Genotype × Environment Interactions for Mineral Concentration in Grain of Organically Grown Spring Wheat

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ABSTRACT

Genotype × environment (G×E) interactions for Ca, Cu, Fe, Mg, Mn, P, and Zn concentrations are not well understood, particularly in the context of organic farming systems. The objectives of this study were to: (i) investigate G×E interactions for mineral nutrient concentration in organically grown wheat; and, (ii) assess whether grain mineral concentration is a broadly or narrowly adapted trait when grown in contrasting environments over time. We evaluated 18 spring wheat (*Triticum aestivum* L.) cultivars on three organic farms in Washington State for mineral concentration and for grain yield in 2008 and 2009. The G×Year (Y) interactions were found for grain yield and all minerals except Fe, Mn, and P and G×Location (L) interactions were found for grain yield and all minerals except Fe. The G×E (G×L×Y) interactions were found for grain yield and all minerals except for Mn. Grain yield was not consistently correlated with mineral nutrients across years and locations. Among minerals, Mg:P, P:Zn, and Mg:Zn were positively correlated in at least five of six site-years, suggesting the potential for simultaneous selection of these minerals. Grain mineral concentrations of Cu, Fe, and P showed relatively broad adaptation across years when compared with Ca and Mg concentrations. Fewer cultivars were broadly adapted spatially than temporally for stable levels of mineral concentration. Several cultivars had relatively high concentrations of two or more minerals across locations, indicating the potential for farmer utilization of broadly adapted cultivars and varietal blends that will significantly increase grain mineral concentration.

Agricultural systems in many countries are often unable to provide sufficient micronutrients to effectively meet the needs of their growing populations (Welch, 2002). This is partly due to the increase in grain yield in numerous agricultural systems over the past 50 yr as a result of increased chemical fertilizer application, which has inadvertently resulted in reduced micronutrient concentrations (Khoshgoftarmanesh et al., 2010). Decreasing micronutrient malnutrition globally was a vital component of three of the eight Millennium Development goals of the General Assembly of the UN in 2001 (UN, 2000), and a concerted worldwide endeavor is currently underway to develop cereal crop cultivars with enhanced nutritional value (Bouis, 1996; Graham et al., 2001; Khoshgoftarmanesh et al., 2010; Stein et al., 2007; White and Broadley, 2009). Organic grain growers in western Washington State tend to market their grain domestically, mostly on a local/regional scale. Often they work closely with bakers and millers to ensure grain quality from year to year and cultivar to cultivar. Conversations among researchers, farmers, and bakers have demonstrated the need and consumer desire for locally grown whole wheat products with a higher nutritional value.

Organic plant breeding programs have the opportunity to address many challenges besides increasing production in organic agriculture. Increasing the nutritional value of crop cultivars should be a high priority for agricultural scientists working in organic systems. A survey conducted by the Organic Farming Research Foundation reported that 56% of respondents ranked the relationship between organic farming practices and the nutritional value of food as among the highest research priorities for organic field crops (Walz, 2004). Nutrient-use efficiency has been shown to be higher in organic systems than conventional systems, though N and P uptake and grain yield increased with nutrient input regardless of the system effects (Hildermann et al., 2010).

In the wheat species *Triticum aestivum, T. monococcum, T. dicoccum,* and *T. turgidum*, genotypic response for mineral concentration has been shown to vary across environments (Gomez-Becerra et al., 2010; Hussain et al., 2010; Joshi et al., 2010; Morgounov et al., 2007; Oury et al., 2006). For example, significant G×E interactions for Fe and Zn concentrations have been reported in wheat across the mega-environment of the East Gangetic plains that include Nepal and Bangladesh, India (Joshi et al., 2010). Additionally, Gomez-Becerra et al. (2010) found that in five locations across Turkey and Israel, some genotypes had the capacity for high mineral concentration under favorable environments, while other genotypes were stable under both favorable and marginal environments for particular minerals.

To optimize grain yield and nutritional value in crop cultivars, it is important to identify and understand the physical and climatic

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Abbreviations: CEC, cation exchange capacity; G×E, genotype × environment interactions, G×L, genotype × location interactions; G×Y, genotype × year interactions.

characteristics of the target locations, including temperature, rainfall patterns, soil type, and soil fertility (Chatrath et al., 2007; Ortiz-Monasterio et al., 2007). Joshi et al. (2010) showed that maximum temperature before flowering and relative humidity and rainfall after flowering influenced Fe concentration in the grain. Rainfall, however, after flowering and minimum temperature before and after flowering appeared to play a significant role in determining Zn concentration (Joshi et al., 2010).

Little is known about the interactions of soil mineral composition and crop cultivar on influencing nutritional quality in cereal grains. Soil erosion, leaching, expansion of crop production onto marginal environments, and changes in fertilizer inputs from manure-based to chemical-based inputs have led to increased incidences of soil micronutrient deficiencies (Fageria et al., 2002). Genotypes differ in their ability to extract minerals from different soil depths. For example, some cultivars have the capacity to exploit Zn from the top 30cm while others have greater Zn in the grain when Zn level is higher in the 30- to 60-cm depth (Joshi et al., 2010). In soils which are deficient in particular nutrients, genotypes which store a higher concentration of that nutrient in the seed can have a fitness advantage when grown in similar conditions (Bonfil and Kafkafi, 2000). Chatzav et al. (2010) suggested that populations which originate from Zn-deficient soils have evolved mechanisms that allow for more Zn to accumulate in their grain, thus enhancing seedling germination and establishment.

Some studies have shown that there is no genetic trade-off between mineral concentration and grain yield in wheat, suggesting that simultaneous selection for these traits has a good probability of success (Graham et al., 2001; Murphy et al., 2008a; Rengel, 2001). On the other hand, several other studies have found trade-offs between mineral concentration and grain yield, which may have to be taken into consideration when breeding for these traits (Garvin et al., 2006; Heitholt et al., 1990; Zhao et al., 2009). Many historical wheat cultivars and landraces contain significantly higher micronutrient concentrations than modern cultivars (Fan et al., 2008; Garvin et al., 2006; Hussain et al., 2010; Murphy et al., 2008a); however, Hildermann et al. (2010) found that modern cultivars (selected under both organic and conventional systems) tended to have higher N-use efficiency than historical cultivars. When testing for mineral concentration and grain yield in organic systems, it is therefore useful to include both historical and modern wheat cultivars in the experimental design. The objectives of this study were to: (i) investigate G×E interactions for and concentrations of Ca, Cu, Fe, Mg, Mn, P, and Zn in organically grown wheat; and, (ii) assess whether grain mineral concentration is a broadly or narrowly adapted trait when grown in contrasting environments over time.

MATERIALS AND METHODS

Experimental Design

Eighteen historical and modern spring wheat cultivars were planted in replicated field trials. Nine of the 18 cultivars were modern (1985–2005) and nine were historical (1917–1965). Historical cultivars included Beaver, Cadet, Canus, Comet, Idaed, Reliance, Ruby, Spinckota, and White Marquis. Modern cultivars included Alpowa, Kelse, Louise, Penewawa, Scarlet, Wakanz, Wawawai, Westbred Express, and Zak. Nine of the 18 cultivars were in the hard red wheat market class and nine designated as soft white wheat. Cultivars were chosen based on previous studies which described significant variation for mineral concentration and relatively high grain yield within their respective market class (Murphy et al., 2008a, 2008b).

Table 1. Total monthly precipitation and average monthly temperature at three locations in western Washington in 2008 and 2009.

	Jubilee Farm		Evergreen State College Farm		Nash's Farm	
Month	2008	2009	2008	2009	2008	2009
			Total monthly precipitation, mm			
May	22.1	96.3	3.0	77.0	11.9	25.9
June	52.6	6.9	24.0	0.0	28.7	3.3
July	14.7	4.1	0.0	4.0	4.3	3.6
August	67.1	20.1	27.0	29.0	30.2	4.1
September	20.1	59.7	0.0	24.0	0.0	16.3
Total	176.5	186.9	54.0	134.0	75.2	53.1
			Average monthly temperature, °C			
May	13.2	13.2	12.2	11.6	9.6	10.6
June	14.5	17.4	13.1	15.9	10.5	13.7
July	18.1	20.6	16.5	18.6	11.7	14.6
August	19.2	19.3	17.1	16.5	11.2	14.0
September	16.0	17.0	14.2	14.8	12.3	13.6
Mean	16.2	17.5	14.6	15.5	11.1	13.3

Field trials were conducted on three certified organic locations in western Washington in 2008 and 2009. Jubilee Farm is located near the town of Carnation, WA in King County (47°38′ 52″ N, 121°54′50″ W). Evergreen State College Organic Farm is located at the Evergreen State College in Olympia, WA in Thurston County (47°2′17″ N, 122°53′57″ W). Nash's Organic Farm is located in Sequim, WA, in Clallam County (48°4′46″ N, 123°6′7″ W). These locations were chosen to represent a range of climatic conditions and rainfall zones within the mega-environment of western Washington State. Total monthly precipitation and average temperature for each site year are shown in Table 1.

Spring wheat cultivars were planted in mid-May in 2008 and 2009 in randomized complete block designs with three replicates. Plots consisted of two rows, 2.5 m long and 0.75 m wide. Plots were kept weed free throughout the growing season by frequent hand weeding. Yellow rust of wheat, caused by pathogenic races of *Puccinia striiformis*, was the only disease found in the plots. No attempt to control the disease was made. Plots were harvested by hand and threshed using a Vogel bundle thresher (Bill's Welding, Pullman, WA).

Soil samples were taken in the spring at planting and early summer (first week of July) each year at each of the three locations to a depth of 150 mm. As wheat is considered by regional farmers to be a low-input crop in relation to common rotational crops, primarily high-value vegetables, no fertilizations were performed before planting. At the request of the cooperating farmers, soil fertility was based on the residual nutrients incorporated before the preceding vegetable crop in the previous year. Ten subsamples from each location were pooled into a single sample, dried, and tested according to McGeehan (2009) at the University of Idaho Analytical Laboratory (Moscow, ID) for nitrate N + nitrite N, N ammonia, available P, available K, Zn, Cu, Mn, Fe, percent organic matter, pH, and cation exchange capacity (CEC). The relatively low temperatures during the growing season in western Washington typically result in spring wheat getting harvested approximately 4 to 6 wk later

Table 2. Grain concentrations of Ca, Cu, Fe, Mg, Mn, P, Zn, and grain yield of 18 spring wheat cultivars grown at three locations in western Washington in 2008.

Cultivar	Ca			Cu			Fe			Mg			Mn			P			Zn			Grain yield		
	J†	E	N	J	E	N	J	E	N	J	E	N	J	E	N	J	E	N	J	E	N	J	E	N
	mg kg^{-1}																					g m^{-2}		
Alpowa	331	331	434	4.93	3.80	3.93	50.3	32.2	26.4	1275	1118	1304	17.8	30.7	15.1	3590	2848	4409	43.1	35.9	27.1	439	105	443
Beaver	354	314	504	5.57	4.33	4.60	53.4	29.9	34.9	1345	1145	1356	28.9	41.4	26.6	3904	3167	4252	55.9	38.1	37.9	347	95	337
Cadet	254	250	346	5.90	5.13	4.40	52.5	35.8	31.9	1391	1300	1313	20.2	39.1	23.8	4258	3582	4099	52.2	46.7	35.9	396	134	343
Canus	484	405	530	6.30	4.33	5.13	62.4	40.0	43.2	1447	1190	1413	32.7	40.7	24.4	3977	3116	4322	67.6	38.3	35.7	475	77	496
Comet	355	288	452	4.90	4.00	4.93	44.7	34.8	39.5	1185	1077	1297	26.1	36.1	26.0	3647	2943	4120	49.3	37.5	47.4	340	89	119
Idaed	326	259	343	6.17	4.80	4.50	51.5	36.4	36.4	1187	1008	1143	21.1	30.6	24.2	3630	2765	3734	45.6	35.9	31.4	342	76	357
Kelse	453	320	680	5.77	5.27	5.17	53.4	31.1	31.6	1280	1147	1225	26.6	42.0	19.1	3808	3238	3867	49.9	33.6	27.7	495	77	705
Louise	389	331	519	5.83	5.23	4.80	50.8	29.4	56.7	1162	1042	1212	28.5	29.1	21.5	3535	2364	3870	45.5	31.6	31.1	559	105	569
Penewawa	435	341	531	5.80	4.10	5.27	35.0	28.3	35.7	1214	1076	1345	26.6	33.8	21.0	3575	2832	4215	44.3	32.5	35.6	528	91	303
Reliance	445	383	513	5.43	4.30	4.83	54.3	32.7	37.2	1233	1083	1283	24.0	34.5	17.9	3867	2945	4112	50.4	33.5	32.7	371	89	417
Ruby	389	305	490	5.53	4.80	4.60	91.2	35.7	37.1	1291	1150	1222	24.6	32.5	23.0	4005	3164	3927	55.8	35.4	37.4	320	70	274
Scarlet	361	308	553	5.43	3.97	5.13	52.0	34.9	38.6	1296	1161	1275	19.3	33.3	20.2	3553	3037	3805	43.6	30.1	30.9	580	135	334
Spinkota	329	275	430	5.07	3.83	4.10	63.0	43.8	41.9	1456	1208	1312	25.4	35.4	22.7	4146	2903	4217	59.2	39.6	39.5	326	109	289
Wakanz	435	375	533	5.53	4.37	5.30	45.5	37.4	34.7	1148	1165	1257	19.5	34.4	21.1	3524	2855	3716	38.7	39.1	33.5	364	112	380
Wawawai	387	275	527	6.23	4.13	5.33	83.3	32.5	36.0	1203	1092	1205	27.8	38.4	22.2	3377	2790	3615	46.9	36	32.8	474	127	259
Westbred Express	366	353	485	6.00	4.67	4.60	74.3	31.6	35.0	1393	1114	1327	26.9	49.8	22.6	3734	2844	3783	48.6	30.2	28.5	492	77	521
White Marquis	393	357	466	6.60	4.33	5.06	42.5	30.6	34.0	1275	1074	1142	26.4	25.3	25.9	4105	3162	3741	58.1	35.8	35.7	353	108	387
Zak	502	367	680	5.97	4.30	5.47	51.4	37.0	42.6	1205	1049	1338	25.8	30.1	18.2	3495	2512	3986	41.5	25.5	31.4	462	147	285
Mean	388	324	501	5.72	4.43	4.84	56.2	34.1	37.4	1277	1122	1276	24.9	35.4	22.0	3763	2948	3988	49.8	35.3	34.0	426	101	379
LSD (0.05)	41	32	102	0.30	0.24	0.49	29.3	3.2	19.5	100	66	94	14.4	3.2	5.7	298	242	417	6.4	3.5	7.1	112	29	78

† J = Jubilee Farm, E = Evergreen College State Farm, N = Nash's Farm.

than in eastern Washington. Therefore, the early summer soil tests accurately reflect mineral concentrations and other soil quality indicators (pH and CEC) that will continue to play an important role in plant health in western Washington.

Mineral Concentration Analysis

Mineral analyses were conducted on grain samples from all replicates at each location in 2008 and 2009. Mineral concentrations were measured at the USDA-ARS Grand Forks Human Nutrition Research Center in Grand Forks, ND. Grain samples were individually ground to a powder (60 s) in a laboratory grinder (Janke & Kunkel, Model 20S3) with a stainless steel chamber and blade. Approximately 0.6 g of each grain sample was weighed and placed in triplicate into separate Pyrex beakers. Watchglasses were placed on the beakers and the samples were ashed in a muffle furnace at 200°C for 2 h and at 490°C for 12 h. The ash was dissolved in 10 mL of concentrated nitric acid (J.T. Baker Instra-Analyzed) and heated on a hotplate at 120°C, refluxing for 2 h. Three milliliters of 30% hydrogen peroxide (J.T. Baker) were slowly added to each beaker, and the samples were allowed to dry. Samples were ashed again in a muffle furnace following the procedure outlined above. The resulting white ash was dissolved in 2 mL of 6 M HCl (J.T. Baker Instra-Analyzed) with heating and subsequently diluted to 10 mL with deionized water. Samples were analyzed simultaneously for Ca, Cu, Fe, Mg, Mn, P, and Zn by inductively coupled argon plasma (ICAP) techniques by using a PerkinElmer 3300 instrument. Four durum wheat standards (National Institute of Standards and Technology, Gaithersburg, MD) and four acid blanks were run with each batch of samples.

Statistical Analyses

Treatment effects were tested by ANOVA using PROC GLM (SAS Institute, Cary, NC) for all measurements. Genotype was considered a fixed effect as the cultivars were specifically chosen based on past performance; location, year, and replications were considered random effects. To attempt to determine whether grain mineral concentration is a broadly or narrowly adapted trait when grown in contrasting environments over time, simple main effects of each genotype were tested using PROC GLM. Specifically, significance was assessed using the "slice" function (in SAS parlance) for each effect (year or location) of each individual mineral and grain yield. This is similar to running one-way ANOVAs for each location and year, with the slice function holding all variables other than the target variable constant and testing this effect for significance. 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). Pearson's correlation coefficients were calculated based on mean trait values and were used to estimate phenotypic relationships between traits of interest. Unless otherwise stated, significance was assessed at the 0.05 probability level.

RESULTS AND DISCUSSION

Genetic Variation for Grain Mineral Concentration and Yield

Significant differences were found among the cultivars across locations and years for concentrations of all minerals in the wheat grain and for grain yield (Tables 2 and 3). Similarly, a wide range of variation for mineral concentration in wheat, wheat amphiploids, and wheat relatives has been reported in previous studies (Cakmak et al., 1999; Chatzav et al., 2010;

Table 3. Grain concentrations of Ca, Cu, Fe, Mg, Mn, P, Zn, and grain yield of 18 spring wheat cultivars grown at three locations in western Washington in 2009.

Cultivar	Ca			Cu			Fe			Mg			Mn			P			Zn			Grain yield		
	J†	E	N	J	E	N	J	E	N	J	E	N	J	E	N	J	E	N	J	E	N	J	E	N
	mg kg^{-1}																					g m^{-2}		
Alpowa	340	278	348	4.60	4.50	4.73	38.3	31.6	31.9	1352	1389	1284	22.5	44.2	20.7	3300	3820	3524	45.8	37.1	30.9	245	98	176
Beaver	373	276	387	5.50	4.63	5.40	48.9	32.7	36.7	1553	1286	1499	34.2	47.8	30.6	4089	3433	4095	63.2	40.5	47.3	158	100	69
Cadet	245	231	364	5.70	4.90	5.40	57.3	36.1	36.0	1531	1275	1489	21.8	38.2	29.4	4061	3458	4196	61.1	41.0	46.5	145	98	95
Canus	528	382	461	5.70	4.73	6.07	58.2	40.1	47.2	1604	1340	1516	24.2	51.8	31.9	4244	3823	4395	61.3	44.4	45.8	263	144	253
Comet	367	305	414	6.03	4.30	5.43	62.5	38.2	45.4	1605	1111	1422	32.7	38.6	33.1	4347	2929	3854	66.2	35.4	48.9	281	109	17
Idaed	294	257	375	5.67	5.07	5.30	55.3	38.8	36.5	1337	1288	1341	26.5	45.3	28.5	3601	3425	3561	53.3	36.3	43.0	248	124	41
Kelse	502	371	425	6.07	5.47	5.63	53.5	37.7	33.9	1490	1488	1289	32.0	48.4	26.3	3972	4128	3731	57.2	35.5	33.2	275	119	294
Louise	450	340	359	5.37	4.43	4.80	45.7	33.2	33.4	1374	1357	1180	23.2	47.4	25.8	3553	3869	3432	47.2	37.2	36.1	348	110	214
Penewawa	380	311	416	6.40	3.87	4.90	50.0	29.3	29.4	1601	1163	1230	33.3	43.2	20.9	4059	3025	3415	48.6	32.0	34.7	209	116	105
Reliance	438	381	394	5.03	4.30	4.63	58.1	36.1	39.3	1418	1303	1164	26.7	44.7	22.2	3846	3823	3507	52.3	37.4	34.8	185	171	290
Ruby	333	330	399	5.50	4.67	5.50	50.3	37.4	72.7	1479	1307	1338	34.2	41.5	29.1	4119	2763	3793	61.5	37.8	46.3	193	82	29
Scarlet	368	313	368	5.20	4.47	4.40	47.2	38.2	51.1	1411	1251	1332	16.4	46.8	22.4	3498	3126	3515	46.1	36.8	39.1	301	133	131
Spinkota	294	289	328	5.17	3.97	5.03	71.7	46.8	57.1	1604	1401	1446	35.0	47.1	31.8	4124	3794	4396	70.5	45.9	59.0	210	175	194
Wakanz	413	342	410	5.37	3.97	5.27	45.4	35.8	37.6	1242	1166	1197	28.8	42.4	28.4	3363	3227	3372	44.1	31.1	32.0	na	119	319
Wawawai	315	267	331	5.30	4.53	5.70	46.1	32.6	38.5	1327	1327	1281	41.7	46.3	28.1	3525	3525	3650	46.6	35.5	45.4	307	145	101
Westbred Express	332	309	398	5.90	4.30	5.60	53.7	39.3	40.1	1550	1290	1355	36.6	54.3	31.0	3801	3315	3573	58.7	35.2	36.1	216	111	205
White Marquis	370	333	406	6.00	5.30	5.50	47.2	34.0	32.6	1362	1295	1215	33.8	40.0	28.6	3997	3979	3886	61.7	43.7	44.8	219	174	235
Zak	459	346	430	6.00	4.47	4.87	52.4	37.7	38.5	1372	1265	1174	30.2	47.6	26.8	3292	3361	3179	48.3	31.7	30.2	167	193	145
Mean	378	315	390	5.58	4.55	5.23	52.3	36.4	41.0	1456	1295	1320	29.7	45.3	27.5	3822	3490	3726	55.2	37.5	40.8	234	129	162
LSD (0.05)	23	41	36	0.34	0.66	0.60	3.3	4.6	20.4	95	167	141	10.2	11.2	7.3	264	818	344	10.1	6.5	7.4	73	103	87

† J = Jubilee Farm, E = Evergreen College State Farm, N = Nash's Farm.

Hussain et al., 2010; Monasterio and Graham, 2000; Murphy et al., 2009; Zhao et al., 2009).

Several cultivars had particularly high concentrations of certain minerals across all environments (Tables 2 and 3). For example, Spinckota had among the highest concentrations of both Fe and Zn, as well as relatively high concentrations of Mg, Mn, and P. Canus ranked in the top five for all mineral nutrients and grain yield across both years. Also, Kelse was among the highest ranking cultivars for Ca, Cu, and grain yield, and Westbred Express for Fe, Mg, Mn, and grain yield. Other cultivars, including Beaver, Cadet, Wawawai, and Zak were among the highest ranked for at least two different mineral nutrients across both years. Alternatively, the soft white cultivars Penewawa and Alpowa had relatively low values for all traits. This corresponds with results from tomato (*Lycopersicon esculentum* Mill.) experiments that showed that when grown in organic systems, choice of tomato cultivar provided the simplest and most effective method of increasing quality and nutritional value (Aldrich et al., 2010).

Grain yield and Ca concentration were significantly higher across locations in 2008 than 2009 (Tables 2 and 3). Conversely, concentrations of Cu, Fe, Mg, Mn, P, and Zn were all significantly higher across locations in 2009 than in 2008 (Tables 2 and 3). This indicates that a trade-off may exist between yield and mineral concentration due to the dilution effect (Davis, 2009; Fan et al., 2008) across environmentally-induced yearly fluctuations. However, when cultivars were analyzed individually, exceptions were often observed, including cultivars with high yield and high mineral concentrations and other cultivars with low yield and low mineral concentration. Caution should be used when deciphering the results for grain yield, as plot size at each location was minimal and not necessarily representative of field-scale grain production.

Significant differences for mineral concentration and grain yield were consistently found each year across locations (Tables 2 and 3). Nash's Farm had the highest values for the macronutrients Ca and P, and low levels of Mn. Evergreen State College Organic Farm had the highest concentrations of Mn and relatively low values of Ca, Cu, Fe, Mg, and grain yield. Jubilee Farm had the highest values for the micronutrients Cu, Fe, and Zn and for the macronutrients Mg and P. In 2009, grain yield was significantly higher at Jubilee Farm than at the other locations (Table 3). Crops grown in the field with manure applications tend to produce grain with higher Fe and Zn values than crops grown with chemical fertilizer (Monasterio and Graham, 2000). This was the case with Jubilee Farm, which of the three locations, was the only farm that used composted manure applications to improve soil fertility.

With the exception of Mn, high levels of micronutrients in the grain were not necessarily associated with high concentrations of micronutrients in the soil. For example, Cu concentration was lower in the soil at each location in 2009 compared with 2008 (Table 4), however, only Jubilee Farm showed a slight decrease in grain Cu concentration over the same time period (Tables 2 and 3). Alternatively, grain harvested at Evergreen and Nash's Farm had higher Cu concentration in 2009 compared with 2008 (Tables 2 and 3), despite significantly lower soil Cu concentration in 2009 (Table 4). An inverse relationship between soil mineral concentration and grain mineral concentration was found at all locations for P and Zn, and at Evergreen Farm for Mn, in 2009 compared to 2008 (Tables 2, 3, and 4). This corresponds with the results found by Chatzav et al. (2010), which showed a negative correlation between soil Zn concentration and grain Zn concentration in wild

Table 4. Mean values ± standard error for soil tests nitrate N + nitrite-N, N-ammonia, available K, available P, Zn, Cu, Mn, and Fe, organic matter, cation exchange capacity (CEC), and pH in 2008 and 2009 based on spring and summer soil tests combined across three locations.

	Jubilee		Evergreen		Nash	
Soil test	2008	2009	2008	2009	2008	2009
Nitrate + Nitrite, mg kg^{-1}	54 ± 14.0	45 ± 21.0	15.1 ± 6.9	39.5 ± 0.5	80.5 ± 19.5	29.5 ± 13.5
N-Ammonia, mg kg^{-1}	7.55 ± 1.15	6.05 ± 0.15	12.00 ± 2.00	4.95 ± 1.05	5.55 ± 0.35	3.25 ± 1.55
Potassium, mg kg^{-1}	395 ± 65	275 ± 65	82 ± 10	88 ± 12	295 ± 15	49 ± 7
Phosphorus, mg kg^{-1}	16.2 ± 1.3	14.6 ± 10.5	15.0 ± 3.0	9.2 ± 0.3	55.0 ± 8.0	4.4 ± 0.6
Zinc , mg kg^{-1}	3. 50 ± 1.10	1.75 ± 0.35	4.85 ± 1.85	1.50 ± 0.40	1.30 ± 0.00	0.68 ± 0.04
Copper, mg kg^{-1}	6.40 ± 0.00	3.17 ± 2.43	6.50 ± 2.40	1.25 ± 0.05	3.70 ± 0.00	2.10 ± 0.20
Manganese, mg kg^{-1}	7.9 ± 0.5	8.3 ± 1.7	26.5 ± 1.5	14.2 ± 5.9	11.5 ± 1.5	16.1 ± 10.9
Iron, mg kg^{-1}	180 ± 10.0	83 ± 17.5	120 ± 10.0	93 ± 7.5	130 ± 0.0	94 ± 16.0
Organic matter, %	4.55 ± 0.25	6.05 ± 1.65	6.90 ± 0.00	6.60 ± 0.10	3.35 ± 0.15	3.0 ± 0.10
CEC, cmol kg^{-1}	22.0 ± 1.0	24.0 ± 2.0	24.0 ± 2.0	25.5 ± 0.5	18.5 ± 0.5	22.5 ± 0.5
pH	5.6 ± 0.0	6.4 ± 0.7	5.6 ± 0.1	6.0 ± 0.7	6.9 ± 0.1	6.2 ± 0.2

emmer wheat in Israel. In our study, high concentrations of Mn in the soil corresponded to high Mn grain concentration at Evergreen Farm. Soil K concentrations were high at Jubilee and at Nash's in 2008 which can lead to deficiencies in other cations, but this did not seem to be the case in our study. Recommended soil values for P were low at all locations in both years with the exception of Nash's in 2008, but values for Cu, Zn, and Fe were all sufficient (Marx et al., 1999). Because soil micronutrient levels were sufficient, other factors such as rainfall and temperature may have had a greater impact on grain nutrient concentration than soil concentration.

The decrease in overall grain yield from 2008 to 2009 was driven by yield decreases at Nash and Jubilee Farm and is likely associated with the decrease in ammonia N and nitrates + nitrates. Evergreen Farm was the only location to show a yield increase from 2008 to 2009, which corresponds with the increase in nitrate N + nitrite N at Evergreen Farm over the same time period (Table 4).

Grain yield was not consistently correlated with mineral nutrients across years and locations (Table 5). Among minerals, Mg:P showed the strongest association, with positive correlations each site year ranging from 0.72 to 0.91 (Table 5). P:Zn likewise were positively correlated at each site year and Mg:Zn in five of six site-years. Chatzav et al. (2010) reported positive correlations between grain Zn and Fe among 128 wild emmer wheat accessions, which correspond with other reports in emmer (Cakmak et al., 2004; Peleg et al., 2008) and domesticated wheat (Morgounov et al., 2007; Zhao et al., 2009). However, many reports of correlations among minerals have varied substantially (Gomez-Becerra et al., 2010; McDonald et

Table 5. Pearson correlation coefficients for grain yield and concentration in the grain of Ca, Cu, Fe, Mg, Mn, P, and Zn for 2008 and 2009 at each of three locations (Loc).

		Cu		Fe		Mg		Mn		P		Zn		Yield	
Mineral	Loc†	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009
Ca	J	0.25	0.05	–0.01	–0.09	–0.15	–0.01	0.03	–0.18	–0.18	–0.01	–0.08	–0.18	0.19	0.37**
	E	–0.08	0.11	–0.15	0.1	–0.11	0.17	–0.06	0.33*	–0.08	0.27	–0.39**	0.05	–0.12	0.21
	N	0.65***	0.23	0.25	0.01	0.26	0.04	–0.30*	0.18	0.04	–0.02	–0.25	–0.29*	0.21	0.24
Cu	J			0.11	0.24	0.01	0.29*	0.13	0.27*	0.06	0.34*	0.19	0.22	0.21	–0.07
	E			0.05	–0.03	0.35**	0.25	0.38**	0.00	0.67***	0.21	0.24	0.43**	–0.19	–0.18
	N			0.21	0.22	0.15	0.62***	0.10	0.33*	–0.03	0.51***	0.12	0.46***	–0.06	–0.14
Fe	J					0.27*	0.60***	0.01	0.18	0.22	0.59***	0.16	0.64***	–0.04	–0.21
	E					0.43**	0.21	0.05	0.27*	0.07	0.13	0.37**	0.35**	0.09	0.15
	N					0.10	0.29*	0.10	0.41**	–0.03	0.24	0.06	0.33*	–0.02	–0.19
Mg	J							–0.06	0.18	0.75***	0.84***	0.55***	0.61***	–0.08	–0.21
	E							0.41**	0.40**	0.72***	0.91***	0.66***	0.35*	0.10	0.34*
	N							–0.06	0.40**	0.75***	0.84***	0.19	0.70***	–0.09	–0.37**
Mn	J									–0.10	0.20	0.52***	0.33*	0.22	–0.48***
	E									0.29*	0.29*	0.21	0.25	–0.17	–0.02
	N									–0.14	0.49***	0.67***	0.39**	–0.30*	–0.05
P	J											0.60***	0.72***	–0.40**	–0.15
	E											0.59***	0.32*	–0.15	0.44***
	N											0.26	0.76***	–0.11	–0.10
Zn	J													–0.17	–0.28*
	E													–0.03	–0.05
	N													–0.57***	–0.42

* Significant at $P < 0.05$.

** Significant a $P < 0.01$.

*** Significant at $P < 0.001$.

† J = Jubilee Farm, E = Evergreen College State Farm, N = Nash's Farm.

Table 6. Analyses of variance showing P values for genotype (G), location (L), and year (Y) main effects and their interactions for Ca, Cu, Fe, Mg, Mn, P, Zn concentration, and grain yield (yield).

Source	df	Ca	Cu	Fe	Mg	Mn	P	Zn	Yield
G	17	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
L	2	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
G×L	34	<0.0001	<0.0001	0.2211	<0.0001	0.0027	0.0051	<0.0001	<0.0001
Y	1	<0.0001	<0.0001	0.5538	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
G×Y	17	<0.0001	<0.0001	0.3183	0.0054	0.8367	0.1004	0.0156	<0.0001
L×Y	2	<0.0001	<0.0001	0.0154	<0.0001	0.0032	<0.0001	0.0006	<0.0001
G×L×Y†	34	<0.0001	<0.0001	0.0003	<0.0001	0.4088	<0.0001	0.003	<0.0001

† G×L×Y is also used as G×E within the text.

al., 2008; Morgounov et al., 2007; Murphy et al., 2009; Shi et al., 2008). Thus, indirect selection for any one mineral based on the concentration of another mineral may not be efficient due to correlations that may fluctuate considerably based on changes in genotypic, environmental, or climatic conditions.

Genotype × Environment Interaction

Significant G×E (G×L×Y) interactions were found for all traits except for Mn concentration (Table 6). Significant G×Y interactions existed for Ca, Cu, Mg, Zn, and grain yield (Table 6). Grain yield and all minerals except Fe had significant G×L interactions (Table 6). Similarly, Chatzav et al. (2010), found significant G×E interactions for all minerals tested except Fe in emmer. However, other studies have found significant G×E interactions for Fe (Joshi et al., 2010; Oury et al., 2006). Joshi et al. (2010) found that Fe and Zn concentrations were highly unstable across years and cultivars. However, Fe was much more stable across locations than Zn (Joshi et al., 2010). It is possible that within the target environment of western Washington state, enough environmental and temporal homogeneity exists that Fe will remain relatively stable. Based on results from other studies however, this does not appear to be normal for mega-environments.

Using ANOVA, each cultivar was tested individually for G×E as a statistical measure of its broad adaptation across years and locations (Table 7). In general, fewer cultivars were broadly adapted spatially than temporally. In particular, all of the cultivars tested had significantly different values across locations for Ca, Cu, Zn, and grain yield and all but one cultivar had significantly different values across locations for Mn (White Marquis) and P (Kelse). Four cultivars each for Fe (Penewawa, Scarlet, Wakanz, and White Marquis) and Mg (Alpowa, Louise, Wakanz, and Wawawai) showed stability across locations. In five environments across Turkey and Israel, S had relatively high stability; Cu, Zn, and Mn exhibited medium stability; and, Ca, Mg, Fe, P, and K exhibited low stability (Gomez-Becerra et al., 2010).

Particular traits, including grain mineral concentrations of Cu, Fe, and P showed broader adaptation across years. All cultivars except Wawawai showed no significant differences for Fe concentration across years. Copper and P concentrations had 11 and 12 cultivars with no significant differences, respectively, over years. Traits with only one cultivar that showed no significant differences over time were Mg and grain yield. Interestingly, this cultivar, Wakanz, was found as not having significant differences in five of the eight traits over years and two of the four traits that had cultivars with no significant differences over locations. Conversely, Spinckota, the cultivar with the highest concentrations of Fe and Zn had significant differences across locations and years for all traits tested. These results indicate it may be possible that broadly adapted cultivars (i.e., Wakanz) and narrowly adapted cultivars (i.e., Spinckota) can be identified for mineral concentration. However, from the results of this study it is difficult to determine whether a lack of significant differences for a particular trait in certain cultivars truly signifies broad adaptation. Therefore, more targeted research is needed to determine whether grain mineral concentration is a broadly or narrowly adapted trait when grown in contrasting environments over time.

Yield stability over time is often a higher breeding priority in organic breeding programs than yield potential in optimal climatic and agronomic conditions (Jones et al., 2010; Löschenberger et al., 2008; Wolfe et al., 2008). Regardless of the environmental and soil differences present at each farm and the climatic differences from year-to-year variation, certain cultivars exhibited broad adaptation for certain traits (Table 7) and have higher concentrations of particular minerals along with a higher grain yield than other cultivars. The highest yielding hard red cultivar, Kelse, and soft white cultivar Louise, should be reliably high yielding across all locations in western Washington. Additionally, Kelse had among the highest concentrations of Ca and Cu and a high protein content, so growers looking for a high yielding cultivar with high levels of particular nutrients may consider growing Kelse in their rotation. Alternatively, Canus was among the highest yielding cultivars, though significantly lower yielding than either Kelse or Louise, and was among the top five cultivars in this study for each mineral tested.

Due to climatic and environmental variations that occur from year to year and location to location, a strategy of blending

Table 7. Cultivars identified for each trait do not show significant differences* across years and/or locations.†

Trait	Years	Locations
Ca	Cadet, Canus, Comet, Idaed	none‡
Cu	Cadet, Canus, Louise, Penewawa, Reliance, Ruby, Scarlet, Wakanz, Wawawai, Westbred Express, Zak	none
Fe	Alpowa, Beaver, Cadet, Canus, Comet, Idaed, Kelse, Louise, Penewawa, Reliance, Ruby, Scarlet, Wakanz, Westbred Express, White Marquis, Zak	Penewawa, Scarlet, Wakanz, White Marquis
Mg	Wakanz	Alpowa, Louise, Wakanz, Wawawai
Mn	Beaver, Cadet, Canus, Comet, Penewawa, Scarlet	White Marquis
P	Alpowa, Beaver, Cadet, Comet, Idaed, Penewawa, Reliance, Ruby, Scarlet, Wakanz, Westbred Express, Zak	Kelse
Zn	Alpowa, Canus, Louise, Penewawa, Reliance, Wakanz, Wawawai	none
Yield	Wakanz	none

* Significance is assessed at $P < 0.05$.

† This is an indication of broad adaptation, both in individual traits and in individual cultivars.

‡ "None" indicates that all cultivars showed significant differences across locations for the trait indicated.

cultivars with key traits of interest may have a positive impact on stability in organic grain farming systems. Blending cultivars increases genetic diversity in the field and has the potential to improve farming systems by: (i) preventing disease outbreaks and/or reducing damage from pests and diseases; (ii) improving yield stability over fluctuating climatic conditions; (iii) yielding a more nutritionally complete grain crop; and/or, (iv) capturing more sunlight and nutrients and perhaps thereby increasing grain yield (Finckh et al., 2000; Finckh and Mundt, 1993; Kaut et al., 2009; Phillips and Wolfe, 2005; Smithson and Lenne, 1996).

There are several combinations of cultivars from those tested in this study that would result in potentially high-yielding blends with enhanced nutritional value. For example, a blend of the hard red cultivars Kelse and Spinckota should combine the high yield potential and short stature of Kelse with the moderate yielding, tall-strawed cultivar Spinckota. The combination of these two cultivars would result in grain high in all mineral nutrients tested in this study. Both have excellent test weights, similar and high protein contents, and are resistant (Kelse) and moderately resistant (Spinckota) to the current races of the stripe rust pathogen (data not shown). Likewise, the blend of hard red wheat cultivars Canus and Westbred Express would combine two cultivars of different heights, with the shorter wheat able to help keep the taller wheat from lodging. Again, both cultivars have high test weight and protein contents and moderate to high resistance to stripe rust. This blend should produce grain of high mineral concentrations for all mineral nutrients tested with enhanced yield stability.

A three-way blend of soft white wheat cultivars Louise, Beaver, and Wawawai could likewise show agronomic and nutritional potential. Louise and Wawawai are modern wheats with moderate plant height and Beaver is an historical cultivar with slightly taller straw and moderate grain yield. Louise has very high grain yields and could potentially compose 50% of this blend to help ensure that the end product would be profitable. Beaver and Wawawai complement each other in grain mineral concentration, together providing high levels of Fe, Mg, Mn, P, and Zn. All are moderately resistant to stripe rust and Louise and Wawawai have good test weight scores (data not shown). These cultivar blends may be the most economical and effective means available to farmers throughout western Washington State for growing wheat crops with maximal mineral concentration in spite of the G×E interactions which were shown to prevail within this mega-environment.

CONCLUSIONS

Genotypic differences exist for grain mineral concentration and grain yield in western Washington State and these differences can be exploited by growers to best optimize their farming and marketing needs. Identifying cultivars with high levels of specific mineral nutrients allows farmers to choose cultivars and/or cultivar blends that maximize the grain yield and nutritional content of the grain they market. The combination of organic farming with nutritionally dense wheat cultivars in specific regions may result in the successful establishment of value-added, locally produced and marketed, grain-integrated farming systems. Soil characteristics and fertility of individual farms play an important role in determining grain mineral concentration, though the influences of farming system, soil, and environment is difficult to pinpoint as many different factors are involved in influencing the nutritional value of wheat. Within one mega-environment, in this case western Washington State, G×E interactions were found for all traits except Mn concentration. It is impractical to select cultivars specific to each microenvironment within western Washington; therefore, carefully selected cultivar blends may prove most beneficial to farmers to optimize the mineral concentration of their yearly wheat crop.

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