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Increasing grain yield while maintaining baking quality in Canada Western Red Spring wheat

Pierre Hucl^a, Connie Briggs^a, Steve Shirtliffe^a, Brian Beres^b, Dean Spaner^c, Adam Dyck^d, and Guillermo Gerard^a

^aDepartment of Plant Sciences and Crop Development Centre, University of Saskatchewan, 51 Campus Dr., Saskatoon, SK S7N 5A8, Canada; ^bAgriculture and Agri-Food Canada, Sustainable Production Systems, Lethbridge Research Centre, 5403 1st Avenue South, Lethbridge, AB T1J 4B1, Canada; ^cDepartment of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB T6G 2P5, Canada; ^dWarburtons Foods Ltd., St Francois Xavier, MB R4L 1A9, Canada

Corresponding author: **Guillermo Gerard** (email: guillermosgerard@gmail.com)

Abstract

Grain protein concentration (GPC) is considered one of the most important quality factors, and it has remained a major culling criterion in the Canadian wheat cultivar development and registration process. However, grain protein composition also plays a critical role in determining the end-use quality of cereal-based products. The objective of this study was to determine whether high-yielding, lower protein Canada Western Red Spring (CWRS) analog wheat lines can exhibit acceptable baking properties (comparable with CWRS cultivars) under contrasting soil nitrogen levels. Five CWRS-analog lines together with four CWRS and one Canada Prairie Spring Red wheat cultivar, representing wide ranges of quality and grain yield potential, were assessed for agronomic and quality traits in multi-environment trials at three locations and five nitrogen fertilizer rates. CWRS analog lines produced significantly higher grain yield and, on average, 0.9% less GPC than the CWRS cultivars. Despite the lower GPC, CWRS-analog lines such as W07786 exhibited suitable and stable baking performance across all nitrogen levels. Based on the genotype \times trait biplot analysis, CWRS-analog baking properties were mainly associated with sodium dodecyl sulfate sedimentation and flour water absorption. Our findings revealed that it is possible to develop wheat cultivars with up to 15% higher grain yield than modern CWRS cultivars and comparable end-use characteristics by reducing current GPC requirements (by up to 1%) while simultaneously selecting for improved baking attributes. This would facilitate an increase in CWRS grain yield genetic gains while maintaining favorable end-use quality and improving the crop competitiveness in western Canada.

Key words: Canada Western red spring, grain yield, grain protein concentration, baking quality, *Triticum aestivum* L.

Résumé

On estime que la teneur en protéines du grain (TPG) est l'un des paramètres les plus importants de la qualité et elle demeure un des principaux critères d'élimination dans le processus canadien de développement et d'homologation de cultivars pour le blé. Cependant, la composition des protéines dans le grain joue elle aussi un rôle primordial dans la détermination de la qualité des produits céréaliers en fonction de leur usage final. Les auteurs voulaient établir si les lignées analogues au blé roux de printemps de l'Ouest canadien (CWRS) à haut rendement mais à plus faible teneur en protéines présentent des propriétés boulangères acceptables (c.-à-d. comparables à celles du blé CWRS) en présence d'une concentration d'azote contrastante dans le sol. À cette fin, ils ont évalué les caractères agronomiques et qualitatifs de cinq lignées analogues au CWRS, de quatre variétés du CWRS et d'un cultivar de blé roux de printemps Canada Prairie correspondant à un large éventail sur les plans de la qualité et du rendement grainier. Les évaluations se sont déroulées dans des conditions environnementales variées, à trois endroits, et à cinq taux de fertilisation avec des engrais azotés. Les lignées analogues au CWRS ont donné un rendement grainier sensiblement plus élevé et une TPG d'en moyenne 0,9 % inférieure à celle des variétés de CWRS. Malgré la plus faible TPG, les lignées analogues comme W07786 présentaient des propriétés boulangères adéquates et stables, à tous les taux de fertilisation. L'analyse par diagramme de double projection (génotype \times caractère) révèle que les propriétés boulangères des lignées analogues au CWRS sont essentiellement associées à la sédimentation SDS et à l'absorption de l'eau par la farine. Les résultats indiquent qu'on pourrait développer des cultivars de blé donnant jusqu'à 15 % plus de grain que les variétés contemporaines de CWRS mais aux caractéristiques comparables pour l'usage final si on diminuait (de jusqu'à un pour cent) les

exigences actuelles relatives à la TPG tout en sélectionnant de meilleures qualités boulangères. De cette façon, on augmenterait génétiquement le rendement grainier du CWRS tout en préservant une qualité adéquate selon la destination finale et en rehaussant la compétitivité de cette culture dans l'Ouest canadien. [Traduit par la Rédaction]

Mots-clés : blé roux de printemps de l'Ouest canadien, rendement grainier, teneur en protéines du grain, qualité boulangère, *Triticum aestivum* L.

Introduction

Globally, the primary focus of wheat breeding programs has been to increase grain yield, improve end-use quality, and disease resistance along with wide adaptability to different environments (Curtis et al. 2002). High grain yield and suitable bread-making quality are essential for the commercial value of wheat and determine the farmers' return on investment (Oury and Godin 2007). However, the simultaneous selection and development of high-yielding varieties capable of maintaining suitable bread-making quality properties are complicated by the frequently observed negative correlation between grain yield and the major quality criterion grain protein concentration (GPC; Simmonds 1995).

Canadian wheat is known around the globe for its versatility, strength, milling characteristics, and superior end product quality. Wheat varieties are segregated into nine market classes based on end-use suitability parameters of GPC, gluten strength, and kernel color (DePauw et al. 2011). Particularly, in Canada Western Red Spring (CWRS), the major wheat class grown in Canada, progress in breeding for grain yield has been sharply curtailed by very stringent high-protein requirements for the market class. Currently, the tolerance for grain and flour protein deviation from the mean of the check cultivars is relatively narrow. A deviation in excess of -0.9% over 2 years typically results in promising wheat experimental lines that are subsequently not released for commercial production. Despite the stringent high-protein requirements set for the class together with the undesirable negative correlation between GPC and grain yield, CWRS annual genetic gains ranging from 0.28% to 0.75% per year, which represents 12.4 and $23 \text{ kg} \cdot \text{ha}^{-1} \cdot \text{year}^{-1}$, respectively, have been reported (Hucl et al. 2015; Iqbal et al. 2016). These genetic improvements represent significant grain yield progress; however, to enhance wheat's competitiveness related to other crops, it is likely that CWRS wheat will have to achieve a greater rate of genetic gain.

Though CWRS wheat is suitable for multiple uses, the primary end-use being targeted by the class is the production of yeast-fermented, high-volume pan-breads whether used alone or in blends (Iqbal et al. 2016). The preparation of such products requires flours with superior baking properties, which must have the ability to produce bread with a large loaf volume and good crumb texture (Rózyło and Laskowski 2011; Gabriel et al. 2017). GPC is widely used as the main parameter in evaluating the quality of wheat products since it can be quickly and easily assessed, which in turn makes it attractive to economically assess experimental lines (Sieber et al. 2015). However, wheat quality is complex, largely dependent on its end-use and determined not only by protein concentration but also by protein composition (Chaudhary et al. 2016). In fact, flours with the same protein concentration do not necessarily produce products of similar end-use qual-

ity, where the correlation between protein concentration and bread-making tests is often relatively poor compared to other quality-related measurements (Fossati et al. 2010). It is widely accepted that allelic diversity as well as the protein composition (glutenin-to-gliadin and high molecular weight glutenin subunits (HMW-GS)/low molecular weight glutenin subunits (LMW-GS) ratios) are associated with different end-use qualities of wheat (Branlard et al. 2001; He et al. 2005). Thus, in several breeding programs high molecular weight glutenin subunits, such as $5 + 10$ (*Glu-D1d* allele), $B \times 7^{OE}$ (*Glu-B1* allele), or $Ax-1$ and $Ax-2^*$ (*Glu-A1* allele) associated with improved baking properties, have been frequently used to simultaneously increase quality and grain yield (He et al. 2005; Ikeda et al. 2007; Liu et al. 2010; Vancini et al. 2019).

Recent studies have identified modern wheat varieties with a 1% – 2% lesser protein concentration performing equally (baking volume) compared to other varieties with high crude protein composition (Zörb et al. 2018; Shewry et al. 2020). The suitable bread-making performance reported with a lower GPC has been associated with an increased glutenin-to-gliadin ratio, which resulted in greater dough elasticity. Thus, an alternative to increase grain yield genetic gains in the CWRS wheat class is to relax the GPC requirement for cultivar registration while selecting for quality profiles associated with improved baking properties.

Nitrogen fertilization is an additional factor affecting wheat quality. The application of N fertilizer usually increases quality parameters such as protein concentration, sedimentation volume, and gluten content but also affects the composition and ratios of glutenin-to-gliadin and HMW/LMW-GS, strongly influencing wheat quality performance (Xue et al. 2016; García-Molina and Barro 2017; Song et al. 2020; Zhen et al. 2020). Such effects have been reported to differ between genotypes and amount of nitrogen applied (Zhang et al. 2008; Shi et al. 2010). In western Canada, there has been a substantial increase in N fertilizer use for agricultural production since the 1980s, which has been associated with increases in grain yield, with potential effects on crop quality as well (Canada 2020). The objective of this study was to determine whether higher yielding, lower protein CWRS wheat prototype lines can exhibit acceptable baking properties (comparable with CWRS cultivars) under contrasting nitrogen conditions.

Materials and methods

Plant material

Five CWRS-analog experimental lines developed by the University of Saskatchewan Crop Development Centre (CDC, <https://cdc.usask.ca>) along with the CWRS cultivars CDC Teal (Hughes and Hucl 1993), CDC Utmost, CDC Stanley, CDC

Table 1. Precipitation and irrigation during the 2013, 2014, and 2015 wheat growing seasons (May to August).

Location	Latitude (°)	Longitude (°)	Year	Precipitation (mm)	Irrigation (mm)
Edmonton	53.5	−113.5	2013	267.0	–
Lethbridge	49.7	−112.8	2013	348.0	114.0
Saskatoon	52.2	−106.6	2013	199.5	–
Edmonton	53.5	−113.5	2014	216.0	–
Lethbridge	49.7	−112.8	2014	212.2	177.8
Saskatoon	52.2	−106.6	2014	281.0	–
Edmonton	53.5	−113.5	2015	137.8	–
Lethbridge	49.7	−112.8	2015	112.8	139.7
Saskatoon	52.2	−106.6	2015	185.0	–

Plentiful, and the Canada Prairie Spring Red (CPSR) cultivar 5702PR were used in the field trials. These cultivars represent a wide range of dough strengths, protein concentration, and grain yield potential. CDC Teal was a long-term check in Canada CWRS wheat registration tests and is considered a desirable target for dough strength. The grain yield of CDC Teal is similar to that of AC Barrie, representing older, long-term baseline genetics in Canadian breeding programs. CDC Utmost was one of the highest yielding CWRS wheat cultivars in Canada western regional trials and is an orange wheat blossom midge (*Sitodiplosis mosellana* Géhin) *Sm1* resistance gene carrier. CDC Stanley and CDC Plentiful have high yield potential despite lacking *Sm1*. All CWRS cultivars carry the 5 + 10 subunit and the null allele (*e*) at the *Glu-D1* and *Glu-A3* locus, respectively. In addition, CDC Teal, CDC Utmost, and CDC Plentiful harbored the B × 7 glutenin overexpression (OE) variant (*Glu-B1*) that confers stronger dough properties. 5702PR is a CPSR wheat that was included as it represents the likely yield target for future CWRS wheat cultivars. 5702PR also carries the 5 + 10 subunit and the B × 7 OE mutation, as do most of the recently released CPSR cultivars. The CDC CWRS-analog experimental lines have yield potential similar to that of 5702PR in comparative yield trials accompanied by lower protein levels than CWRS wheat cultivars. The CDC CWRS-analog lines carry the 5 + 10 and 7 + 9 subunits at *Glu-D1* and *Glu-B1* locus, respectively. Except for W07859 (null allele carrier), all the CWRS-analog lines had the *d* allele at the *Glu-A3* locus (Fig. S1).

Field trials and experimental design

The field trials were carried out at three locations in western Canada: Saskatoon, Edmonton, and Lethbridge, during the years 2013, 2014, and 2015 (Table 1). The experiments at Saskatoon and Edmonton were conducted under rainfed condition, while at Lethbridge irrigation was used. The experiments were arranged in a split-plot design with four replicates. The main plots were assigned to five nitrogen fertilization levels: N0 (0 kg N·ha^{−1}), N70 (70 kg N·ha^{−1}), N140 (140 kg N·ha^{−1}), N210 (210 kg N·ha^{−1}), and N280 (280 kg N·ha^{−1}), applied as granulated urea via side banding at sowing and the sub-plots to wheat genotypes. The plot dimensions at the three sites were as follows: 4.1 m² at Lethbridge, 4.5 m² at Edmonton, and 11.1 m² at Saskatoon, using a seeding rate of 300 seeds·m^{−2} at all three sites. Two plots of spring wheat

cultivar CDC Go were used as a border between each main plot to avoid the nitrogen diffusion from the higher fertilizer rate to the lower rate treatments. The trials were sown in mid to late May, and standard herbicides were used for weed control.

Agronomic traits and small-scale quality assessments

Standard agronomic data were collected from the field trials. Plant height (PH) was recorded at physiological maturity from the soil surface to the tip of the spike excluding awns. Days to maturity (DM) were recorded as the number of days from planting until 95% of the peduncles in each plot had turned yellow. Plots were harvested using a plot combine and grain samples were dried (moisture content of 10 ± 1%) and weighed. Grain yield (GY) was the total weight of seed yield in each plot divided by the plot area and expressed as Mg·ha^{−1}. Small-scale quality tests were conducted on the field trial samples in the CDC Grain Innovation Laboratory (GIL). Test weight (TW), Kernel hardness index (KHI), GPC, Falling number (FN), and sodium dodecyl sulfate (SDS) sedimentation volume were measured on each plot (i.e., 600 samples per year). Test weight was measured by using a 0.5 hL cup; KHI was determined with a Perten SKCS 4100 single-kernel characterization system (Perten Instruments North America, Springfield, IL, USA); GPC was measured using combustion nitrogen analysis (AACCI Method 46–30.01) using a LECO model FP-528 (Leco Corp, St. Joseph, MI, USA). The SDS sedimentation volume was measured by using the method proposed by Axford et al. (1978) and the Hagberg Falling number was determined according to the AACCI Method 56–81.04. For post-milling measurements, reps 1 and 2 were used, resulting in 300 samples being subjected to more labor-intensive measurements for each year. A Brabender Quadrumat Jr flour mill was used to produce flour from reps 1 and 2 from each site/year. Flour milling yield (FY; 100 g basis), flour protein concentration (FIPro), protein loss (Ploss), flour ash content (FIAsh), and color were assessed using AACCI Methods 26–50.01, 46–30.01, 08–01.01, and 14–30.01, respectively. The dough mixing properties, flour water absorption (FAB), dough development time (DDT), mixing tolerance index (MTI), and dough development stability (STA) were evaluated using a Perten microDoughLab (63 rpm).

Table 2. Fixed-effect *F*-tests of agronomic and small-scale quality measurements for 10 wheat genotypes grown under five nitrogen rates in nine environments.

Fixed effects sources	df	Agronomic traits			Kernel properties				
		PH	DM	GY	TW	GPC	SDS	FN	KHI
Genotype	9	73.8***	17.4***	4.4***	10.9***	29.5***	15.4***	3.3*	68.1***
N rate	4	32.1***	163.1***	2.6*	1.2 ns	6.5**	2.8*	0.2 ns	3.9*
Genotype × N rate	36	0.0 ns	0.0 ns	1.5*	2.2**	1.3 ns	3.0***	1.2 ns	2.1***

Fixed effects sources	df	Flour properties				Dough properties			
		FY	FlPro	PLoss	FlAsh	L*	a*	b*	FAB
Genotype	9	56.8***	48.1***	87.9***	5.0***	29.2***	10.0***	53.7***	50.6***
N rate	4	0.9 ns	6.9***	17.8***	1.5 ns	3.7*	0.6 ns	0.8 ns	8.0***
Genotype × N rate	36	1.0 ns	1.0 ns	0.6 ns	1.3 ns	1.0 ns	1.0 ns	2.0**	2.0**

Note: *, **, ***: significant at $p < 0.05$, $p < 0.01$, $p < 0.001$, respectively. ns, not significant.

PH, plant height; DM, days to maturity; GY, grain yield; TW, test weight; GPC, grain protein concentration; SDS, sodium dodecyl sulfate sedimentation volume; FN, Falling number; KHI, kernel hardness index; FY, flour yield; FlPro, flour protein concentration; PLoss, flour protein loss; FlAsh, flour ash concentration; L*, a* and b*, flour color parameters lightness, redness, and yellowness, respectively; FAB, flour water absorption; DDT, dough development time; MTI, mixing tolerance index; STA, dough development stability.

Baking assessments

Based on GIL-conducted quality evaluations and the field trial outcomes, a sub-set of seven genotypes was selected in consultation with Warburtons Foods Ltd. for more intensive baking assessments using their pilot-scale protocols at the Canadian International Grains Institute (CIGI), which were designed to mimic the process used in a Warburtons commercial bakery. For each of the selected genotypes, a composite sample (10 kg per sample) was made across the four repetitions for each nitrogen fertilization level and from the sites where a more pronounced response to the N fertilizer rate was measured (Saskatoon and Edmonton in 2013 and 2014 and Saskatoon and Lethbridge in 2015). A Buhler Laboratory Mill (MLU-202) with three breaks and three reductions was used to mill the wheat. A straight-grade white flour was produced by combining appropriate milling streams. Pan bread was prepared using the Warburtons commercial white bread formulation, and doughs were mixed and molded using a Warburtons Proprietary Tweedy Mixer and Proprietary Molder. The doughs were then proofed (80% RH; 42 °C) to a height of 0.7 mm below the top edge of the pan (measurement taken at the center of the pan). Breads were baked using a Picard reel oven (Drummondville, QC). The following baking properties were measured: baking absorption (BkAbs) determined as the amount of water required to form a soft dough, mix time (MT) determined as the time required to achieve optimum gluten development in the dough, proof time (PrT) determined as the amount of time required for the dough to achieve a set height in the pan, and Loaf Height (LfhT) determined as the height of the baked bread (measurement taken in the center of the loaf). The following day, one loaf of each bread was cut in half crosswise and scored using 10-point scales for the following parameters: crumb color (Cc-Col) determined visually with a higher score given to loaves with a whiter and brighter crumb color, texture (Tx) determined visually with a higher score given to loaves with good cell shape, cell uniformity, and cell fineness; softness (Soft) determined by finger compression of the crumb with a higher score given to loaves with a soft crumb, and crumb strength

(CrSr) determined by rubbing the surface of the crumb with the fingers with a higher score given to loaves that had good crumb strength.

Statistical analysis

Linear mixed models to evaluate the effects of genotype and N rate treatments on agronomic and quality traits were fitted using PROC MIXED in SAS version 9.4. Genotype and N rate treatments were considered fixed effects, whereas environments (combination of year and site) and repetitions were considered random effects. The covtest option was specified in the PROC MIXED statement to estimate the variance components. Least square means were separated using the PDIF option of LSMEANS in SAS PROC MIXED, setting the probability of Type I error or α level (α) = 0.05. Mean comparisons among cultivars were conducted by Fisher's protected least significant differences (LSD) based on Student's *t* distribution. Relationships among quality properties and genotypes were studied by the genotype × trait (GT) biplot method (Yan et al. 2001). The GT biplot was created based on the first two principal components (PC) resulting from singular value decomposition (SVD) of the standardized GT table.

Results

Agronomic traits

The statistical analysis revealed significant differences among genotypes in grain yield, days to maturity, and plant height ($p < 0.001$). The N rate and genotype × N rate interaction effects were only significant ($p < 0.05$) for grain yield, indicating that the responses of different genotypes to N rates were different (Table 2). Overall, the grain yield of CWRS-analog lines was significantly higher than that of CWRS cultivars. Line W07847 had the highest yield (4.7 Mg·ha⁻¹), which was 6.8% and 14.6% higher than the grain yield of CDC Stanley (4.4 Mg·ha⁻¹) and CDC Teal (4.1 Mg·ha⁻¹), the highest and lowest yielding CWRS cultivars, respectively. In addition,

Table 3. Least square means of agronomic and small-scale quality measurements for 10 wheat genotypes grown under five nitrogen rates in nine environments.

Fixed effects sources	Agronomic traits				Kernel properties							
	PH (cm)	DM (day)	GY (Mg·ha ⁻¹)	TW (kg·hL ⁻¹)	GPC (%)	SDS (mL)	FN (sec)	KHI				
Genotype												
CDC Teal	89.8a	93.9c	4.1c	78.9b	15.1a	72.8ab	409.0ab	61.3d				
CDC Utmost	88.3ab	94.5c	4.2bc	79.2b	14.6b	73.3ab	411.7ab	62.6cd				
CDC Stanley	89.7a	96.3b	4.4b	79.2b	14.7b	66.7cd	408.5ab	63.5c				
CDC Plentiful	86.7b	94.1c	4.2bc	80.4a	14.7b	68.3c	415.0a	65.6bc				
CWRS Lines	88.6ab	94.7c	4.2bc	79.4b	14.8b	70.3bc	411.0ab	63.2c				
5702PR	82.0c	97.3ab	4.6ab	75.8c	13.3e	70.3bc	392.4b	56.3e				
W07786	81.4c	97.9a	4.5ab	80.2ab	13.9cd	72.0b	371.7c	64.7bc				
W07838	78.3d	96.1b	4.3bc	80.4a	13.7d	72.3ab	389.4bc	66.7b				
W07847	76.2d	96.9ab	4.7a	79.8ab	13.9cd	73.3ab	390.4bc	74.8a				
W07859	71.0e	96.9ab	4.2bc	80.2ab	13.5de	66.1d	392.0bc	73.8a				
W07876	76.7d	97.8ab	4.6ab	79.6ab	14.2c	74.2a	390.1bc	75.6a				
CWRS analog	76.7d	97.1ab	4.4ab	80.0ab	13.8cd	71.6b	386.7bc	71.1b				
N rate (kg·ha ⁻¹)												
N0	79.4c	93.7e	3.9b	79.6a	13.1b	68.8b	397.7a	63.7b				
N70	81.7b	95.8d	4.3ab	79.4a	13.9b	70.8ab	398.7a	66.7a				
N140	82.9a	96.6c	4.5a	79.2a	14.2ab	71.4a	395.8a	67.4a				
N210	83.1a	97.2b	4.5a	79.3a	14.7a	71.8a	395.2a	67.1a				
N280	82.7a	97.6a	4.6a	79.3a	14.9a	71.8a	397.7a	67.6a				
Fixed effects sources	Flour properties							Dough properties				
	FY (%)	FIPro (%)	PLoss	FIAsh (%)	L*	a*	b*	FAB (%)	DDT (min)	MTI	STA (min)	
Genotype												
CDC Teal	70.0b	14.9a	0.14f	0.46ab	85.6bc	1.0cd	13.6c	64.4b	4.2b	39.6bc	11.1ab	
CDC Utmost	69.4c	14.5b	0.04g	0.44bc	86.2a	0.9d	12.3f	65.0a	4.9a	34.1c	10.6ab	
CDC Stanley	71.5a	14.5b	0.23e	0.44bc	85.6bc	1.1bc	14.0b	63.1c	3.6bc	50.0ab	7.8c	
CDC Plentiful	69.9bc	14.2c	0.44c	0.46ab	85.8b	0.9cd	13.3d	63.4c	4.1b	42.0b	8.8bc	
CWRS Lines	70.2b	14.5b	0.21e	0.45b	85.8b	1.0cd	13.3d	64.0b	4.2b	41.4b	9.6bc	
5702PR	71.3a	12.6e	0.66a	0.44bc	85.6c	1.0cd	12.9e	60.5e	4.7ab	41.4b	10.8ab	
W07786	69.8bc	13.4d	0.35d	0.45b	85.5cd	1.1c	13.3de	63.5c	3.8bc	51.2a	7.8c	
W07838	70.2b	13.2d	0.42c	0.44bc	85.5cd	1.2bc	14.4a	62.3d	4.6ab	44.2b	10.2b	
W07847	67.1d	13.3d	0.62ab	0.43c	84.9e	1.3b	13.5cd	64.7ab	3.2c	38.1bc	9.7b	
W07859	69.2c	12.9e	0.59b	0.47a	85.4d	1.1bc	13.0e	63.7bc	2.9c	48.2ab	7.1c	
W07876	67.3d	13.5d	0.61ab	0.43c	84.9e	1.4a	13.9b	64.3b	3.3c	33.7c	12.3a	
CWRS analog	68.7c	13.3d	0.54b	0.44bc	85.2d	1.2bc	13.6c	63.7bc	3.6bc	43.0b	9.4bc	
N rate (kg·ha ⁻¹)												
N0	69.5a	12.4b	0.67a	0.46a	85.8a	1.1a	13.5a	62.5c	3.2b	48.7a	7.2b	
N70	69.4a	13.3b	0.69b	0.45ab	85.5b	1.1a	13.5a	63.3b	3.9a	44.3ab	9.2a	
N140	69.1a	13.7ab	0.59b	0.45ab	85.4b	1.1a	13.5a	63.7ab	4.0a	40.3b	9.8a	
N210	69.9a	14.4a	0.62b	0.44b	85.4b	1.1a	13.4a	63.8ab	4.3a	39.6b	11.1a	
N280	69.8a	14.6a	0.48c	0.44b	85.4 b	1.1a	13.3a	64.0a	4.4a	38.4b	10.8a	

Notes: Means followed by the same letter code are not statistically significantly different as per Fisher's least significant difference (LSD) at $p = 0.05$.

PH, plant height; DM, days to maturity; GY, grain yield; TW, test weight; GPC, grain protein concentration; SDS, sodium dodecyl sulfate sedimentation volume; FN, Falling number; KHI, kernel hardness index; FY, flour yield; FIPro, flour protein concentration; Ploss, flour protein loss; FIash, flour ash concentration; L*, a*, and b*, flour color parameters lightness, redness, and yellowness, respectively; FAB, flour water absorption; DDT, dough development time; MTI, mixing tolerance index; STA, dough development stability.

W07847 yielded 2.2% higher than the CPSR cultivar 5702PR (4.6 Mg·ha⁻¹), although the difference was not statistically significant (Table 3). CWRS-analog lines also exhibited later maturity and shorter PH compared to the CWRS cultivars. CWRS-analog lines, on average, reached physiological matu-

urity 2 days later and were 12 cm shorter than CWRS cultivars, respectively (Table 3). Regarding N rates, GY appeared to increase with N supply; however, a differential increase rate was observed among the genotypes (Table S1). Overall, the highest grain yields were observed at N280 (4.6 Mg·ha⁻¹),

Table 4. Fixed-effect *F*-tests of CIGI baking properties for seven wheat genotypes grown under five nitrogen rates in six environments.

Fixed effects sources	df	Baking properties								
		BkAbs	MT	PrT	Lfht	Lfht:GPC	Tx	Soft	CrStr	CrCol
Genotype	6	16.63**	6.57*	10.55*	8.53**	2.79*	4.03**	3.24 ns	1.23 ns	6.01***
N rate	4	43.98***	74.27***	2.46 ns	20.60***	53.50***	64.86***	35.24 ns	19.91***	58.11***
Genotype × N rate	24	1.67*	0.93 ns	2.49***	1.75*	0.23 ns	1.73*	2.55***	1.22 ns	2.20**

Note: *, **, ***: significant at $p < 0.05$, $p < 0.01$, $p < 0.001$, respectively. ns, not significant.

BkAbs, baking absorption; MT, mix time; PrT, proof time; Lfht, loaf height; Lfht:GPC, loaf volume per percentage point of protein; Tx, texture; Soft, softness; CrStr, crumb strength; CrCol, crumb color.

N210 (4.5 Mg·ha⁻¹), and N140 (4.5 Mg·ha⁻¹) with N0 producing the lowest grain yield (3.9 Mg·ha⁻¹).

Kernel properties

Significant differences among genotypes were observed for all evaluated kernel properties. In addition, KHI, GPC, and SDS sedimentation volume were significantly affected by the N rate, while for TW, KHI, and SDS a significant genotype × N rate interaction was also observed (Table 2). The CWRS cultivars had significantly higher GPC than the CWRS-analog lines, and the CPSR cultivar 5702PR. GPC for CWRS cultivars was, on average, 0.93 and 1.12% higher than the experimental lines and 5702PR, respectively. The cultivars CDC Teal and W07876 with 15.1% and 14.2% exhibited the highest GPC values among CWRS cultivars and CWRS-analog lines, respectively (Table 3). Increased N supply significantly increased GPC from 13.1% to 14.9% with the application of N0 and N280, respectively. A similar pattern among genotypes was observed for FN, where CDC Plentiful and W07786 had the highest (415 sec) and lowest (372 sec) values, respectively. Despite the lower FN values observed in CWRS-analog lines, all of them had FN values > 370 sec. Overall, the KHI of CWRS-analog lines was significantly higher than for the CWRS cultivars and 5702PR. For this trait, a significant genotype × N rate interaction was observed. The cultivars 5702PR, W07838, and W07859 exhibited a significant increase in response to N from N0 to N70, while for the remaining genotypes the increase in N levels did not significantly affect KHI values. For SDS, W07876 had the highest value (74.2 mL), but it was not significantly higher compared to W07847 (73.3 mL), CDC Utmost (73.3 mL), CDC Teal (72.8 mL), and W07838 (72.3 mL). A significant interaction for SDS resulted because only two cultivars, 5702PR and W07876, exhibited increases in SDS due to an increase in N level (Table S1).

Flour properties

Significant differences among genotypes were observed for all flour properties. Flour protein concentration, Ploss, and L* (brightness) were significantly affected by the N rate, while for b* (yellowness) a significant cultivar × N rate interaction was observed due to a differential genotype response to the N rate (Table 2). Overall, CWRS cultivars had higher values than CWRS-analog lines for FY and L*; however, lines such as W07838 and W07786 did not exhibit significant differences from CWRS cultivars for both traits, respectively (Table 2). The flour protein concentration followed the same trend as

GPC, with values ranging from 12.6% (5207PR) to 14.9% (CDC Teal). Overall, CWRS cultivars had an average FIPPro that was 1.3% and 1.9% units higher than the CWRS-analog lines and 5702PR, respectively. For flour extraction, values ranged from 67.1% to 71.5%. Protein loss ranged from 0.04% (CDC Teal) to 0.66% (5702PR), with CWRS-analog lines exhibiting, on average, higher values than the CWRS cultivars. However, lines such as W07786 had lower Ploss values than the CWRS cultivar CDC Plentiful (Table 3). Flour L* values ranged from 86.2 units for CDC Utmost to 84.9 units for lines W07876 and W07847. Flour a* values (“redness”) ranged from 1.4 (W07876) to 0.9 (CDC Utmost) units whereas b* values ranged from 14.4 (W07838) to 12.3 (CDC Utmost) units (Table 3). Increased N rates significantly increased FIPPro and reduced the L* values, while no significant N effects were evident for the other flour properties (Table 3).

Dough properties

Dough properties exhibited significant differences among genotypes, nitrogen levels, and the interaction between genotype and nitrogen level (Table 2). No clear trend was observed between CWRS cultivars and CWRS-analog lines for dough properties. Flour water absorption values ranged from 60.5% to 65.0% with cultivars CDC Utmost (65.0%), W07847 (64.7%), and CDC Teal (64.4%) exhibiting the highest values. Dough development time values range from 2.9 (W07859) to 4.9 (CDC Utmost) units whereas MTI values range from 33.7 (W07876) to 51.2 (W07786). The STA values ranged from 7.1 to 12.3 min, with W07876 and CDC Teal exhibiting the highest STA values (Table 3). Nitrogen fertilization affected all dough properties; however, these effects differed among genotypes (Table S1).

Baking properties

Significant differences among genotypes were observed for BkAbs, MT, PrT, LfHt, loaf height per unit protein (Lfht: GPC), Tx, and CrCol. Baking absorption, MT, LfHt, Lfht: GPC, Tx, CrStr, and CrCol were also significantly affected by the N rate, while a significant genotype × N rate interaction was observed for BkAbs, PrT, LfHt, Tx, Soft, and CrCol (Table 4). The genotype × N rate interaction indicated that baking properties of the genotypes responded differently to the N rate. While significant decreases in LfHt, Soft, and CrCol were observed for the CWRS cultivars through increasing N rates, the CWRS-analog line W07786 did not show a significant response to the N rate (Fig. 1). Thus, the baking properties of W07786 did not differ significantly compared to the CWRS

Fig. 1. Mean values of LfHt: loaf height; Soft: softness; CrCol: crumb color; Tx: texture; BkAbs: baking absorption for the genotype \times N rate interaction vs. GPC (%): grain protein concentration.

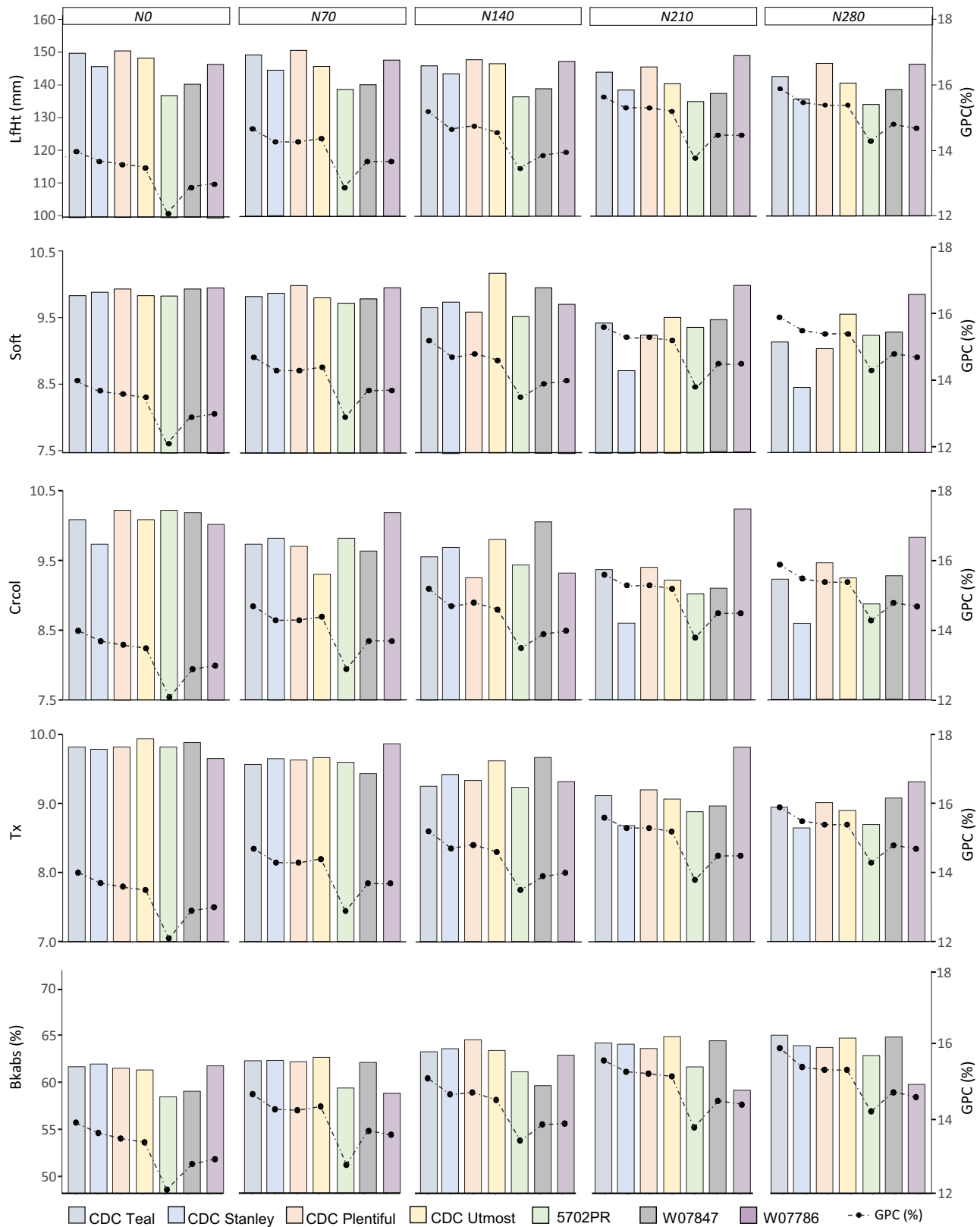


Table 5. Least square means of CIGI baking properties for seven wheat genotypes grown under five nitrogen rates in six environments.

Fixed effects sources	Baking properties								
	BkAbs (%)	MT (s)	PrT (min)	Lfht (mm)	Lfht:GPC	CrCol	Tx	Soft	CrStr
Genotype									
CDC Teal	63.2a	209.2a	41.9bc	146.0ab	9.7b	9.6b	9.3b	9.6ab	9.4a
CDC Utmost	63.2a	209.9a	45.3b	144.2ab	9.9b	9.6b	9.4b	9.8a	9.3a
CDC Stanley	63.3a	199.9ab	46.9b	141.3b	9.6b	9.1c	9.2b	9.3b	9.2a
CDC Plentiful	62.9a	204.8ab	37.9c	147.9a	10.1ab	9.7b	9.4b	9.6ab	9.4a
CWRS lines	63.1a	205.9a	43.0bc	144.8ab	9.8b	9.5b	9.3b	9.6ab	9.3a
5702PR	60.7b	196.5ab	46.0b	136.2c	10.3ab	9.5b	9.2b	9.5ab	9.2a
W07786	59.3c	176.4b	50.7ab	147.2a	10.6a	10.1a	9.7a	9.9a	9.6a
W07847	63.2a	188.4b	53.2a	139.0bc	10b	9.5b	9.3b	9.6ab	9.4a
CWRS analog	61.2b	182.4b	51.9ab	143.1ab	10.3ab	9.8ab	9.5ab	9.7ab	9.5a
N rate (kg·ha ⁻¹)									
N0	60.8d	188.3e	45.4b	145.3a	11.0a	10.1a	9.8a	9.88a	9.8a
N70	61.4c	191.6d	45.6b	145.2a	10.4b	9.7b	9.6b	9.84a	9.7ab
N140	62.6b	197.5c	45.4b	143.6b	10.0b	9.6c	9.4c	9.76a	9.5b
N210	63.1a	203.9b	46.3ab	141.2c	9.5c	9.3d	9.1d	9.38b	9.0c
N280	63.5a	208.0a	46.9a	140.3c	9.3c	9.2d	8.9d	9.22c	8.9c

Note: Means followed by the same letter code are not statistically significantly different as per Fisher's least significant difference (LSD) at $p = 0.05$.

BkAbs, baking absorption; MT, mix time; PrT, proof time; Lfht, loaf height; Lfht:GPC, loaf volume per percentage point of protein; Tx, texture; Soft, softness; CrStr, crumb strength; CrCol, crumb color.

cultivars at N0, but it had higher values for most of the baking parameters (except BkAbs) with increasing N level, particularly under N210 and N280 N rates. In addition, W07786 had a significantly higher Lfht: GPC ratio than most of the CWRS cultivars, although no significant genotype \times N rate interaction was observed for this trait. Loaf height values range from 133.8 at N280 (5702PR) to 150.7 mm at N70 (CDC Utmost), Softness ranged from 8.45 at N280 (CDC Stanley) to 10.17 at N70 (CDC Utmost), whereas CrCol values ranged from 8.6 at N280 (CDC Stanley) to 10.22 at N0 (5702PR). Increased N levels negatively affected Tx for all cultivars ($p = 0.05$); however, W07786 only exhibited significant reductions at N280. Texture values ranged from 8.6 in CDC Stanley at N280 to 9.9 in CDC Utmost at N0 (Table 5). Baking absorption and MT were the only two baking properties positively affected by the N fertilizer rate, except for W07786, which did not show significant BkAbs differences among N levels (Fig. 1).

Association among traits and genotypes

To summarize the overall genotype performances and their interaction with quality traits ($G \times T$), we conducted a GT bi-plot analysis. The first two principal components explained 40.3% and 23.8% of the total standardized variation of the data, respectively (Fig. 2). Trait relationships revealed that GPC and FIPPro had positive associations with dough properties STA and DDT, MT, and BkAbs while exhibiting a negative relationship with MTI and PrT. Baking properties, Softness, CrCol, Tx, and CrStr were strongly associated with each other, while LfHt was positively associated with FAB and SDS. CWRS wheat cultivars CDC Teal, CDC Utmost, and CDC Plentiful were associated with GPC and the dough properties DDT and STA, while the CWRS-analog line W07786 was associated with improved baking properties, SDS, and FAB. W07847 had

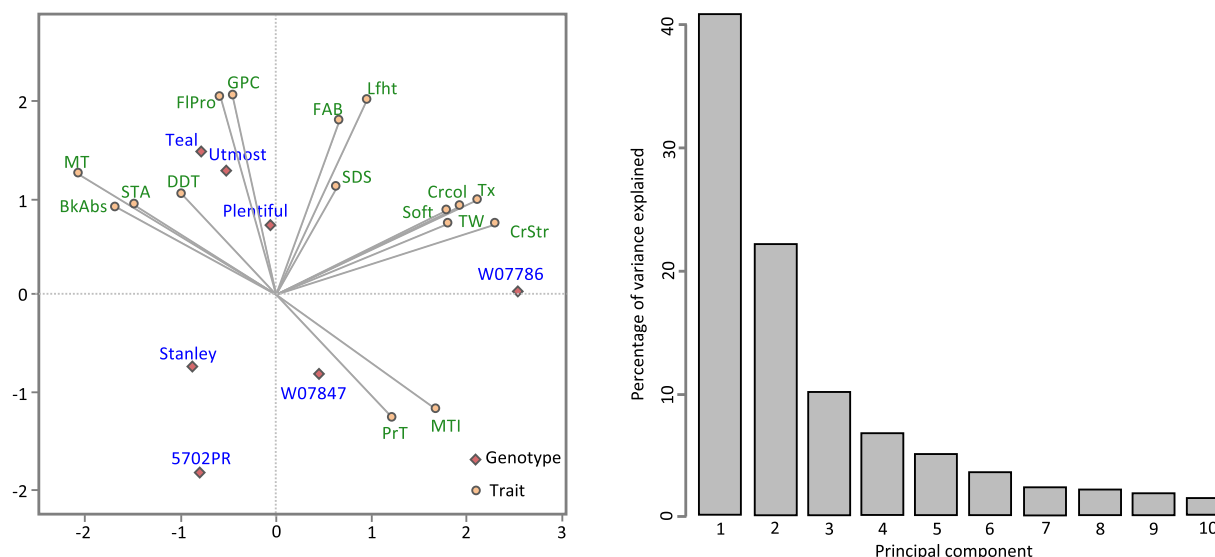
higher PrT values, while CDC Stanley and 5702PR did not show a strong association with any of the quality traits under evaluation (Fig. 2).

Discussion

In the Canadian wheat grading system, wheat cultivars are evaluated and segregated into nine market classes based on end-use suitability parameters of GPC, gluten strength, and kernel color (DePauw et al. 2011). Especially for the CWRS wheat class, high protein concentration is considered to be the single most important quality factor and it remains a major culling criterion in the Canadian wheat cultivar development and registration process. As a result, genetic gains in grain yield have been curtailed due to the frequently observed negative correlation between grain yield and GPC (Simmonds 1995). However, it has been demonstrated that wheat quality is complex and determined not only by GPC but also by protein composition (Chaudhary et al. 2016). For the CWRS class, whose primary end-use is the production of yeast-fermented high-volume pan-breads, baking properties such as loaf volume, determined by a standard baking test, together with texture and the characteristics of the crumb are the most important quality parameters (Gabriel et al. 2017).

In the present study, CWRS analog lines had higher grain yield (up to 15%) coupled with reduced plant height relative to the CWRS cultivars. On average, the CWRS analog lines had lower FN and increased Ploss compared to the CWRS cultivars; however, both traits were within acceptable ranges, while no clear trends were observed for dough properties (Table 3). Higher yielding CWRS analog lines exhibited, on average, 0.9%-unit lower GPC than commercial CWRS cultivars; however, lines such as W07786 outperformed the com-

Fig. 2. Genotype by trait (GT) biplot for seven genotypes and sixteen quality traits based on singular value decomposition of trait-standardized data ("Scaling = 1, Centering = 2") and trait-focused singular value partition ("SVP = 2").



mercial cultivars in the baking quality assessment. These results suggest differences in protein quality, which would allow CWRS analog lines to exhibit desirable baking properties even with protein values lower than those observed in current CWRS cultivars. According to the SDS-PAGE analysis, CWRS cultivars carry the null allele *e*, while CWRS analog lines carry the *d* allele at the *Glu-A3* locus. The *Glu-A3e* allele is usually associated with negative effects on wheat quality, while the *Glu-A3d* allele has been reported to make significant contributions to wheat quality (Ikeda et al. 2007; Jin et al. 2013). Wheat cultivars with lower GPC but with a desirable baking quality profile indicating improved protein quality have been previously reported (Waterer and Evans 1985; Zörb et al. 2018; Shewry et al. 2020).

In the GT biplot analysis, baking properties such as Lfht, Soft, CrCol, and Tx were mainly associated with SDS and FAB, both traits being reported as protein and baking quality indicators (Kent 1983; Waterer and Evans 1985; Kaushik et al. 2015). Previous studies covering a wide range of bread-making qualities showed that SDS sedimentation volume was a superior test in predicting loaf volumes of bread produced by both mechanical development (Chorleywood) and long fermentation procedures (Preston et al. 1982; Dhaka and Khatkar 2015). In contrast, GPC did not show a strong association with baking properties but rather was associated with dough properties. Thus, even though GPC is widely used as the main criterion in predicting wheat quality (Gabriel et al. 2017; Xue et al. 2019) it is not necessarily a reliable indicator of baking quality when used alone or without consideration of other quality traits. Moreover, our results emphasize the high relevance of protein quality when developing new cultivars as demonstrated by the strong association between SDS and baking properties such as loaf volume in baking tests.

The effects of nitrogen fertilization on wheat quality have been extensively investigated (Guarda et al. 2004; Xue et al.

2019; Gagliardi et al. 2020; Song et al. 2020). In our study, a significant nitrogen effect was observed in several evaluated traits (Tables 2 and 4). Application of N fertilizer increased GY as well as quality parameters such as GPC, SDS sedimentation values, and the dough properties FAB, DDT, and STA. These results are consistent with previous findings (Guarda et al. 2004; Xue et al. 2019; Song et al. 2020). In contrast, negative effects were associated with the increased N level on baking properties such as Lfht, Tx, Soft, and CrCol. However, such effects varied among genotypes, once again suggesting differences in protein quality. Specifically, increased N fertilization had a negative impact on CWRS cultivar baking performance, while CWRS analog lines such as W07786 did not exhibit significant differences among N rates (Fig. 1). Studies of the wheat quality response to increased N level using several wheat cultivars have shown that N treatment increased not only GY, GPC, SDS, and gluten content but could have to changes in the grain protein composition increasing the glutenin/gliadin and HMW-GS/LMW-GS ratios, which has been shown to strongly influencing wheat quality performance (Xue et al. 2016; García-Molina and Barro 2017; Zhong et al. 2019; Song et al. 2020). Such effects have been reported to differ among genotypes and amount of nitrogen applied (Zhang et al. 2008; Shi et al. 2010). The gluten composition and ratio play a vital role in determining loaf volume and the appearance and crumb structure of cereal-based products (Goesaert et al. 2005; Delcour et al. 2012). Gliadins confer dough viscosity and extensibility, while glutenin is responsible for dough strength and elasticity (Wieser 2007). Particularly for loaf volume, a balanced glutenin/gliadin ratio is important to ensure higher volumes, since the dough needs to be sufficiently extensible to respond to gas pressure generated during the fermentation process but also strong enough to resist collapse (Fleitas et al. 2018).

The nature of the genotype-by-fertilizer rate interaction observed for most of the baking properties evalu-

ated in our study suggested that a possible increase in the glutenin/gliadin ratio in CWRS cultivars, associated with N supply, might have increased the dough tenacity and that insufficient gluten elasticity led to lower bread loaf volume. These results are in agreement with those reported by [Park et al. \(2014\)](#), who reported increased glutenin/gliadin and HMW-GS/LMW-GS ratios in bread wheat cultivars with greater N supply. [Ames et al. \(2003\)](#) also reported a negative nitrogen effect on gluten strength for durum wheat cultivars with stronger gluten properties. A very high glutenin/gliadin ratio can lead to extra strong (“bucky”) dough formation, which may prevent the expansion of gas cells, hence resulting in a reduced loaf volume ([Pechanek et al. 1997](#); [Riaz et al. 2019](#)). In contrast, the CWRS analog line W07786, exhibiting a significantly higher yield, was able to maintain a desirable quality performance across N levels, even with lower GPC. In addition, W07786 had a loaf height per unit protein significantly ($p = 0.05$) higher than CWRS cultivars. This line, like most of the CWRS analog lines tested, carries the *d* allele at the *Glu-A3* locus. The presence of this allele is associated with increased extensibility and elongation of the dough ([Branlard et al. 2001](#); [Park et al. 2011](#)) which would explain the high loaf volume even with increased dough tenacity (with high N supply). Suitable bread-making performance in lower GPC cultivars associated with greater dough elasticity has been previously reported ([Zörb et al. 2018](#); [Shewry et al. 2020](#)).

The development of improved cultivars exhibiting quality stability across a wide range of available N levels is a key requirement for wheat breeders and processors since it allows their use across many environmental conditions. This type of genotype could also contribute to improved nitrogen use efficiency, increased economic return, and reduced environmental pollution ([Belete et al. 2018](#)). The results obtained through the methodology used in this study demonstrated that lines with lower GPC values than current CWRS cultivars can exhibit analogous baking properties, even under widely contrasting nitrogen fertilization levels. However, as the quality parameters can be affected by the methodology used, future work using additional approaches is needed to confirm and validate our findings. As the CWRS analog line had grain yields up to 15% higher than CWRS cultivars, an expansion of the acceptable GPC lower limit for lines with an improved quality profile would facilitate a significant increase in the genetic gains for grain yield while maintaining end-use quality and result in greater competitiveness of the crop.

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Author information

Author notes

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Supplementary material

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