, and chalkiness among cycles grown in two organic environments with three replications per environment at West Madison Agricultural Research Station in 2023. Means are averaged over harvest dates and tasters. Within a trait (column), means that share the same letter are not significantly different by a Tukey's Honest Significant Difference test at a significance level of 0.05.

Cycle	*Sweetness*	*Tenderness*	*Chalkiness*
0	3.09 a	3.23 a	3.17 a
1	3.14 a	3.04 ab	2.99 ab
2	2.93 a	2.89 b	2.97 ab
3	2.92 a	2.58 c	2.85 b

Table 3.4. Estimated marginal mean ratings of tenderness and chalkiness at two harvest dates, 19 and 24 days after pollination (DAP), among cycles of selection grown in two organic environments with three replications per environment at West Madison Agricultural Research Station in 2023. Means are averaged over tasters. Within a trait (column), means that share the same letter are not significantly different by a Tukey's Honest Significant Difference test at a significance level of 0.05.

Cycle	*Tenderness at 19 DAP*	*Tenderness at 24 DAP*	*Chalkiness at 19 DAP*	*Chalkiness at 24 DAP*
0	3.88 a	2.51 a	3.72 a	2.66 a
1	3.64 ab	2.43 a	3.60 a	2.50 ab
2	3.43 bc	2.36 a	3.50 a	2.29 ab
3	3.25 c	1.94 b	3.49 a	2.24 b

Table 3.5. Intercepts, significant linear and quadratic coefficients, and coefficients of determination (R^2) for response to selection among four cycles grown in two organic environments with three replications per environment at West Madison Agricultural Research Station in 2023. *,**,*** correspond to significant at .05, .01, .001 probability levels, respectively. ns = not significant.

Model Term	*Tenderness*	*Chalkiness*	*Ear Width*	*Ear Length*
Intercept	2.937	2.995	3.749	23.109
Linear Coefficient	-0.465***	-0.224**	-0.197**	-0.711**
Quadratic Coefficient	ns	Ns	ns	ns
R^2	0.71	0.39	0.72	0.76

3.4.3 Ear Length and Width

C0 had ears that were wider and longer than C3 (Table 3.6). Selection resulted in ears that were 0.30 cm narrower and 1.3 cm shorter on average in C3 compared to C0. Significant linear trends over cycles of selection existed for ear width and ear length (Table 3.5). Like tenderness and chalkiness, the indirect response to selection for the ear size traits was negatively linear across cycles, which is an undesirable direction for all four traits. The linear models explained most of the variation in cycle means for tenderness (0.71), ear width (0.72), and ear length (0.76) (Table 3.5).

Table 3.6. Estimated marginal means of ear length and ear width over four cycles of selection grown in two organic environments with three replications per environment at West Madison Agricultural Research Station (WMARS) in 2023. Within a trait (column), means that share the same letter are not significantly different by a Tukey's Honest Significant Difference test at a significance level of 0.05.

Cycle	*Ear Width (cm)*	*Ear Length (cm)*
0	3.87 a	23.7 a
1	3.77 ab	22.8 ab
2	3.78 ab	23.5 a
3	3.58 b	22.4 b

3.4.4 Total Soluble Solids

Cycle was not a significant source of variation for TSS despite direct selection on this trait. Dissection of the variance components of TSS revealed that most of the variation in this trait is due to error in these four populations (cycles) (Table 3.7). Entry mean heritability was 0.08 for TSS at 19 DAP, and 0 for TSS at 24 DAP and for TSS averaged over harvest dates (Table 3.7). It is interesting that error variance was twice as high at 24 DAP compared to 19 DAP for this trait (Table 3.7). In general, total carbohydrate content and total polysaccharide content are expected to be higher at 24 DAP, while total sugar content and moisture are expected to be lower at 24 DAP, compared to 19 DAP, in field corn genotypes (Creech, 1965). Perhaps it

is due to the lower levels of sugar content or moisture and/or the higher levels of insoluble carbohydrates that cause more imprecision in the measurement at 24 DAP compared to 19 DAP. Similarly, heritability was 0 for total sugar, sucrose, glucose, and fructose, as there was no difference in the cycle means for these traits (Table 3.7). Realized heritability for TSS was -0.24 for the first two cycles of selection and 0.27 for the third cycle of selection (Table 3.8).

Table 3.7. The variance components estimated for total soluble solids (TSS) averaged over two harvest dates, TSS at 19 days after pollination (DAP), and TSS at 24 DAP, total sugar, sucrose, glucose, and fructose for four cycles of selection grown in two organic environments with three replications per environment at West Madison Agricultural Research Station. Heritability was calculated as $H^2 = V_g / V_g + V_{ge}/2 + V_{error}/2*3$ with 2 and 3 referring to the number of environments and replications within environment. For total soluble solids collected over two harvest dates, the denominator of V_{error} is 2*3*2.

Variance Component	TSS	TSS at 19 DAP	TSS at 24 DAP	Total Sugar	Sucrose	Glucose	Fructose
Genotype	0	0.04619	0	0	0	0	0
Environment	1.64×10^{-9}	0.14649	0	2.86358	0.22502	0.43284	0.24197
Genotype x Environment	0.21657	0.15987	0.19891	0.09503	0	0.0261	0
Harvest Date	0.04023	NA	NA	NA	NA	NA	NA
Replication (Environment)	0.00567	0.00052	0	0	0	0	0
Error	4.2501	2.88851	5.57596	1.37181	0.83007	0.26647	2.27142
Entry Mean Heritability	0	0.08	0	0	0	0	0

Table 3.8. Realized heritability for total soluble solids (TSS) at 24 days after pollination (DAP) for four cycles of selection grown in two organic environments with three replications per environment at West Madison Agricultural Research Station. Response to selection = mean of cycle x – mean of cycle x+1. Selection differential = mean of selected plants – mean of population. Realized heritability = response to selection / selection differential. Means are arithmetic means from raw phenotypic data.

Cycle	Population Mean	Mean of Selected Plants	Response to Selection	Selection Differential	Realized Heritability
0	11.07	13.58	-0.59	2.51	-0.24
1	10.48	12.93	-0.58	2.45	-0.24
2	9.9	13.45	0.96	3.55	0.27
3	10.86				

3.4.5 Correlations among Traits

TSS at 19 DAP moderately positively correlates with TSS at 24 DAP (Figure 3.2). TSS at 24 DAP was moderately negatively correlated with tenderness and kernel moisture at 24 DAP (Figure 3.2). TSS did not significantly correlate with any other quality trait. Total sugar at 24 DAP was strongly positively correlated with glucose and fructose at 24 DAP and moderately positively correlated with sucrose at 24 DAP (Figure 3.2). Total sugar at 24 DAP was moderately positively correlated with ratings of chalkiness at the same harvest date, where higher ratings of chalkiness were desirable (low chalkiness) (Figure 3.2). Glucose and fructose at 24 DAP were strongly positively correlated with chalkiness at 24 DAP, but sucrose was not significantly correlated with chalkiness (Figure 3.2). None of the sugar content traits were significantly correlated with sweetness ratings except for glucose and fructose at 24 DAP were weakly negatively correlated with sweetness at the same harvest date (Figure 3.2). Among the quality trait ratings, within a harvest date, each of the three traits were strongly correlated with one another (Figure 3.2). Kernel moisture at 24 DAP was strongly correlated with total sugar at 24 DAP, moderately correlated with sucrose at 24 DAP, and moderately correlated with sweetness at 19 DAP (Figure 3.2).

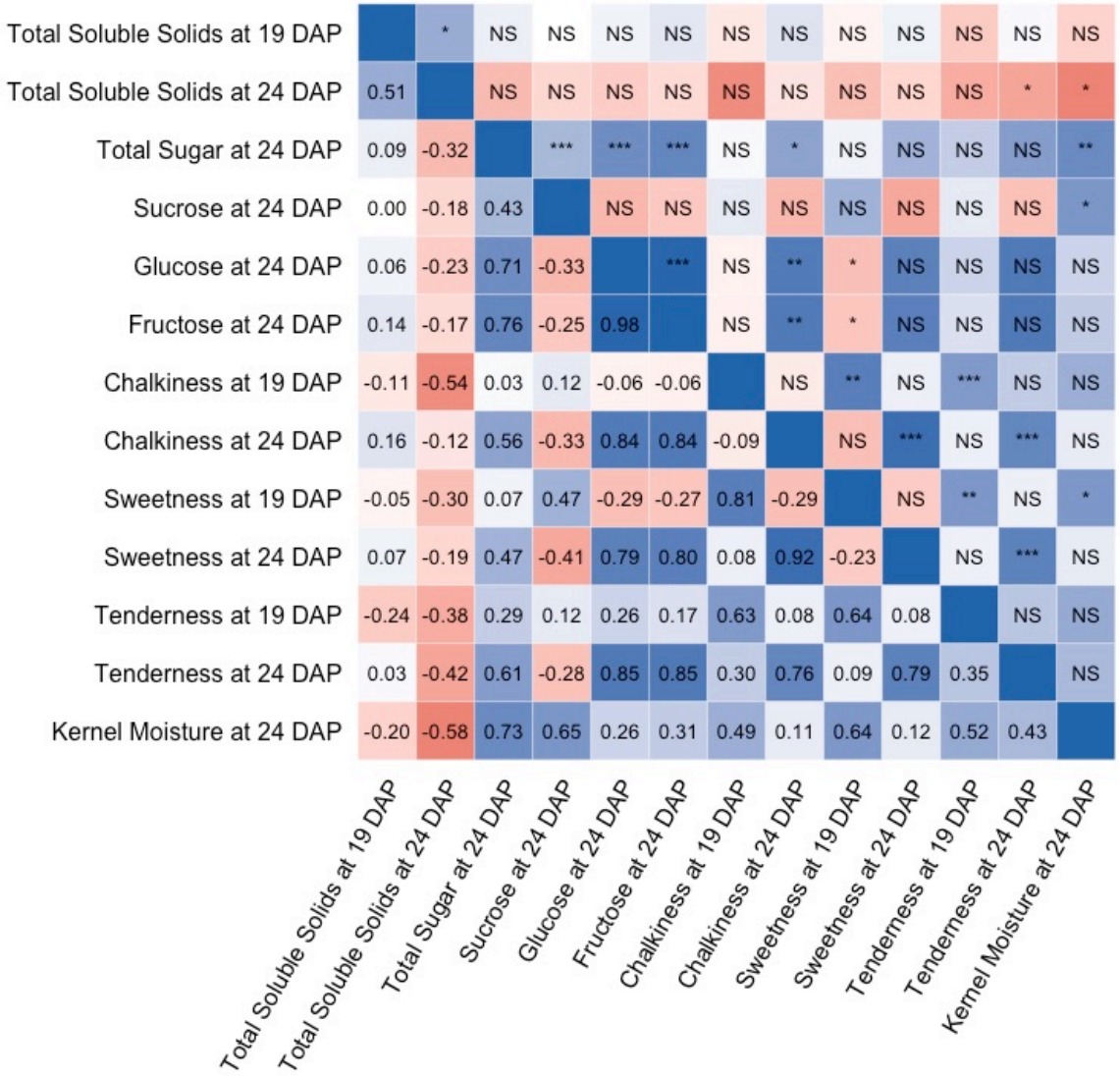

Figure 3.2. Pearson correlation coefficients (lower diagonals) and significance (upper diagonals, *,**,*** correspond to 0.05, 0.01, 0.001 probability levels, respectively) among traits measured at two harvest dates, 19 and 24 days after pollination (DAP) among four cycles of selection grown in two organic environments with three replications per environment at West Madison Agricultural Research Station in 2023. Cells are colored white for correlation coefficients of 0, colored blue for positive correlation coefficients, with increasing saturation as coefficient approaches 1, and colored red for negative correlation coefficients, with increasing saturation as coefficient approaches -1. NS = not significant.

3.4.6 Coefficient of Inbreeding

Selection intensity ranged from 9.60% to 10.86% over cycles of selection (Table 3.9). After three cycles of selection, the rate of inbreeding was 5.9%, assuming an F' of 0 in C1 (Table 3.9).

Table 3.9. The population size, number of families selected per cycle, selection intensity per cycle, and the cumulative coefficient of inbreeding over cycles of recurrent selection in the population. To form each cycle, ~10% of self-pollinated (S_1) families with the highest total soluble solids at 24 days after pollination were selected. †The population size refers to the number of S_1 families that were evaluated each cycle, while >100 ears were self-pollinated each evaluation cycle, only ears with >50% pollinated kernels per ear were evaluated. ∴The number of S_1 families that were recombined to form the next cycle. ‡The cumulative inbreeding over cycles, assuming an F' of 0 in cycle 1.

Year	*Cycle*	*Population Size*†	*S_1 Families Selected*∴	*Selection Intensity (%)*	*Wright's Coefficient of Inbreeding*‡
2020	1	92	10	10.86	0.025
2021	2	125	12	9.60	0.045
2022	3	173	17	9.83	0.059

3.5 Conclusion

Direct selection on TSS did not change the TSS, total sugar, sucrose, fructose, or glucose content among cycles, nor did selection change the fresh harvest window in the population in the desired direction. The failure is likely primarily due to the low heritability for TSS, particularly the heritability of TSS at 24 DAP ($h^2 = 0$), which was the timepoint at which selections were made to form the next cycle. The realized heritability for TSS at 24 DAP was low across all cycles of selection. The realized heritability for TSS was negative for the first two cycles of selection ($h^2 = -0.24$) and positive for the third cycle of selection ($h^2 = 0.27$). Selection response on quantitatively controlled traits can be low in early cycles of selection, especially if the alleles impacting the trait are at low frequencies in the base population. It is possible that more cycles of selection are needed to see differences in population means for this trait. Variance components

revealed that variability in TSS in the populations was almost entirely due to error, not genetic differences. Furthermore, the correlation among TSS, sugar content, and eating quality traits were nonexistent or negative. Specifically, TSS at 24 DAP was moderately negatively correlated with kernel moisture and tenderness at 24 DAP, both attributes that contribute to a wider harvest window when elevated. In a study using *shrunken2* sweet corn, TSS was found to have a low proportion of variance due to additive variance and a high proportion due to error variance, similar to the results observed in this study (Solomon et al., 2012). Together, these results demonstrate that selection on TSS was not an effective method to select for a wider fresh harvest window in this population over four cycles of selection.

Indirect responses to selection did occur. Specifically, the tenderness, chalkiness, ear length, and ear width were reduced in C3 relative to C0. These changes were significantly negatively linear across cycles. All four of these changes are undesirable. The reduction in ear width and length could be attributed to inbreeding depression. A coefficient of inbreeding of 0.059 is low, but even low levels of inbreeding can have deleterious effects in maize. Burton et al. (1978) reported that variation for response to inbreeding depression is trait and genotype dependent, with significant inbreeding depression for yield occurring at inbreeding levels as low as F = 0.125, but no inbreeding depression for ear number at inbreeding levels as high as F = 0.3125 in the genotypes studied (Burton et al., 1978). Reduced ear diameter and ear length are well-established consequences of inbreeding in maize (Hallauer & Miranda, 1988).

There were few significant correlations among sugar content traits and eating quality ratings. Importantly, however, total sugar at 24 DAP was moderately positively correlated with chalkiness at 24 DAP (Figure 3.2). Chalkiness is a trait that describes the starch content in sweet x field (vegetable) corn populations, and more desirable taster ratings for this trait correlate with

total sugar content. This is evidence that selection using bite tests could be effective in reducing the undesirable chalkiness, and increasing the total sugar content, of vegetable corn populations and therefore positively impacting the fresh harvest quality. Recurrent selection using bite tests has been effective in improving eating quality in sweet corn (Shelton & Tracy, 2015). Kernel moisture was strongly positively correlated with total sugar and sucrose, which is an association well documented in the literature (Ledenčan et al., 2022; Soberalske & Andrew, 1978; Tracy, 1994). Given this association, and that quantifying moisture content is more expedient and less costly than quantifying sugar content, future work could focus on recurrent selection on kernel moisture to widen the harvest window in vegetable corn populations.

Taster was a significant source of variation for tenderness and chalkiness. Differences among taster ratings likely contributed to reduced power to detect significant differences in population means and correlations among sugar content traits and quality ratings. Differences among taster ratings can be due to a variety of factors, among them the inherent variability in the perception of taste or taster fatigue over the course of multiple weeks of tasting (Hasin-Brumshtein et al., 2009; Klee, 2010). Due to the perishability of sweet corn, providing a standard check over the course of multiple weeks is logistically difficult and a standard check variety was not available at each tasting, which could have contributed to variability among taster ratings (Carneiro et al., 2020). It is generally better to have more tasters when tasters are untrained in order to account for variability in ratings and maintain power to detect any genetic differences among samples (Dawson & Healy, 2018). The limited number of ears per plot precluded including additional tasters. Anecdotally, tasters often commented that among ears within a plot there was variation perceived for the three quality traits, which is not surprising given that ears are from variable populations. Therefore, future work should aim to taste more ears per cycle

and/or recruit more tasters. Other studies cite these same challenges, variability within the cultivar, perishability, limited samples, and inability for all cultivars to be tasted at once, in assessing quality in breeding programs (Carneiro et al., 2020; Hagenguth et al., 2022). However, the perceived variability for quality traits is an indicator that improvement could be possible for these quality traits in these populations.

3.6 Chapter Three References

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4 Chapter Four: Trial of Performance and Uniformity of Open Pollinated Sweet Corn Varieties under Organic Management

4.1 Abstract

Organic growers surveyed in Wisconsin identify open pollinated sweet corn varieties as important in their systems but in need of improvement in vigor and uniformity. Nationally, surveys of organic growers for the State of Organic Seed Report found that sweet corn breeding should target yield, flavor, disease resistance, and germination. A trial of experimental and commercially available open pollinated sweet corn varieties determined the uniformity of flowering time, tested trialing methodology for this trait, and determined the performance of varieties in organic environments in Madison, WI for a suite of traits relevant to growers and consumers. Differences in the uniformity of flowering time existed among varieties. A variety released in 2023, 'Quick Kiss', was as uniform in silk emergence as the open pollinated check variety, as was 'Who Gets Kissed', a variety released in 2014, and 'Who Gets Kissed Too', an experimental variety. The trial determined that standard methodology for evaluating mid silk date functions for open pollinated varieties as a tool to decide harvest date but does not quantify the variability inherent in open pollinated variety flowering time. The method of recording flowering on a per plant basis is the only way to assess variance and is useful for driving decision making and assessing efficacy of selection efforts. Two generations of mass selection for earlier and more uniform flowering time has been ineffective in improving the variability in flowering for Who Gets Kissed Too relative to Who Gets Kissed. Selection efficiency for this trait could be

improved by better controlling for environmental variation, using methods like stratified mass selection, or by increasing the amount of additive variance expressed among offspring by developing full-sib or selfed families. Most varieties performed as well as the hybrid and open pollinated variety checks for stand counts, husk traits, tip fill, and percent marketable ears. Many varieties outperformed the open pollinated check for number of kernel rows and row configuration. Who Gets Kissed had longer ears than the hybrid and open pollinated variety checks. Many varieties performed as well as the hybrid check for eating quality traits. But notably, experimental varieties Who Gets Kissed Too and 'Olympic Sweet', and a variety released in 2022, 'Honey Badger', had a significantly higher ratings for sweetness at both harvest dates compared to the bottom three varieties in the trial. Within harvest date, all *se1* varieties, except for Candy Mountain, performed just as well as the hybrid check for sweetness. Additionally, all varieties, except for Who Gets Kissed, performed as well as the hybrid check for holding tenderness across both harvest dates. Trialing methods for determining the uniformity of traits like flowering time and eating quality of open pollinated varieties could be improved by first quantifying the variability inherent in the variety via measuring a large sample of ears in multiple environments, information which in turn could be used to inform selection to improve the uniformity of varieties, to better serve the needs of growers.

4.2 Introduction

The commercial corn industry in the United States today is dominated by hybrid cultivars, but there are many advantages to continuing to maintain and breed open pollinated varieties (OPs or OPVs) (Duvick, 2005). Salient among these advantages are the ability for continued improvement through on-farm breeding, allowing specific adaptation at the farm or regional level or to management regimes (Masuka et al., 2017; Zystro et al., 2021). Farmers can save seed

from OPVs, which in addition to varietal improvement can also reduce input costs and reliance on the formal seed sector, providing an insurance that the variety will remain available to grow. Additionally, OPVs are genetically diverse and are a living source of genetic variation for traits relevant today or traits that might be in the future, such as resistance to emerging pests and diseases (Dhliwayo et al., 2014; Mutinda et al., 2018; Warburton et al., 2008).

Participatory plant breeding (PPB) is the process of farmer led breeding in collaboration with professional plant breeders and other stakeholders in the food system, whereby generally farmers develop the breeding goals and selection takes place on farm (Rhoades & Booth, 1982). The benefits to PPB include increased farmer adoption of new varieties due to farmer involvement from inception to finished variety, high selection efficiency especially in low input systems or environments which the formal seed sector does not serve, and the sharing of power between breeders, growers, and other constituents (Ceccarelli & Grando, 2022). While PPB has its roots in the Global South, it has gained traction in the Global North as well (Colley et al., 2021; Colley et al., 2022). Due to the decentralization of the breeding process, PPB is well suited to breeding for diverse organic environments as well as adapting varieties to the changing climate (Ceccarelli, 2015). There have been several documented PPB projects with corn, including the development of 'Who Gets Kissed', a variety used in this research (Ceccarelli & Grando, 2019; Colley et al., 2021; Shelton & Tracy, 2015; Witcombe et al., 1996).

Taken together, these advantages illustrate how OPVs can provide alternatives to hybrids for organic growers whose needs might not be met by inbred-hybrid breeding programs. A tenet in plant breeding is to breed in the environment of intended use (Falconer, 1952). While organic environments differ from their conventional counterparts in significant ways, including in fertility and pest management, farming systems whether conventional or organic span a spectrum

of practices and levels of inputs (Wolfe et al., 2008). The use of soluble fertilizers and pesticides as is typical under conventional management homogenizes the growing environment. Organic growers often rely upon the mineralization of organic matter for fertility as well as employ practices such as crop rotation, trap or companion plantings, or host plant resistance to break pest and disease cycles (Lammerts van Bueren et al., 2011). Research in cereals indicates that breeding varieties for organic systems via direct selection in organic systems often results in higher gains when grown in organic systems compared to indirect selection under conventional management (Murphy et al., 2007; Wolfe et al., 2008). On the other hand, Lorenzana & Bernardo (2008) found in field corn, traits like grain yield have higher estimates of heritability in conventional systems and therefore indirect selection in conventional environments for performance in organic environments is more efficient. Others argue that rank change genotype by environment interactions between conventional and organic environments warrant separate breeding programs or at the minimum early testing in organic environments to identify promising breeding lines (Burger et al., 2008). Revilla et al. (2015) highlighted that decisions of resource allocation can be trait specific. They found no interaction between genotype performance in organic versus conventional environments for corn grain yield or moisture, but significant interactions for many quality traits, like grain density and kernel weight (Revilla et al., 2015). Therefore, context is important. The genotypes and environments tested impact performance and calculations of heritability, and genetic correlations among environments or management systems are trait specific, so decisions about resource allocation are made within these contexts. The diversity of management practices and market needs under the organic umbrella can warrant decentralized or farmer led breeding, which can use OPVs as parental

material, taking advantage of the heterozygosity and heterogeneity inherent in maize OPVs to breed for specific adaptation.

However, a disadvantage of OPVs compared to hybrids is a lack of uniformity for traits of importance to growers or consumers. Hybrids are genetically homogenous and any variation within a cultivar is due to the environment, while OPVs can exhibit a range of variation for traits within a variety. In comparison to OPVs, hybrids can be advantageous where uniformity is required, such as for mechanical harvest where consistent ear height is needed or for markets that require stringent quality standards. Therefore, OPV breeders must strike a balance between allowing enough variation to remain for future breeding within an OPV, while also approaching a level of uniformity for traits like germination, eating quality, and flowering time.

Uneven germination can cause difficulty in weed management, particularly in organic systems that rely upon mechanical cultivation, due to differences in the timing of canopy closure or plant height. Poor germination can be caused by a variety of biotic and abiotic factors, among them pathogen attack and poor seedling vigor. Sweet corn has high sugar levels in the endosperm which negatively affect germination, yet consumers prefer sweet corn that is very sweet and tender (Viesselmann et al., 2014; Zhang et al., 2019). Tenderness is conferred by a thin pericarp, but the pericarp also functions as a defense barrier against pathogens and splitting due to osmatic pressure during development (Tracy & Galinat, 1987). Finding a balance between these two important traits, germination and eating quality, is a challenge for sweet corn breeders.

Uniform flowering time is important for growers for a variety of reasons. Principally, flowering time is directly related to eating quality. Sweet corn is harvested when sugar levels are at their peak, at about 21-23 days after pollination (DAP) depending on the variety and environment. If every plant flowers at relatively the same time, determining when to harvest is

easy. Additionally, harvesting a field all at once, instead of picking and choosing the ripest ears, is most efficient from a labor perspective. Lastly, sweet corn has a long growing season compared to many other vegetable crops and provides only one to two harvests (ears) per plant; therefore, many farming operations seek to turn over the sweet corn field space and plant another crop as quickly as possible. Therefore, uniformity in flowering time, and thus harvest time, facilitates efficiency in crop planning and marketing as well.

The need for improvement of sweet corn germination and OPV uniformity, while maintaining high eating quality, are priorities identified by organic growers. A majority of organic growers in Wisconsin surveyed in 2015 prefer sweet corn OPVs over hybrids and believe breeding organic OPVs should be a priority (Lyon et al., 2015). But these respondents also identified that relative to hybrids, OPVs have a lack of vigor and uniformity, and therefore marketability. Nationally, organic growers identified yield, quality, and field emergence as breeding priorities in sweet corn in surveys conducted for the State of Organic Seed Report (Colley et al., 2022). The Wisconsin Sweet Corn Breeding and Genetics program breeds OPVs under organic conditions for organic growers in Wisconsin. Yet there is a gap in understanding if breeding efforts to improve uniformity of traits like flowering time have been successful and if typical trialing methods are effectively quantifying the variability within a variety for traits of interest to growers and consumers.

A variety trial determined the performance of ten commercially available and experimental varieties in organic environments in Wisconsin. Specifically, the trial characterized the uniformity of flowering time within an OPV through two methods of assessing these traits compared to hybrid and OPV checks. The trial also evaluated the performance of OPVs for a suite of qualitative and quantitative traits of importance to growers and consumers, including

plant and ear height, ear length and width, number of kernel rows, row configuration, tip fill, husk appearance and protection, percentage of marketable ears, and eating quality at two harvest dates. The Wisconsin Sweet Corn Breeding and Genetics Program released in 2023 a new OPV, 'Quick Kiss,' based on the performance of this variety in this trial, and is evaluating another variety, 'Who Gets Kissed Too,' for potential release.

4.3 Materials and Methods

4.3.1 Germplasm Development

There were nine OPVs evaluated in the trial. These include five populations developed in the Wisconsin Sweet Corn Breeding and Genetics program, one developed by Organic Seed Alliance (OSA) in Chimacum, WA, and three commercially available populations. Of the five developed in Wisconsin, four were bred under organic conditions ('Quick Kiss', 'Honey Badger', 'Who Gets Kissed (WGK)', 'Who Gets Kissed Too (WGK Too)') and one was partially bred under organic conditions, ('Lindsey Meyer Blue x Howling Mob', (LMB x HM)). 'Honey Badger' is a synthetic variety bred by Jared Zystro. WGK is a product of PPB among breeders Adrienne Shelton, Bill Tracy, John Navazio, Jared Zystro, and OSA, farmer Martin Diffley, and was released in partnership with High Mowing Seed Company in 2014 (Colley et al., 2022; Shelton & Tracy, 2015). The PPB project that developed WGK also concurrently developed a second population that was five days earlier in flowering. 'Olympic Sweet', developed by OSA, was bred via PPB on three organic farms in Washington using the early population as the parental material (Colley et al., 2022). The Wisconsin breeding program also used the early population to develop Quick Kiss by selecting for lodging resistance and uniform flowering time for five generations. WGK Too was bred out of WGK, by selection and recombining of plants homozygous for the *se1* allele in the winter of 2020/2021 and selecting more uniform and earlier

flowering time in the summers of 2021 and 2022. The relatedness of Olympic Sweet and Quick Kiss and, separately, WGK and WGK Too, provide a platform for comparison of these varieties and an ability for the success of selection efforts to be evaluated.

Of the commercially available populations, 'Top Hat', was bred by independent breeder Jonathan Spero of Lupine Knoll Farm and seed was purchased from Siskiyou Seeds in 2022 and from Hudson Valley Seed Company in 2023 due to the variety being out of stock at Siskiyou Seeds (*Corn, Top Hat, Sweet*, n.d.; *Top Hat Sweet Corn*, n.d.). Adaptive Seeds sells 'Candy Mountain', which was bred in Montana (*Sweet Corn, Candy Mountain (Organic)*, n.d.). Lastly, 'Howling Mob', is a variety that has been on the market since the early 1900s and is a standard or check variety for morphological and phenological traits among the OPVs. Howling Mob would not be considered a check variety for the experimental OPVs in this trial for quality traits because it is a different endosperm type. A hybrid variety, 'Temptation' from Seminis Seeds, is often used by organic producers and was included in the trial as a check for both uniformity of traits as well as a standard for *se1* eating quality (Colley et al., 2022). Temptation, Quick Kiss, Honey Badger, Olympic Sweet, and WGK Too are homozygous *se1* (*su1su1se1se1*). While WGK is heterozygous for *se1* (*su1su1Se1se1*). Top Hat is described by Siskiyou Seeds as "mostly sugary enhanced" and Candy Mountain is described by Adaptive Seeds as "mixed sugary enhanced (SE) and normal sugary (SU) kernels" on their respective websites (*Corn, Top Hat, Sweet*, n.d.; *Sweet Corn, Candy Mountain (Organic)*, n.d.). Finally, LMB x HM and Howling Mob do not contain the recessive *se1* allele but are homozygous *su1* (*su1su1Se1Se1*).

WGK is commercially available, but breeders seed from the Wisconsin breeding program was planted in this trial, not purchased seed. Similarly, as of 2022 and 2023, respectively, Honey Badger and Quick Kiss have also been released, but breeders seed was planted in the trial. Photos

of representative ears of each of the varieties can be found in the Appendix at the end of the chapter (Appendix Figures A7-1 – A7-10).

4.3.2 Experimental Design

The trial was conducted at West Madison Agricultural Research Station (WMARS) which has a Plano silt loam (fine-silty, mixed mesic Typic Argiudoll) soil type. The trial was planted in a randomized complete block design (RCBD) in 2022 and 2023 with two organic environments per year and two replications per environment with ten entries. At WMARS in the summer of 2022, the entries were planted in 6-row plots, and in 2023 the entries were planted in 4-row plots. Plots were planted in rows 3.5 m long, with 0.76 m between rows, and an alley of 0.91 m between plots. Plots were direct seeded with twenty-five seeds per row then subsequently thinned to twelve plants per row at the V5 growth stage. The plants were allowed to open pollinate.

4.3.3 Trait Evaluation

Data collected on agronomic and morphological traits included stand counts and plant and ear height. Stand counts were taken prior to thinning at V5 stage. Plant and ear heights were taken by the method described in Chapter Three on four random plants per row, excluding the first and last plants in a row, from the middle four rows per plot in 2022 and from all four rows per plot in 2023.

Data collection on flowering differed between years. In 2022, flowering time was taken on a per plant basis to evaluate the uniformity of flowering time within a variety. Each plant in the six-row plot was tagged and the start of pollen shed (anthesis) and the emergence of silk (pistillate flowering) were recorded for each plant. Due to labor constraints in 2023, flowering

time data was recorded at five timepoints on a per plot basis: the date when the first plant per plot began shedding pollen, the date when the first plant per plot had silk emergence, the date when 50% of the plot had pollen shedding and silks emerged (hereafter referred to as the mid-silk date), the date when the last plant in a plot began shedding pollen, and the date when the last plant in a plot had silk emergence. In both years, pollen shed was recorded as beginning when at least one third of central spike of the tassel had emerged anthers. Silk emergence was recorded as beginning when silks had emerged on the upper most ear. Calendar dates were converted to accumulated growing degree days (GDD) after planting using a base of 50 degrees Fahrenheit. Weather data were collected from a weather station situated at "Verona, West Mad Ag Sta" published at www.newa.cornell.edu (New York State Integrated Pest Management and College of Agriculture and Life Sciences at Cornell University, 2023). Individual plant data from 2022 was used to calculate the corresponding five timepoints collected in 2023 to compare flowering across years.

Ten ears, excluding the first and last ear per row, were harvested from two of the four middle rows of the plot in 2022, or from two of the middle rows of the plot in 2023, at each harvest date. These ears were used to evaluate eating quality and ear and husk traits, which included husk appearance, husk protection, row configuration, tip fill, percentage of marketable ears, ear length, and ear width. The first harvest was at 20 days after the average mid silk date for the four middle rows of the plot in 2022, or at 20 days after the mid silk date of the plot in 2023, hereafter referred to as 20 days after pollination (DAP). Ten ears were harvested five days later at 25 DAP. The same evaluation was performed on both harvests. Traits were measured using the methods described in Chapter Three. Percentage of marketable ears is the ratio of ears harvested to those that were deemed marketable, which was having adequate kernel and tip fill

and being at least 6” in length. Ear length and ear width were recorded as a single score per harvest per plot that was an average length and width of the marketable ears within plot. Husk appearance, husk protection, row configuration, and tip fill were given a single score for an average evaluation of the ten harvested ears.

Lastly, on each harvest date two marketable and representative ears of the plot were evaluated for eating quality and given an average per plot rating for sweetness, tenderness, and overall liking on a 1-5 scale, where 1 was low or poor and 5 was high or excellent. Sweetness was scored from 1 (no perceptible sweetness and/or undesirable off flavors) to 5 (very sweet flavor), with 3 as acceptable sweetness. Tenderness is scored from 1 (tough, hard to bite through) to 5 (very tender and not chewy) with 3 as some initial resistance to biting perceived. Overall liking is scored from 1 (I do not want to bite this ear again and I would not purchase it) to 5 (I want to bite this ear again and I would purchase it) with 3 as acceptable.

4.3.4 Statistical Analysis

A linear model was built using the lm() command from the R stats package in R via backward model selection (R Core Team, 2021). Models were evaluated using the Akaike information criterion (AIC) and adjusted R^2 as criteria to choose the best fit model. The location was WMARS in both years of the trial and ‘environment’ represents a location-year combination. The model treated environment, replication nested in environment, variety, and harvest date (DAP) as fixed effects. The model was:

$$Y_{ijkl} = \mu + variety_i + environment_j + replication(environment)_{jk} + harvest\ date_l + (variety\ x\ environment)_{ij} + (variety\ x\ harvest\ date)_{il} + (environment\ x\ harvest\ date)_{jl} + (variety\ x\ environment\ x\ harvest\ date)_{ijl} + e_{ijkl} \quad (1)$$

Where Y_{ijkl} is the phenotypic value measured for variety *i* in environment *j* and replication *k* on harvest date *l,* μ is the grand mean, $variety_i$ is the effect of variety *i,* $environment_j$ is the effect of environment *j,* $replication(environment)_{jk}$ is the effect of replication *k* nested in environment *j,* $harvest\ date_l$ is the effect of harvest date *l,* $(variety\ x\ environment)_{ij}$ is the effect of the interaction between variety *i* and environment *j,* $(environment\ x\ harvest\ date)_{jl}$ is the effect of the interaction between environment *j* and harvest date *l,* $(variety\ x\ environment\ x\ harvest\ date)_{ijl}$ is the effect of the interaction among variety *i*, environment *j*, and harvest date *l,* and e_{ijkl} is the random error term. A separate model, without harvest date, was built for traits that were not collected over harvest dates, including flowering traits, plant and ear heights, and stand counts. The model included the same terms as model 1 but without the harvest date main effect term and without the interaction terms with harvest date. Outliers were checked using the rosnerTest() command from the EnvStats package in R (Millard, 2013). Model assumptions, including normality and equal variance of the residuals, were verified graphically. The flowering data collected on a per plant basis in 2022 had unequal variances among varieties. A generalized least square model using the command gls() from R package nlme was fit that accommodates unequal variances and a Dunnett T3 correction used for pairwise comparisons which accommodates unequal variances and sample sizes (Pinheiro & Bates, 2023). Analysis of variance (ANOVA) for all traits was conducted on plot means, with F-tests on the mean squares used to determine significant sources of variation. In instances of (variety x environment) interactions, spearman's rank correlations were calculated to determine if the interaction was due to a change in magnitude or a change in rank among varieties across environments. Post hoc pairwise tests were conducted using the emmeans() command from the Emmeans package in R with p-values adjusted using Tukey's Honest Significant Difference and

the confidence intervals adjusted by the Dunn-Sidak correction for multiple comparisons with an alpha level of significance of 0.05 (Lenth, 2022).

4.4 Results and Discussion

4.4.1 Analysis of Variance

Variety was a significant source of variation for all traits in ANOVA (Table 4.1). While Environment was a significant source of variation for all traits except for sweetness, tip fill, row configuration, first pollen shed, first silk emergence, last pollen shed and mid silk (Table 4.1). A variety x environment interaction was significant for all traits except for ear length, ear width, row configuration, husk protection, ear height, and all five flowering traits (Table 4.1). Harvest Date was a significant source of variation for eating quality traits -- sweetness, tenderness, and overall liking -- as well as percentage of marketable ears and ear width (Table 4.1). A variety x harvest date interaction was not a significant source of variation for any trait, but an environment x harvest date interaction was a significant source of variation for sweetness and overall liking (Table 4.1). Lastly, a three-way interaction among variety, environment, and harvest date was a significant source of variation for overall liking, row count, tip fill, and husk appearance (Table 4.1).

Spearman correlations generally had a high and significant coefficient for most traits, except for percent marketable ears and husk appearance (Appendix Table A8-1). Percent marketable ears had a high correlation among environments in 2022 (rho = 0.93, p-value $\leq$ 0.001) but a low correlation among all other combinations of environments. Similarly, husk appearance had a high correlation among environments within years (rho = 0.80, p-value $\leq$ 0.01 in 2022; rho = 0.81, p-value $\leq$ 0.01 in 2023) but a low correlation among all other combinations of environments. Given that the varieties, except for Temptation, are OPVs, some of the differential

performance across environments could be due to variability within variety. For these reasons and for the sake of clarity, results will be presented averaged over environments except for percent marketable ears and husk appearance, which will be averaged over environments within years.

	Source of Variation							
Trait	Variety	Environment	(Variety x Environment)	Replication(Environment)	Harvest Date	(Variety x Harvest Date)	(Environment x Harvest Date)	(Variety x Harvest Date x Environment)
Overall Liking	***	***	***	ns	***	ns	***	*
Sweetness	***	ns	**	ns	***	ns	**	ns
Tenderness	***	***	*	ns	***	ns	ns	ns
Percent Marketable Ears	***	***	***	ns	*	ns	ns	ns
Row Count	***	***	*	ns	ns	ns	ns	*
Ear Length	***	**	ns	*	ns	ns	ns	ns
Ear Width	***	***	ns	ns	***	ns	ns	ns
Tip Fill	***	ns	***	ns	ns	ns	ns	*
Row Configuration	***	ns	ns	**	ns	ns	ns	ns
Husk Appearance	***	**	**	ns	ns	ns	ns	*
Husk Protection	***	**	ns	ns	ns	ns	ns	ns
Ear Height	***	***	ns	ns	NA	NA	NA	NA
Plant Height	***	***	***	ns	NA	NA	NA	NA
Stand (percent per plot)	***	***	***	ns	NA	NA	NA	NA
First Pollen Shed	***	ns	ns	ns	NA	NA	NA	NA
First Silk Emergence	***	ns	ns	ns	NA	NA	NA	NA
Last Pollen Shed	***	ns	ns	ns	NA	NA	NA	NA
Last Silk Emergence	***	**	ns	ns	NA	NA	NA	NA
Mid Silk	***	ns	ns	ns	NA	NA	NA	NA

Table 4.1. Significance of F tests from analysis of variance for traits of ten varieties from a trial conducted at West Madison Agricultural Research Station in four organic environments with two replications per environment. *,**,*** correspond to significant at .05, .01, and .001 probability levels, respectively. ns = not significant, NA = not applicable.

4.4.2 Flowering Traits

Measured in all four environments, Candy Mountain, Olympic Sweet, LMB x HM, Quick Kiss and Honey Badger were among the earliest varieties to begin flowering, for both pollen shed and silk emergence (Appendix Table A8-2). WGK, WGK Too, and Top Hat were among the latest varieties for both first pollen shed and silk emergence. In pairwise comparisons for mid-silk date, Candy Mountain was not earlier than Temptation, Quick Kiss, or Olympic Sweet, but was earlier than both the *su1* varieties, as well as Top Hat, Honey Badger, WGK, and WGK Too (Appendix Table A8-2). WGK, WGK Too, and Top Hat were the latest for mid silk date, but Top Hat was not significantly later than Howling Mob. Similar trends existed for last plant per plot to begin pollen shed and silk emergence, though there were fewer significant differences among varieties for these traits. The beginning of pollen shed is highly positively correlated with first silk emergence (Figure 4.1). The mid silk date is also highly positively correlated with both traits (Figure 4.1). Similarly, there was a strong positive correlation between the last plant to start shedding pollen and the last plant with silk emergence in a plot, as this often occurs on the same plant (Figure 4.1).

Figure 4.1. Pearson correlation coefficients among agronomic, morphological, and phenological traits from ten varieties of sweet corn grown at West Madison Agricultural Research Station in four organic environments with two replications per environment in 2022 and 2023. *,**,*** correspond to significant at .05, .01, and .001 probability levels, respectively. NS = not significant. Cells are colored white for correlation coefficients of 0, colored blue for positive correlation coefficients, with increasing saturation as coefficient approaches 1, and colored red for negative correlation coefficients, with increasing saturation as coefficient approaches -1.

The Wisconsin breeding program was interested in comparing the two methodologies of recording flowering data to determine if the less laborious method employed in 2023 yielded similar results to the method employed in 2022. The typical method used for evaluating flowering time is the method used in 2023: a visual inspection of the plot where a mid-silk date is called without necessarily counting the number of flowering plants to ensure exactly half are flowering. Harvest dates are then planned based on the mid silk date. Given the variability in

flowering among plants within an OPV, it is useful to compare the two methods to determine which is best to adopt for OPVs moving forward. While error and environmental effects across years confound direct comparison, the methods yielded very similar results (Table 4.2). The largest difference between the mean GDD of a variety between the two years and methods was 44 GDD and the smallest difference was 5 GDD (Table 4.2). The average GDD accumulation per day at WMARS in the months of May-August in 2022 and 2023 is 19 to 20 GDD (New York State Integrated Pest Management and College of Agriculture and Life Sciences at Cornell University, 2023). Therefore, the methods differed at most by about two calendar days. These data suggest that visually calling a mid-silk date is a sufficient method for OPVs that yields similar results to recording data on a per plant basis. While the mid-silk date is useful for harvest planning and the beginning and ending of flowering on a per plot basis collected in 2023 provides information about the range flowering time, just this information alone, does not, however, provide an understanding of the variability within an OPV for flowering time.

Table 4.2. Mean and standard error of accumulated growing degree days (GDD) on the day that 50% of plants within a plot were both shedding pollen and had silk emergence (mid-silk date) for varieties grown at West Madison Agricultural Research Station in two organic environments in 2022 and two organic environments in 2023 with two replications per environment. There were four plots evaluated in each year. The difference in GDD means between years (= mean in 2022 – mean in 2023) are displayed.

Growing Degree Day Accumulation at the Mid-Silk Date in Environments grown in 2022 and 2023					
Identity	*2022 Mean (GDD)*	*2022 Standard Error (GDD)*	*2023 Mean (GDD)*	*2023 Standard Error (GDD)*	*Difference in Year Means (GDD)*
Candy Mountain	993	4.75	1004	7.74	-11
Quick Kiss	1012	10.53	1017	17.95	-5
Olympic Sweet	10145	15.32	1030	12.37	-15
Temptation	1019	8.12	1006	12.44	13
LMB x HM	1019	8.12	1063	4.09	-44
Honey Badger	1058	5.05	1039	6.84	19
Howling Mob	1076	8.08	1101	4.56	-25
Top Hat	1112	8.11	1091	5.35	21

WGK	1123	3.75	1147	0.58	-24
WGK Too	1135	11.02	1118	21.98	17

A tight flowering window is important to growers. Large variance in flowering time complicates decision making at harvest time, as one plant might be at peak eating quality while another is immature. To calculate variance, the method used in 2022 is needed. Temptation has the narrowest interquartile range (IQR) for both traits, pollen shed and silk emergence, compared to all the OPVs (Figure 4.2 and 4.3). For silk emergence, WGK had the same IQR as the OPV standard, Howling Mob, which had the second smallest IQR after Temptation (Figure 4.2). WGK Too had a very similar IQR, 56 growing degree days (GDD), as Howling Mob and WGK, which were 54 GDD. Candy Mountain had the largest IQR for silk emergence, 95 GDD, and Honey Badger had the second largest, 83 GDD (Figure 4.2).

For beginning pollen shed, after Temptation, Quick Kiss, Olympic Sweet, and WGK had the same and second smallest IQRs, which were 16 GDD shorter than Howling Mobs' IQR (Figure 4.3). The only variety with a wider IQR than Howling Mob for pollen shed was Honey Badger. Having a large IQR for silk emergence is not desirable, because the timing of pollination of the silks determines the timing of optimal eating quality. Diverse timing will result in variable quality if the ears are harvested on the same day. Therefore, greater uniformity for this trait is important for growers. On the other hand, having a large IQR for anthesis is perhaps desirable, resulting in an increased period of pollen shed. It is noteworthy that the OPV standard, Howling Mob had one of the smallest IQRs for silk emergence but one of the largest IQRs for pollen shed.

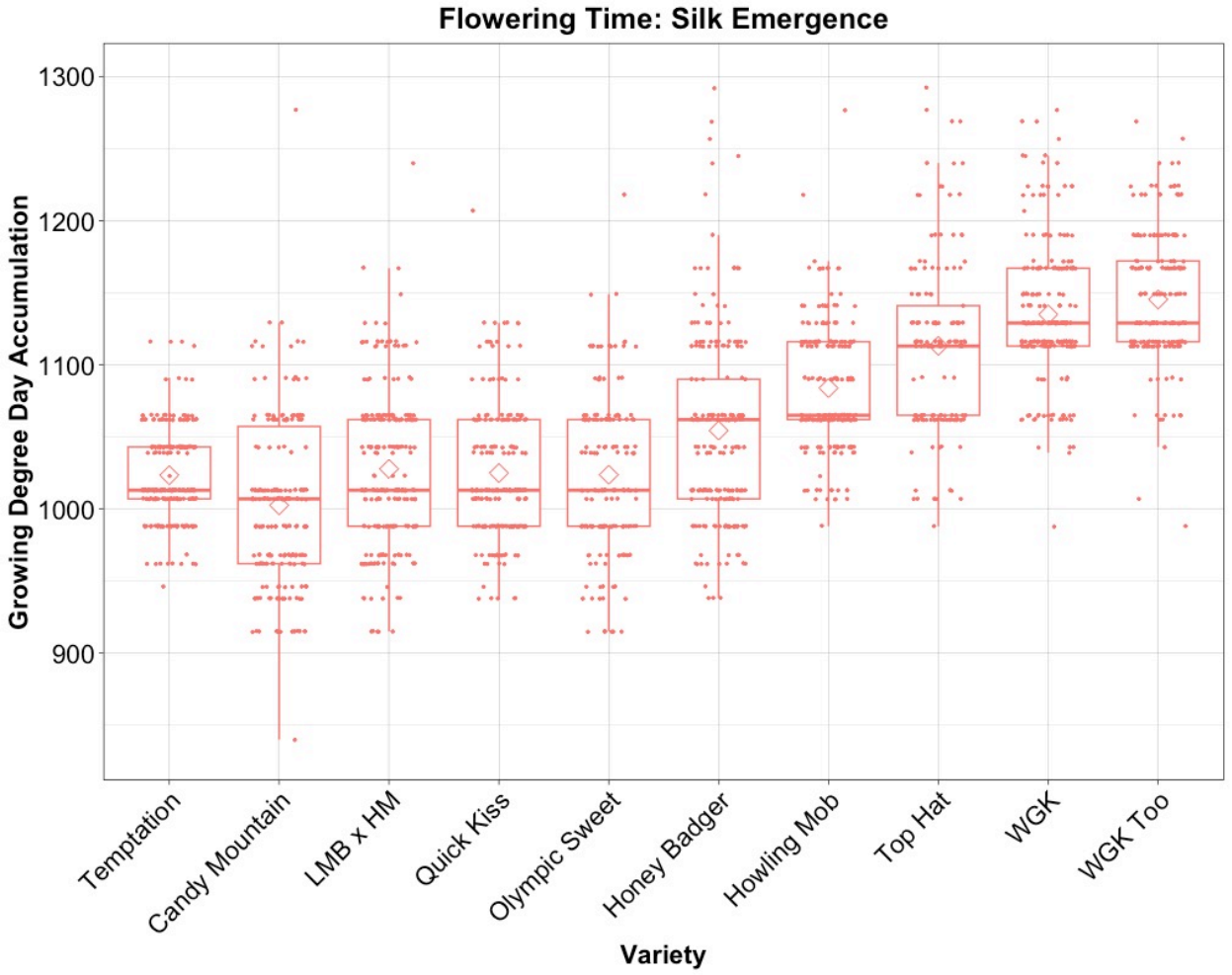


Figure 4.2. Boxplots of accumulated growing degree days (GDD) on the day of silk emergence for varieties grown at West Madison Agricultural Research Station in two organic environments with two replications per environment in 2022. Points represent individual plants measured.

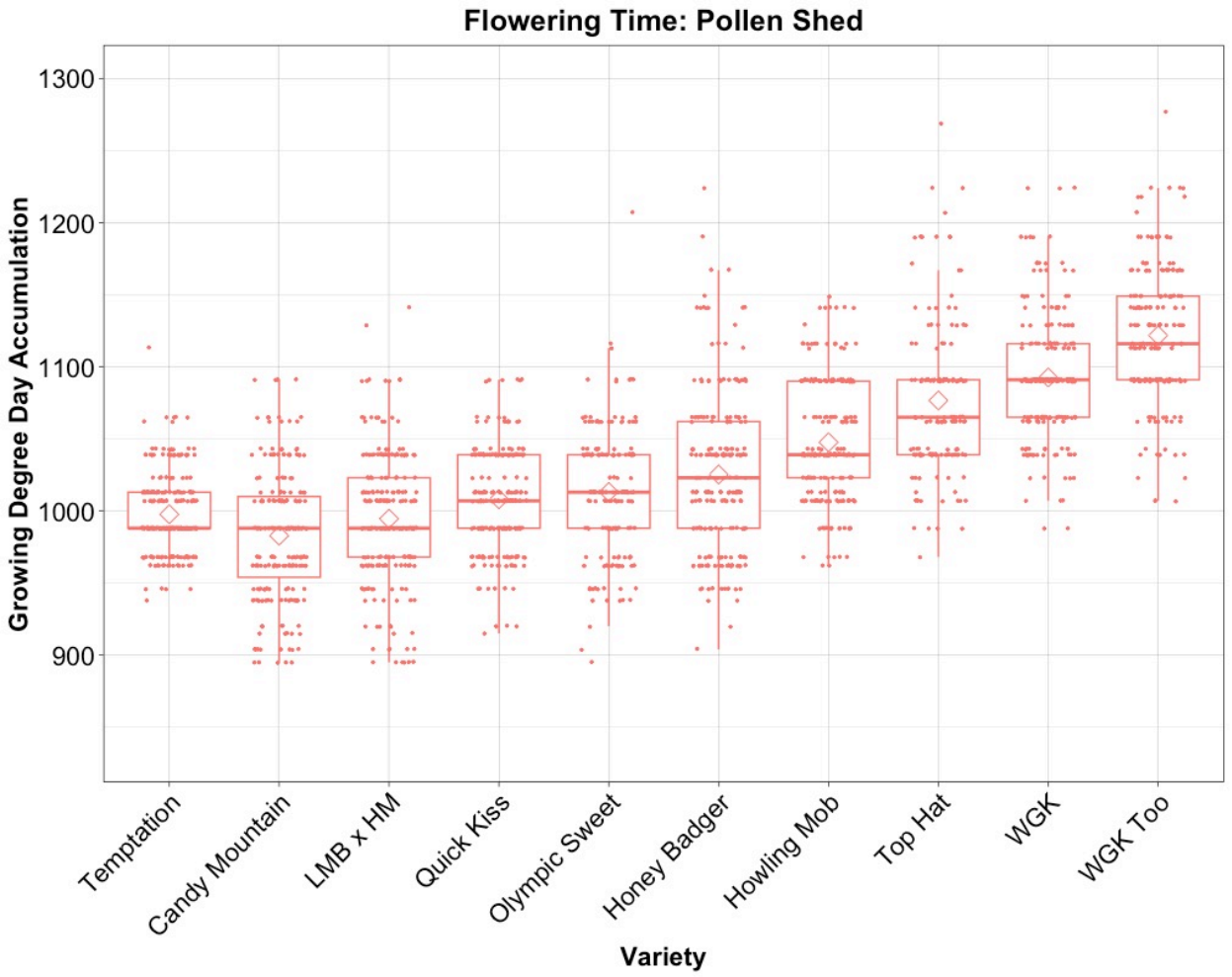
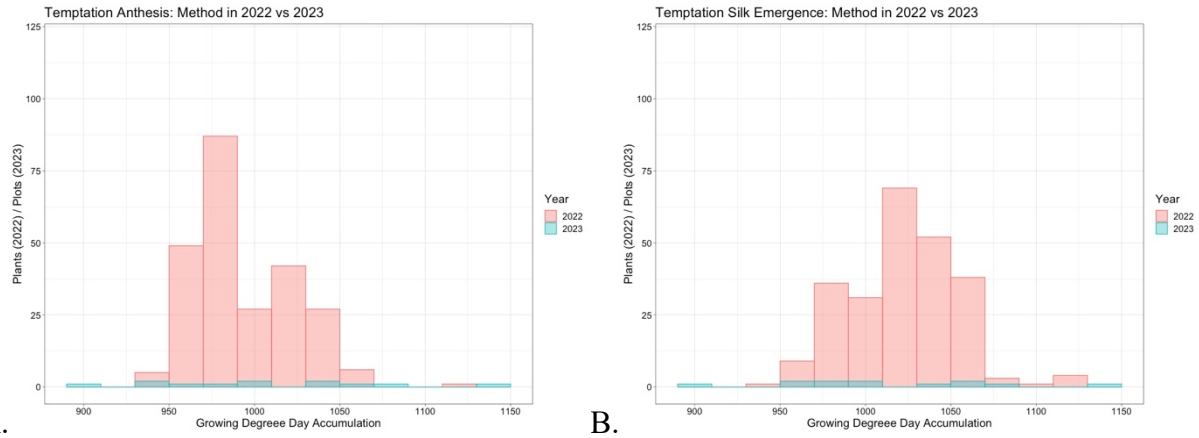


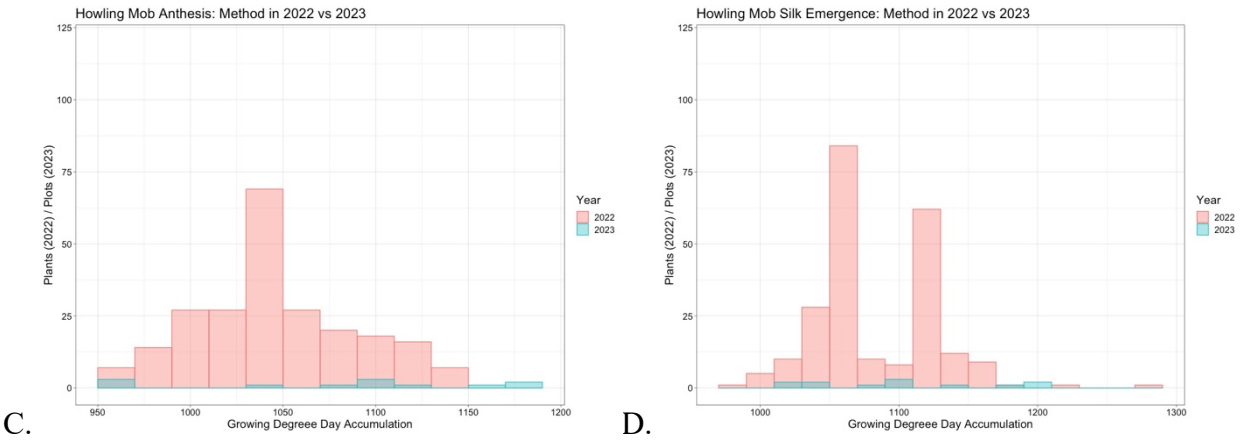
Figure 4.3. Boxplots of accumulated growing degree days (GDD) on the day of first pollen shed (anthesis) for varieties grown at West Madison Agricultural Research Station in two organic environments with two replications per environment in 2022. Points represent individual plants measured.

While IQR describes the spread of the middle 50% of the data, the trial sought to characterize the full range of variability for flowering time within varieties, as the tails of the distribution impact the eating quality of the harvest as well. Variance for silk emergence ranged from 945 to 4106 GDD and for pollen shed 722 to 2997 GDD among varieties in the trial (Appendix Tables A8-3 and A8-4). Unsurprisingly, Temptation, the commercial hybrid, had the lowest variance for both first pollen shed and silk emergence. Given that plants within a single cross hybrid are genetically identical, any variance in flowering time is due to the environment.

Quick Kiss and Howling Mob were second and third least variable for first pollen shed and silk emergence. Quick Kiss had lower variability than the OPV check for pollen shed but higher variability for silk emergence. WGK also had a similar variance to the OPV check for silk emergence. While Howling Mob had one of the largest IQRs for pollen shed it also had one of the lowest variances in the trial for this trait, indicating that this variety had a larger spread in the middle 50% of the distribution compared to other varieties but does not have as many extreme values compared to other varieties. On the other hand, Howling Mob ranked second least variable behind Temptation for silk emergence, as well as had the second smallest IQR, with most plants flowering within two intervals of 20 GDD (Figure 4.4). Howling Mob therefore represents what could be considered an ideotype for flowering time for OPVs. While the other check variety, Temptation, has the lowest IQR and variance, or a very tight distribution overall (Figure 4.4). Additionally, while collection of flowering on a per plant basis provides information about the variability of flowering within variety, method used in 2023 only captures the range of flowering time, providing information about the extremes, but cannot provide information about variability (Figure 4.4).



A. B.



C. D.

Figure 4.4. Histograms of anthesis (A and C) and silk emergence (B and D) for 'Temptation' (A and B) and 'Howling Mob' (C and D) grown in two organic environments, in 2022 and 2023, respectively, at West Madison Agricultural Research Station with two replications per environment. Red bars are data recorded on a per plant basis in 2022 while blue bars are data recorded on a plot basis in 2023. Data in 2023 reflects the first and last plant to begin anthesis and silk emergence per plot, respectively, and the mid flowering date. The y axis represents the number of plants for data in 2022 and the number of plots in 2023. Histogram bin widths are 20 Growing Degree Days (GDD).

Importantly, WGK Too, in addition to WGK and Quick Kiss, had levels of variation that did not differ from Howling Mob in pairwise Levene's Tests for equal variances of silk emergence (Table 4.3). All the other OPVs in the trial were more variable than Howling Mob for silk emergence, and none of the OPVs were as uniform as Temptation for the two flowering traits. WGK and WGK Too did not differ from one another for silk emergence variability, despite WGK Too undergoing two generations of mass selection for a tighter, more uniform flowering window. Olympic Sweet and Quick Kiss did differ in silk emergence variability, which could be due to the selection in Quick Kiss for more uniform flowering, or due to selection in Olympic Sweet that directly or indirectly impacted the flowering uniformity, or to other factors such as drift.

For pollen shed variability, Quick Kiss, WGK, LMB x HM, Olympic Sweet, and Candy Mountain were not different from Howling Mob (Appendix Table A8-5). WGK Too was more variable than Howling Mob and WGK for pollen shed variability, again, despite selection efforts

otherwise. WGK and Quick Kiss did not differ, and both were less variable than Olympic Sweet and Candy Mountain for this trait.

Top Hat and Honey Badger were ninth and tenth, respectively, as the most variable for both flowering traits (Appendix Tables A8-3 and A8-4). Top Hat and Olympic Sweet were the poorest varieties for stand counts averaged over all environments (Appendix Table A8-6). Top Hat had the fewest plants evaluated for flowering traits in 2022 environments among the ten varieties in the trial and thus the largest standard error (Appendix Tables A8-3 and A8-4). While the differences in sample size and unequal variance were accounted for in the model built for pairwise comparisons among flowering trait means, it is important to note that sample size is in the denominator of the equation to calculate variance. Thus, the comparisons made between variances should be considered jointly with the sample size. Most importantly, though, a grower is impacted by variability in flowering time regardless of, or more precisely in combination with, the stand, so considering these traits in conjunction with one another is important when recommending varieties for Wisconsin growers.

Table 4.3. Pairwise Levene's Tests for Equality of Variances with a null hypothesis of equal variance among varieties for silk emergence collected on a per plant basis grown at West Madison Agricultural Research Station in two organic environments in 2022 with two replications per environment. *,**,*** correspond to significant at .05, .01, and .001 probability levels, respectively.

Pairwise Levene's Test for Equality of Variances in Silk Emergence		
Variety Pair	*F-Value*	*P-Value*
Howling Mob – Temptation	15.195	<0.001 ***
Quick Kiss – Temptation	16.938	<0.001 ***
WGK Too – Temptation	20.385	<0.001 ***
WGK – Temptation	14.011	<0.001 ***
LMB x HM – Temptation	49.211	<0.001 ***
Olympic Sweet – Temptation	56.838	<0.001 ***
Candy Mountain – Temptation	62.180	<0.001 ***
Top Hat – Temptation	49.845	<0.001 ***
Honey Badger – Temptation	69.621	<0.001 ***
Quick Kiss – Howling Mob	0.0219	0.8825
WGK Too – Howling Mob	0.3832	0.5362

WGK – Howling Mob	0.0022	0.9623
LMB x HM – Howling Mob	8.1208	0.00457 **
Olympic Sweet – Howling Mob	10.728	0.001149 **
Candy Mountain – Howling Mob	13.212	<0.001 ***
Top Hat – Howling Mob	10.783	0.00112 **
Honey Badger – Howling Mob	20.243	<0.001 ***
WGK Too – Quick Kiss	0.2304	0.6315
WGK – Quick Kiss	0.0369	0.8478
WGK Too – WGK	0.4232	0.5157
Olympic Sweet – Quick Kiss	9.976	0.001712 **

4.4.3 Agronomic & Morphological Traits

Percent stand within plot ranged from 48% to 89% (Appendix Table A8-6). There were differences in weather between the two years of the trial that directly impacted water availability at planting. May of 2023 was the fourth driest May on record, since records began 1895, in Wisconsin (Vavrus, 2023). The two environments planted in May of 2023 had to be irrigated after planting due to poor emergence, which is highly unusual in the history of the Wisconsin Sweet Corn Breeding Program. Environments planted in 2022 were not irrigated after planting. The seed planted in both years of the trial of Quick Kiss, WGK Too, Honey Badger, and WGK were grown in the same seed environment and treated the same in seed post-harvest processing. There were no significant differences among these varieties for stand. The seed from the other varieties in the trial came from different seed environments and therefore direct comparisons for this trait are not possible. Plots were thinned after stand counts to a uniform density and most of the other traits in the trial are relatively unaffected by low population density.

Howling Mob had the tallest plants and ears, averaging 172cm tall with ears at 66cm (Figure 4.5). WGK, WGK Too, LMB x HM, and Temptation were varieties with the second most tall plants and ears. Olympic Sweet and Quick Kiss had the shortest ear height and Olympic Sweet had the shortest plant height. Olympic Sweet averaged 121cm tall with ears at 38cm and

Quick Kiss at 131cm tall with ears at 42cm. Quick Kiss was not significantly shorter in plant and ear height from Top Hat, Honey Badger, and Candy Mountain. Plant and ear height were moderately positively correlated with first pollen shed and first silk emergence (Figure 4.6). The shortest and tallest varieties were among those that were earlier and later flowering, respectively. The selection for earlier and more uniform flowering in WGK Too has not changed the plant and ear height relative to WGK. Similarly, the selection of Olympic Sweet and Quick Kiss has not significantly changed the ear height relative to each other, but Olympic Sweet did have significantly shorter plants in these environments.

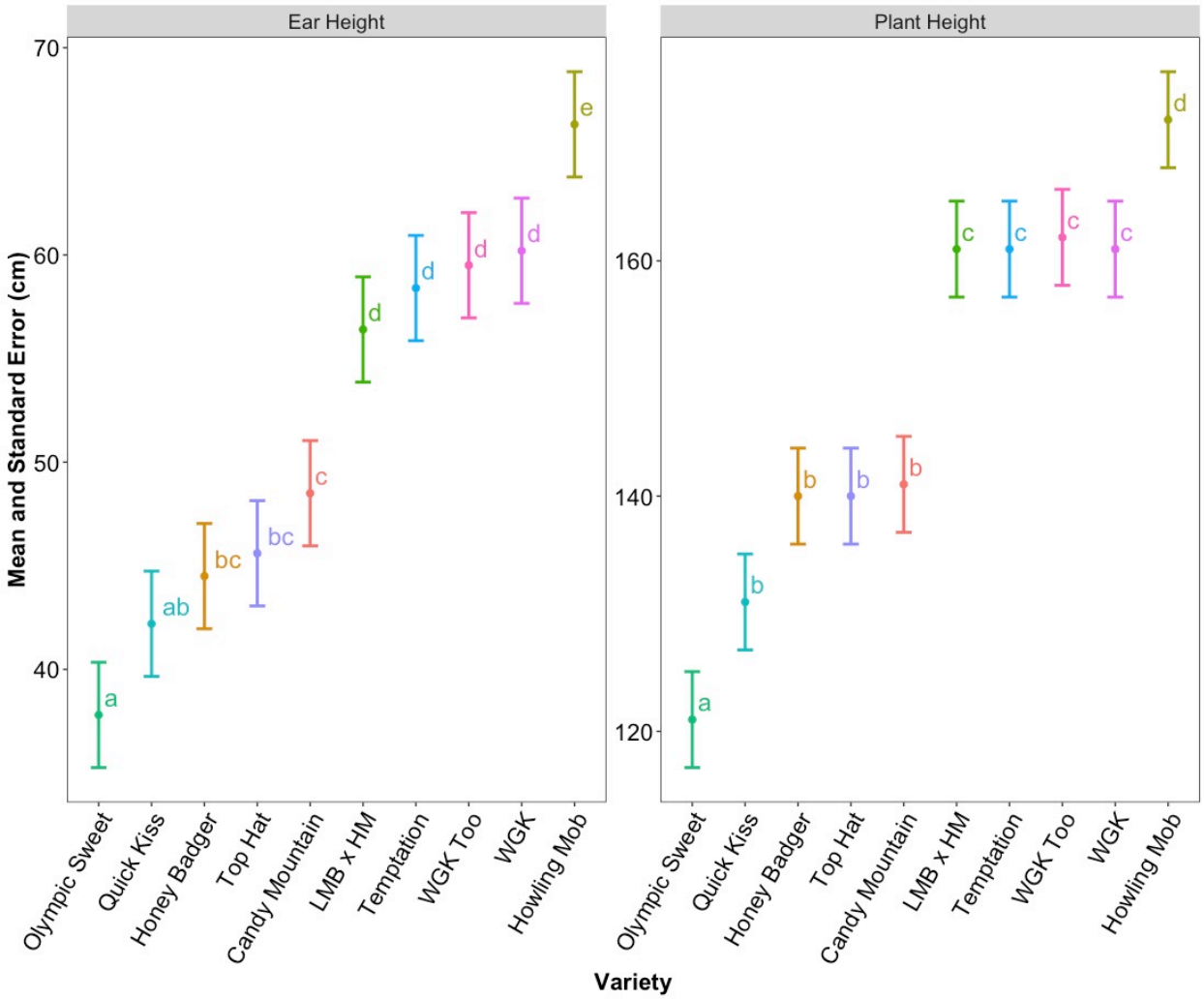


Figure 4.5. Mean and standard error of plant (right panel) and ear (left panel) heights for ten sweet corn varieties grown at West Madison Agricultural Research Station in four organic environments with two replications per environment in 2022 and 2023. Means (within trait) that share the same letter are not significantly different by a Tukey's Honest Significant Difference test at a significance level of 0.05.

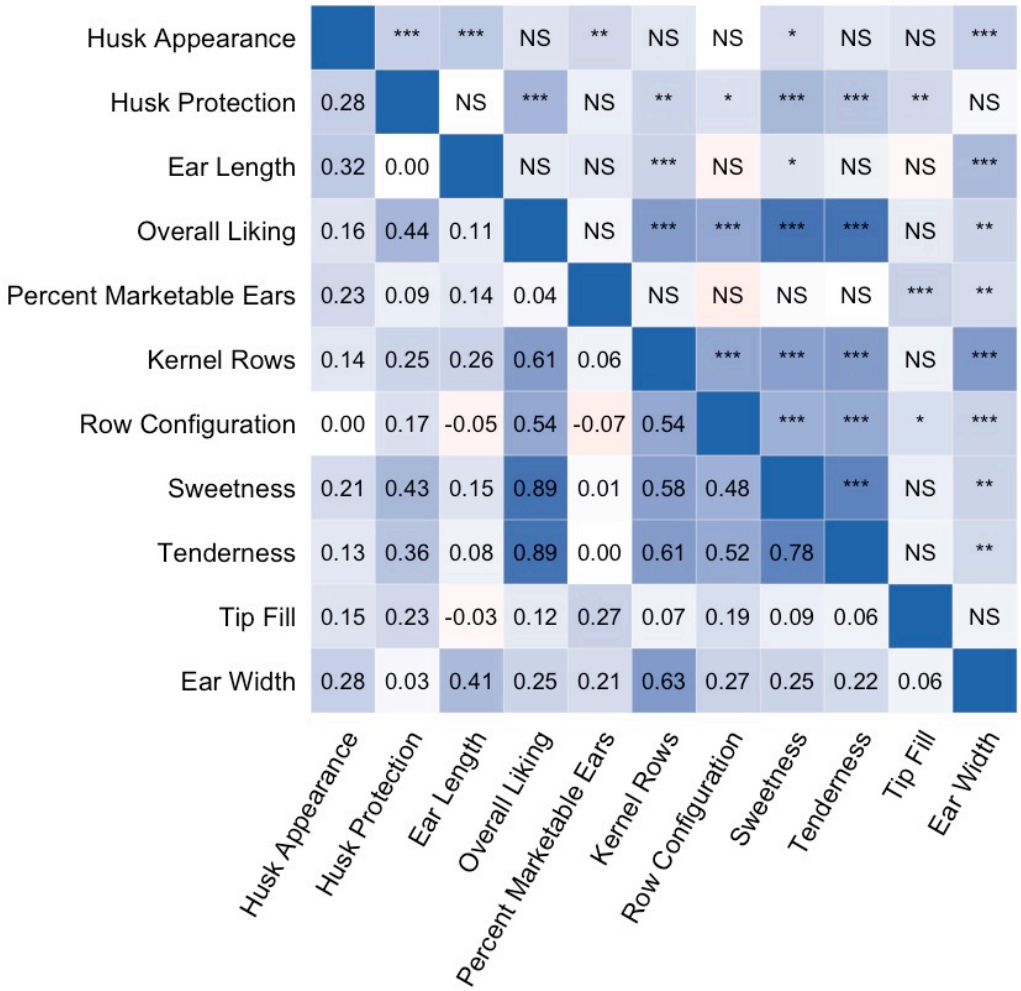


Figure 4.6. Pearson correlation coefficients among eating quality and ear and husk morphological traits from ten varieties of sweet corn grown at West Madison Agricultural Research Station in four organic environments with two replications per environment in 2022 and 2023. *,**,*** correspond to significant at .05, .01, and .001 probability levels, respectively. NS = not significant. Cells are colored white for correlation coefficients of 0, colored blue for positive correlation coefficients, with increasing saturation as coefficient approaches 1, and colored red for negative correlation coefficients, with increasing saturation as coefficient approaches -1.

Ear length and width are important traits for consumers, who often purchase sweet corn at a price per ear, regardless of the size. WGK had among the longest and widest ears in the trial, with ears measuring 20.1 cm long and 4.93 cm wide on average (Appendix Tables A8-7 and A8-

8). WGK had significantly longer ears than the commercial hybrid, Temptation, with 1.4 cm longer ears on average, but not significantly wider ears than Temptation. Honey Badger, WGK Too, and Howling Mob were not different from WGK for mean ear length. Additionally, WGK was not significantly wider than Olympic Sweet, Honey Badger, Quick Kiss, or Temptation, but was wider than WGK Too, LMB x HM, Candy Mountain, Top Hat and Howling Mob. LMB x HM ears averaged 4.22 cm in width, which was not different from Candy Mountain.

Tip Fill is a trait that holds both aesthetic value as well as contributes to kernel yield, in terms of the number of full, plump kernels on an ear. Top Hat along with Temptation were the varieties with the best tip fill, though Top Hat was not significantly different from LMB x HM (Appendix Table A8-9). Candy Mountain and Quick Kiss had the poorest tip fill (Appendix Table A8-9). Number of kernel rows is also a component of kernel yield, and more kernel rows are desirable. All the OPVs, except for LMB x HM, outperformed the OPV check, Howling Mob, for number of kernel rows (Appendix Table A8-10). While Howling Mob had eleven kernel rows on average, most OPVs had more than thirteen. Like ear width, a positively correlated trait, WGK had the highest mean number of kernel rows, with 15.38 on average, but was not different from Temptation, Honey Badger, or WGK Too (Appendix Table A8-10; Figure 4.6). LMB x HM had the fewest kernel rows with 9 on average. Interestingly, number of kernel rows was moderately positively correlated with overall liking, which is likely driven by the *su1* varieties having lower numbers of kernel rows while also having lower eating quality overall (Figure 4.6).

Row configuration is a rating of the appearance and straightness of the kernel rows. The *su1* varieties, Howling Mob and LMB x HM, both exhibited an undesirable trait of gaps between the kernel rows and therefore scored the lowest in the trial for row configuration (Appendix

Figures A7-5 and A7-10; Appendix Table A8-11). All the other OPVs, except Honey Badger and Candy Mountain, were no different than hybrid check for this trait. Row configuration was also moderately positively correlated with overall liking, likely for the same reason as number of kernel rows (Figure 4.6).

There were few significant differences for husk appearance. Candy Mountain had significantly poorer husk appearance than Olympic Sweet, Howling Mob, WGK, and WGK Too in 2022 (Appendix Table A8-12). While in 2023, Candy Mountain was the poorest in the trial for this trait (Appendix Table A8-12). Many of the top performing varieties for this trait were not significantly different from one another. Overall, most varieties performed on par with both the hybrid (Temptation) and OPV (Howling Mob) checks for this trait (Appendix Table A8-12).

Husk protection might be an important trait in organic environments where there are few options for effectively managing insect predation (Moore & Tracy, 2019). Honey Badger, WGK, Top Hat, and WGK Too performed just as well as the hybrid check for husk protection (Appendix Table A8-13). Interestingly, the OPV check Howling Mob was rated among the poorest for this trait, but only four varieties had better husk protection (Appendix Table A8-13). The Wisconsin breeding program actively selects for good husk protection. Husk protection was moderately positively correlated with overall liking and sweetness, again likely due to *su1* varieties scoring lower for husk protection and overall liking (Figure 4.6).

Percent marketable ears ranged from 59% to 99% in 2022 and 88% to 96% in 2023 (Appendix Table A8-14). There were no significant differences among varieties in pairwise comparisons for this trait in 2023. In 2022, Temptation had among the highest percentage of marketable ears along with WGK, WGK Too, Howling Mob, LMB x HM, and Top Hat (Appendix Table A8-14).

4.4.4 Eating Quality Traits

Averaged over all varieties in the trial, the sweetness rating at the early harvest date (20 DAP) was 3.3, which was significantly higher than the average sweetness rating at the late harvest date (25 DAP), 2.7. The same was true for tenderness, where the average tenderness rating at the early harvest date was 3.5, which was significantly higher than the rating at the late harvest date of 2.8. There was a similar trend for overall liking, though the means were not significantly different between the early (3.2) and late (2.6) harvest dates.

Averaged over harvest dates, all the *se1* varieties performed better than Candy Mountain and the *su1* varieties for overall liking (Appendix Table A8-15). Except for these three varieties, the OPVs were just as liked overall as Temptation. The same trend existed for sweetness and for tenderness averaged over harvest dates (Appendix Tables A8-16 and A8-17).

The ability for a variety to maintain high quality over a range of harvest dates, or to have a wide harvest window, is very desirable. Sweet corn quality is notoriously ephemeral, yet modern breeding has made significant advances in isolating and combining endosperm mutations to improve quality, storability, and harvest windows. Research has established that *su1* varieties have lower eating quality by modern standards and this trial confirmed that. The least liked varieties overall were the *su1* varieties, Howling Mob and LMB x HM, along with Candy Mountain (Figure 4.7). These three varieties were also rated the lowest at both harvest dates, 20 and 25 DAP, with ratings spanning from 1 to 2 out of 5 on average. Except for Candy Mountain at 20 DAP was not significantly different from WGK at 25 DAP (Figure 4.7). All of the other varieties performed just as well as the hybrid check for overall liking at both harvest dates (Figure 4.7).

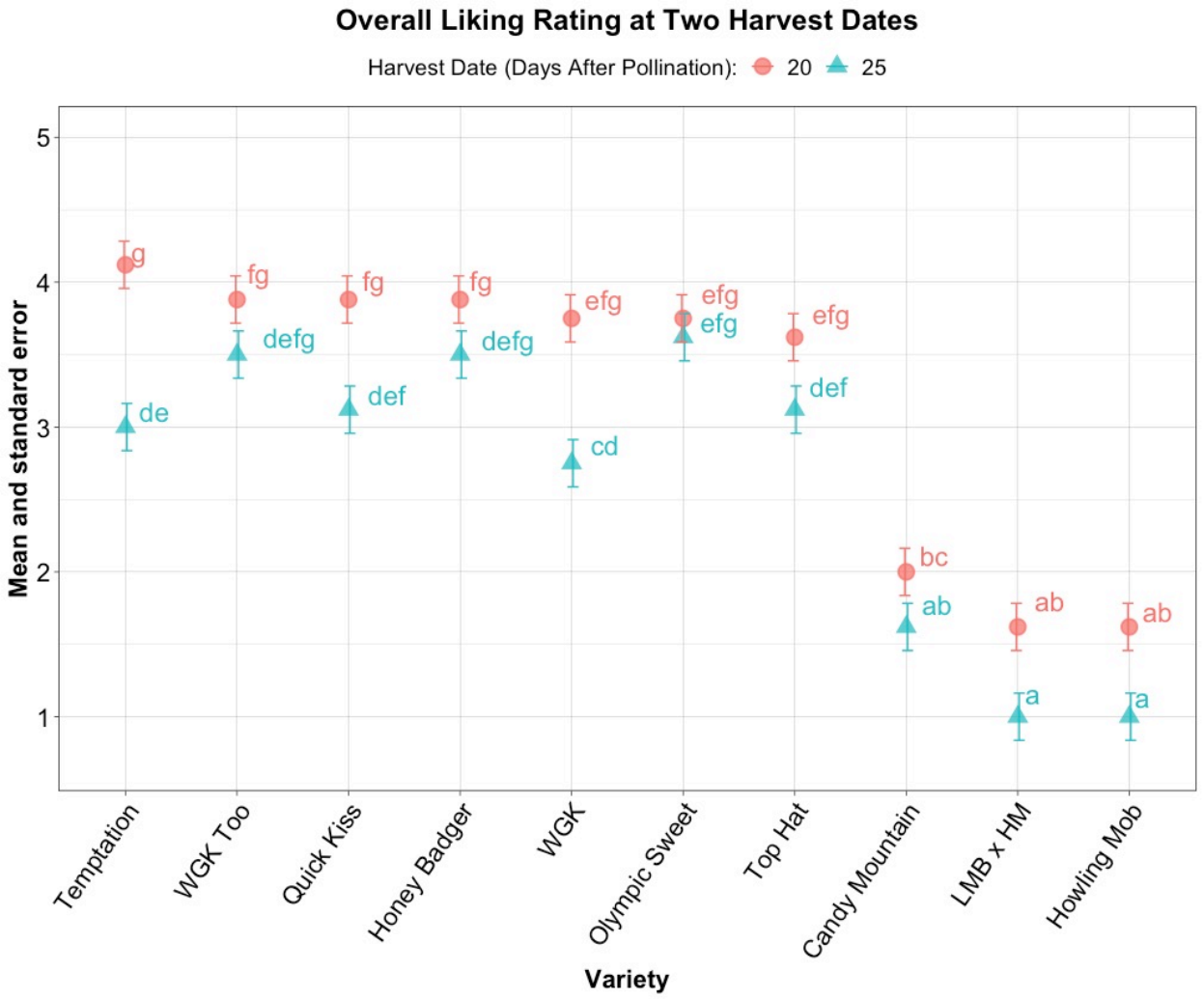


Figure 4.7. Mean and standard error for overall liking rating (1-5) over two harvest dates, 20 and 25 days after pollination, for ten varieties of sweet corn grown at West Madison Agricultural Research Station in four organic environments with two replications per environment in 2022 and 2023. Means that share the same letter are not significantly different by a Tukey's Honest Significant Difference test at a significance level of 0.05.

The three varieties rated the lowest for overall liking were also rated the among the lowest for sweetness at both harvest dates (Figure 4.8). Overall liking was strongly positively correlated with sweetness (Figure 4.6). Most notably, Olympic Sweet, WGK Too, and Honey Badger were the only three varieties that had ratings significantly higher than the bottom three varieties at *both* harvest dates. Within variety, there were no significant differences in rating

between the two harvest dates. This stability in sweetness across harvest dates is a very desirable trait.

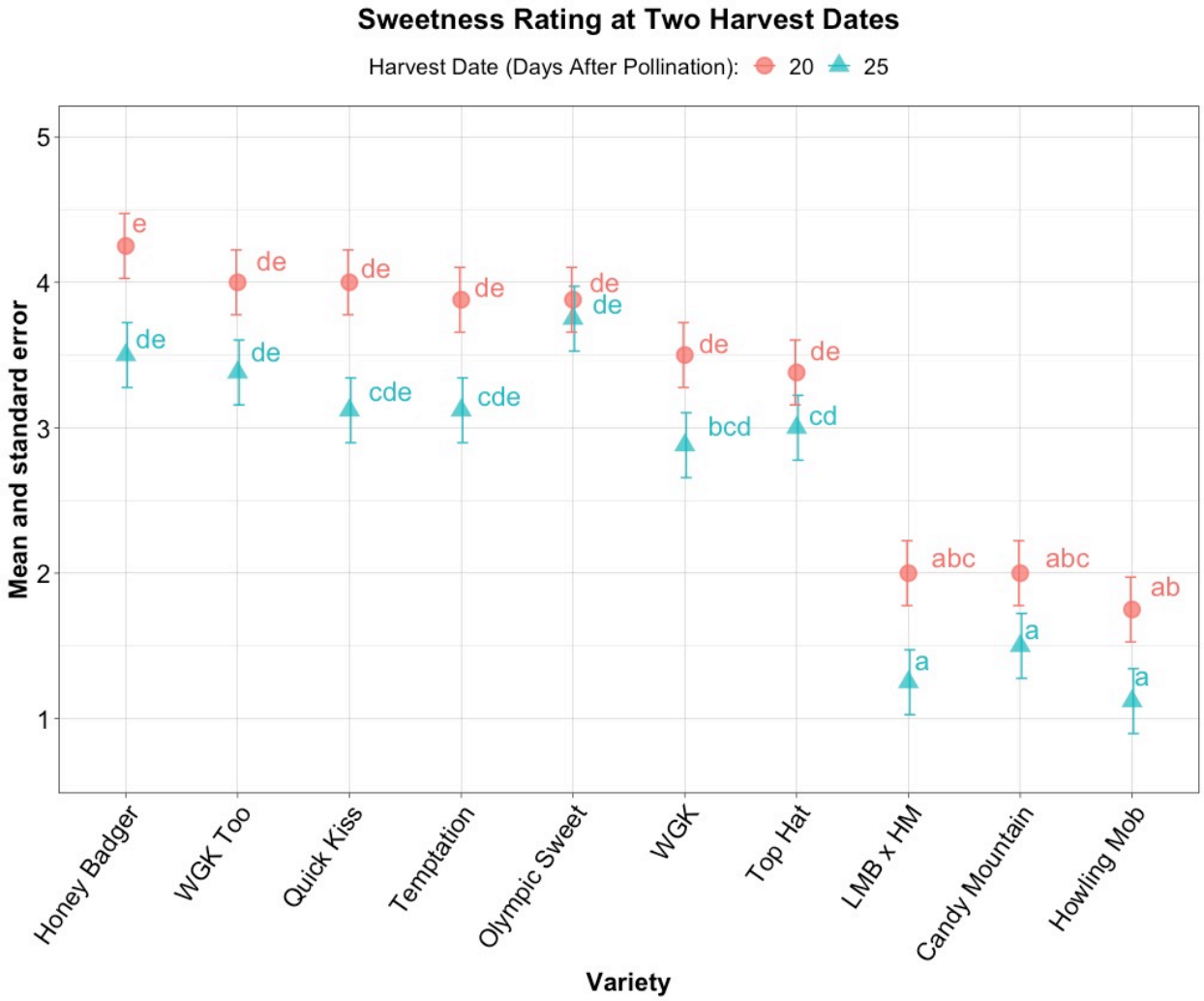


Figure 4.8. Mean and standard error for sweetness rating (1-5) over two harvest dates, 20 and 25 days after pollination, for ten varieties of sweet corn grown at West Madison Agricultural Research Station in four organic environments with two replications per environment in 2022 and 2023. Means that share the same letter are not significantly different by a Tukey's Honest Significant Difference test at a significance level of 0.05.

The *su1* varieties at both harvest dates and Candy Mountain at 25 DAP scored the lowest in the trial for tenderness ratings (Figure 4.9). Tenderness was strongly positively correlated with both overall liking and sweetness (Figure 4.6). While WGK at 25DAP and Candy Mountain at both harvest dates were not different from Howling Mob at 20DAP, all the other *se1* varieties

outperformed Howling Mob at both harvest dates for tenderness, as expected given the difference in endosperm type (Figure 4.9). Except for WGK, all the varieties performed just as well as the hybrid check in holding their tenderness across harvest dates (Figure 4.9).

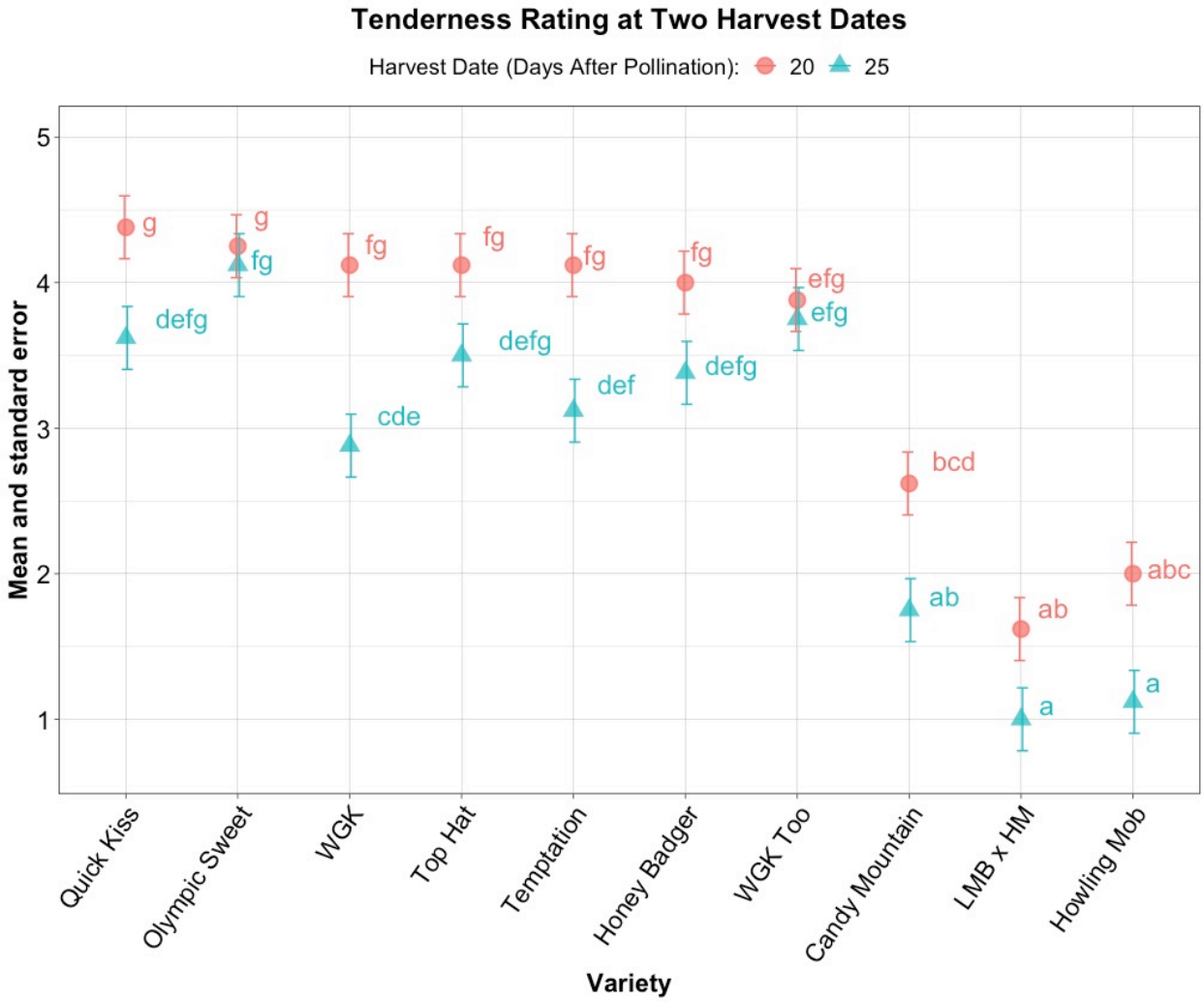


Figure 4.9. Mean and standard error for tenderness rating (1-5) over two harvest dates, 20 and 25 days after pollination, for ten varieties of sweet corn grown at West Madison Agricultural Research Station in four organic environments with two replications per environment in 2022 and 2023. Means that share the same letter are not significantly different by a Tukey's Honest Significant Difference test at a significance level of 0.05.

4.5 Conclusion

This trial identified varieties that performed well for many traits in the environments measured, often on par or better than hybrid and OPV checks for both quantitative and qualitative traits, as well as areas where varieties could be improved. In the environments and years tested, among the OPVs, Quick Kiss is a desirable variety for combining both earliness and low variability for flowering time. Early varieties have the advantages of allowing a grower to be first to market with sweet corn, allowing the field space to be turned over and used for another crop more quickly, and avoiding disease and insect pressures that may build as the season progresses. Olympic Sweet was also early and had very high quality, scoring among the best varieties in the trial for sweetness and tenderness as well as holding these high trait values across harvest dates. Olympic Sweet was very variable for flowering time, ranking eighth of ten for silk emergence variability. Similarly, Honey Badger was among the sweetest in the trial but was also the most variable for silk emergence. The data from this trial indicates that improvement is needed in the flowering window of Honey Badger and Olympic Sweet to move them closer to the variability of the OPV standard, an opportunity for breeding work to continue on these high-quality varieties.

If a late OPV is preferred, WGK and WGK Too were among late varieties that also had low variance for flowering time and performed well for quality traits, except for WGK's holding capacity for tenderness across harvest dates, which was poorer than all other varieties in the trial. Given that Quick Kiss and WGK or WGK Too had significantly different flowering times, they could conceivably be planted at the same time to provide two succession harvests of sweet corn. Quick Kiss, WGK, and WGK Too had variability in flowering time that was no different than the OPV check, Howling Mob. WGK, WGK Too, and Honey Badger were also desirable for ear

length and Olympic Sweet, Honey Badger, and WGK had good ear width. Additionally, Top Hat had excellent tip fill and row configuration paired with high quality and later season maturity.

This trial determined that the selection for more uniform flowering time in WGK Too relative to WGK has not been effective. The variation for silk emergence and pollen shed are not different between varieties as determined by pairwise Levene's Tests. The selection to improve uniformity of flowering was conducted as mass selection, where rather than sib mating the population at least three times over the course of eight days, which is the typical method the Wisconsin program uses for maintaining open pollinated populations, WGK Too was only sib mated once, as soon as least 100 plants had flowered. The hypothesis was that this selection method would move WGK Too to be earlier and more uniform in flowering among plants. Perhaps more generations of selection are needed to see improvement. In the future, selection efficiency could be improved by better controlling for environmental variation. This could be achieved through a stratified mass selection scheme (Bernardo, 2014). Selection efficiency could also be improved by creating full-sib, half-sib, or selfed families to increase the amount of additive variance that is expressed among offspring and allow for families to be replicated in multiple environments (Bernardo, 2020).

The trial determined that recording flowering time on a per plant basis is the best way to understand the uniformity of flowering time. Recording a "visual" mid silk date does return similar mid silk dates as the per plant method and this information is useful for determining harvest time per plot. Recording the beginning and end of flowering does provide data on the range of flowering time but this isn't as useful as knowing how the flowering time is distributed within a variety, as the beginning and end dates could be extreme outliers. The information gleaned from the variability of flowering time can guide future selection efforts. While the trial

evaluated eating quality via tasting two marketable and representative ears per plot and recording an average rating, significant variation existed for eating quality within harvest date and plot. This variability and/or too few ears sampled, could be why the trial lacked power to differentiate many varieties for eating quality traits. Future trials could explore a method of quantifying the variability of eating quality within variety in the same way that flowering time was quantified. This could be accomplished by self-pollinating a large number of ears per variety, then tasting every ear per plot on set harvest dates in multiple environments. Given that growers in Wisconsin and nationally have indicated that uniformity is a breeding priority, efforts should be made to improve the uniformity of both flowering time and eating quality in order to also improve marketability. Future selection work for improved uniformity in eating quality could be done in tandem with selection for uniform flowering time.

4.6 Chapter Four References

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II. Appendix Tables A8: Trait Means

Table A8-1. Spearman correlations for traits with significant variety x environment interactions in ANOVA for varieties grown at West Madison Agricultural Research Station four organic environments with two replications per environment in 2022 and 2023. Spearman correlations are shown among environments, averaged over all other factors, or among harvest dates within environments, averaged over all other factors. Environment codes are as follows: 1 = 2022 Early, 2 = 2022 Late, 3 = 2023 Early, 4 = 2023 Late. Harvest Date codes are as follows: 20 DAP = 20 Days after pollination, the early harvest date and 25 DAP = 25 Days after pollination, the late harvest date. *,**,*** correspond to significant at .05, .01, and .001 probability levels, respectively.

Spearman Rank Correlations		
Environment	Trait	Spearman's rho
1 – 2	Overall Liking	0.87***
1 – 3	Overall Liking	0.62*
1 – 4	Overall Liking	0.71*
2 – 3	Overall Liking	0.61
2 – 4	Overall Liking	0.71*
3 – 4	Overall Liking	0.78**
1 – 2	Sweetness	0.88***
1 – 3	Sweetness	0.70*
1 – 4	Sweetness	0.88***
2 – 3	Sweetness	0.61
2 – 4	Sweetness	0.75*
3 – 4	Sweetness	0.91***
1 – 2	Tenderness	0.90***
1 – 3	Tenderness	0.76*
1 – 4	Tenderness	0.84**
2 – 3	Tenderness	0.81**
2 – 4	Tenderness	0.80**
3 – 4	Tenderness	0.89***
1 – 2	% Marketable Ears	0.93***
1 – 3	% Marketable Ears	0.46
1 – 4	% Marketable Ears	0.31
2 – 3	% Marketable Ears	0.42
2 – 4	% Marketable Ears	0.47
3 – 4	% Marketable Ears	0.22
1 – 2	Number of Kernel Rows	0.87**
1 – 3	Number of Kernel Rows	0.80**
1 – 4	Number of Kernel Rows	0.83**
2 – 3	Number of Kernel Rows	0.76*
2 – 4	Number of Kernel Rows	0.83**
3 – 4	Number of Kernel Rows	0.74*
1 – 2	Tip Fill	0.83**
1 – 3	Tip Fill	0.74*

1 – 4	Tip Fill	0.62*
2 – 3	Tip Fill	0.89***
2 – 4	Tip Fill	0.67*
3 – 4	Tip Fill	0.72*
1 – 2	Husk Appearance	0.80**
1 – 3	Husk Appearance	0.38
1 – 4	Husk Appearance	0.49
2 – 3	Husk Appearance	0.64*
2 – 4	Husk Appearance	0.61
3 – 4	Husk Appearance	0.81**
1 – 2	Plant Height	0.85**
1 – 3	Plant Height	0.89***
1 – 4	Plant Height	0.82**
2 – 3	Plant Height	0.84**
2 – 4	Plant Height	0.94***
3 – 4	Plant Height	0.88***
1 – 2	Stand (% per plot)	0.92***
1 – 3	Stand (% per plot)	0.66*
1 – 4	Stand (% per plot)	0.79**
2 – 3	Stand (% per plot)	0.57
2 – 4	Stand (% per plot)	0.73*
3 – 4	Stand (% per plot)	0.85**
1 (20 DAP) – 2 (20 DAP)	Overall Liking	0.93***
1 (20 DAP) – 3 (20 DAP)	Overall Liking	0.76*
1 (20 DAP) – 4 (20 DAP)	Overall Liking	0.76*
2 (20 DAP) – 3 (20 DAP)	Overall Liking	0.76*
2 (20 DAP) – 4 (20 DAP)	Overall Liking	0.73*
3 (20 DAP) – 4 (20 DAP)	Overall Liking	0.78**
1 (25 DAP) – 2 (25 DAP)	Overall Liking	0.87***
1 (25 DAP) – 3 (25 DAP)	Overall Liking	0.81**
1 (25 DAP) – 4 (25 DAP)	Overall Liking	0.87**
2 (25 DAP) – 3 (25 DAP)	Overall Liking	0.60
2 (25 DAP) – 4 (25 DAP)	Overall Liking	0.82**
3 (25 DAP) – 4 (25 DAP)	Overall Liking	0.90***
1 (20 DAP) – 2 (20 DAP)	Sweetness	0.65*
1 (20 DAP) – 3 (20 DAP)	Sweetness	0.85**
1 (20 DAP) – 4 (20 DAP)	Sweetness	0.79**
2 (20 DAP) – 3 (20 DAP)	Sweetness	0.72*
2 (20 DAP) – 4 (20 DAP)	Sweetness	0.84**
3 (20 DAP) – 4 (20 DAP)	Sweetness	0.87**
1 (25 DAP) – 2 (25 DAP)	Sweetness	0.85**
1 (25 DAP) – 3 (25 DAP)	Sweetness	0.87**
1 (25 DAP) – 4 (25 DAP)	Sweetness	0.84**
2 (25 DAP) – 3 (25 DAP)	Sweetness	0.77**
2 (25 DAP) – 4 (25 DAP)	Sweetness	0.71*

3 (25 DAP) – 4 (25 DAP)	Sweetness	0.95***
1 (20 DAP) – 2 (20 DAP)	Number of Kernel Rows	0.94***
1 (20 DAP) – 3 (20 DAP)	Number of Kernel Rows	0.63*
1 (20 DAP) – 4 (20 DAP)	Number of Kernel Rows	0.76*
2 (20 DAP) – 3 (20 DAP)	Number of Kernel Rows	0.75*
2 (20 DAP) – 4 (20 DAP)	Number of Kernel Rows	0.77**
3 (20 DAP) – 4 (20 DAP)	Number of Kernel Rows	0.75*
1 (25 DAP) – 2 (25 DAP)	Number of Kernel Rows	0.91***
1 (25 DAP) – 3 (25 DAP)	Number of Kernel Rows	0.88***
1 (25 DAP) – 4 (25 DAP)	Number of Kernel Rows	0.73*
2 (25 DAP) – 3 (25 DAP)	Number of Kernel Rows	0.86**
2 (25 DAP) – 4 (25 DAP)	Number of Kernel Rows	0.91***
3 (25 DAP) – 4 (25 DAP)	Number of Kernel Rows	0.76*
1 (20 DAP) – 2 (20 DAP)	Tip Fill	0.93***
1 (20 DAP) – 3 (20 DAP)	Tip Fill	0.63*
1 (20 DAP) – 4 (20 DAP)	Tip Fill	0.82**
2 (20 DAP) – 3 (20 DAP)	Tip Fill	0.57
2 (20 DAP) – 4 (20 DAP)	Tip Fill	0.61
3 (20 DAP) – 4 (20 DAP)	Tip Fill	0.70*
1 (25 DAP) – 2 (25 DAP)	Tip Fill	0.78**
1 (25 DAP) – 3 (25 DAP)	Tip Fill	0.78**
1 (25 DAP) – 4 (25 DAP)	Tip Fill	0.74*
2 (25 DAP) – 3 (25 DAP)	Tip Fill	0.77**
2 (25 DAP) – 4 (25 DAP)	Tip Fill	0.73*
3 (25 DAP) – 4 (25 DAP)	Tip Fill	0.70*
1 (20 DAP) – 2 (20 DAP)	Husk Appearance	0.80**
1 (20 DAP) – 3 (20 DAP)	Husk Appearance	0.60
1 (20 DAP) – 4 (20 DAP)	Husk Appearance	0.76*
2 (20 DAP) – 3 (20 DAP)	Husk Appearance	0.65*
2 (20 DAP) – 4 (20 DAP)	Husk Appearance	0.69*
3 (20 DAP) – 4 (20 DAP)	Husk Appearance	0.85**
1 (25 DAP) – 2 (25 DAP)	Husk Appearance	0.73*
1 (25 DAP) – 3 (25 DAP)	Husk Appearance	0.61
1 (25 DAP) – 4 (25 DAP)	Husk Appearance	0.76*
2 (25 DAP) – 3 (25 DAP)	Husk Appearance	0.82**
2 (25 DAP) – 4 (25 DAP)	Husk Appearance	0.71*
3 (25 DAP) – 4 (25 DAP)	Husk Appearance	0.81**

Table A8-2. Mean of accumulated growing degree days (GDD) on five flowering traits (First pollen shed per plot, first silk emergence per plot, mid silk date, last plant to begin pollen shed per plot, last plant with silk emergence per plot) as well as the range of silk emergence and pollen shed (Last Plant – First Plant) for ten varieties grown at West Madison Agricultural Research Station in four organic environments with two replications per environment in 2022 and 2023. Within a column, means that share the same letter are not significantly different by a

Tukey's Honest Significant Difference test at a significance level of 0.05. Statistics are averaged over environments and replications.

Growing Degree Accumulation of Five Flowering Traits							
Variety	*First Pollen Shed per Plot (GDD)*	*First Silk Emergence per Plot (GDD)*	*Mid Silk Date per Plot (GDD)*	*Last Plant to Begin Pollen Shed per Plot (GDD)*	*Last Plant with Silk Emergence per Plot (GDD)*	*Range in Silk Emergence (GDD)*	*Range in Pollen Shed (GDD)*
Candy Mountain	889 a	908 a	998 a	1066 a	1118 ab	210	178
Olympic Sweet	915 ab	928 ab	1022 ab	1114 abcd	1155 abc	227	200
LMB x HM	915 ab	951 b	1041 b	1102 abc	1132 ab	181	187
Quick Kiss	916 ab	939 ab	1014 ab	1078 ab	1112 ab	173	196
Honey Badger	923 ab	943 ab	1048 b	1129 bcd	1168 abc	224	245
Temptation	940 bc	958 bc	1012 ab	1079 ab	1095 a	137	155
Howling Mob	974 cd	1016 de	1088 c	1148 cde	1187 bcd	171	213
Top Hat	980 cde	999 cd	1102 cd	1176 def	1230 cd	231	250
WGK	1014 de	1042 e	1135 d	1208 ef	1254 d	213	240
WGK Too	1016 e	1037 de	1127 d	1217 f	1229 cd	192	213

Table A8-3. Summary statistics (estimated marginal means, standard error, variance, minimum value, maximum value, and number of plants measured (n)) for accumulated growing degree days (GDD) on the day of first pollen shed for each plant within a plot for varieties grown at West Madison Agricultural Research Station in two organic environments with two replications per environment in 2022. Means that share the same letter are not statistically different at significance level of 0.05 using the Dunnett T3 correction for multiple comparisons with unequal variance.

Growing Degree Day Accumulation of First Pollen Shed per Plant						
Variety	*Mean (GDD)*	*Standard Error (GDD)*	*Variance (GDD)*	*Minimum (GDD)*	*Maximum (GDD)*	*N (plants)*
Candy Mountain	983 a	2.97	1975.95	895	1091	211
LMB x HM	995 ab	2.83	1972.17	895	1141	232
Temptation	998 bc	2.76	722.15	938	1113	244
Quick Kiss	1008 c	2.91	1247.25	915	1091	220
Olympic Sweet	1011 c	3.49	2075.63	895	1207	165
Honey Badger	1025 d	2.94	2996.77	904	1224	216
Howling Mob	1048 e	2.83	1654.46	962	1149	232
Top Hat	1076 f	3.51	2814.28	968	1269	154
WGK	1092 g	2.84	1730.00	988	1224	230
WGK Too	1122 h	3.13	2159.13	1007	1277	195

Table A8-4. Summary statistics (estimated marginal means, standard error, variance, minimum value, maximum value, and number of plants measured (n)) for accumulated growing degree days (GDD) on the day of first silk emergence for each plant within a plot for varieties grown at West Madison Agricultural Research Station in two organic environments with two replications per environment in 2022. Means that share the same letter are not statistically different at significance level of 0.05 using the Dunnett T3 correction for multiple comparisons with unequal variance.

Growing Degree Day Accumulation of First Silk Emergence per Plant						
Variety	*Mean (GDD)*	*Standard Error (GDD)*	*Variance (GDD)*	*Minimum (GDD)*	*Maximum (GDD)*	*N (plants)*
Candy Mountain	1003 a	3.44	3249.72	840	1277	210
Temptation	1023 b	3.19	945.39	946	1116	244
Olympic Sweet	1023 b	4.04	2970.65	915	1218	164
Quick Kiss	1024 b	3.36	1981.51	938	1207	220
LMB x HM	1028 b	3.24	2791.16	915	1240	236
Honey Badger	1055 c	3.39	4105.72	938	1292	217
Howling Mob	1084 d	3.27	1797.63	988	1277	232
Top Hat	1112 e	4.11	3634.00	988	1292	149
WGK	1135 f	3.28	2187.31	988	1277	230
WGK Too	1145 f	3.63	2022.66	988	1269	193

Table A8-5. Pairwise Levene's Tests for Equality of Variances with a null hypothesis of equal variance among varieties for anthesis collected on a per plant basis grown at West Madison Agricultural Research Station in two organic environments in 2022 with two replications per environment. *,**,*** correspond to significant at .05, .01, and .001 probability levels, respectively.

Pairwise Levene's Test for Equality of Variances in Anthesis		
Variety Pair	*F-Value*	*P-Value*
Howling Mob – Temptation	24.634	9.683e-07 ***
Quick Kiss – Temptation	15.961	7.524e-05***
WGK Too – Temptation	48.914	1.008e-11***
WGK – Temptation	15.429	9.846e-05***
LMB x HM – Temptation	34.457	8.199e-09***
Olympic Sweet – Temptation	40.413	5.521e-10***
Candy Mountain – Temptation	51.279	3.26e-12***
Top Hat – Temptation	47.559	2.12e-11***
Honey Badger – Temptation	77.752	2.2e-16***
Quick Kiss – Howling Mob	1.8734	0.1718
WGK Too – Howling Mob	4.0644	0.04443*
WGK – Howling Mob	0.4884	0.485
LMB x HM – Howling Mob	0.9748	0.324
Olympic Sweet – Howling Mob	2.3456	0.1264
Candy Mountain – Howling Mob	3.4654	0.0633

Top Hat – Howling Mob	7.1716	0.007725**
Honey Badger – Howling Mob	16.275	6.445e-05***
WGK Too – WGK	6.6951	0.01*
WGK – Quick Kiss	0.299	0.5848
Olympic Sweet – Quick Kiss	9.7627	0.003266**
LMB x HM – Quick Kiss	5.6531	0.01784*
Candy Mountain – Quick Kiss	11.779	0.0006572***
WGK – LMB x HM	2.6474	0.1044
WGK – Olympic Sweet	4.3752	0.03711*
WGK – Candy Mountain	6.135	0.01363*
LMB x HM – Olympic Sweet	0.3432	0.5583
LMB x HM – Candy Mountain	0.6603	0.4169
Olympic Sweet – Candy Mountain	0.0241	0.8768

Table A8-6. Estimated marginal means and standard error of percent stand within plot (=V5 plants emerged/kernels planted) for varieties grown at West Madison Agricultural Research Station in four environments with two replications per environment in 2022 and 2023. Means that share the same letter are not significantly different by a Tukey's Honest Significant Difference test at a significance level of 0.05.

Percent Stand within Plot			
Variety	*Mean (percent)*	*Standard Error (percent)*	*N (plots)*
Olympic Sweet	0.48 a	0.019	8
Top Hat	0.53 a	0.019	8
WGK Too	0.63 b	0.019	8
Candy Mountain	0.69 b	0.019	8
Honey Badger	0.70 b	0.019	8
Quick Kiss	0.71 b	0.019	8
WGK	0.72 b	0.019	8
Temptation	0.84 c	0.019	8
Howling Mob	0.89 c	0.019	8
LMB x HM	0.89 c	0.019	8

Table A8-7. Estimated marginal means and standard error of ear length for varieties grown at West Madison Agricultural Research Station in four organic environments with two replications per environment in 2022 and 2023. Means that share the same letter are not significantly different by a Tukey's Honest Significant Difference test at a significance level of 0.05.

Ear Length			
Variety	*Mean (cm)*	*Standard Error (cm)*	*N (plots)*
Candy Mountain	17.7 a	0.21	16
LMB x HM	18.3 ab	0.21	16
Olympic Sweet	18.6 ab	0.21	16
Top Hat	18.6 ab	0.21	16
Temptation	18.7 bc	0.21	16
Quick Kiss	18.8 bcd	0.21	16
Honey Badger	19.6 cde	0.21	16

WGK Too	19.7 cde	0.21	16
Howling Mob	19.7 de	0.21	16
WGK	20.1 e	0.21	16

Table A8-8. Estimated marginal means and standard error of ear width for varieties grown at West Madison Agricultural Research Station in four organic environments with two replications per environment in 2022 and 2023. Means that share the same letter are not significantly different by a Tukey's Honest Significant Difference test at a significance level of 0.05.

Ear Width			
Variety	*Mean (cm)*	*Standard Error (cm)*	*N (plots)*
LMB x HM	4.22 a	0.047	16
Candy Mountain	4.36 ab	0.047	16
Top Hat	4.45 b	0.047	16
Howling Mob	4.57 bc	0.047	16
WGK Too	4.71 cd	0.047	16
Quick Kiss	4.74 cde	0.047	16
Honey Badger	4.83 de	0.047	16
Olympic Sweet	4.8 3de	0.047	16
Temptation	4.88 de	0.047	16
WGK	4.93 e	0.047	16

Table A8-9. Estimated marginal means and standard error of tip fill for varieties grown at West Madison Agricultural Research Station in four organic environments with two replications per environment in 2022 and 2023. Means that share the same letter are not significantly different by a Tukey's Honest Significant Difference test at a significance level of 0.05.

Tip Fill			
Variety	*Mean (1-5 rating)*	*Standard Error (1-5 rating)*	*N (plots)*
Candy Mountain	2.62 a	0.142	16
Quick Kiss	2.69 a	0.142	16
Olympic Sweet	3.38 b	0.142	16
Honey Badger	3.44 b	0.142	16
WGK Too	3.44 b	0.142	16
Howling Mob	3.50 b	0.142	16
WGK	3.69 b	0.142	16
LMB x HM	3.81 bc	0.142	16
Top Hat	4.44 cd	0.142	16
Temptation	4.81 d	0.142	16

Table A8-10. Estimated marginal means and standard error of number of kernel rows for varieties grown at West Madison Agricultural Research Station in four organic environments with two replications per environment in 2022 and 2023. Means that share the same letter are not significantly different by a Tukey's Honest Significant Difference test at a significance level of 0.05.

Number of Kernel Rows

Variety	*Mean (number of kernel rows per ear)*	*Standard Error (number of kernel rows per ear)*	*N (plots)*
LMB x HM	9.06 a	0.242	16
Howling Mob	10.97 b	0.258	15
Candy Mountain	12.56 c	0.242	16
Olympic Sweet	13.94 d	0.242	16
Quick Kiss	13.94 d	0.242	16
Top Hat	14.19 de	0.242	16
WGK Too	14.62 def	0.242	16
Honey Badger	14.88 def	0.242	16
Temptation	15.25 ef	0.242	16
WGK	15.38 f	0.242	16

Table A8-11. Estimated marginal means and standard error of row configuration for varieties grown at West Madison Agricultural Research Station in four organic environments with two replications per environment in 2022 and 2023. Means that share the same letter are not significantly different by a Tukey's Honest Significant Difference test at a significance level of 0.05.

Row Configuration			
Variety	*Mean (1-5 rating)*	*Standard Error (1-5 rating)*	*N (plots)*
Howling Mob	2.12 a	0.136	16
LMB x HM	2.44 a	0.136	16
Honey Badger	3.50 b	0.136	16
Candy Mountain	3.56 b	0.136	16
Quick Kiss	3.69 bc	0.136	16
WGK	3.69 bc	0.136	16
Olympic Sweet	3.94 bc	0.136	16
WGK Too	3.94 bc	0.136	16
Top Hat	4.00 bc	0.136	16
Temptation	4.25 c	0.136	16

Table A8-12. Estimated marginal means and standard error of husk appearance for varieties grown at West Madison Agricultural Research Station in four organic environments with two replications per environment in 2022 and 2023. Means that share the same letter are not significantly different by a Tukey's Honest Significant Difference test at a significance level of 0.05.

Husk Appearance					
Variety	*Mean (2022) (1-5 rating)*	*Standard Error (2022)*	*Mean (2023) (1-5 rating)*	*Standard Error (2023)*	*N (plots/year)*
Candy Mountain	2.50 a	0.167	2.00a	0.235	8
LMB x HM	3.25 ab	0.167	4.00 bc	0.235	8

Temptation	3.25 ab	0.167	4.95 c	0.235	8
Honey Badger	3.38 abc	0.167	3.12 b	0.235	8
Top Hat	3.50 abc	0.167	3.38 bc	0.235	8
Quick Kiss	3.62 abc	0.167	3.62 bc	0.235	8
Olympic Sweet	3.75 bc	0.167	4.00 bc	0.235	8
Howling Mob	4.00 bc	0.167	3.38 bc	0.235	8
WGK	4.38 bc	0.167	3.75 bc	0.235	8
WGK Too	4.50 c	0.167	3.25 bc	0.235	8

Table A8-13. Estimated marginal means and standard error of husk protection for varieties grown at West Madison Agricultural Research Station in four organic environments with two replications per environment in 2022 and 2023. Means that share the same letter are not significantly different by a Tukey's Honest Significant Difference test at a significance level of 0.05.

Husk Protection			
Variety	*Mean (1-5 rating)*	*Standard Error (1-5 rating)*	*N (plots)*
Candy Mountain	2.75 a	0.171	16
Howling Mob	3.19 ab	0.171	16
LMB x HM	3.50 abc	0.171	16
Olympic Sweet	3.50 abc	0.171	16
Quick Kiss	3.50 abc	0.171	16
Honey Badger	3.88 bcd	0.171	16
WGK	4.06 cd	0.171	16
Top Hat	4.19 cd	0.171	16
Temptation	4.44 d	0.171	16
WGK Too	4.50 d	0.171	16

Table A8-14. Estimated marginal means and standard error of percent marketable ears for varieties grown at West Madison Agricultural Research Station in two years (2022 and 2023) with two organic environments per year and two replications per environment. Means that share the same letter are not significantly different by a Tukey's Honest Significant Difference test at a significance level of 0.05.

Percent Marketable Ears					
Variety	*Mean (2022) (percent)*	*Standard Error (2022)*	*Mean (2023) (percent)*	*Standard Error (2023)*	*N (plots/year)*
Candy Mountain	0.59 a	0.471	0.88 a	0.0472	8
Honey Badger	0.72 ab	0.606	0.86 a	0.0472	8
Olympic Sweet	0.73 abc	0.616	0.95 a	0.0472	8
Quick Kiss	0.75 abcd	0.634	0.88 a	0.0472	8
Top Hat	0.85 bcde	0.734	0.83 a	0.0472	8
LMB x HM	0.91 cde	0.796	0.88 a	0.0472	8
Howling Mob	0.93 de	0.809	0.85 a	0.0472	8

WGK Too	0.93 de	0.809	0.89 a	0.0472	8
WGK	0.98 e	0.859	0.93 a	0.0472	8
Temptation	0.99 e	0.871	0.96 a	0.0472	8

Table A8-15. Estimated marginal means and standard error of overall liking averaged over two harvest dates for varieties grown at West Madison Agricultural Research Station in four organic environments with two replications per environment in 2022 and 2023. Means that share the same letter are not significantly different by a Tukey's Honest Significant Difference test at a significance level of 0.05.

Overall Liking			
Variety	*Mean (1-5 rating)*	*Standard Error (1-5 rating)*	*N (plots)*
Howling Mob	1.31 a	0.115	16
LMB x HM	1.31 a	0.115	16
Candy Mountain	1.81 a	0.115	16
WGK	3.25 b	0.115	16
Top Hat	3.38 b	0.115	16
Quick Kiss	3.50 b	0.115	16
Temptation	3.56 b	0.115	16
Honey Badger	3.69 b	0.115	16
Olympic Sweet	3.69 b	0.115	16
WGK Too	3.69 b	0.115	16

Table A8-16. Estimated marginal means and standard error of sweetness averaged over two harvest dates for varieties grown at West Madison Agricultural Research Station in four organic environments with two replications per environment in 2022 and 2023. Means that share the same letter are not significantly different by a Tukey's Honest Significant Difference test at a significance level of 0.05.

Sweetness			
Variety	*Mean (1-5 rating)*	*Standard Error (1-5 rating)*	*N (plots)*
Howling Mob	1.44 a	0.157	16
LMB x HM	1.62 a	0.157	16
Candy Mountain	1.75 a	0.157	16
Top Hat	3.19 b	0.157	16
WGK	3.19 b	0.157	16
Temptation	3.50 b	0.157	16
Quick Kiss	3.56 b	0.157	16
WGK Too	3.69 b	0.157	16
Olympic Sweet	3.81 b	0.157	16
Honey Badger	3.88 b	0.157	16

Table A8-17. Estimated marginal means and standard error of tenderness averaged over two harvest dates for varieties grown at West Madison Agricultural Research Station in four organic environments with two replications per environment in 2022 and 2023. Means that share the

same letter are not significantly different by a Tukey's Honest Significant Difference test at a significance level of 0.05.

Tenderness			
Variety	*Mean (1-5 rating)*	*Standard Error (1-5 rating)*	*N (plots)*
LMB x HM	1.31 a	0.153	16
Howling Mob	1.56 ab	0.153	16
Candy Mountain	2.19 b	0.153	16
WGK	3.50 c	0.153	16
Temptation	3.62 c	0.153	16
Honey Badger	3.69 c	0.153	16
Top Hat	3.81 c	0.153	16
WGK Too	3.81 c	0.153	16
Quick Kiss	4.00 c	0.153	16
Olympic Sweet	4.19 c	0.153	16