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Investigating Parental Effects on End-Use Quality in Hard Winter Wheat (Triticum aestivum L.) Hybrids

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Investigating Parental Effects on End-Use Quality in Hard Winter Wheat (*Triticum aestivum L.)* Hybrids

By

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INVESTIGATING PARENTAL EFFECTS ON END-USE QUALITY IN HARD WINTER WHEAT (*Triticum aestivum L.*) HYBRIDS

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To optimize the performance and marketing of hybrid wheat, breeders need to understand the impact parents have on end-use quality. The goal of this study was to investigate the inheritance of end-use quality traits of hard winter wheat reciprocal hybrids produced by Easterly (2017). The 2016 analysis included 71 reciprocal hybrid combinations from 13 parents and the 2017 analysis included 79 reciprocal hybrid combinations from 14 parents. The reciprocals were composed of crosses between the top performing and bottom performing parents with respect to end-use quality as quantified by a Mixograph, a SDS sedimentation assay, and a SDS-SRC hybrid assay. The Mixograph was digitalized using Mixsmart® software provided by the National Manufacturing Company (National Manufacturing Company) and two Mixograph analyses were derived to determine the quality of the hybrid; the mix peak time (min) analysis, and the gluten strength {(%TQ*min)/grams of protein} x tolerance (unitless) analysis. In general, the Mixograph analyses of the reciprocals indicated the dough strength of the hybrid tended to reflect the dough strength of the female parent as hybrids that combined a strong female with a strong male and hybrids that combined a strong female with a weak male had relatively strong dough strength. Conversely, the hybrids that combined a weak female with a weak male and a weak female with a strong male

exhibited relatively weak dough strength. The maternal effects could be due to higher rates of self-pollination than initially thought, or due to the genetic composition of the triploid endosperm, which is composed of 2N maternal and 1N paternal DNA. Easterly indicated that one parent, NE07531 was well sterilized, and hybrids NE07531 was the female parent appeared to have mid-parent dough strength, suggesting that at least in some cases, true hybrids were produced. Irrespective of any reciprocal cross differences, it appeared in some cases a single high quality parent can potentially mask end-use quality deficiencies of the other parent in the performance of the hybrid, but this need further work to determine.

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CHAPTER 1

INVESTIGATING PARENTAL EFFECTS ON END-USE QUALITY IN HARD WINTER WHEAT (*Triticum aestivum L.*) HYBRIDS

ABSTRACT

To optimize the performance and marketing of hybrid wheat, breeders need to understand the impact parents have on end-use quality. The goal of this study was to investigate the inheritance of end-use quality traits of hard winter wheat reciprocal hybrids produced by Easterly (2017). The 2016 analysis included 71 reciprocal hybrid combinations from 13 parents and the 2017 analysis included 79 reciprocal hybrid combinations from 14 parents. The reciprocals were composed of crosses between the top performing and bottom performing parents with respect to end-use quality as quantified by a Mixograph, a SDS sedimentation assay, and a SDS-SRC hybrid assay. The Mixograph was digitalized using Mixsmart® software provided by the National Manufacturing Company (National Manufacturing Company) and two Mixograph analyses were derived to determine the quality of the hybrid; the mix peak time (min) analysis, and the gluten strength {(%TQ*min)/grams of protein} x tolerance (unitless) analysis. In general, the Mixograph analyses of the reciprocals indicated the dough strength of the hybrid tended to reflect the dough strength of the female parent as hybrids that combined a strong female with a strong male and hybrids that combined a strong female with a weak male had relatively strong dough strength. Conversely, the hybrids that combined a weak female with a weak male and a weak female with a strong male exhibited relatively weak

dough strength. The maternal effects could be due to higher rates of self-pollination than initially thought, or due to the genetic composition of the triploid endosperm, which is composed of 2N maternal and 1N paternal DNA. Easterly indicated that one parent, NE07531 was well sterilized, and hybrids NE07531 was the female parent appeared to have mid-parent dough strength, suggesting that at least in some cases, true hybrids were produced. Irrespective of any reciprocal cross differences, it appeared in some cases a single high-quality parent can potentially mask end-use quality deficiencies of the other parent in the performance of the hybrid, but this need further work to determine.

INTRODUCTION

As the population of the world increases, there becomes an increased demand to raise the production of crops and the foods from which they are made. The Declaration of the World Summit on Food Security (FAO, 2009) predicted that world food production will have to increase by 70 percent from 2009 to 2050 to securely feed an estimated 9 billion people. Globally, as the third highest cereal crop produced (FAO, 2015), wheat (*Triticum spp*.) is critical to attaining a plentiful food supply. Hybrid wheat is a strategy that offers a means of increasing production because similar to other hybrid crops, grain yields and resistance to abiotic and biotic stresses are generally boosted based on heterosis(Longin *et al.*, 2012, 2013, Tester *et al*., 2010, Whitford *et al.*, 2013). However, long-term success in hybrid wheat breeding hinges on breeders creating and maintaining heterotic pools of parents that when crossed, result in the highest performing hybrids for grain yield and end-use quality (Zhao *et al*., 2015, Pickett, 1993).

In addition to creating hybrids that deliver optimal grain yields, hybrid wheat breeders have the added challenge of meeting end-use quality requirements and delivering ahigh-quality product (Guzman *et al*., 2016). This can be problematic since quantifying end-use quality often requiressourcing seed that may not be available at the early generation stages of a hybrid seed program (Gross *et al*., 2007). Ideally, breeders can also use marker or genomic assisted selection (MAS and GAS respectively) to screen early generations of hybrid material for end-use quality (Liu *et al.*, 2016, Goutam *et al.*, 2013, Whitford *et al.*, 2013, Guzman *et al.*, 2016), but few markers exist to account for the many genes that determine the complex mixing characteristics intrinsic to different wheat flours. Furthermore, there is a general lack of research into the effects of parents

on end-use quality, and heterosis for quality traits in hybrid hard winter wheat (Morojele and Labuschagne, 2013, McNeal *et al.,* 1968)

Perhaps the most effective way of addressing these research gaps with respect to hybrid wheat end-use quality will be through collaborations between public universities and industry. This study represents just such a collaborative effort, and it was undertaken by Bayer CropScience (now BASF) and the University of Nebraska-Lincoln. The objective was to investigate heterosis and parental effects on end-use quality in hybrid hard winter wheat produced by Easterly (2017).

In comparison to other crops, wheat's floral biology has made it more challenging to produce and analyze seed from hybrids for end-use quality. Common or bread wheat (*T. aestivum* L.*).* is naturally cleistogamous, or self-pollinating, which makes outcrossing and commercial hybrid seed production more difficult(Pickett 1993, Sarkar *et al*. 2013, Whitford *et al*., 2013, Longin *et al*., 2012). Overcoming the tendency to naturally self-pollinate is key to successfully producing hybrid wheat seed, and it requires; 1) The development of male and female parents with floral traits that enable outcrossing, and 2) the implementation of a robust and reliable fertility control system (Whitford *et al.,* 2013, Singh *et al.,* 2010, Pickett, 1993, Mette *et al.,* 2015). Regardless of the obstacles to produce and maintain wheat hybrids, successfully marketing such hybrids will require end-use quality to equal, or exceed the quality of pure-line bred and released cultivars.

LITERATURE REVIEW PART 1: FLORAL ARCHITECTURE, FERTILITY CONTROL SYSTEMS, DOUBLE FERTILIZATION

Floral Architecture

As previously stated, male and female parents in hybrid wheat programs must exhibit traits that promote outcrossing. Specifically, male parents must possess good anther extrusion, anther size, pollen longevity, pollen quantity, and most importantly the pollination period must nick, or sync with the female parent's flowering period (Boeven *et al*., 2016, Khan *et al*., 1973). Due to the short longevity of wheat pollen, about 10-30 minutes (de Vries, 1971), having female parents that also nick with the male parent is essential, and can be improved by developing female cultivars with longer flowering periods and more receptive stigmas (Khan *et al*., 1973, Pickett, 1993, Boeven *et al.*, 2018). However, elite germplasms that exhibit strong male floral traits are rare and fertility control systems are designed to be genotype independent, which expands the range of potential female parents. As a result, hybrid wheat programs typically have fewer potential male parents than female parents (Liu *et al.*, 2016) and most research focuses on developing cultivars with better male traits like anther extrusion (Garst, 2017).

Several studies (Okada *et al*., 2018, Boeven *et al.*, 2016, Langer *et al*., 2014, de Vries 1971, Garst, 2017) have reported phenotypic variation in male floral traits that would encourage out-crossing, so there is hope for breeders to improve these traits through traditional breeding approaches. Additionally, Boeven *et al.* (2018) correlated visual anther extrusion with female seed set, thus showing an opportunity to develop male cultivars by selecting for visual anther extrusion. Okada *et al.* (2018) has also

shown that after flowering, unfertilized wheat ovaries push open the wheat flower in a second effort to be fertilized, which is both a helpful indication to breeders that whatever chosen fertility control mechanism was effective and acts to help expose the stigma to neighboring pollen donors. It is critical that research programs and seed companies continue capitalizing on the phenotypic diversity of wheat floral traits to improve both male and female floral characteristics to ensure a reliable seed set in hybrids.

Fertility Control Systems

The other component of attaining a reliable hybrid seed set in wheat is a stable and robust fertility control system. This prevents self-pollination and guarantees only hybrid seed is produced on a commercial scale. Several extensive reviews have been published about the fertility control systems that are used in hybrid wheat seed production (Whitford et al. 2013, Singh et al. 2010, Mette M.F. 2015, Longin C.F.H. 2012, Pickett A.A. 1993, de Vries A. 1971,). The cytoplasmic male sterility (CMS) system and the chemical hybridizing agents (CHAs) are currently the most promising and commonly used fertility control systems and will be the focus of this section.

CMS System

Kihara (1951) was the first to report CMS in wheat and sparked the effort to develop the first hybrid wheat seed production system based on the CMS model (Wilson and Ross, 1962). *Triticum a.* CMS systems rely on transposons in mitochondrial DNA that results in the inability to produce male pollen (Hanson *et al*., 2004), thus inducing male sterility. Fertility restoration in the resulting hybrid is facilitated with nuclear fertility restorer (Rf) genes that encode proteins that negate the effects of the CMS genes

and restore fertility in the offspring. This represents a critical step in wheat hybrid seed production. The CMS system requires 3 lines designated the A, B, and R line, with their genetic properties listed below (Singh *et al.,* 2010, Whitford *et al*., 2013, Pickett 1993).

- **A-line** - female parent that carries the cytoplasmic male sterility gene, male sterile
- **B-line** - maintainer line that has the same nuclear DNA as A-line but does not carry the CMS gene, male fertile
- **R-line** – male parent that carries Rf genes

The Rf genes are classified as either sporophytic or gametophytic, depending on which tissue the restorative affects manifest in (Horn 2006). In hybrid breeding, sporophytic Rf genes are more practical because they restore fertility to the female pollen producing cells, resulting in 100% restored pollen viability (Pickett 1993). If the same hybrid was produced with a Rf gene that acted gametophytically, male fertility would only be restored to the gametes that contained the Rf gene, and only 50% of the resulting pollen would be viable, which would be unacceptable.

Unfortunately, the CMS system is still complex and commercial efforts to create a CMS system for hybrid wheat seed production have largely been unsuccessful (Singh *et al.,* 2010). Complications arise because several major Rf genes are needed to restore fertility (Bahl *et al.*, 1973), and these genes must be present in the crop genome and must not be linked with deleterious mutations. Also, effectiveness of certain CMS systems can be dependent on environmental conditions like temperature and photoperiod (Kaul 1988), resulting in incomplete sterility and shriveling of the F1 seed. Finally, the hybrids

produced must capture enough heterosis to justify the additional expenses associated with establishing and maintaining a CMS program. However, our understanding of Rf genes is improving (Martin *et al.*, 2008) and sequencing and marker technologies are allowing breeders to backcross Rf genes into elite cultivars more efficiently. These improvements are making CMS systems for hybrid wheat seed production more feasible.

CHA System

Limitations in the CMS system have sparked a significant amount of effort to develop chemical methods to induce male sterility in wheat without compromising female fertility. These compounds that cause male sterility in a crop are called chemical hybridizing agents, or CHAs, and are mainly used for hybrid seed production. CHA hybridizing systems eliminate many of the problems associated with CMS systems; mainly that there is no need for a maintenance line (B-line), does not require any pre-breeding to introduce the Rf or CMS genes into the R and A-lines respectively, is fast and relatively easy to implement, and allows for a much wider range of parental combinations (Singh *et al.*, 2010). However, there is an extensive list of requirements that a CHA must meet before it can be commercially used (Singh *et al.*, 2010, Whitford *et al.*, 2013)

- female fertility must remain unaffected
- genotype independent
- systemic activity and persistence that allows treated plants to be in varying stages of maturity and still be effective
- wide application period to account for weather delays like rain or wind
- non-phytotoxic and non-mutagenic

- environmentally safe
- economic to synthesize
- practical to apply
- low dosage to ensure reasonable margins are still attainable
- Must not affect F1 seed quality and seedling or plant vigor

Current CHAs meet most of these qualifications but are still limited due to narrow windows of application. Utilizing a CHA system poses a significant financial risk to seed production companies because success hinges on unpredictable factors like wind and rain, which can delay application. Application delays can result in failure to inhibit self-pollination, which ultimately signifies a failure to produce a hybrid. Croisor®100 (EFSA 2010) was the CHA that was used to produce the hybrids that were used in our analysis (Easterly, 2017), and has an approximate window of application of 2-3 days. Other CHAs have application windows of up to 5 or 6 days, but at the cost of being slightly more toxic.

Ultimately, the resources available to the researcher or seed producer will determine what fertility control system is used. Also, as transgenic systems become more widely adopted, de Block *et al.* (1997) has developed a *barnase/barstar* transgenic hybrid seed production strategy. Kempe *et al.* (2014) improved this system by establishing a split-gene method, allowing for only the female plant to be transformed instead of both parents, which considerably reduces the initial investment costs. Hybrid wheat breeders must keep these systems in mind moving forward.

Double-Fertilization

Wheat (*Triticum aestivum L.*) is a flowering plant, which means that it undergoes double-fertilization during its reproductive cycle. This process begins when a male pollen lands on a receptive stigma and the pollen germinates, initiating the growth of the pollen tube (Dumas and Rogowsky, 2008). The pollen tube proceeds to deposit two male sperm cells into the ovule of the flower; one pollen fertilizes the egg cell and result in a diploid embryo, while the other sperm fertilizes the two polar nuclei cells which eventually become the triploid endosperm. Zhang (2016) demonstrated that corn kernel size was affected by the maternal tissue of the corn and could be a result of the triploid endosperm tissue. Hybrid wheat kernels may similarly be impacted; however, it is the F_2 seed that is harvested from the F_1 hybrid that is used to make final bread products. There would have to be a genetic mechanism like imprinting that would cause the F_1 maternal tissue to pass on any traits that would be exhibited in the F_2 kernels.

LITERATURE REVIEW PART 2: HYBRID WHEAT END-USE QUALITY

Wheat end-use quality is complex because it is partially determined by the user and the product to be produced from the flour, meaning the end-use is usually a result of the growing environment and food markets being targeted(Guzman *et al.,* 2016). Australia and Canada have end-use quality scales that categorize wheats based on protein content (Blakeney *et al.,* 2009) and quality performance compared to a standard check(Bushuk *et al*., 1978). End-use qualities that millers are mainly concerned with optimizing are physical properties of the grain, such as; kernel hardness, size, and density (test weight), which have a significant impact on flour yield and quality (Matsuo and Dexter, 1980, Morris, 2002). Food companies and bakers require flour that has good dough-mixing and baking properties, so they can produce and deliver a consistent product to consumers. Finally, aside from the food product type, the consumer is generally interested in purchasing and eating safe food products that are delicious and nutritious. To successfully develop hybrid wheat cultivars that meet these demands, it is necessary to understand how parents contribute to quality in the F_2 harvested seed generation.

Unfortunately, adequately quantifying end-use quality in hybrid wheat often requires significantly more seed than is typically available in the early stages of breeding programs (Goutam *et al.*, 2013). To overcome this limitation, various assays and instruments have been developed that can utilize relatively small amounts of grain and provide important information on the physical and biochemical end-use properties of flour (AACC 2010, Lorenzo *et al.,* 1987, Moonen *et al.,* 1982, Seabourn *et al.,* 2012, Martinant *et al.,* 1998, Gaines *et al.,* 1996, Perten Instruments North America,

Springfield, IL). However, these assays have limitations, particularly when analyzing cultivars with high protein content (Seabourn *et al.,* 2012). Until now, breeders and cereal chemists have had little opportunity or need to consider how various parental combinations in an F_1 might impact the end-use quality of final food products made by food producers. As stated earlier, this collaboration represents such an opportunity to meet the growing need to address this question.

Kernel hardness (Pasha *et al*. 2010, Morris 2002), gliadins (Sozinov and Poperelya, 1980, 1982, Pogna *et al.*, 1982, 1990, Branlard *et al*., 2001, Metakovsky *et al.*, 1997b,c, Wrigley *et al.*, 1982, Killermann and Zimmermann, 2000, Shewry *et al.*, 2003), and glutenins (Payne *et al.*, 1979, 1981b, 1987a, 1987b, 1987c, Payne and Lawrence, 1983, Payne P. I., 1987, Pogna *et al.*, 1988, Lawrence *et al.*, 1988, MacRitchie *et al*., 1990, Gupta and MacRitchie, 1995, Killermann and Zimmermann, 2000, Shewry *et al*., 2003, Pasha *et al.*, 2010, Rodriguez-Quijano *et al*., 1996b, Rousset *et al*., 1992, Lorenzo *et al.,* 1987, Cooper *et al*., 2016,) are wheat seed traits demonstrated to determine end-use quality. A few studies have attempted to document the heterosis for kernel hardness and gluten quality expressed by wheat hybrids (Morojele and Labuschagne, 2013) as well as the effects that maternal tissue has on hybrid wheat end-use quality (McNeal *et al.,* 1968).Gaining an understanding of the heterosis for quality traits, and the impact of parents on hybrid wheat end-use quality should assist breeders in selecting, and utilizing genomic selection (Liu *et al.*, 2016, Goutam *et al.*, 2013, Guzman *et al.*, 2016) to potentially predict the parents that optimize performance in various food products.

Physical and Chemical Properties Associated with Wheat Quality

Wheat quality is generally determined by its physical and chemical properties, and Goutam *et al.* (2013) provides a comprehensive list of the traits associated with those properties.

Some physical properties are:

- Grain appearance score
- Kernel or grain hardness
- Vitreousness of kernel
- 1000 kernel weight
- Test Weight
- Kernel size and shape

Whereas, some chemical properties are:

- Protein content (NIR)
- Protein quality and sedimentation test
- Assays that measure dough mixing (i.e. Mixograph) or viscoelastic properties (i.e. Alveograph)

Morphological traits (grain kernel size, test weight, 1000 kernel weight), and grain appearance (absence of grain damage) are important to producers because in many situations, these determine the market grade and the price received (Guzman *et al.*, 2016). These traits are also important to millers because kernel morphology can determine flour yield, and damaged or shriveled seed often reduces flour yield. Besides kernel morphology, hardness is a major determinant of end-use quality in bread wheat (Pasha *et*

al., 2010,) because when compared with soft kernels, when hard kernels are milled, they produce more broken starch chains which absorb more water and result in an increase in loaf volume (Morris, 2002).

Kernel Hardness

Morris (2002) describes the genetic mechanism responsible for kernel texture, or hardness. The wild-type puroindoline proteins a and b confer a soft kernel texture; whereas, the absence of a functional puroindoline protein (either due to a null allele or mutation) results in a hard kernel texture. It is necessary for millers and other wheat handlers to be able to measure hardness and other individual kernel traits in a high-throughput manner, which is why Perten Instruments (Perten Instruments North America Inc., Springfield, IL) developed a single-kernel characterizations system; the SKCS 4100. The SKCS 4100 measures kernel weight (mg), diameter (mm), moisture (%), and kernel hardness (HI). The SKCS is fitted with a teethed rotor that crushes the seed, and an algorithm that results in the measurements (Gaines *et al.* 1996). This method only uses 300 seeds and is a useful tool for collecting end-use quality data in about two or three minutes..

Once the grain has been milled and processed into flour, the focus of quality shifts from physical properties of the kernel to the chemical properties of the proteins in the endosperm. Among these proteins, gluten has a significant impact on end-use quality and is comprised mainly of glutenins and gliadins. These proteins polymerize into massive networks during dough-formation, and they enable the unique extensibility and viscoelastic properties exhibited by the wheat dough (Shewry *et al.*, 2003, Gale, 2005,

Payne, 1987, Goutam *et al.,* 2013, Pogna *et al*., 1988). Glutenins are grouped into high-molecular weight subunits (HMW-GS) and low-molecular weight subunits (LMW-GS), and gliadins can be separated and designated as α, β, γ, or ω, based on the method developed by Bushuk and Zillman (1978).

Gliadins

Gliadins are alcohol soluble and glutenins are alcohol insoluble and are made up of monomeric proteins, while glutenins are polymeric (Shewry *et al.,* 2003)*Gli-1 and Gli-2* are the loci responsible for encoding gliadins, and variation at these loci is associated with differences in bread-making quality and gluten strength (Sozinov and Poperelya, 1980, 1982, Pogna *et al*., 1982, Wrigley *et al*., 1982, Metakovsky *et al*., 1997b,c,). Shewry *et al*. (2003) points out that the SDS sedimentation assay is widely used to determine breadmaking quality. In this assay, larger sedimentation volumes are caused by the formation of a mesh, or gel, that is solely comprised of insoluble glutenin proteins (Moonen *et al*., 1982, Payne *et al*., 1987c). This demonstrates that the positive association between the *Gli-1* allele and loaf volume is a result of genes encoding LMW subunits at *Glu-3*. *Gli-2* was also shown to express C-type LMW-GS (Masci *et al.*, 2002), which is additional evidence that gliadins, and the variation expressed by the gliadin genes are important to determining end-use quality.Understanding the relationship between gliadins and end-use quality enables hybrid wheat breeders to use gliadin markers to select for improved end-use quality. It also emphasizes the importance of gluten to quality, and the need to use gliadins and glutenins to select for hybrid wheat quality.

Glutenins: HMW-GS and LMW-GS

Payne *et al*. (1979) were the first to identify HMW-GS as a source of genetic variation for bread-making quality. Subsequent research has shown that *Glu-A1, Glu-B1,* and *Glu-D1* loci encode the HMW-GS, with *Glu-B1* and *Glu-D1* being the most influential to determining end-use quality (Lawrence and Shepherd, 1980, Payne and Lawrence, 1983, Zheng *et al.*, 2009, Cooper *et al*., 2016, Goutam *et al*., 2013). The *Glu-1B* and *Glu-1D* loci encode compound proteins, meaning each encodes up to two protein subunits. Different alleles at these loci can have a significant impact on dough strength and baking quality. For example, the *Glu-D1d* allele that encodes the Dx5 + Dy10 subunits confers superior dough strength and loaf volume compared with the *Glu-D1a* allele that encodes Dx2 + Dy12 subunits which have a deleterious effect on quality (Payne *et al*., 1987c, Cooper *et al.*, 2016, Butow *et al*., 2003, Ammar *et al*., 1997, Kolster *et al.*, 1991). Butow *et al.* (2003) also demonstrated that the *Glu-B1a1* allele ($Bx7^{OE}$ +By8) is associated with increased dough strength, likely due to an overexpressed Bx7 subunit compared to the more common *Glu-B1b* allele (Bx7 + By8).

The *Glu-3* loci alsohas a significant impact on gluten strength and dough extensibility (Payne *et al.*, 1984, 1987a, Gupta and Shepherd, 1988, Pogna *et al.*, 1988, Cornish *et al.*, 2001), and the LMW-GS are classified into 3 groups; B-type, C-type, and D-type. The D- and C- groups of LMW-GS have not been studied in detail, but they are highly similar in sequence to ω-type and α/γ-type gliadins respectively (Shewry *et al*., 2003). The C- and D-type LMW-GS are thought to be a result of mutations in gliadin alleles that resulted in the inclusion of cysteine residues, which enables them to form

disulfide bonds and polymeric proteins (Shewry *et al.*, 2003). B-group glutenins make up most of the LMW-GS, but they do not show any similarities in sequences with gliadins.

Polymeric proteins, which are interconnected via disulfide bonds are foundational in establishing the gluten network largely responsible for determining end-use quality. Gupta et al. (1995) used lines developed by Gupta and Shepherd (1993) carrying either single, double, or triple translocations that systemically eliminated LMW-GS to determine the effect they had on quality. Eliminating LMW-GS impacted size distribution of the polymeric proteins and resulted in reduced gluten quality. Furthermore, they created lines that eliminated all HMW-GS and demonstrated that the LMW-GS were not as critical as HMW-GS in determining polymer size. There have also been reports of epistatic interactions between the *Glu-A1, Glu-B1,* and *Glu-D1* alleles. Kolster *et al.* (1991) showed that the effects of the *Glu-A1* and *Glu-B1* alleles were dependent on the allele present at the *Glu-D1* loci. Epistatic relationships have also been identified between the *Glu*-1 and *Glu-3* loci (Gupta and MacRitchie, 1994). Hybrid wheat offers a new opportunity to use these epistatic interactions to potentially mask the expression of deleterious end-use quality alleles and optimize the quality in the final product.

Previous Literature Addressing Heterosis and Maternal Effects on Wheat Quality

To make sound crossing decisions, hybrid wheat breeders must consider the effects of heterotic interactions between genes associated with end-use quality, and how maternal tissue will influence gluten quality. The literature available addressing these two interactions are limited, but studies have been conducted to measure heterosis and

maternal effects potentially impacting end-use hybrid wheat quality, (Morojele and Labuschagne, 2013, McNeal *et al.,* 1968, Borghi and Perenzin, 1994).

Using 5 parents, each expressing different end-use quality, Morojele and Labuschagne (2013) produced 38 F_1 and F_2 progeny from a 5 x 5 diallel crossing strategy and estimated mid-parent and best-parent heterosis for 7 quality characteristics, including; break flour yield (BFY), flour protein content (FPC), mixograph development time (MDT), SDS sedimentation value (SDS), kernel weight (kw), kernel diameter (kd), and kernel hardness (HI), however, they did not investigate any reciprocal effects. There was a significant difference amongst the F_1 and F_2 progeny for all 7 of the traits. The F_1 hybrids showed positive mid-parent heterosis in SDS volume, HI, BFY, and kw. In the F_2 progeny, they reported positive mid-parent heterosis for MDT, HI, and SDS volume, and positive best-parent heterosis for MDT, HI, kw and kd. The F_2 quality is much more important than the quality of the actual hybrid F_1 seed, because it is the F_2 seed that is milled to produce food products

Borghi and Perenzin (1994) similarly used a diallel crossing scheme composed of 7 parents to create 21 F1 hybrids. They observed significant heterosis in agronomic traits such as: yield, plant height, and time to heading. They did not include reciprocal cross hybrids in the analysis. They also observed significantly improved alveographic parameters (P) and P/L ratio, signifying an increased grain value of up to 30 % in commercial hybrid production. They concluded hybrids show significant potential to boost farmer profits by providing better quality grain.

McNeal *et al.* (1968) made reciprocal crosses using four parents expressing a range of quality for baking and milling. They also evaluated F_2 seed to determine if there was a maternal effect on quality. This is reasonable to investigate because the endosperm and the embryo of a wheat kernel are two separate organs, and the endosperm is technically a triploid organ with 2N from the mother parent and 1N from the male (Shewry *et al.,* 2003). Since the endosperm contains all the gluten proteins, there's potential for quality to be influenced by the extra dose of female parent. Thus, it's important to examine if there's a maternal influence on quality because it could affect the direction of the cross. McNeal *et al.* (1968) concluded their investigation saying, '*These data indicate that reciprocal crosses have little if any effect on quality characteristics.'*

Lorenzo *et al.* 1987 showed that high sedimentation values using the sodium dodecyl sulphate sedimentation test (SDS-sedimentation) correlated with higher loaf volume, an important quality trait determined by gluten, particularly HMW-GS. This assay used less material than was needed for bake assays and could be performed at a high-throughput. Seabourn *et al.* 2012 introduced the SDS-SRC (sodium dodecyl sulfate-solvent retention capacity) assay which had an even higher correlation to loaf volume compared with the SDS assay. It also used very little material and could be used to evaluate end-use quality traits in early generation materials at high-throughput.

Another instrument that is routinely used to identify gluten strength and select for its expression is the Mixograph (Martinant *et al.,* 1998), which uses an aggressive mixing action to separate weak and strong dough and determine mixing time, strength, and tolerance. Also, in addition to the Perten SKCS 4100, there is also a Perten infrared

reflectance (NIR) instruments that can quickly and accurately measure protein content in grain and flour (Perten Instruments North America, Springfield, IL).

The assays that were used by McNeal *et al.* (1968) were limited to wheat and flour protein content (via ion-binding method), milling yield (Brabender quadruplex), sedimentation (Zeleny *et al.*, 1960), farinograph and grain yield. Considering this is one of the only studies done on end-use quality looking solely at reciprocal hybrid wheat crosses, maternal influence on end-use quality in hybrid wheat is poorly understood. Maternal impact on end-use quality is important for hybrid wheat breeders to understand because in a CMS hybridization system the only difference between the reciprocal cross is the cytoplasm of the parents. If there are interaction between cytoplasmic genes of the two parents that affect quality, then it would be important to consider which parent is used as the female parent.This justifies our objectives to investigate the influence of maternal tissue on end-use quality using reciprocal hybrid wheat crosses. It is also a good opportunity to look at mid-parent and better-parent heterosis in hybrid wheat, another topic of crucial importance.

MATERIALS AND METHODS

Seed Sources and Production of Hybrids:

A diallel crossing plan similar to Borghi and Perenzing (1994), Morojele and Labuschagne (2013), and McNeat *et al.* (1968) was implemented to achieve yield trial hybrid combinations and the combinations that were evaluated for quality. Twenty-six genotypes were selected and used as the parents to produce the F_1 hybrids within a 26 x 25 diallel crossing block located near Mead, NE, at the Agricultural Research and Development Center (ARDC). One parent was only included in the 2015 crossing block (NE10478-1), and it was replaced by Harry in the 2016 crossing block. The F_1 seed was produced in the summer of 2015 and 2016, planted in the fall of 2015 and 2016, and harvested in the summer of 2016 and 2017. The parents consisted of germplasm developed by the University of Nebraska-Lincoln and Texas A&M University and they represented a diversity of expression for end-use quality traits.

The hybrid trials that provided the seed for quality evaluations were designed as described by Easterly (2017). The trials were planted to an augmented design with replicated checks, and they included over 800 plots and over 600 F_1 hybrids representing 313 reciprocal crosses. The resulting F_2 seed harvested from the yield trials was subsequently used for quality analyses.

Male-sterility within the diallel blocks was achieved by applying Croisor 100® (active ingredient - sintofen; 1-(4-chlorophenyl)-5-(2-methoxyethoxy)-4-oxo-1,4-dihydrocinnoline-3) (Saaten-Union Recherche, City, France; Easterly 2017) CHA. According to Easterly (2017), roughly three weeks post-CHA application, female

genotypes from four crossing blocks a year were selected and three to five heads were covered with pollen impermeable bags and secured with tape and bamboo stakes. After the grain filling period and prior to harvest the bagged heads were removed and threshed individually, and the seed number was recorded, indicating how successful the CHA application was. In 2015 there was an average of 5.7 seeds per spike, and in 2016 there were 2.6 seeds per spike. In 2016, it was determined that approximately 64% of the females were completely male-sterile; whereas in 2015, approximately 38% were completely male sterile. The improvement in the level of sterility achieved by the CHA in 2016 was likely due to the CHA being applied more efficiently (Easterly, 2017). To focus on evaluating a representative group of hybrids that combined parents considered to be classified as weak and strong for end-use quality, all parents were first tested and ranked for end-use quality. Parental testing included: Mixograph analyses, SDS assays, and SDS-SRC hybrid assays as described in the *Analytical Methods and Instruments*. The parent lines were subsequently grouped as the strongest (top ~25%) and weakest (bottom ~25%) parents **(Table 2**) by visually comparing the Mixographs to known quality checks, with Overland representing a poor quality check and Freeman representing a good quality check. Although all of the assays had similar results, the Mixograph represented the most robust assay for determining gluten strength, therefore, the Mixograph results were weighed more heavily than the other assays. Thirteen parents in total were categorized in 2016 (six strong and seven weak) and 14 parents were categorized in 2017 (seven strong and seven weak), and the hybrids produced between those representative parents were all analyzed. Therefore, in 2016, each parent was represented in a maximum of twelve

hybrids along with their reciprocals, and in 2017, each parent was represented in a maximum of thirteen hybrids along with their reciprocals.

Hybrids and their reciprocals were categorized prior to evaluation based on the *apriori* determination of the parents as being either strong, or weak for end-use quality. Consequently, hybrids and their reciprocals were in turn categorized as representative of: 1) strong x strong, 2) weak by weak, and 3) weak x strong, and 4) strong x weak combinations **(**List of hybrids evaluated for 2016 and 2017 can be found in appendix A). In 2016, 149 hybrid samples were analyzed, of which 144 were represented by a reciprocal combination. In 2017, 167 hybrid samples were analyzed, of which 158 were represented by a reciprocal combination. Evaluations were performed in the Quality Lab located within the Bayer Cropscience wheat research stationat Beaver Crossing, NE. The SDS Sedimentation and SDS-SRC Hybrid Assay Control Compilation (Appendix E) shows a compilation of SDS sedimentation values (mL) and weight value percentatge using a control variety. The CV of the SDS sedimentation control compilation was 1.70, while the CV for the SDS-SRC hybrid assay was 2.43, indicating that there was relatively little variation in the performance of an assay across samples. assays have a high repeatability. Also, according to the Mixsmart® Handbook, the mid-line integrals section as reported by this assay is one of the most reproducible parts of a Mixograph analysis (Walker and Walker, 2004). In the present study, the Mixograph data were important to the evaluation of hybrid end-use quality, and because of the known reproducibility of the assay, a large sampling of hybrid combinations was evaluated in deference to repeatedly evaluating the same hybrid combinations.

Analytical Methods and Instruments:

Analytical procedures to determine the end-use quality of hybrids were developed by the American Association of Cereal Chemists (AACC 2010) and USDA (Seabourn *et al.*, 2012). The AACC method 39-25.01 was performed to determine whole grain protein content (%) using a Perten DA 7250 NIR (Perten Instruments North America, Springfield, IL), and AACC method 39-11.01 was used to determine flour protein (%), moisture (%), and ash (%) content using a Perten Inframatic 9500 (Perten Instruments, Springfield, IL.). A Perten SKCS 4100 was used to perform AACC method 55-31.01 and determine single-kernel traits, including kernel mass (mg), hardness index (HI), kernel diameter (mm), and kernel moisture (%). The SKCS is fitted with a teethed rotor that crushes the seed, and it employs an algorithm that results in the data that is output for each measurement (Gaines et al. 1996). The procedure was modified such that only 100, instead of the typical 300 kernels were analyzed.

The UDY cyclone sample mill (UDY corporation, Fort Collins, CO) was used to grind samples for the AACC method 56-70.01 sodium dodecyl sulfate (SDS) analyses as well as for the SDS-SRC hybrid assay described by Seabourn *et al.* (2012). The SDS sedimentation test was used to determine the sedimentation value (mL), and the SDS-SRC hybrid assay was used to determine the weight value %.. The sedimentation assay followed the AACC method 56-70.01 with slight modifications. Weight values were calculated using the following equation from Seabourn *et al.* (2012).

Equation 1

$$Weight\ Value\ (\%) = \left(\left(\frac{Pellet\ weight}{Flour\ weight} \right) x \left\{ \left[\frac{86}{100 - Flour\ Moisture\ \%} \right] \right\} - 1 \right) x\ 100$$

Flour samples were produced using a slightly modified version of the AACC method 26-50.01, Brabender Quadrumat Jr. (Quadruplex) method for milling wheat (C. W. Brabender® Instruments, South Hackensack, NJ). Instead of using the sieve provided in the Quadrumat Jr., samples were sifted using the Sampl-sifter produced by Great Western Manufacturing (Great Western Manufacturing Inc. Co., Leavenworth, KS) and separated into three partitions; the bran, middlings, and flour. Among these partitions, only the flour was retained.

Mixograph data was collected on the 35-gram mixograph (National Manufacturing Company, Lincoln, NE) that followed AACC method 54-40.02.Mixograms were visually ranked and digitally analyzed using the MIXSMART® for windows: Computerized Data Acquisition and Analysis For the Mixograph (Walker and Walker, 2004). Values collected by the MIXSMART® used in our analysis included peak height (% torque), peak development time (min), peak time integral (min*% torque), peak width (% torque), right of peak width (min), and right of peak height (% torque).

Two other values were used in our analysis and are derived from the MIXSMART data; one is a measurement of tolerance to overmixing and the other is a measure of gluten strength per gram of protein. Tolerance can be calculated as shown in **equation 2** (method developed by Dr. Kaufman at Bayer Crop Science (now BASF), personal communication) while gluten strength per gram of protein was developed by the author (**equation 3**). Because the tolerance equation takes the reciprocal of the amount of strength lost, samples that lose very little or no strength result in tolerance values that approach infinity. These equations represent an effort to assign more objective

measurements to determining gluten quality. Visual scoring and parameters determined using the Mixsmart® data were used to rank parents. The following sections describe the methods used to evaluate heterosis and the maternal effect in the hybrids.

Equation 2

$$Tolerance = 1/|\left\{\frac{(MPH * MPW) - (RPH * RPW)}{MPH * MPW}\right\}|$$

Equation 3

$$Strength\left(\frac{\%TQ * Min}{grams\ of\ protein}\right) = \frac{MPI}{Grams\ of\ Protein}$$

Where MPH = Midline-peak height, MPW = Midline-peak width, RPH = Right of peak height, RPW = Right of peak width, MPI = Mid-line peak integral

Graphical Analysis: By collecting data on many reciprocal crosses and graphing them all together, we can observe how the end-use quality of the hybrids compare to the quality of the parents. and determine if there is any maternal effect on end-use quality in hybrid wheat as possibly represented by the detection of reciprocal effects The SDS sedimentation assay and SDS-SRC assay are graphed with the sedimentation value (mL) and weight value (%) on the y-axis and the protein content (%) on the x-axis. Since sedimentation value and weight value are greatly impacted by overall protein content (Seabourn *et al.*, 2012), this allows us to evaluate samples in terms of protein functionality as a function of protein quantity. The Mixograph should be the most effective tool at separating the difference in protein functionality among the hybrids. The

Mixograph analysis is presented in two ways; the first is more traditional and displays peak mix development time on the y-axis and flour protein content (%) on the x-axis. The second representation of the Mixograph data utilize the strength and tolerance terms derived from **equations 3 and 4** and has the strength value plotted on the y-axis and the tolerance value on the x-axis. When looking at the graphs, samples that have larger strength values and larger tolerance values are more desirable than samples with smaller strength or tolerance value.

Calculating Maternal Effect: To meet our objective of determining if there is a reciprocal effect on the end-use quality of F2 progeny we had to determine if the hybrids, along with their reciprocal crosses, performed identically in end-use quality assays and physical kernel measurements.. Our alternative hypothesis is that the direction of the cross does impact the trait of the hybrid, or H_A: A x B $\neq$ B x A. We tested our hypothesis using a t-test between the mean values of the hybrids when parent A was used as a female, and the mean values of the reciprocal hybrids when parent A was used as the male. We performed a t-test for each parent (thirteen in 2016 and fourteen in 2017) on kernel hardness (HI), kernel diameter (mm), kernel weight (mg), grain protein (%), SDS sedimentation value (mL), weight value (%), and mix peak time (Min) test using the add-in data analysis tools available in the 2013 edition of Microsoft excel using an α of .05. The degrees of freedom for each t-test was dependent on how many reciprocal crosses were completed in the analysis because hybrids without a reciprocal were not included in the analysis. P-values of less than .05 signify a significant difference between the average performance of the hybrids depending on the direction of the cross.

RESULTS AND DISCUSSION

Determining Strong and Weak Parents for 2016 and 2017

It was essential that the parents that we selected were distinctive from each other because it allowed us to examine how the quality of the hybrid and reciprocal compared to the quality of the parents. Mixsmart® digital data was collected and analyzed for both years, and Mixsmart® digital pictures were collected on the 2017 year. Digital pictures of a strong (Freeman, **Fig. 1**) and a weak (Goodstreak, **Fig. 2**) Mixograph illustrate the dough strength profiles of two parental cultivars, and the type of data obtained in the determination of whether the dough is considered strong, or weak in performance. The Freeman Mixograph had a smooth and steady development up to the peak time of 5.51 Min, while the Goodstreak Mixograph had a steep and rapid development to the peak time of 2.43 Min. In addition, the Freeman Mixograph appeared to be more tolerant to overmixing based on the visual assessment of the Mixograph, while the Goodstreak Mixograph quickly reduced in strength and appeared have little tolerance to overmixing. In 2016, the weakest parents based on these Mixograph standards were determined to be; Goodstreak, Overland, TX10D2063, TX10D2230, TX11D3129, TX12M4063, TX12M4065; whereas, the strongest performing parents were; Freeman, LCH13NEDH_11_24, NE07531, NE09517_1, Wesley, and Settler_CL, (**Table 2).** In 2017, the weakest performing 25% were determined to be; Goodstreak, Overland, TX09D1172, TX10D2063, TX10D2363, TX11D3129, TX12M4063; whereas, the strongest performing 25% were; Freeman, LCH13NEDH_11_24, NE07531, NE09517_1, Robidoux, Settler_CL, and Harry (**Table 2).**

Goodstreak, Overland, TX10D2063, TX11D3129, and TX12M4063 were visually identified as bottom performing parents in both years of testing, while Freeman, LCH13NEDH_11_24, NE07531, and NE09517_1 were identified as top performing parents in both years. Based on the visual assessment of the parent Mixographs, Goodstreak and LCH13NEDH_11_24 were visually identified as the weakest and strongest, respectively. Additionally, it is important to note that in some cases, the difference between a strong and an intermediate or a weak and an intermediate parent Mixograph was subtle, meaning that identifying the parents that performed in the top 10 % and bottom 10 % can be done easily by visual analysis, but selecting the top 25 % and bottom 25 % is more time consuming. In 2016 Robidoux and NE10683 performed well relative to the entire pool of parents but were classified as intermediate performing parents due to the small discrepancies in their Mixograph curves. In 2017, several parents including Ruth, NE10683, TX12M4065, and TX12M4004 also appeared to have desirable quality, but Harry was ranked over these cultivars based on subtle differences in the Mixograph. Ultimately, visually assessing each parent Mixograph relative to a pool of parent Mixographs was time consuming and subjective to the experience of the scorer. This highlights the need for a better analytical tool to assess Mixographs and the extensive data collected by the Mixsmart® software presented an opportunity to increase efficiency and improve accuracy of scoring Mixographs One suggestion to improve this method would be to establish specific benchmarks for strength, tolerance, or mix peak time based on check cultivars with known end-use quality, such as Overland for poor quality and Freeman for strong quality. Any parent cultivar performing lower or higher than these standards could then be classified as strong or weak accordingly.

After classifying the parent Mixographs visually, it was important to compare the results to the SDS sedimentation assay and SDS-SRC hybrid assay results. The SDS sedimentation assay and the SDS-SRC hybrid assay can be biased by high protein content samples because high protein quantity can overcompensate for poor protein quality (Seabourn *et al.* 2012). Evaluating the sedimentation volume (mL) and weight value (%) as a function of protein content (%) was suggested to reduce this potential bias by creating a sedimentation and weight value ratio that adjusts for both protein performance and quantity. The SDS sedimentation assay results for the 2016 and 2017 parents were graphed to show the performance difference between the strong parents and the weak parents (**Fig. 3** and **Fig. 4**). Strong parents consistently are represented as data points above the dashed green line, indicating that it has desirable quality. Alternatively, the weak parents generally are represented as data points below the red dashed line, indicating they have undesirable dough quality. Graphs representing the SDS-SRC hybrid assay results for 2016 and 2017 enable a similar separation of the categories (**Fig. 5** and **Fig. 6**).

The 2016 parents were ranked from 1 to 25 based on their performance as assessed by the SDS sedimentation and the SDS-SRC assay. These rankings are shown in relation to the visual Mixograph determination of dough strength for each genotype (**Table 3)**. The strong parents NE09517-1, Settler CL, Wesley, Freeman, LCH13NEDH-11-24, and NE10589 ranked in the top ten for the SDS sedimentation assay and the SDS-SRC hybrid assay, while the weak parents ranked in the bottom ten for both assays. Assessments of the parents of hybrids tested in 2017 similarly demonstrated that the strong parents were ranked in the top ten for both assays and the weak parents, with the

exception of Goodstreak, ranked in the bottom half.(**Table 4)**. Agreement between the SDS sedimentation assay and the SDS-SRC assay in the ranking of parents indicates that the Mixograph profiles are able to adequately distinguish between genotypes with different dough mixing characteristics.

Developing an Objective Assessment Tool Using Mixsmart® Parameters

With the strong and weak parents categorized, our second intent was to make an objective assessment of dough strength based on Mixographs. The most important parameters to bakers and food producers are the mix peak development time (min) and tolerance to overmixing. The mix peak time is the time it takes for the gluten network to develop to peak strength and represents an important quality indicator. Additionally, Walker and Walker (2004) stated that one of the most repeatable measurements in the Mixograph analysis was the midline integral, which is interpreted as the amount of work needed for the dough to reach peak development. The data collected by Mixsmart® was used to graph the Mixographs for mix peak time (min), tolerance, and strength (%TQ*Min/grams of protein) as opposed to visually assessing each hybrid Mixograph and comparing them to the quality of the parents individually.

Bar graphs represent the mix peak time of the parents used in the 2016 (**Fig. 7**) and the 2017 (**Fig. 8**) analysis. In 2016, the strong parents had mix peak times ranging from 3.5 minutes (NE10589) to 5.9 minutes (LCH13NEDH_11_24), while the weak parents had mix peak times ranging from 1.97 minutes (Goodstreak) to 3.06 (TX12M4065), showing no overlap in mix peak time between the strong and weak parents (**Fig. 7**). In 2017, the mix peak time of the parents identified with strong quality ranged from 4.34 minutes (Harry) to 8.13 minutes (LCH13NEDH_11_24), while the mix

peak time of the weak quality parents ranged from 2.43 minutes (Goodstreak) to 3.87 minutes (TX12M4063). There was excellent separation between the strong and weak dough strength parents (**Fig. 8**).

Mix peak time data collected by Mixsmart® was effective at elucidating the differences in performance between the strongest and weakest dough quality genotypes. However, it is important to note that there was some overlap between the intermediate performing cultivars and the strong and weak parents in both years. Specifically, in 2016, Robidoux (4.34 min), TX11D3008 (3.74 min), NE07531 (3.74 min), and TX12M4004 (3.59 min) were categorized as having intermediate performance based on the visual analysis of the Mixographs, despite having a longer mix peak time than NE10589 (3.50 min). Additionally, TX09D1172 (2.99 min) and TX10D2363 (2.77 min) were both categorized as having intermediate quality based on the visual analysis, despite having shorter mix peak times than TX12M4063 (3.09 min). Furthermore, in 2017 Harry (4.34 min) was determined to have strong dough strength based on the visual analysis, but also a slower mix peak time than seven cultivars that were categorized as having intermediate quality; including; TX12M4065 (4.94 min), TX12M4004 (4.87 min), TX10D2230 (4.71 min), Ruth (4.60 min), PSB13NEDH_15_58W (4.58 min), TX11D3112 (4.47 min), and NE10683 (4.38 min). Lastly, TX11D3026 (3.63 min) and Wesley (3.25 min) were both classified as having intermediate quality based on the visual analysis, despite having slower mix peak times than TX12M4063 (3.09 min). While the mix peak time analysis effectively identifies the strongest and weakest performing cultivars, it is limited in separating cultivars that have similar quality. This conclusion is partially due to the fact that the mix peak development time does not consider the tolerance of the variety to

overmixing, which is carefully examined while assessing the Mixograph visually. An analysis that quantifies dough strength as well as tolerance to overmixing could potentially be a valuable analytical tool in quantifying end-use dough quality in wheat.

Using the Mixsmart® data, the Mixograph strength x tolerance analysis was developed that considers gluten strength (**Eq. 2,** {(%TQ*Min)/grams of protein}) as well as tolerance to overmixing (**Eq. 3**, unitless). Comparing the results of the Mixograph strength x tolerance analysis to the mix peak time analysis and visual assessment of the parent Mixographs helped us determine the value of this assay as an analytical tool in evaluating end-use quality. The 2016 strong quality parents had strength values ranging from 29.7 ({%TQ*Min}/grams of protein, NE10589) to 45.7 ({%TQ*Min}/grams of protein, LCH13NEDH_11_24) and tolerance values ranging from 1.6 (NE10589) to 26.7 (LCH13NEDH_11_24) (**Fig. 9.1** and **Fig. 9.2**). The 2016 weak quality parents ranged from Goodstreak which had the lowest strength of 13.64 ({%TQ*Min}/grams of protein) to TX10D2230 with a strength of 21.88 ({%TQ*Min}/grams of protein), while Goodstreak also had the lowest tolerance value of 1.18 ranging to TX10D2230 with a tolerance of 1.46 (**Fig. 9.1** and **Fig. 9.2**). In 2017, the parents that were visually categorized as strong had strength values ranging from 36.0 ({%TQ*Min}/grams of protein, Harry) to 54.3 ({%TQ*Min}/grams of protein, LCH13NEDH_11_24), and the same group in this category had tolerance values ranging from 3.3 (Robidoux) to 7.1 (Freeman, **Fig. 10**). The weak parents of 2017 had strength values ranging from 16.6 ({%TQ*Min}/grams of protein, Goodstreak) to 28.9 ({%TQ*Min}/grams of protein TX12M4063), and tolerance values ranging from 1.26 (TX10D2063) to 1.87 (TX09D1172, **Fig. 10**). The Mixograph strength x tolerance analysis also supports our

visual analysis by identifying Goodstreak as the having the weakest gluten strength and LCH13NEDH_11_24 as has having the strongest gluten strength for samples derived from both years. Use of the Mixograph strength x tolerance parameter is an effective means of at separating the weak quality from thestrong quality parents.

Another critical observation is how NE10589, which was visually scored as a top performing parent in 2016, was tightly grouped with three parents that were visually categorized as having intermediate strength; and included TX11D3008, TX12M4004, and TX11D3026 (**Fig. 9.1** and **Fig. 9.2**). Visually comparing these specific parents, including NE10589, they all appeared to perform very similarly. NE10589 was selected over the other cultivars because it appeared to have slightly better tolerance, but ultimately it was very challenging to distinguish. This implies the Mixograph strength x tolerance analysis is comparable to a visual assessment performed by an experienced laboratory technician. A similar result was observed with Harry in 2017 (**Fig. 10**). Harry was designated a top performing parent based on the visual assessment but performed similarly to four other parents, including TX12M4004, TX12M4065, Ruth, and NE10683, which were categorized as having intermediate quality. The digital photos of the Mixographs for Harry, TX12M4004, TX12M4065, Ruth, and NE10683 provides additional evidence that the Mixograph strength x tolerance analysis is comparable to a visual assessment of the Mixograph (**Fig. 11**). After making these observations, it was determined that the Mixograph strength x tolerance analysis would make a valuable tool in making comparisons between the quality of the hybrid, the quality of the reciprocal, and the quality of the parents. With a better understanding of how the Mixsmart® data can be used to represent the Mixograph profile, it is possible to establish standard

thresholds of strength and tolerance that can be used to categorize the end-use quality of the cultivars based on the Mixograph.

Using the Mixograph Strength x Tolerance and Mix Peak Time To Evaluate Inheritance of Reciprocal Hybrids

After collecting the hybrid Mixograph data, we used the Mixograph strength x tolerance analysis that we developed to examine how the quality performance of the reciprocal hybrids compared with the parents. Specifically, it was important that we determine if there was a reciprocal effect on quality, or if the hybrids performed close to the mid-parent value, or both. The Mixograph strength x tolerance analysis was applied to all of the hybrids, and to compare the quality of the reciprocals to the quality of the parents. Appendix F shows the numbers assigned to each parent cultivar categorized.

We initially focused our analysis on several cultivars to evaluate their reciprocal hybrids to see if there were any notable patterns among their Mixographs, a strategy that we then expanded to include all the hybrids evaluated in the analysis. The first reciprocal hybrids that we isolated to evaluate were crossed using the cultivar TX12M4063 as either the female (P1) or the male (P2) parent. TX12M4063 performed in the bottom 25 % in both years of our analysis, additionally, Easterly (2017) reported TX12M4063 to have a mean seed count of 27 seeds per self-bag, indicating it wasn't sterilized well and that the hybrids may have high levels of self-pollination. The 2016 Mixograph strength x tolerance analysis of the reciprocal hybrids with TX12M4063 designated as the P1 parent indicates that the P1xP2 hybrids (blue) are tightly grouped with the TX12M4063 parent, indicating the P1xP2 hybrids all had poor dough strength similar to TX12M4063 (**Fig. 12**). Alternatively, the P2xP1 reciprocal hybrids (green) appear to have strong and weak

performing hybrids that mirror the dough strength of the P2 parent (**Fig. 12**). The 2017 Mixograph strength x tolerance results showed a similar trend were most of the P1xP2 hybrids performed poorly and were tightly associated to the TX12M4063 parent, with the exception of the hybrids between Goodstreak and Robidoux (**Fig. 13**). The TX12M4063 x Robidoux hybrid appeared to have improved tolerance compared to the TX12M4063 parent and worse strength than the Robidoux parent. In addition, the TX12M4063 x Goodstreak hybrid falls between both of the parents on the graph. These results suggest that the Mixograph strength x tolerance analysis detected a difference between the TX12M4063 reciprocal hybrids, and that the hybrid dough strength tended to reflect the quality of the female parent. This may have been caused by human or sprayer error applying the CHA unsuccessfully or the line being hard to sterilize, resulting in hybrids partially composed of unsterilized female seed, which influenced the quality of the hybrid enough to be detected by the Mixograph strength x tolerance analysis. Making physical blends of the parents in different ratios and then performing the Mixograph on those blends could help determine the effect of having self-pollinated female seed in the hybrid. Furthermore, comparing the Mixograph of the blends to the hybrid and reciprocal Mixograph that are verified hybrids (comparing plant height of hybrid compared to parents) could help elucidate the relationship between the end-use quality of the hybrid and the parents.

It was then important for us to examine the Mixograph mix peak time (min) of the reciprocal hybrids of TX12M4063 so that a comparison between the two Mixographs analyses could be made. A paired t-test was set up to see if there was a reciprocal effect mix peak times between the P1xP2 and the P2xP1 hybrids when TX12M4063 was

designated as the P1 parent ($\alpha = .05$). The 2016 analysis showed the average mix peak time (min) of the P1xP2 hybrids was 2.75 min while the average of the P2xP1 hybrids was 3.53 min, with a p-value = .020 (**Table 5**), indicating a significant difference. This supports the conclusion made by the 2016 Mixograph strength x tolerance analysis of the TX12M4063 reciprocal hybrids. The paired t-test of the 2017 Mixograph mix peak time (min) analysis with TX12M4063 designated as the P1 parent showed that P1xP2 hybrids had an average mix peak time of 4.25 min while the P2xP1 hybrids had an average mix peak time of 4.36 min, with a p-value of .700 (**Table 6**). This also supports previous conclusion that the 2017 hybrids were made more successfully. Also, the TX12M4063 x LCH13NEDH_11_24 reciprocal hybrids and the TX12M4063 x NE09517_1 reciprocal hybrid appear to have large differences in their mix peak times, with the hybrid reflecting the mix peak time of the female parent (**Table 6**). This would indicate that the reciprocal effect caused by higher levels of self-pollination were only detectable when there was a large difference in the mix peak time (min) of the parents, as was the case with both the LCH13NEDH_11_24 and NE09517_1 parent. As suggested earlier, this concept could potentially be tested by artificially making blends of parental seed to determine the effect that self-pollinated female seed can have on the Mixograph profile.

Next, we evaluated the reciprocal hybrids with NE07531 designated as the P1 parent, which performed in the top 25 % in the 2017 year of our analysis and Easterly (2017) reported had a mean seed count of four seeds per spike per self-bag, meaning there was higher confidence that NE07531 was sterilized and produced true hybrids. Determining if the dough strength of the P1xP2 hybrids reflected the mid-parent value of the parents or NE07531 would help elucidate the nature of the relationship between the

quality of the parents and the quality of the hybrids. The 2017 Mixograph strength x tolerance analysis for the reciprocal hybrids with NE07531 designated as the P1 parent showed that the P1xP2 hybrids (blue) tended to fall between the P1 and P2 parent, reflecting the mid-parent performance (**Fig. 14**). This was especially apparent with the hybrids between parents of opposing strength, i.e. the NE07531 x Goodstreak, NE07531 x Overland, NE07531 x TX09D1172, NE07531 x TX11D3129, and NE07531 x TX12M4063 hybrids. These results suggest that the purer hybrids had dough strength that reflected the mid-parent value, which is what we predicted and what previous research has indicated (McNeal *et al.*, 1968, Morojele and Labuschagne, 2013).

To further our analysis, a paired t-test was set up between the average Mixograph mix peak time (min) of the P1xP2 and the P2xP1 hybrids with NE07531 designated as the P1 parent ($\alpha = .05$). The average mix peak time (min) of the P1xP2 hybrids was 5.09 min with the average of the reciprocal P2xP1 hybrids being 4.35 min on average, indicating the difference between the groups was significantly different with a p-value = .000864 (**Table 7**). The NE07531 x Goodstreak hybrid had a mix peak time close to the mid-parent value, indicating that it was a true hybrid, while its reciprocal Goodstreak x NE07531 had a mix peak time (min) mirroring Goodstreak, indicating it may not have been a pure hybrid. Furthermore, NE07531 x Freeman, NE07531 x Harry, NE07531 x Overland, and NE07531 x Settler_CL had very similar mix peak time values to their reciprocal hybrids, and they that were close to the mid-parent value, suggesting the crosses may have been made successfully. NE07531 x Robidoux had a mix peak time that was a roughly a minute longer than either of the parents, indicating there may have been some heterosis in the cross. The mix peak time (min) of the hybrids NE07531 x

TX09D1172, NE07531 x TX11D3129, and NE07531 x TX12M4063 tended to reflect the mix peak time of NE07531, while the reciprocal crosses had mix peak time (min) values that reflected the mix peak time (min) of the respective female parent used. It is possible that the mean seed count of NE07531 underestimated the amount of unsterilized female in the hybrids. Alternatively, NE07531 could have been successfully sterilized and the maternal effect detected by our analysis could be due to the triploid endosperm, which is composed of 2N maternal and 1N paternal genetic material. Lastly, other agronomic characteristics may have caused the hybridization to be unsuccessful such as different maturity rates. Considering that the TX cultivars and the NE07531 cultivar were developed in different growing regions adapted to different maturity rates, it is reasonable to hypothesize that the hybridization between these cultivars may have failed because they did not nick. The results imply the Mixograph analyses are valuable in making observations about the relationship between the dough strength, tolerance, and mix peak times of hybrids and their parents.

Using the Mixograph strength x tolerance analysis and mix peak time (min) to Evaluate General Inheritance Pattern of Dough Quality for a Yield Trial

Using the guidelines developed by evaluating the TX12M4063 and NE07531 reciprocal hybrids, our next step was to apply the same analytical technique to all the hybrids to get a general idea of how the Mixograph quality indicators were inherited among the hybrids. The hybrids were put into four categories based on the visual performance of the male and female Mixographs; hybrids with two weak parents (weak/weak - coded as red), hybrids with two strong parents (strong/strong - coded as green), hybrids crossed strong female to weak male (strong/weak – coded as blue), and

hybrids crossed weak female to strong male (weak/strong – coded as orange). We hypothesized that the strong/strong hybrids (green) should perform strongly on the analyses and the weak/weak (red) hybrids should perform poorly on the analyses. We also hypothesized the reciprocal hybrids composed of parents with opposing dough strength (strong/weak – blue and weak/strong – orange) should perform similarly. However, the TX12M4063 and NE07531 analyses suggested the hybrid Mixographs could reflect the female parent, indicating either failure to sterilize the female or a maternal influence due to the triploid endosperm.

Using the color-coding system as described, the Mixograph strength x tolerance analysis was performed on the 2016 hybrids by graphing the hybrids with the strength {(%TQ*Min)/grams of protein} on the Y-axis and tolerance to overmixing on the X-axis (unitless). To visualize the relationship between the hybrids and the parents, the parents were included in the analysis with the strong parents coded as purple and the weak parent coded as yellow. The results show the strong/strong hybrids had relatively strong dough strength and tolerance (similar to the strong parents) while the weak/weak hybrids had relatively poor dough strength and tolerance (similar to the weak parents, **Fig 15.1** and **Fig 15.2**). This supported our hypothesis that crossing parents with strong dough strength together would result in strong hybrids and crossing parents with weak dough strength results in weak hybrids. Contrary to the hypothesis, the results also indicated the strong/weak hybrids and reciprocal weak/strong hybrids did not appear to perform similarly and did not overlap, instead, the hybrid quality appeared to be more influenced by the quality of the female parent used in the cross (**Fig 15.1** and **Fig 15.2**). Specifically, the strong/weak hybrids appeared to have relatively strong quality while the weak/strong

hybrids appeared to have relatively weak quality. The 2017 hybrid Mixograph strength x tolerance analysis had similar results; the strong/strong hybrids appeared to have relatively strong quality while the weak/weak hybrids appeared to have relatively weak quality (**Fig 16.1** and **Fig 16.2**). Additionally, the strong/weak and the reciprocal weak/strong hybrids did not appear to overlap, instead the performance of the hybrid tended to reflect the performance of the female parent (**Fig 16.1** and **Fig 16.2**). This suggests there could have been higher levels of self-pollination among the female parents than detected, or the maternal effect on dough strength detected by the Mixograph strength x tolerance analysis could be due to the triploid endosperm. However, it appeared in some cases a single high quality parent can potentially mask end-use quality deficiencies of the other parent in the performance of the hybrid.

Paired t-tests were performed on the 2016 and 2017 hybrids to determine if there was a reciprocal effect on dough strength {(%TQ*Min)/g} and tolerance ($\alpha = .05$). In 2016, hybrids of nine of the 13 cultivars showed a reciprocal effect for dough strength {(%TQ*Min)/g}; including Freeman, Goodstreak, LCH13NEDH-11-24, NE09517_1, Settler_CL, TX10D2063, TX10D2230, TX11D3129, TX12M4063 (**Table 8**). Additionally, hybrids from four of the 13 cultivars did not show a significant reciprocal effect on dough strength; including NE10589, Overland, TX12M4065, and Wesley (**Table 8**). In 2017, hybrids from ten of the 14 cultivars showed a reciprocal effect for dough strength {(%TQ*Min)/g}; including Goodstreak, Harry, LCH13NEDH-11-24, NE07531, NE09517_1, Overland, Settler_CL, TX09D1172, TX10D2063, TX11D3129 (**Table 9**). Also, hybrids from four of the 14 cultivars did not show a significant reciprocal effect in mean dough strength, and those included; Freeman, Robidoux,

TX10D2363, and TX12M4063 (**Table 9**). A significant reciprocal effect for dough strength {(%TQ*Min)/g} was detected among the hybrids from Goodstreak, LCH13NEDH-11-24, NE09517_1, Settler_CL, TX10D2063, and TX11D3129 in both 2016 and 2017. Hybrids from Robidoux and TX10D2363 were not analyzed in the 2016 year, but hybrids from both Freeman and TX12M4063 showed a significant reciprocal effect in 2016 but not in 2017. In terms of tolerance, only hybrids of TX10D2230 and TX12M4063 from 2016 and hybrids from Goodstreak and TX12M4063 from 2017 appeared to have a significant a reciprocal effect (**Table 10** – 2016, **Table 11** – 2017). The small amount of significance is likely due to the large variation in tolerance between the samples. The tolerance is a necessary part of the analysis because it quantifies a critical quality indicator, however, further work must be done to improve the tolerance parameter so more powerful comparisons can be made. Lastly, for each significant difference that was detected, the mean of the P1xP2 hybrids reflected the P1 variety, while the mean of the P2xP1 parents reflected the mean of the P2 parents, indicating that the female influenced the quality of the hybrid more than the male. This has the same implications as the Mixograph strength x tolerance analysis; the maternal effect on quality detected by our analysis could be due to higher levels of self-pollination or it could be the effect of the triploid endosperm.

The paired t-tests of the 2016 and 2017 hybrid Mixograph strength x tolerance analysis verifies the observations that the hybrid quality, when composed of parents with differing quality, tends to be more heavily influenced by the quality of the female than the quality of the male. It appears that the Mixograph strength x tolerance analysis may be a sensitive analytical tool in detecting reciprocal effects in hybrid wheat quality. This

reciprocal effect could be the result of high levels of female self-pollination, either due to some sterilization issues or other agronomic or genetic factors (plant height, nicking, etc.). To the interest of hybrid wheat breeders, this data also indicates that female quality may be more important in determining the quality of the hybrid than male quality, assuming our results aren't solely due to high levels of female self-pollination. In a hybrid wheat breeding program, there are typically many fewer potential male parents than female parents because a good male, in terms of making a hybrid, requires complex floral architecture that is difficult to select for. This belief has led to the philosophy that the program can't afford to have poor quality male parents, restricting the number of potential male cultivars. These results imply that it is possible to hide the poor quality of a cultivar by crossing it as a male to a female with strong quality, giving the breeder more options in parental selection.

Continuing our analysis of the hybrid Mixograph data, the Mixograph mix peak time (min) analysis was also performed on the 2016 and 2017 hybrids to help validate the findings made by the Mixograph strength x tolerance analysis. Hybrids with two strong quality parents were coded as green (strong/strong), hybrids with two weak quality parents were coded as red (weak/weak), hybrids composed of a strong quality female crossed to a weak quality male were coded as blue (strong/weak), and the reciprocal hybrid composed of a weak quality female crossed with a strong quality male were coded as orange (weak/strong). The mix peak time (min) of the hybrids was plotted on the Y-axis against the flour protein (%) on the X-axis. This would allow us to make comparisons between the quality of the hybrids and the parents to be made based on the functionality of the protein.

The results of the 2016 hybrid mix peak time (min) analysis showed that the strong/strong hybrids and the strong/weak hybrids appeared to perform similarly to the strong quality parents, with all but 1 of the hybrids having mix peak times ranging from 3 minutes to 6.24 minutes (**Fig. 17**). The strong/strong hybrids performed as we hypothesized and appeared to have similar mix peak times (min) to the strong parents, while the strong/weak hybrids appeared to outperform the weak/strong reciprocal hybrids. The weak/strong hybrids and the weak/weak hybrids were also grouped together with mix peak times ranging from 1.87 minutes to 3.7 minutes, indicating that the hybrids had similar peak times to the weak parents and performed relatively poorly (**Fig. 17**). In general, the 2016 strong/strong hybrids and strong/weak hybrids had higher mix peak times than the weak/weak hybrids and the weak/strong hybrids. This supports the observations made by the Mixograph strength x tolerance analysis, indicating the female parent had more of an influence over mix peak time (min) than the male parent. The single strong/strong hybrid with poor performance was NE09517_1 x Wesley, indicating a human error may have occurred in the harvesting, processing, sampling, or testing of the seed because both NE09517_1 and Wesley had strong quality with mix peak times of 4.99 minutes and 4.76 minutes, respectively. It is unlikely that the hybrid of two parents with strong quality would inherit relatively poor quality, especially considering the reciprocal Wesley x NE09517_1 reciprocal had a mix peak time of 4.55 minutes.

Paired t-tests were set up between the mean mix peak time (min) of the P1xP2 and P2xP1 reciprocals with respect to each variety (P1) to determine if the reciprocal effect that we observed on the 2016 hybrids was significant ($\alpha = .05$, **Table 12**). The results indicated a significant difference in the mix peak time (min) of the reciprocal

hybrids of 8 of 13 cultivars; including Freeman, Goodstreak, LCH13NEDH-11-24, NE09517-1, Overland, Settler_CL, TX10D2063, and TX12M4063. Additionally, 5 of 13 cultivars showed no significant difference or reciprocal effect between the hybrids; including NE10589, TX10D2230, TX11D3129, TX12M4065, and Wesley (**Table 12**). Of the 8 cultivars that a reciprocal effect was detected, the mean of the P1xP2 hybrids tended to reflect the P1 parent more than the P2 parent, indicating that the mix peak time of the hybrid was more influenced by the female parent used in the cross.

It was then important to see if the 2017 hybrid mix peak time (Min) results supported the findings made in the 2016 hybrids because the sterility data collected by Easterly (2017) indicated that mean seed count per self-bag was 5.7 seeds for the 2016 hybrids and 2.6 seeds per head in 2017 hybrids. The 2017 hybrid Mixograph mix peak time (min) analysis showed similar result to the 2016 hybrids; the strong/strong hybrids and strong/weak hybrids appear to be grouped together and have similar mix peak times (min) to the strong parents, ranging from 3.78 minutes to 8.03 minutes. The weak/weak hybrids and weak/strong hybrids also appear to be grouped together and have similar mix peak times (min) to the weak parents, ranging from 2.47 minutes to 4.79 minutes (**Fig. 18)**. The paired t-test results for the 2017 hybrid mix peak times indicated that all the cultivars except for Harry, Robidoux, TX10D2363, and TX12M4063 exhibited a significant reciprocal effect (**Table 13**). Additionally, of the cultivars that did have a significant reciprocal effect, the mean of the P1xP2 hybrids tended to reflect the P1 parent while the mean of the P2xP1 hybrids tended to reflect the mean of the P2 parents. The Mixograph mix peak time analysis and the strength x tolerance analysis both indicated that hybrids composed of strong parents will have strong quality and hybrids

composed of weak parents will have weak quality, which we hypothesized. Contrary to our hypothesis, there appeared to be a reciprocal effect in some of the hybrids composed of parents with opposing quality, with the quality of the hybrid being more influenced by the quality of the female parent.

Lastly, it was important to identify specific reciprocal pairs that exhibited a significant ($\alpha = .05$) reciprocal effect for Mixograph strength {(%TQ*Min)/grams of protein}, tolerance, and mix peak time. In 2016 the results indicated there was an eight percent reciprocal effect for Mixograph strength {(%TQ*Min)/grams of protein}, a one percent reciprocal effect for tolerance, and an 11 % reciprocal effect for mix peak time (Min, **Table 14**). Specifically, six of 71 reciprocal pairs exhibited a significant ($\alpha = .05$) reciprocal effect for Mixograph strength [{(%TQ*Min)/grams of protein}, **Table 15**], 1 of 71 reciprocal pairs exhibited a reciprocal effect for Mixograph tolerance (**Table 16)**, and 8 of 71 reciprocal pairs exhibited a reciprocal effect for Mixograph mix peak time (Min, **Table 17**). The 2017 results indicated there was an eight percent reciprocal effect for Mixograph strength {(%TQ*Min)/grams of protein}, a one percent reciprocal effect for tolerance, and a six percent reciprocal effect for mix peak time (Min, **Table 18**). Specifically, six of 79 reciprocal pairs exhibited a reciprocal effect for Mixograph strength [{(%TQ*Min)/grams of protein} **Table 19**], one of 79 reciprocal pairs for Mixograph tolerance (**Table 20**), and five of 79 reciprocal pairs for Mixograph mix peak time (Min, **Table 21**).

These results support the implications that the Mixograph analyses used in this study may be sensitive analytical tools in detecting reciprocal effects in hybrid wheat quality. These analyses include the Mixograph strength {(%TQ*Min)/grams of protein} x

tolerance analysis and the Mixograph mix peak time (min) analysis. This data also indicates a parent with poor quality could potentially be masked if it is crossed as a male to a female with strong quality, but this requires further study. The reciprocal effect detected by our analyses could be the result of high levels of self-pollination among the females, or it could be due to the triploid endosperm composed of 2N maternal and 1N paternal genetic material considering it was a maternal reciprocal effect.

Using the Mixograph Analyses to Identify Cultivars that Appeared to be Successfully Sterilized but Exhibited Reciprocal Effect

The next step of our analysis was to isolate the hybrids from cultivars that appeared to be sterilized but still exhibited a reciprocal effect, specifically, hybrids that reflected the quality of female parent regardless of what male parent was used in the cross. Hybrids from Goodstreak were reviewed first because there was a clear pattern when visually assessing the Mixographs that indicated when Goodstreak was used as the female parent, the hybrid Mixograph was nearly identical to the unsterilized parent Goodstreak's Mixograph. This result was interesting because Easterly (2017) indicated that Goodstreak had a mean seed count of 4 seeds per self-bag, showing the CHA was successful. The Mixograph strength x tolerance analysis of the Goodstreak (P1) reciprocals showed that the performance of the P1xP2 hybrids were tightly associated to the Goodstreak parent, indicating all the P1xP2 hybrids had similar, poor quality (**Fig. 19**). This would suggest that even though the CHA sterilization of Goodstreak was successful, there was some other characteristic that made Goodstreak fail as a female parent. Interestingly, the performance of the P2xP1 reciprocal hybrids appear to perform close to the mid-parent value, particularly among the hybrids with a strong quality female

parent (**Fig. 19**). Additionally, the Mixograph strength x tolerance analysis of the 2017 Goodstreak (P1) reciprocals had similar results; the P1xP2 hybrids performed almost identically to Goodstreak while the P2xP1 hybrids appeared to have quality that reflected the mid-parent value, especially in the hybrids where the female parent had strong quality (**Fig. 20**).

A paired t-test between the mean mix peak time (min) of the 2016 and 2017 Goodstreak (P1) reciprocals was conducted to see if the perceived reciprocal effect was significant ($\alpha = .05$). The 2016 results showed that the average mix peak time (min) of the P1xP2 hybrids was 2.05 minutes with a variance of .0090, while the average mix peak time (min) of the P2xP1 hybrids was 3.18 minutes with a variance of .50 (p-value = .000221) , indicating the reciprocal effect detected was significant (**Table 22**). In general, the P1xP2 hybrid Mixographs mix peak times ranged from 1.87 minutes to 2.17 minutes, similar to Goodstreak which had a mix peak time of 1.97 minutes. Focusing on the P2xP1 results, the hybrids where Goodstreak was crossed to a strong quality female resulted in a hybrid that performed near the mid-parent value, indicating that Goodstreak had a negative impact on quality. The results of the paired t-test of the 2017 Goodstreak (P1) reciprocals show the P1xP2 hybrid mean mix peak time was 2.61 minutes with a variance of .0084, and the P2xP1 hybrid mean mix peak time was 3.65 minutes with a variance of .50 (p-value - .00032), indicating a significant effect (**Table 23**). As the variance of the P1xP2 hybrids indicates the performance of these hybrids was almost identical, supporting the observations that hybrids with Goodstreak as the female parent have quality similar to Goodstreak. However, similar to the 2016 results, the 2017 P2xP1

hybrids appeared to perform close to the mid-parent value, particularly when the female parent had strong quality

These Mixograph analyses indicated that in the pool of hybrids analyzed, Goodstreak appeared to nick more effectively when used as the male parent in the hybrid than when it was used as the female parent, even though evidence measured the CHA sterilization of Goodstreak was successful (Easterly, 2017). There must be some other agronomic factor that inhibited hybridization when Goodstreak was used as the female parent, but also made it a good male parent. Speculating, this could be due to the relatively tall plant height of Goodstreak; the pollen from the other male parents did not overcome the height gap to pollinate Goodstreak, resulting in P1xP2 hybrids with a high composition of self-pollinated Goodstreak seed. Alternatively, when used as the male, the tall plant height would put Goodstreak in an advantageous position to pollinate the female parent, resulting in true hybrids. This emphasizes the importance of carefully considering all the physical characteristics of a cultivar when deciding whether to use it as a female or a male, and that the Mixograph strength x tolerance and mix peak time analyses may be able to assist hybrid breeders in making selection decisions. In addition, the results imply that the true hybrid quality performance should lie somewhere between the two parents, while the P1xP2 hybrids indicate that unsuccessful hybrids will tend to reflect the quality of the female parent and exhibit a reciprocal effect.

Reciprocal hybrids from LCH13NEDH_11_24 were also studied because the visual Mixograph of the LCH13NEDH_11_24 parent was very strong and distinctive, which would make its effect on the hybrid easy to observe. Easterly (2017) reported the cultivar had a mean seed count of 3 seeds, indicating the CHA was successful in

sterilizing the hybrids when it was used as a female parent. However, many of the P1xP2 hybrid Mixographs appeared to have a strong resemblance to LCH13NEDH_11_24 (P1). Applying the Mixograph strength x tolerance analysis to the 2016 and 2017 LCH13NEDH_11_24 (P1) reciprocal hybrids helped us objectively make comparisons of the quality of the hybrids to the parents. In general, the P1xP2 hybrids had strong quality and displayed relatively high strength {(%TQ*Min)/grams of protein} values (similar to LCH13NEDH_11_24), but had reduced tolerance in all P1xP2 hybrids, suggesting hybrids were made but the tolerance was more affected than the strength {(%TQ*Min)/grams of protein (**Fig. 21)**. Additionally, the P2xP1 reciprocal hybrids where LCH13NEDH_11_24 was crossed to a weak quality female appeared to all have poor quality and did not perform near the P1xP2 reciprocal, indicating that LCH13NEDH_11_24 may have been a poor male parent. The 2017 Mixograph strength x tolerance analysis of the LCH13NEDH_11_24 (P1) reciprocals showed that in general, the P1xP2 hybrids had strong quality performance relative to the P2xP1 hybrids, especially the P2xP1 hybrids where the P2 parent had weak quality (**Fig. 22**). The LCH13NEDH_11_24 x Goodstreak was a notable hybrid in the 2017 year because it appeared to perform at the mid-parent value for both the tolerance and strength {(%TQ*Min)/grams of protein} and provides strong evidence this was a true hybrid. However, the P1xP2 hybrids appear to have stronger quality than the P2xP1 hybrids, suggesting there was a reciprocal effect.

To supplement our analysis, we used the Mixograph mix peak time (min) of the 2016 and 2017 LCH13NEDH_11_24 reciprocal hybrids to set up a paired t-test between the P1xP2 and P2xP1 reciprocals to determine if there was a significant effect ($\alpha = .05$).

The 2016 results show the mean mix peak time of the P1xP2 hybrids was 5.40 minutes with a variance of .39, and the mean mix peak time of the P2xP1 hybrids was 3.68 minutes with a variance of 1.12 (p-value =0.0028, **Table 24**). This suggests that among the hybrids we analyzed, when LCH13NEDH_11_24 (P1) was used as the female parent, the P1xP2 had significantly longer mix peak times on average than the P2xP1 reciprocals. The 2017 analysis showed the P1xP2 hybrids had a mean mix peak time of 5.91 minutes with a variance of 1.53, and the P2xP1 hybrids had a mean mix peak time of 3.90 minutes with a variance of 1.60 (p-value = .013, **Table 25**). The notable LCH13NEDH_11_24 x Goodstreak hybrid in 2017 had a mix peak time of 3.78 minutes, a mid-parent value that fell closer to Goodstreak (2.43 minutes) than LCH13NEDH_11_24 (8.13 minutes), further indicating this was a purer hybrid.

The Mixograph analyses of the Goodstreak and LCH13NEDH_11_24 reciprocals helped us understand why there appeared to be a reciprocal effect on the quality of our hybrids in the general analysis of all the hybrids. If the Mixograph analyses are sensitive to detecting higher levels of self-pollination, our observations could indicate that similarly to Goodstreak, there was some other factor such as late fertile tillers that biased the results. Additionally, we have provided evidence that our analysis can detect what we believe to be purer hybrids by evaluating the quality performance of the hybrid with respect to the two parents, but these findings will require further work.

Evaluating Reciprocal Hybrids Using the SDS Sedimentation Assay, SDS-SRC Hybrid Assay, and Kernel Hardness

During the SDS sedimentation assay, insoluble glutenin proteins form a gel-like network that settle at various rates determined by the functionality and quantity of protein

in the sample. In that sense, not all sedimentation values are created equal. Consider two samples (A and B) that both have a sedimentation value of 15 mL, but sample A has 11% protein content and sample B has 15% protein content. We would conclude that the gel-like mesh of glutenins formed by sample A was stronger than that formed by sample B, indicating better quality protein. The SDS-SRC hybrid assay is like the SDS sedimentation assay in that it uses the same reagents (minus the dye) to form an insoluble glutenin network, but it differs in that an additional centrifugation step is added to force those glutenins into a pellet. The moisture value % is determined by the amount of moisture retained by the pellet and was shown by Seabourn *et al.* (2012) to be positively correlated with loaf volume. They also concluded that poor protein quality could be masked with high protein quantity, so to avoid this, all our sedimentation values and weight values were also considered with protein content

Although it was determined that the Mixograph analyses were more representative than the SDS sedimentation, the SDS-SRC hybrid assay, and the SKCS, it was still important to see if the same observations of quality inheritance can be made using these other assays. The hybrids were color-coded using the same system described in the Mixograph analyses. The SDS sedimentation assay results are graphed with the sedimentation value (mL) on the Y-axis and the grain protein % on the X-axis. Samples that are at or above the green dotted line are considered strong, while samples at or below the red dotted line are considered weak, and samples in the middle are neither. The 2016 hybrids SDS sedimentation data showed that nearly all of the strong/strong and strong/weak hybrids are at or above the green dotted line, which reflected the quality of the strong parents (**Fig. 23**). Also, most of the weak/weak and weak/strong hybrids fell at or below the red

dotted line, with only a handful of samples falling in the space between the dotted lines. These results supported our previous observations on the Mixograph data in that the strength of the hybrids appears to reflect or be influenced more by the quality of the female parent than the male parent.

Paired t-tests were conducted on the 2016 hybrids to determine if there was a significant reciprocal effect ($\alpha = .05$). These results showed that the reciprocals from eight of the 13 parents; including Freeman, LCH13NEDH-11-24, NE09517-1, Settler CL, TX10D2230, TX11D3129, TX12M4063, and TX12M4065 exhibited a significant reciprocal effect in the SDS sedimentation ratio (**Table 26)**. In every case there was a significant p-value, the mean of the hybrids were always closer to the female parent indicating that the female parent was having a greater impact on end-use quality than the male parent. Also, hybrids from five (Goodstreak, NE10589, Overland, TX10D2063, and Wesley) of the 13 parents did not show a significant reciprocal effect (**Table 26**). It was notable that there was not a detectable difference in the SDS sedimentation data for Goodstreak, even though there were major reciprocal effects detected by the Mixograph.

When evaluating the 2017 hybrids SDS sedimentation results, the strength of the hybrids appeared to be stronger compared to the 2016 hybrids, similar to the parent analyses (**Fig. 24**). While the quality in general seems better in 2017, there is still a clear grouping between the strong/strong and the strong/weak hybrids and the strong parents, as well an association between the weak/weak and weak/strong hybrids and the weak parents. These results were similar to the 2016 hybrid SDS sedimentation analysis results and the 2016 and 2017 hybrid Mixograph data, again suggesting there was a female impact on the hybrid quality. It is also important to mention that the strong/weak (blue)

and weak/strong (orange) hybrids appear to overlap more in the 2017 analysis than in the 2016 analysis, supporting the observations for grain yield made by Easterly (2017).

The paired t-tests of the 2017 hybrids were set up to detect a difference between the means of the reciprocal hybrids ($\alpha = .05$) and determined that hybrids from seven (Goodstreak, LCH13NEDH-11-24, NE07531, NE09517_1, TX09D1172, TX11D3129, and TX12M4063) of the 14 parents showed a significant reciprocal effect (**Table 27)**. The hybrids from the seven other parents (Freeman, Harry, Overland, Robidoux, Settler-_CL, TX10D2063, and TX10D2363), did not show any reciprocal effect on the SDS sedimentation assay (**Table 27**). Considering both years, hybrids from LCH13NEDH-11-24, NE09517-1, TX11D3129, and TX12M4063 showed a significant reciprocal effect for the sedimentation ratio. Additionally, in every case there was a significant reciprocal effect, the mean of the sedimentation ratio of the hybrids was always closer to the female parent indicating a maternal influence on hybrid quality. These results supported the previous findings made using the Mixograph analyses.

The graphical analysis of the SDS-SRC hybrid assay for the 2016 hybrids showed that the hybrids with strong female parents were grouped together and generally reflected the quality of the strong parents (**Fig. 25**). In contrast, the hybrids with weak female parents were grouped together and reflected the quality of the weak parents (**Fig. 25**). The blue and orange dots were reciprocal hybrids that should be overlapping if there were no maternal effects, but the graph appears to be showing a reciprocal effect where the female parent is having a greater influence on quality than the male parent. This supports the findings of the mixograph and the SDS sedimentation assays. The paired t-tests ($\alpha = .05$) for reciprocal hybrids found that hybrids from four (LCH13NEDH_11_24, NE09517_1,

TX12M4063, and TX12M4065) of the 13 parents had significant reciprocal effects (**Table 28**). Hybrids from the remaining nine (Freeman, Goodstreak, NE10589, Overland, Settler _CL, TX10D2063, TX10D2230, TX11D3129, and Wesley) of 13 parents did not show significant reciprocal effect.

In 2017, the graphical analysis shows that the hybrids are more integrated and overlapping than the 2016 hybrids, which was expected based on previous observations (**Figure 26**). Again, paired t-tests ($\alpha = .05$) were set up to determine if there was reciprocal effect between mean weight value of the hybrids for each variety. The results showed that hybrids from five (LCH13NEDH-11-24, Overland, TX10D2363, TX12M4063, and TX11D3129) of the 14 parents had a reciprocal effect (**Table 29**). Hybrids from the other nine (Freeman, Goodstreak, Harry, NE07531, NE09517-1, Robidoux, Settler-CL, TX09D1172, TX10D2063, and TX11D3129) parents did not exhibit a reciprocal effect among the hybrids of these cultivars. Interestingly, a significant reciprocal effect was detected in the hybrids from both LCH13NEDH-11-24 and TX12M4063 in both 2016 and 2017.

We also investigated how the physical kernel traits of the hybrids related to the parents, specifically to see if there was a reciprocal effect on any of the kernel traits. Paired t-tests ($\alpha = .05$) were set up on the 2016 hybrids to determine if there was a reciprocal effect on the hardness. The results showed that there was a significant difference between the hybrids of six (Freeman, Goodstreak, Overland, TX10D2230, TX11D3129, TX12M4065) of the 13 cultivars analyzed (**Table 30**), indicating a potential reciprocal effect. Alternatively, the reciprocal hybrids from the other seven (LCH13NEDH-11-24, NE09517-1, NE10589, Settler-CL, and TX10D2063,

TX12M4063, and Wesley) of the 13 parents were not significantly different (**Table 30**). Additionally, the hybrids that exhibited a reciprocal effect tended to reflect the hardness of the female parent. This is especially apparent between the hybrids when the softest (TX11D3129 and Freeman) and hardest (TX10D2230 and TX12M4065) cultivars were used as the female parent in the cross. For example, the average kernel hardness between the hybrids where TX11D3129 (HI = 59.59) was used as the female parent was 62.92, but the average hardness between the hybrids when it was used as the male parent was 79.38 (**Table 31**).

Identical paired t-tests ($\alpha = .05$) were performed on the 2017 hybrids to determine if there was a reciprocal effect detected on kernel hardness. The results showed that hybrids from four (Freeman, TX09D1172, TX11D3129, and TX12M4063) of the 14 cultivars evaluated showed a reciprocal effect (**Table 23**). The hybrids from the remaining ten cultivars (Goodstreak, Harry, LCH13NEDH-11-24, NE07531, NE09517-1, Overland, Robidoux, Settler-CL, TX10D2063, and TX10D2363) were not significantly different (**Table 23**). Interestingly, Freeman and TX11D3129 were the softest parent cultivars of both years, and in both years our results showed that there was a significant reciprocal difference between hardness of their hybrids. Furthermore, the average kernel hardness of the hybrids when Freeman and TX11D3129 were used as the female parent in the cross were significantly softer, suggesting that the female parent could have a greater impact on kernel hardness. These results support the previous findings of the Mixograph analyses that that the reciprocal effect could be due to high levels of self-pollination or could be the result of the genetic composition of the triploid endosperm. The SKCS calculates hardness based on an average of 100 kernels, and if there are more

self-pollinated female kernels in the hybrid, then this would cause the average hardness of the hybrid to reflect the hardness of the female.

CONCLUSION

The goal of this project was to analyze hybrid wheat cultivars produced by Easterly (2017) to determine how the quality of those hybrids compared to the parents, i.e. if the quality of the hybrid was better than (high parent heterosis), worse than (low parent heterosis), similar to the parents or intermediate between the parent used in the cross. We hypothesized that hybrids produced by two strong quality parents would be strong, hybrids produced by two weak quality parents would be weak, and hybrids produced by a weak and strong parent would have intermediate quality if the additive gene model was correct. Additionally, we hypothesized that there should be no difference in quality performance between reciprocal hybrids (P1xP2 = P2xP1).

First, the top performing 25 % and bottom performing 25 % of parents based on quality were determined by visually assessing the parent Mixographs for dough strength and tolerance to overmixing. Then, we developed objective analytical tools to evaluate the Mixographs by comparing our visual assessment to important quality indicators provided by the Mixsmart ® data collection software. These Mixograph analyses were called the strength x tolerance analysis and the mix peak time analysis and were critical in allowing us to make important, objective comparisons between the quality of the parents and the quality of the reciprocals. We initially evaluated the reciprocals of TX12M4063 (mean seed count = 27) and NE07531 (mean seed count = 4) using the Mixograph analyses to get a better understanding of how quality is inherited when CHA sterilization is not or is effective. In the case of TX12M4063, the results of the Mixograph analyses indicated that when CHA sterilization is ineffective, the quality of the P1xP2 hybrid will reflect the P1 (female) parent. In contrast, the Mixograph analyses

of the NE07531 reciprocals indicated the quality of the P1xP2 hybrid should reflect the mid-parent quality performance, although a reciprocal effect was still detected among the NE07531 reciprocals.

Using the guidelines developed evaluating the TX12M4063 and NE07531 reciprocal hybrids, the general performance of the hybrids were evaluated using the same Mixograph analyses. The results supported our hypothesis that hybrids with two strong quality parents will have strong quality, and hybrids with two weak quality parents will have weak quality. However, some of the hybrids composed of parents with opposing quality exhibited a reciprocal effect, resulting in hybrids whose quality reflected the quality of the female parent used in the cross. In 2016, 6 of 71 reciprocal pairs exhibited a significant ($\alpha = .05$) reciprocal effect for Mixograph strength {(%TQ*Min)/grams of protein}, 1 of 71 reciprocal pairs exhibited a reciprocal effect for Mixograph tolerance, and 8 of 71 reciprocal pairs exhibited a reciprocal effect for Mixograph mix peak time (min). In 2017, 6 of 79 reciprocal pairs exhibited a reciprocal effect for Mixograph strength {(%TQ*Min)/grams of protein}, 1 of 79 reciprocal pairs for Mixograph tolerance, and 5 of 79 reciprocal pairs for Mixograph mix peak time (min). These results were contrary to what we hypothesized and indicated that there were some reciprocal effects, although, they were detected at a low frequency. The maternal effects detected by the Mixograph analyses could be due to higher rates of self-pollination than initially thought, or it could be due to the genetic composition of the triploid endosperm.Also, it appeared in some cases a single high quality parent can potentially mask end-use quality deficiencies of the other parent in the performance of the hybrid, but this need further work to determine. This indicates that although these analyses have potential to be

powerful analytical tools in evaluating Mixographs, they require further experimentation to make better comparisons about the quality inheritance in hybrid wheat.

Although the Mixograph analyses were not sensitive enough to make significant statements about hybrids with similar quality, the Mixograph analyses of the reciprocals from the strongest (LCH13NEDH_11_24) and weakest (Goodstreak) helped us make valuable observations. The Mixograph analyses of the Goodstreak reciprocals indicated that when Goodstreak was used as the female (P1), the hybrids were very similar in quality to Goodstreak, even Easterly (2017) concluded Goodstreak was successfully sterilized with the CHA. This suggests that Goodstreak may have other agronomic characteristics that make it a difficult to use as a female parent but also indicated it produced true hybrids when used as a male parent. The LCH13NEDH_11_24 reciprocal hybrids also provided us with an example of a variety that appeared to be sterilized by the CHA but still exhibited a reciprocal effect. The analyses were likely able to detect this effect with these parents more easily because they were at the most extreme ends of quality, and these effects would not have been detectable with the majority of hybrids whose parents were of similar quality.

REFERENCES

A. E. Walker and C. E. Walker. 2004. Documentation and User's Instructions for MIXSMART® for Windows: Computerized Data Acquisition and Analysis For the Mixograph.

Ahmad M. 2000. Molecular marker-assisted selection of HMW glutenin alleles related to wheat bread quality by PCR-generated DNA markers. *Theor. App. Genet.* **101**:892-896.

American Association of Cereal Chemists, 2010. Approved Methods of the AACC (St. Paul, MN, USA).

Ammar K., Lukaszewski A. J., Banowetz G.M. 1997. Effect of *Glu*-D1(5+10) on gluten strength and polymeric protein composition in durum wheat. *Cereal Foods World* **42:**610.

Bahl P. N., Maan S. S. 1973. Chromosomal location of fertility-restoring genes in six lines of common wheat. *Crop Science*. **13**: 317-320.

Blakeney A. B., Cracknell R. L., Crosbie G. B., Jefferies S. P., Miskelly D. M., O'Brien L., Panozzo J. F., Suter D. A. I., Solah S., Watts T., Westcott T., Williams R. M. 2009. Understanding Australian Wheat Quality. Grains Research and Development Corporation.

Boeven H. G., Philipp & Würschum, Tobias & Rudloff, Julia & Ebmeyer, Erhard & Longin, Friedrich. 2018. Hybrid seed set in wheat is a complex trait but can be improved indirectly by selection for male floral traits. *Euphytica*. 214. 110. 10.1007/s10681-018-2188-1.

Boeven, P. H. G., Longin, C. F. H., Leiser, W. L. et al. 2016. Genetic architecture of male floral traits required for hybrid wheat breeding. *Theoretical and Applied Genetics* **129**: 2343-2357. https://doi.org/10.1007/s00122-016-2771-6.

Borghi B, Perenzin M. 1994. Diallel analysis to predict heterosis and combining ability for grain yield, yield components and bread-making quality in bread wheat (T. aestivum). *TAG Theoretical and Applied Genetics* **89**, 975-981.

Branlard G., Darvedet M., Saccomano R., Lagoutte F., Gourdon J. 2001. Genetic diversity of wheat storage proteins and bread wheat quality. *Euphytica*. **119:**59-67.

Bushuk W., Zillman R. R. 1978. Wheat Cultivar Identification by Gliadin Electrophoregrams. 1. Apparatus, Method, and Nomenclature. *Canadian Journal of Plant Science*. **58**(2): 505-515. https://doi.org/10.4141/cjps78-076.

Butow B. J., Ma W., Gale K. R., Cornish G. B., Rampling L., Larroque O., Morell M. K., Bekes F. 2003. Molecular discrimination of Bx7 alleles demonstrates that a highly expressed high-molecular-weight glutenin allele has a major impact on wheat dough flour strength. *Theor. Appl. Genet.* **107**(8):1524-1532. doi:10.1007/s00122-003-1396-8.

Cockerham C. C. 1961. Implications of Genetic Variances in a Hybrid Breeding Program. *Crop Science.* **1**:**47-52**.

Cooper J. K., Stromberger J. A., Morris C. F., Bai G., Haley S. D. 2016. End-Use Quality and Agronomic Characteristics Associated with the *Glu-B1al* High-Molecular-Weight Glutenin Allele in U.S. Hard Winter Wheat. *Crop Science*. **56**:2348-2353. doi:10.2135/cropsci2015.10.0610.

Cornish G. B., Bekes F., Allen H. M., Martin J. M. 2001. Flour proteins linked to quality traits in an Australian doubled haploid wheat population. *Aust. J. Agric. Res.* **52:**1339-1348.

C. W. Brabender ® Instruments, Inc. C. W. Brabender Instruments, Inc., South Hackensack, NJ.

De Block, M., Debrouwer, D. and Moens, T. 1997. The development of nuclear male sterility system in wheat. Expression of the barnase gene under the control of tapetum specific promoter. *Theor Appl Genet* (1997) **95**: 125-131 https://doi.org/10.1007/s001220050540.

de Vries A. 1971. Flowering biology of wheat, particularly in view of hybrid seed production – a review. *Euphytica*. **20**:152-170.

Dixon L., Bencivenga S., Boden S. 2018. A new opening for wheat seed production. *Journal of Experimental Biology*. **69**(3):341-343.

Driscoll, C. J. 1985. Modified XYZ system of producing hybrid wheat. *Crop Sci.* **25:** 115-116.

Driscoll, C. J. 1977. Registration of Cornerstone male-sterile wheat germplasm. *Crop Sci.* **17:** 190.

Driscoll, C. J. 1972. X Y Z System of Producing Hybrid Wheat. *Crop Sci*. **12**: 516-517. doi:10.2135/cropsci1972.0011183X001200040039x.

Dumas, C. and Rogowsky P. 2008. Fertilization and early seed formation. *Comptes Rendus Biologies*. **331**(10):715-725. https://doi.org/10.1016/j.crvi.2008.07.013.

Easterly A. 2017. Developing Hybrid Wheat for the Great Plains: Addressing Hybridization, Heterosis, and the Heterotic Pool Development. Ph.D. Dissertation University of Nebraska – Lincoln.

EFSA. 2010. Conclusion on the peer review of the pesticide risk assessment of the active substance sintofen. *European Food Safety Authority Journal.* 8, 1931.

FAO. 2015. FAO statistical pocketbook. World food and agriculture 2015. Rome: Food and Agricultural Organization of the United States.

Food and Agriculture Organization (FAO) of the United Nations, Declaration of the World Summit on Food Security, Rome, 16-18 November 2009 (www.fao.org/wsfs/world-summit/en/).

Gaines C. S., Finney P. F., Fleege L. M., and Andrews L. C. 1996. Predicting a Hardness Measurement Using the Single-Kernel Characterization System. *Cereal Chem.* **73**(2):278-283.

Gale K. R. 2005. Diagnostic DNA markers for quality traits in wheat. *J cereal Sci.* **41**: 181-192.

Garst N. 2017. Assessing Anther Extrusion and its Effect on US Hard Winter Wheat (Triticum aestivum L.) Hybrid Seed Production. University of Nebraska-Lincoln.

Giroux M. J., Morris C. F. 1998. Wheat grain hardness results from highly conserved mutations in the friabilin components puroindoline a and b. *Proc. Natl. Acad. Sci.* **95:**6262-6266.

Goutam U., Kukreja S., Tiwari R., Chaudhury A., Gupta R. K., Dholakia B. B., Yadav R. 2013. Biotechnological approaches for grain quality improvement in wheat: Present status and future possibilities. *Australian Journal of Crop Science*. **7**(4):469-483.

Great Western Manufactruing Co. Inc. Great Western Manufactruing Co. Inc., Leavenworth, KS.

Gross C., Bervas E., Chanliaud G., Gharmet G. 2007. Genetic analysis of bread making quality scores in bread wheat using a recombinant inbred line population. *Theor. Appl. Genet.* **115**: 313-323.

Gupta R. B., Be´ke´s F., and Wrigley C. W. 1991. Prediction of physical dough properties from glutenin subunit composition in bread wheats: Correlation study. *Cereal Chem*. **68:**328–333.

Gupta R. B. and MacRitchie F. 1994. Allelic variation at glutenin subunit and gliadin loci *Glu*-1, *Glu*-3 and *Gli*-1 of common wheats. 2. Biochemical basis of the allelic effects on dough properties. *J. Cereal Sci.* **19:**19-29. **Gupta R. B., and Shepherd K.W.** 1993.

Production of multiple wheat-rye 1RS translocation stocks and genetic analysis of LMW subunits of glutenin and gliadins in wheats using these stocks. *Theor. Appl. Genet.* **85:**719-728.

Gupta R. B., Popineau Y., Lefebvre J., Cornec M., Lawrence G. J., and MacRitchie F. 1995. Biochemical basis of flour properties in bread wheats. II. Changes in polymeric protein formation and dough/gluten properties associated with the loss of low M_r or high M_r glutenin subunits. *J. Cereal Sci*. **21:**103–116.

Gupta R. B. and Shepherd K. W. 1988. Low molecular weight glutenin subunits in wheat: Their variation, inheritance and association with breadmaking quality. In "Proceedings of the 7th International Wheat Genetics Symposium," pp. 943-949. Institute of Plant Science Research, Cambridge.

Gupta R. B., Singh N. K., and Shepherd N. K. 1989. The cumulative effect of allelic variation in LMW and HMW glutenin subunits on dough properties in progeny of two bread wheats. *Theor. Appl. Genet.* **77:**57-64.

Guzman C., Pena R., Singh R., Autrique E., Dreisigacker S., Crossa J., Rutkoski J., Poland J., Battenfield S. 2016. Wheat quality improvement at CIMMYT and the use of genomic selection on it. *Applied and Translational Genomics*. **11:**3-8. doi:10.1016/j.atg.2016.10.004.

Halford N. G., Field J. M., Blair H., Urwin P., Moore K., Robert L., Thompson R., Flavell R. B., Tatham A. S., and Shewry P. R. 1992. Analysis of HMW glutenin subunits encoded by chromosome 1A of bread wheat (Triticum aestivum L.) indicates quantitative effects on grain quality. *Theor. Appl. Genet*. **83:**373–378.

Hanson M. R., Bentolila S. 2004. Interactions of Mitochondrial and Nuclear Genes that Affect Male Gametophyte Development. *The Plant Cell*. **16 [suppl 1]:**154-169 doi:10.1105/tpc.015966.

Horn R. 2006. Recombination: cytoplasmic male sterility and fertility restoration in higher plants: In: Esser K., Luttge U., Beyschlag W., Murata J., eds. *Progress in Botany*. **67**. Berlin: Springer, 31-52.

Jiang Y., Schmidt R. H, Zhao Y., Reif J. C. 2017. A quantitative genetic framework highlights the role of epistatic effects for grain-yield heterosis in bread wheat. *Nature genetics*. **49**(12):1741-1746. doi: 10.1038/ng.3974.

Kaul M. L. H. 1988. Male sterility in higher plants. In: Frankel R., Grossman M., Maliga P., eds. *Monographs*. *Theoretical and applied genetics.* **10**. Berlin, Heidelberg, New York: Springer, 775-797.

Kempe K., Rubtsova M., Gils M. 2014. Split-gene system for hybrid wheat seed production. *Proc. Natl. Acad. Sci. USA*. **111**(25):9097-9102. https://doi.org/10.1073/pnas.1402836111

Khan M. N., Heyne E. G., Arp A. L. 1973. Pollen distribution and seed set on *Triticum aestivum* L. *Crop Science.* **13:**223-226.

Kihara H. 1951. Substitution of nucleus and its effects on genome manifestation. *Cytologia.* **16**:177-193.

Killermann B., and Zimmermann G. 2000. Relationship between allelic variation of *Glu*-1, *Glu*-3 and *Gli*-1 prolamin loci and baking quality in doubled haploid wheat populations. In ‘‘Wheat Gluten’’ (P. R. Shewry and A. S. Tatham, eds.), pp. 66–70. *Royal Society of Chemistry*, Cambridge.

Kolster P., Van Eeuwijk F. A., Van Gelder W. M. J. 1991. Additive and epistatic effects of allelic variations at the high molecular weight glutenin subunit loci determining the bread making quality of breeding lines of wheat. *Euphytica.* **55**:227-285.

Langer M., Simon & Longin, Friedrich & Würschum, Tobias. 2014. Phenotypic evaluation of floral and flowering traits with relevance for hybrid breeding in wheat (Triticum aestivum L.). *Plant Breeding*. 133. 10.1111/pbr.12192.

Lawrence G. J., MacRitchie F., and Wrigley C. W. 1988. Dough and baking quality of wheat lines deficient in glutenin subunits controlled by the *Glu*-A1, *Glu*-B1 and *Glu*-D1 loci. *J. Cereal Sci*. **7:**109–112.

Lawrence G. J. and Shepherd K. W. 1980. Variation in glutenin protein subunits of wheat. *Aust. J. Biol. Sci*. **33:**221–233.

Liu G., Zhao Y., Gowda M., Longin C. F. H., Reif J. C., Mette M. F. 2016 Predicting Hybrid Performances for Quality Traits through Genomic-Assisted Approaches in Central European Wheat. *PLoS ONE* **11**(7): e0158635. https://doi.org/10.1371/journal.pone.0158635

Ljubicic N., Petrovic S., Kostić M., Dimitrijevic M., Hristov N., Kondic A., Jevtic R. 2017. Diallel analysis of some important grain yield traits in bread wheat crosses. *Turkish Journal of Field Crops*. 22. 1-1. 10.17557/tjfc.297681.

Longin, C. F. H., Gowda, M., Mühleisen, J. et al. 2013. Hybrid wheat: quantitative genetic parameters and consequences for the design of breeding programs. *Theor Appl Genet* **126**: 2791. https://doi.org/10.1007/s00122-013-2172-z

Longin C. F. H., Muhleisen J., Maurer H. P., Zhang H., Gowda M., Reif J. C. 2012. Hybrid breeding in autogamous cereals. *Theoretical and Applied Genetics*. **125**(6):1087-1096.

Lorenzo A., Kronstad W. E., and Vieira L. G. E. 1987. Relationships between high molecular weight glutenin subunits and loaf volume in wheat as measured by sodium dodecyl sulphate sedimentation test. *Crop Sci*. **27:**253–257.

Luo C., Griffin W. B., Branlard G., and McNeil D. L. 2001. Comparison of low- and high molecular weight glutenin allele effects on flour quality. *Theor. Appl. Genet.* **102**:1088–1098.

MacRitchie F., duCros D. L., and Wrigley C. W. 1990. Flour polypeptides related to wheat quality. *Adv. Cereal Sci. Technol*. **10**:79–145.

Martin A. C., Atienza S., Ramirez M., Barro F., Martin A. 2008. Male fertility restoration of wheat in *Hordeum chilense* cytoplasm is associated with 6HchS chromosome addition. *Australian Journal of Agricultural Research* **59**, 206-213.

Martinant J. P., Nicolasa Y., Bouguennec A., Popineaub Y., Saulnierc L., Branlard G. 1998. Relationships between mixograph parameters and indices of wheat grain quality. *J. Cereal Sci.* **27**: 179-189.

Masci S., Rovelli L., Kasarda D. D., Vensel W. H., and Lafiandra D. 2002. Characterization and chromosomal localization of C-type low-molecular-weight glutenin subunits in the bread wheat cultivar Chinese Spring. *Theor. Appl. Genet.* **104:**422-428.

McNeal F. H., Berg M. A., Watson C. A. 1968. Reciprocal Crosses and Their Influence on Wheat Quality. *Crop Sci*. **8**:485-487. doi:10.2135/cropsci1968.0011183X000800040027

Metakovsky E. V., Annichiarico P., Boggini G., and Pogna N. E. 1997b. Relationships between gliadin alleles and dough strength in Italian common wheats. *J. Cereal Sci.* **25:** 229–236.

Metakovsky E. V., Felix I., and Branlard G. 1997c. Association between dough quality (W value) and certain gliadin alleles in French common wheat cultivars. *J. Cereal Sci.* **26:**371–373.

Mette M. F., Gils M. Longin C. F. H., Reif J. C. 2015. Hybrid breeding in wheat. In: Ogihara Y., Takumi S., Handa H., eds. Advances in wheat genetics: from genome to field. Tokyo: Springer.

Moonen J. H. E., Scheepstra A., and Graveland A. 1982. Use of SDS-sedimentation test and SDS-polyacrylamide gel electrophoresis for screening breeders' samples of wheat for breadmaking quality. *Euphytica.* **31:**677–690.

Morojele M. E., Labuschagne M. T. 2013. Heterotic Performance of Quality Characteristics of Bread Wheat Cultivars. *African Crop Science Journal*. **21**(4):283-289.

Morris C. F. 2002. Puroindolines: the molecular genetic basis of wheat grain hardness. *Plant Mol. Biol.* **48**: 633-647.

National Manufacturing Company. National Manufacturing Company., Lincoln, NE.

Okada T, Jayasinghe JEARM, Nansamba M, et al. 2018. Unfertilized ovary pushes wheat flower open for cross-pollination. *Journal of Experimental Botany*. **69**(3):399-412. doi:10.1093/jxb/erx410.

Pasha I., Anjum F. M., Morris C.F. 2010. Grain Hardness: A Major Determinant of Wheat Quality. *Food Sci Tech Int.* **16**:512-522.

Payne P. I. 1987. Genetics of wheat storage proteins and the effect of allelic variation on bread-making quality. *Annu Rev Plant Physiol.* **38**:141-153

Payne P. I., Corfield K. G., and Blackman J. A. 1979. Identification of a high-molecular weight subunit of glutenin whose presence correlates with breadmaking quality in wheats of related pedigree. *Theor. Appl. Genet*. **60:**229–236.

Payne P. I., Corfield K. G., Holt L. M., and Blackman J. A. 1981b. Correlations between the inheritance of certain high-molecular weight subunits of glutenin and breadmaking quality in progenies of six crosses of bread wheat. *J. Sci. Food Agric*. **32:**51–60.

Payne P. I., Holt L. M., Harinder K., McCartney D. P., and Lawrence G. J. 1987b. The use of near-isogenic lines with different HMW glutenin subunit composition in studying breadmaking quality and glutenin structure. In ‘‘Proceedings of the 3rd International Gluten Workshop’’ (R. D. Lasztity and F. Be´ke´s, eds.), pp. 216–226.

Payne P. I., Jackson E. A., Holt L. M. 1984. The association between γ-gliadin 45 and gluten strength in durum wheat cultivars: a direct causal effect or the result of genetic linkage. *J. Cereal Sci.* **2:**73-81

Payne P. I., and Lawrence G. J. 1983. Catalogue of alleles for the complex gene loci, Glu-A1, Glu-B1 and Glu-D1 which code for high-molecular-weight subunits of glutenin in hexaploid wheat. *Cereal Res. Commun*. **11:**29–35.

Payne P. I., Nightingale M. A., Krattiger A. F., and Holt L. M. (1987a). The relationship between the HMW glutenin subunit composition and the breadmaking quality of British-grown wheat cultivars. *J. Sci. Food Agric.* **40:**51–65.

Payne P. I., Seekings J. A., Worland A. J., Jarvis M. G., and Holt L. M. 1987c. Allelic variation of glutenin subunits and gliadins and its effects on breadmaking quality in wheat: Analysis of F_5 progeny from ‘‘Chinese Spring’’ ‘‘Chinese Spring (Hope 1A).’’ J. Cereal Sci. 6, 103–118.

Perten Instruments North America Inc. Perten Instruments North America., Inc., Springfield, IL.

Pickett A. A. 1993. Hybrid wheat: Results and problems. Adv. Plant Breed., Suppl. J. Plant Breed. 15, 1-259.

Pogna N. E., Autran J. C., Mellini F., Lafiandra D., Feillet P. 1990. Chromosome 1B encoded gliadins and glutenin subunits in durum wheat: Genetics and relationship to gluten strength. *J. Cereal Sci.* **11**:15-34

Pogna N. E., Boggini G., Corbellini M., Cattaneo M., Dal Belin Peruffo A. 1982. Association between gliadin electrophoretic bands and quality in common wheat. *Can. J. Plant Sci.* **4**:913-918.

Pogna N. E., Lafiandra D., Feillet P., Autran J.-C. 1988. Evidence for direct causal effect of low molecular weight subunits of glutenins on gluten viscoelasticity in durum wheats. *J Cereal Sci.* **7:**211-214.

Potrykus, I. (2010) Lessons from the 'Humanitarian Golden Rice' project: regulation prevents development of public good genetically engineered crop products. *NewBiotechnology.* **27**(5) pp. 466-472.

Rodriguez-Quijano M. and Carrillo J. M. 1996b. Relationship between allelic variation of *Glu-1* and *Gli-1/Glu-3* prolamin loci and gluten strength in hexaploid wheat. *Euphytica.* **91:**141-148.

Rousset M., Carrillo J. M., Qualset C. O., Kasarda D. D. 1992. Use of recombinant inbred lines of wheat for study of associations of high-molecular-weight glutenin subunit alles to quantitative traits. 2. Milling and bread-baking quality. *Theor. Appl. Genet.* **83:**403-412.

Sarkar B., and Roy B. 2013. Achievement and Letdown in Hybrid Wheat. 241-262.

Seabourn B. W., Xiao Z. S., Tilley M., Herald T. J., Park S. 2012. A Rapid, Small-Scale Sedimentation Method to Predict Breadmaking Quality of Hard Winter Wheat. *Crop Science.* **52**:1306-1315. doi:10.2135/cropsci2011.01.0210.

Shewry P. R., Nigel H., Domenico L. (2003). Genetics of Wheat Gluten Proteins. Advances in genetics. 49. 111-84. 10.1016/S0065-2660(03)01003-4.

Shull G. H. 1948. What is" heterosis"? Genetics **33**, 439.

Singh S. K., Chatrath R., Mishra, B. 2010. Perspective of hybrid wheat research: A review. Indian Journal of Agricultural Sciences. 80. 1013-27.

Sleper D. A. and Poehlman J. M. 2006. Breeding Field Crops. Blackwell Publishing, Iowa.

Sozinov A. A. and Poperelya F. A. 1980. Genetic classification of prolamines and its use for plant breeding. *Ann. Technol. Agric.* (Paris) **29:**229-245.

Sozinov A. A. and Poperelya F. A. 1982. Polymorphism of prolamines and variability of grain quality. *Qualitas Planatarum Plant Foods Num. Nutr.* **31:**243-249.

Tester M., Langridge P., 2010. Breeding technologies to increase crop production in a changing world. *Science*. **327**(5967):818-822.

UDY Corporation. UDY Corporation., Fort Collins, CO.

Whitford R., Fleury D., Reif J. C., Garcia M., Okada T., Korzun V., Langridge P. 2013. Hybrid breeding in wheat: technologies to improve hybrid wheat seed production. *Journal of Experimental Botany.* **64**: 5411–5428.

Wilson J. A., Ross W. M. 1962. Male sterility interaction of *Triticum aestivum* nucleus and *Triticum timopheevi* cytoplasm. *Wheat Information Service*. **14**:415-421

Wrigley C. W., Robinson P. J., Williams W. T. 1982. Association between individual gliadin proteins and quality, agronomic and morphological attributes of wheat cultivars. *Aust. J. Agric. Res.* **33:**409-418.**Yuan Q., Deng Z., Peng T., Tian J.** 2012. QTL-based analysis of heterosis for number of grains per spike in wheat using DH and immortalized F2 populations. *Euphytica*. **188:**387-395.

Zeleny L., Greenaway W. T., Gurney G. M., Fifield C. C., and Lebsock K. 1960. Sedimentation value as an index of dough-mixing characteristics in early-generation wheat selection. *Cereal Chem.* **37:**673-678.

Zhang X., Hirsch C., Sekhon R., de Leon N., Kaeppler S. 2016. Evidence for maternal control of seed size in maize from phenotypic and transcriptional analysis. *J. of Exp Bot*. **67**(6): 1907-1917. https://doi.org/10.1093/jxb/erw006

Zhao Y., Li Z., Liu G., Jiang Y., Maurer H. P., Wurschum T., Mock H. P., Matros A., Ebmeyer E., Schachschneider R., Kazman E., Schacht J., Gowda M., Longin C., Reif J. 2015. Genome-based establishment of a high-yielding heterotic pattern for hybrid wheat breeding. *Proc. Natl. Acad. Sci. USA* **112**(51): 15624–15629. doi:10.1073/pnas.1514547112.

Zheng S., Byrne P. F., Bai G., Shan X., Reid S. D., Haley S. D., Seabourn B. W. 2009. Association analysis reveals effects of wheat glutenin alleles and rye translocations on dough-mixing properties. *J. Cereal Sci.* **50**(2):283-290. doi:10.1016?j.jcs.2009.06.008.

Zhou K. J., Wang S. H., Feng Y. Q., Liu Z. X., Wang G. X. 2006. The 4E-ms system of producing hybrid wheat. *Crop Science.* **46**: 250-255.

Figures and Tables

Table 1. Parent Cultivars Used to Produce F1 Hybrids.

Cultivars were selected by Easterly (2017) to provide a diverse range of genetics to achieve maximum heterosis. All cultivars except NE10589 and Harry were grown each year, which were only included in the 2015 and 2016 year, respectively.

Parent	Years Used as Parent	Breeding Program Origin
FREEMAN	15, 16	UNL
GOODSTREAK	15, 16	UNL
LCH13NEDH-11-24	15, 16	UNL
NE07531	15, 16	UNL
NE09517-1	15, 16	UNL
NE10683	15, 16	UNL
OVERLAND	15, 16	UNL
PANHANDLE	15, 16	UNL
PSB13NEDH-15-58W	15, 16	UNL
ROBIDOUX	15, 16	UNL
SETTLER CL	15, 16	UNL
TX09D1172	15, 16	Texas A&M
TX10D2063	15, 16	Texas A&M
TX10D2230	15, 16	Texas A&M
TX10D2363	15, 16	Texas A&M
TX11D3008	15, 16	Texas A&M
TX11D3026	15, 16	Texas A&M
TX11D3049	15, 16	Texas A&M
TX11D3112	15, 16	Texas A&M
TX11D3129	15, 16	Texas A&M
TX12M4004	15, 16	Texas A&M
TX12M4063	15, 16	Texas A&M
TX12M4065	15, 16	Texas A&M
WESLEY	15, 16	UNL
NE10589	15	UNL
HARRY	16	UNL

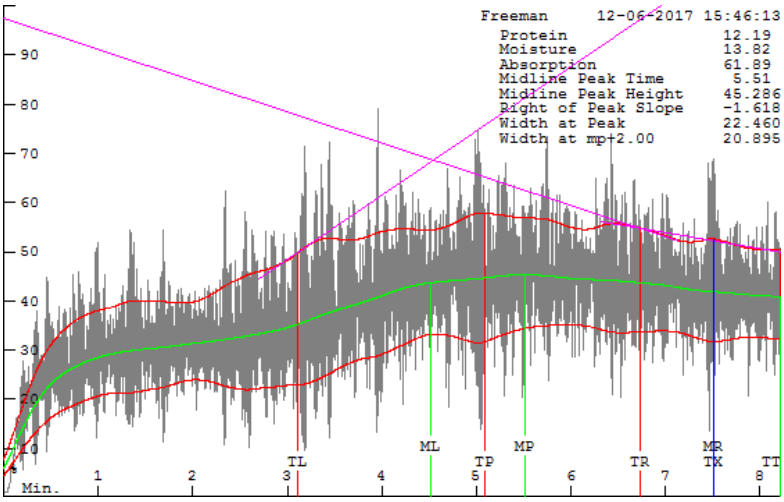


Figure 1. Freeman Mixograph (2017) - Example of Strong Performing Cultivar. Digital picture of Freeman parent mixogram from 2017 captured by MIXSMART® software. The Y-axis represents % torque (rotational force of dough across pins) and the X-axis represents time (min). Important parameters include midline peak time (MPT), which represents the time it takes to achieve maximum mixing resistance. The height and the width of the line at peak time (midline peak height and midline peak width, or MPH and MPW) are also important because they indicate the strength of the gluten network at that point. The midline peak integral or MPI (%TQ*min) is the area under the green line from the beginning of the curve until the midline peak time and represents the amount of work put into mixing the dough up to that point.

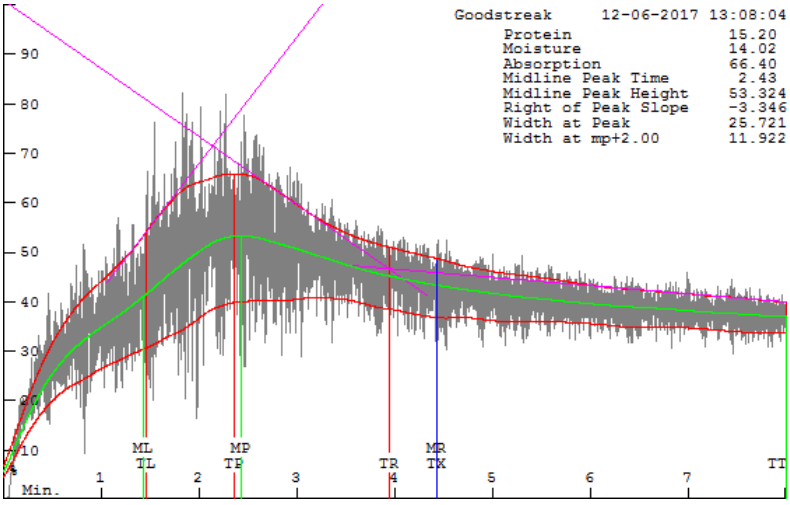


Figure 2. Goodstreak Mixograph (2017) - Example of Weak Performing Cultivar. Digital picture of Goodstreak parent mixogram from 2017 captured by MIXSMART® software. Currently visually scoring the mixograms is currently the most widely used scoring system, but the advancement in digital analysis has enabled us to explore more objective scoring methods, such as using the tolerance and strength equations (**equations 2 and 3).**

Table 2. Parental Quality Evaluation Results: Strongest and Weakest Parents. Shows the strongest and weakest parents in terms of end-use quality from the 2016 and 2017 trials and represent the parents of the hybrids selected for analysis. They were evaluated using the procedures described in the *Analytical Methods and Instruments* section. Parents highlighted yellow were identified as strong or weak in both years and include four strong parents and five weak parents.

2016		2017	
Strong Parents	Weak Parents	Strong Parents	Weak Parents
FREEMAN	GOODSTREAK	FREEMAN	GOODSTREAK
LCH13NEDH-11-24	OVERLAND	LCH13NEDH_11_24	OVERLAND
NE09517-1	TX10D2063	NE07531	TX09D1172
SETTLER CL	TX10D2230	NE09517_1	TX10D2063
WESLEY	TX11D3129	ROBIDOUX	TX10D2363
NE10589	TX12M4063	SETTLER_CL	TX11D3129
	TX12M4065	HARRY	TX12M4063

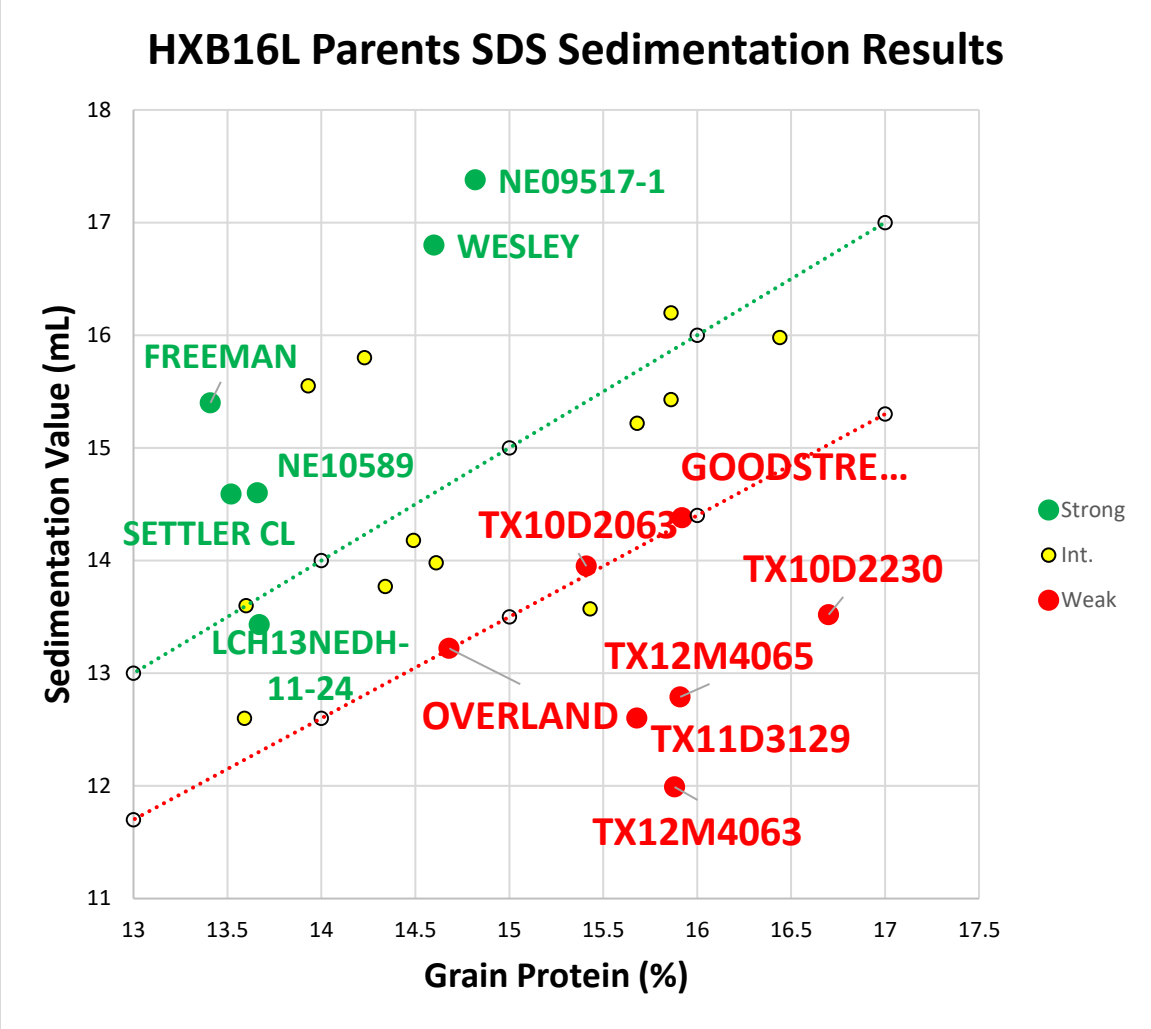


Figure 3. 2016 Parent Cultivar SDS Sedimentation Assay Results.
Represents the 2016 parent samples that were analyzed using the SDS sedimentation assay. The Y-axis is the sedimentation value (mL) and the X-axis is the grain protein %. Samples that are at or above the green dotted line are considered desirable, samples that are at or below the red dotted line are considered undesirable, while samples in the middle are neither. The parents determined to be the strongest via visual assessment of the Mixographs are shown in green and the weakest parents are shown in red, while the intermediate parents are shown in yellow.

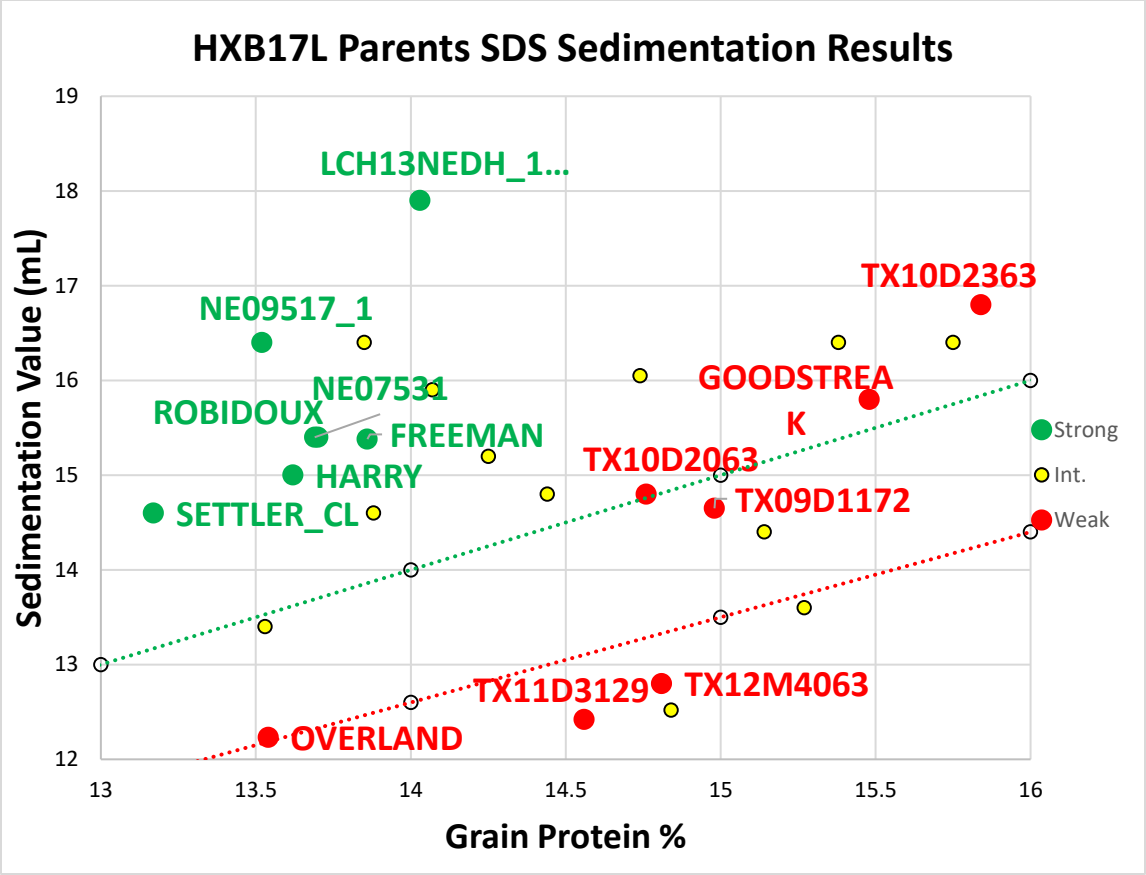


Figure 4. 2017 Parent Cultivar SDS Sedimentation Assay Results.
Represents the 2017 parent samples that were selected and analyzed using the SDS sedimentation assay. The Y-axis is the sedimentation value (mL) and the X-axis is the grain protein %. Samples that are at or above the green dotted line are considered desirable, samples that are at or below the red dotted line are considered undesirable, while samples in the middle are neither. The parents determined to be the strongest via visual assessment of the Mixographs are shown in green and the weakest parents are shown in red, while the intermediate parents are shown in yellow.

Table 3. 2016 Parent Performance Comparisons by Mixograph, SDS Sedimentation, and SDS-SRC Hybrid Assays.

Displays ranking of cultivars based on end-use quality as measured by each assay, including the visual Mixograph assessment, SDS sedimentation rank, and SDS-SRC rank. Green cells indicate top six performing cultivars while red cells indicate bottom seven performing cultivars.

Variety	Mixograph Visual Assessment	SDS Ratio	SDS Sed. Rank	Weight Value Ratio	SDS-SRC Rank
WESLEY	Strong	1.151	2	21.21	3
SETTLER CL	Strong	1.079	6	21.23	2
FREEMAN	Strong	1.148	3	21.04	4
NE09517-1	Strong	1.173	1	22.83	1
NE10589	Strong	1.069	7	20.61	6
LCH13NEDH-11-24	Strong	0.982	10	20.84	5
ROBIDOUX	Intermediate	1.116	4	20.28	8
NE07531	Intermediate	1.110	5	20.11	9
TX11D3049	Intermediate	1.021	8	19.86	10
TX12M4004	Intermediate	0.979	11	19.67	12
NE10683	Intermediate	0.927	17	19.74	11
TX11D3112	Intermediate	0.957	16	18.88	13
TX09D1172	Intermediate	0.973	12	18.75	15
PSB13NEDH-15-58W	Intermediate	1.000	9	20.39	7
TX11D3008	Intermediate	0.879	21	17.83	18
TX11D3026	Intermediate	0.960	15	18.82	14
PANHANDLE	Intermediate	0.971	14	18.41	16
TX10D2363	Intermediate	0.972	13	17.13	22
OVERLAND	Weak	0.901	20	17.28	21
TX12M4065	Weak	0.804	23	14.85	24
TX10D2063	Weak	0.905	18	17.95	17
TX12M4063	Weak	0.755	25	14.41	25
TX10D2230	Weak	0.810	22	15.26	23
GOODSTREAK	Weak	0.903	19	17.36	20
TX11D3129	Weak	0.804	24	17.51	19

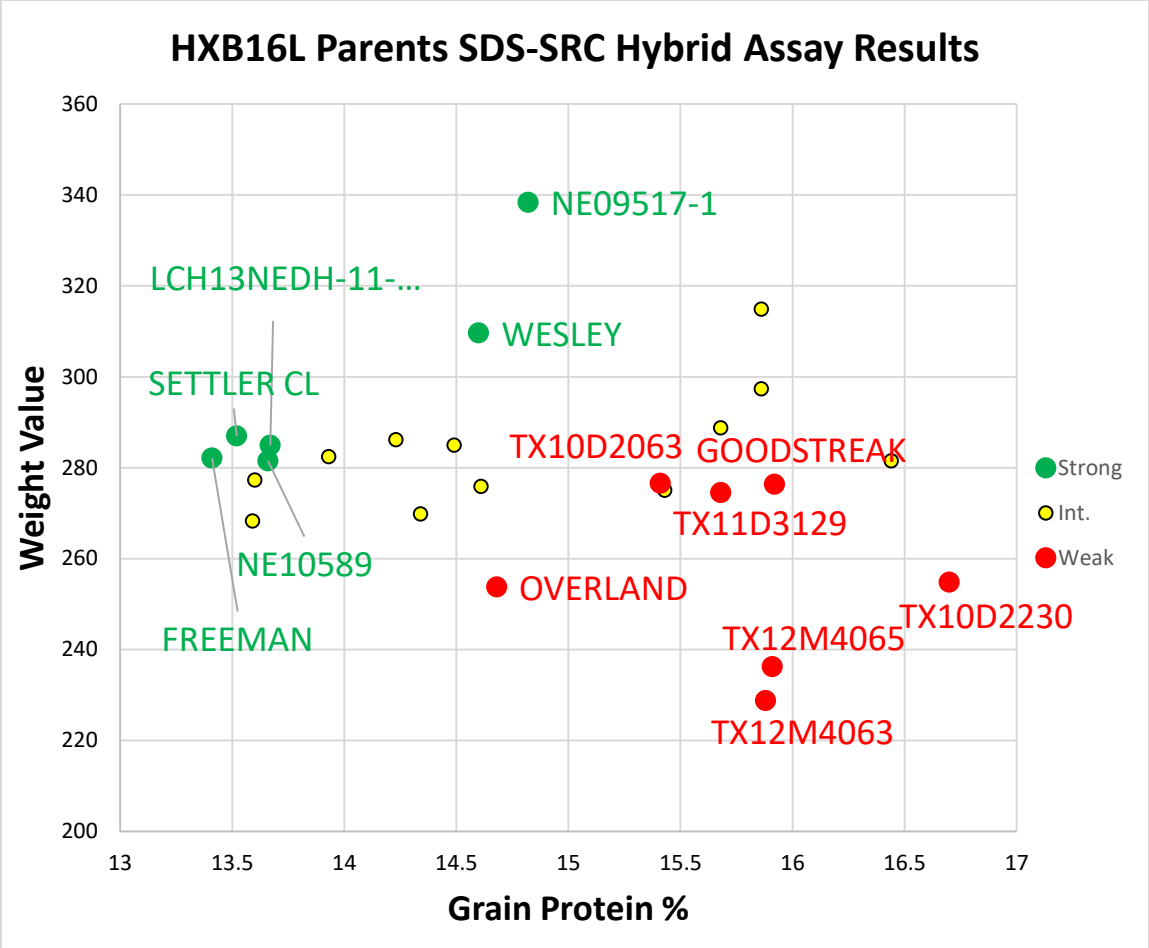


Figure 5. 2016 Parent Cultivar SDS-SRC Hybrid Assay Results.
Represents the SDS-SRC hybrid assay of the 2016 parents. The Y-axis represents the weight value % as calculated by **equation 1**, and the X-axis represents grain protein %. The parents determined to be the strongest via visual assessment of the Mixographs are shown in green and the weakest parents are shown in red, while the intermediate parents are shown in yellow.

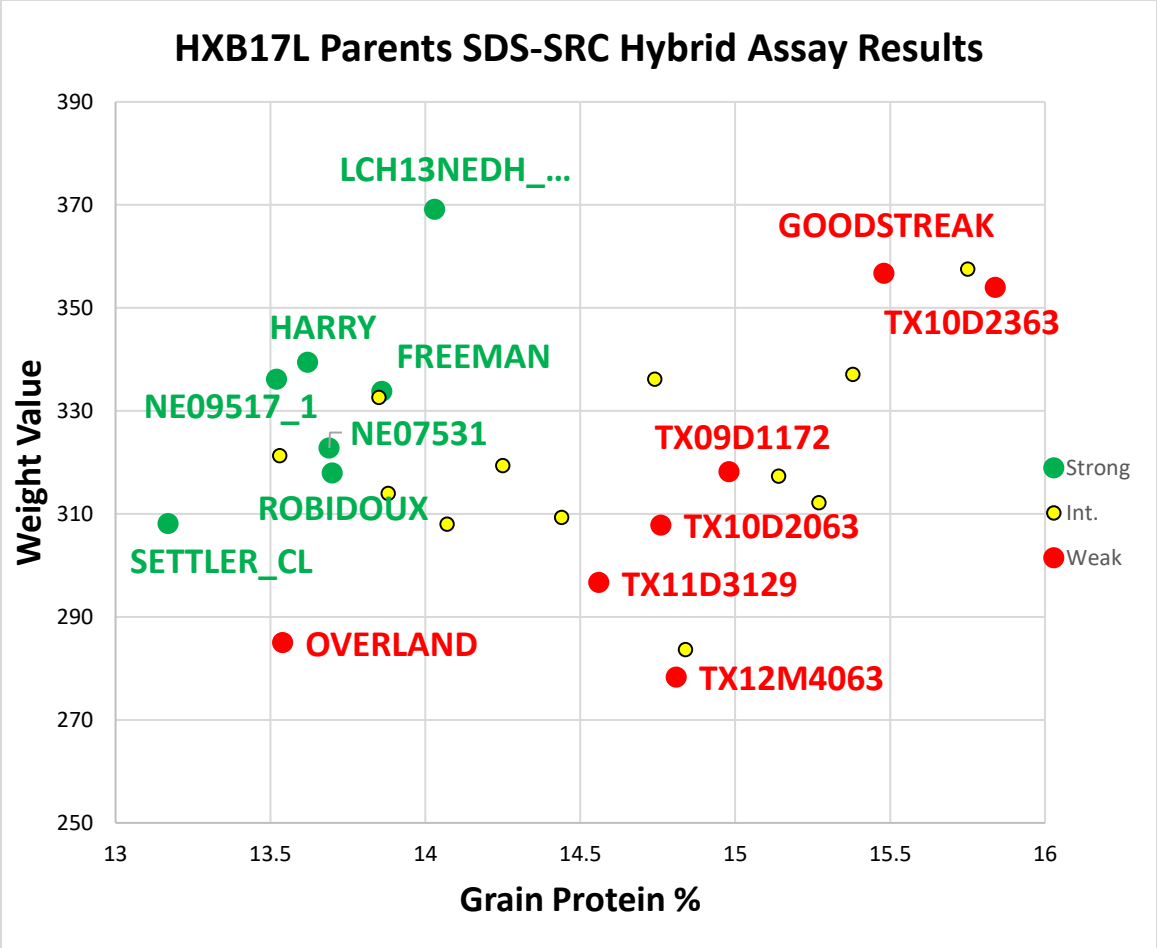


Figure 6. 2017 Parent Cultivar SDS-SRC Hybrid Assay Results.
Represents the 2017 parents SDS-SRC hybrid assay results. The Y-axis represents the weight value % as calculated by **equation 1**, and the X-axis represents grain protein %. The parents determined to be the strongest via visual assessment of the Mixographs are shown in green and the weakest parents are shown in red, while the intermediate parents are shown in yellow.

Table 4. 2017 Parent Performance Comparisons by Mixograph, SDS Sedimentation, and SDS-SRC Hybrid Assays.

Displays ranking of cultivars based on end-use quality as measured by each assay, including the visual Mixograph assessment, SDS sedimentation rank, and SDS-SRC rank. Green cells indicate top seven performing cultivars while red cells indicate bottom seven performing cultivars.

Variety	Mixograph Visual Score	SDS Ratio	SDS Sed. Rank	Weight Value Ratio	SDS-SRC Rank
FREEMAN	Strong	1.110	7	24.08	4
NE09517_1	Strong	1.213	2	24.86	3
NE07531	Strong	1.125	5	23.58	7
HARRY	Strong	1.101	9	24.92	2
LCH13NEDH_11_24	Strong	1.276	1	26.31	1
ROBIDOUX	Strong	1.124	6	23.20	9
SETTLER_CL	Strong	1.109	8	23.40	8
RUTH	Intermediate	1.184	3	24.02	5
WESLEY	Intermediate	1.066	12	21.92	16
NE10683	Intermediate	0.990	19	23.75	6
TX12M4004	Intermediate	1.130	4	21.89	17
TX11D3049	Intermediate	1.041	15	22.70	12
TX11D3026	Intermediate	1.025	16	21.42	18
TX11D3112	Intermediate	1.067	11	22.41	14
PANHANDLE	Intermediate	1.089	10	22.81	11
PSB13NEDH_15_58W	Intermediate	1.052	14	22.62	13
TX10D2230	Intermediate	0.951	21	20.96	21
TX12M4065	Intermediate	0.844	26	19.11	25
TX11D3008	Intermediate	0.891	23	20.44	23
TX09D1172	Weak	0.978	20	21.24	19
OVERLAND	Weak	0.903	22	21.05	20
TX12M4063	Weak	0.864	24	18.79	26
TX10D2363	Weak	1.061	13	22.35	15
TX10D2063	Weak	1.003	18	20.85	22
TX11D3129	Weak	0.853	25	20.37	24
GOODSTREAK	Weak	1.021	17	23.04	10

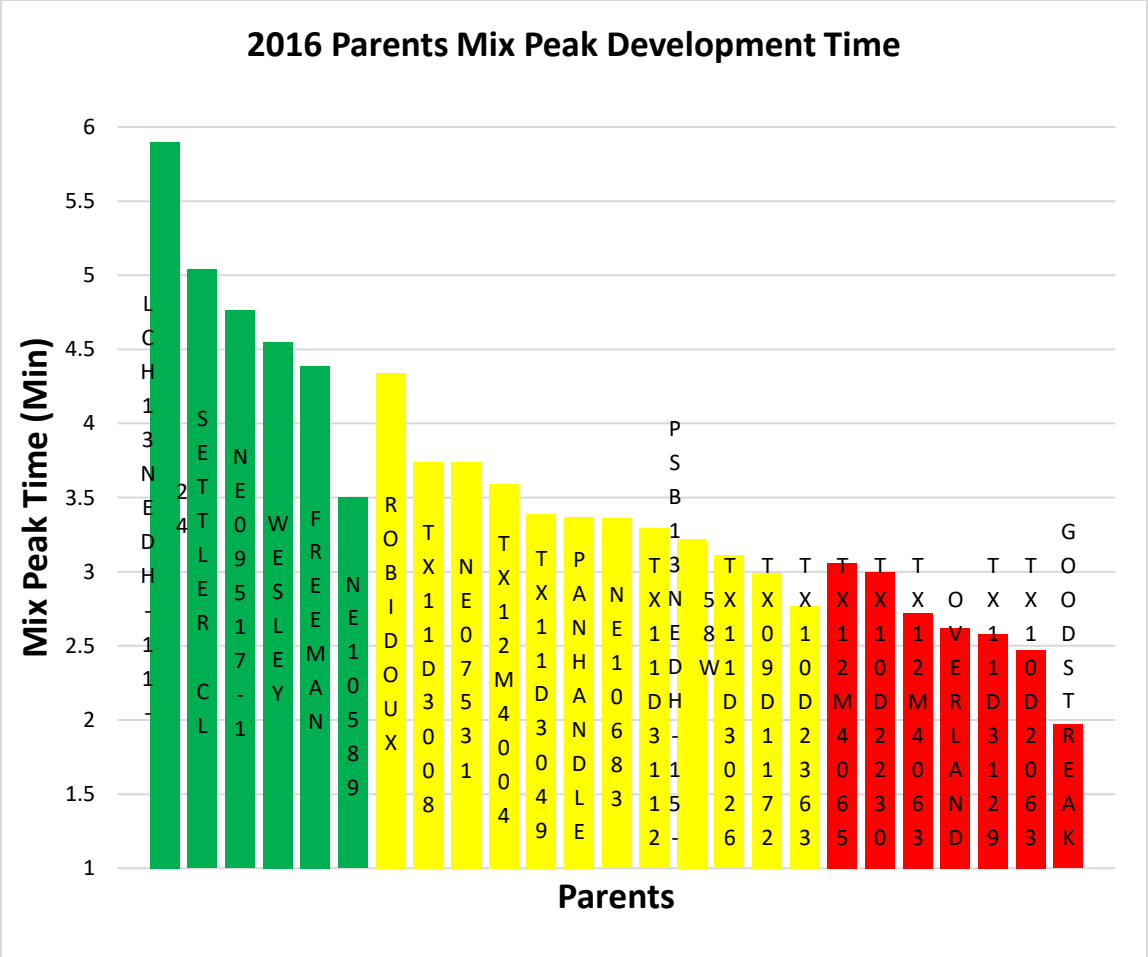


Figure 7. 2016 Parent Cultivar Mixograph Mix Peak Development Times. Displays the Mixograph mix peak time (min) of the 25 parent cultivars used in the 2016 analysis. The parents determined to be the strongest via visual assessment of the Mixographs are shown in green and the weakest parents are shown in red, while the intermediate parents are shown in yellow.

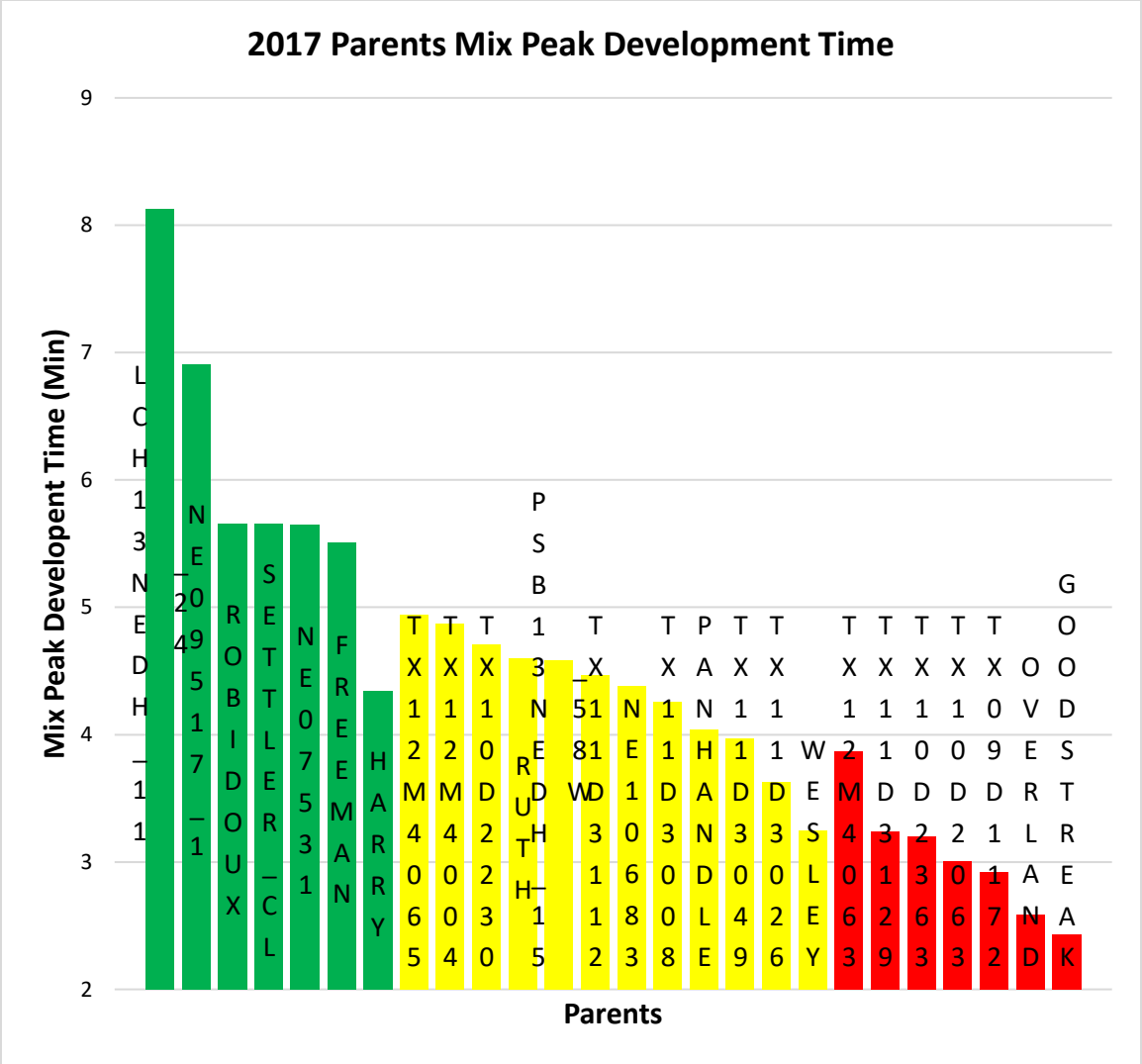


Figure 8. 2017 Parent Cultivar Mixograph Mix Peak Development Times.
Displays the Mixograph mix peak time (min) of the 26 parent cultivars used in the 2017 analysis. The parents determined to be the strongest via visual assessment of the Mixographs are shown in green and the weakest parents are shown in red, while the intermediate parents are shown in yellow.

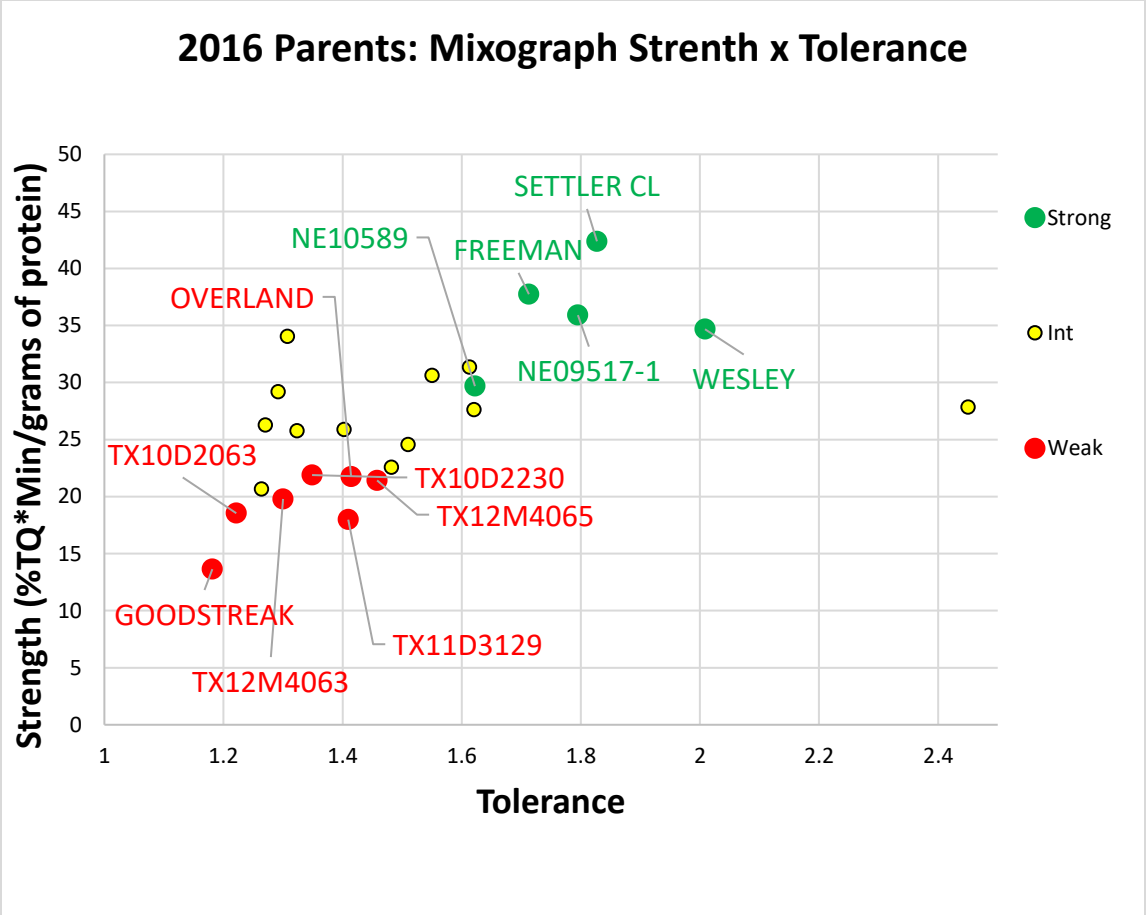


Figure 9.1 2016 Parent Cultivar Mixograph Strength x Tolerance Analysis – Excluding LCH13NEDH_11_24.

The Y-axis represents the strength and is calculated using **equation 3,** the units for strength are {(%torque*minutes)/g}. The X-axis represents the tolerance to overmixing and was calculated using **equation 2**, it is a unitless measurement. The parents determined to be the strongest via visual assessment of the Mixographs are shown in green and the weakest parents are shown in red, while the intermediate parents are shown in yellow. Cultivars that are higher and towards the right of the graph are more desirable than cultivars that appear lower and to the left. The parent variety LCH13NEDH_11_24 was excluded from this graph. **Figure 9.2** includes LCH13NEDH_11_24 and shows how it compares to the other parents.

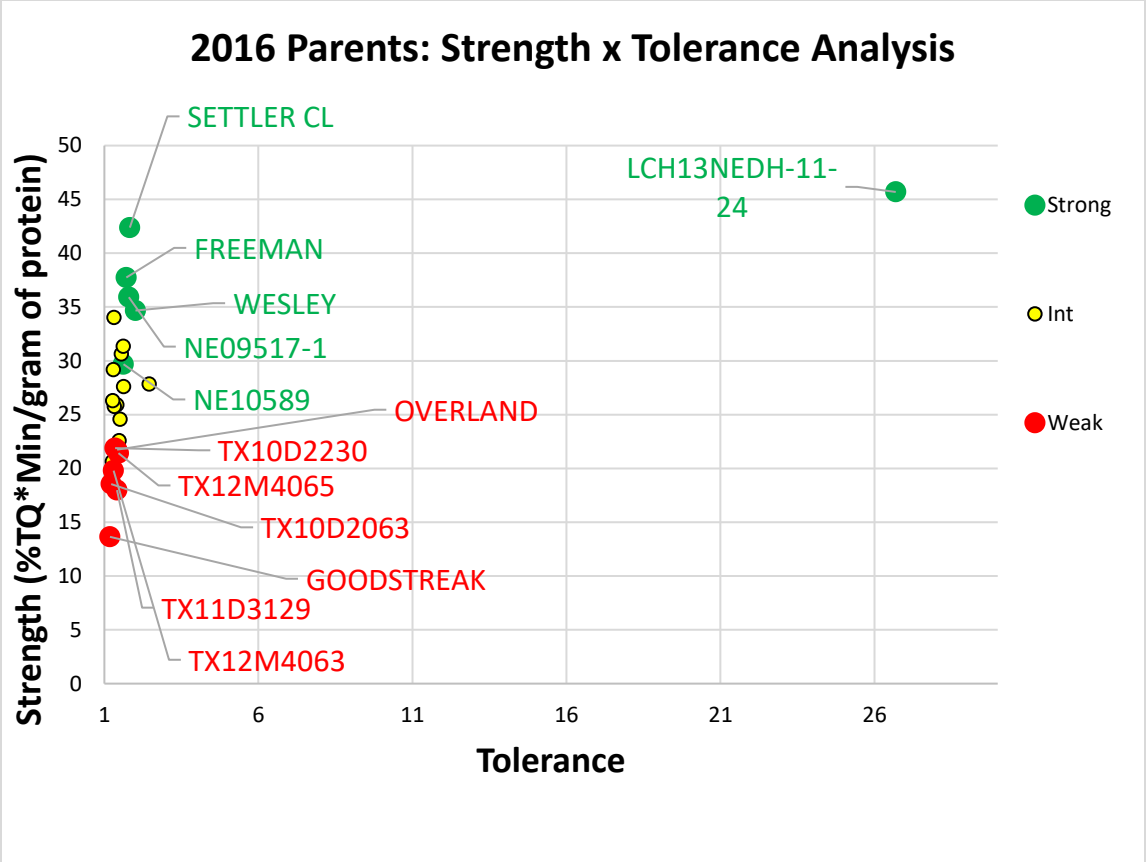


Figure 9.2 2016 Parent Cultivar Mixograph Strength x Tolerance Analysis. The parent variety LCH13NEDH_11_24 lost no strength after mix peak time, and therefore had a significantly higher tolerance than the other parents. LCH13NEDH_11_24 was the strongest and most tolerant parent with a tolerance of 26.7 and a strength value of 45.7 {(%TQ*min)/g}. The worst parent in terms of strength and tolerance was Goodstreak, with scores of 13.6 ({%TQ*min)/g} and 1.2, respectively. The parents determined to be the strongest via visual assessment of the Mixographs are shown in green and the weakest parents are shown in red, while the intermediate parents are shown in yellow. Cultivars that are higher and towards the right of the graph are more desirable than cultivars that appear lower and to the left.

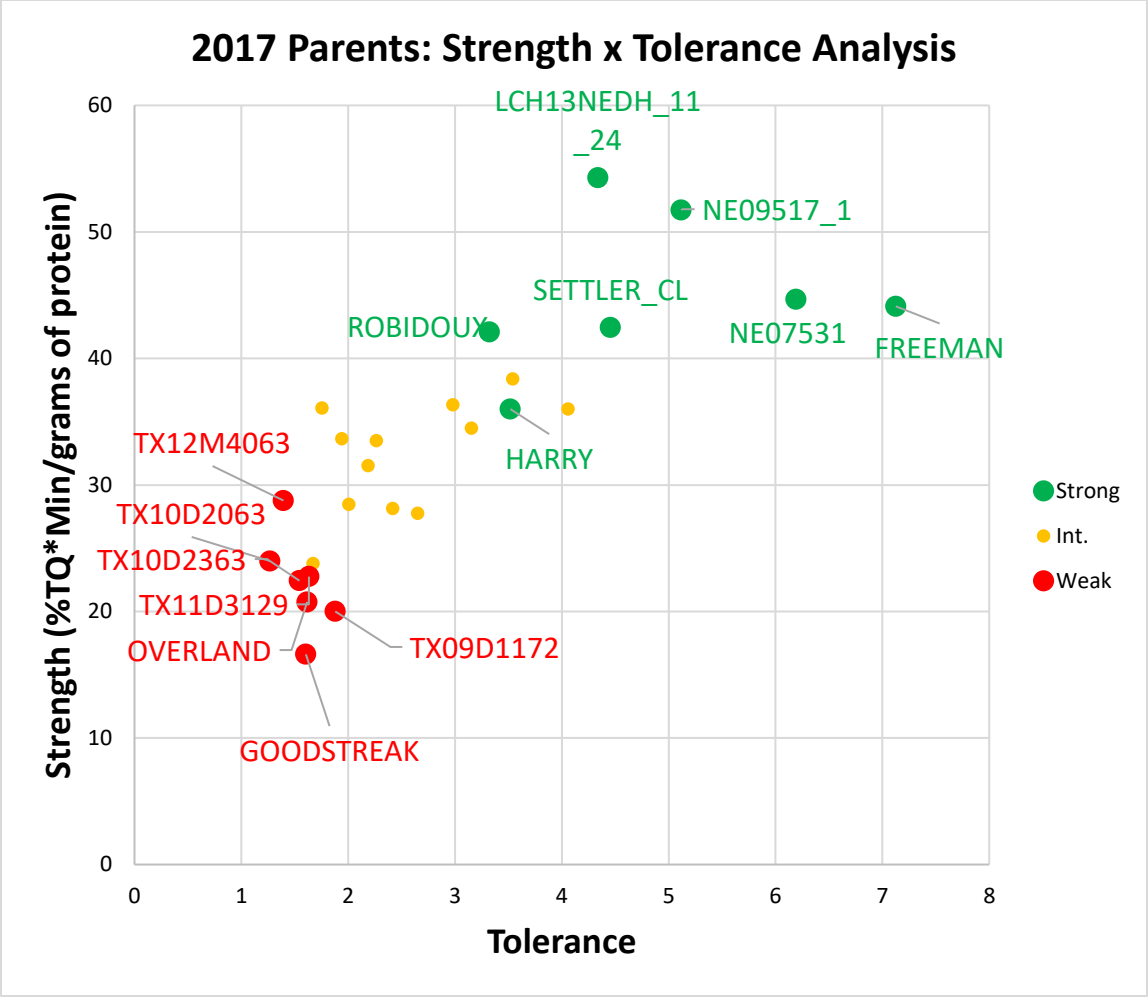
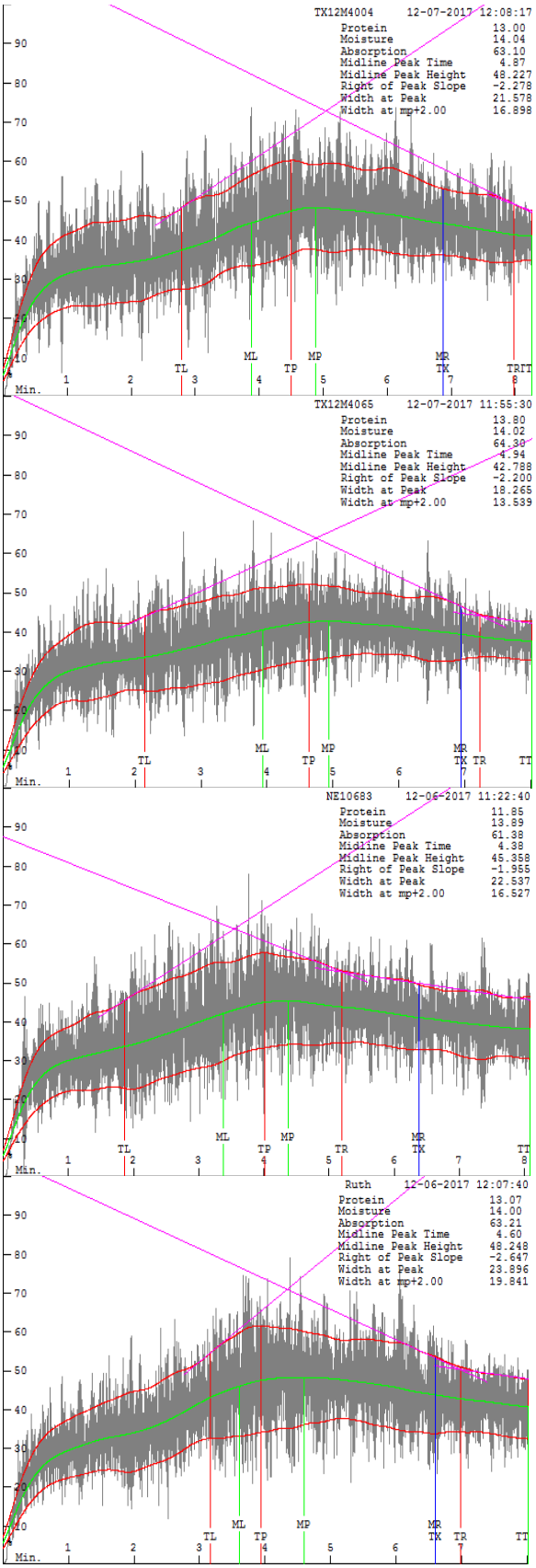


Figure 10. 2017 Parent Cultivar Mixograph Strength x Tolerance Analysis.

The Y-axis represents the strength, or the amount of work put into the dough to develop it to peak time, per gram of protein, and is calculated using **equation 3**. The units for strength are {(%torque*minutes)/g}. The X-axis represents the tolerance to overmixing and is a unitless ratio calculated using **equation 2**. The parents determined to be the strongest via visual assessment of the Mixographs are shown in green and the weakest parents are shown in red, while the intermediate parents are shown in yellow Cultivars that are higher and towards the right of the graph are more desirable than cultivars that appear lower and to the left.



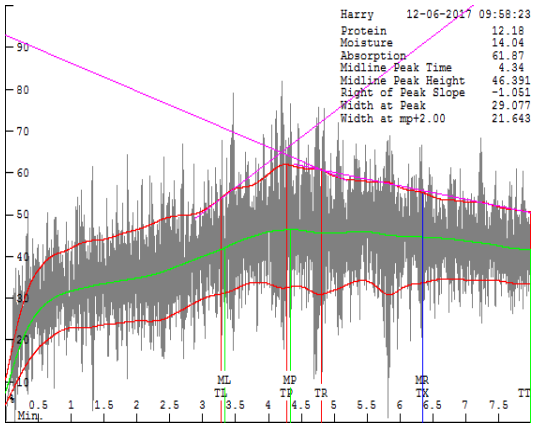


Figure 11. Comparing Cultivars with Similar Dough Strength as Measured by the Mixograph Strength x Tolerance Analysis.
Displayed is the 2017 Harry Mixograph (right, strong) compared to the TX12M4004, TX11M4065, NE10683, and Ruth Mixographs (left, intermediate). These Mixographs were scored similarly by the Mixograph strength x tolerance analysis, and they all appear to be visually similar, emphasizing the sensitivity of the strength x tolerance assay.

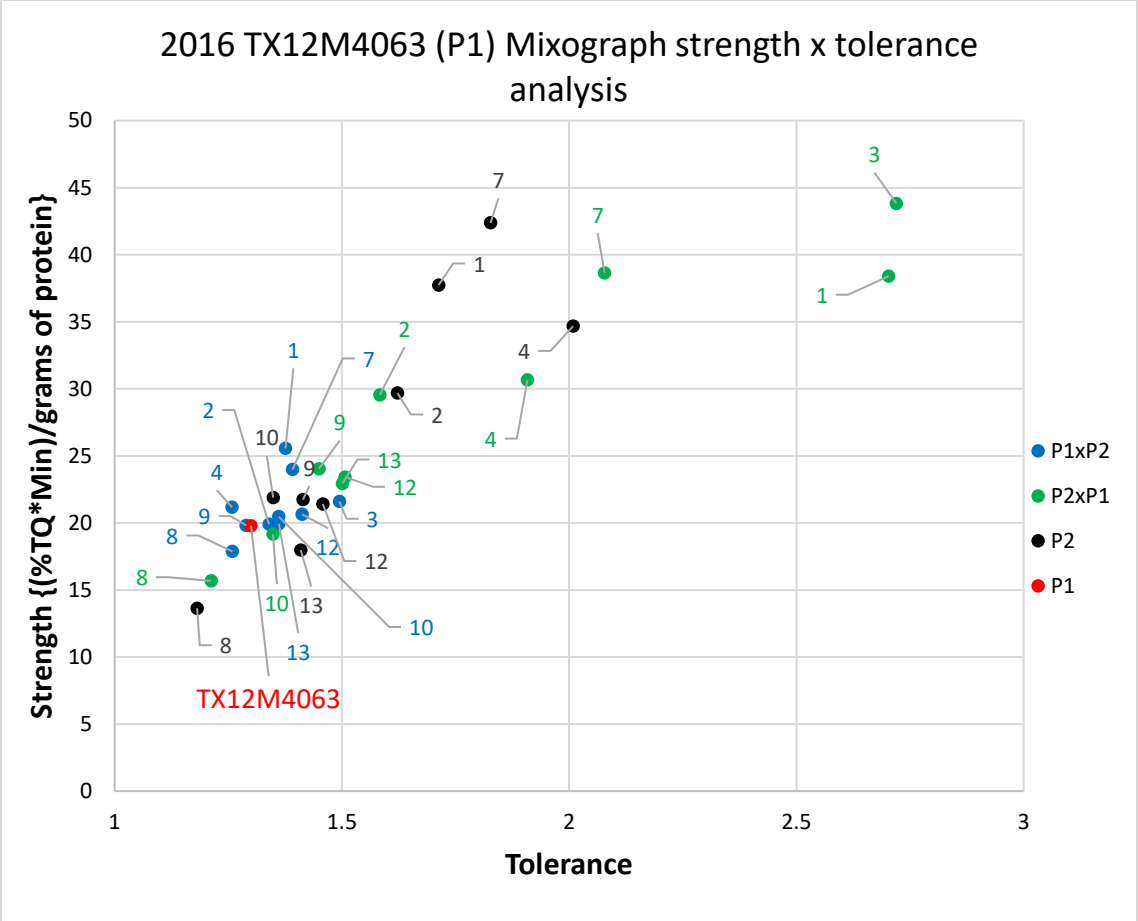


Figure 12. Mixograph Strength x Tolerance Analysis evaluating 2016 TX12M4063 (P1) reciprocal hybrids.
TX12M4063 as the P1 parent was coded in red, P1xP2 hybrids with TX12M4063 as the female parent were coded blue, P2xP1 reciprocals with TX12M4063 as male parent were coded green, P2 parents used in the crosses are coded in black. TX12M4063 was sterilized poorly and appears to have P1xP2 hybrids that reflect the unsterilized TX12M4063 parent.

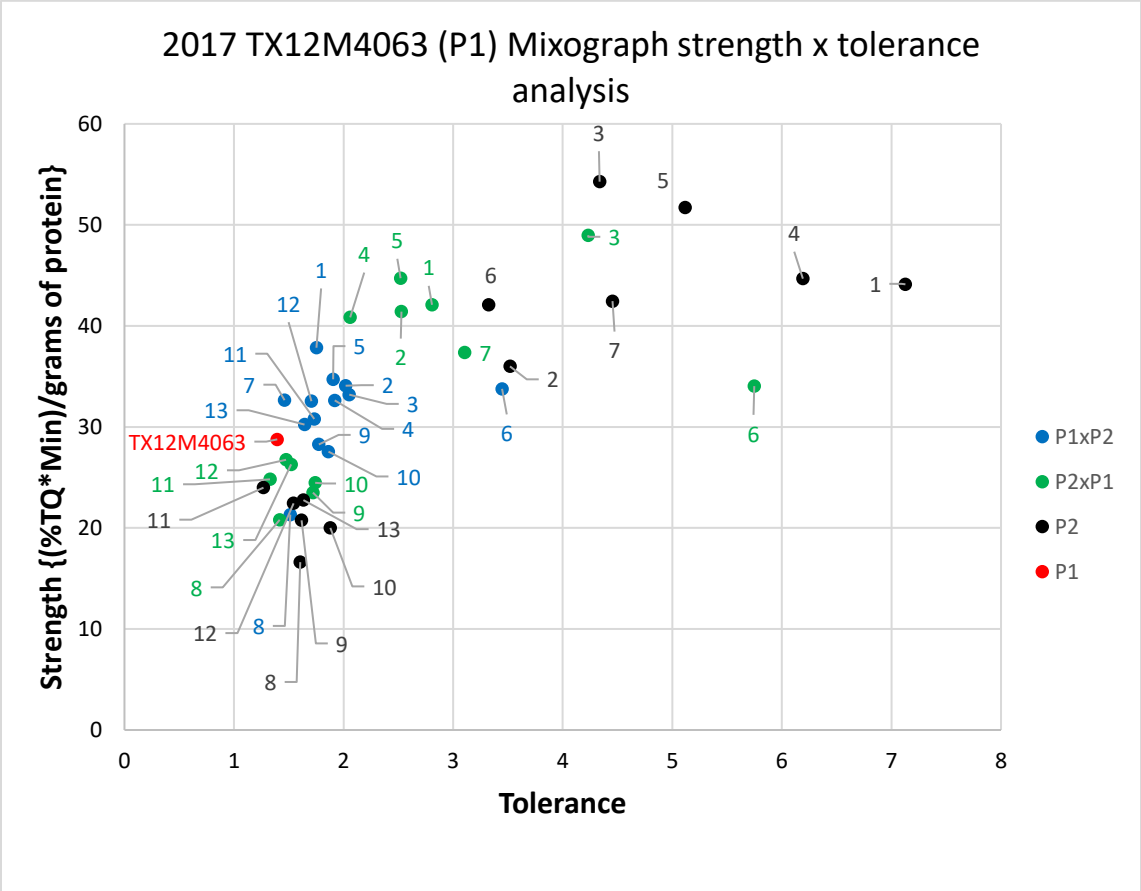


Figure 13. Mixograph Strength x Tolerance Analysis evaluating 2017 TX12M4063 (P1) reciprocal hybrids.
TX12M4063 as the P1 parent was coded in red, P1xP2 hybrids with TX12M4063 as the female parent were coded blue, P2xP1 reciprocals with TX12M4063 as male parent were coded green, P2 parents used in the crosses are coded in black. TX12M4063 was sterilized poorly and appears to have P1xP2 hybrids that reflect the unsterilized TX12M4063 parent.

Table 5. Mixograph Mix Peak Time Analysis of the 2016 TX12M4063 Reciprocal Hybrids.

Displays the mix peak time (Min) of TX12M4063 (P1), the P2 parents, and the P1xP2 and P2xP1 reciprocals to investigate how the parents and the hybrids compare. Paired t-test indicates a significant difference ($\alpha = .05$) between the mean mix peak time of the P1xP2 and P2xP1 reciprocals, indicating a maternal effect possibly due to self-fertilization of female.

TX12M4063 (P1)	P2	TX12M4063 (P1) Mix Peak Time (Min)	P1xP2 Mix Peak Time (Min)	P2xP1 Mix Peak Time (Min)	P2 Mix Peak Time (Min)
TX12M4063	SETTLER CL	2.72	3.18	4.48	5.04
TX12M4063	TX11D3129	2.72	2.59	2.79	2.58
TX12M4063	TX10D2230	2.72	2.74	2.88	3
TX12M4063	GOODSTREAK	2.72	2.49	2.12	1.97
TX12M4063	LCH13NEDH-11-24	2.72	2.73	5.32	5.9
TX12M4063	OVERLAND	2.72	2.54	2.73	2.62
TX12M4063	TX12M4065	2.72	2.75	2.98	3.06
TX12M4063	NE10589	2.72	2.62	3.46	3.5
TX12M4063	WESLEY	2.72	2.78	4.15	4.55
TX12M4063	FREEMAN	2.72	3.03	4.35	4.39

Mean	2.745	3.526
Variance	0.046517	1.007649
Observations	10	10
t Stat	-2.81883	
P(T<=t) two-tail	0.020085	
t Critical two-tail	2.262157	

Table 6. Mixograph Mix Peak Time Analysis of the 2017 TX12M4063 Reciprocal Hybrids.

Displays the mix peak time (Min) of TX12M4063 (P1), the P2 parents, and the P1xP2 and P2xP1 reciprocals to investigate how the parents and the hybrids compare. Paired t-test indicates no significant difference ($\alpha = .05$) between the mean mix peak times of the P1xP2 and P2xP1 reciprocals. Indicates hybrids could have been made better in 2017 or that the quality of the parents were too similar to detect a difference between the reciprocals.

TX12M4063 (P1)	P2	TX12M4063 (P1) Mix Peak Time (Min)	P1xP2 Mix Peak Time (Min)	P2xP1 Mix Peak Time (Min)	P2 Mix Peak Time (Min)
TX12M4063	FREEMAN	3.87	4.68	5.49	5.51
TX12M4063	GOODSTREAK	3.87	3.09	2.71	2.43
TX12M4063	HARRY	3.87	4.76	5.14	4.34
TX12M4063	LCH13NEDH_11_24	3.87	4.69	6.77	8.13
TX12M4063	NE07531	3.87	4.32	5.36	5.65
TX12M4063	NE09517_1	3.87	4.79	5.92	6.91
TX12M4063	OVERLAND	3.87	3.71	2.92	2.59
TX12M4063	ROBIDOUX	3.87	4.58	4.39	5.66
TX12M4063	SETTLER_CL	3.87	4.38	5.06	5.66
TX12M4063	TX09D1172	3.87	3.88	3.37	2.92
TX12M4063	TX10D2063	3.87	4.18	2.95	3.01
TX12M4063	TX10D2363	3.87	4.25	3.22	3.2
TX12M4063	TX11D3129	3.87	4	3.42	3.24

Mean	4.254615	4.363077
Variance	0.241844	1.797106
Observations	13	13
t Stat	-0.39487	
P(T<=t) two-tail	0.699868	
t Critical two-tail	2.178813	

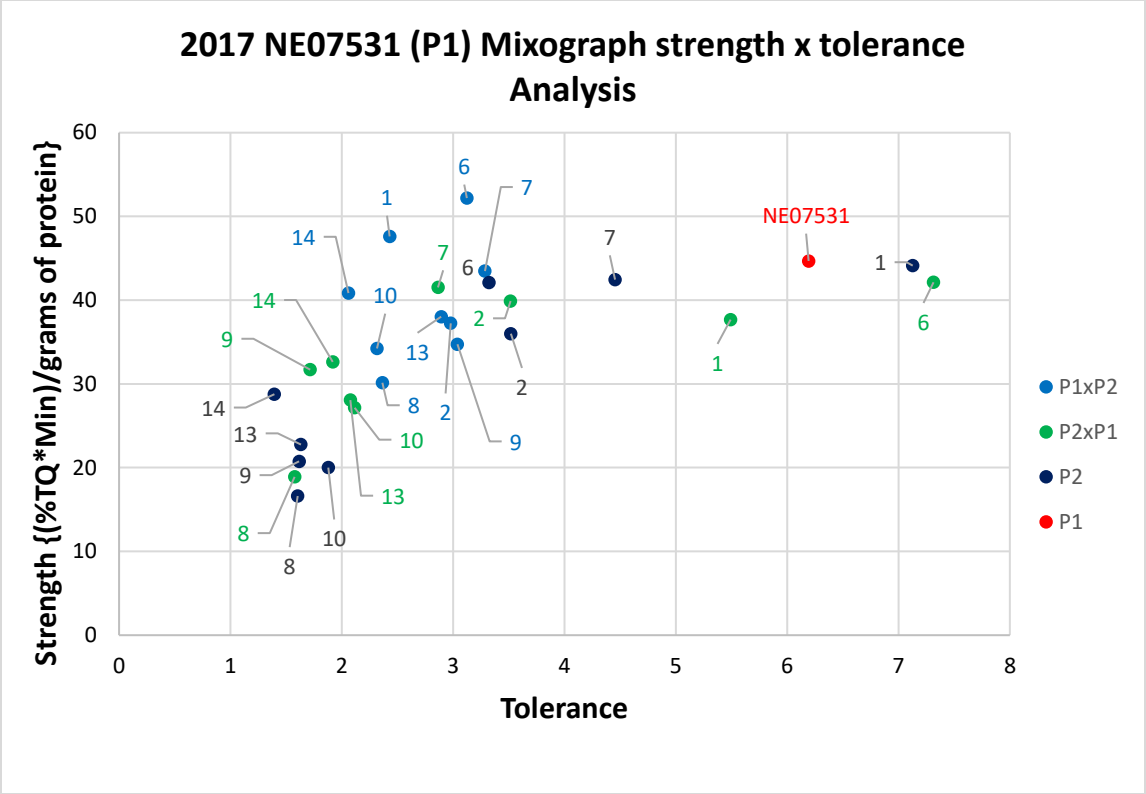


Figure 14. Mixograph Strength x Tolerance Analysis evaluating 2017 NE07531 (P1) reciprocal hybrids.

Displays the mix peak time (Min) of NE07531 (P1), the P2 parents, and the P1xP2 and P2xP1 reciprocals to investigate how the parents and the hybrids compare. NE07531 was sterilized well and P1xP2 hybrids appear to be performing near mid-parent value, especially hybrids with P2 parents eight, nine, ten, 13, and 14.

Table 7. Mixograph Mix Peak Time Analysis of the 2017 NE07531 Reciprocal Hybrids.

Displays the mix peak time (Min) of NE07531 (P1), the P2 parents, and the P1xP2 and P2xP1 reciprocals to investigate how the parents and the hybrids compare. Paired t-test indicates a significant difference ($\alpha = .05$) between the mean mix peak times of the P1xP2 and P2xP1 reciprocals.

NE07531 (P1)	P2	NE07531 (P1) Mix Peak Time (Min)	P1xP2 Mix Peak Time (Min)	P2xP1 Mix Peak Time (Min)	P2 Mix Peak Time (Min)
NE07531	FREEMAN	5.65	5.51	4.89	5.51
NE07531	GOODSTREAK	5.65	3.9	2.77	2.43
NE07531	HARRY	5.65	4.87	5.03	4.34
NE07531	OVERLAND	5.65	4.13	3.66	2.59
NE07531	ROBIDOUX	5.65	6.56	5.5	5.66
NE07531	SETTLER_CL	5.65	5.76	5.27	5.66
NE07531	TX09D1172	5.65	4.69	3.77	2.92
NE07531	TX11D3129	5.65	5.07	3.91	3.24
NE07531	TX12M4063	5.65	5.36	4.32	3.87

Mean	5.094444	4.346667
Variance	0.675678	0.803275
Observations	9	9
t Stat	5.159209	
P(T<=t) two-tail	0.000864	
t Critical two-tail	2.306004	

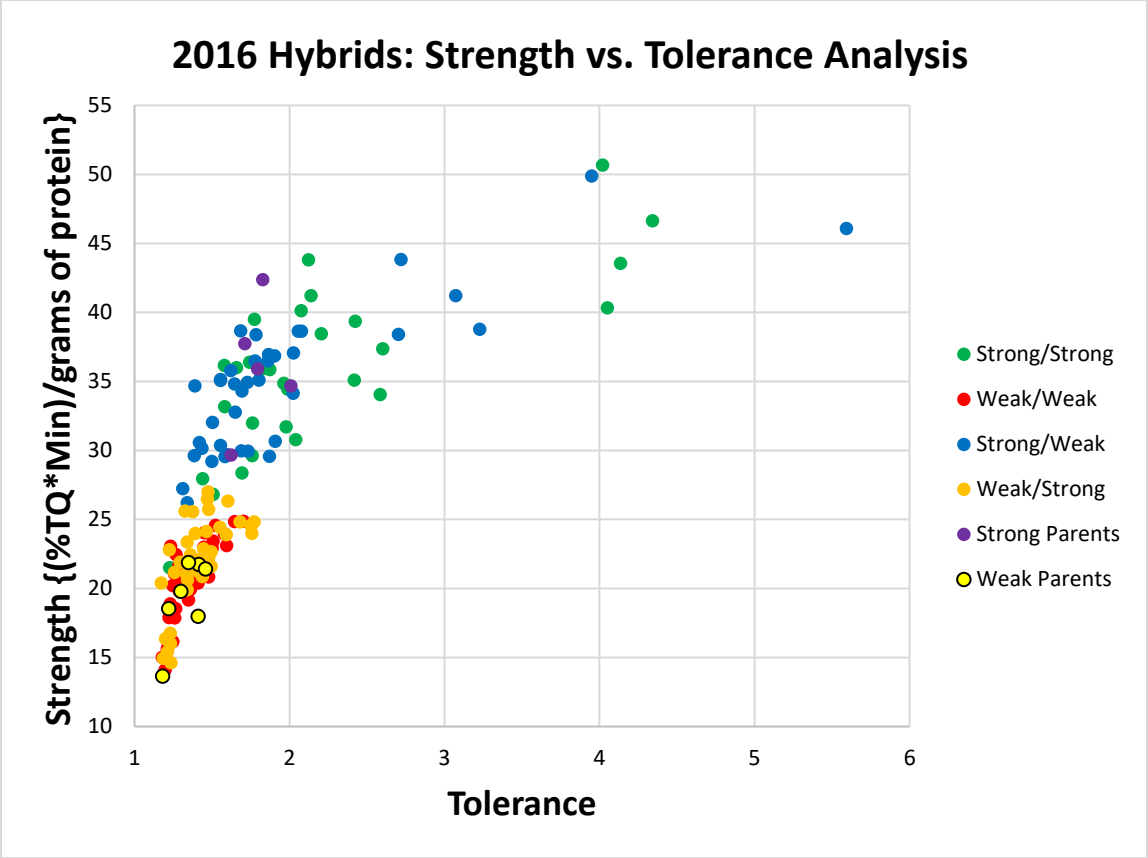


Figure 15.1 Mixograph Strength vs. Tolerance Analysis of 2016 Hybrids – Tolerance range from 1 – 6.

These graphs show the relationship between the strength and tolerance variables as calculated by **equation 2** and **equation 3**. The Y-axis represents the strength, or the amount of work put into the dough to develop it to peak time, per gram of protein. The units for strength are {(%torque*minutes)/g}. The X-axis represents tolerance to overmixing and is unitless. Strong parents are shown in purple while weak parents are shown in yellow to help show the relationship between the hybrids and the parents. Only samples that fell within the tolerance range of 1 to 6 are shown here, **Figure 9.2** shows the results of all the hybrid samples (includes the outlier)

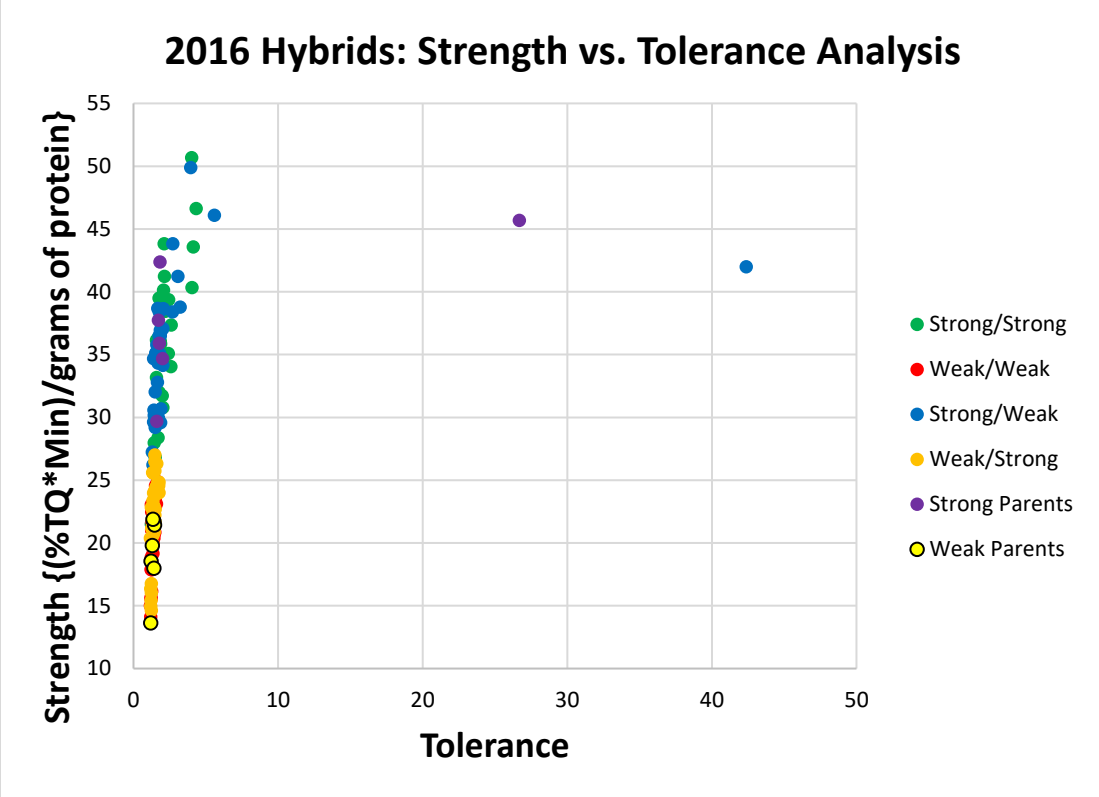


Figure 15.2 Mixograph Strength vs. Tolerance Analysis of 2016 Hybrids – All Samples

These graphs show the relationship between the strength and tolerance variables as calculated by **equation 2** and **equation 3**. The Y-axis represents the strength, or the amount of work put into the dough to develop it to peak time, per gram of protein. The units for strength are {(%torque*minutes)/g}. The X-axis represents the tolerance to overmixing and is unitless. Strong parents are shown in purple while weak parents are shown in yellow to help show the relationship between the hybrids and the parents. All samples are included in this graph.

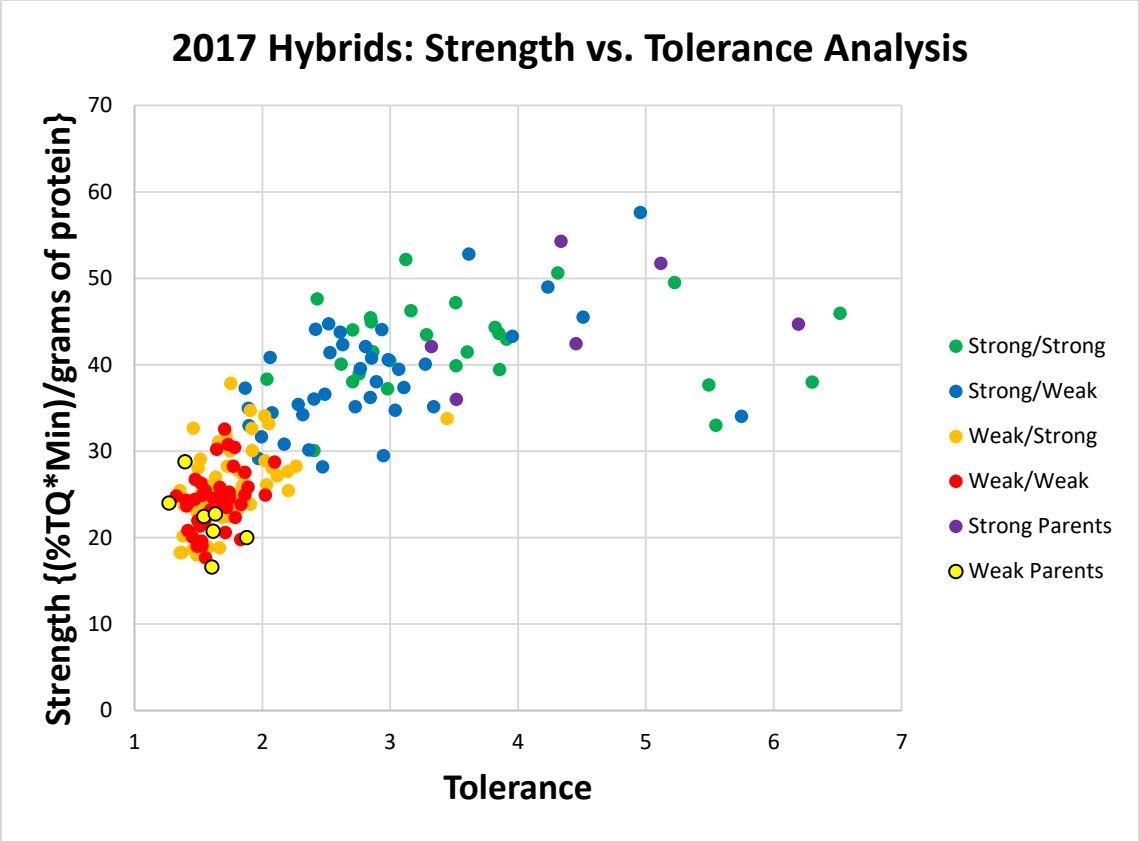


Figure 16.1 2017 Hybrids Mixograph Strength vs. Tolerance Analysis of 2017 Hybrids - Tolerance range 1-7.

These graphs show the relationship between the strength and tolerance variables as calculated by **equation 2** and **equation 3**. The Y-axis represents the strength, or the amount of work put into the dough to develop it to peak time, per gram of protein. The units for strength are {(%torque*minutes)/g}. The X-axis represents the tolerance to overmixing and is a unitless ratio. Cultivars that are higher and towards the right of the graph are more desirable than cultivars that appear lower and to the left. The strong parents are shown in purple and the weak parents are shown in yellow to show the relationship between the quality of the parents and the hybrids. This graph is omitting the samples that have a tolerance above 7.

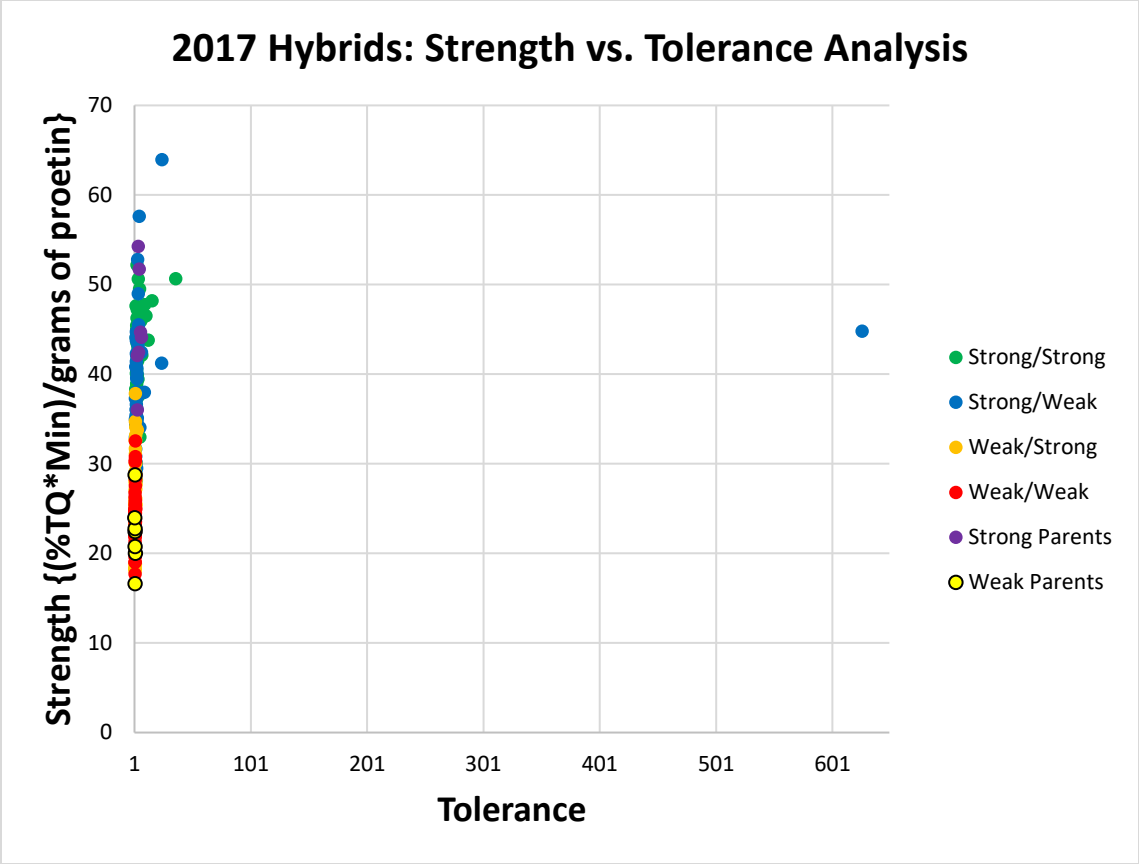


Figure 16.2 Mixograph Strength vs. Tolerance Analysis of 2017 Hybrids - All Samples.

Figure 19.2 shows the strength vs. tolerance analysis results for all the hybrid samples, including the outliers. Because the tolerance equation takes the reciprocal of the amount of strength lost, samples that lose very little or no strength result in tolerance values that approach infinity. The strong parents are shown in purple and the weak parents are shown in yellow to show the relationship between the quality of the parents and the hybrids.

Table 8. Paired t-test Results for Mixograph Strength of the 2016 Hybrids.
Paired t-tests ($\alpha = .05$) between mean strength {(%TQ*min)/grams of protein} of reciprocal hybrids for each parent cultivar used in the 2016 analysis. P1 indicates the female reference parent, P1xP2 are hybrids with P1 as the female parent, and P2xP1 are the reciprocals with P1 as the male parent. In addition to the difference in mean, the difference in variance also suggests large maternal effect.

Variety (P1)	P1 Strength {(%TQ)*Min/ grams of protein}	Mean P1xP2 Hybrids	Mean P2xP1 Hybrids	Variance P1xP2	Variance P2xP1	P-Value
FREEMAN	37.7	36.0	29.0	2.3	79.6	0.02161
GOODSTREAK	13.6	15.5	25.9	0.6	47.6	0.00041
LCH13NEDH-11-24	45.7	43.8	30.2	22.3	96.3	0.00385
NE09517-1	35.9	37.3	26.5	18.0	51.5	0.00009
NE10589	29.7	29.6	27.3	5.5	76.0	0.33859
OVERLAND	21.7	23.4	28.4	1.4	80.9	0.09990
SETTLER CL	42.4	35.2	27.1	8.2	51.0	0.00102
TX10D2063	18.5	20.7	30.0	1.2	93.0	0.01158
TX10D2230	21.9	21.5	27.4	3.9	64.0	0.01975
TX11D3129	18.0	21.9	27.0	1.6	50.9	0.03286
TX12M4063	19.8	21.1	28.6	4.9	85.5	0.01260
TX12M4065	21.4	24.7	28.5	2.2	100.0	0.19081
WESLEY	34.7	31.7	28.1	21.0	98.1	0.22587

Table 9. Paired t-test Results for Mixograph Strength of the 2017 Hybrids.
Paired t-tests (α = .05) between mean strength of reciprocal hybrids for each parent cultivar used in the 2017 analysis. P1 indicates the female reference parent, P1xP2 are hybrids with P1 as the female parent, and P2xP1 are the reciprocals with P1 as the male parent. In addition to the difference in mean, the difference in variance also suggests large maternal effect.

Variety (P1)	P1 Strength {(%TQ*Min)/ grams of protein}	Mean – P1xP2 Hybrids	Mean – P2xP1 Hybrids	Variance (P1xP2)	Variance (P2xP1)	P-Value
FREEMAN	44.1	40.5	36.0	35.19	107.85	0.1153
GOODSTREAK	16.6	19.0	27.7	0.80	39.23	0.0005
HARRY	36.0	39.3	31.3	19.37	91.84	0.0269
LCH13NEDH-11-24	54.3	44.8	30.3	78.84	110.50	0.0195
NE07531	44.7	39.8	33.3	48.59	60.47	0.0032
NE09517_1	51.7	43.3	30.9	38.26	93.65	0.0029
OVERLAND	20.8	25.8	31.8	14.72	64.97	0.0176
ROBIDOUX	42.1	37.6	35.7	21.28	99.96	0.4425
SETTLER_CL	42.5	38.9	31.6	20.67	79.26	0.0068
TX09D1172	20.0	25.6	31.6	6.68	70.46	0.0205
TX10D2063	24.0	23.9	32.6	1.45	123.94	0.0291
TX10D2363	22.4	25.4	32.2	1.87	117.48	0.0674
TX11D3129	22.8	25.6	32.6	3.05	75.14	0.0109
TX12M4063	28.8	31.5	33.5	16.72	90.56	0.3092

Table 10. Paired t-test Results for Mixograph Tolerance of the 2016 Hybrids. Paired t-tests ($\alpha = .05$) between mean tolerance of reciprocal hybrids for each parent cultivar used in the 2016 analysis. P1 indicates the female reference parent, P1xP2 are hybrids with P1 as the female parent, and P2xP1 are the reciprocals with P1 as the male parent. In addition to the difference in mean, the difference in variance also suggests large maternal effect, although LCH13NEDH_11_24 had P1xP2 hybrids with widely varying tolerance values.

Variety (P1)	P1 Tolerance	Mean – P1xP2 Hybrids	Mean – P2xP1 Hybrids	Variance (P1xP2)	Variance (P2xP1)	P-Value
FREEMAN	1.71	1.97	1.86	0.125	0.763	0.69581
GOODSTREAK	1.18	1.21	1.57	0.000	0.341	0.06517
LCH13NEDH-11-24	26.69	7.62	1.83	150.101	0.201	0.17090
NE09517-1	1.80	1.80	1.57	0.086	0.157	0.10671
NE10589	1.62	1.64	1.82	0.077	0.619	0.28774
OVERLAND	1.41	1.56	5.62	0.018	166.793	0.34426
SETTLER CL	1.83	1.84	1.72	0.058	0.575	0.48996
TX10D2063	1.22	1.29	1.96	0.004	1.711	0.13782
TX10D2230	1.35	1.33	1.62	0.007	0.081	0.00337
TX11D3129	1.41	1.41	1.49	0.004	0.028	0.21321
TX12M4063	1.30	1.35	1.80	0.005	0.294	0.02042
TX12M4065	1.46	1.55	1.67	0.018	0.604	0.61601
WESLEY	2.01	1.75	1.74	0.150	0.603	0.93933

Table 11. Paired t-test Results for Mixograph Tolerance of the 2017 Hybrids.
Paired t-tests ($\alpha = .05$) between mean tolerance of reciprocal hybrids for each parent cultivar used in the 2017 analysis. P1 indicates the female reference parent, P1xP2 are hybrids with P1 as the female parent, and P2xP1 are the reciprocals with P1 as the male parent. The tolerance values had a very wide range, resulting in unusually high mean and variance estimates in some varieties, including LCH13NEDH_11_24 and Freeman.

Variety (P1)	P1 Tolerance	Mean – P1xP2 Hybrids	Mean P2xP1 Hybrids	Variance (P1xP2)	Variance (P2xP1)	P-Value
FREEMAN	7.13	6.95	3.52	90.504	15.538	0.2620
GOODSTREAK	1.60	1.51	1.99	0.017	0.242	0.0073
HARRY	3.52	4.82	2.67	38.413	2.217	0.2806
LCH13NEDH-11-24	4.34	92.80	3.32	55392.970	11.747	0.3546
NE07531	6.19	2.72	3.18	0.186	3.913	0.4905
NE09517_1	5.12	2.96	5.39	0.652	118.957	0.5008
OVERLAND	1.62	1.75	3.93	0.021	38.135	0.2281
ROBIDOUX	3.32	4.62	3.42	5.573	10.297	0.3720
SETTLER_CL	4.45	4.35	2.44	17.064	1.728	0.1940
TX09D1172	1.88	1.85	50.11	0.067	29995.870	0.3349
TX10D2063	1.27	1.52	2.65	0.018	3.179	0.0683
TX10D2363	1.54	1.53	3.08	0.014	6.018	0.0721
TX11D3129	1.63	1.85	2.29	0.032	0.642	0.0569

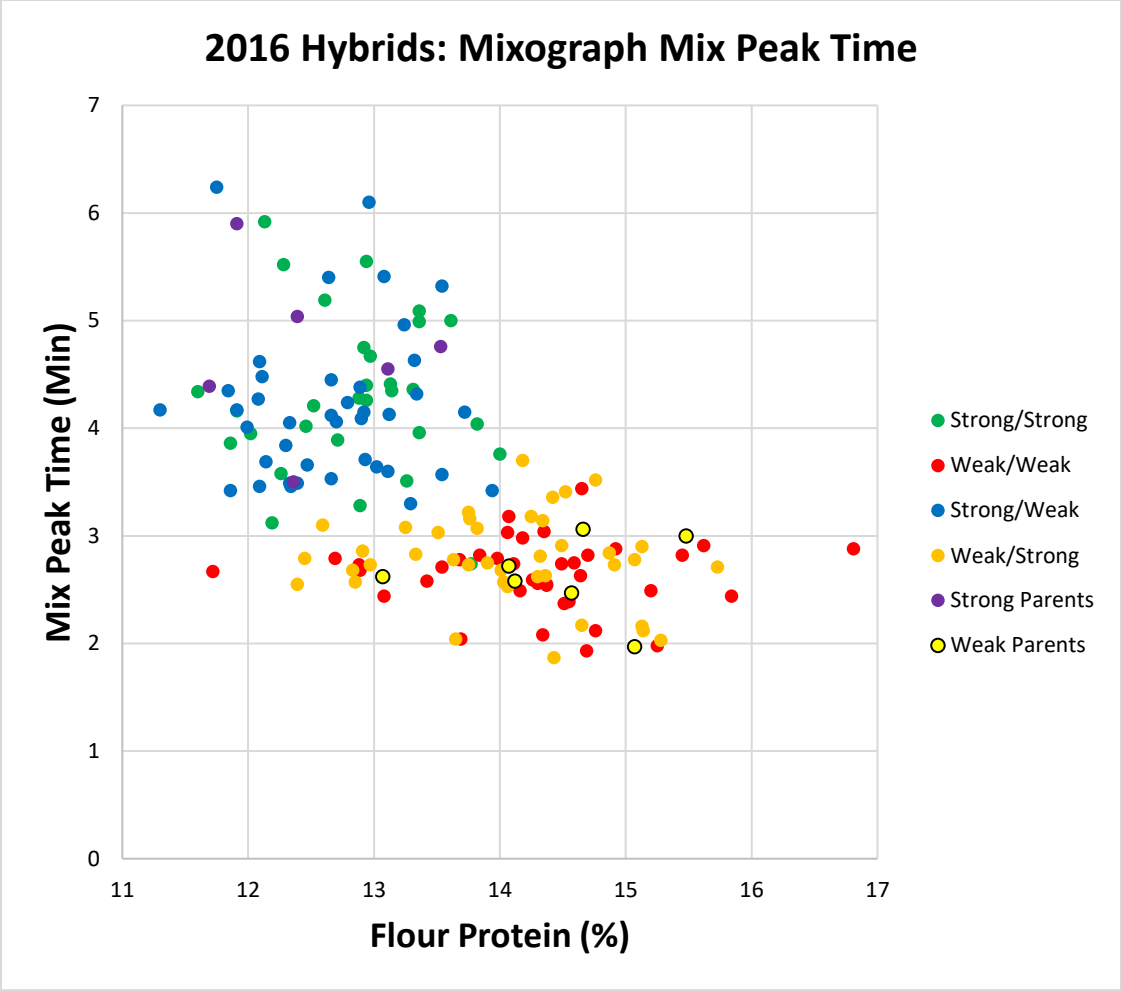


Figure 17. Mixograph Mix Peak Time Analysis of 2016 Hybrids.
This graph shows the peak mix development time for the 2016 hybrid selections as plotted against flour protein %. The mix peak time (min) is displayed on the Y-axis while the flour protein % is plotted X-axis. The strong parents are shown in purple and the weak parents are shown in yellow and help show the relationship between the quality of the parents and the hybrid.

Table 12. Paired t-test Results for Mixograph Mix Peak Time of the 2016 Hybrids. Paired t-tests (α = .05) between mean Mixograph mix peak time (Min) of reciprocal hybrids for each parent cultivar used in the 2016 analysis. P1 indicates the female reference parent, P1xP2 are hybrids with P1 as the female parent, and P2xP1 are the reciprocals with P1 as the male parent. In addition to the difference in mean, the difference in variance also suggests large maternal effect.

Variety (P1)	P1 Mix Peak Time (Min)	Mean – P1xP2 Hybrids	Mean – P2xP1 Hybrids	Variance (P1xP2)	Variance (P2xP1)	df	P-Value
FREEMAN	4.39	4.13	3.51	0.048	1.045	11	0.04667
GOODSTREAK	1.97	2.05	3.18	0.009	0.502	10	0.00022
LCH13NEDH-11-24	5.90	5.40	3.68	0.390	1.115	9	0.00284
NE09517-1	4.76	4.52	3.23	0.248	0.549	10	0.00009
NE10589	3.50	3.48	3.40	0.047	1.074	11	0.78791
OVERLAND	2.62	2.68	3.51	0.010	1.011	9	0.02200
SETTLER CL	5.04	4.21	3.37	0.117	0.724	11	0.00136
TX10D2063	2.47	2.59	3.67	0.015	1.374	9	0.01275
TX10D2230	3.00	2.86	3.34	0.051	0.823	10	0.08547
TX11D3129	2.58	2.85	3.27	0.023	0.597	9	0.07806
TX12M4063	2.72	2.75	3.53	0.047	1.008	9	0.02009
TX12M4065	3.06	3.19	3.53	0.064	1.325	10	0.28295
WESLEY	4.55	3.82	3.49	0.192	1.238	11	0.34977

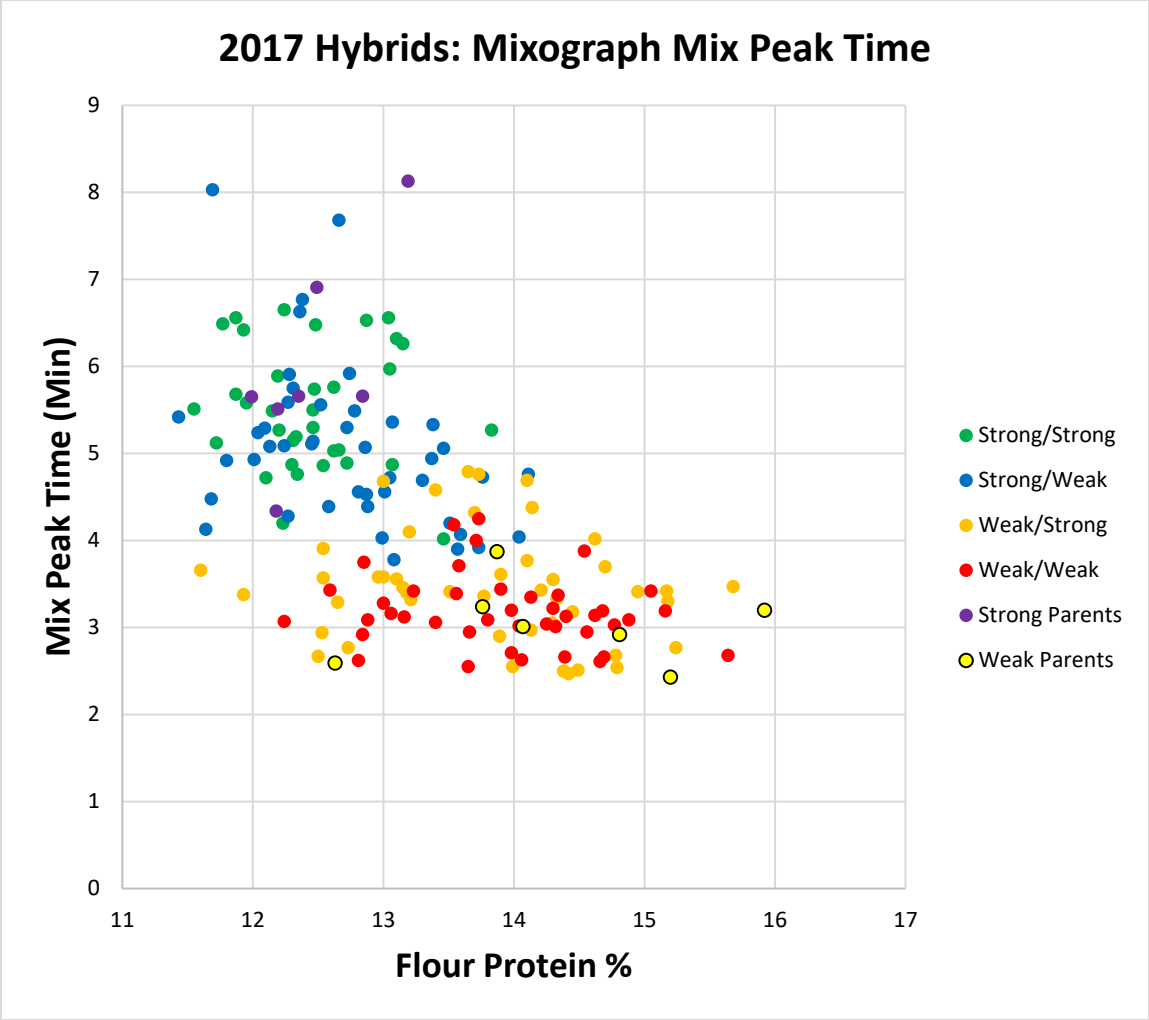


Figure 18. Mixograph Mix Peak Time Analysis of 2017 Hybrids

This graph shows the peak mix development time for the 2016 hybrid selections as plotted against flour protein %. The mix peak time (min) is displayed on the Y-axis while the flour protein % is plotted X-axis. The strong parents are shown in purple and the weak parents are shown in yellow to show the relationship between the quality of the parents and the hybrids.

Table 13. Paired t-test Results for Mixograph Mix Peak Time of the 2017 Hybrids. Paired t-tests ($\alpha = .05$) between mean strength of reciprocal hybrids for each parent cultivar used in the 2016 analysis. P1 indicates the female reference parent, P1xP2 are hybrids with P1 as the female parent, and P2xP1 are the reciprocals with P1 as the male parent. In addition to the difference in mean, the difference in variance also suggests large maternal effect.

Variety (P1)	P1 Mix Peak Time (Min)	Mean – P1xP2 Hybrids	Mean – P2xP1 Hybrids	Variance (P1xP2)	Variance (P2xP1)	P-Value
FREEMAN	5.51	5.06	4.49	0.482	1.629	0.0364
GOODSTREAK	2.43	2.61	3.65	0.008	0.503	0.0002
HARRY	4.34	4.94	4.11	0.191	1.487	0.0531
LCH13NEDH_11_24	8.13	5.91	3.90	1.534	1.604	0.0128
NE07531	5.65	5.09	4.35	0.676	0.803	0.0009
NE09517_1	6.91	5.70	4.07	0.616	1.506	0.0011
OVERLAND	2.59	3.07	4.02	0.136	0.935	0.0041
ROBIDOUX	5.66	4.85	4.56	0.280	1.550	0.3230
SETTLER_CL	5.66	4.92	4.10	0.266	1.443	0.0207
TX09D1172	2.92	3.39	4.12	0.070	1.035	0.0150
TX10D2063	3.01	3.06	4.22	0.017	2.104	0.0242
TX10D2363	3.20	3.32	4.09	0.033	1.590	0.0665
TX11D3129	3.24	3.45	4.25	0.084	1.206	0.0164
TX12M4063	3.87	4.25	4.36	0.242	1.797	0.6999

Table 14. 2016 Overall Summary of Hybrid Pairs that Exhibited Reciprocal Effect as Determined by the Mixograph Strength, Tolerance, and Mix Peak Time.
Provides an overall summary of the number of hybrid pairs that exhibited significant (α = .05) reciprocal effect detected using the Mixograph strength {(%TQ*Min)/grams of protein}, tolerance, and mix peak time (Min) in the 2016 hybrid analysis.

Variety	Reciprocal Hybrids Tested	Strength ({%TQ*Min}/ grams of protein)	Tolerance	Mix Peak Time (Min)
FREEMAN	12	0	0	0
GOODSTREAK	11	1	0	1
LCH13NEDH-11-24	10	5	1	5
NE09517-1	11	1	0	3
NE10589	12	0	0	0
OVERLAND	10	1	1	1
SETTLER CL	12	0	0	0
TX10D2063	10	1	0	2
TX10D2230	11	0	0	1
TX11D3129	10	0	0	0
TX12M4063	10	1	0	1
TX12M4065	11	1	0	1
WESLEY	12	1	0	1
Hybrids With Reciprocal Effect		12	2	16
Total Hybrids		142	142	142
Reciprocal Effect %		8%	1%	11%

Table 15. 2016 Hybrids Exhibiting Reciprocal Effect for Mixograph Strength. Listed are the 12 hybrids from the 2016 Mixograph strength {(%TQ*Min/grams of protein} analysis that exhibited a significant ($\alpha = .05$) reciprocal effect. P1 is the female parent, P1xP2 is hybrid with P1 as the female parent and P2 as the male parent, and P2xP1 is the reciprocal, and P2 is the male parent.

F. Parent (P1)	M. Parent (P2)	Female (P1) Strength	Hybrid (P1xP2) Strength	Reciprocal (P2xP1) Strength	Male (P2) Strength
GOODSTREAK	LCH13NEDH-11-24	13.6	16.0	38.8	45.7
LCH13NEDH-11-24	TX10D2063	45.7	46.1	19.7	18.5
LCH13NEDH-11-24	TX12M4063	45.7	43.8	21.6	19.8
LCH13NEDH-11-24	GOODSTREAK	45.7	38.8	16.0	13.6
LCH13NEDH-11-24	OVERLAND	45.7	42.0	24.8	21.7
LCH13NEDH-11-24	TX12M4065	45.7	49.9	25.7	21.4
NE09517-1	WESLEY	35.9	38.4	21.5	34.7
OVERLAND	LCH13NEDH-11-24	21.7	24.8	42.0	45.7
TX10D2063	LCH13NEDH-11-24	18.5	19.7	46.1	45.7
TX12M4063	LCH13NEDH-11-24	19.8	21.6	43.8	45.7
TX12M4065	LCH13NEDH-11-24	21.4	25.7	49.9	45.7
WESLEY	NE09517-1	34.7	21.5	38.4	35.9

Table 16. 2016 Hybrids Exhibiting Reciprocal Effect for Mixograph Tolerance. Listed are the two hybrids from the 2016 Mixograph tolerance analysis that exhibited a significant ($\alpha = .05$) reciprocal effect. P1 is the female parent, P1xP2 is hybrid with P1 as the female parent and P2 as the male parent, and P2xP1 is the reciprocal, and P2 is the male parent.

F. Parent (P1)	M. Parent (P2)	Female (P1) Tolerance	Hybrid (P1xP2) Tolerance	Reciprocal (P2xP1) Tolerance	Male (P2) Tolerance
LCH13NEDH-11-24	OVERLAND	26.68944	42.37288	1.680983	1.414261
OVERLAND	LCH13NEDH-11-24	1.414261	1.680983	42.37288	26.68944

Table 17. 2016 Hybrids Exhibiting Reciprocal Effect for Mix Peak Time.
Listed are the 16 hybrids from the 2016 Mixograph mix peak time (min) analysis that exhibited a significant ($\alpha = .05$) reciprocal effect. P1 is the female parent, P1xP2 is hybrid with P1 as the female parent and P2 as the male parent, and P2xP1 is the reciprocal, and P2 is the male parent.

F. Parent (P1)	M. Parent (P2)	Female (P1) Mix Peak Time	Hybrid (P1xP2) Mix Peak Time	Reciprocal (P2xP1) Mix Peak Time	Male (P2) Mix Peak Time
GOODSTREAK	LCH13NEDH-11-24	1.97	2.16	4.62	5.9
LCH13NEDH-11-24	TX10D2063	5.9	6.1	2.71	2.47
LCH13NEDH-11-24	TX12M4063	5.9	5.32	2.73	2.72
LCH13NEDH-11-24	GOODSTREAK	5.9	4.62	2.16	1.97
LCH13NEDH-11-24	OVERLAND	5.9	5.4	2.86	2.62
LCH13NEDH-11-24	TX12M4065	5.9	6.24	3.36	3.06
NE09517-1	TX10D2230	4.76	4.96	2.9	3
NE09517-1	TX10D2063	4.76	4.63	2.68	2.47
NE09517-1	WESLEY	4.76	4.99	2.74	4.55
OVERLAND	LCH13NEDH-11-24	2.62	2.86	5.4	5.9
TX10D2063	NE09517-1	2.47	2.68	4.63	4.76
TX10D2063	LCH13NEDH-11-24	2.47	2.71	6.1	5.9
TX10D2230	NE09517-1	3	2.9	4.96	4.76
TX12M4063	LCH13NEDH-11-24	2.72	2.73	5.32	5.9
TX12M4065	LCH13NEDH-11-24	3.06	3.36	6.24	5.9
WESLEY	NE09517-1	4.55	2.74	4.99	4.76

Table 18. 2017 Overall Summary of Hybrid Pairs that Exhibited Reciprocal Effect as Determined by the Mixograph Strength, Tolerance, and Mix Peak Time.
Provides an overall summary of the number of hybrid pairs that exhibited significant (α = .05) reciprocal effect detected using the Mixograph strength {(%TQ*Min)/grams of protein}, tolerance, and mix peak time (Min) in the 2017 hybrid analysis.

Variety	Reciprocal Hybrids Tested	Strength ({%TQ*Min}/ grams of protein)	Tolerance	Mix Peak Time (Min)
FREEMAN	13	1	0	0
GOODSTREAK	13	1	0	0
HARRY	12	2	0	1
LCH13NEDH_11_24	7	2	1	3
NE07531	9	0	0	0
NE09517_1	10	2	0	2
OVERLAND	13	1	0	1
ROBIDOUX	11	0	0	0
SETTLER_CL	11	0	0	0
TX09D1172	13	0	1	1
TX10D2063	11	1	0	1
TX10D2363	10	2	0	1
TX11D3129	12	0	0	0
TX12M4063	13	0	0	0
Hybrids With Reciprocal Effect		12	2	10
Total Hybrids		158	158	158
Reciprocal Effect %		8%	1%	6%

Table 19. 2017 Hybrids Exhibiting Reciprocal Effect for Mixograph Strength. Listed are the 12 hybrids from the 2017 Mixograph strength {(%TQ*Min/grams of protein} analysis that exhibited a significant ($\alpha = .05$) reciprocal effect. P1 is the female parent, P1xP2 is hybrid with P1 as the female parent and P2 as the male parent, and P2xP1 is the reciprocal, and P2 is the male parent.

F. Parent (P1)	M. Parent (P2)	Female (P1) Strength	Hybrid (P1xP2) Strength	Reciprocal (P2xP1) Strength	Male (P2) Strength
FREEMAN	TX10D2363	44.1	45.5	26.1	22.4
GOODSTREAK	HARRY	16.6	18.8	42.3	36.0
HARRY	GOODSTREAK	36.0	42.3	18.8	16.6
HARRY	NE09517_1	36.0	30.1	50.6	51.7
LCH13NEDH_11_24	OVERLAND	54.3	44.1	23.4	20.8
LCH13NEDH_11_24	TX10D2063	54.3	57.6	23.3	24.0
NE09517_1	HARRY	51.7	50.6	30.1	36.0
NE09517_1	TX10D2363	51.7	52.8	25.0	22.4
OVERLAND	LCH13NEDH-11-24	20.8	23.4	44.1	54.3
TX10D2063	LCH13NEDH-11-24	24.0	23.3	57.6	54.3
TX10D2363	FREEMAN	22.4	26.1	45.5	44.1
TX10D2363	NE09517_1	22.4	25.0	52.8	51.7

Table 20. 2017 Hybrids Exhibiting Reciprocal Effect for Mixograph Tolerance. Listed are the two hybrids from the 2017 Mixograph tolerance analysis that exhibited a significant ($\alpha = .05$) reciprocal effect. P1 is the female parent, P1xP2 is hybrid with P1 as the female parent and P2 as the male parent, and P2xP1 is the reciprocal, and P2 is the male parent.

F. Parent (P1)	M. Parent (P2)	Female (P1) Tolerance	Hybrid (P1xP2) Tolerance	Reciprocal (P2xP1) Tolerance	Male (P2) Tolerance
LCH13NEDH-11-24	TX09D1172	4.33	626	1.84	1.87
TX09D1172	LCH13NEDH-11-24	1.87	1.84	626	4.33

Table 21. 2017 Hybrids Exhibiting Reciprocal Effect for Mix Peak Time.
Listed are the ten hybrids from the 2017 Mixograph mix peak time (min) analysis that exhibited a significant ($\alpha = .05$) reciprocal effect. P1 is the female parent, P1xP2 is hybrid with P1 as the female parent and P2 as the male parent, and P2xP1 is the reciprocal, and P2 is the male parent.

F. Parent (P1)	M. Parent (P2)	Female (P1) Mix Peak Time	Hybrid (P1xP2) Mix Peak Time	Reciprocal (P2xP1) Mix Peak Time	Male (P2) Mix Peak Time
HARRY	NE09517_1	4.34	4.02	6.65	6.91
LCH13NEDH-11-24	OVERLAND	8.13	5.56	2.77	2.59
LCH13NEDH-11-24	TX09D1172	8.13	5.91	3.43	2.92
LCH13NEDH-11-24	TX10D2063	8.13	7.68	3.03	3.01
NE09517_1	HARRY	6.91	6.65	4.02	4.34
NE09517_1	TX10D2363	6.91	6.63	3.47	3.2
OVERLAND	LCH13NEDH-11-24	2.59	2.77	5.56	8.13
TX09D1172	LCH13NEDH-11-24	2.92	3.43	5.91	8.13
TX10D2063	LCH13NEDH-11-24	3.01	3.03	7.68	8.13
TX10D2363	NE09517_1	3.2	3.47	6.63	6.91

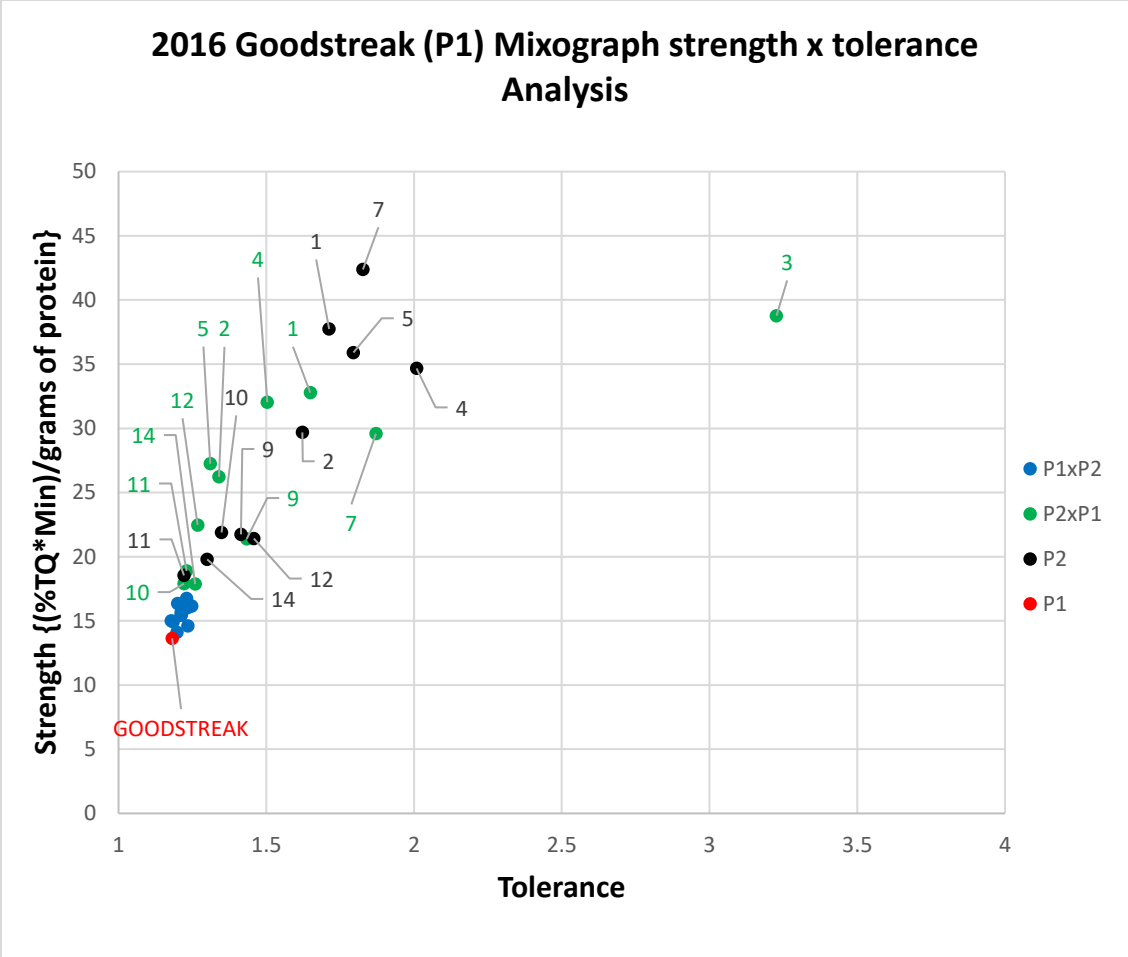


Figure 19. Mixograph strength x tolerance analysis evaluating 2016 Goodstreak (P1) reciprocal hybrids.

The Mixograph strength x tolerance analysis of the Goodstreak reciprocal hybrids from 2016 with the strength {(%TQ*Min)/grams of protein} graphed on the Y-axis and the tolerance graphed on the X-axis. P1 represents Goodstreak (coded in red), P1xP2 are hybrids with Goodstreak as the female parent (coded in blue), P2xP1 are the reciprocals with Goodstreak as the male parent (coded in green), and the P2 parents are coded in black.

Table 22. Mixograph Mix Peak Time Analysis of the 2016 Goodstreak Reciprocal Hybrids.

Shows the Mixograph mix peak time (Min.) of the Goodstreak reciprocal hybrids from the 2016 analysis. P1 is Goodstreak, P1xP2 are the hybrids with Goodstreak as the female parent and P2 as the male parent, P2xP1 is the reciprocal, and P2 is the male parent. P-value of paired t-test ($\alpha = .05$) indicates significant reciprocal difference between the mean mix peak time (Min) of the P1xP2 hybrids and the P2xP1 reciprocals.

P1 (female)	P2 (male)	Goodstreak (P1)	P1xP2 hybrid mix peak time (min)	P2xP1 hybrid mix peak time (min)	P2 mix peak time (min)
GOODSTREAK	TX10D2230	1.97	2.04	2.44	3
GOODSTREAK	FREEMAN	1.97	2.17	3.69	4.39
GOODSTREAK	NE09517-1	1.97	2.03	3.42	4.76
GOODSTREAK	SETTLER CL	1.97	2.04	3.64	5.04
GOODSTREAK	WESLEY	1.97	2.12	3.66	4.55
GOODSTREAK	TX10D2063	1.97	1.93	2.37	2.47
GOODSTREAK	TX12M4063	1.97	2.12	2.49	2.72
GOODSTREAK	LCH13NEDH-11-24	1.97	2.16	4.62	5.9
GOODSTREAK	TX12M4065	1.97	2.08	2.82	3.06
GOODSTREAK	NE10589	1.97	1.87	3.3	3.5
GOODSTREAK	OVERLAND	1.97	1.98	2.58	2.62

Mean	2.049091	3.184545
Variance	0.008949	0.501687
t Stat	-5.62204	
P(T<=t) two-tail	0.000221	

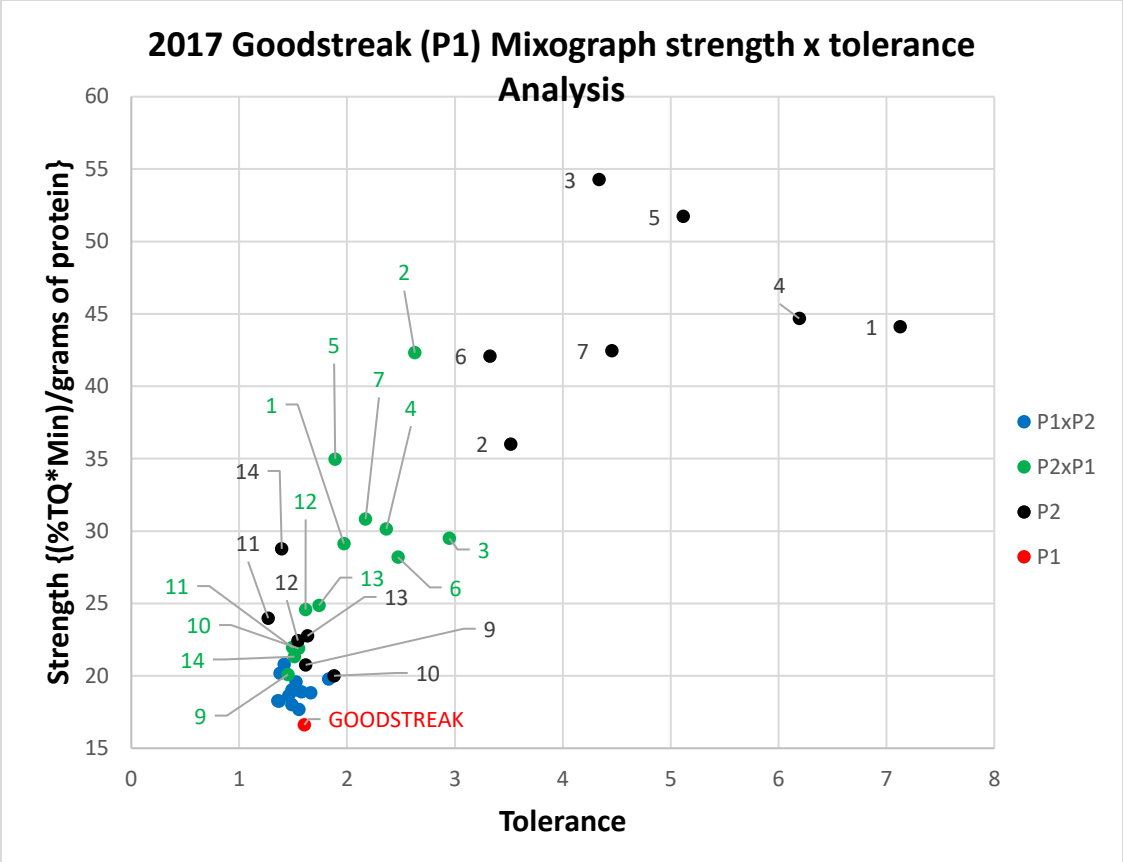


Figure 20. Mixograph strength x tolerance analysis evaluating 2017 Goodstreak (P1) reciprocal hybrids.

The Mixograph strength x tolerance analysis of the Goodstreak reciprocal hybrids from 2017 with the strength {(%TQ*Min)/grams of protein} graphed on the Y-axis and the tolerance graphed on the X-axis. P1 represents Goodstreak (coded in red), P1xP2 are hybrids with Goodstreak as the female parent (coded in blue), P2xP1 are the reciprocals with Goodstreak as the male parent (coded in green), and the P2 parents are coded in black.

Table 23. Mixograph Mix Peak Time Analysis of the 2017 Goodstreak Reciprocal Hybrids.

Shows the Mixograph mix peak time (Min.) of the Goodstreak reciprocal hybrids from the 2017 analysis. P1 is Goodstreak, P1xP2 are the hybrids with Goodstreak as the female parent and P2 as the male parent, P2xP1 is the reciprocal, and P2 is the male parent. P-value of paired t-test ($\alpha = .05$) indicates significant reciprocal difference between the mean mix peak time (Min) of the P1xP2 hybrids and the P2xP1 reciprocals.

P1 (female)	P2 (male)	Goodstreak (P1) mix peak time (min)	P1xP2 hybrid mix peak time (min)	P2xP1 hybrid mix peak time (min)	P2 mix peak time (min)
GOODSTREAK	FREEMAN	2.43	2.55	3.92	5.51
GOODSTREAK	HARRY	2.43	2.68	4.92	4.34
GOODSTREAK	LCH13NEDH-11-24	2.43	2.5	3.78	8.13
GOODSTREAK	NE07531	2.43	2.77	3.9	5.65
GOODSTREAK	NE09517_1	2.43	2.47	4.73	6.91
GOODSTREAK	OVERLAND	2.43	2.66	2.55	2.59
GOODSTREAK	ROBIDOUX	2.43	2.54	4.04	5.66
GOODSTREAK	SETTLER_CL	2.43	2.51	4.07	5.66
GOODSTREAK	TX09D1172	2.43	2.68	3.01	2.92
GOODSTREAK	TX10D2063	2.43	2.61	2.95	3.01
GOODSTREAK	TX10D2363	2.43	2.63	3.19	3.2
GOODSTREAK	TX11D3129	2.43	2.66	3.28	3.24
GOODSTREAK	TX12M4063	2.43	2.71	3.09	3.87

Mean	2.613077	3.648462
Variance	0.008406	0.502647
t Stat	-4.97983	
P(T<=t) two-tail	0.00032	

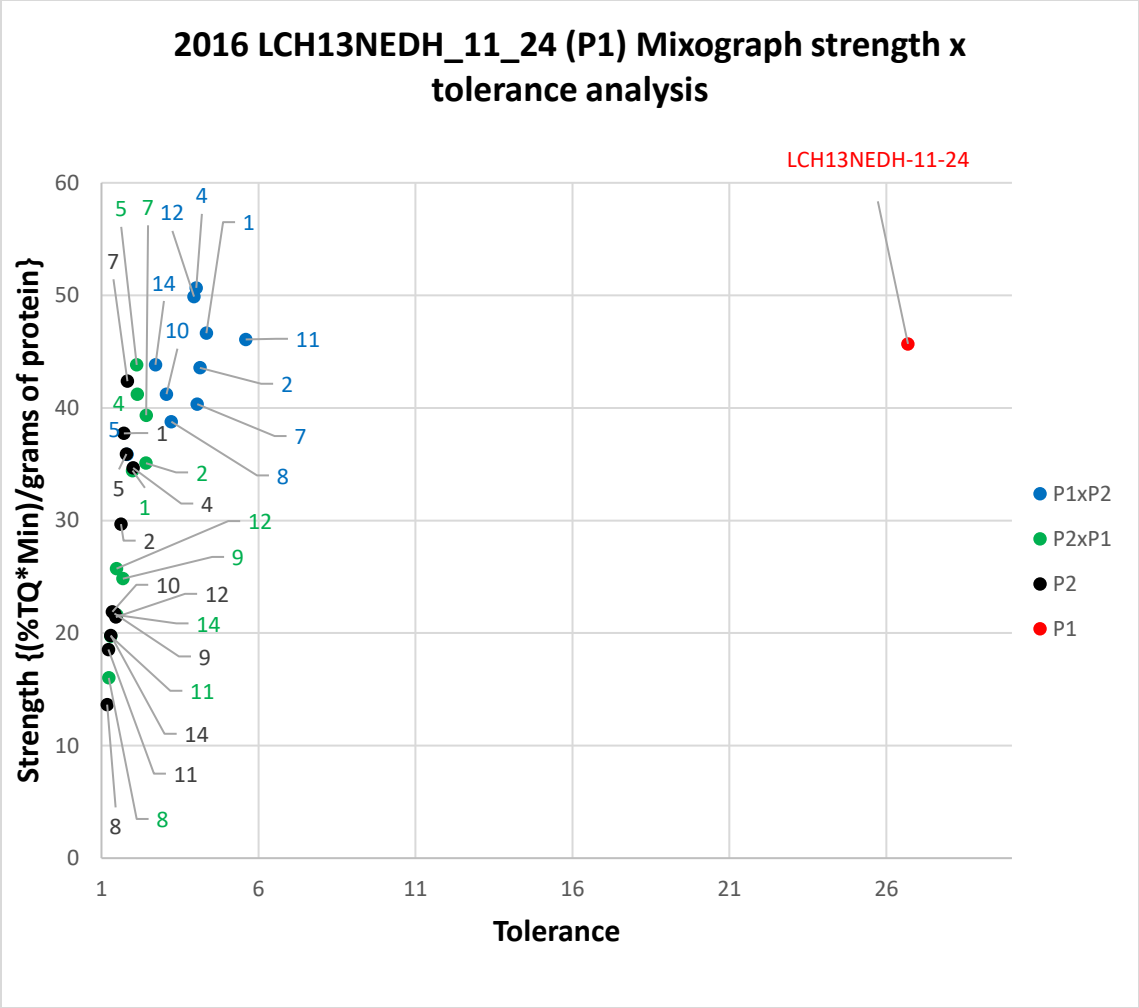


Figure 21. Mixograph Strength x Tolerance Analysis Evaluating 2016 LCH13NEDH_11_24 (P1) Reciprocal Hybrids.

The Mixograph strength x tolerance analysis of the LCH13NEDH_11_24 reciprocal hybrids from 2016 with the strength {(%TQ*Min)/grams of protein} graphed on the Y-axis and the tolerance graphed on the X-axis. P1 represents LCH13NEDH_11_24 (coded in red), P1xP2 are hybrids with LCH13NEDH_11_24 as the female parent (coded in blue), P2xP1 are the reciprocals with LCH13NEDH_11_24 as the male parent (coded in green), and the P2 parents are coded in black.

Table 24. Mixograph Mix Peak Time Analysis of the 2016 LCH13NEDH_11_24 Reciprocal Hybrids.

Mixograph mix peak time (Min.) of the LCH13NEDH_11_24 reciprocal hybrids from the 2016 analysis. P1 is LCH13NEDH_11_24, P1xP2 are the hybrids with LCH13NEDH_11_24 as the female parent and P2 as the male parent, P2xP1 is the reciprocal, and P2 is the male parent. P-value of paired t-test ($\alpha = .05$) indicates significant reciprocal difference between the mean mix peak time (Min) of the P1xP2 hybrids and the P2xP1 reciprocals. Highlighted hybrid was not included in paired t-test.

P1 (female)	P2 (male)	LCH13NEDH-11-24 (P1)	P1xP2 hybrid mix peak time (min)	P2xP1 hybrid mix peak time (min)	P2 mix peak time (min)
LCH13NEDH-11-24	SETTLER CL	5.9	5.09	5	5.04
LCH13NEDH-11-24	TX10D2063	5.9	6.1	2.71	2.47
LCH13NEDH-11-24	TX12M4063	5.9	5.32	2.73	2.72
LCH13NEDH-11-24	NE10589	5.9	5.55	4.02	3.5
LCH13NEDH-11-24	WESLEY	5.9	5.92	4.34	4.55
LCH13NEDH-11-24	NE09517-1	5.9	4.26	5.19	4.76
LCH13NEDH-11-24	GOODSTREAK	5.9	4.62	2.16	1.97
LCH13NEDH-11-24	FREEMAN	5.9	5.52	4.41	4.39
LCH13NEDH-11-24	OVERLAND	5.9	5.4	2.86	2.62
LCH13NEDH-11-24	TX12M4065	5.9	6.24	3.36	3.06
LCH13NEDH-11-24	TX10D2230	5.9	5.41	#N/A	3

Mean	5.402	3.678
Variance	0.390373	1.115018
t Stat	4.061196	
P(T<=t) two-tail	0.002837	

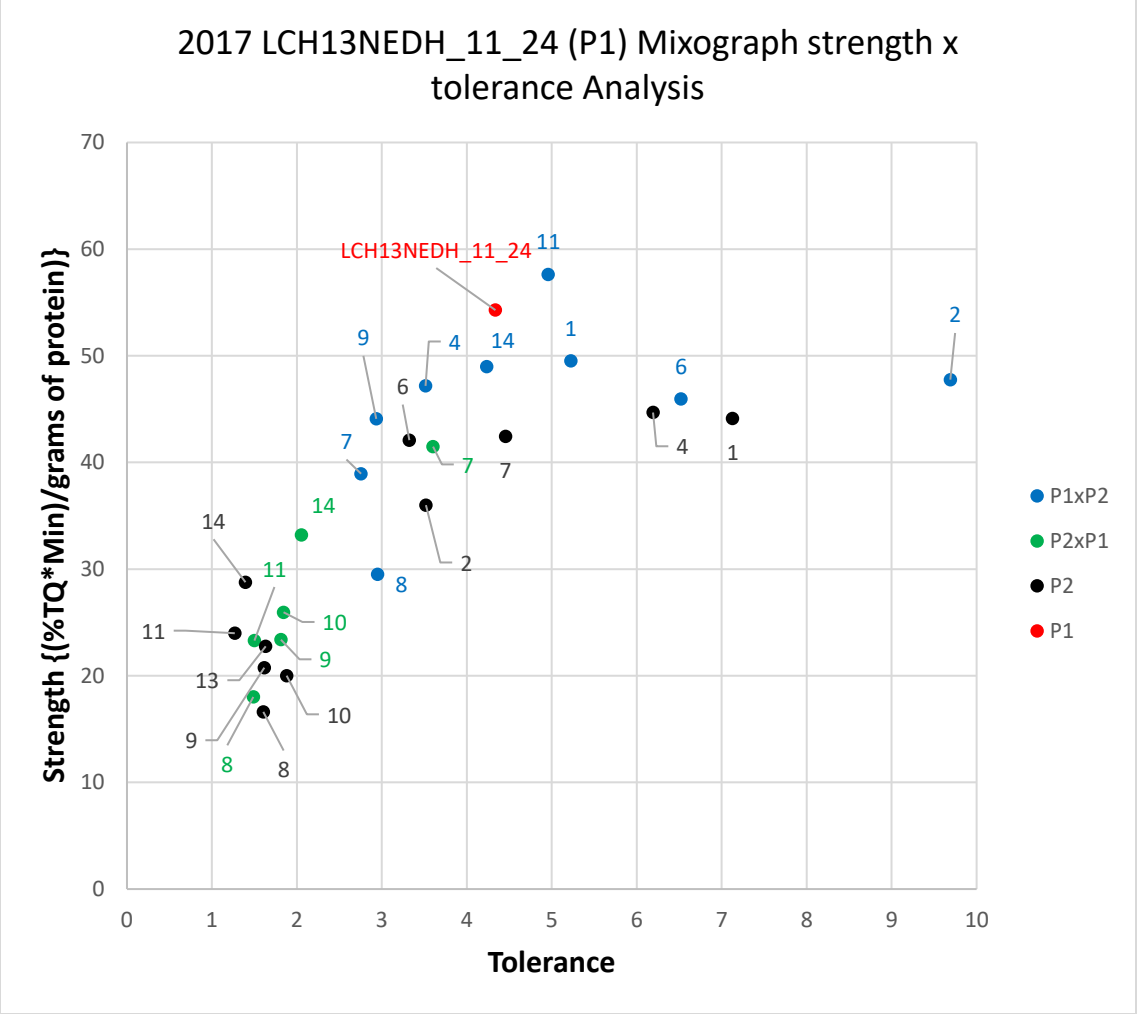


Figure 22. Mixograph Strength x Tolerance Analysis Evaluating 2017 LCH13NEDH_11_24 (P1) Reciprocal Hybrids.

The Mixograph strength x tolerance analysis of the LCH13NEDH_11_24 reciprocal hybrids from 2017 with the strength {(%TQ*Min)/grams of protein} graphed on the Y-axis and the tolerance graphed on the X-axis. P1 represents LCH13NEDH_11_24 (coded in red), P1xP2 are hybrids with LCH13NEDH_11_24 as the female parent (coded in blue), P2xP1 are the reciprocals with LCH13NEDH_11_24 as the male parent (coded in green), and the P2 parents are coded in black.

Table 25. Mixograph Mix Peak Time Analysis of the 2017 LCH13NEDH_11_24 Reciprocal Hybrids.

Mixograph mix peak time (Min.) of the LCH13NEDH_11_24 reciprocal hybrids from the 2017 analysis. P1 is LCH13NEDH_11_24, P1xP2 are the hybrids with LCH13NEDH_11_24 as the female parent and P2 as the male parent, P2xP1 is the reciprocal, and P2 is the male parent. P-value of paired t-test ($\alpha = .05$) indicates significant reciprocal difference between the mean mix peak time (Min) of the P1xP2 hybrids and the P2xP1 reciprocals. Highlighted hybrid was not included in paired t-test. Highlighted hybrids were not included in paired t-test ($\alpha = .05$).

P1 (female)	P2 (male)	LCH13NEDH_11_24 (P1) mix peak time (min)	P1xP2 hybrid mix peak time (min)	P2xP1 hybrid mix peak time (min)	P2 mix peak time (min)
LCH13NEDH-11-24	FREEMAN	8.13	6.42	5.58	5.51
LCH13NEDH-11-24	GOODSTREAK	8.13	3.78	2.5	2.43
LCH13NEDH-11-24	HARRY	8.13	6.56	#N/A	4.34
LCH13NEDH-11-24	NE07531	8.13	6.53	#N/A	5.65
LCH13NEDH-11-24	OVERLAND	8.13	5.56	2.77	2.59
LCH13NEDH-11-24	ROBIDOUX	8.13	6.48	#N/A	5.66
LCH13NEDH-11-24	SETTLER_CL	8.13	5.27	5.3	5.66
LCH13NEDH-11-24	TX09D1172	8.13	5.91	3.43	2.92
LCH13NEDH-11-24	TX10D2063	8.13	7.68	3.03	3.01
LCH13NEDH-11-24	TX11D3129	8.13	8.03	#N/A	3.24
LCH13NEDH-11-24	TX12M4063	8.13	6.77	4.69	3.87

Mean	5.912857	3.9
Variance	1.53359	1.603533
t Stat	3.503032	
P(T<=t) two-tail	0.012779	

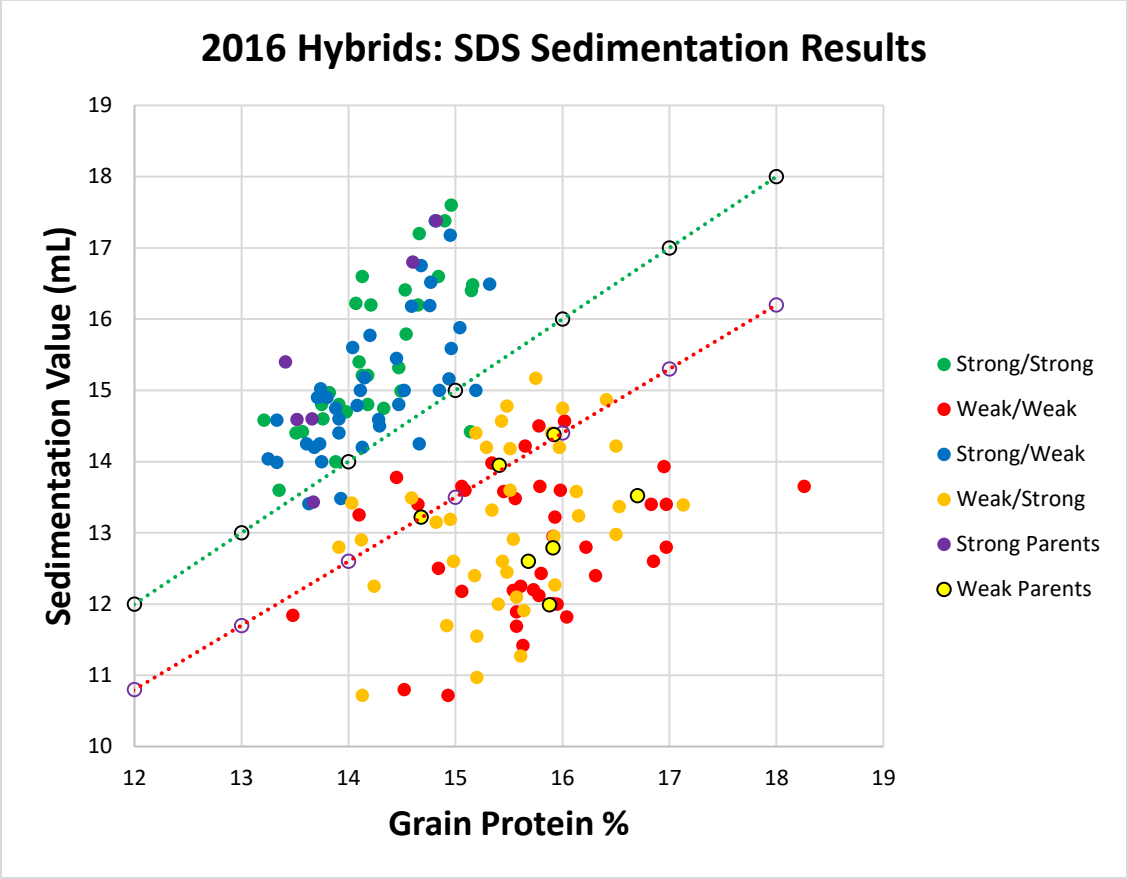


Figure 23. SDS Sedimentation Assay Results of 2016 Hybrids.
Represents the 2016 hybrid samples that were selected and analyzed using the SDS sedimentation assay. The Y-axis is the sedimentation value (mL) and the X-axis is the grain protein %. The legend is written in traditional pedigree notation with the left side representing the quality of female and right side represents quality of male. Samples that are at or above the green dotted line are considered desirable, samples that are at or below the red dotted line are considered undesirable, while samples in the middle are neither.

Table 26. Paired t-test Results for SDS Sedimentation Ratio of 2016 Hybrids.
Paired t-tests for the 2016 SDS sedimentation assay results, including the sedimentation ratio (mL/% protein) of each parent (P1), the mean sedimentation ratio of the hybrids crossed P1xP2, the reciprocal sedimentation ratio, the variance among those means, the degrees of freedom, and the p-value ($\alpha = .05$).

Cultivar (P1)	P1 SDS Sed. Ratio (mL/%)	Mean – P1xP2 Hybrids	Mean – P2xP1 Reciprocals	Variance (P1xP2)	Variance (P2xP1)	P-Value
FREEMAN	1.15	1.08	0.97	0.0009	0.0172	0.01243
GOODSTREAK	0.90	0.89	0.94	0.0010	0.0134	0.14351
LCH13NEDH-11-24	0.98	1.07	0.99	0.0025	0.0168	0.03802
NE09517-1	1.17	1.14	0.94	0.0017	0.0146	0.00018
NE10589	1.07	1.03	0.95	0.0011	0.0220	0.08707
OVERLAND	0.90	0.90	0.97	0.0013	0.0237	0.22249
SETTLER CL	1.08	1.04	0.94	0.0013	0.0161	0.00515
TX10D2063	0.91	0.92	0.96	0.0008	0.0225	0.37734
TX10D2230	0.81	0.79	0.95	0.0014	0.0224	0.00304
TX11D3129	0.80	0.78	0.90	0.0020	0.0192	0.00647
TX12M4063	0.76	0.79	0.93	0.0019	0.0202	0.01082
TX12M4065	0.80	0.78	0.93	0.0009	0.0174	0.00110
WESLEY	1.15	1.06	0.93	0.0025	0.0222	0.02246

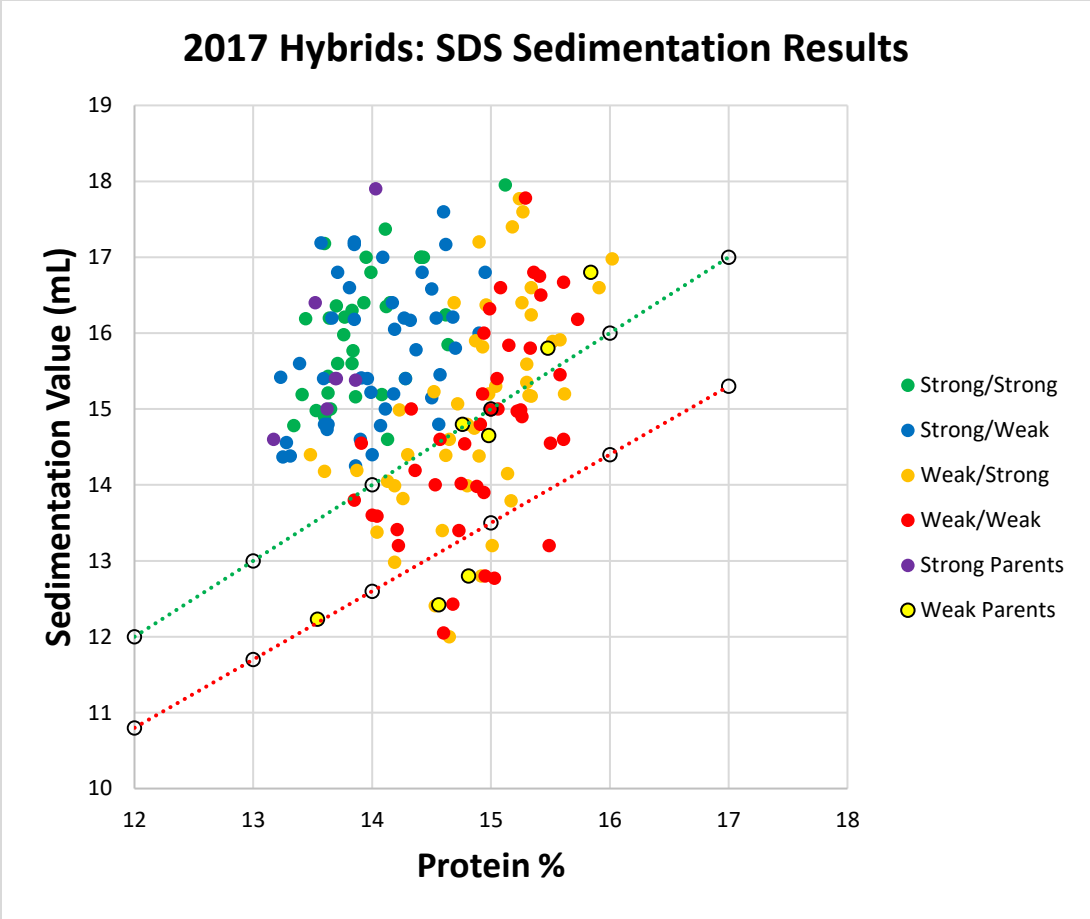


Figure 24. SDS Sedimentation Assay Results of 2017 Hybrids.
Represents the 2017 hybrid samples that were selected and analyzed using the SDS sedimentation assay. The Y-axis is the sedimentation value (mL) and the X-axis is the grain protein %. The legend is written in traditional pedigree notation with the left side representing the quality of female and right side represents quality of male. Samples that are at or above the green dotted line are considered desirable, samples that are at or below the red dotted line are considered undesirable, while samples in the middle are neither. The strong parents are shown in purple and the weak parents are shown in yellow to show the relationship between the quality of the parents and the hybrids.

Table 27. Paired t-test Results for SDS Sedimentation Ratio of 2017 Hybrids.
Paired t-tests for the 2017 SDS sedimentation assay results, including the sedimentation ratio (mL/% protein) of each parent (P1), the mean sedimentation ratio of the hybrids crossed P1xP2, the reciprocal sedimentation ratio, the variance among those means, the degrees of freedom, and the p-value ($\alpha = .05$).

Cultivar (P1)	P1 SDS Sed. Ratio (mL/%)	Mean – P1xP2 Hybrids	Mean – P2xP1 Reciprocals	Variance (P1xP2)	Variance (P2xP1)	P-Value
FREEMAN	1.11	1.09	1.07	0.0021	0.0081	0.4371
GOODSTREAK	1.02	1.03	1.09	0.0010	0.0066	0.0228
HARRY	1.10	1.14	1.08	0.0025	0.0113	0.1000
LCH13NEDH-11-24	1.28	1.21	1.01	0.0013	0.0025	0.0005
NE07531	1.12	1.14	1.08	0.0022	0.0069	0.0272
NE09517_1	1.21	1.18	1.03	0.0036	0.0063	0.0003
OVERLAND	0.90	0.99	1.05	0.0020	0.0109	0.0733
ROBIDOUX	1.12	1.11	1.08	0.0015	0.0109	0.2937
SETTLER_CL	1.11	1.09	1.04	0.0011	0.0167	0.1894
TX09D1172	0.98	1.01	1.07	0.0019	0.0103	0.0308
TX10D2063	1.00	1.01	1.03	0.0010	0.0072	0.3556
TX10D2363	1.06	1.10	1.03	0.0016	0.0103	0.0933
TX11D3129	0.85	0.89	1.05	0.0029	0.0095	0.0002
TX12M4063	0.86	0.94	1.05	0.0021	0.0118	0.0019

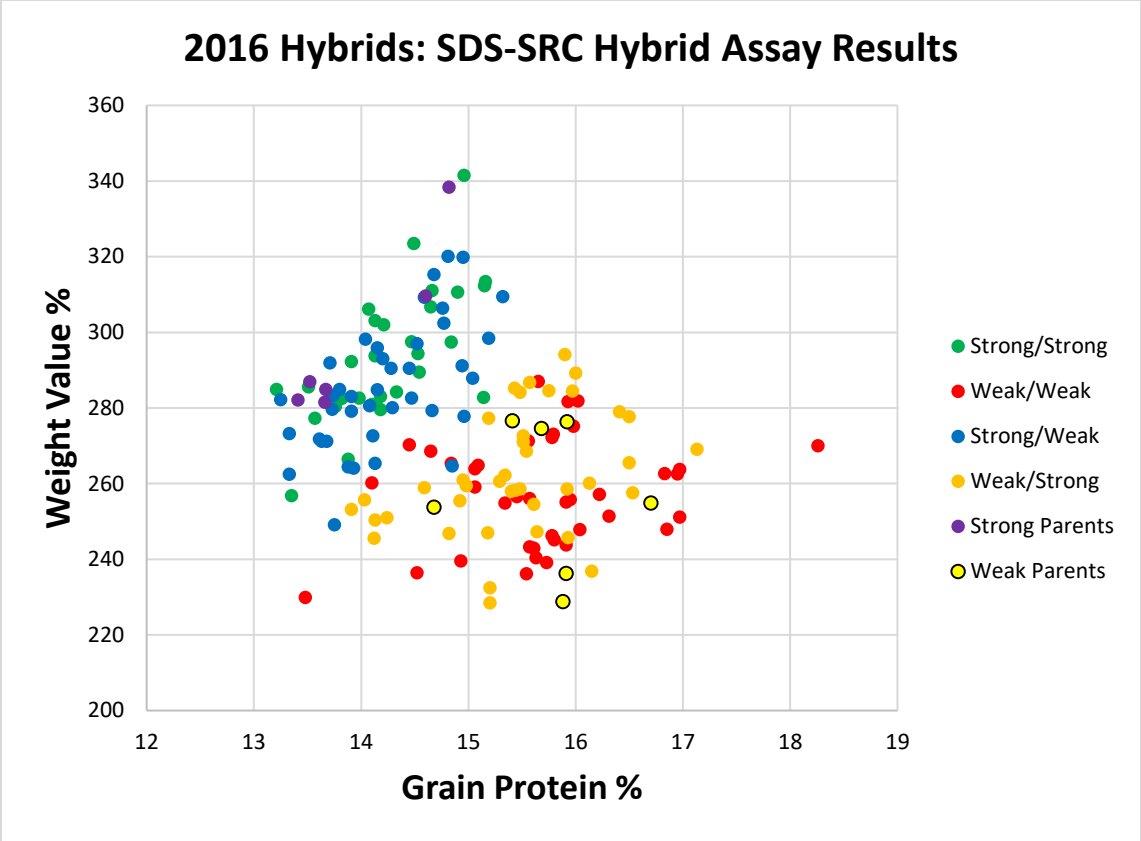


Figure 25. SDS-SRC Hybrid Assay Results of 2016 Hybrids.
Represents the SDS-SRC hybrid assay that was described by Seabourn *et al.* (2012) and the USDA in Manhattan, KS. The purpose of this assay is to measure the same protein functionality that the SDS assay measures but is faster and more closely correlated to loaf volume. The Y-axis represents the weight value % as calculated by **equation 1**, and the X-axis represents grain protein %. The strong parents are shown in purple and the weak parents are shown in yellow and help show the relationship between the end-use quality of the parents and the hybrid.

Table 28. Paired t-test Results for the SDS-SRC Hybrid Assay of 2016 Hybrids.
Paired t-tests for the 2016 SDS-SRC hybrid assay results, including the weight value % of each parent (P1), the mean weight value % of the hybrids crossed P1xP2, the reciprocal mean weight value %, the variance among those means, the degrees of freedom, and the p-value ($\alpha = .05$).

Cultivar (P1)	P1 Weight Value %	Mean – P1xP2 Hybrids	Mean – P2xP1 Reciprocals	Variance (P1xP2)	Variance (P2xP1)	P-Value
FREEMAN	282.1	286.8	276.7	47.5	311.6	0.10356
GOODSTREAK	276.4	277.1	275.3	154.7	252.0	0.64249
LCH13NEDH-11-24	284.9	292.6	278.2	83.6	320.0	0.02651
NE09517-1	338.3	312.5	272.0	159.5	422.9	0.00007
NE10589	281.5	270.2	282.3	131.5	789.6	0.22615
OVERLAND	253.7	253.5	273.6	124.0	576.8	0.05812
SETTLER CL	287.0	281.7	271.4	220.7	526.9	0.15597
TX10D2063	276.6	273.1	277.2	104.4	525.4	0.55766
TX10D2230	254.8	262.5	266.7	71.1	677.1	0.55797
TX11D3129	274.6	254.3	265.5	90.2	513.8	0.14723
TX12M4063	228.8	249.8	270.9	235.7	531.1	0.04218
TX12M4065	236.2	246.8	270.5	105.7	333.6	0.00255
WESLEY	309.6	292.1	275.7	223.0	790.4	0.10019

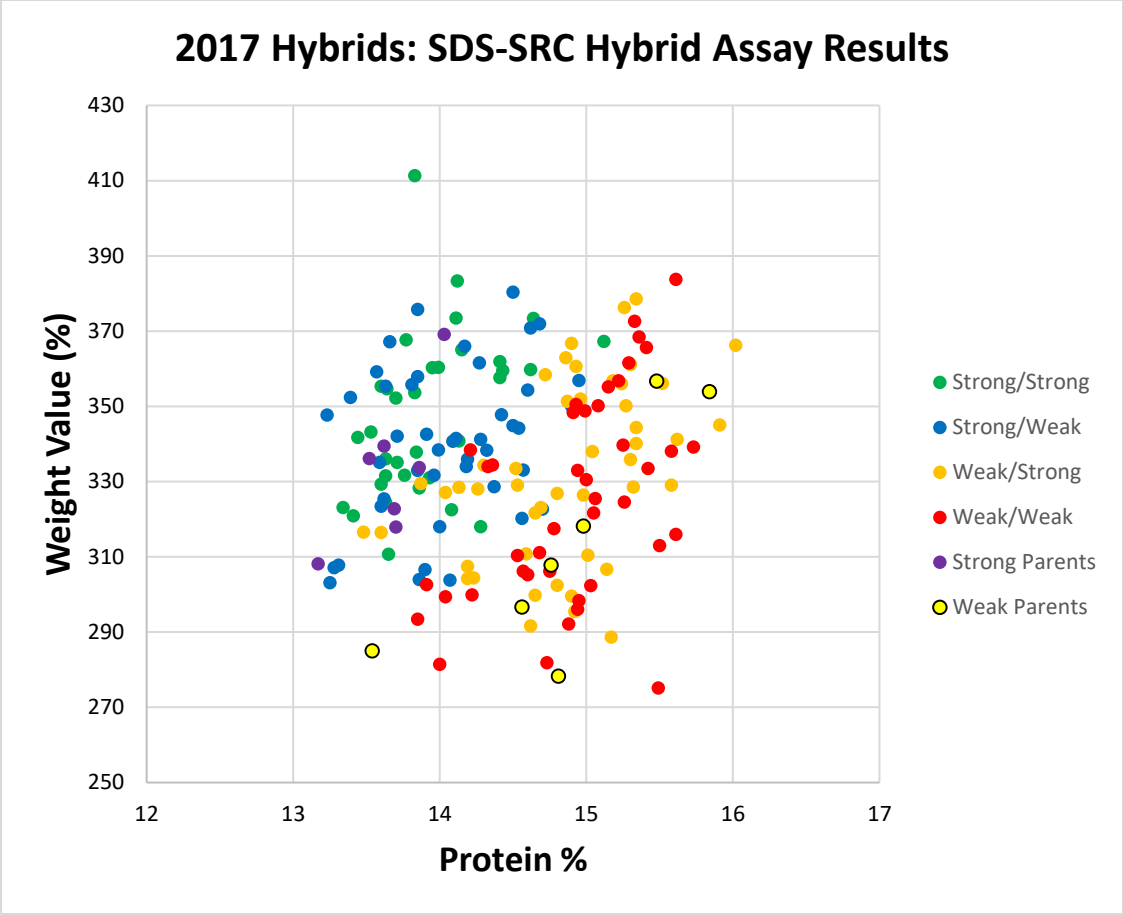


Figure 26. SDS-SRC Hybrid Assay Results of 2017 Hybrids.
Represents the SDS-SRC hybrid assay that was described by Seabourn *et al.* (2012) and the USDA in Manhattan, KS. The Y-axis represents the weight value % as calculated by **equation 1**, and the X-axis represents grain protein %. The strong parents are shown in purple and the weak parents are shown in yellow to show the relationship between the quality of the parents and the hybrids.

Table 29. Paired t-test Results for the SDS-SRC Hybrid Assay of 2017 Hybrids.

Paired t-tests for the 2017 SDS-SRC hybrid assay results, including the weight value % of each parent (P1), the mean weight value % of the hybrids crossed P1xP2, the reciprocal mean weight value %, the variance among those means, the degrees of freedom, and the p-value ($\alpha = .05$).

Cultivar (P1)	Cultivar Weight Value %	Mean P1xP2 Hybrids	Mean – P2xP1 Reciprocals	Variance (P1xP2)	Variance (P2xP1)	P-Value
FREEMAN	333.7	339.3	339.8	456.2	208.8	0.9486
GOODSTREAK	356.7	361.0	350.9	249.1	171.4	0.0722
HARRY	339.4	356.1	338.1	402.1	607.7	0.1014
LCH13NEDH_11_24	369.1	353.4	327.5	161.3	466.6	0.0172
NE07531	322.8	347.9	348.8	490.0	1133.0	0.9490
NE09517_1	336.2	348.1	338.4	116.5	556.8	0.1730
OVERLAND	285.0	311.4	328.9	280.9	531.9	0.0020
ROBIDOUX	317.9	340.1	337.9	836.0	419.6	0.8462
SETTLER_CL	308.1	324.7	337.8	187.2	977.7	0.2270
TX09D1172	318.2	334.0	341.6	165.5	820.1	0.2545
TX10D2063	307.8	330.2	319.5	129.8	783.0	0.1985
TX10D2363	353.9	354.6	323.8	125.9	475.4	0.0023
TX11D3129	296.6	312.7	334.8	212.5	718.2	0.0217
TX12M4063	278.3	301.3	326.5	277.9	472.9	0.0037

Table 30. Paired t-test Results for Kernel Hardness of 2016 Hybrids.
The 2016 hybrids kernel hardness (HI) paired t-test results ($\alpha = .05$), including the mean hardness of each parent variety (P1), the mean hardness of the hybrids crossed P1xP2, the reciprocal mean hardness, the variance among those means, the degrees of freedom, and the p-value. Means were taken over 100 seeds.

Cultivar (P1)	Variety Hardness (HI)	Mean – P1xP2 Hybrids	Mean – P2xP1 Reciprocal	Variance (P1xP2)	Variance (P2xP1)	P-Value
FREEMAN	63.88	67.95	76.34	23.17	52.76	0.00292
GOODSTREAK	75.65	72.08	76.66	5.70	32.18	0.01435
LCH13NEDH-11-24	77.35	79.59	78.81	10.13	58.54	0.74680
NE09517-1	77.07	75.18	76.43	2.54	51.57	0.52142
NE10589	82.63	82.49	78.91	9.98	81.77	0.13383
OVERLAND	87.66	83.68	75.98	9.84	46.57	0.00346
SETTLER CL	81.10	78.05	78.05	13.52	58.49	0.99754
TX10D2063	80.44	80.00	77.65	3.97	78.13	0.40251
TX10D2230	90.22	83.55	77.20	14.95	66.86	0.04714
TX11D3129	59.56	62.92	79.38	0.88	49.80	0.00003
TX12M4063	82.99	78.36	76.62	3.71	66.22	0.47846
TX12M4065	92.04	88.15	76.98	5.33	40.16	0.00040
WESLEY	77.42	74.51	77.77	17.26	43.82	0.15749

Table 31. Paired t-test Results for Kernel Hardness of 2017 Hybrids.
The 2017 hybrids kernel hardness (HI) paired t-test results ($\alpha = .05$), including the mean hardness of each parent variety (P1), the mean hardness of the hybrids crossed P1xP2, the reciprocal mean hardness, the variance among those means, the degrees of freedom, and the p-value. Means were taken over 100 seeds.

Cultivar (P1)	Variety Hardness (HI)	Mean – P1xP2 Hybrids	Mean – P2xP1 Reciprocal	Variance (P1xP2)	Variance (P2xP1)	P-Value
FREEMAN	43.86	49.49	53.45	6.87	31.14	0.0342
GOODSTREAK	52.18	54.52	54.35	6.29	31.92	0.9261
HARRY	52.07	54.68	58.15	17.61	29.07	0.0718
LCH13NEDH-11-24	61.06	60.11	59.88	6.17	79.18	0.9418
NE07531	47.73	53.59	54.73	27.73	29.96	0.5537
NE09517_1	53.57	56.94	57.03	15.42	43.59	0.9671
OVERLAND	61.86	61.08	57.78	9.75	34.37	0.0535
ROBIDOUX	57.81	57.53	56.25	9.77	30.88	0.4291
SETTLER_CL	58.29	57.84	57.93	10.07	52.34	0.9628
TX09D1172	60.37	61.43	56.02	19.40	27.42	0.0050
TX10D2063	60.83	59.28	57.23	5.82	44.59	0.3173
TX10D2363	50.36	56.13	58.18	11.65	37.43	0.2455
TX11D3129	44.12	46.74	55.75	13.09	18.06	0.0000
TX12M4063	63.01	65.38	59.83	25.13	30.43	0.0013

Appendix A

Hybrid Selections from 2016.

Table 3 represents the hybrids harvested in 2016 that were selected for analysis. Hybrids fell into 1 of 4 categories; Category 1 - both parents exhibited strong quality traits (green), Category 2 - both parents exhibited weak quality traits (red), Category 3 - the male parent exhibited strong quality traits and the female parent exhibited weak quality traits (orange), and lastly, Category 4 - the male parent exhibited weak quality traits and the female parent exhibited strong quality traits (blue). Also identifies if reciprocal cross was included in analysis.

Short Pedigree	Female Quality	Male Quality	Category	Reciprocal Included?
FREEMAN/LCH13NEDH-11-24	Strong	Strong	1	Yes
FREEMAN/NE09517-1	Strong	Strong	1	Yes
FREEMAN/NE10589	Strong	Strong	1	Yes
FREEMAN/SETTLER CL	Strong	Strong	1	Yes
FREEMAN/WESLEY	Strong	Strong	1	Yes
LCH13NEDH-11-24/FREEMAN	Strong	Strong	1	Yes
LCH13NEDH-11-24/NE09517-1	Strong	Strong	1	Yes
LCH13NEDH-11-24/NE10589	Strong	Strong	1	Yes
LCH13NEDH-11-24/SETTLER CL	Strong	Strong	1	Yes
LCH13NEDH-11-24/WESLEY	Strong	Strong	1	Yes
NE09517-1/FREEMAN	Strong	Strong	1	Yes
NE09517-1/LCH13NEDH-11-24	Strong	Strong	1	Yes
NE09517-1/NE10589	Strong	Strong	1	Yes
NE09517-1/SETTLER CL	Strong	Strong	1	Yes
NE09517-1/WESLEY	Strong	Strong	1	Yes
NE10589/FREEMAN	Strong	Strong	1	Yes
NE10589/LCH13NEDH-11-24	Strong	Strong	1	Yes
NE10589/NE09517-1	Strong	Strong	1	Yes
NE10589/SETTLER CL	Strong	Strong	1	Yes
NE10589/WESLEY	Strong	Strong	1	Yes
SETTLER CL/FREEMAN	Strong	Strong	1	Yes
SETTLER CL/LCH13NEDH-11-24	Strong	Strong	1	Yes
SETTLER CL/NE09517-1	Strong	Strong	1	Yes
SETTLER CL/NE10589	Strong	Strong	1	Yes
SETTLER CL/WESLEY	Strong	Strong	1	Yes
WESLEY/FREEMAN	Strong	Strong	1	Yes
WESLEY/LCH13NEDH-11-24	Strong	Strong	1	Yes
WESLEY/NE09517-1	Strong	Strong	1	Yes
WESLEY/NE10589	Strong	Strong	1	Yes
WESLEY/SETTLER CL	Strong	Strong	1	Yes
GOODSTREAK/OVERLAND	Weak	Weak	2	Yes

Short Pedigree	Female Quality	Male Quality	Category	Reciprocal Included?
GOODSTREAK/TX10D2063	Weak	Weak	2	Yes
GOODSTREAK/TX10D2230	Weak	Weak	2	Yes
GOODSTREAK/TX12M4063	Weak	Weak	2	Yes
GOODSTREAK/TX12M4065	Weak	Weak	2	Yes
OVERLAND/GOODSTREAK	Weak	Weak	2	Yes
OVERLAND/TX10D2063	Weak	Weak	2	No
OVERLAND/TX10D2230	Weak	Weak	2	Yes
OVERLAND/TX11D3129	Weak	Weak	2	Yes
OVERLAND/TX12M4063	Weak	Weak	2	Yes
TX10D2063/GOODSTREAK	Weak	Weak	2	Yes
TX10D2063/TX10D2230	Weak	Weak	2	Yes
TX10D2063/TX11D3129	Weak	Weak	2	Yes
TX10D2063/TX12M4063	Weak	Weak	2	Yes
TX10D2063/TX12M4065	Weak	Weak	2	Yes
TX10D2230/GOODSTREAK	Weak	Weak	2	Yes
TX10D2230/OVERLAND	Weak	Weak	2	Yes
TX10D2230/TX10D2063	Weak	Weak	2	Yes
TX10D2230/TX11D3129	Weak	Weak	2	Yes
TX10D2230/TX12M4063	Weak	Weak	2	Yes
TX10D2230/TX12M4065	Weak	Weak	2	Yes
TX11D3129/GOODSTREAK	Weak	Weak	2	No
TX11D3129/OVERLAND	Weak	Weak	2	Yes
TX11D3129/TX10D2063	Weak	Weak	2	Yes
TX11D3129/TX10D2230	Weak	Weak	2	Yes
TX11D3129/TX12M4063	Weak	Weak	2	Yes
TX11D3129/TX12M4065	Weak	Weak	2	Yes
TX12M4063/GOODSTREAK	Weak	Weak	2	Yes
TX12M4063/OVERLAND	Weak	Weak	2	Yes
TX12M4063/TX10D2063	Weak	Weak	2	Yes
TX12M4063/TX10D2230	Weak	Weak	2	Yes
TX12M4063/TX11D3129	Weak	Weak	2	Yes
TX12M4063/TX12M4065	Weak	Weak	2	Yes
TX12M4065/GOODSTREAK	Weak	Weak	2	Yes
TX12M4065/OVERLAND	Weak	Weak	2	No
TX12M4065/TX10D2063	Weak	Weak	2	Yes
TX12M4065/TX10D2230	Weak	Weak	2	Yes
TX12M4065/TX11D3129	Weak	Weak	2	Yes
TX12M4065/TX12M4063	Weak	Weak	2	Yes
GOODSTREAK/FREEMAN	Weak	Strong	3	Yes
GOODSTREAK/LCH13NEDH-11-24	Weak	Strong	3	Yes
GOODSTREAK/NE09517-1	Weak	Strong	3	Yes

Short Pedigree	Female Quality	Male Quality	Category	Reciprocal Included?
GOODSTREAK/NE10589	Weak	Strong	3	Yes
GOODSTREAK/SETTLER CL	Weak	Strong	3	Yes
GOODSTREAK/WESLEY	Weak	Strong	3	Yes
OVERLAND/FREEMAN	Weak	Strong	3	Yes
OVERLAND/LCH13NEDH-11-24	Weak	Strong	3	Yes
OVERLAND/NE09517-1	Weak	Strong	3	Yes
OVERLAND/NE10589	Weak	Strong	3	Yes
OVERLAND/SETTLER CL	Weak	Strong	3	Yes
OVERLAND/WESLEY	Weak	Strong	3	Yes
TX10D2063/FREEMAN	Weak	Strong	3	Yes
TX10D2063/LCH13NEDH-11-24	Weak	Strong	3	Yes
TX10D2063/NE09517-1	Weak	Strong	3	Yes
TX10D2063/NE10589	Weak	Strong	3	Yes
TX10D2063/SETTLER CL	Weak	Strong	3	Yes
TX10D2063/WESLEY	Weak	Strong	3	Yes
TX10D2230/FREEMAN	Weak	Strong	3	Yes
TX10D2230/NE09517-1	Weak	Strong	3	Yes
TX10D2230/NE10589	Weak	Strong	3	Yes
TX10D2230/SETTLER CL	Weak	Strong	3	Yes
TX10D2230/WESLEY	Weak	Strong	3	Yes
TX11D3129/FREEMAN	Weak	Strong	3	Yes
TX11D3129/LCH13NEDH-11-24	Weak	Strong	3	No
TX11D3129/NE09517-1	Weak	Strong	3	Yes
TX11D3129/NE10589	Weak	Strong	3	Yes
TX11D3129/SETTLER CL	Weak	Strong	3	Yes
TX11D3129/WESLEY	Weak	Strong	3	Yes
TX12M4063/FREEMAN	Weak	Strong	3	Yes
TX12M4063/LCH13NEDH-11-24	Weak	Strong	3	Yes
TX12M4063/NE10589	Weak	Strong	3	Yes
TX12M4063/SETTLER CL	Weak	Strong	3	Yes
TX12M4063/WESLEY	Weak	Strong	3	Yes
TX12M4065/FREEMAN	Weak	Strong	3	Yes
TX12M4065/LCH13NEDH-11-24	Weak	Strong	3	Yes
TX12M4065/NE09517-1	Weak	Strong	3	Yes
TX12M4065/NE10589	Weak	Strong	3	Yes
TX12M4065/SETTLER CL	Weak	Strong	3	Yes
TX12M4065/WESLEY	Weak	Strong	3	Yes
FREEMAN/GOODSTREAK	Strong	Weak	4	Yes
FREEMAN/OVERLAND	Strong	Weak	4	Yes
FREEMAN/TX10D2063	Strong	Weak	4	Yes
FREEMAN/TX10D2230	Strong	Weak	4	Yes

FREEMAN/TX11D3129	Strong	Weak	4	Yes
FREEMAN/TX12M4063	Strong	Weak	4	Yes

Short Pedigree	Female Quality	Male Quality	Category	Reciprocal Included?
FREEMAN/TX12M4065	Strong	Weak	4	Yes
LCH13NEDH-11-24/GOODSTREAK	Strong	Weak	4	Yes
LCH13NEDH-11-24/OVERLAND	Strong	Weak	4	Yes
LCH13NEDH-11-24/TX10D2063	Strong	Weak	4	Yes
LCH13NEDH-11-24/TX10D2230	Strong	Weak	4	No
LCH13NEDH-11-24/TX12M4063	Strong	Weak	4	Yes
LCH13NEDH-11-24/TX12M4065	Strong	Weak	4	Yes
NE09517-1/GOODSTREAK	Strong	Weak	4	Yes
NE09517-1/OVERLAND	Strong	Weak	4	Yes
NE09517-1/TX10D2063	Strong	Weak	4	Yes
NE09517-1/TX10D2230	Strong	Weak	4	Yes
NE09517-1/TX11D3129	Strong	Weak	4	Yes
NE09517-1/TX12M4065	Strong	Weak	4	Yes
NE10589/GOODSTREAK	Strong	Weak	4	Yes
NE10589/OVERLAND	Strong	Weak	4	Yes
NE10589/TX10D2063	Strong	Weak	4	Yes
NE10589/TX10D2230	Strong	Weak	4	Yes
NE10589/TX11D3129	Strong	Weak	4	Yes
NE10589/TX12M4063	Strong	Weak	4	Yes
NE10589/TX12M4065	Strong	Weak	4	Yes
SETTLER CL/GOODSTREAK	Strong	Weak	4	Yes
SETTLER CL/OVERLAND	Strong	Weak	4	Yes
SETTLER CL/TX10D2063	Strong	Weak	4	Yes
SETTLER CL/TX10D2230	Strong	Weak	4	Yes
SETTLER CL/TX11D3129	Strong	Weak	4	Yes
SETTLER CL/TX12M4063	Strong	Weak	4	Yes
SETTLER CL/TX12M4065	Strong	Weak	4	Yes
WESLEY/GOODSTREAK	Strong	Weak	4	Yes
WESLEY/OVERLAND	Strong	Weak	4	Yes
WESLEY/TX10D2063	Strong	Weak	4	Yes
WESLEY/TX10D2230	Strong	Weak	4	Yes
WESLEY/TX11D3129	Strong	Weak	4	Yes
WESLEY/TX12M4063	Strong	Weak	4	Yes
WESLEY/TX12M4065	Strong	Weak	4	Yes

Appendix B
Hybrid Selections from 2017.
Table 4 represents hybrids harvested in 2017 that were selected for analysis. Hybrids fell into 1 of 4 categories; Category 1 - both parents exhibited strong quality traits (green), Category 2 - both parents exhibited weak quality traits (red), Category 3 - the male parent exhibited strong quality traits and the female parent exhibited weak quality traits (orange), and lastly, Category 4 - the male parent exhibited weak quality traits and the female parent exhibited strong quality traits (blue). Also identifies if reciprocal cross was included in analysis.

Short Pedigree	Female Quality	Male Quality	Category	Reciprocal Included?
FREEMAN/HARRY	Strong	Strong	1	Yes
FREEMAN/LCH13NEDH_11_24	Strong	Strong	1	Yes
FREEMAN/NE07531	Strong	Strong	1	Yes
FREEMAN/NE09517_1	Strong	Strong	1	Yes
FREEMAN/ROBIDOUX	Strong	Strong	1	Yes
FREEMAN/SETTLER_CL	Strong	Strong	1	Yes
HARRY/FREEMAN	Strong	Strong	1	Yes
HARRY/NE07531	Strong	Strong	1	Yes
HARRY/NE09517_1	Strong	Strong	1	Yes
HARRY/ROBIDOUX	Strong	Strong	1	Yes
HARRY/SETTLER_CL	Strong	Strong	1	Yes
LCH13NEDH_11_24/FREEMAN	Strong	Strong	1	Yes
LCH13NEDH_11_24/HARRY	Strong	Strong	1	No
LCH13NEDH_11_24/NE07531	Strong	Strong	1	No
LCH13NEDH_11_24/ROBIDOUX	Strong	Strong	1	No
LCH13NEDH_11_24/SETTLER_CL	Strong	Strong	1	Yes
NE07531/FREEMAN	Strong	Strong	1	Yes
NE07531/HARRY	Strong	Strong	1	Yes
NE07531/ROBIDOUX	Strong	Strong	1	Yes
NE07531/SETTLER_CL	Strong	Strong	1	Yes
NE09517_1/FREEMAN	Strong	Strong	1	Yes
NE09517_1/HARRY	Strong	Strong	1	Yes
NE09517_1/ROBIDOUX	Strong	Strong	1	Yes
NE09517_1/SETTLER_CL	Strong	Strong	1	No
ROBIDOUX/FREEMAN	Strong	Strong	1	Yes
ROBIDOUX/HARRY	Strong	Strong	1	Yes
ROBIDOUX/NE07531	Strong	Strong	1	Yes
ROBIDOUX/NE09517_1	Strong	Strong	1	Yes
ROBIDOUX/SETTLER_CL	Strong	Strong	1	Yes
SETTLER_CL/FREEMAN	Strong	Strong	1	Yes
SETTLER_CL/HARRY	Strong	Strong	1	Yes

Short Pedigree	Female Quality	Male Quality	Category	Reciprocal Included?
SETTLER_CL/LCH13NEDH_11_24	Strong	Strong	1	Yes
SETTLER_CL/NE07531	Strong	Strong	1	Yes
SETTLER_CL/ROBIDOUX	Strong	Strong	1	Yes
GOODSTREAK/OVERLAND	Weak	Weak	2	Yes
GOODSTREAK/TX09D1172	Weak	Weak	2	Yes
GOODSTREAK/TX10D2063	Weak	Weak	2	Yes
GOODSTREAK/TX10D2363	Weak	Weak	2	Yes
GOODSTREAK/TX11D3129	Weak	Weak	2	Yes
GOODSTREAK/TX12M4063	Weak	Weak	2	Yes
OVERLAND/GOODSTREAK	Weak	Weak	2	Yes
OVERLAND/TX09D1172	Weak	Weak	2	Yes
OVERLAND/TX10D2063	Weak	Weak	2	Yes
OVERLAND/TX10D2363	Weak	Weak	2	Yes
OVERLAND/TX11D3129	Weak	Weak	2	Yes
OVERLAND/TX12M4063	Weak	Weak	2	Yes
TX09D1172/GOODSTREAK	Weak	Weak	2	Yes
TX09D1172/OVERLAND	Weak	Weak	2	Yes
TX09D1172/TX10D2063	Weak	Weak	2	Yes
TX09D1172/TX10D2363	Weak	Weak	2	Yes
TX09D1172/TX11D3129	Weak	Weak	2	Yes
TX09D1172/TX12M4063	Weak	Weak	2	Yes
TX10D2063/GOODSTREAK	Weak	Weak	2	Yes
TX10D2063/OVERLAND	Weak	Weak	2	Yes
TX10D2063/TX09D1172	Weak	Weak	2	Yes
TX10D2063/TX10D2363	Weak	Weak	2	Yes
TX10D2063/TX11D3129	Weak	Weak	2	Yes
TX10D2063/TX12M4063	Weak	Weak	2	Yes
TX10D2363/GOODSTREAK	Weak	Weak	2	Yes
TX10D2363/OVERLAND	Weak	Weak	2	Yes
TX10D2363/TX09D1172	Weak	Weak	2	Yes
TX10D2363/TX10D2063	Weak	Weak	2	Yes
TX10D2363/TX11D3129	Weak	Weak	2	Yes
TX10D2363/TX12M4063	Weak	Weak	2	Yes
TX11D3129/GOODSTREAK	Weak	Weak	2	Yes
TX11D3129/OVERLAND	Weak	Weak	2	Yes
TX11D3129/TX09D1172	Weak	Weak	2	Yes
TX11D3129/TX10D2063	Weak	Weak	2	Yes
TX11D3129/TX10D2363	Weak	Weak	2	Yes
TX11D3129/TX12M4063	Weak	Weak	2	Yes
TX12M4063/GOODSTREAK	Weak	Weak	2	Yes
TX12M4063/OVERLAND	Weak	Weak	2	Yes

Short Pedigree	Female Quality	Male Quality	Category	Reciprocal Included?
TX12M4063/TX09D1172	Weak	Weak	2	Yes
TX12M4063/TX10D2063	Weak	Weak	2	Yes
TX12M4063/TX10D2363	Weak	Weak	2	Yes
TX12M4063/TX11D3129	Weak	Weak	2	Yes
GOODSTREAK/FREEMAN	Weak	Strong	3	Yes
GOODSTREAK/HARRY	Weak	Strong	3	Yes
GOODSTREAK/LCH13NEDH_11_24	Weak	Strong	3	Yes
GOODSTREAK/NE07531	Weak	Strong	3	Yes
GOODSTREAK/NE09517_1	Weak	Strong	3	Yes
GOODSTREAK/ROBIDOUX	Weak	Strong	3	Yes
GOODSTREAK/SETTLER_CL	Weak	Strong	3	Yes
OVERLAND/FREEMAN	Weak	Strong	3	Yes
OVERLAND/HARRY	Weak	Strong	3	Yes
OVERLAND/LCH13NEDH_11_24	Weak	Strong	3	Yes
OVERLAND/NE07531	Weak	Strong	3	Yes
OVERLAND/NE09517_1	Weak	Strong	3	Yes
OVERLAND/ROBIDOUX	Weak	Strong	3	Yes
OVERLAND/SETTLER_CL	Weak	Strong	3	Yes
TX09D1172/FREEMAN	Weak	Strong	3	Yes
TX09D1172/HARRY	Weak	Strong	3	Yes
TX09D1172/LCH13NEDH_11_24	Weak	Strong	3	Yes
TX09D1172/NE07531	Weak	Strong	3	Yes
TX09D1172/NE09517_1	Weak	Strong	3	Yes
TX09D1172/ROBIDOUX	Weak	Strong	3	Yes
TX09D1172/SETTLER_CL	Weak	Strong	3	Yes
TX10D2063/FREEMAN	Weak	Strong	3	Yes
TX10D2063/HARRY	Weak	Strong	3	Yes
TX10D2063/LCH13NEDH_11_24	Weak	Strong	3	Yes
TX10D2063/NE07531	Weak	Strong	3	No
TX10D2063/NE09517_1	Weak	Strong	3	Yes
TX10D2063/ROBIDOUX	Weak	Strong	3	No
TX10D2063/SETTLER_CL	Weak	Strong	3	Yes
TX10D2363/FREEMAN	Weak	Strong	3	Yes
TX10D2363/HARRY	Weak	Strong	3	Yes
TX10D2363/NE07531	Weak	Strong	3	No
TX10D2363/NE09517_1	Weak	Strong	3	Yes
TX10D2363/ROBIDOUX	Weak	Strong	3	Yes
TX10D2363/SETTLER_CL	Weak	Strong	3	No
TX11D3129/FREEMAN	Weak	Strong	3	Yes
TX11D3129/HARRY	Weak	Strong	3	Yes
TX11D3129/NE07531	Weak	Strong	3	Yes

Short Pedigree	Female Quality	Male Quality	Category	Reciprocal Included?
TX11D3129/NE09517_1	Weak	Strong	3	Yes
TX11D3129/ROBIDOUX	Weak	Strong	3	Yes
TX11D3129/SETTLER_CL	Weak	Strong	3	Yes
TX12M4063/FREEMAN	Weak	Strong	3	Yes
TX12M4063/HARRY	Weak	Strong	3	Yes
TX12M4063/LCH13NEDH_11_24	Weak	Strong	3	Yes
TX12M4063/NE07531	Weak	Strong	3	Yes
TX12M4063/NE09517_1	Weak	Strong	3	Yes
TX12M4063/ROBIDOUX	Weak	Strong	3	Yes
TX12M4063/SETTLER_CL	Weak	Strong	3	Yes
FREEMAN/GOODSTREAK	Strong	Weak	4	Yes
FREEMAN/OVERLAND	Strong	Weak	4	Yes
FREEMAN/TX09D1172	Strong	Weak	4	Yes
FREEMAN/TX10D2063	Strong	Weak	4	Yes
FREEMAN/TX10D2363	Strong	Weak	4	Yes
FREEMAN/TX11D3129	Strong	Weak	4	Yes
FREEMAN/TX12M4063	Strong	Weak	4	Yes
HARRY/GOODSTREAK	Strong	Weak	4	Yes
HARRY/OVERLAND	Strong	Weak	4	Yes
HARRY/TX09D1172	Strong	Weak	4	Yes
HARRY/TX10D2063	Strong	Weak	4	Yes
HARRY/TX10D2363	Strong	Weak	4	Yes
HARRY/TX11D3129	Strong	Weak	4	Yes
HARRY/TX12M4063	Strong	Weak	4	Yes
LCH13NEDH_11_24/GOODSTREAK	Strong	Weak	4	Yes
LCH13NEDH_11_24/OVERLAND	Strong	Weak	4	Yes
LCH13NEDH_11_24/TX09D1172	Strong	Weak	4	Yes
LCH13NEDH_11_24/TX10D2063	Strong	Weak	4	Yes
LCH13NEDH_11_24/TX11D3129	Strong	Weak	4	No
LCH13NEDH_11_24/TX12M4063	Strong	Weak	4	Yes
NE07531/GOODSTREAK	Strong	Weak	4	Yes
NE07531/OVERLAND	Strong	Weak	4	Yes
NE07531/TX09D1172	Strong	Weak	4	Yes
NE07531/TX11D3129	Strong	Weak	4	Yes
NE07531/TX12M4063	Strong	Weak	4	Yes
NE09517_1/GOODSTREAK	Strong	Weak	4	Yes
NE09517_1/OVERLAND	Strong	Weak	4	Yes
NE09517_1/TX09D1172	Strong	Weak	4	Yes
NE09517_1/TX10D2063	Strong	Weak	4	Yes
NE09517_1/TX10D2363	Strong	Weak	4	Yes
NE09517_1/TX11D3129	Strong	Weak	4	Yes

NE09517_1/TX12M4063	Strong	Weak	4	Yes
ROBIDOUX/GOODSTREAK	Strong	Weak	4	Yes

Short Pedigree	Female Quality	Male Quality	Category	Reciprocal Included?
ROBIDOUX/OVERLAND	Strong	Weak	4	Yes
ROBIDOUX/TX09D1172	Strong	Weak	4	yes
ROBIDOUX/TX10D2363	Strong	Weak	4	Yes
ROBIDOUX/TX11D3129	Strong	Weak	4	Yes
ROBIDOUX/TX12M4063	Strong	Weak	4	Yes
SETTLER_CL/GOODSTREAK	Strong	Weak	4	Yes
SETTLER_CL/OVERLAND	Strong	Weak	4	Yes
SETTLER_CL/TX09D1172	Strong	Weak	4	Yes
SETTLER_CL/TX10D2063	Strong	Weak	4	Yes
SETTLER_CL/TX11D3129	Strong	Weak	4	Yes
SETTLER_CL/TX12M4063	Strong	Weak	4	Yes

Appendix C
2016 Parent Grain Protein and SKCS data.
Table 5 shows the grain protein % (moisture fixed at 12%), mean kernel hardness index (HI), mean kernel diameter (mm), and mean kernel weight (mg) of the 2016 parents. The kernel data represents the average of 100 seeds. The average, max, min, and standard deviations of protein, hardness, diameter, and weight are displayed below the table.

Sample Name	Protein % (Fixed @ 12% Moisture)	Hardness Index (HI)	kernel diameter (mm)	kernel weight (mg)	S.D. kernel weight
FREEMAN	13.4	63.9	2.7	33.4	10.3
GOODSTREAK	15.9	75.6	2.7	29.7	8.7
LCH13NEDH-11-24	13.7	77.3	2.6	29.7	7.6
NE07531	14.2	73.9	2.7	31.2	10.7
NE09517-1	14.8	77.1	2.8	32.7	9.6
NE10589	13.7	82.6	2.8	31.1	9.0
NE10683	13.6	64.1	2.7	32.1	10.1
OVERLAND	14.7	87.7	2.6	26.6	9.2
PANHANDLE	15.7	74.3	2.7	32.5	9.3
PSB13NEDH-15-58W	13.6	74.5	2.7	32.1	13.1
ROBIDOUX	13.9	76.9	2.7	30.4	10.3
SETTLER CL	13.5	81.1	2.8	32.9	10.2
TX09D1172	15.9	86.6	2.8	32.1	10.1
TX10D2063	15.4	80.4	2.6	28.5	7.9
TX10D2230	16.7	90.2	2.8	34.1	10.9
TX10D2363	16.4	72.8	2.8	31.3	8.8
TX11D3008	15.4	95.2	2.6	28.8	9.6
TX11D3026	14.3	93.7	2.8	31.6	8.1
TX11D3049	15.9	79.0	2.7	29.5	10.0
TX11D3112	14.6	87.8	2.8	31.9	9.4
TX11D3129	15.7	59.6	2.8	32.7	8.9
TX12M4004	14.5	72.1	2.9	35.3	9.6
TX12M4063	15.9	83.0	2.7	31.5	11.2
TX12M4065	15.9	92.0	2.8	34.7	8.8
WESLEY	14.6	77.4	2.6	29.8	10.4
Average	14.9	79.2	2.7	31.4	
Max	16.7	95.2	2.9	35.3	
Min	13.4	59.6	2.6	26.3	
S.D.	1.02	9.19	.07	2.02	

Appendix D

2017 Parent Grain Protein and SKCS data.

Physical characteristics of parents including; grain protein % (with grain moisture fixed at 12%), mean kernel hardness index (HI), mean kernel diameter (mm), and mean kernel weight (mg). Under the graph the average, max, min, and standard deviation of protein, hardness, diameter, and weight are listed.

Sample Name	Protein % (Fixed @ 12% Moisture)	Hardness Index (HI)	kernel diameter (mm)	kernel weight (mg)
TX11D3008	15.3	83.0	2.6	27.4
WESLEY	15.4	54.0	2.9	35.9
TX12M4004	14.1	60.3	2.8	33.6
FREEMAN	13.9	43.9	2.7	30.5
TX10D2230	15.1	70.3	2.9	35.6
TX12M4065	14.8	78.1	2.6	27.3
TX09D1172	15.0	60.4	2.8	35.0
OVERLAND	13.5	61.9	2.6	29.8
NE09517_1	13.5	53.6	2.7	31.7
RUTH	13.9	64.6	2.8	31.4
NE07531	13.7	47.7	2.8	33.0
TX12M4063	14.8	63.0	2.9	35.0
HARRY	13.6	52.1	2.6	29.4
TX10D2363	15.8	50.4	2.9	34.6
TX11D3049	15.8	61.3	2.7	30.1
TX11D3026	14.4	76.8	2.7	31.2
LCH13NEDH-11-24	14.0	61.1	2.5	26.3
TX10D2063	14.8	60.8	2.8	30.9
ROBIDOUX	13.7	57.8	2.7	28.8
TX11D3112	14.3	69.3	2.8	32.7
PANHANDLE	14.7	56.9	2.7	32.1
SETTLER_CL	13.2	58.3	2.7	30.9
TX11D3129	14.6	44.1	2.6	28.8
PSB13NEDH-15-58W	13.9	61.3	2.8	31.7
GOODSTREAK	15.5	52.2	2.8	31.8
NE10683	13.5	43.1	2.8	33.9
Average	14.4	59.5	2.7	31.5
Max	15.8	83.0	2.9	35.9
Min	13.2	43.1	2.5	26.3
S.D.	0.77	10.24	.11	2.62

Appendix E

SDS Sedimentation and SDS-SRC Hybrid Assay Control Compilation.

These two tables represent a compilation of the SDS-SRC and SDS sedimentation results of the control variety. The low CV values for both assays (SDS-SRC – 2.42, SDS sedimentation – 1.70) indicates that both assays are highly repeatable and justifies our decision not to perform duplicates in our assays.

SDS-SRC Hybrid Assay Control Compilation	
ID	Weight Value %
QL control	315.8362
QL control	328.0245
QL control	322.7049
QL control	310.6239
QL control	305.6304
QL control	312.4452
QL control	327.6450
QL control	319.5693
QL control	311.1345
QL control	323.3741
Average	317.6988
St. Dev.	7.718490981
CV	2.42949957

SDS Sedimentation Assay Control Compilation	
ID	Sedimentation Volume (mL)
QL control	13.38
QL control	13.25
QL control	13.59
QL control	13.18
QL control	13
QL control	13.43
QL control	13.2
QL control	13.5
QL control	13.6
QL control	13.62
QL control	13
QL control	13.55
Average	13.35833333
St. Dev.	0.227309694
CV	1.701632144

Appendix F

Numbers assigned to parent cultivars used for hybrid analyses and comparisons.

Listed are the numbers assigned to the parents categorized in the 2016 and 2017 analyses. This was used to make comparisons between reciprocals and parent cultivars.

2016

Cultivar	Number
Freeman	1
NE10589	2
LCH13NEDH_11_24	3
Wesley	4
NE09517_1	5
Settler_CL	7
Goodstreak	8
Overland	9
TX10D2230	10
TX10D2063	11
TX12M4065	12
TX11D3129	13
TX12M4063	14

2017

Cultivar	Number
Freeman	1
Harry	2
LCH13NEDH_11_24	3
NE07531	4
NE09517_1	5
Robidoux	6
Settler_CL	7
Goodstreak	8
Overland	9
TX09D1172	10
TX10D2063	11
TX10D2363	12
TX11D3129	13
TX12M4063	14