Genetic responses in milling, flour quality, and wheat sensitivity traits to grain yield improvement in U.S. hard winter wheat

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A rising global population necessitates continued genetic improvement of wheat (Triticum spp.), but not without monitoring of unintended consequences to processors and consumers. Our objectives were to re-establish trends of genetic progress in agronomic and milling traits using a generational meter stick as the timeline rather than cultivar release date, and to measure correlated responses in flour quality and human wheat-sensitivity indicators. Grain yield and kernel size showed stepwise increases over cycles, whereas wheat protein content decreased by 1.1 g/100 g. Reduced protein content, however, did not result in lower dough strength pertinent to bread baking. A novel method of directly testing gluten elasticity via the compression-recovery test indicated a general increase in gluten strength, whereas the ratio of total polymeric to total monomeric proteins remained stable. Also showing no change with genetic progress in yield were flour levels of gluten epitopes within the key immunotoxic 33-mer peptide. The oligosaccharide fructan, present in milled and wholemeal flours, increased with increasing grain yield potential. While yield improvement in U.S. bread wheat was not accompanied by a decline in gluten strength or systematic shift in a key wheat sensitivity parameter, the unanticipated rise in total fructans does implicate potentially new dietary concerns.

1. Introduction

A rapidly growing world population is making yield gains in common wheat (Triticum aestivum L.) immediately critical, with the added challenge of avoiding further pressure on already strained natural resources, and while heeding the concerns of a more apprehensive public with partial understanding of modern agriculture. Under that pressure, wheat breeding programs must do more than lay claims to genetic gains in grain yield; rather, they must generate yield gains while avoiding unintended consequences of specific interest to growers, users, and consumers.

Consumers are concerned about the origin of, and production practices attached to, their food and specifically, what is in their food. This concern is manifested in an evolving consumer food narrative toward food products containing natural ingredients, and thus lacking oxidizing agents, dough conditioners, and other additives the baking industry would have used to compensate for performance deficiencies inherent to the wheat itself.

This culture change has brought about a two-sided “gluten crisis” gripping the entire wheat supply chain. On the one hand are consumers who hold the perception, right or wrong, that “today’s wheat is not our grandfather’s wheat” for various reasons, but most notably due to a...
perceived change in the amount or kind of gluten that is arguably causing wheat to become less healthy to consume. Partly in response to that perception, the U.S. gluten-free food market reached a value of $2.7B in 2018 (https://www.researchandmarkets.com/research/szb7b3/the_u_s?w=5; verified Jan. 15, 2020), while the market hardly existed before 2011. On the other hand, parts of the U.S. baking industry have voiced concerns that the processing quality of today’s bread wheat has declined, a belief only aggravated by fewer options available from added ingredients (https://www.bakingbusiness.com/articles/46848-the-impending-gluten-crisis; https://www.world-grain.com/articles/11678-wands-calls-for-better-collaboration-in-wheat-industry-to-boost-quality; verified Jan. 15, 2020).

Considering processing quality first, U.S. bread wheat breeders to varying degrees do consider certain quality parameters along with grain yield and yield-influencing traits such as pest resistance. Most often they base selection decisions on the mixograph, which requires less flour and run-time than the more commonly used alveograph and farinograph prevalent in the milling and baking industry. Though standard curves are used for reference, the mixing tolerance score can be subjective depending on operator experience. Fortunately, computer-generated parameters estimated from a mixogram can be used to quantify specific components such as stability and bandwidth, although these parameters are reported less often. The compression-recovery (CORE) test provides a direct indicator of gluten strength by measuring the degree of recovery from deformation in compressed gluten samples (Chapman et al., 2012). Although it remains novel to the wheat breeding community, the CORE test is relatively quick and requires a minimal amount of flour.

The consumer side of the gluten crisis is centered on the perceived notion that wheat, specifically gluten, is less healthy to consume compared to wheat from a century ago. Part of this concern is fueled by the rise in celiac disease (CD) and other forms of gluten intolerance (Lohi et al., 2007), yet without indisputable reasons to account for this rise. The 33-mer peptide fragment from α-gliadin is widely considered the most important CD-immunogenic peptide, because it harbors three CD-active epitopes and is highly resistant to proteases in the human digestive system (Schalk et al., 2017). While attempts have been made to resolve the impact of plant breeding on the 33-mer levels in wheat accessions (Escarnot et al., 2018), diminution for key determinants of the phenotype, such as environmental (E) and genotype-environmental (GE) interactions, and disconnect between unimproved and improved genetics, shadow those results.

Non-celiac wheat sensitivity (NCWS) is a disorder that leads consumers to avoid gluten-containing food products at a rate far exceeding the frequency at which CD or NCWS are thought to exist (Lebowohl et al., 2015; https://news.gallup.com/poll/184307/one-five-americans-incude-gluten-free-foods-diet.aspx; verified Feb. 1, 2020). Fermentable oligo-, di-, mono-saccharides and polyols (FODMAPs) could be to blame for causing irritable bowel syndrome (IBS) symptoms often mistaken as a gluten intolerance response (Skodje et al., 2018). Wheat contains the oligosaccharide fructan, which occurs in various forms of carbohydrate polymers that are almost exclusively composed of β-linked fructose. These polymers serve as a carbohydrate reserve in plant metabolic functions.

Fructans exert positive health effects, particularly the more highly polymerized fructans that are not digested or absorbed in the small intestine and instead move to the colon to selectively stimulate the growth of beneficial bifidobacteria (Gibson et al., 1995). On the other hand, this poor absorption of FODMAPs may cause osmotic stress within the colon (Ziegler et al., 2016). Rapid fermentation by microbes in the colon can sometimes cause substantial gas formation (Gibson and Shepherd, 2005). Both the poor absorption with osmotic stress and the rapid fermentation of FODMAPs can induce luminal distention and gastrointestinal symptoms (Biesiekierski and Iven, 2015). With rising evidence that FODMAPs, and fructans specifically, may cause some of the symptoms normally linked to NCWS, it is important to determine how grain fructan content responds to yield improvement.

This study was designed to quantify true genetic changes for end-use quality parameters, epitopes of the 33-mer recognized by the G-12 monoclonal antibody, and fructan content in response to genetically-driven, incremental increases in grain yield. In particular, we set out to quantify selection responses using a panel of wheat genotypes organized by known parent-offspring relationships into distinct breeding cycles, contrary to the simpler and conventional method of estimating trait responses among random cultivars differentiated only by their year of release. Duration of a breeding cycle can vary widely depending on the number of years consumed for inbred line development (i.e., breeding method) and for line evaluation and purification, either case causing asynchrony between genealogical position and year of release.

2. Materials and methods

2.1. Genetic materials

Specific hard red winter (HRW) wheat cultivars and elite experimental lines (n = 28–30) were selected for the simple but crucial reason they were bred for or adapted to the same geography (U.S. southern Great Plains) in which they would be evaluated herein. Thus, unusual performance could not be imputed to poor adaptation. Some cultivars were selected for historical relevance, that is, to represent milestones in breeding advancements and dominance of planted hectares in the southern Plains, such as ‘Triumph’, ‘Newton’, ‘Chisholm’, ‘Karl’, ‘Duster’, ‘Jagger’, ‘Jagalene’, and ‘Gallagher’.

Cultivars were further selected based on their known parent-offspring relationships along three primary lineages from the common heirloom landrace cultivar ‘Turkey’ (Fig. 1, Table S1). Relationships were deduced from pedigrees accessible via public databases such as the U.S. National Plant Germplasm System, or for non-released genotypes, from unpublished data. Complex pedigrees more common for older cultivars in breeding cycles 1 and 2 allowed multiple genetic paths back to Turkey, but for the purpose of breeding cycle alignment in Fig. 1, the shortest route was usually taken. Thus, HRW cultivars currently in production are, at a minimum, six breeding cycles or generations removed from the HRW ancestor, Turkey. All cultivars were developed by Kansas State University or Oklahoma State University, in addition to ‘Jagalene’ (formerly Agripro Biosciences, Inc.), Triumph (private breeder, Oklahoma), and Turkey and ‘Khar kok’ (landrace introductions).

2.2. Methods

2.2.1. Nursery design and management

Field trials were established at the Oklahoma Agricultural Experiment Station (OAES) Agronomy Research Station in Stillwater, Oklahoma and at the OAES North Central Research Station near Lahoma, Oklahoma in 2015 and repeated through 2018. Each site-year was conducted as a randomized complete block design with two replicates and 28 (years 2015, 2016) or 30 genotypes per replicate (years 2017, 2018). Two genotypes in breeding cycles 1 and 2 allowed multiple genetic paths back to Turkey, but for the purpose of breeding cycle alignment in Fig. 1, the shortest route was usually taken. Thus, HRW cultivars currently in production are, at a minimum, six breeding cycles or generations removed from the HRW ancestor, Turkey. All cultivars were developed by Kansas State University or Oklahoma State University, in addition to ‘Jagalene’ (formerly Agripro Biosciences, Inc.), Triumph (private breeder, Oklahoma), and Turkey and ‘Khar kok’ (landrace introductions).

2.2.2. Determination of milling and kernel characteristics

Clean grain samples, with no detectable preharvest moisture damage or postharvest insect damage, were evaluated using approved methods and standards set by AAC International. Wheat protein (14% moisture basis) was measured using near infrared reflectance spectroscopy. Kernel weight, kernel diameter, and hardness index were measured using the Perten Single Kernel Characterization System 4100 (SKS; Perten Instruments, Segelkorp, Sweden). Flour yield was determined by
experimental milling using Method 26.22.01 and a Brabender Quadramat Senior mill (C.W. Brabender, South Hackensack, NJ).

2.2.3. Determination of gluten, dough, and flour quality

Physical dough tests were conducted with a computer-assisted mixograph using a 10-g bowl (AACC method 54.40.02) and further described in Supplemental Material.

Compression-recovery (CORE) tests were conducted to measure the elastic recovery of gluten following compression, another indicator of gluten strength. A gluten recovery index was determined using the same flour samples as for the mixograph, following the procedure described by Chapman et al. (2012). Triplicate subsamples were tested per experimental unit. Shaped gluten samples were allowed to rest for one to 2 min before loading into the Gluten CORE Analyzer (Perten Instruments, AB, Huddinge, Sweden), then compressed for 5 s with a force of 8N, followed by a 55-s recovery period.

A further test of gluten strength was the ratio of total polymeric protein (TPP) to total monomeric protein (TMP), using the protein extraction procedures described by Gupta et al. (1993). The protein extract was analyzed using size exclusion chromatography with an Agilent 1100 HPLC (Agilent Technologies, Palo Alto, CA), equipped with a 300 x 7.8 mm Biosep-SEC-s4000 column (Phenomenex, Torrance, CA). The chromatograms were manually integrated into two peaks, with the area of the first peak corresponding to polymeric proteins and the area of the second peak corresponding to monomeric proteins.

2.2.4. Gluten analysis using the G12 ELISA

Milled flour samples were analyzed on an as-is basis for concentration of gluten-specific epitopes recognized by the G-12 monoclonal antibody. Five grams flour were subjected to the AgraQuant Gluten G12 ELISA test (Romer Labs, Inc., Union, MO), following the protocol in AACC method 38–52.01 originally outlined in Halbmayr-Jech et al. (2012), and further described in Supplemental Material.

2.2.5. Fructan analyses

Total fructan content was determined in milled flour and wholemeal flour (dry basis) from the 2017 and 2018 experiments. Remnant flour samples used for flour quality tests provided the milled flour samples, whereas ground whole wheat kernels (15 g) using an Udy Cyclone Lab Sample mill (UDY Corporation, Fort Collins, CO) provided the wholemeal flour samples. Fructan analyses were conducted using the Megazyme K-FRUC Fructan Assay Kit (Megazyme, Co. Wicklow, Ireland).

2.3. Statistical analysis

Statistical analysis was conducted in SAS v9.4 (SAS Institute, Cary, NC). All traits were analyzed using linear mixed models methods. Restricted maximum likelihood estimates (REML) of the variance components for the random effects of the environment (E), genotype (G), and GE interactions were obtained, and significance of the variance components was determined by the relatively conservative Wald test. For the ELISA and fructan traits, additional variance components were estimated within subsets of the breeding cycles representing heirloom genotypes (breeding cycles 0–3) and contemporary genotypes (breeding cycles 4–6).

Trait responses were regressed on the number of generations removed from the common ancestor (breeding cycle). The general chi-square (GCS) score provided a gauge for data overdispersion. Traits with GCS scores greater than one were subjected to square root or natural logarithmic transformations to reduce data overdispersion.

The relationship between grain yield and protein was obtained by regressing wheat protein content on yield in a linear mixed model. The residuals generated from this analysis were further used in a regression on breeding cycle number. The residuals were treated as a trait to test for protein sensitivity to, or a metabolic cost associated with, higher grain yields. A more negative slope indicates greater protein sensitivity to higher grain yield. All tests of significance were conducted at the nominal 0.05 level.

3. Results and discussion

3.1. Agronomic traits

While the central motivation behind this research was to document certain rheological and biochemical changes that have occurred indirectly across multiple iterations of yield-focused selection, it is important to first establish any agronomic shifts as a framework for interpreting the indirect responses. After all, miniscule changes in the primary trait (yield) would call for a different interpretation of any shift in a secondary quality or sensitivity trait.

Surveying the estimated variance across all lineages and breeding
cycles, the genotypic effect was significant for grain yield (Table 1), and for days to heading and plant height (data not shown). The genotype x environment (GE) effect for grain yield was highly significant (Table 1), underscoring the importance of estimating selection response across a natural range of environmental conditions to arrive at a robust gauge of genetic change. Genotypic effects were not partitioned among the three lineages. Instead, the rationale was to utilize the three lineages in the analysis as they were intended in the experimental design: as an integral, organized form of genetic replication to strengthen response estimates across multiple parent-offspring relationships that are challenging to visualize in genetic-gain experiments designed from random cultivars.

The positive selection response for grain yield was highly significant, as determined by linear regression on breeding cycle (BC) using a natural logarithmic transformation to correct for overdispersion (Fig. 2A). In original units, the cultivar ‘Bentley’ (BC 6) had the highest mean grain yield, 4393 ± 342 (standard error) kg ha⁻¹, across all environments. ‘Kharkof’ (BC 0) had the lowest average grain yield, 2197 ± 177 kg ha⁻¹. Because the grain yield response was transformed, the regression coefficient is virtually uninterpretable (Pek et al., 2017). Therefore, reporting the gain in grain yield per breeding cycle (a regression coefficient) relative to the baseline heirloom cultivar Turkey would be inappropriate, because regression coefficients cannot be inverse transformed (Pek et al., 2017). Only the response value for each breeding cycle can be inverse transformed. Thus grain yield increased about 77% from cycle 0 to 6, which corresponds to wheat improvement in the U.S.

Table 1
Variance component estimates from REML and significance levels for random effects of 28–30 genotypes evaluated for two to 3 years at Stillwater and Lahoma, OK.

| Source of variation | Grain yielda | Test weight | Kernel weightb | Kernel diameter |
|---------------------|--------------|-------------|----------------|-----------------|
|                     | (kg ha⁻¹)²   | (kg hl⁻¹)² | (mg kernel⁻¹)² | mm²             |
| Environment (E)     | 0.05         | 1.21        | 0.06           | 0.02            |
| Genotype (G)        | 0.03***      | 0.34*       | 0.02***        | 0.004***        |
| GxE                  | 0.01***      | 0.74***     | 0.004*         | 0.000***        |
| Residual            | 0.02         | 0.61        | 0.02           | 0.004           |

| Source of variation | Wheat protein | Flour yielda | Hardness index | Adjusted SDS sedimentation vol |
|---------------------|---------------|--------------|----------------|-----------------------------|
|                     | (g/100 g)²    | (g/100 g)²   | ml²            |                             |
| Environment (E)     | 1.44          | 0.01         | 0.06           | 0.18                        |
| Genotype (G)        | 0.31***       | 0.02***      | 0.31***        | 0.31***                     |
| GxE                  | 0.11***       | 0.002        | 0.31***        | 0.10*                       |
| Residual            | 0.20          | 0.02         | 0.10           | 0.52                        |

| Source of variation | Mixing tolerance | Mixograph bandwidtha | Mixograph stability | CORE recovery indexa |
|---------------------|-------------------|----------------------|---------------------|---------------------|
|                     | (1-10)²           | mm²                  | (%)²               |                    |
| Environment (E)     | 0.35              | 0.07                 | 0.18               | 0.03               |
| Genotype (G)        | 0.46***           | 0.05                 | 0.32***            | 0.17***            |
| GxE                  | 0.17***           | 0.09***              | 0.10***            | 0.03**             |
| Residual            | 0.41              | 0.22                 | 0.06               | 0.98               |

| Source of variation | TPP/TMP | Gluten concentrationb | Milled flour fructans | Whole meal fructans |
|---------------------|---------|-----------------------|-----------------------|---------------------|
|                     | ppm²    | (g/100 g)²            | (g/100 g)²            |                    |
| Environment (E)     | 0.04    | 0.05                  | 0.04                  | 0.03               |
| Genotype (G)        | 0.005***| 0.03**                | 0.02***               | 0.03***            |
| GxE                  | 0.001*  | 0                   | 0.003                 | 0.01               |
| Residual            | 0.002   | 0.07                  | 0.01                  | 0.02               |

a, b Square root and natural logarithmic transformation used to estimate selection response, respectively.

*, **, *** Significant at p < 0.05, 0.01, and 0.001 respectively.


table 1
Variance component estimates from REML and significance levels for random effects of 28-30 genotypes evaluated for two to 3 years at Stillwater and Lahoma, OK.

3.2. Wheat and milling characteristics

Against this backdrop of a stepwise, consistent rise in yielding ability since the dawn of HRW wheat breeding, any associated changes in other characteristics can be considered consequential by direct (i.e., breeders intentionally applied selection pressure though hardly ever by strict truncation) or indirect means. For the latter, selection pressure would not have been applied, but a genetic association with yield or other selected traits caused a correlated selection response. In some cases, wheat breeders would have been unaware of these underlying associations, because the measuring tools were not widely available or there was no a posteriori reason to suspect a genetic relationship with the targeted traits.

Grain volume weight was certainly within breeders’ sights, but was targeted within a highly variable window of acceptability depending on the breeder. Grain volume weight increased 0.22 kg hl⁻¹ BC⁻¹ (Table 2), which counters reports by Khalil et al. (2002) for a similar but older genetic panel of HRW cultivars and Hucl et al. (2015) for Canadian spring wheat, both of whom found no significant response. Concomitant increases in volume weight and grain yield provide dual benefit in the form of a producer premium in some crop years and greater capacity for extractable flour for the miller.

Significant genotypic and GE effects were typically present (Table 1) for other common milling traits. Kernel weight significantly increased across breeding cycles (Fig. 2B). Given that kernel weight is one component of grain yield, it is not surprising to see their parallel selection responses, though wheat yield gains worldwide have not consistently shown a kernel weight dependency (Rudd, 2009). Genetic gains are more likely found in net weight per spike, that is, the product of kernel number per spike and kernel weight (Khalil et al., 2002). Our focus in this study was on kernel weight, because larger kernel weight (and size) carries obvious benefit to both producer and miller. Winter wheat breeding programs in the U.S. Great Plains consider >30 mg an acceptable target level for kernel weight. All 17 contemporary genotypes in the last two breeding cycles averaged 30 mg or higher across environments. Kernel weight response was mirrored by the response in southern Plains from the 1920s to current day. The tight-fitting linear selection response shown in Fig. 2A indicates genetic improvement in HRW wheat yield has occurred with no apparent genetic plateau looming.

Previous studies by Donmez et al. (2001), Khalil et al. (2002), Fufa et al. (2005), and Hucl et al. (2015), among several others, reported an increase in grain yield on a year-of-release basis, whereas we reported on a per-breeding cycle basis. Year of cultivar release does not accurately align filial connections among lineages, as in the case for Duster in BC 4 which was released 12 years later than its counterpart Jagger (Fig. 1). Cultivars in BC 5 varied by as much as 10 years in release date (Jagalene vs. Gallagher). The length of a breeding cycle, or the amount of time from commercialization of the parent to that of its offspring, can be highly variable, especially today as wheat breeders employ conventional inbred line development strategies versus the more rapid doubled haploid strategy in the same breeding program.

In agreement with previously reported results (Donmez et al. 2001; Fufa et al. 2005), negative responses in heading date and plant height (not shown) reflected selection for shorter stature wheat lines and higher priority for earlier maturity. Days to heading decreased 0.06 sqrt(days) BC⁻¹, and plant height decreased 0.11 sqrt(cm) BC⁻¹ (P < 0.001). Kharkof (BC 0) had the tallest average height (102 ± 5.7 cm) and the latest heading date (124 ± 2 days), whereas ‘Lonerider’ (BC 6) was shortest (77 ± 3.5 cm) and Triumph (BC 1) was earliest (107 ± 3 days). Even the shortest stature recorded for Lonerider equates to just short of waist height for the American female with average adult height of 163 cm (https://www.medicalnewstoday.com/articles/321132.php; verified Jan. 13, 2020), contrary to popular press accounts falsely claiming modern wheat to be much shorter (see Jones, 2012).

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Fig. 2. Selection responses for grain yield (A), kernel weight (B), wheat protein, 14% moisture basis (C), mixing tolerance (D), mixograph bandwidth (E), CORE index (F), TPP/TMP ratio (G), gluten, as is basis (H), milled flour fructans, dry basis (I), and wholemeal fructans, dry basis (J) across six winter wheat breeding cycles. Test of significance for the regression coefficient ($H_0: b = 0$) is shown by the $p$ value.
SKCS kernel diameter (Table 2).

Kernel hardness index increased significantly, though erratically, across breeding cycles (Table 2), an unexpected shift given the lack of intra-class directional selection pressure. Breeding programs typically target values for HRW wheat between 60 and 90 on a SKCS scale of -20 to 120. This slight upick in hardness was not associated with a significant response in flour yield (Table 2), measured with a small experimental mill. New HRW cultivars are opportunistically selected for higher flour yield, but usually not at the expense of a competitive yield level or overall fitness.

An incremental decrease in wheat protein content of 0.2 g/100 g per breeding cycle (Fig. 2C) netted a total loss of 1.1 g/100 g from BC 0 to BC 6. Other reported trends in bread wheat protein content are mixed, with some studies indicating losses (Fufa et al., 2005), no change (Khalil et al., 2002), or gains (Cox et al., 1989; Hucl et al., 2015). All trials were managed so that higher yielding genotypes should not have experienced insufficient nitrogen, which would have caused a negative bias in wheat protein content. While wheat protein content has decreased throughout the modern wheat breeding era, the current level has not fallen below the 12.0 g/100 g threshold typically targeted by U.S. HRW wheat breeding programs (Fig. 2C). In BC 6, wheat protein content varied from 12.4 ± 0.6 g/100 g (Lonerider) to 13.4 ± 0.3 g/100 g (OK11311F). Consistent with their divergent selection responses, the genetic correlation for grain yield versus grain protein was negative (r = −0.27), as reported in previous studies (Fufa et al., 2005).

The loss in wheat protein content across breeding cycles leads to the question, have contemporary cultivars in the more advanced cycles become more protein-sensitive, that is, are contemporary high yielding genotypes more inclined to lose wheat protein as environment productivity increases than foundational or heirloom cultivars? To address this question, linear regression was performed for wheat protein content versus grain yield among the 176 genotype x site-year combinations (Fig. 3A). The resulting deviations from regression in wheat protein content or residuals, were then plotted against breeding cycle (Fig. 3B). Cultivars with a positive residual, by inference, accumulate wheat protein at levels higher than expected for their yielding ability. Residuals from the protein-yield relationship were indeed dependent on breeding cycle, indicated by the significant (P < 0.05) negative slope in Fig. 3B. A closer examination of Fig. 3B, however, revealed that while there has been a decline from the heirloom cultivars, the modern cultivars in breeding cycles 5 and 6 showed both positive and negative mean deviations. While the modern cultivars could be at an inherent disadvantage for protein accumulation due to a dilution effect of grain nitrogen, it appears that any disadvantage that exists currently may be cultivar-specific and thus not a sweeping cause for alarm.

3.3. Gluten, dough, and flour functionality

The milling and baking industry commonly uses the alveograph and farinograph to measure dough behaviors respectively as resistance to expansion and resistance to mixing. While these tests are satisfactory stalwarts in commercial practice, they lack agility for use in wheat research programs often limited by small flour quantity or burdened by large sample load, especially in the early inbreeding generations of developing a new wheat cultivar. Instead, U.S. wheat breeders continue to rely on the less consuming mixograph, which heavily supported cultivar development in breeding cycles 3 to 6. Our evaluation of selection response, therefore, focused on the conventional mixograph parameters, a protein-adjusted determination of SDS sedimentation volume as an indirect measurement of the presence of larger protein components, a direct measurement of the proportion of polymeric (larger) proteins as the TFP/TMP ratio, and a direct measurement of gluten elasticity via the CORE recovery index, all of which produced significant genotypic and GE variance components (Table 1).

Even with the previously mentioned decline in wheat protein content, mixing tolerance rating increased (Fig. 2D), the mixograph stability index decreased (Table 2), and mixograph bandwidth showed no change (Fig. 2E). Although the linear response in adjusted SDS sedimentation volume was significant across all breeding cycles (Table 2), a sudden and temporary spike in sedimentation volume for breeding cycles 2 and 3 largely influenced the negative linear response (data not shown). Opposite the bandwidth, a lower mixograph stability index indicates greater mixing tolerance, assuming sufficient protein to allow proper gluten development. Altogether, these parameters indicate no change to a slight improvement in flour quality in HRW wheat over time. For both tolerance score and mixograph bandwidth, a spike occurred in selection response at BC 3 due mostly to cultivar ‘Karl 92’, with a mean tolerance score of 3.1 ± 0.2 units, bandwidth of 19.5 ± 1.5 mm, and stability value of 6.3 ± 0.7. Karl 92 and its immediate predecessor Karl were known throughout the breeding and end-user communities as outstanding sources of high protein and dough strength. High stability values

### Table 2

| Trait (unit)                | Mean of breeding cycle 0 | Regression coefficient | P > t     |
|-----------------------------|--------------------------|------------------------|-----------|
| Volume weight (kg hl⁻¹)     | 75.2                     | 0.22                   | 0.001     |
| SKCS kernel diameter (mm)   | 2.6                      | 0.03                   | <0.001    |
| SKCS hardness index         | 7.7                      | 0.10                   | <0.001    |
| Flour yield (g/100 g)       | 7.7*                     | 0.003                  | 0.642     |
| Mixograph stability         | 3.3                      | −0.18                  | <0.001    |
| Adjusted SDS sedimentation  | 7.1                      | −0.07                  | 0.014     |

* Square root transformation used to estimate selection response.

### Fig. 3.

Residuals generated from the linear regression of wheat protein content (14% moisture basis) versus grain yield for 30 winter wheat genotypes replicated within six Oklahoma environments (A), then plotted as a correlated trait response across breeding cycles (BC) (B).
indicative of a steeper descent in the mixograph curve, and thus poor tolerance to overmixing, were common among the older cultivars of BC’s 1 and 2.

Using a square root transformation, the compression recovery index increased across breeding cycles (Fig. 2F). This increase occurred solely as an indirect response to selection for mixograph, sedimentation volume, and baking characteristics, because CORE testing would not have been available for selection and cultivar development. Greater recovery indicates greater gluten elasticity conferred by polymer characteristics rather than rheological properties of dough. Nevertheless, a notable spike in the recovery index occurred in BC 3 as it did for mixograph properties, where the common driver of dough strength and gluten elasticity was Karl 92 (57.2 ± 0.8% recovery). Some contemporary entries were noted for having gluten elasticity on the same level as Karl 92, including ‘Spirit Rider’ (56.9 ± 0.9%) and Jagalene (58.2 ± 0.8%). Mixograph bandwidth showed a positive genetic correlation with the recovery index, indicating that a common indicator of dough strength coincided with this less exploited indicator of gluten strength. The magnitude of the correlation ($r = 0.28$), however, encourages positive selection for both parameters rather than one as a substitute for the other.

Knowledge of gluten composition provides a broad interpretation of bread dough functionality, which must encompass a balance of elastic and viscous rheological properties. One such assessment is derived from the ratio of total polymeric proteins (TPP) to total monomeric proteins (TMP), or quasi-ratio of the glutenin-to-gliadin contents. The monomeric gliadins mostly confer cohesive and extensibility properties in the gluten system, whereas the polymeric glutenins confer strength and elasticity of gluten; the ratio, or balance, of the two thus confers dough strength and extensibility. The TPP/TMP ratio showed no significant linear trend in Fig. 2G, indicating that the balance of glutenins to gliadins has not shifted systematically in the past 100 years of HRW wheat breeding. Therefore, dough strength has not changed due to broad-scale gluten composition pattern changes in HRW wheat, even in the presence of a unit decline (1.1 g/100 g) over all breeding cycles in wheat protein content to the mean current level of 12.9 ± 0.1 g/100 g.

### 3.4. Sensitivity traits

Presently, three major adverse reactions are related to the consumption of wheat: celiac disease, wheat allergy, and NCWS (Dale et al., 2019). We chose two biochemical components most often associated with seemingly disparate but potentially convergent causal elements of two of these known adverse reactions among humans: fructans measured as total concentration of fructo-oligosaccharides and fructan polysaccharide, and the gluten ELISA. The sequences recognized by the G-12 antibody are regarded as a key driver of immunogenicity in gluten (Arentz-Hansen et al., 2002). Fructans, which have no immunogenic potential, and besides their prebiotic role as a fermentable carbohydrate, may induce IBS symptoms when consumed in excess amounts or by individuals with a fructan intolerance (Fedewa and Rao, 2014), and thus may be one of the potential sources of NCWS.

In contrast to the aforementioned traits, GE interactions were nonexistent for gluten content and milled flour fructan content whereas their genotypic variances were highly significant (Table 1), a variance pattern highly conducive to selection. We found no genetic trend in gluten content, expressed in ppm flour, in response to a definitive rise in wheat yields (Fig. 2H). Escamot et al. (2018) used A1 and G12 antibodies to assay for all copies of the epitope associated with the 33-mer peptide, and reported no discernible difference among cultivars of disparate origin. While any amount of dietary gluten, and specifically sequences found within the 33-mer peptide, is toxic to celiac patients, we show no evidence that an increase in the epitopes common to the 33-mer is an unintended consequence of decades of dedicated yield improvement and competency for leavened bread products. Continued monitoring in other market classes of U.S. wheat is needed to form the critical genetic basis to refute popular claims that modern wheat breeding has led to cultivars directly linked to our food supply with elevated levels of immunogenic proteins.

Further partitioning of the gluten phenotypic variation revealed significant genetic variation among contemporary cultivars (Table 3), thus providing the necessary fuel to drive selection for reduced gluten presence independent of yield. Nowhere was the dispersion in gluten concentration more evident than between the two half-sibs, Gallagher and ‘Iba’, from BC 5. Iba produced the lowest gluten concentration (103.9 ± 15.3 ppm) in the contemporary group, whereas Gallagher produced the highest, and twice that level (209.1 ± 14.0 ppm), making it an undesirable resource for selection. This disparity combined with their close genetic relationship supports the case that gluten concentration, as measured by the G-12 antibody, may be controlled by relatively few genes in HRW wheat. Previous studies have shown that the 33-mer is controlled by few alpha-gliadins on chromosome 6D (Molberg et al., 2005).

In contrast to gluten patterns, milled flour fructan content increased across breeding cycle (Fig. 2I), or proportionately 30% from BC 0 to BC 6. Jagalene in BC 5 produced the highest flour fructan content (1.13 ± 0.08 g/100 g), and Kharkof produced the lowest (0.46 ± 0.04 g/100 g). A noticeable spike again was observed in BC 3, in which all three members (Chisholm, Karl, Karl 92) produced similar flour fructan contents averaging 0.97 g/100 g.

Even with the anomaly of BC 3, the steady increase in fructan content cannot be overlooked for potential cause and future dietary implications. The observed selection response was clearly indirect, or an unintended consequence, because wheat breeders in the southern Plains never applied direct selection pressure for grain or flour fructans. What then would serve as a common denominator to genetic gains in grain yield and fructans deposited in the grain? At this point, one cannot rule out the mathematical aberration, or compensatory effect, of the observed decline in the protein fraction with a corresponding rise in the non-starch carbohydrate fraction. Additionally, fructans may play a positive role during osmotic stress by enhancing remobilization of carbon reserves from vegetative tissues to the grain (Yang et al., 2004), a mechanism ostensibly beneficial to germplasm targeted for a region prone to periods of chronic water stress in the southern Great Plains.

To our knowledge, this study is the first to quantify differences in fructan content in an historic North American bread wheat panel featuring incremental changes in yielding ability, particularly at this level of environment sampling. Previous studies reported wholemeal fructan contents for modern wheat cultivars, varying from about 0.8 to 2.3 g/100 g (Huynh et al., 2008; Veenstra et al., 2019). Wholemeal samples will have a greater fructan content than milled flour, but our analysis showed identical indirect selection responses for milled flour versus wholemeal fructan content (Fig. 2J).

The observed increase in fructan content in contemporary cultivars must be considered in light of human dietary consequences. Presently, a reported fructan tolerance level for individuals with a FODMAP response of $0.05$ and $0.01$ respectively.

### Table 3

| Source of variation | Gluten | Milled flour fructans |
|---------------------|--------|-----------------------|
|                     | Heirloom | Modern | Heirloom | Modern |
|                     | (ln(ppm)) | (g/100 g) | (ln(ppm)) | (g/100 g) |
| Environment         | 0.06 | 0.04 | 0.03 | 0.04 |
| Genotype            | 0.02 | 0.04** | 0.04** | 0.02** |
| GxE                  | 0 | 0.001** | 0.01 | 0.001 |
| Residual            | 0.07 | 0.01 | 0.07 | 0.01 |

*Significant at p < 0.05 and 0.01 respectively.

Natural logarithmic transformation applied to response.
sensitivity is 0.3 g per serving (Varney et al., 2017). In 2017, U.S. per capita consumption averaged 59.8 kg flour per year (https://www.ers.usda.gov/topics/crops/wheat/wheat-sector-at-a-glance/; verified Jan. 15, 2020), or 0.16 kg per day. A total fructan content of 1 g/100 g equates to 1.63 g fructans per day. At three meals per day, this amounts to 0.54 g fructans per meal, assuming one serving of milled wheat flour per meal. Even this low consumption rate is nearly two times the current purported tolerance level. For the majority of the population with no FODMAP or fructan intolerance, these escalated levels likely cause no concern. However, wheat breeders might consider the feasibility of reversing this upward trend in fructans, considering that an estimated 24% of IBS sufferers have symptoms linked to fructan sensitivity (Böhm et al., 2013). Baking methods and dough fermentation time also may play a role in reducing the amount of fructans present in the consumable end product (Ziegler et al., 2016; Menezes et al., 2019).

In addition to the positive effects to the wheat plant, fructans have proven to be beneficial to the majority of humans. Fructans, especially the inulin type, are generally accepted as prebiotics since their fermentation leads to favorable changes in microbiota composition in the gastrointestinal tract, thus conferring digestive health benefits (Roberfroid et al., 2010). Couple the health benefits to humans and the stress tolerance to plants, and it becomes apparent why wheat breeders may select for increased fructan content (Veenstra et al., 2019), or maintain current levels with further increases in grain yield. However, studies to date largely do not consider the possible negative effect fructans can have on a portion of the population.

The ability to select bidirectionally for either high-fructan wheat or low-fructan wheat hinges on the necessary genetic diversity in various germplasm pools. The analysis of variance in Table 3 indicated significant genotypic effects among heirloom or contemporary genotypes, thus allowing breeders to select for either increased or decreased fructan content. Further research on the dietary implications and benefits, and the benefits to the wheat plant itself, will enable wheat breeders to make better informed selection goals for fructans.

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Appendix A. Supplementary data
Supplementary data to this article can be found online at https://doi.org/10.1016/j.cja.2020.102986.

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