A Comprehensive Genotype and Environment Assessment of Wheat Grain Ash Content in Oregon and Washington: Analysis of Variation

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ABSTRACT

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A comprehensive analysis of the variation in wheat grain ash content has not been previously conducted. This study assessed the relative contribution of genotype and environment to variation in ash content, with a particular aim of ascertaining the potential for manipulating the trait using contemporary adapted germplasm. A total of 2,240 samples were drawn from four years of multilocation field plots grown in the wheat production areas of Oregon and Washington states. Genotypes included commercial cultivars and advanced breeding lines of soft and hard winter, and soft and hard spring wheats with red and white kernel color, several soft white club wheats, and one soft white spring waxy wheat cultivar. In addition to ash, protein content, test weight, and Single Kernel Characterization System kernel hardness, weight and size were also measured. In total, 20 separate fully balanced ANOVA results were conducted. Whole model R^2 values were highly significant, 0.62–0.91. Nineteen of the 20 ANOVA results indicated significant genotype effects, but the

effects were not large. In contrast, environment effects were always highly significant with F values often one to two orders of magnitude larger than the genotype F values. The grand mean for all samples was 1.368% ash. For individual data sets, genotype means across environments varied ≈0.1–0.3% ash. The genotypes judged noteworthy because they had the highest least squares mean ash content were OR9900553 and ClearFirst soft white winter, NuHills hard red winter, Waxy-Pen and Cataldo soft white spring, and WA8010 and Lochsa hard spring wheats. Genotypes with lowest least squares mean ash were Edwin (club) soft white winter, OR2040073H hard red winter, WA7952 soft white spring, and WA8038 hard spring wheats. In conclusion, wheat grain ash is more greatly influenced by crop year and location than by genotype. However, sufficient genotype variation is present to plausibly manipulate this grain trait through traditional plant breeding.

Inorganic minerals are a minor constituent of wheat (*Triticum* spp.) grain, on the order of 1–2% by weight. Interest in the mineral content of wheat grain and flour lies in three areas: 1) flour mill performance, 2) flour quality, and 3) nutrition. The general consensus among cereal chemists is that ash has little to no relationship to flour baking performance per se. Yet ash content of flours may be among the most pervasive specifications imposed upon millers. As C.O. Swanson (1932, 1948) stated, "The primary value of the "ash" determination is to measure the thoroughness of the separation of the bran coat from the endosperm. It serves this purpose better than any other determination, and as such is indispensable in mill control. We know, but we need to emphasize that a high ash may be due to faulty milling or long extraction, but also to the fact that the endosperm of some wheat is higher in ash than in other wheat. The high ash is due to season, soil, and wheat variety." The utility of ash in mill control resides in the fact that bran tissues, especially the aleurone, are much higher in ash concentration than the central endosperm, which is on the order of 0.25–0.35%. The difference in concentration between two tissues may be as high as 45-fold (Morris et al 1945; Hinton 1959). Sur-

prisingly, considering the long-standing importance of ash to the cereal chemistry community, a survey of *Cereal Chemistry* from 1934 to the present identified few relevant reports on varietal differences in whole wheat ash. Anecdotally, it seems that it is widely "known" that cultivars differ in wheat ash and that environment also plays a major role (Swanson 1932, 1948; Posner 1991; Posner and Hibbs 1997).

Nordgren and Andrews (1941) reported that wheat ash among a set of six U.S. hard red spring wheat cultivars grown at four Minnesota locations was 1.63–2.28%. Cultivar mean values were 1.82–2.02%, whereas location means were 1.77–2.08%. Four winter wheat cultivars grown at four locations in Kansas and Nebraska were 1.57–1.77% ash (cultivar means), and 1.56–1.85% ash (location means). Seven hard red spring wheat cultivars grown in Winnipeg had ash contents of 1.44–1.81%. El Gindy et al (1957) studied the response of Pawnee hard red winter, Seneca soft red winter, and Cornell 595 soft white winter wheat cultivars to three fertilizer regimes in two soil types near Wooster, Ohio. ANOVA indicated that "The amount of ash in the whole wheat was influenced by cultivar more than anything else." Dikeman et al (1982) examined composites derived from the same individual beginning hard red winter wheat grain lots harvested in two different years. One series of composites included individual cultivars (pooled across locations), the other series included individual locations (pooled across cultivars). The varietal composites ranges were from lows of 1.82–1.69% to highs of 2.01–1.99% ash (years 1 and 2, respectively). The location composites ranges were 1.68–2.14%, and from 1.51–1.93% ash (years 1 and 2, respectively). Ayoub et al (1994) studied the response of four hard red spring wheat cultivars to N fertilization over two years and two sites in Quebec, Canada. N fertilization decreased ash content in three of four site-years with individual treatment mean values of 1.9–3.8% (high N, location 1, 1991 vs. zero N, location 2, 1990). Cultivar means across four site-years and fertilizer regimens were 2.16–2.40% ash, with significant differences in three of the four site-years.

Morris et al (1945) and Hinton (1959) conducted detailed analyses of the ash distribution of the wheat kernel. Morris et al (1945) examined multilocation and multiyear composites of Tenmarq hard red winter and Trumbull soft red winter wheat kernels

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and reported whole kernel ash values of 1.54 and 1.71%, respectively. Hinton (1959) reported on the ash content of 19 wheat samples from nine countries from around the world, the range was 1.38–1.91%.

A number of studies have examined the content of individual minerals in wheat grain (for example, see those cited by Peterson et al 1986) due to the importance that mineral nutrition plays in human health. Peterson et al (1986) analyzed 10 different minerals among a set of 27 world wheat cultivars. The ratio of environmental variance (σ^2_e) to genetic variance (σ^2_g), which is an indicator of the relative influence of environment versus genotype, was 6.76 for Ca to 0.66 for Mg. For comparison, grain protein content had a σ^2_e/σ^2_g ratio of 7.34, indicating that it is much more highly influenced by environment than by genotype. Although the studies have some relevance to wheat ash, in that ash is the mineral content of wheat, they do little to directly address the issue of genetic and environmental variation in whole wheat ash.

In summary, an assessment of the role of genotype and environment in variation for whole wheat ash is largely lacking. The present study examined a large number of wheat genotypes adapted to the U.S. Pacific Northwest that were grown side-by-side in multiple field plot nurseries in 2004-2007 (year of harvest).

Genotypes encompassed soft white winter common, soft white spring common, soft white club (winter and spring types), hard red winter, hard red spring, and hard white (winter and spring types). Genotypes were derived from ≈23 public and private breeding programs and included commercially grown cultivars and the most advanced breeding lines from these programs.

MATERIALS AND METHODS

A total of 2,240 grain samples were analyzed for whole grain ash and protein contents, test weight, and Single Kernel Characterization System 4100 kernel hardness, weight, and size. These samples originated from small plots (on the order of 3.3–7.5 m² depending on location) using common cultural practices as part of the Oregon State University (OSU) Oregon Winter Wheat Elite Yield Trials (OWEYT) and Washington State University (WSU) Cereal Variety Testing (CVT) Program. Genotypes were organized into four separate nurseries: soft winter, hard winter, soft spring, and hard spring. This classification ignores grain color (white or red) and spike morphology (club or lax head). The exact composition of each of these four nurseries varied according to state and crop year. Eltan soft white winter wheat was additionally included in the Washington hard winter nurseries; soft and hard spring wheat nurseries were only grown in Washington. Nurseries and samples were identified by crop year (crop year refers to the year of harvest; winter wheats were planted in the fall of the preceding calendar year). The crop years were 2004-2007 (Tables I–IV).

For a given state-crop year-nursery combination (for example, Oregon 2004 Soft Winter; Table I), all locations had an identical nursery composition (i.e., the same set of genotypes, in this case n = 22). The primary purpose of these nurseries was to provide agronomic and yield data on the most advanced breeding lines and commercial cultivars to farmers and plant breeders. Tables I–IV list for each state-crop year-nursery the number of genotypes and the locations in which they were grown. e-Xtra Supplementary Tables A and B list the individual genotypes for each state-crop year-nursery, and the specific locations where each nursery was grown. In total, the study included 101 unique environments (an environment is defined here as a combination of a geographical location and harvest year) from Oregon and Washington states. Many of the geographical locations (identified by a nearby place name) were used over multiple crop years (although the exact location of field plots varied due to crop rotation and other factors).

Grain samples were combine-harvested, cleaned, and an aliquot was ground to pass a 0.5-mm screen in a Udy cyclone mill. A subsample (≈2.5 g precisely weighed) of this ground whole grain was analyzed for moisture and ash using a TGA-601 thermogravimetric oven (Leco, St. Joseph, MI). Moisture was determined after a 1-hr hold at 150°C and ash after a 3-hr hold at 600°C gravimetrically. Throughout this report, ash is expressed on a 12% moisture basis. Clean grain was analyzed for bulk density (test weight) (Approved Method 55-10) (AACC International 2000), protein (Dumas combustion nitrogen × 5.7; Leco FP-528) (AACC Approved Method 46-30). A 300-kernel sample was analyzed using the SKCS 4100 (Perten Instruments, Springfield, IL) (AACC Approved Method 55-31).

In total, 20 separate fully balanced ANOVA tests were conducted using the general linear models (GLM) procedure (SAS v. 9.1.3) (SAS Institute, Cary, NC) according to state-crop year-nursery (see Tables I–IV). Type III mean squares were used to test genotype and environment model components (fixed). Genotype and environment variance components (random) were estimated using the REML estimation method in SAS Proc MIXED (covariance parameters estimates, standard errors and Z score significance levels). The REML Proc MIXED routine was also used to identify the extreme highest and lowest ash genotypes for a given nursery across all state-crop year combinations, based on least squares means (genotype fixed, environment random). Genotype and environment partial correlation coefficients (Pearson, r) were estimated using the SAS Proc GLM one-way multivariate routine across all state-crop year combinations for each nursery. Partial correlations were generated using the PRINTE option in the MANOVA statement from the error SS&CP matrix.

RESULTS

General Observations

A fairly large number of wheat genotypes (13–36) were included in each of the four nurseries each year. Each nursery was grown in three to six locations in either Oregon or Washington state (Tables I–IV). Each location, in combination with a specific crop year, represents a unique growing environment. The genotypes represented cultivars currently in commercial production or advanced breeding lines that were either in the Washington State Crop Improvement Association Foundation Seed Service or were of sufficient promise to be considered for release.

Balanced ANOVA for each state-crop year-nursery returned whole model R^2 of 0.62–0.91 (average of 0.81). Whole model F values in all instances were highly significant ($P < 0.0004$, data not shown). These results indicated that the simple factorial models were robust and that significant differences were present. Attention was thus turned to the model components of genotype and environment (the G-by-E interaction term could not be directly analyzed and was part of the error term).

In general, all but one ANOVA (hard spring WA 2007) returned significant genotype effects, but the effects were not large (Tables I–IV). Genotype F values were 1.61–6.78. In contrast, environment effects were always highly significant with much larger F values, often an order of magnitude larger than genotype F values, and at times approaching two orders of magnitude larger (Tables I–IV).

Over the entire sample set ($n = 2,240$) the average ash content for wheat grain was 1.368%. The means for each state-crop year-nursery are presented in Tables I–IV and ranged from 1.153% (hard winter WA 2005) to 1.532% (hard spring WA 2006). For individual state-crop year-nurseries, genotype means across environments varied ≈0.1–0.3% ash (genotype maximum minus minimum). Given the LSD values at α = 0.05 (Tables I–IV), the genotypic range encompassed from ≈1.7 to ≈4 LSD values. Considering environment means over genotypes for each state-crop year-nursery, maximum minus minimum differences were greater

than for genotypes, generally at ≈0.3–0.4% ash (minimum difference was 0.084%, maximum difference was 0.510%). In this case, considering the LSD values, the environment range often encompassed 6–10 LSD values or more (data not shown).

Variance components for genotype and environment for each of the state-crop year combinations for all four nurseries indicated that genotype was always a considerably smaller source of variation compared with environment (Tables I–IV). The ratio of the two variances ($\sigma_{Genotype}/\sigma_{Environment}$) was always <1 with a range of 0.03–0.645. Although a few of the variance ratios were ≈0.5, most were <0.2 and half were <0.1. These results indicate that the role of the environment was always a greater source of variation than genotype for wheat ash and in most instances 10–20 fold greater.

Specific Genotype Effects

As noted above, there existed significant genotype differences for all but one state-crop year-nursery data sets (Tables I–IV). An initial analysis was conducted based on these two-way ANOVA that used Duncan's multiple range test to identify extreme high and low ash genotypes (data not shown). Because this analysis produced a very large number of genotype comparisons, and that the genotypes varied across crop years (excluding checks), an alternative strategy was developed. Using Proc MIXED and

REML, all of the genotypes for each of the state-crop year combinations were combined (for each of the four nurseries) and least squares means (LS means) were calculated, these being adjusted for the marginal means of each environment. After ranking by LS means, the extreme six highest and six lowest ash genotypes were identified; these are listed in Table V due to their potential interest as genetic resources, i.e., genetic sources of high and low ash grain. The entire set of genotypes and their LS mean ash content is listed in e-Xtra Supplemental Table C.

Among the 89 unique soft white winter and club wheat genotypes, OR9900553 had the highest LS mean ash content at 1.46%, followed by ClearFirst, three experimental lines and Bitterroot (Table V). WA8020 was the sixth highest ranked with an ash content of 1.42% At the other extreme, Edwin club wheat cultivar was lowest ash at 1.26%. The other five lowest ash soft winter genotypes included experimental breeding lines from the WSU, OSU, AgriPro, Idaho and USDA-ARS breeding programs. ARS 97278-2 was sixth lowest ash with an ash content of 1.28%. There were 59 hard red and white winter wheat genotypes in the study (Eltan soft white was also included in this nursery). The highest LS mean ash content genotype was NuHills at 1.54%, followed by three breeding lines, Weston (a mixed hardness red winter) (Morris and King 2002) and WA8025 at 1.38% ash (Table V).

TABLE I
Analysis of Variance[a] Whole Model R^2 Values, Model Component F Values, Sample Statistics, and Variance Components for Wheat Grain Ash Content of Soft White Winter Common and Club Wheat Genotypes Grown in Oregon (OR) and Washington (WA) States During Various Crop Years

Component	OR 2004	OR 2006	OR 2007	WA 2004	WA 2005	WA 2006	WA 2007
Genotypes, n	22	27	23	34	27	36	22
Environments, n	6	6	6	6	6	6	5
Whole model R^2	0.81	0.85	0.84	0.85	0.91	0.83	0.80
Genotype F value	1.87*	4.77***	1.81*	4.23***	5.01***	3.48***	2.30*
Environment F value	81.2***	118.3***	104.5***	165.5***	247.0***	144.5***	69.8***
Grand mean (%)	1.434	1.415	1.321	1.344	1.304	1.377	1.235
Genotype max (%)	1.511	1.505	1.385	1.508	1.402	1.545	1.320
Genotype min (%)	1.343	1.323	1.245	1.262	1.235	1.280	1.164
LSD$_{(0.05)}$	0.098	0.064	0.078	0.080	0.059	0.071	0.081
Environment max (%)	1.719	1.606	1.595	1.605	1.600	1.600	1.385
Environment min (%)	1.268	1.242	1.201	1.185	1.194	1.258	1.119
$\sigma_{Genotype}$	0.00106	0.00199	0.000624	0.00267	0.00178	0.00187	0.00108
$\sigma_{Genotype}$ SE	0.000723	0.000696	0.000432	0.000866	0.000619	0.000610	0.000603
$\sigma_{Environment}$	0.0267	0.0137	0.0207	0.024	0.0241	0.00436	0.0129
$\sigma_{Environment}$ SE	0.171	0.00870	0.0132	0.0153	0.0153	0.00315	0.00931
$\sigma_{Genotype}/\sigma_{Environment}$	0.0397	0.146	0.0302	0.111	0.0740	0.429	0.0833

[a] Significance: ns > 0.05; * < 0.05; ** < 0.001; *** < 0.0001.

TABLE II
Analysis of Variance[a] Whole Model R^2 Values, Model Component F Values, Sample Statistics, and Variance Components for Wheat Grain Ash Content of Hard Red and White Winter Wheat Genotypes Grown in Oregon (OR) and Washington (WA) States During Various Crop Years

Component	OR 2006	WA 2004	WA 2005	WA 2006	WA 2007
Genotypes, n	19	20	18	19	23
Environments, n	3	6	3	3	5
Whole model R^2	0.88	0.78	0.68	0.67	0.62
Genotype F value	2.33*	6.54***	2.10*	1.71*	3.30***
Environment F value	111.8***	40.96***	18.02***	40.11***	17.37***
Grand mean (%)	1.347	1.342	1.153	1.391	1.204
Genotype max (%)	1.496	1.556	1.243	1.496	1.352
Genotype min (%)	1.216	1.233	1.110	1.276	1.114
LSD$_{(0.05)}$	0.135	0.097	0.075	0.105	0.100
Environment max (%)	1.572	1.491	1.205	1.543	1.310
Environment min (%)	1.226	1.171	1.121	1.262	1.140
$\sigma_{Genotype}$	0.00295	0.00659	0.000754	0.001006	0.00293
$\sigma_{Genotype}$ SE	0.00179	0.00259	0.000521	0.000711	0.00128
$\sigma_{Environment}$	0.0383	0.015	0.00195	0.0106	0.00454
$\sigma_{Environment}$ SE	0.0386	0.00972	0.00206	0.00767	0.00341
$\sigma_{Genotype}/\sigma_{Environment}$	0.0769	0.439	0.387	0.095	0.645

[a] ns > 0.05; * < 0.05; ** < 0.001; *** < 0.0001

The lowest hard winter was OR2040073H at 1.15% ash, followed by two sister lines of the same pedigree (WI88-052/Tomahawk/3/OR943576; OR943576 is Mildress/CI14482//Yamhill/Hyslop/3/Rondezvous). Two Idaho breeding lines and ACS52025 rounded out the lowest six genotypes. OR2040073H notably had the lowest LS mean ash content of any genotype in the study; however it should be pointed out that this value is confounded with the environments where each of the four nurseries were grown. It does, however, illustrate the low level of ash that is attainable in this production region.

The two spring wheat nurseries had higher overall ash content compared with the winter nurseries. Whether this is due to genetics or to the locations where the wheat plots were grown, or to a general difference in growth and development (for example, spring wheats generally fill grain and mature later in the season compared with winter wheats) cannot be delineated in the present study. Nevertheless, of the 33 soft white spring genotypes, Waxy-Pen, a full waxy, zero amylose cultivar had the highest LS mean ash at 1.57%. Waxy-Pen is a near-isogenic backcross derivative of Penawawa (Morris and King 2007); Penawawa was not included in the present study. This places Waxy-Pen as the overall highest LS mean ash content genotype in the entire study. The second highest ash content soft spring genotype was Cataldo followed by

four experimental breeding lines. WA7986 (sixth highest) had 1.49% ash. At the low-end range of ash for soft white spring genotypes were six experimental lines, ranging from the lowest at 1.28% (WA7952) to 1.34% (ID599). Lastly, the hard spring wheat nursery had as its highest LS mean ash entry the breeding line WA8010 (1.55%). Lochsa, Alta Blanca, Buck Pronto (an Argentine introduction), and two experimental lines rounded out the highest six. The lowest ash hard spring was WA8038 (1.35%) followed by another Washington breeding line and Otis. Two other Washington and one Idaho breeding lines accounted for the remaining three lowest ash genotypes.

Correlations Among Grain Traits

Pearson genotypic and environment partial correlation coefficients were calculated between wheat grain ash content and other grain and kernel traits, which included test weight, protein, and SKCS hardness, hardness standard deviation, kernel weight, kernel weight standard deviation, kernel diameter, and kernel diameter standard deviation on all individual grain samples, for the four nurseries. Generally, none of the correlations were especially high, indicating that inherent differences in ash content were not in large part a reflection of gross kernel morphological differences (either genetic or environmentally induced, such as shriveling). It

TABLE III
Analysis of Variance[a] Whole Model R^2 Values, Model Component F Values, Sample Statistics, and Variance Components
for Wheat Grain Ash Content of Soft White Spring Common and Club Wheat Genotypes
Grown in Washington (WA) State During Various Crop Years

Component	WA 2004	WA 2005	WA 2006	WA 2007
Genotypes, n	13	14	13	16
Environments, n	6	5	5	5
Whole model R^2	0.79	0.85	0.89	0.78
Genotype F value	3.05*	6.78***	4.75***	3.22**
Environment F value	36.75***	52.51***	81.76***	41.46***
Grand mean (%)	1.272	1.498	1.438	1.435
Genotype max (%)	1.337	1.650	1.548	1.562
Genotype min (%)	1.210	1.328	1.326	1.342
LSD$_{(0.05)}$	0.072	0.081	0.093	0.097
Environment max (%)	1.415	1.704	1.673	1.629
Environment min (%)	1.150	1.396	1.209	1.289
$\sigma_{Genotype}$	0.00132	0.00470	0.00419	0.00261
$\sigma_{Genotype\ SE}$	0.00081	0.00217	0.00224	0.00139
$\sigma_{Environment}$	0.0106	0.0150	0.0332	0.0149
$\sigma_{Environment\ SE}$	0.00688	0.0108	0.0238	0.0108
$\sigma_{Genotype}/\sigma_{Environment}$	0.124	0.314	0.126	0.175

[a] ns > 0.05; * < 0.05; ** < 0.001; *** < 0.0001

TABLE IV
Analysis of Variance[a] Whole Model R^2 Values, Model Component F Values, Sample Statistics, and Variance Components
for Wheat Grain Ash Content of Hard Red and White Spring Wheat Genotypes
Grown in Washington (WA) State During Various Crop Years

Component	WA 2004	WA 2005	WA 2006	WA 2007
Genotypes, n	17	21	25	26
Environments, n	5	5	4	5
Whole model R^2	0.91	0.89	0.90	0.67
Genotype F value	2.34*	3.08**	2.08*	1.61 ns
Environment F value	154.0***	144.9***	207.2***	41.39***
Grand mean (%)	1.388	1.525	1.532	1.405
Genotype max (%)	1.472	1.594	1.618	1.476
Genotype min (%)	1.316	1.448	1.410	1.296
LSD$_{(0.05)}$	0.086	0.075	0.096	0.101
Environment max (%)	1.642	1.808	1.705	1.551
Environment min (%)	1.132	1.418	1.289	1.305
$\sigma_{Genotype}$	0.00124	0.00149	0.00126	0.000796
$\sigma_{Genotype\ SE}$	0.000782	0.000723	0.000728	0.000619
$\sigma_{Environment}$	0.0415	0.0257	0.0386	0.0101
$\sigma_{Environment\ SE}$	0.0296	0.0183	0.0317	0.00729
$\sigma_{Genotype}/\sigma_{Environment}$	0.0298	0.0578	0.0326	0.0788

[a] ns > 0.05; * < 0.05; ** < 0.001; *** < 0.0001

should be noted that very low test weight (<U.S. No. 2) environments were not included in the study a priori. Those correlations that seemed to be the most informative are presented in Table VI. Of those correlations not shown, the three highest were SKCS kernel hardness and its standard deviation for genotypic effects for the soft white spring nursery (r = 0.33 and 0.29, respectively), and SKCS kernel diameter for genotypic effects for the soft winter wheat nursery (r = 0.30).

Ash tended to be negatively correlated with test weight, with r of –0.1 to –0.4, suggesting that lower test had some minor relationship with higher ash (Table VI). Genotypic and environment correlations were more-or-less similar with no consistent trend across nurseries. The coefficients for the soft winter and soft spring wheat nurseries were highest, explaining ≈6–16% of the variation (r^2). Wheat grain protein content, on the other hand, showed a distinct difference between the genotypic and environment correlations, the environment correlations always being larger. The genotypic correlations between ash and protein were low and variable, suggesting independence of traits. The environment correlations, however, were all similar at 0.30–0.46. This correlation could plausibly reflect either environmental conditions that affects both traits or, perhaps more likely, a reflection of mineral nutrition, fertilization, or other soil effects. Kernel weight correlations were variable and generally not significant; among the two winter wheat sets, there was a small positive genotypic relationship between kernel weight and ash, indicating that there exists genotypes with heavier kernels and higher ash. The environment correlations were mostly negative but not significant at P <0.001, suggesting that variation in kernel weight was not related to ash content. Interestingly, variability in kernel weight (standard deviation) was relatively high for the two winter wheat nurseries (r = 0.33 and 0.41) indicating that more variable kernel weights were positively correlated to higher ash content. If the higher kernel weight standard deviation was due to a greater number of smaller kernels (which would have a higher bran-to-endosperm ratio), it was not reflected in the mean kernel weight correlations, which were positive in sign.

DISCUSSION

The ash content of wheat grain produced in the primary production zones of Washington and Oregon states averaged ≈1.37% across all market classes, individual genotypes, specific environments and multiple crop years. For each of the four classes, the ash content standard deviation was consistently 0.16–0.18%. Therefore, ≈ 95% of all grain samples would lie within an interval of two times the standard deviation (0.32–0.36%). Generally, the ash contents encountered were lower than those reported in Minnesota, Kansas, Nebraska, and Manitoba (Nordgren and Andrews 1941), in composites from the Central and Southern U.S. Great Plains (Dikeman et al 1982), and Quebec (Ayoub et al 1994). The extent to which this trend is due to soil, climate, management, or genetics is at present unknown.

Of the variation in wheat ash that was encountered, the greater proportion was due to environment, that is, unique combinations of geographic location and crop year (Tables I–IV). This source of variation was often an order of magnitude or greater than that attributable to genotype. Nevertheless, there was clear support for genetic differences among cultivars and advanced breeding lines in all classes: winter, spring, hard, soft, white, red, club, and

TABLE V
Wheat Genotypes Ranked by Highest and Lowest Least Squares Mean Grain Ash[a]
Across Years and Environments in Oregon (OR) and Washington (WA) States

Soft White Winter Common and Club	Hard Red and White Winter	Soft White Spring Common and Club	Hard Red and White Spring
OR9900553	NuHills	Waxy-Pen	WA8010
ClearFirst	ARS3501	Cataldo	SX1504B
BZ6WM02-1154	W96-054W	ARS055303	Lochsa
ORH0100920	BZ9W02-2032	WA8059	Alta Blanca
Bitterroot	Weston	WA8008	BZ999-592
WA8020	WA8025	WA7986	Buck Pronto
ARS97278-2	ID641	ID599	WA7957
ID620	ACS52025	WA7961	ID592
99X1009-23	ID621	WA8039	WA7930
ORH010837	OR2040075H	WA8007	Otis
WA7934	OR2040074H	WA7920	WA7953
Edwin	OR2040073H	WA7952	WA8038

[a] Only the 6 most extreme genotypes are listed for highest (upper group) and lowest (lower group) least squares mean grain ash, ranked high to low in each grouping.

TABLE VI
Genotypic and Environment Correlations (r)[a] of Wheat Grain Ash Content with Kernel Traits[b]

	Soft White Winter	Hard Winter	Soft Spring	Hard Spring
Sample size	1124	403	293	420
Test weight correlation coefficient				
Genotypic	–0.32**	–0.11	–0.26**	–0.17*
Environment	–0.24**	–0.10	–0.40**	–0.12
Wheat protein correlation coefficient				
Genotypic	0.20**	–0.10	0.07	0.17*
Environment	0.32**	0.41**	0.46**	0.30**
SKCS kernel weight correlation coefficient				
Genotypic	0.11*	0.21**	–0.08	–0.07
Environment	–0.04	–0.11	–0.20	0.07
SKCS kernel weight standard deviation				
Genotypic	0.33**	0.41**	0.20	0.18
Environment	0.08	0.06	–0.05	0.01

[a] *, Indicates correlation is significance at 0.001–0.0001; **, indicates significance at <0.0001; all others have a P value > 0.001.
[b] SKCS, Single Kernel Characterization System.

common (Tables I–V). Differences were not great, however; generally on the order of 0.1–0.3%.

Analysis of the ash content of genotypes by nursery, but across all possible crop years and locations, using REML permitted the direct comparison of genotypes by least squares means (Table V and e-Xtra Supplementary Table C). The genotypes judged noteworthy because they had the highest LS mean ash content were OR9900553 and ClearFirst soft white winter, NuHills hard red winter, Waxy-Pen, and Cataldo soft white spring, and WA8010 and Lochsa hard spring wheats. Genotypes with lowest LS mean ash were Edwin (club) soft white winter, OR2040073H hard red winter, WA7952 soft white spring, and WA8038 hard spring wheats. It would be of particular interest to resolve further the genetic, biochemical, and physiological basis for these observed genotype differences.

Variation in wheat ash was generally only occasionally and to a minor extent related to other potentially confounding grain and kernel traits (Table VI). For example, samples with high ash were not highly correlated with low test weight, low kernel weight or small kernel diameter (Table VI and data not shown). The most notable relationships were 1) negative correlations between ash and test weight for the two soft wheat nurseries (genetic and environmental), indicating high ash was somewhat related to low test weight, 2) environmental correlations between ash and protein, indicating that locations that produced high protein also produced higher ash, and 3) positive genotypic correlations for the two winter nurseries between kernel weight and variability and ash. The possible basis for this last observation was not readily known.

Returning to the question posed by Swanson (1932, 1948), "Is there any relief from ash?", the present study indicates that from a breeding and genetic standpoint based on a very large set of diverse wheat genotypes originating from a large number of breeding programs the answer must be "very little". Generally, the extreme genotype values averaged across environments were on the order of a couple tenths of 1% ash. Clearly the single biggest reduction in wheat ash would result from originating grain from low ash environments. Whether these are consistent from a geographical (and practical) aspect is at present unknown. Also, at this time it is not possible to delineate a location and the farming and management practices applied there. We may agree with Swanson that the best use of ash in the field of cereal chemistry is, "to measure the thoroughness of the separation of the bran coat from the endosperm." Ash is a grain trait of wheat that appears to have limited but nevertheless heritable variation. In the future, it will be of interest to resolve further the individual components of ash from a nutritional and plant biological standpoint.

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