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Nutritional and quality characteristics expressed in 31 perennial wheat breeding lines

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Abstract

Soil erosion due to annual cropping on highly erodible farmland is a major ecological concern in the wheat growing regions of Washington State. In response to requests from farmers, the winter wheat breeding program at Washington State University has been developing perennial wheat selected from crosses between wild wheatgrass species and commonly grown annual wheat cultivars. In 2005/06, we conducted field trials of the most promising perennial wheat breeding lines derived from interspecific crosses between tall wheatgrass (*Thinopyrum elongatum*) and bread wheat (*Triticum aestivum*). Thirty-one perennial breeding lines and two annual winter wheat cultivars were evaluated for nutritional value in the form of grain mineral concentration, multiple baking and milling quality traits, and ease of grain threshability. The objective of this study was to identify the strengths and weaknesses of these post-harvest traits in the perennial wheat lines derived from these interspecific crosses. Mineral nutrient concentrations in the perennial lines were 44, 40, 24, 23, 32, 30 and 33% higher than the annual control cultivars for calcium, copper, iron, magnesium, manganese, phosphorus and zinc, respectively. The annual cultivars had a higher grain mineral content per unit area of land than the perennial lines, due primarily to the higher grain yields of the annual cultivars. Compared to the annual wheat cultivars, the perennial lines produced grain with smaller seed size, lower test weight and reduced flour yield, mix time and loaf volume. Protein content was 3.5–4.5% higher in the perennial lines than in the annual cultivars. The threshability index (TI) ranged from 0.63 to 0.89 in the perennials ($\mu = 0.75$); significantly lower than the mean TI of the annual cultivars ($\mu = 0.97$). The significant genotype × location interaction found for TI suggests that the variation in annual precipitation positively influenced some perennial lines to express greater threshability. In addition to transferring traits important to the perennial growth habit in wheat, the wild wheatgrass species also introduced beneficial characteristics (i.e. increased protein and mineral concentration) and deleterious traits (poor threshing grain and inferior baking qualities). This research gives researchers a platform from which to direct further research and selection in the development of perennial wheat.

Key words: perennial grains, wheat, agroecology, yield, mineral concentration, specialty crops, protein, threshability

Advances in cereal-based agricultural systems over the 40-year period from 1960 to 2000 helped to effectively reduce the global percentage of chronically malnourished people from 60 to 17%[1]. The elevated yields inherent in these high-input 'Green Revolution' systems also alleviated ecological land-use problems by reportedly keeping 1.2 billion hectares of largely fragile ecosystems out of agricultural production[1]. However, modern farming systems based on annual cropping systems are widely considered to be a contributing factor to multiple environmental problems, including the loss of genetic diversity and biological diversity, soil erosion and pollution from fertilizers and pesticides[2,3]. For example, coastal dead zones, exacerbated by runoff of nitrogenous fertilizers, have spread exponentially since the 1960s, the beginning of the Green Revolution era, and now total over 245,000 km^2 in area[3]. Additionally, water erosion alone causes approximately 31.5 t ha^{-1} of soil loss per year in wheat-based cropping systems in the Palouse region of Washington State[4]. Due to the potential ecological benefits of growing perennial-based cereals as a solution to these global agroecological problems, research into the development and agronomic production of perennial cereal crops is emerging across the US, China and Australia[5–7].

The large increases in the percentage of people suffering from micronutrient malnutrition over the past four decades coincide with the global expansion of high-yielding, input-responsive cereal cultivars[8,9]. Although grain yields have significantly increased post-Green Revolution, global food systems are not providing people with sufficient micronutrients[9–12]. Currently, over 40% of the world's population is micronutrient deficient; the dietary intake of iron (Fe) of more than two billion people worldwide is inadequate[13–17]. Notably, the mineral concentration of annual wheat varieties has shown a steady general decline over the past 50+ years[18].

Improving the nutritional value of wheat has potential to be realized through wide crosses utilizing wild wheatgrass species[19–22]. Although this study focuses on perennial wheat lines with *Thinopyrum elongatum* in the pedigree, multiple wild species in addition to *T. elongatum* can be used as parents in the development of perennial wheat. These species can be chosen based on chromosome number, and on traits including local adaptation, disease resistance, drought tolerance and growth habit[23]. Wild relatives of wheat confer beneficial characteristics to the development of perennial wheat; however, they can also be a source of negative traits, particularly those relating to end-use quality and grain threshability.

In addition to addressing the important agronomic traits of grain yield and regrowth after harvest, challenges inherent in the development of perennial wheat include selection of cultivars with enhanced nutritional value, acceptable baking and milling quality and free threshing grain. The objectives of this study were to evaluate grain concentration of calcium (Ca), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), phosphorus (P) and zinc (Zn); several end-use quality traits important to baking and milling properties; and grain threshability in 31 perennial wheat lines and two annual wheat cultivars in three locations in Washington State. Each location represents a potential target environment for perennial wheat due to excessive soil loss from wind and/or water erosion. We discuss aspects of the current state of perennial wheat development and identify key traits to target for genetic improvement.

Materials and Methods

Mineral and quality analyses

Mineral analyses of Ca, Cu, Fe, Mg, Mn, P and Zn were performed at the Grand Forks Human Nutrition Research Center in North Dakota using methods reported by Murphy et al.[18]. Mineral analyses were conducted on all genotypes at each location. End-use quality traits, including test weight, kernel hardness and whole wheat protein were measured on all perennial lines from each location post-harvest according to AACC Approved Methods 55-10, 55-31 and 46-30, respectively[24]. Quality traits including flour yield, flour ash, flour protein, mixograph absorption, mixing time and loaf volume of pan bread were determined on both annual wheat cultivars and on five randomly chosen perennial breeding lines from Ritzville and Pullman. Ash and protein content, mixograph absorption, mixing time and bread baking quality were determined according to AACC Approved Methods 08-01, 46-30, 54-40A and 10-10B, respectively[24]. Seed size and weight were measured on all lines in Kahlotus and Ritzville. Kernel hardness was tested on all genotypes at each location and test weight was evaluated at Ritzville and Pullman.

Experimental design

In this study, perennial is defined as a plant exhibiting post-sexual cycle regrowth for a minimum of two cycles[25]. All perennial lines used in this study were shown to be perennial in previous breeding trials. Thirty-one F_5 perennial wheat breeding lines were derived from a *T. elongatum*/Chinese Spring/Madsen population using a modified-bulk pedigree selection method. Genotypes were selected as single plants from a second year perennial bulk population grown in Pullman, WA from 2000 to 2002, and seed increased during the 2002–2003 and 2003–2004 field seasons. Selection was based on regrowth after harvest, winter survival after regrowth and subsequent seed set.

The 31 perennial genotypes were grown with two annual hard red winter wheat cultivars, 'Finley' and 'Bauermeister', at three rainfed locations in Washington State (Kahlotus, Ritzville and Pullman) in 2005/06 using a randomized complete block design with four replicates per location, as described in Murphy et al.[26]. Briefly, plots were 2.5 m long and 1.25 m wide and consisted of four rows at 30 cm spacing at Ritzville and Kahlotus and seven rows at 18 cm spacing at Pullman. Seeding rate was approximately 45 kg ha^{-1} at Ritzville and Kahlotus and 85 kg ha^{-1} at Pullman. Plots were fertilized with 46.0 kg ha^{-1} of N at Ritzville and Kahlotus. In Pullman, 100.8 kg ha^{-1} of N, 22.7 kg ha^{-1} of phosphate and 17.0 kg ha^{-1} of sulfur were incorporated into the soil within a week of planting. These fertilizer treatments reflect the locally prevalent application rates for annual wheat.

Plots were harvested at grain maturity with a Hege plot combine (Niederlassung, Germany) with stainless steel sieves and cleaned with a Hege seed cleaner with stainless steel sieves. A threshability index (TI) was estimated by dividing initial grain yield before cleaning by grain yield after a uniform cleaning process. The cleaning process removes seed with tenacious glumes that were still attached to the rachis while conserving only the threshed grain.

Analysis of variance in PROC GLM (SAS Institute, Cary, NC) was used to analyze data threshability and mineral concentration and to test for genotype × location interactions. Location and genotype were considered fixed.

Table 1. Grain yield (g plot^{-1}), regrowth (% regrowing plants plot^{-1}), threshability index (TI), thousand kernel weight (TKW) and mineral concentrations (μg g^{-1}) for calcium (Ca), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), phosphorus (P) and zinc (Zn) reported for annual wheat cultivars (A) and perennial breeding lines (P) across three locations in Washington State (Kahlotus, Ritzville and Pullman) in 2005/06. Trait mean and standard error (SE) are shown at the bottom of the table.

Entry	Habit	Yield	Regrowth	TI	TKW	Ca	Cu	Fe	Mg	Mn	P	Zn
Finley	A	1318	0	0.98	32.5	368	3.93	33.4	1185	32.5	2885	20.3
Bauermeister	A	1207	0	0.96	34.5	392	3.86	38.1	1264	36.3	2986	21.6
P-0001	P	602	42.9	0.67	27.8	719	7.04	48.4	1612	48.5	4300	30.7
P-0002	P	613	45.8	0.68	28.7	679	6.46	47.9	1562	53.4	4068	31.6
P-0003	P	573	35.8	0.70	31.3	689	6.57	49.5	1585	51.4	4291	30.6
P-0004	P	476	33.8	0.71	28.3	777	6.71	51.8	1695	50.2	4755	33.9
P-0005	P	528	35.8	0.66	30.8	740	6.88	49.6	1642	52.9	4433	31.5
P-0006	P	539	63.8	0.65	29.1	728	6.28	47.6	1562	53.5	4172	34.8
P-0007	P	495	62.1	0.72	29.4	746	6.80	47.5	1573	54.1	4244	31.5
P-0008	P	597	59.2	0.63	30.7	723	6.67	52.6	1690	55.1	4523	34.1
P-0009	P	469	45.4	0.89	26.4	600	6.16	42.6	1422	46.6	3604	28.2
P-0010	P	494	35.0	0.78	26.7	633	6.02	45.5	1540	46.9	3858	28.9
P-0011	P	588	41.3	0.82	27.8	684	7.21	49.5	1644	53.7	4378	35.0
P-0012	P	475	48.8	0.75	29.4	604	6.23	46.7	1584	51.1	4166	33.0
P-0013	P	590	47.9	0.67	25.4	638	5.53	42.0	1448	40.0	3907	28.0
P-0014	P	634	22.5	0.67	28.0	654	6.43	46.9	1625	50.7	4204	30.5
P-0015	P	500	42.9	0.70	29.9	683	6.26	47.5	1621	51.8	4372	33.1
P-0016	P	603	41.7	0.65	27.2	690	6.38	45.3	1609	51.0	4205	31.0
P-0017	P	639	59.6	0.79	31.3	624	6.32	47.2	1605	50.2	4343	29.1
P-0018	P	472	75.4	0.79	30.3	729	6.13	45.8	1645	49.7	4446	27.6
P-0019	P	791	40.0	0.81	27.2	699	6.20	46.9	1622	49.8	4254	28.5
P-0020	P	517	38.3	0.84	26.8	725	6.45	47.9	1552	50.3	4198	30.4
P-0021	P	549	49.2	0.84	27.6	683	6.48	46.0	1565	49.8	4214	31.4
P-0022	P	457	51.3	0.70	30.3	656	6.48	46.1	1613	52.9	4228	30.4
P-0023	P	625	30.8	0.77	26.0	703	6.75	46.1	1607	54.8	4266	34.5
P-0024	P	452	52.5	0.79	27.8	718	6.44	46.5	1603	49.1	4223	31.4
P-0025	P	509	22.1	0.74	28.0	655	6.64	45.8	1601	53.3	4229	32.0
P-0026	P	523	25.4	0.87	28.7	642	6.12	42.1	1499	46.6	3993	30.4
P-0027	P	623	51.7	0.86	27.7	696	6.42	45.4	1618	50.3	4393	31.2
P-0028	P	520	49.6	0.83	26.1	613	6.66	44.8	1600	51.3	4113	32.4
P-0029	P	469	48.3	0.76	27.7	731	7.35	48.8	1690	55.9	4424	34.0
P-0030	P	578	41.7	0.72	26.2	716	6.68	47.8	1582	52.0	4117	31.2
P-0031	P	602	39.6	0.70	27.6	630	6.30	44.1	1496	50.4	3821	26.7
Mean (A)	A	1262	0	0.97	33.5	380	3.90	35.8	1224	34.4	2936	20.9
SE (A)	A	56	0	0.01	1.0	12	0.04	2.4	40	1.9	51	0.6
Mean (P)	P	552	44.5	0.75	28.3	684	6.49	46.8	1591	50.9	4218	31.2
SE (P)	P	13	2.2	0.01	0.3	8	0.06	0.4	11	0.6	40	0.4

Levene's test was used to test for homogeneity of variance across locations and normality was checked using the Shapiro–Wilk test in PROC Univariate (SAS Institute).

Fifteen soil subsamples were randomly collected to a depth of 30.5 cm and pooled for analysis at each location. Soil organic matter, pH, and available Cu, Fe, Mn, P and N were determined by the University of Idaho analytical soil testing laboratory.

Results

Grain mineral concentration

Micronutrient concentrations for Cu, Fe, Mn and Zn were 40, 24, 32 and 33% higher, respectively, in the perennial grain than in the annual grain (Table 1). Macronutrient concentration followed the same pattern, with Ca, Mg and P being 44, 23 and 30% higher, respectively, in the perennial lines (Table 1). Differences were found among genotypes for all minerals tested ($P < 0.001$).

Soil organic matter was 1.6, 2.0 and 2.6 for Kahlotus, Ritzville and Pullman, respectively. Soil pH was 5.5% for Kahlotus and Ritzville and 5.1% for Pullman. Cation exchange capacity was 14, 15 and 29 cmol(+) kg^{-1} respectively for Kahlotus, Ritzville and Pullman. Available soil mineral concentrations of Cu, Fe, Mn, N (nitrate + nitrite), N (ammonia) and P for each location are shown in Figure 1. Available potassium (K) was 650, 720 and 190 μg g^{-1} for Kahlotus, Ritzville and Pullman, respectively.

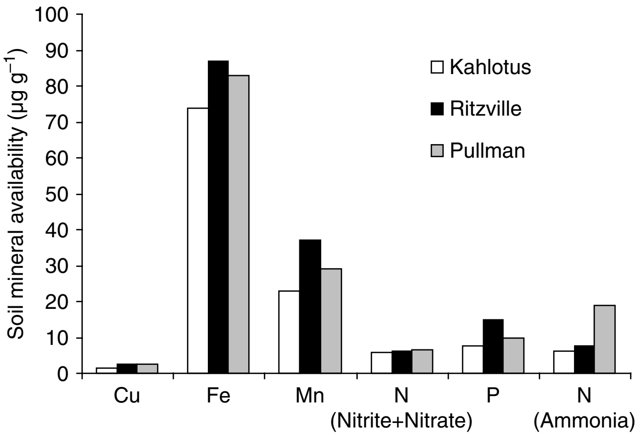

Figure 1. Soil bioavailability of copper (Cu), iron (Fe), manganese (Mn), nitrogen (N) with nitrites + nitrates, phosphorus and N in the form of ammonia at three locations (Kahlotus, Ritzville and Pullman) in Washington State.

Location had a significant effect on grain mineral concentration for all minerals tested. The perennial grain from Kahlotus had the highest level of all mineral nutrients except Mn (Fig. 2). Soil available Mn was accordingly lowest at this location (Fig. 1). All minerals were moderately to highly correlated with each other ($P < 0.05$) (Table 2).

Trait means across locations for each genotype are reported for grain yield, regrowth, TI, thousand kernel weight (TKW) and the seven minerals tested (Table 1). To estimate total mineral content of Cu, Fe and Zn per unit area for each genotype, we multiplied mineral concentration (µg g^{-1}) by grain yield per plot (g plot^{-1}), and divided by 1000. The annual cultivars had a mean of 4.92 g plot^{-1} Cu (range = 4.65–5.18), 45.0 g plot^{-1} Fe (range = 44.1–46.0), and 26.38 g plot^{-1} Zn (range = 26.02–26.73). The perennials had a mean of 3.58 g plot^{-1} Cu (range = 2.89–4.90), 25.9 g plot^{-1} Fe (range 20.0–37.1) and 17.19 g plot^{-1} Zn (range = 13.05–22.56).

Grain yield per plot was negatively correlated with grain mineral concentration for all minerals when the annual cultivars were included in the analysis (Table 2). When the correlation analysis included only the perennial breeding lines, no associations were found between grain yield and mineral concentration for any minerals (Table 2). When annuals were included in the analysis, TKW was negatively correlated with Ca, Cu and Zn; however, in the absence of the annual cultivars, TKW was positively associated with Mg, Mn and P (Table 2).

End-use quality

The perennial lines produced smaller and lighter kernels than the annual cultivars, resulting in decreased test weight (Tables 3 and 4). The perennial lines generally exhibited lower flour yield, loaf volume and mix time; greater whole wheat protein and flour protein content; and, similar ash content of flour compared to the annual cultivars (Table 3). Kernel hardness of the perennial lines was 47.8% lower than what is typically observed in hard wheat, while protein content of the perennial lines was 3.5–4.5% greater than the annual hard wheat cultivars (Table 4).

Whole wheat protein content ranged from 14.0 to 16.5% in Pullman and 15.2 to 17.6% in Ritzville for the perennial lines. For the annual cultivars, 'Bauermeister' had 11.6 and 12.7% whole wheat protein in Pullman and Ritzville, respectively, and 'Finley' had 12.4 and 12.7% whole wheat protein at the respective locations (Table 3). The annual hard red cultivars had greater kernel hardness, seed weight and seed size than the perennial lines (Table 4).

Threshability

The mean TI in the annual cultivars was 0.97. This was considerably higher than the perennial lines, which ranged from 0.63 to 0.89, with a mean of 0.75 (Table 1). A TI of 1.00 would indicate that all the glumes had been separated from the grain during harvest. A TI of 0.0 would indicate that the grain had very tenacious glumes and the seed would not separate from these glumes through standard mechanical harvest. A significant genotype × location interaction was found for TI ($P < 0.0001$). TI was positively

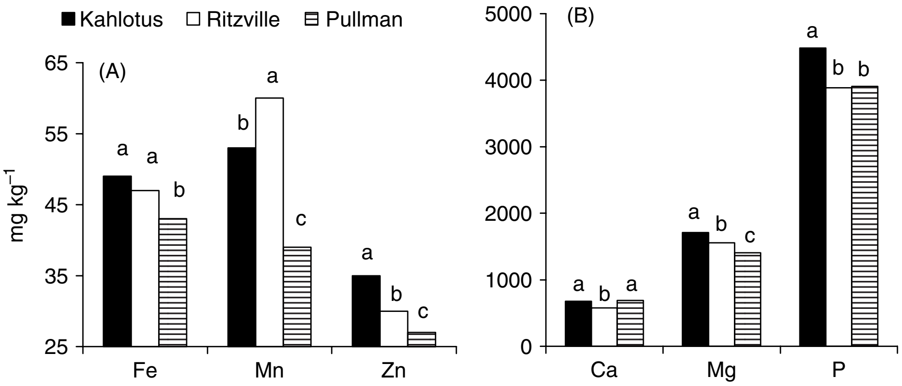

Figure 2. Effect of location (Kahlotus, Ritzville and Pullman) on micronutrient (A) and macronutrient (B) concentration (mg kg^{-1} dry weight) in perennial grain grown in Washington State. Lower-case letters above the bars indicate significant differences ($P < 0.05$) among locations for each nutrient tested.

Table 2. Correlations between grain yield, regrowth after harvest, threshability index (TI), thousand kernel weight (TKW) and mineral concentrations of calcium (Ca), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), phosphorus (P) and zinc (Zn) among annual and perennial breeding lines. Correlations below the leading diagonal include the annual wheat cultivars; correlations above the leading diagonal represent the perennial breeding lines only. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

	Yield	Regrowth	TI	TKW	Ca	Cu	Fe	Mg	Mn	P	Zn
Yield		−0.18	−0.12	−0.14	−0.03	−0.07	0.04	0.05	−0.02	0.02	−0.16
Regrowth	−0.67***		−0.03	0.32	0.18	−0.10	0.08	0.10	0.08	0.16	−0.04
TI	0.52**	−0.43*		−0.34	−0.30	−0.09	−0.43*	−0.26	−0.23	−0.24	−0.18
TKW	0.52**	−0.24	0.16		0.18	0.12	0.47	0.39*	0.37*	0.48**	0.11
Ca	−0.80***	0.65***	−0.64***	−0.45**		0.49**	0.67***	0.59***	0.41*	0.70***	0.40*
Cu	−0.82***	0.56***	−0.56***	−0.50**	0.87***		0.64***	0.63***	0.73***	0.53**	0.61***
Fe	−0.68***	0.54**	−0.67***	−0.20	0.86***	0.85***		0.77***	0.63***	0.77***	0.58***
Mg	−0.75***	0.60***	−0.62***	−0.32	0.88***	0.89***	0.91***		0.65***	0.91***	0.56*
Mn	−0.74***	0.58***	−0.59***	−0.31	0.81***	0.91***	0.85***	0.88***		0.51**	0.66***
P	−0.75***	0.62***	−0.61***	−0.29	0.91***	0.86***	0.90***	0.97***	0.83***		0.55**
Zn	−0.74***	0.50**	−0.55***	−0.41*	0.79***	0.86***	0.82***	0.84***	0.87***	0.83***	

Table 3. End-use quality characteristics including test weight (Twt), single kernel hardness (SKHard), whole wheat protein (UWprt), flour yield (FYeld), flour ash (Fl Ash), flour protein (FProt), mixture absorption (MAbs), mix time (MTime) and loaf volume (LVol), measured for the two annual wheat cultivars ('Bauermeister' and 'Finley') and four perennial breeding lines in two locations (Pullman and Ritzville).

Variety	Location	Twt	SKHard	UWPrt	FYeld	Fl Ash	FProt	MAbs	MTime	LVol
Bauermeister	Pullman	60.2	70.3 ± 17.8	11.6	71.5	0.43	10.0	59.0	4.0	745
	Ritzville	60.1	75.9 ± 13.6	12.7	72.8	0.43	11.7	60.7	3.9	925
Finley	Pullman	62.7	71.2 ± 16.8	12.4	72.7	0.38	11.5	61.3	3.3	925
	Ritzville	62.9	74.3 ± 13.8	12.7	75.9	0.42	12.0	61.4	2.2	930
PF4R1-0009	Pullman	56.0	71.0 ± 20.2	14.0	62.1	0.46	12.3	57.9	3.7	755
	Ritzville	54.3	54.0 ± 17.0	15.2	61.8	0.48	13.6	60.3	2.8	820
PF4R1-0014	Pullman	56.3	50.1 ± 19.7	14.9	57.3	0.44	12.8	57.2	1.5	765
	Ritzville	55.4	38.6 ± 14.9	17.0	58.8	0.45	15.1	61.0	1.8	810
PF4R1-0019	Pullman	57.9	61.0 ± 18.1	14.8	58.9	0.39	12.2	56.3	2.2	775
	Ritzville	57.4	49.0 ± 16.0	16.2	59.2	0.40	13.9	58.3	1.2	795
PF4R1-0021	Pullman	54.6	45.0 ± 20.0	16.5	59.6	0.43	13.2	56.4	1.3	765
	Ritzville	55.7	35.1 ± 16.6	17.6	60.1	0.45	15.6	61.7	1.5	790
PF3R1-0030	Pullman	55.5	52.9 ± 19.6	15.2	59.8	0.42	13.3	56.4	1.7	705
	Ritzville	54.2	38.1 ± 18.6	17.3	62.0	0.45	15.5	60.4	1.4	760

Table 4. Quality characteristics, including test weight, percent whole wheat protein, single kernel hardness, thousand kernel seed weight (g) and single kernel seed size (mm), were compared between annual wheat cultivars and perennial breeding lines grown at three locations in Washington State (na = data unavailable for these tests in respective location).

Location	Growth habit	Test weight	Protein	Kernel hardness	Seed weight	Seed size
Kahlotus	Annual	na	14.5	70.9 ± 13.8	38.5 ± 7.5	2.55 ± 0.40
	Perennial	na	18.7	45.9 ± 17.3	25.4 ± 5.6	1.72 ± 0.34
Ritzville	Annual	61.5	12.7	75.1 ± 13.7	36.5 ± 7.9	2.50 ± 0.40
	Perennial	55.8	17.2	40.4 ± 16.2	26.4 ± 5.1	1.76 ± 0.33
Pullman	Annual	61.5	12	70.8 ± 17.3	na	na
	Perennial	56	15.5	47.8 ± 19.2	na	na

associated with yield and negatively correlated with regrowth when the annual cultivars were included in the analysis (Table 2). No phenotypic associations were found between either TI and yield or TI and regrowth when only the perennial lines were included in the analysis (Table 2).

Discussion

Grain mineral concentration

Cereal crops have the potential to provide a significant increase in the overall micronutrient availability for much of the world's population without access to diverse food crops[27]. The increased mineral nutrient concentration in the perennial grain is most likely derived from wheatgrass species *T. elongatum*. Accordingly, Uauy et al.[20] found increased Fe and Zn concentrations in synthetic hexaploids developed from tetraploid durum and the diploid wheat ancestor, *Aegilops tauchii*. The elevated concentrations of micronutrients in the perennial grain may be a result of improved scavenging ability of the larger roots of the perennial lines[28]; however, the total mineral content per unit area of cultivated land is significantly lower in the perennial grain than in the annual grain. This is predominantly due to the much higher grain yield in the annual cultivars.

Another reason for the enhanced micronutrient concentration in the perennial grain may be that the smaller grain size is directly related to the increased mineral concentration. This is known as the 'dilution effect'. The mineral concentration of the grain would decrease if a corresponding increase in grain size was due only to an increase in the endosperm and not the bran or germ, where the majority of the minerals are located. We found significant differences in TKW among the perennial lines (Table 1), and TKW was positively correlated with mineral concentration for Mg, Mn and P (Table 2). No relationship was found between TKW and the mineral concentrations of Ca, Cu, Fe or Zn among the perennial lines (Table 1). When the annual cultivars were included in the correlation analysis, however, TKW was negatively associated with Ca, Cu and Zn (Table 2).

This provides contradictory evidence regarding the dilution effect theory and is in part based on the issue of whether comparing the grain composition between annuals and perennials is an appropriate test of the dilution effect theory. For example, perennial and annual wheat should likely be considered two different crop species (much the same as wheat and triticale), as they differ in chromosome number and designation. Therefore, correlations among the perennial lines only (annuals excluded) may provide the most accurate account of the actual relationships between TKW and mineral concentration. Future research is needed to fully understand the roles that scavenging ability and the dilution effect play in achieving perennial grain with enhanced mineral nutrition and improved grain yield.

Grain mineral concentration is dependent to a great extent upon various soil properties, including soil organic matter, pH and the bioavailability of minerals in the soil[29–31]. Soils with a low pH have been shown to reduce uptake of the macronutrients Ca and Mg and to increase uptake of the micronutrients Zn, Mn and Fe[32]. The lack of a positive correlation between soil nutrient availability and grain nutrient concentration across all cultivars indicates that other environmental factors such as drought may have contributed to higher grain nutrient concentrations. In soybeans, plant stress induced by drought resulted in an increase in the accumulation of P, K, Ca, Mo, Mn, Cu and Zn, and may be an important response in drought stress tolerance[33]. The more drought-prone environments represented by Kahlotus and Ritzville generally showed higher levels of micronutrients in the grain than Pullman, the region with higher annual precipitation (Fig. 2).

End-use quality

Despite the much higher protein content compared to the annual cultivars, the perennial lines produced a smaller loaf volume of bread (Tables 3 and 4), indicating the weak protein strength and inferior gluten quality of these perennial wheat lines for baking bread. P-0021 had the highest whole wheat protein among the two annual cultivars and four perennial lines tested in Ritzville and Pullman (Table 3). Though the protein is not of bread baking quality, its enhanced concentration in the grain could have a significant dietary impact for human and livestock consumption. High protein livestock feed is important for many animals and perennial wheat could contribute to local production of high protein grain to complement mixed legume species in regions where soybeans are unable to grow, expensive to import or unavailable.

With the exception of one line (P-0009), most of the perennial lines showed similar water absorption and shorter mixing time in comparison to the annual cultivars. The relatively low flour yield found in the perennial lines was probably due to the low test weight and smaller kernels of the grain. These traits are important for baking quality and the variation among the perennial lines for these traits indicates the potential for genetic improvement.

Further improvements in grain characteristics, milling quality and protein strength of the perennial lines are needed before acceptable utilization as hard red hard wheat for baking is possible. The potential for perennial wheat in the soft white market class, however, is greater. Soft white wheat is the predominant market class in the Pacific Northwest, and is used for pastries, white Asian noodles, cookies, cereal flakes and flatbreads. In general, the quality of these products does not depend on protein strength and typical bread making characteristics like gluten quality and loaf volume. With this marketing option in mind, we have shifted a percentage of the perennial wheat breeding priorities to the selection of perennial lines with potential in the soft white market class.

Threshability and seed shattering

Thinopyrum species have tenacious glumes, a trait controlled by recessive mutations at the *Tg* loci, dominant modifying genes at the Q locus and additional modifying mutations at several other loci[34,35]. These tenacious glumes were transferred to a portion of the perennial progeny, making mechanical harvest less efficient. Though early domesticated wheat, with non-shattering, indehiscent spikes, have been found dating back to ~9250 years BP[36], the wild wheatgrass species we use in our perennial wheat breeding program have typically not been subject to artificial selection for either tenacious glumes or indehiscent spikes. Therefore, we anticipate selection for these traits to be of importance both during pre-breeding of wild species and selection of perennial wheat hybrids.

The TI range of 0.63–0.89 in the perennial lines indicates that the genes for threshability are segregating, and the population as a whole is tending to improve for this trait. Unlike selection for nutritional value and end-use quality traits, selection for increased threshability has potential for further improvement through the use of evolutionary breeding methods[37,38]. The significant genotype × location interaction for TI found in this study may be due to the environmental effect of abundant moisture at Pullman which reduced threshability.

We observed that plants with shorter spikes did not have the brittle rachis trait that we have found in many wild wheatgrass species and in perennial wheat hybrids with longer spikes (unpublished data). In the tetraploid emmer wheat, shattering is determined by the *Br* (brittle rachis) loci on chromosomes 3A and 3B[34,39]. In rice, Li et al.[40] showed that a single amino acid change is primarily responsible for the loss of shattering. The cultivated allele of the *sh4* (shattering4) gene confers both a non-shattering trait and allows for easy threshability of the grain. This suggests the potential for a rapid removal of the shattering trait from the perennial wheat genome. The brittle rachis trait, though possibly exploited in the Neolithic era bridging the hunter/gatherer era and the dawn of agriculture through ground gathering of shattered cereal spikelets[41], is a characteristic that does not conform to efficiencies needed for harvesting in modern agriculture.

Future Research in Perennial Wheat

Though the ability to transfer genes from perennial wild wheat species that confer the post-harvest regrowth trait is elegantly simple, there exist additional characteristics necessary for the successful establishment of perennial wheat[25]. These agronomic traits can vary by region and by agroecosystem and include disease resistance; drought, heat and cold tolerance; winter and/or summer dormancy; regrowth vigor and timing; and carbohydrate allocations in the root and crown[42–44].

This study focused on the post-harvest characteristics of mineral concentration, end-use quality and grain threshability. In addition to multiple agronomic traits, baking and milling quality and grain threshability must be improved before widespread adoption of perennial wheat by farmers, millers and bakers will occur. Though not a requirement for the successful development of a perennial wheat cultivar, increased grain mineral concentration is a reportedly beneficial trait currently found in perennial wheat breeding lines that should continue to be exploited and selected for in subsequent perennial wheat breeding evaluations.

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