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Evaluation of environment and cultivar impact on lentil protein, starch, mineral nutrients, and yield

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Abstract

Lentil (*Lens culinaris* Medik.) is an important source of protein, starch, and mineral nutrients in many parts of the world. However, the impact of environment and cultivar on the enrichment of these nutrients is not well understood. Four lentil cultivars ('Avondale', 'CDC Richlea', 'CDC Maxim', and 'CDC Imvincible') varying in color, seed size, and maturity were evaluated at five Montana locations with diverse climatic and soil conditions over 3 yr. Significant cultivar, location, and year effects were found for yield, protein, starch, and minerals. Grain protein concentration was the highest at Moccasin (262 g kg^{-1}) and lowest at Richland (246 g kg^{-1}), whereas starch concentration was the highest at Richland (455 g kg^{-1}) and lowest at Moccasin (441 g kg^{-1}). Among cultivars, Avondale was the top yielding cultivar (1965 kg ha^{-1}) and adaptable to most of the environments; CDC Imvincible was the top protein producer (265 g kg^{-1}); and CDC Richlea is the leading starch producer (456 g kg^{-1}). Grain protein concentration was negatively correlated with starch. Lentil grains varied in nutrient concentrations across locations, with the northcentral Montana region producing 10- to 20-times greater selenium concentration than other locations. CDC Maxim had the highest iron (62.1 mg kg^{-1}) and zinc (31.5 mg kg^{-1}) concentrations. Seed protein concentration was positively correlated with phosphorus, sulfur, copper, and boron. Seed starch is positively correlated with magnesium and manganese. Results suggest that plant breeding and production site selection could enrich lentil nutrient concentrations to help combat malnutrition in the world.

Abbreviations: DM, dry matter; GGE, genotype main effect and genotype × environment biplot.

1 | INTRODUCTION

Lentil (*Lens culinaris* Medik), a member of the *Leguminosae* family, is an important food component in most parts of the world (Faris et al., 2013). It is among the earliest domesticated plant species from the Middle East (Cokkizgin & Shtaya, 2013), having been cultivated for 10,000 yr (Rizvi et al., 2019). Lentil is relatively drought-tolerant (Cokkizgin & Shtaya, 2013; Joshi et al., 2017) and has an important role in human and animal diets as well as in sustaining agricultural production systems. Canada is the top lentil producer with a total production of 2.17 million metric tons, followed by India (1.23 million tons), Australia (0.53 million tons), Turkey (0.35 million tons), and the USA (0.25 million tons) in 2018 (FAOSTAT, 2019).

Lentil has become an important rotational crop for wheat (*Triticum aestivum* L.) production systems in dryland regions such as the Northern Great Plains of the United States, facilitating the movement away from fallow to cereal–legume annual cropping (Chen et al., 2012). Inclusion of lentil makes grass weed control easier, breaks disease cycles, improves water use efficiency and enhances yield (Chen et al., 2012; Miller et al., 2015; Peterson et al., 1996; Sileshi, et al., 2011), as well as offers the opportunity for improved economic returns (Tanaka et al., 2005). Incorporation of lentil and other annual legumes also reduces nitrogen fertilizer needs (Reckling et al., 2016; Stagnari et al., 2017), thereby reducing energy input because nitrogen (N) is the biggest energy input in crop production (Afshar & Chen, 2015; Afshar et al., 2015). These benefits partially explain the increase in lentil production in Montana from 60,703 ha in 2005 to 202,343 ha in 2018 (USDA-NASS, 2019).

Lentil is an excellent source of dietary protein and starch (Joshi et al., 2017; Rathod & Annapure, 2016) and is a primary protein source in many parts of the world including the Middle East and India (Khazaei et al., 2019). Research has shown that plant-based protein diets are associated with health benefits (Chauveau & Lasseur, 2019; Kahleova et al., 2018; Krajcovicova-Kudlackova et al., 2005). The demand for human consumption of plant-based protein will continue to grow in the future (Pihlanto et al., 2017). Previous studies demonstrated that protein concentration in lentil varies by cultivar (Ghumman et al., 2016; Ninou et al., 2019; Nosworthy et al., 2018; Wang & Daun, 2006), climate (Erskine et al., 1985; Fatima et al., 2018; Sharaan et al., 2003), soil (Huang et al., 2016; Zeidan, 2007), and agricultural practices (Gill, 2013; Mandal et al., 2018). Wang and Daun (2006) found that protein concentration in lentil varied between 243 and 302 g kg^{-1} dry matter (DM), whereas in another study protein concentration ranged from 252 to 293 g kg^{-1} DM (Wang et al., 2008). Lentil is also an excellent source of complex carbohydrates (Siva et al., 2019), and a good source of dietary fiber which is a valuable component of a healthy diet (de Almeida Costa et al., 2006; Phillips, 1993). Starch content in lentil varies between 350 and 650 g kg^{-1} (Jood et al., 1998; Wang & Daun, 2004).

Core Ideas

- Lentil grain produced at different locations showed significant differences in nutrient concentrations.
- Different cultivars showed different capacities of enriching protein and minerals.
- Protein is negatively correlated with starch and positively correlated with certain mineral elements.
- Plant breeding and production site selection could enhance lentil nutrient concentrations.

Furthermore, lentil is a good source of mineral nutrients (Vandemark et al., 2018). Mineral nutrients are important to human health and mineral malnutrition is a major global health problem (Zafar et al., 2018; Dewan, 2016). Enhancing mineral content in food grains has received increased attention and can contribute to food and nutrition security in developing countries (Mutuku et al., 2020; Faris, 2013; Thavarajah et al., 2011; Vandemark et al., 2018). Iron (Fe) is an important part of hemoglobin, a protein that carries oxygen to body tissues. Globally, an estimated 50% of anemia cases are likely attributed to Fe deficiency (Stoltzfus, 2003), which makes the body more susceptible to other diseases (Hassan et al., 2016). About 33% of the world's population suffers from zinc (Zn) deficiency which is characterized by growth retardation and impaired immune function (Prasad, 2004). Selenium (Se) is another important micronutrient that is linked to cancer prevention (National Academy of Sciences, 2004). Grains grown at eight locations in Saskatchewan, Canada varied in total Fe and Zn concentrations among 19 lentil genotypes, with estimated broad-sense heritability of 64 and 68% for Fe and Zn, respectively (Thavarajah et al., 2009). Significant cultivar and location variations were observed in analyzing the micronutrient content of lentil and other pulse crops (Ray et al., 2014). Vandemark et al. (2018) found location, year, and their interactions with genotype had greater impacts than genotype on mineral concentrations of chickpea (*Cicer arietinum*) and lentil in the U.S. Pacific Northwest.

The objective of this study was to evaluate nutrient concentrations of lentil grains, including protein, starch, macro- and micronutrients, as they are influenced by cultivars and geographical locations in the Northern Great Plains where the majority of U.S. lentils are produced. Although mineral concentrations of selected lentil grains grown in the U.S. Pacific Northwest and in Saskatchewan, Canada have been reported (Ray et al., 2014; Vandemark et al., 2018), the nutrient levels of lentil grains grown in the U.S. Northern Great Plains have not been studied. More importantly, there is little research to link lentil yield, protein, and starch to mineral concentrations. Information on cultivar selection and production site identification for consistent production of lentil grains with high protein and mineral concentrations is needed for growers in the major lentil producting regions. The broad-sense heritability data based on multiple locations and years provided an indication of potential plant breeding for selecting nutrient enriched traits.

2 | MATERIALS & METHODS

2.1 | Site description

A lentil cultivar trial was executed at five locations across Montana: four Montana State University Agricultural Research Centers located in Bozeman (45.645933 lat, −111.069232 long, elevation 1364 m), Conrad (48.308599 lat, −111.924749 long, elevation 1068 m), Havre (48.499032 lat, −109.807326 long, elevation 756 m), and Moccasin (47.059779 lat, −109.952239 long, elevation 1251 m), and an on-farm trial at Richland (48.728142 lat, −106.062768 long, elevation 819) in Valley County, MT, in 2017, 2018, and 2019. Details for each site including temperature and selected agronomic practices are summarized in Table 1. Climate data were obtained from the on-site weather station at each Agricultural Research Center and from the nearest weather station for the on-farm location (29 km from the on-farm site).

The locations varied in terms of soil and weather conditions. The soils are Amsterdam silt loam in Bozeman, Telstad loam and Hillon clay loam in Havre, Scobey-Kevin clay loam in Conrad, Danvers-Judith clay loam in Moccasin, and Farnuf-Reeder loam in Richland. Year-to-year seasonal variation in precipitation was substantial not only among locations but also within a location over time (Figure 1). For example, Havre received 57 mm in 2017 but 115 mm in 2018, compared with Moccasin which received 198 mm in 2017 and 290 mm in 2018, respectively. Overall, average precipitation was lower in Havre and was higher in Moccasin than other locations, respectively (Figure. 1). The mean air temperature also varied among the locations and among the years within a location, which affected planting date and likely plant growth (Table 1).

2.2 | Experimental design and field management

All field experiments were arranged in a randomized complete block with four replications. Plot sizes ranged from 5 to 9 m^2, with a row spacing of 18 to 30 cm, based on the planter configuration at different locations. Four cultivars from a larger lentil adaptation trial were selected in the current study on the basis of seed color and size: 'Avondale' (green, medium seed size), 'CDC Richlea' (green, medium seed size), 'CDC Imvincible' (green, small seed size), and 'CDC Maxim' (red, small seed size). Seed sizes of 60 mg $seed^{-1}$ and above are considered large, 45–60 mg $seed^{-1}$ as medium, 34–45 mg $seed^{-1}$ as small, and less than 34 mg $seed^{-1}$ as extra small (Barker, 2020). All four cultivars were planted at each of the five locations in 2017, 2018, and 2019.

Seeds were treated with Obvius Fungicide (BASF Corporation) at a rate of 0.18 g a.i. kg^{-1} seed and Cruiser 5FS Insecticide (Syngenta Crop Protection) at a rate of 0.5 g a.i. kg^{-1} seed prior to planting. Rhizobial inoculant (Primo GX2, Verdesian Life Sciences) was mixed with seed at planting with the manufacturer recommended rate. Seeds were planted at 4 cm depth at a rate of 129 seeds m^{-2}. Lentils were planted following wheat, barley (*Hordeum vulgare* L.), alfalfa (*Medicago sativa* L.), or chemical fallow, depending on the location and year. Planting and harvesting dates are provided in Table 1. Lentil grains were harvested using a Wintersteiger Classic plot combine (Wintersteiger) at maturity.

2.3 | Analysis of seed protein, starch, and minerals

Lentil grain samples (40 g) were uniformly milled using a UDY cyclone sample mill (UDY Corporation) to pass through a 0.5-mm screen. Moisture content of the seed flours was determined gravimetrically by weighing 1.0-g samples prior to and following a 48-h oven-drying at 65 ˚C (Jones, 1990). All subsequent results for seed protein and starch concentrations are presented on a DM basis. Total seed N was measured on 2-mg seed flour samples by the Pregl-Dumas method on a Perkin Elmer 2400 Series II CHNS/O Elemental Analyzer (PerkinElmer) in CHN mode using acetanilide as the standard. Seed protein concentration was calculated by multiplying the total grain N concentration to a N-to-protein conversion factor of 6.25. Grain starch concentration was determined using the Megazyme Total Starch Assay Kit (AA/AMG; Megazyme) based on the Rapid Total Starch (RTS) method. Assays were performed using 100 mg of each grain flour and absorbance was measured at a 510-nm wavelength using a UV-1800 Spectrophotometer (Shimadzu Corporation). Mineral nutrients were analyzed by inductively coupled plasma (ICP) method in a commercial lab (Agvise Laboratories, Northwood, ND).

2.4 | Statistical analysis

Data were subjected to analysis of variance (ANOVA) using a mixed model using SAS software (SAS Institute., 2009)

TABLE 1 Summary of mean temperatures and agronomic history at each location in 2017, 2018 and 2019

Location[a]	Year	Mean temperature Apr.	May	June	July	Aug.	Sept.	$N–P_2O_5–K_2O$	Previous crop[b]	Seeding date	Harvest date
		°C						kg ha^{-1}			
Bozeman	2017	6.6	11.5	15.9	21.8	19.4	13.1	–	F	30 Apr.	9 Aug.
	2018	5.4	13.3	15.2	19.2	18.0	13.2	–	B	27 Apr.	29 Aug.
	2019	6.1	9.5	15.1	18.2	18.8	14.4	–	SW	7 May	3 Sept.
Conrad	2017	5.1	12.0	16.0	22.0	19.0	9.6	12–22–22	F	5 Apr.	3 Aug.
	2018	1.9	13.8	14.9	19.2	17.2	13.3	11–22–20	F	30 Apr.	7 Aug.
	2019	4.9	8.3	14.9	17.8	17.8	9.0	0–22–22	NA	23 Apr.	27 Aug.
Havre	2017	6.9	12.4	17.7	23.1	19.6	14.2	–	WW	7 Apr.	18 July
	2018	2.2	14.9	17.4	20.8	19.5	12.3	–	F,B	29 Apr.	15 Aug.
	2019	7.0	9.7	16.3	19.7	19.6	13.4	–	F	16 Apr.	1 Aug.
Moccasin	2017	5.7	11.3	15.5	22.0	18.8	13.3	–	B	19 Apr.	26 July
	2018	3.0	12.8	14.7	18.6	18.7	12.8	22–34–22	FB	9 May	7 Sept.
	2019	5.4	8.1	14.3	18.0	18.0	13.3	11–17–11	Alf/G	19 Apr.	15 Aug.
Richland	2017	2.8	9.1	13.5	19.7	15.6	10.7	–	F	26 Apr.	8 Aug.
	2018	-1.6	11.8	14.4	17.0	15.9	7.7	–	C	8 May	16 Aug.
	2019	3.3	7.3	13.8	17.4	15.0	10.1	–	SW	29 May	21 Aug.

[a] Weather Station closest to Richland field site: OPHEIM 12 SSE, MT USA USC00246238.

[b] Alf/G, alfalfa/grass; B, barley; C, canola; F, fallow; FB, forage barley; SW, spring wheat; WW, winter wheat; NA, not available.

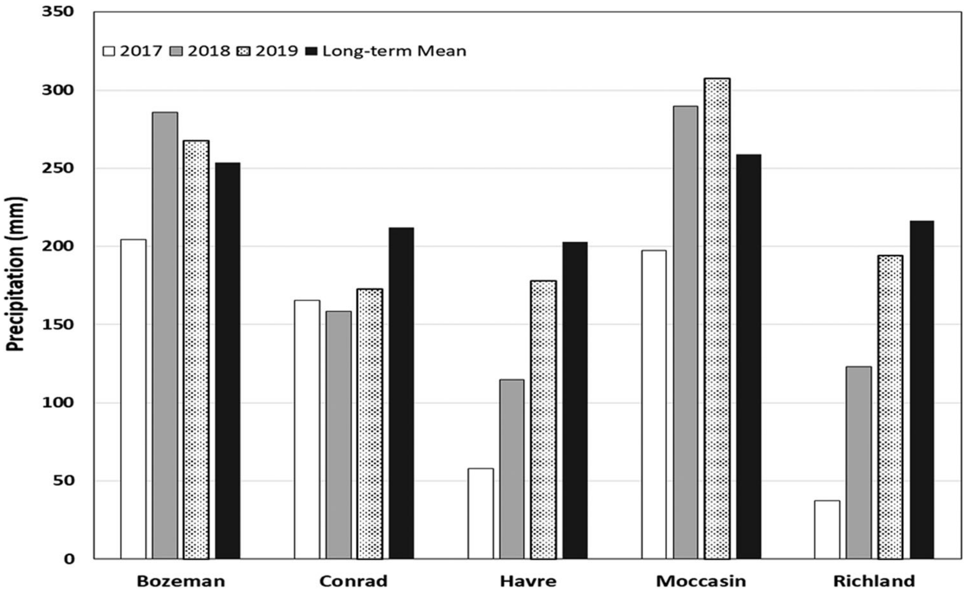

FIGURE 1 Cumulative in-season precipitation from April to August from 2017 to 2019 and long-term means (Apr.–Aug.) at the different locations in Montana

TABLE 2 Environments and cultivars used in the multi-environment evaluations for yield, protein, starch, and minerals

Environment	Code	Year	Location	Cultivar	Code	Name
1	E1	2019	Bozeman	1	G1	Avondale
2	E2	2019	Conrad	2	G2	CDC Imvincible
3	E3	2019	Havre	3	G3	CDC Maxim
4	E4	2019	Moccasin	4	G4	CDC Richlea
5	E5	2019	Richland			
6	E6	2018	Bozeman			
7	E7	2018	Conrad			
8	E8	2018	Havre			
9	E9	2018	Moccasin			
10	E10	2018	Richland			
11	E11	2017	Bozeman			
12	E12	2017	Conrad			
13	E13	2017	Havre			
14	E14	2017	Moccasin			
15	E15	2017	Richland			

for a randomized complete block design (RCBD) considering block as random effect, and year, cultivar, and location as fixed effects. Means were compared using protected Fisher's least significant difference (LSD) test at $P \leq .05$. To explore genotype × environment (G × E) interactions, each location within every year was considered a separate environment resulting in a total of 15 environments (Table 2). Biplot graphical analyses described by (Yan & Hunt, 2001; Yan et al., 2001) were conducted using GenStat 19th edition (VSN International) to rank genotype, genotype adaptation to environment, and identify mega-environments. The genotype main effect and G × E biplot (GGE-biplot; Yan, 2001) was generated with the first two principal components (PCs). Pearson's correlation analyses were conducted for yield, protein, starch, and mineral elements using SAS IML Studio 15.1 software (SAS Institute., Cary, NC, 2009). Broad-sense heritability (H^2, i.e., the proportion of genotypic variation [Vg] over the total phenotypic variation [Vp]), was calculated from the appropriate error mean squares in the ANOVA (Thavarajah et al., 2009).

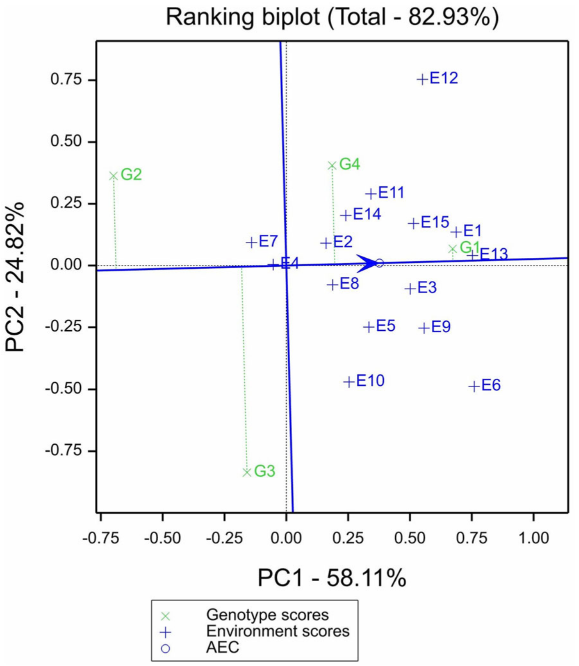

FIGURE 2 Environment and cultivar biplot showing the ranking and stability of yield. Cultivars located far from the *x*-axis have the strongest genotype × environment interaction and low stability

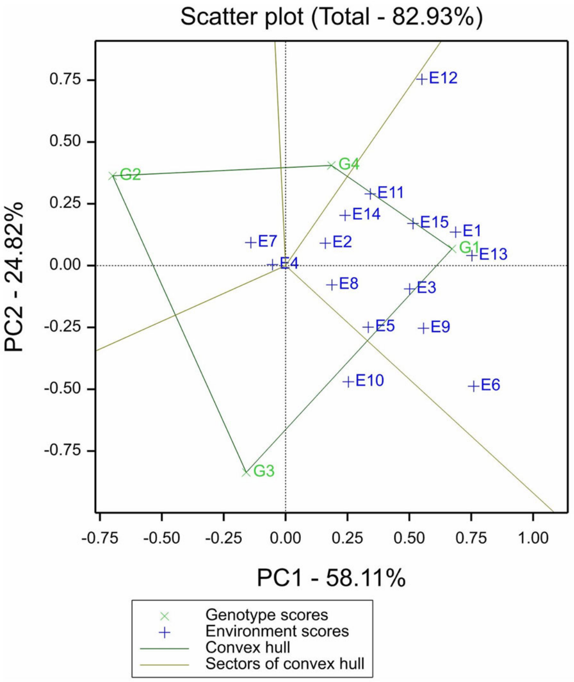

FIGURE 3 Environment and cultivar biplot showing the variety adaptation to environments

3 | RESULTS

3.1 | Lentil seed yield affected by cultivar and environment

Location, cultivar, location × cultivar, year × cultivar, and the location × cultivar × year interaction affected lentil grain yield ($P < .01$). Overall, grain yield was highest in 2019 (2,215 kg ha^{-1}) and lowest in 2017 (1,470 kg ha^{-1}) because of drought (Figure 1). Lentil yield at locations was greatest at Bozeman (2,194 kg ha^{-1}) followed by Richland (1,925 kg ha^{-1}), then Conrad (1,711 kg ha^{-1}), then Moccasin (1,660 kg ha^{-1}) and Havre (1,610 kg ha^{-1}). Cultivar × environment (location–year) interactions were displayed in the biplot (Figure 2) generated from environment × cultivar interactions using the 15 environments listed in Table 2.

Yan and Kang (2003) defined an ideal genotype as being the highest yielding genotype across test environments with stable performance. Figure 2 shows that Avondale (G1) had the highest and most stable yield (closest to the *x*-axis in the biplot), followed by CDC Richlea (G4). Avondale was the top performing cultivar in 12 of the 15 test environments for yield (Figure 3). All environments (except E4, E7, and E10) were located in the sector along with Avondale (G1). CDC Richlea (G4) was positioned at the edge of the sector in the biplot, indicating an adaptability similar to that of Avondale. These results indicate the potential of selecting a well-adapted, high yielding cultivar for different regions in Montana and possibly elsewhere in the Northern Great Plains. The estimated broad-sense heritability for yield was .31 (Table 3).

3.2 | Lentil protein and starch affected by cultivar and environment

Seed protein and starch concentrations were significantly affected by year, location, and cultivar (Table 3). Grain protein concentration was lower in 2018 (245 g kg^{-1}) than in 2017 (256 g kg^{-1}) and 2019 (258 g kg^{-1}). Averaged over years and cultivars, the protein concentration at Moccasin (262 g kg^{-1}) and Havre (260 g kg^{-1}) were higher than other locations (246 to 250 g kg^{-1}). Although the location × cultivar and year × location × cultivar interactions were significant (Table 3), the interactions were mainly due to the magnitude rather than cross interactions. The cultivar explained a large portion of the variations with a broad-sense heritability of .62 for protein. Averaged across years and locations, CDC Imvincible produced grain with the highest protein concentration (265 g kg^{-1}) followed by CDC Maxim (G3) at 258 g kg^{-1}, whereas CDC Richlea (G4) and Avondale (G1) had the lowest grain protein concentrations (246 and 248 g kg^{-1}).

Lentil grain starch concentration was higher in 2017 (448 g kg^{-1}) than in 2018 and 2019 (445 g kg^{-1}; Table 3). Averaged over years and cultivars, starch concentration was the highest at Richland (455 g kg^{-1}) and lowest at Moccasin (441 g kg^{-1}). CDC Richlea produced the highest (456 g kg^{-1}) and CDC Maxim produced the lowest (436 g kg^{-1}) starch con-

TABLE 3 Analysis of variance on the effects the separate means of year, cultivar, and location on yield, protein, starch, and nutrient contents of lentil cultivars

Source of variation	*df*	Yield	Protein	Starch	P	K	Ca	Fe	Zn	S	Mn	Cu	Mg	B	Se
		kg ha^{-1}			——g kg^{-1}——			— mg kg^{-1} —		g kg^{-1}	— mg kg^{-1} —		g kg^{-1}	— mg kg^{-1} —	
Year (Y)	2	4.1E+07**	7.9E+03**	6.5E+03**	2.5E+08**	1.5E+09**	7.8E+06**	6.1E+04**	1.8E+04**	5.1E+07**	7.9E+03**	3.9E+04**	2.9E+07**	3.3E+01**	9.4E+02**
Location (L)	4	4.9E+06**	3.3E+01**	1.6E+01**	8.5E+06**	1.0E+07**	2.9E+05**	8.8E+01**	2.7E+03**	1.0E+06**	3.0E+02**	6.1E+01	1.6E+04**	1.7E+01**	1.9E+01**
Y × L	8	3.5E+06**	1.2E+01**	1.6E+01**	5.0E+06**	3.6E+06**	1.6E+04**	1.1E+02**	4.4E+02**	4.0E+05**	7.1E+01**	5.0E+01	1.8E+04**	7.0E+00**	1.1E+00**
Rep (Y × L)	45	7.8E+03**	7.7E-01	1.3E-01	8.4E+04	1.4E+05	1.5E+03	1.8E+01	8.7E+00	1.5E+04	5.9E+00	2.6E+01	7.2E+03	4.9E-01	1.7E-02
Cultivar (C)	3	3.9E+05**	2.4E+01**	1.1E+01**	1.2E+06**	3.3E+06**	3.5E+04**	3.3E+02**	8.7E+01**	2.5E+05**	1.0E+00	1.4E+02**	1.6E+05**	1.4E+01**	3.5E+00**
Y × C	6	1.0E+05**	2.1E+00**	1.4E+01**	1.4E+05	4.5E+05	1.3E+04**	6.2E+01*	1.7E+01	4.1E+04**	1.7E+01*	1.2E+02**	2.4E+04**	3.3E+00**	3.3E+00**
L × C	12	5.3E+04**	1.7E+00**	4.7E+00**	7.5E+04	9.0E+04	7.4E+03**	2.2E+01	8.1E+00	1.3E+04	2.6E+00	2.4E+01	3.1E+03	7.2E-01	1.2E-01
Y × L × C	24	3.6E+04**	1.3E+00**	3.7E+00**	8.8E+04	2.7E+05*	5.6E+03*	2.9E+01*	9.3E+00	1.3E+04	1.9E+00	3.4E+01	7.4E+03**	1.1E+00	2.2E-01
H^2[a]		0.31	0.62	0.07	0.70	0.57	0.27	0.47	0.53	0.51	0	0.14	0.51	0.46	0.14
Year															
2017		1470c	256a	448a	3.4b	8.6b	0.67a	57.9	27.7b	1.6a	11.5b	7.5b	1.30a	9.2	0.7b
2018		1775b	245b	445b	3.8a	9.0a	0.64b	57.4	34.5a	1.6a	11.6b	8.4a	1.19b	9.0	1.4a
2019		2215a	258a	445b	3.5b	8.9a	0.70a	58.3	28.1b	1.5b	13.1a	7.4b	1.18b	9.1	0.5b
Location															
Bozeman		2194a	249b	446b	3.2c	8.1d	0.85a	65.3a	27.9b	1.3b	16.1a	7.1	1.24b	7.9c	0.1c
Richland		1925b	246b	455a	3.4bc	9.2a	0.63bc	56.6b	38.0a	1.7a	13.0b	7.4	1.20c	9.0b	0.3c
Conrad		1711c	250b	445bc	2.9d	8.6c	0.66bc	54.6b	16.9d	1.6a	10.9c	6.8	1.22bc	9.4b	0.9b
Mocassin		1660cd	262a	441d	4.1a	9.1a	0.67b	56.0b	36.2a	1.6a	10.9c	8.4	1.20c	9.0b	0.1c
Havre		1610d	260a	442cd	3.5b	8.9b	0.62c	58.8ab	23.4c	1.6a	10.8c	8.1	1.27a	9.9a	2.4a
Cultivar															
Avondale		1965a	248c	449b	3.3b	9.1a	0.67b	58.8ab	27.7d	1.5c	12.5	6.9c	1.27a	8.7c	0.8b
CDC Richlea		1849b	246c	456a	3.4b	8.9b	0.68b	54.1b	28.8c	1.5c	12.3	7.3b	1.29a	8.5c	0.7c
CDC Maxim		1786c	258b	443c	3.7a	8.5d	0.72a	62.1a	31.5a	1.7a	11.8	8.1a	1.19b	9.2b	0.8b
CDC Imvincible		1671d	265a	436d	3.6a	8.7c	0.63c	56.7ab	29.8b	1.6b	12.0	8.3a	1.16c	10.1a	0.9a

Note. Within a column, source of variation, values not sharing a common letter are significantly different at the .05 probability level according to the least significant difference test.

[a] H^2 is the broad-sense heritability.

*Significant at the .05 probability level. **Significant at the .01 probability level.

centrations (Table 3). The ranking of locations and cultivars for starch concentrations was opposite the grain protein concentration. Environment (location-year) explained more variation than cultivar on starch concentration. The broad-sense heritability was only .07 for starch (Table 3).

3.3 | Lentil mineral concentrations affected by cultivar and environment

All mineral concentrations in lentil grain were significantly affected by environment (location–year) and cultivar selection (Table 3), with two exceptions. Cultivar selection did not affect Mn concentration ($P > .05$) and Cu concentration was unaffected by location ($P > .05$; Table 3). The range in mineral concentrations among the cultivars were from 3.3 to 3.7 g kg^{-1} for phosphorus (P), 8.5 to 9.1 g kg^{-1} for potassium (K), 0.63 to 0.72 g kg^{-1} for calcium (Ca), 54.1 to 62.1 mg kg^{-1} for iron (Fe), 27.2 to 31.5 mg kg^{-1} for Zn, 1.5 to 1.7 g kg^{-1} for sulfur (S), 11.8 to 12.5 mg kg^{-1} for manganese (Mn), 6.9 to 8.3 mg kg^{-1} for copper (Cu), 1.16 to 1.29 g kg^{-1} for magnesium (Mg), 8.5 to 10.1 mg kg^{-1} for boron (B), and 0.7 to 0.9 mg kg^{-1} for selenium (Se), respectively.

No cultivar × location interactions were found in this study, except for Ca, indicating that cultivars ranked similarly in each of the individual locations in terms of the macro- and mineral concentrations, except for Ca (Table 3). Macro- and micronutrient concentrations varied greatly among locations (Table 3). For examples, grain Ca (0.85 g kg^{-1}), Fe (65.3 mg kg^{-1}), and Mn (16.1 mg kg^{-1}) concentrations were significantly higher in Bozeman, and Zn concentration was significantly higher in Richland (38.0 mg kg^{-1}) and Moccasin (36.2 mg kg^{-1}) than other locations. Most notable is the Se concentration of lentil grain at Havre (2.4 mg kg^{-1}) and Conrad (0.9 mg kg^{-1}), which was 10 to 24 times the Se concentration of lentil grain produced at Moccasin and Bozeman (Table 3). CDC Maxim had the highest Fe, Zn, and S concentrations in the grain among the cultivars, whereas CDC Imvincible grain had the greatest Se, P, and Cu concentrations. Cultivar selection explained a considerable amount of the variance in mineral concentrations, except for Mn. The estimated broad-sense heritability was .70, .57, .27, .47, .53, .51, 0, .14, .51, .46, and .14 for P, K, Ca, Fe, Zn, S, Mn, Cu, Mg, B, and Se, respectively. This indicates that there is a potential to enrich lentil grain mineral concentrations, such as Fe and Zn, through plant breeding.

3.4 | Correlations between protein, starch, and mineral elements

Pearson's correlations indicated that lentil yield was positively correlated with grain Mn and Ca concentrations, but negatively correlated to the concentration of protein, S, Mg, Cu, B, and Se (Table 4). Grain protein concentration was positively correlated with P, S, Cu, and B, whereas starch concentration was positively correlated with Mg and Mn concentrations. However, starch concentration was negatively correlated to protein, P, S, Cu, B, and Se. These correlations indicate that targeting lentil production for high grain protein concentration might also result in higher P, S, Cu, and B concentrations. However, yield depression will likely occur because of negative correlation between grain yield and protein concentration. If high starch concentration in grain is desired, separate breeding efforts will be needed to select for cultivars like CDC Richlea that produce grain with higher starch concentration and others like CDC Imvincible producing grain with higher protein concentration.

4 | DISCUSSION

In this study, Avondale (G1) was identified to have the greatest yield and the greatest stability of the four cultivars that were tested (Figure 2) and was adaptable to almost all the tested environments as shown using the GGE biplot (Figure 3). Indeed, Avondale is a recently released cultivar selected for production in the U.S. Northern Great Plains, whereas the other cultivars were developed and released for production in other regions. Prerelease yield trials of Avondale have been conducted at the locations included in this study and demonstrated the superior performance of this cultivar to others that were included in the trials (data not shown). Less adaptability (based on grain yield) of two cultivars (CDC Maxim and CDC Imvincible) indicates a need for multisite and multiyear variety trials for selecting a site-specific as well as a broadly adaptable cultivar. Other researchers have also used GGE biplots to identify desirable genotypes demonstrating yield stability in dry pea (*Pisum sativium* L.; Ito et al., 2016), maize (*Zea mays* L.; Ndhlela et al., 2014), wheat (*Triticum aestivum* L.; Rad et al., 2013; Mohammadi & Amri, 2016), barley (*Hordeum vulgare* L.; Mehari et al., 2014) and sorghum (*Sorghum bicolor* L.; Rao et al., 2011).

Lentil is a major source of plant-based protein globally (Khazaei et al., 2019) and demand for plant-based protein in the human diet is expected to continue to grow (Pihlanto et al., 2017). In this study, CDC Imvincible produced grain with much higher protein concentrations than the other cultivars (Table 3). The broad-sense heritability was estimated at .62 based on the four cultivars grown in five locations for 3 yr, indicating breeding cultivars for higher protein concentration is possible. Other studies have also reported significant differences in protein concentration amongst cultivars (Ghumman et al., 2016; Ninou et al., 2019; Nosworthy et al., 2018; Subedi et al., 2020; Tao et al., 2017; Wang & Daun, 2006).

TABLE 4 Pearson's correlation coefficients and the *P*-values among all studied attributes of four lentil cultivars grown in five locations in three years 2017, 2018, and 2019 ($n = 240$)

	Yield	Protein	Starch	P	K	S	Mg	Zn	Fe	Mn	Cu	B	Ca	Se
Yield	**1**													
Protein	−.23	1												
P value	<.01													
Starch	.11	−.51	1											
P value	.09	<.01												
P	−.05	.40	−.34	1										
P value	.43	<.01	<.01											
K	−.10	−.02	−.03	.55	1									
P value	.15	.74	.63	<.01										
S	−.39	.16	−.22	.38	.54	1								
P value	<.01	.01	<.01	<.01	<.01									
Mg	−.21	.01	.13	.11	.34	.12	1							
P value	<.01	.80	.05	.10	<.01	.06								
Zn	−.02	.03	−.006	.56	.42	.25	−.11	1						
P value	.75	.57	.92	<.01	<.01	<.01	.08							
Fe	.06	.01	−.06	.14	.11	.07	.14	.07	1					
P value	.33	.85	.34	.03	.09	.27	.03	.27						
Mn	.65	−.20	.16	.01	−.07	−.46	.01	.17	.30	1				
P value	<.01	<.01	.01	.86	.28	<.01	.77	.01	<.01					
Cu	−.34	.27	−.36	.47	.22	.39	−.12	.43	.08	−.29	1			
P value	<.01	<.01	<.01	<.01	<.01	<.01	.06	<.01	.22	<.01				
B	−.40	.40	−.35	.25	.13	.32	.05	−.02	−.01	−.24	.35	1		
P value	<.01	<.01	<.01	<.01	.04	<.01	.43	.74	.83	<.01	<.01			
Ca	.33	−.14	.07	−.04	−.21	−.38	.07	.02	.17	.53	−.04	−.25	1	
P value	<.01	.03	.30	.53	<.01	<.01	.29	.76	.01	<.01	.50	<.01		
Se	−.26	−.008	−.13	.11	.13	.19	.21	−.31	.01	−.30	.20	.40	−.25	1
P value	<.01	.90	.04	.09	.04	<.01	<.01	<.01	.79	<.01	<.01	<.01	<.01	

Although genetics influenced grain protein concentration, environment also played a great role in lentil grain protein accumulation. Two environments, Moccasin and Havre, produced higher protein concentrations than the other locations in this study. Further study is needed to explore the mechanism of environmental factors affecting protein concentration. Other studies also reported genetic and environment effects influencing lentil protein concentrations (Erskine et al., 1985; Fatima et al., 2018; Sharaan et al., 2003). Organic and inorganic nitrogen fertilizers have shown positive effects on lentil yields and protein concentrations (Huang et al., 2016; Zeidan, 2007).

Pearson's correlation analysis showed that protein concentration was positively associated with P, S, Cu, and B, implying that producers may produce high protein lentil grain enriched in P, S, Cu, and B. Furthermore, these results imply that farmers may select an environment to produce lentil grains with enhanced protein and mineral nutrient concentrations. In this study, lentil grown at the Havre and Conrad locations in northcentral Montana had a much higher Se concentration (2.4 mg kg^{-1}) than lentil grown in other locations in the state. The Bozeman location in southwest Montana produced the highest Fe concentration (65.3 mg kg^{-1}), whereas the Richland site in northeastern Montana produced the highest Zn concentration (38.0 mg kg^{-1}). It is not clear if high concentration of minerals in lentil grains produced in those locations was resulted from high concentration of the minerals in the soil. However, Thavarajah et al. (2009) reported Fe concentration in lentil grain was not highly correlated to soil total Fe content. A study conducted by Afshar et al. (2020) in eastern Montana showed that two foliar applications of Zn at heading and flowering stages increased wheat grain Zn concentration to above 40 mg kg^{-1}.

In addition to the geological locations, breeding and cultivar selection can also improve macro- and micronutrient concentrations in lentil grain. In this study, CDC Maxim produced the highest Zn (31.5 mg kg^{-1}) and Fe (62.1 mg kg^{-1}) concentrations compared to other cultivars tested. Other studies also found large variation in mineral concentrations among lentil germplasm and commercial cultivars (Vandemark et al., 2018; Sarker et al., 2018). The mineral concentrations in this study are comparable to the lentil grains produced in the U.S. Pacific Northwest (Vandemark et al., 2018), except for Se concentration. The Se concentration was much higher when lentil grain was produced in Havre and Conrad than in the U.S. Pacific Northwest, but the Fe and Zn concentrations were slightly lower in this study. Thavarajah et al. (2009) reported that two small red lentil cultivars grown in Saskatoon, Canada had Fe concentration of 93 to 99 mg kg^{-1} compared to 74 to 77 mg kg^{-1} for two green lentil genotypes. In our study, CDC Maxim, a small red lentil, produced grain with 31.5 mg kg^{-1} Zn and 62.1 mg kg^{-1} Fe, and the Fe concentration of CDC Maxim in this study was lower than the small red lentils grown in Saskatoon reported by Thavarajah et al. (2009). Furthermore, the broad-sense heritability was estimated at .47 for Fe and .53 for Zn in this study (Table 3) compared to .64 for Fe and .68 for Zn reported by Thavarajah et al. (2009). Lower broad-sense heritability in this study was likely due to the small number of cultivars (four cultivars) included in the study.

Mineral malnutrition is one of the major global health problems and enhancing mineral content in lentil grains could bring great benefits to human health in certain parts of the world (Bouhlal et al., 2019; Faris, 2013). An analysis of lentil samples collected in six major lentil producing countries (Thavarajah et al., 2011) showed that lentil produced in Nepal and southern Australia had the highest Se concentrations (0.18 and 0.15 mg kg^{-1}) and lentil grown in Syria and Morocco had the lowest Se concentrations (0.02 and 0.03 mg kg^{-1}). It is recommended that 55 μg of Se per day for human intake is essential for health and cancer prevention in adults (National Academy of Sciences, 2004). In this study, Se concentration in lentil grain was much higher at Havre (2.4 mg kg^{-1}) and Conrad (0.9 mg kg^{-1}) than those reported in Nepal and Australia. The concentration of Se was reported at 0.4 to 0.5 mg kg^{-1} in Saskatchewan-grown lentil grains consistent with the geographic proximity to Havre (Ray et al., 2014). No interactions were observed between cultivar and location for most of the minerals measured in this study, indicating that the ranking for the selected cultivars was similar at each of the individual locations in terms of mineral concentrations. Thavarajah et al. (2009) also reported no genotype × location interaction effects on Fe in lentil grains produced in Saskatchewan, Canada. In another study by Ray et al. (2014) in Saskatchewan, no cultivar × location interaction effects were found for Ca, Zn, Mn, nickel, or Cu. Little cultivar × location interaction indicates that plant breeders may develop a cultivar with high mineral enriching ability for growers to consistently produce high macro- and mineral nutrient concentration lentil grain across geographical locations in the Northern Great Plains. Results in this study indicate that lentil nutrients including protein, starch, and mineral nutrients may be enhanced through plant breeding and production site selection. Since the Northern Great Plains (including the United States and Canada) exports 62.3% of the world's exported lentil (Workman, 2020), this study has a significant implication on food and nutrition security of the world.

AUTHOR CONTRIBUTIONS

Chengci Chen: Conceptualization; Funding acquisition; Project administration; Resources; Supervision; Validation; Writing-review & editing. Fatemeh Etemadi: Writing – original draft. William Franck: Investigation; Writing-review & editing. Sooyoung Franck: Data curation; Investigation. Magdi Abdelhamid: Data curation; software; visualization. Jafar ahmadi: Software. Kevin McPhee: Investigation;

Writing-review & editing. Yesuf Assen Mohammed: Data curation; Investigation. Patick M. Carr: Investigation, Writing-review & editing. Peggy Lamb: Investigation. John Miller: Investigation. Yi Zhou: Software; Visualization. Shahram Torabian: Software; Visualization. Ruijun Qin: Writing-review & editing.

CONFLICT OF INTEREST

The authors report no conflicts of interest.

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