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Worldwide Genetic Diversity for Mineral Element Concentrations in Rice Grain

Shannon R. M Pinson,^{*} Lee Tarpley, Wengui Yan, Kathleen Yeater, Brett Lahner, Elena Yakubova, Xin-Yuan Huang, Min Zhang, Mary Lou Guerinot, and David E. Salt

ABSTRACT

With the aim of identifying rice (*Oryza* spp.) germplasm having enhanced grain nutritional value, the mineral nutrient and trace element concentrations (or ionome) of whole (unmilled) grains from a set of 1763 rice accessions of diverse geographic and genetic origin were evaluated. Seed for analysis of P, Mg, K, S, Ca, As, Cd, Co, Cu, Fe, Mn, Mo, Ni, Rb, Sr, and Zn concentrations by inductively coupled plasma mass spectrometry was produced over 2 yr in Beaumont, TX, under both flooded and unflooded watering regimes. The distributions of all element concentrations analyzed were skewed toward higher concentration. A significant portion of this ionic variation has a genetic basis (broad sense heritabilities 0.14–0.75), indicating an ability to breed for improved grain concentration of all elements except possibly Ni. Variation in grain elemental concentrations was not strongly associated with plant height, heading time, or grain shape, suggesting these physiological factors are not of primary importance in controlling ionic variation in rice grain. Accessions high in specific elements were sometimes found to have similar genetic or geographic origins, suggesting they share a heritable mechanism underlying their enhanced ionomes. For example, accessions with high Ca, Mg, or K were more common in the *indica* than in the *japonica* subgroup; low As was most common among temperate *japonica* accessions; and several lines high in Mo originated in Malaysia or adjacent Brunei.

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Abbreviations: ARO, *aromatic* subgroup; AUS, *aus* subgroup; DHD, days from planting to heading (also known as flowering); GL, grain length; GW, grain width; H^2 , broad sense heritability; ICP–MS, inductively coupled plasma mass spectrometry; IND, *indica* subgroup; LS Mean, least squares mean; PCA, principal component analysis; QTL, quantitative trait locus (QTLs, quantitative trait loci); TEJ, temperate *japonica* subgroup; TRJ, tropical *japonica* subgroup.

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WITH ABOUT HALF the world's human population dependent on rice (*Oryza* spp.) as a staple food, the grain provides the majority of dietary nutrients to billions of people. Unfortunately, rice grains are not dense in mineral nutrients, making nutrient deficiencies common among those dependent on rice as a dietary staple (Grusak and Della Penna, 1999). In addition to dietary supplementation and industrial food fortification, biofortification via the development of new crop cultivars with increased concentration of nutrients in their edible portions has been proposed as a possible solution to alleviate global malnutrition (e.g., Graham et al., 1999, 2001; Gregorio et al., 2000; White and Broadley, 2005; Bouis and Welch, 2010; Masuda et al., 2013; Rawat et al., 2013; Murgia et al., 2013). In fact, studies have documented improved health indicators from human consumption of crops biofortified for iron and β -carotene (Haas et al., 2005; van Jaarsveld et al., 2005; Low et al., 2007; Cercamondi et al., 2013). While most white (milled) rice in U.S. grocery stores is fortified by adding nutrients to the external surface of the milled rice grains (Flour Fortification Initiative, 2012), enhancement of the rice grain's nutritional value through genetic improvements could include both increasing concentrations of desirable elements (e.g., Fe, Zn, or Ca) and decreasing concentrations of undesirable elements (e.g., As or Cd) and could create new marketing strategies for nutritionally enhanced, value-added products. If the biofortification is accomplished through traditional breeding and selection, rather than through genetic engineering, then the nutritionally enhanced crops would not require special regulation or documentation and could thus be deployed more rapidly. Cultivars biofortified through traditional breeding methods would be able to acquire organic certification if grown under organic field conditions, and use of alleles conferring intermediate rather than extreme phenotypes found naturally functional among diverse rice accessions could be used to fine-tune grain element concentrations.

The most prevalent nutrient deficiencies worldwide are Fe and Zn, with estimates that more than 25% of the world's population are at risk of mild to severe deficiency in one or both elements and with each element attributable for the deaths of 0.8 million persons per year (World Health Organization [WHO], 2002, 2009; Maret and Sandstead, 2006). Because of significant interest in biofortifying grains for Fe and Zn, numerous studies have focused on the routes and mechanisms governing accumulation of Fe and Zn in crop grains (for reviews, see Stangoulis, 2010; Masuda et al., 2013). In that the seed serves as an energy and nutrient source for germinating seedlings, grain biofortification might also offer opportunity to enhance crop health. For example, reduced concentrations of P in seed have been associated with reduced germination and seedling vigor (Bolland and Baker, 1988; Robinson et al., 2012;

White and Veneklaas, 2012), though the association may not be obligatory (Rose et al., 2013a). The first step toward breeding rice cultivars with an enhanced elemental composition (or ionome) is to understand the genetic diversity in germplasm collections available to breeders.

Rice is grown under a wide range of environmental conditions around the world. It is produced below sea level as well as in mountainside terraces as much as 1800 m above sea level, in tropical equatorial countries as well in the more temperate areas of Eastern and Western Europe. Although rice is most commonly produced under a shallow flood (8 to 20 cm water) in the United States, on a global view it is also commonly grown under upland (aerated rainfed) conditions and even under deep-water flood (more than 50 cm) in tropical areas that experience a monsoon season. Grassi et al. (2009) showed that a cultivar developed for upland production yielded more when grown flooded, indicating that an aerobic or unflooded production system limits rice yields even in the absence of water stress or drought. It has been shown that the concentrations of various elements in rice grain are so affected by the soil and other aspects of the environment under which a rice grain is produced that grain ionome profiles can be used to distinguish among rices produced in different countries and geographical regions of the world (Kelly et al., 2002; González et al., 2011; Li et al., 2012). This suggests that rice cultivars adapted to geographic regions where a particular element is especially lacking, or present in excess, may express genes for enhanced ability to mine those elements out of the soil and/or partition them to other organs once taken up by the plant's roots or compartmentalized in leaves. While Zn deficiency is widespread throughout the rice-growing regions of the world, deficiencies and toxicities of other elements (e.g., As, Ca, Fe, K, and Mo) are more specific to certain soil types or irrigation conditions. It is likely that landraces and cultivars adapted to these particular geographic regions may contain enhanced ability to absorb, exclude, or detoxify specific elements. One goal of the present study was to compare the grain ionome of 1763 diverse rice accessions to determine if rice accessions adapted to a particular region of the world or if those from a particular ancestral lineage were more likely to produce rice grains with reduced or elevated concentrations of one or more of the 16 elements (P, Mg, K, S, Ca, As, Cd, Co, Cu, Fe, Mn, Mo, Ni, Rb, Sr, Zn) we analyzed.

A second goal of the study was to evaluate correlations between the elements and plant traits, as these can implicate mechanisms of element uptake, transport, and grain accumulation. Because soil redox potential is known to significantly alter the availability of many soil mineral elements to plant uptake, seed from the diverse rice accessions was produced under both flooded and unflooded (flush irrigated) field conditions. While it is generally accepted

that mineral elements may be remobilized from vegetative plant tissues to developing grains (Hocking and Pate, 1977; Himelblau and Amasino, 2001), recent study also indicates that major portions of mineral elements found in seeds have been supplied through root uptake and translocation during grain fill (Sperotto et al., 2012). It is difficult to know how to efficiently improve a process that is not already well understood. Thus, one barrier slowing the breeding and development of biofortified crops is a lack of understanding of the network of physicochemical and regulatory processes that are responsible for directing the element uptake by roots and subsequent translocation of the mineral elements into and out of vegetative tissues before they can be ultimately carried or loaded into developing grains. The third goal of this study, assuming sufficient differentiation existed, was to identify from among the 1763 widely diverse rice accessions those with extreme grain ionomes for use in further studies aimed at identifying genes, physiology, and biochemical factors underlying their differences in grain element concentrations.

MATERIALS AND METHODS

Genetic Materials

A total of 1763 highly diverse rice accessions (each a different genotype) collected from around the world (Supplemental Table S1) were compared for grain element concentrations. A majority (1748) are in the USDA Core Collection, which represents genetic diversity among ~20,000 accessions of rice germplasm originated from 116 countries (Yan et al., 2007, 2010). Among the five subgroup or ancestral lineages of rice (Garris et al., 2005; Zhao et al., 2011; Ren et al., 2013), this study included 578 accessions of the *indica* (IND) accessions, 412 temperate *japonica* (TEJ) accessions, 358 tropical *japonicas* (TRJ), 169 *aus* (AUS), 63 *aromatic* (ARO) accessions, and 183 accessions considered admixtures (<60% of 72 simple sequence repeat markers from any one subgroup) among these subgroups (Agrama et al., 2010).

All *O. sativa* and *O. glaberrima* accessions in the USDA Rice Core Collection were planted, but those that did not produce sufficient quality and quantity of seed for grain analysis in the 2007 fields were eliminated from further study. To evaluate the suitability of identifying quantitative trait loci (QTLs) within preexistent mapping populations, 15 parents of widely studied biparental rice mapping populations were included in the study, 8 of which also represent modern (released after 1980) southern U.S. TRJ rice cultivars (Cybonnet, Cocodrie, Jefferson, Kaybonnet, Lemont, LaGrue, Saber, and Rosemont), and 7 of which were cultivars of international origin (Azucena [TEJ], Dragon Eyeball 100 [IND], Jasmine 85 [IND], Kasalath [IND], RT0034 [IND], TeQing [IND], and Zhe733 [IND]). With 'Gulfmont' and 'Cypress' included in the USDA Core Collection, a total of 10 modern southern U.S. rice cultivars were evaluated for their grain ionomes.

Field-Plot Planting and Management

Seed samples for grain ionomic analyses were produced in small field plots in Beaumont, TX, using the same planting, field management, and harvest methods used in companion studies (Norton et al., 2012, 2014; Zhang et al., 2014). Because availability of many elements in soil is known to be greatly affected by soil redox potential, this rice grain study evaluated grains from the same set of diverse rice accessions grown over 2 yr under both flooded (anaerobic) and unflooded (aerobic, flush irrigated) irrigation schemes. All field plots were planted into soil classified as League clay (fine, smectitic, hypothermic Oxyaquic Dystrudert; Chen et al., 1989). Plots were fertilized 3 to 5 wk before planting with 33.6 kg/ha P and at the time of planting with 73 kg/ha N (as urea). Plots were drill-seeded approximately 2-cm deep into a dry seedbed using a Hege 80 Plot Seeder (Wintersteiger Inc., Salt Lake City, UT). Germination was initiated with the first flush irrigation, applied 1 d after planting. Additional flush irrigations were applied as needed to maintain soil moisture until seedlings of the shorter accessions were approximately 9-cm tall, at which time a 7- to 16-cm depth flood was applied and maintained on the flooded fields until the last plots per field were fully mature and hand-harvested. Unflooded fields received regular flush irrigations (once or twice a week) as necessary to prevent water stress while maintaining aerated soil conditions.

Use of small (13-cm) plot lengths, hereafter called hillplots, allowed each replication to be drill-seeded in a single day into a single paddy or irrigation area (Fig. 1A). Two replications of each Core accession were planted per water treatment (flooded versus unflooded) in both 2007 and 2008 at two different planting dates (replications) per year, with approximately 3 wk between planting dates. Because of storm damage, however, seed for grain analysis was obtained from a total of three flooded replications (both early and late plantings in 2007, early planting only in 2008) and three unflooded replications (early planting only in 2007, both early and late plantings in 2008). Experimental units consisted of a single hillplot per genotype (5 to 6 seeds per plot) per water treatment, with two planting replications per year. Hillplots were arranged in rows 25-cm apart, with each 240-cm field-row containing five individual hillplots, each separated by 47 cm. The relatively wide spacing between hillplots minimized plot-to-plot interactions from either shading or root-zone overlap. Field rows were arranged as five tiers of 100 rows each in a rectangular 28 × 20 m² field area surrounded by an irrigation levee, with a maximum distance of 34 m between plots in opposite corners of each replication. To evaluate variance due to field heterogeneity, 20 plots of check cultivars were planted in a 4 × 5 grid pattern throughout each treatment block (paddy). Each check-plot consisted of three to five hillplots (five planted, not all grew) per each of three check cultivars. Lemont was used to represent typical modern U.S. tropical *japonica* cultivars among the checks. TeQing, a cultivar from China, represented *indica* germplasm having taller plant height, later maturity, more numerous tillers (branches) per plant, and a larger root system than Lemont. The third check cultivar, Dragon Eyeball 100, is one of several rice cultivars valued by consumers in China for the high iron concentration of its unmilled, purple-bran kernels.

Lodging of the tall and/or weak-stemmed accessions was prevented by tying the main culms of each hillplot to

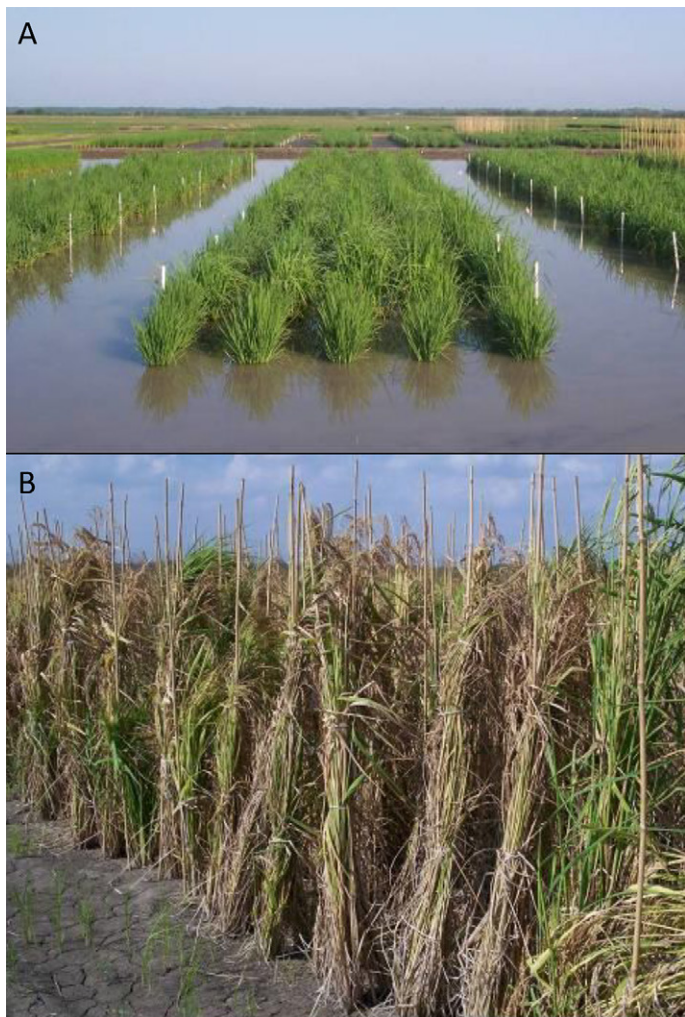


Figure 1. Field plots as observed (a) early and (b) late season, both flooded and unflooded (foreground and background in [a]). To minimize soil variance between plots and allow an entire replication to fit within a single irrigation paddy (field block), small hillplots were used, one per accession per field block. During midseason, plants of hillplots were tied to bamboo stakes to prevent lodging.

a bamboo stake (Fig. 1B). The date of 50% heading was recorded for each hillplot. Days to heading (DHD) was calculated by subtracting the date of first irrigation after planting (0 to 1 d post planting) from the heading date recorded for each plot. Mature panicles were hand-harvested. Rough rice samples were dried to 12% moisture using an ambient-forced-air drier then stored at ambient temperature in sealed plastic boxes until subsampled for phenotypic analysis.

Determination of Element Concentrations in Unmilled Rice Grain

The concentrations of 16 elements were simultaneously determined in samples of brown (unmilled) rice grains using inductively coupled plasma mass spectrometry (ICP-MS). Unmilled rather than white (milled) kernels were evaluated to allow detection of variation in concentrations of elements that primarily accumulate in bran as well as those that accumulate primarily in endosperm and to prevent confounding the data with variance from potential milling differences (Hansen et al.,

2012; Saenchai et al., 2012). The study of whole-grain brown rice makes this study consistent with, and more directly relevant to, biofortification research in other grain crops such as wheat (*Triticum aestivum* L.) (Salunke et al., 2012; Wu et al., 2013; Borrill et al., 2014), maize (*Zea mays* L.) (Baxter et al., 2012a), and pearl millet (*Pennisetum glaucum*) (Cercamondi et al., 2013) as well as prior studies in *Arabidopsis thaliana* (Vreugdenhil et al., 2004; Baxter et al., 2012b) and rice (e.g., Norton et al., 2012, 2014; Kuramata et al., 2013; Zhang et al., 2014). The convention in this report will be to report mineral concentrations as micrograms per gram grain dry weight ($\mu\text{g g}^{-1}$) and to present the five plant macronutrients first (P, Mg, K, S, Ca) in high to low order of their grain concentration, followed by the remaining 11 elements in alphabetical order (As, Cd, Co, Cu, Fe, Mn, Mo, Ni, Rb, Sr, Zn). The methods used to harvest and select seed for grain analysis are described in more detail by Zhang et al. (2014). Methods used to digest the kernels and then analyze them by ICP-MS are described elsewhere (Norton et al., 2012; Zhang et al., 2014). Briefly, 10 to 15 fully mature grains were hand-threshed from the tips of 3 panicles harvested per hillplot, then dehulled using a Satake TH035A sheller (Satake Engineering Co. Ltd.), on which the Zn-containing rubber lining the rollers (Stangoulis, 2010) was replaced with PU40 polyurethane plastic. From this, three complete (unbroken and unmilled) kernels were selected, (approximate weight 0.05 g) and digested with 1.0 mL concentrated HNO_3 in 16×100 mm Pyrex tubes at temperatures stepped from ambient to 110°C over a period of 12 h. Indium (EM Science) was added to the acid to a final concentration of $20 \mu\text{g L}^{-1}$ as an internal standard. Samples were diluted to 10.0 mL and analyzed for their total elemental composition on a PerkinElmer Elan DRCe ICP-MS (PerkinElmer Corp.) using the following stable isotopes: P31, K39, Mg25, S34, Ca43, As75, Cd114, Co59, Cu65, Fe57, Mn55, Mo98, Ni60, Rb85, Sr88, and Zn66 and known natural isotopic abundances. To normalize data between machine runs to correct for drift, portions of the samples were combined and used as a matrix-matched standard, measured after every nine samples. Samples were normalized to the averaged signals of the best-measured elements and weights of seven samples per run (Lahner et al., 2003). All ICP-MS data on rice grain used in this study is publicly available at www.ionomicshub.org (accessed 9 Sept. 2014).

Preliminary Analyses of Effects Due to Water Management Treatment and Spatial Variance within Field Replications

The phenotypic variance observed for each element among the plots of repeated checks was examined further so that knowledge derived among the checks on magnitude of variance due to water treatment, planting time, and within-field spatial effects could be used to select statistical models most appropriate for evaluating the larger (1763 diverse accessions) data set. Using the Fit Model application of JMP version 9.0 (SAS Institute, 2010), the variance of each element response was first assessed on the basis of the partitions of sum of squares in models with Genotype, Water treatment, Year, Replication(within Year) (which also reflected differences in planting time), Tier(within Replication), Column(within Replication), and interaction terms. The water treatment (flooded vs. unflooded) effects were

found to be so proportionally large for all 16 elements (>90.7%) that all other factors in the model had extremely minor effects in comparison. Therefore, flooded and unflooded data were kept separate in the following variance-partitioning analyses.

It was initially anticipated that a 3-wk difference in planting time between the two replications per year would result in a growing season so different that Replication(within Year) effects would be similar to Year effects. This hypothesis proved true with variance analyses indicating Replication effects in flooded and unflooded fields up to 30 times greater than Year effects. When variance was partitioned into Genotype, Replication, Tier(within Replication), Column(within Replication), and interaction effects, the terms reflecting within-field spatial variance (Tier, Column, and Tier \times Column) were insignificant (at $\alpha = 0.05$) for the majority of elements. Even for those elements for which they were statistically significant (As, Co, Fe, Ni, and Rb flooded; Cd, Fe, Mn and Rb unflooded), they were relatively minor, with Genotype, Replication and Genotype \times Replication together accounting for 90 to 97% of the variance attributed to all the model factors combined. In fact, Genotype was the single largest factor for most elements in both flooded and unflooded conditions. The few elements for which environmental factors (Replication and/or Genotype \times Replication) were larger than Genotype effects were Ni and Sr flooded, and As, Ni, and Sr under unflooded conditions.

Means and Comparisons among the 1763 Diverse Rice Accessions

In response to the large effects of water treatment seen among the check plots, and the small size and lack of useful trends observed for the within-field variance, comparisons made among the 1763 accessions were based on least squares means (LS means) calculated across the three replications per water treatment per accession using SAS (version 9.1, SAS Institute 2007). Partitioning of the variances per element into Subgroup, Geographic Region of Origin, and the interaction effects indicated significantly greater effects from Subgroup than Geographic Region of Origin, though both effects were statistically significant. Therefore, the results and discussion section focuses primarily on differences among ancestral subgroups rather than differences among accessions from different global regions, but all comparisons were made and considered during the data interpretations.

In addition to the grain element concentrations and DHD data collected from the 2007 and 2008 Texas hillplots, data on DHD, plant height (Ht, in cm), grain weight, and shape dimensions among the Core accessions from previous Arkansas flooded field evaluations of the USDA Core accessions (Yan et al., 2007) were analyzed. The following statistical analyses were performed using JMP version 9.0 (SAS Institute, 2010). Pearson correlations among the 16 elements, DHD, and grain dimensions included numerous pairwise comparisons, which can increase type I error. Therefore significance thresholds ($r \geq 0.310$ for both flooded and unflooded conditions) were determined using Bonferroni's adjustments (Abdi, 2007). Differences among various data subsets (e.g., water treatments, subgroups, or regions of origin) were tested for significance using ANOVA. The principal component analyses were performed using correlation matrices as input, thus all data were both standardized (Z-scale transformed) and mean-centered; unrotated results are provided.

RESULTS AND DISCUSSION

Trait Averages and Variances among 1763 Rice Accessions Grown Flooded and Unflooded

Data on the LS means, ranges, and variances of the grain element concentrations averaged over the 1763 accessions grown under both flooded and unflooded conditions are presented in Table 1. Soil chemistry and mineral element availability are known to differ between flooded (anaerobic, chemically reduced) and unflooded (oxidized) fields (De Datta, 1987; Daum et al., 2002; Zhang et al., 2004; Arao et al., 2009; Sahrawat, 2012). Some elements are more available for plant uptake when they are chemically reduced than when oxidized in aerobic soil, for example, Fe and As. The element with the largest observed change in grain concentration due to water treatment was As, with 30X higher concentrations in grains produced under flooded conditions than unflooded conditions. The elements next most sensitive to water treatment differences were Cd and Ni, which, in contrast to As, averaged 10X lower concentrations in grains grown under flooded rather than unflooded conditions. When making selections, breeders often seek to use growth conditions that maximize the phenotypic differences among genotypes. For most elements (all except Ni and As), a wider range in grain concentrations (max./min. ratios, Table 1) was observed under unflooded than under flooded conditions, initially suggesting that breeder selections for grain concentration of most elements might be more efficient in unflooded than flooded fields. Phenotypic variance is most useful when it is due to genetic rather than environmental effects. The proportion of total phenotypic variance explained by genotype is known as broad sense heritability (H^2). As a point of reference, maturity (as measured by DHD) is generally considered a highly heritable trait in rice and had $H^2 = 0.9$ under both flooded and unflooded conditions in this study. The heritabilities of the 16 elements ranged from 0.14 to 0.69 under flooded conditions (average 0.49) and from 0.23 to 0.75 under unflooded conditions (avg. 0.57) (Table 1). Fourteen of the 16 elements had $H^2 > 0.5$ under one or both field conditions, indicating that grain element concentrations could be improved through breeder selections conducted on seed produced in one growing condition, if not both, for these 14 elements. Nickel had the lowest H^2 (≤ 0.24) under both flooded and unflooded conditions, followed by P ($H^2 \approx 0.3$ under both conditions). Although genetic variability was statistically significant for both Ni and P, indicating the potential to make breeding improvement, with low relatively heritabilities, breeding progress would be expected to be slower than for elements with higher H^2 .

Partitioning of variance indicated that for all elements except As, Subgroup explained proportionally more variance (1.5 to 16 times more) than Geographic Region of Origin across both flooded and unflooded conditions

Table 1. Summary of grain element concentrations ($\mu\text{g g}^{-1}$) from flooded and unflooded field studies of 1763 rice (*Oryza sativa* L.) accessions. Minimum (min.), maximum (max.), max./min. ratio, mean, standard deviation (SD), coefficient of variance (CV), and heritability (broad sense) based on least squares means calculated for 1763 accessions across three replications per water-irrigation treatment. FI, flooded; Unfl, unflooded.

	FI							Unfl							Ratio of FI/Unfl means
	Min.	Max.	Max./Min. ratio	Mean	SD	CV	Heritability	Min.	Max.	Max./Min. ratio	Mean	SD	CV	Heritability	
P	2985	4734	1.6	3792	249	0.066	0.31	2198	4748	2.2	3455	283	0.082	0.29	1.1
K	2260	4541	2.0	2940	294	0.100	0.36	1565	4308	2.8	2738	295	0.108	0.61	1.1
Mg	1191	2006	1.7	1566	113	0.072	0.57	955	1916	2.0	1411	118	0.084	0.41	1.1
S	520	1757	3.4	1171	160	0.137	0.46	153	2912	19.1	1435	202	0.140	0.65	0.8
Ca	75.3	255.8	3.4	129.4	25.6	0.198	0.56	51.9	502.3	9.7	130.9	29.4	0.224	0.57	1.0
As	0.217	2.610	12.1	0.945	0.312	0.330	0.57	0.001	0.125	125.2	0.030	0.019	0.631	0.64	31.2
Cd	0.002	0.087	40.7	0.010	0.006	0.598	0.24	0.010	1.543	154.3	0.102	0.097	0.951	0.63	0.1
Co	0.010	0.241	23.8	0.052	0.022	0.425	0.57	0.004	0.240	62.5	0.037	0.020	0.555	0.67	1.4
Cu	1.756	5.397	3.1	3.340	0.565	0.169	0.46	3.300	11.354	3.4	6.444	1.100	0.171	0.75	0.5
Fe	1.55	16.58	10.7	11.45	1.40	0.122	0.47	0.09	25.89	287.6	12.04	3.18	0.264	0.73	1.0
Mn	14.35	46.51	3.2	24.72	4.15	0.168	0.58	15.37	76.00	4.9	35.94	7.70	0.214	0.55	0.7
Mo	0.063	2.059	32.4	0.429	0.118	0.274	0.69	0.084	2.006	24.0	0.388	0.105	0.271	0.53	1.1
Ni	0.298	6.036	20.3	1.573	0.495	0.315	0.14	4.073	37.828	9.3	10.963	2.359	0.215	0.23	0.1
Rb	3.93	24.48	6.2	10.78	2.53	0.235	0.64	0.30	35.81	120.9	11.77	4.10	0.348	0.73	0.9
Sr	0.303	1.425	4.7	0.555	0.134	0.241	0.68	0.140	2.035	14.5	0.417	0.121	0.290	0.64	1.3
Zn	15.72	65.01	4.1	24.29	3.55	0.146	0.50	19.34	63.13	3.3	29.99	4.07	0.136	0.47	0.8
DHD	52.5	129.5	2.5	90.4	14.3	0.159	0.91	54.5	118.6	2.2	93.7	13.5	0.144	0.87	1.0

(Supplemental Table S2). This is consistent with the fact that among the 1763 accessions in this study, accessions of multiple subgroups were collected from each of the 14 worldwide regions (Supplemental Fig. 1). In that the IND and AUS rice cultivars are more commonly grown in tropical regions with longer growing seasons than the regions where the more cold-tolerant TEJ accessions predominate, it was not unexpected to find that the TEJ subgroup has the lowest average DHD among the subgroups (Figs. 2 and 3). Because of long-term cultural preferences and breeding selections, the TEJ subgroup also has the widest average grain width (GW), the shortest average grain length (GL), and thus the roundest (smallest GL/GW ratio) grain average among the subgroups (Fig. 4). Because of nonrandom differences among the subgroups, such as described here for grain shape and DHD, all further data analyses were conducted per individual subgroup as well as across all 1763 accessions.

Consistent with known changes in availability of elements in reduced (flooded) soil solution, average grain concentrations of As were higher, and Cd and Ni were lower in rice grains produced on flooded than on unflooded plants (Table 1). While other studies have reported reduced grain Fe under flooded conditions (Zhang et al., 2014), contrary to what one would expect on the basis of increased availability of Fe in soil solution, the mean grain Fe across the 1763 was not significantly different between the two water treatments. It has been suggested that altered grain accumulation of Fe and some other elements under flooded conditions may be due to the iron

plaque (layer of oxidized Fe) that forms on the root surfaces of flooded rice plants and has been shown to increase the uptake or access of some elements (e.g., P, Zhang et al., 1999), but decrease plant uptake or access of others (e.g., Zn [Zhang et al., 1998; Wu et al., 2010], Se [Zhou et al., 2007], and As [Hu et al., 2005; Wu et al., 2012]). In addition to soil chemistry, changes in root morphology are also associated with soil aeration, with rice plants producing more surface-level roots under flooded conditions but deeper roots under unflooded growth conditions (Hoshikawa, 1989; Lafitte et al., 2001; Uga et al., 2012; Rose et al., 2013b). The surface-level roots are primarily in a soil zone that maintains some oxygen content, thus allowing access to mineral elements that are less soluble under the reduced conditions deeper in the flooded soil (De Datta, 1987, Rose et al., 2013b).

Accessions with Elevated Grain Concentrations Were Identified for Each Element

The distributions of each of the 16 elements were positively skewed, with right-hand or positive tail longer than the left (negative) tail, and a population mean greater than (to the right of) both the median and the distribution peak (Figs. 2 and 3; Supplemental Table S1). The rarity of accessions in the extended upper tails (i.e., above the 90th percentiles, Figs. 2 and 3) combined with the 2- to 200-fold range in grain concentration observed among the elements within this set of diverse rice accessions (Table 1) suggest that the rare accessions having exceptionally high

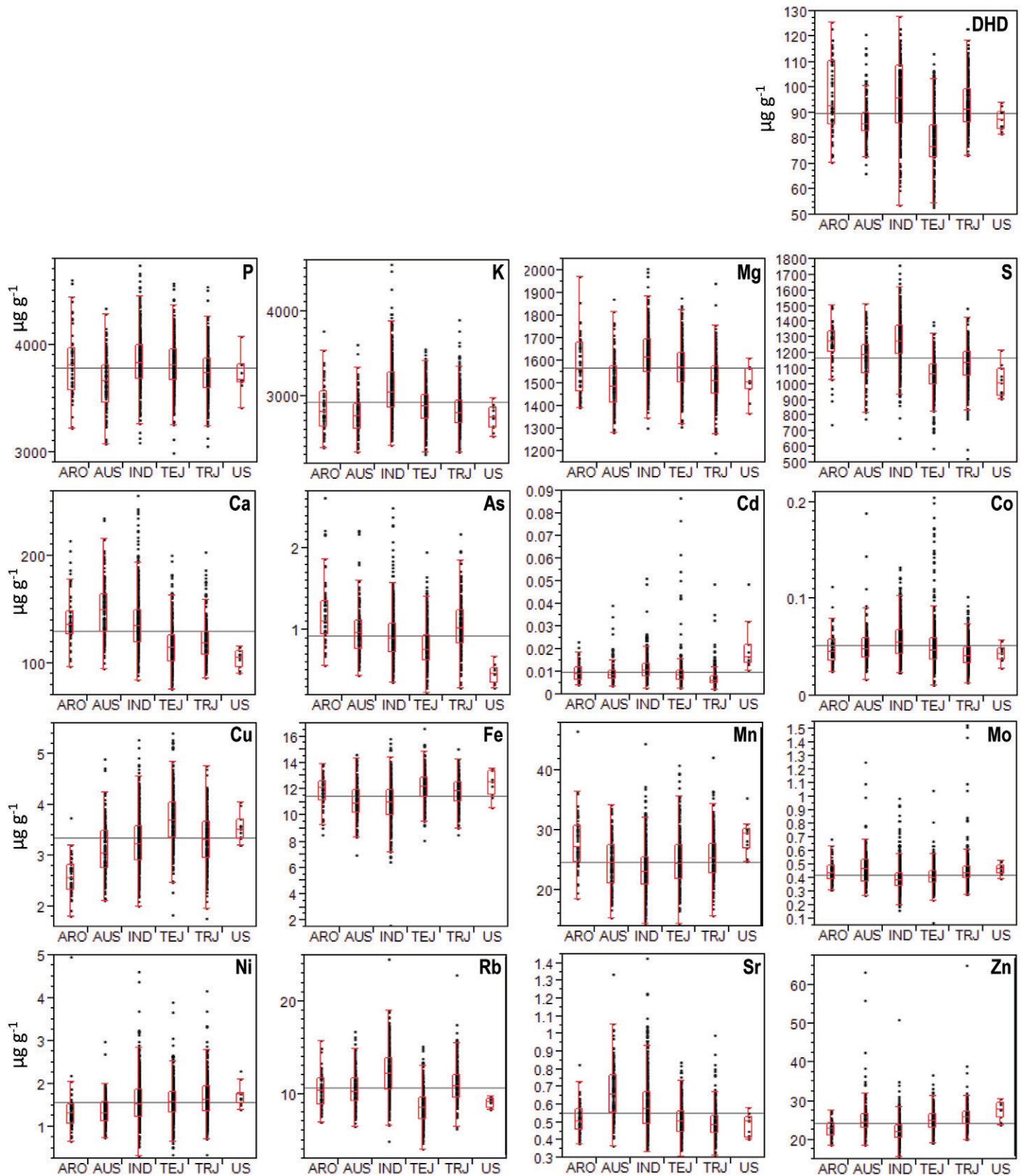


Figure 2. Mean data from 1763 diverse rice (*Oryza sativa* L.) accessions grown flooded and plotted per ancestral lineage or subgrouping. For comparison purposes, data from 10 modern southern U.S. rice cultivars are presented separately as well as included in the tropical *japonica* subgroup. The box represents data between the 25th and 75th percentiles, whiskers (error bars) indicate the 90th and 10th percentiles. Red lines inside the boxes represent the mean. The mean across all subgroups is indicated by the black line. ARO, *aromatic*; AUS, *aus*; IND, *indica*; TEJ, *temperate japonica*; and TRJ, *tropical japonica*.

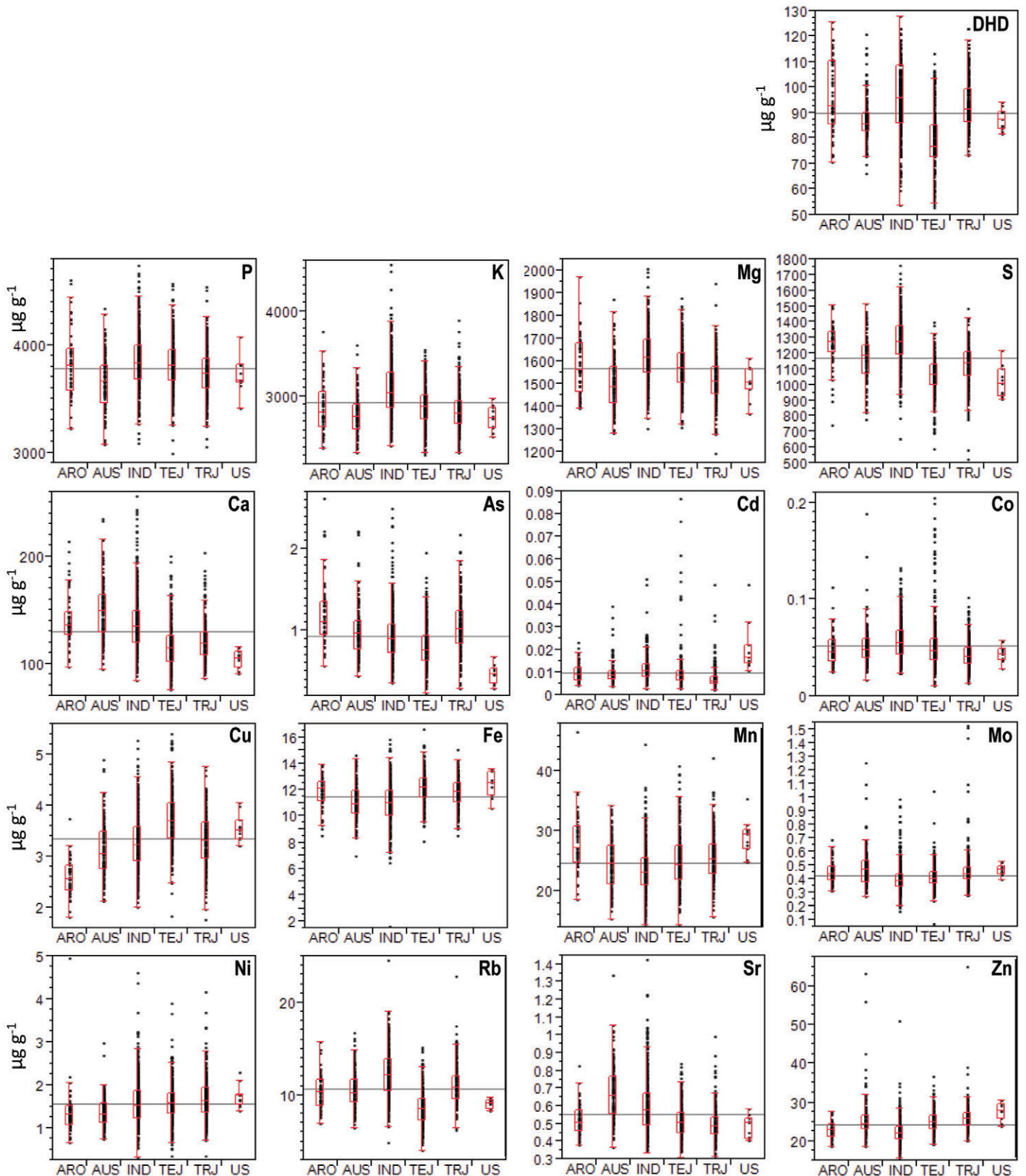


Figure 3. Mean data from 1763 diverse rice (*Oryza sativa* L.) accessions grown unflooded and plotted per ancestral lineage or subgrouping. For comparison purposes, data from 10 modern southern U.S. rice cultivars are presented separately as well as included in the tropical *japonica* subgroup. The box represents data between the 25th and 75th percentiles, whiskers (error bars) indicate the 90th and 10th percentiles. Red lines inside the boxes represent the mean. The mean across all subgroups is indicated by the black line. ARO, aromatic; AUS, aus; IND, indica; TEJ temperate *japonica*; and TRJ, tropical *japonica*.

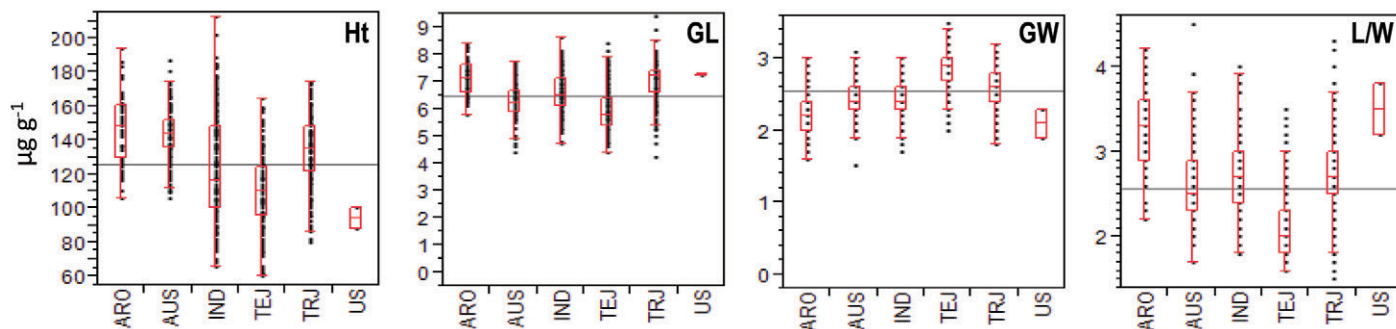


Figure 4. Trait means determined in other USDA Core Collection flooded field studies (Yan et al., 2007) plotted per ancestral lineage or subgrouping. For comparison purposes, data from 10 modern southern U.S. rice (*Oryza sativa* L.) cultivars are presented separately as well as included in the tropical *japonica* subgroup. The box represents data between the 25th and 75th percentiles, whiskers (error bars) indicate the 90th and 10th percentiles. Red lines inside the boxes represent the mean. The mean across all subgroups is indicated by the black line. ARO, aromatic; AUS, aus; IND, indica; TEJ temperate *japonica*; and TRJ, tropical *japonica*.

grain-element concentrations may result from a mutation in one or a few genes of large effect. If the extreme phenotypes were due to a single mutation that was selected for (e.g., favored their adaptability to the soil chemistry characteristic of a particular region or location), then the accessions found extreme for a particular element would be expected to have similar ancestry (subgroup membership) and locations of origin. Indeed, five of the top 10 accessions in terms of mean grain Mo were TRJ accessions collected from either Malaysia or Brunei, adjacent island countries, suggesting they may share a heritable underlying mechanism that enhances grain Mo. These islands have acidic soils, and low pH decreases the bioavailability of Mo (Raun et al., 1998), suggesting that there might be a deficiency of available Mo in the soils of this geographic region. The wide range in heading dates (91 to 116 DHD) among these five high-Mo accessions supports the Pearson correlation data in indicating that heading time is not a determinant of grain Mo. Likewise, wide variations for plant height and grain shape were also noted among these high-Mo accessions from Malaysia and Brunei.

Of the 10 accessions with highest grain Cd under flooded conditions, all were members of the TEJ subgroup, four of which originated from Eastern Europe, and three of which were collected in Hungary. In contrast, a majority of accessions high for grain Cd under unflooded conditions were from the AUS subgroup. None of the accessions contained more than the codex alimentarius limit of $0.4 \mu\text{g g}^{-1}$ Cd when grown flooded, but several accessions of the AUS, IND, and TEJ subgroups exceeded this concentration when grown unflooded. The distributions for grain Cd for all five subgroups were strongly positively skewed under both flooded and unflooded conditions, with a majority of the accessions being desirably low in grain Cd concentration. Interestingly, 5 of the 10 topmost accessions in terms of grain Co were also TEJ from Eastern Europe, though none of the accessions from Eastern Europe were high for both Cd and Co.

Like Cd, genes and mechanisms that reduce accumulation of As in grains are desired. The grain As concentrations in every one of the 1763 accessions was low ($<0.13 \mu\text{g g}^{-1}$, Fig. 3) when grown unflooded, but higher (0.2 to $2.6 \mu\text{g g}^{-1}$, Fig. 2) when grown flooded, focusing concerns about high grain As in rice plants exposed to reduced soil conditions. Among the 10 accessions with highest grain As grown flooded, four of the five subgroups (all except TEJ) are represented, with accessions originating from five different geographic regions (Fig. 6; Supplemental Table S1). In contrast, low grain As showed significant association with ancestral lineage with 7 of the 10 accessions selected for lowest grain As under flooded conditions being TEJ or admixtures with TEJ, and three being TRJ. In contrast, low grain As was uncommon among the IND and AUS subgroups, with two IND but no AUS among the 20 accessions lowest for grain As. In a smaller set (~ 300) of diverse rice accessions grown in Bangladesh, China, and two U.S. sites (Texas and Arkansas), it was noted that the TEJ subgroup had the lowest average grain As (Norton et al., 2012). Interestingly, 2 of the 20 accessions lowest for grain As in the present study were modern U.S. rice cultivars (Gulfmont and Jefferson). In further agreement with Norton et al. (2012), the 10 modern U.S. cultivars observed herein were relatively low for grain As overall (Fig. 2).

Correlations between Elements

The Pearson correlations (r) determined between traits among the 1763 accessions were calculated separately for flooded and unflooded data (Table 2). The correlations between flooded and unflooded LS means per element are shown in the boxes. Some interesting patterns can be seen among the correlations between the various elements. For example, Rb and K are chemical analogs and known to share plant uptake mechanisms, thus the fact that Rb and K are significantly and positively correlated under both flooded ($r = 0.45$) and unflooded ($r = 0.35$) conditions is as expected. The elements Ca and Sr are also chemical analogs and were

Table 2. Correlations between traits in the flooded (upper right matrix) and unflooded (lower left matrix) studies. Correlations between traits (grain element concentrations [$\mu\text{g g}^{-1}$], grain shape, plant height, and days to heading) for the 1763 diverse rice accessions calculated from LS mean data across three field replications per water-irrigation treatment. The correlations for each trait presently observed under both flooded and unflooded conditions are underlined. Bold text indicates significance at $\alpha = 0.05$. Bonferroni adjustments were used to account for the multiple comparisons made. DHD, days from planting to heading.

	Macronutrients													Micronutrients					Data from previous study [†]		
	P	K	Mg	S	Ca	As	Cd	Co	Cu	Fe	Mn	Mo	Ni	Rb	Sr	Zn	DHD	Height	Grain length	Grain width	L/W ratio
P	0.37	0.66	0.72	-0.02	-0.02	0.14	0.01	0.04	-0.09	-0.33	-0.05	-0.15	-0.12	0.21	-0.09	-0.06	0.15	0.02	-0.04	0.03	-0.05
K	0.59	0.54	0.56	0.05	0.28	0.19	-0.01	0.02	-0.19	-0.28	0.11	-0.16	-0.15	0.45	0.15	-0.23	0.33	0.03	-0.09	-0.08	-0.01
Mg	0.76	0.42	0.54	-0.01	0.11	0.16	0.04	0.03	-0.06	-0.23	0.06	-0.26	-0.11	0.22	0.10	-0.25	0.22	-0.08	-0.15	-0.07	-0.04
S	-0.13	-0.19	-0.04	<u>0.29</u>	0.06	0.01	0.08	0.10	-0.16	-0.21	-0.22	-0.12	-0.00	0.27	0.02	-0.19	0.33	0.07	0.258	-0.24	0.29
Ca	0.00	0.09	0.04	-0.12	0.31	0.26	-0.00	0.00	-0.39	-0.17	0.37	-0.12	-0.16	0.26	0.80	-0.19	0.23	0.27	-0.13	-0.24	0.07
As	-0.20	-0.20	-0.10	0.07	-0.01	-0.09	-0.28	-0.11	-0.44	-0.15	0.04	0.09	-0.22	0.38	0.107	-0.08	0.37	0.37	0.09	-0.08	0.10
Cd	0.14	0.14	0.15	0.06	0.07	-0.14	0.49	0.06	0.21	-0.07	0.03	0.02	0.25	-0.02	0.07	-0.10	-0.16	-0.19	-0.08	-0.07	-0.01
Co	0.02	-0.01	-0.09	0.01	-0.04	0.05	0.01	<u>0.17</u>	-0.03	0.02	-0.13	-0.12	0.04	-0.11	0.13	0.09	-0.18	-0.03	-0.03	0.07	-0.08
Cu	-0.19	-0.26	-0.01	0.27	-0.09	0.21	-0.01	-0.00	0.55	0.09	-0.06	-0.17	0.37	-0.25	-0.23	0.10	-0.39	-0.44	-0.15	0.27	-0.26
Fe	-0.37	-0.28	-0.28	0.05	-0.12	0.31	-0.31	0.04	0.35	0.39	0.08	-0.09	0.14	-0.38	0.01	-0.03	-0.44	-0.14	-0.13	0.14	-0.15
Mn	0.07	0.14	0.04	-0.11	0.13	0.19	-0.04	0.19	-0.01	-0.03	0.47	0.00	-0.02	-0.02	0.28	-0.03	0.04	-0.04	-0.04	-0.06	0.04
Mo	-0.07	-0.10	-0.21	-0.04	-0.04	-0.17	0.01	-0.05	-0.16	-0.20	-0.06	0.74	-0.07	0.01	-0.14	0.06	0.02	0.08	0.11	-0.04	0.09
Ni	0.15	0.02	0.17	0.11	0.04	-0.02	0.09	0.13	0.16	0.01	-0.06	-0.05	<u>0.21</u>	-0.04	-0.03	0.03	-0.31	-0.11	0.04	0.14	-0.07
Rb	0.22	0.35	0.19	0.04	0.09	-0.31	0.22	0.07	-0.21	-0.50	-0.10	0.11	0.23	0.62	0.06	-0.18	0.54	0.30	0.19	-0.22	0.23
Sr	-0.09	0.08	0.02	0.04	0.29	0.05	0.21	-0.08	0.10	0.08	-0.02	-0.04	0.07	0.11	0.44	-0.25	-0.05	0.13	-0.20	0.16	-0.03
Zn	0.03	-0.16	-0.03	-0.07	0.02	-0.01	-0.10	0.10	0.14	0.08	-0.02	-0.04	0.07	-0.14	-0.03	0.32	-0.07	0.85	0.24	0.15	-0.04
DHD	0.33	0.40	0.24	-0.09	0.01	-0.48	0.17	0.02	-0.39	-0.50	-0.16	0.17	0.04	0.52	-0.10	-0.07	0.85	0.24	0.25	-0.37	0.38
Height [†]	0.08	0.04	-0.11	-0.10	0.14	-0.09	0.02	0.12	-0.36	-0.31	-0.04	0.13	0.09	0.30	0.04	0.05	0.25	-	0.15	-0.06	0.11
Grain length [†]	0.08	0.01	-0.08	0.15	-0.11	-0.16	-0.01	0.07	-0.20	-0.15	-0.12	0.18	0.04	0.14	-0.19	-0.01	0.27	-	-	-0.27	0.79
Grain width [†]	-0.15	-0.09	-0.17	-0.06	-0.06	0.24	-0.20	0.08	0.20	0.36	0.03	-0.10	0.01	-0.26	-0.04	0.11	-0.33	-	-	-	-0.81
Grain L/W ratio [†]	0.13	0.06	0.05	0.12	-0.03	-0.23	0.11	-0.02	-0.24	-0.30	-0.07	0.16	0.00	0.22	-0.10	-0.07	0.36	-	-	-	-

[†]Plant height and grain shape dimensions (weight, length, width, L/W ratio) were as observed by Yan et al. (2007).

very highly correlated under flooded conditions ($r = 0.80$) but were not correlated ($r = 0.29$) under unflooded conditions. Some of the other correlations observed were less readily explained, such as the positive correlations observed between Rb-As, Mn-Ca, and Cu-Ni and the negative correlations for Cu-Ca, Cu-As, P-Fe, and Rb-Fe under flooded conditions; and the positive correlation between As-Fe and the negative correlations between P-Fe, K-Fe, Cd-Fe, and Rb-As under flooded conditions. The strongest, most consistent element-to-element correlations were observed among the three essential macronutrients P, K, and Mg. These elements are not considered chemical analogs and are not known to share uptake mechanisms. When the data were analyzed separately by ancestral subgroups (data not shown), these elements remained highly correlated across all subgroups, indicating a widespread association among these elements in terms of grain accumulation. Strong positive associations between P, K, and Mg were also reported within two biparental rice mapping populations (Zhang et al., 2014). Significant P-K and P-Mg associations have also been reported in maize kernels (Baxter et al., 2012a) and seed of *A. thaliana* (Vreugdenhil et al., 2004; Baxter et al., 2012b). Among the 1763 accessions, individual exceptions to the strong positive correlations generally seen between grain P, K, and Mg could be found, such as Core accessions 310241 and 311625, which had high grain P but low grain K, and Core 311012 which had high grain K, but low concentrations of P and K.

Principal Components within Water Treatments and Subgroups

Principal component analysis (PCA) reinforced the importance of intercorrelation between P, K, and Mg in terms of grain concentration in that the first principal component (PC1) comprised P, K, and Mg along with Rb, Cu, Fe, and As regardless of flooding treatment (Fig. 5). Interestingly, the loading of each element onto the PC1 was consistent with

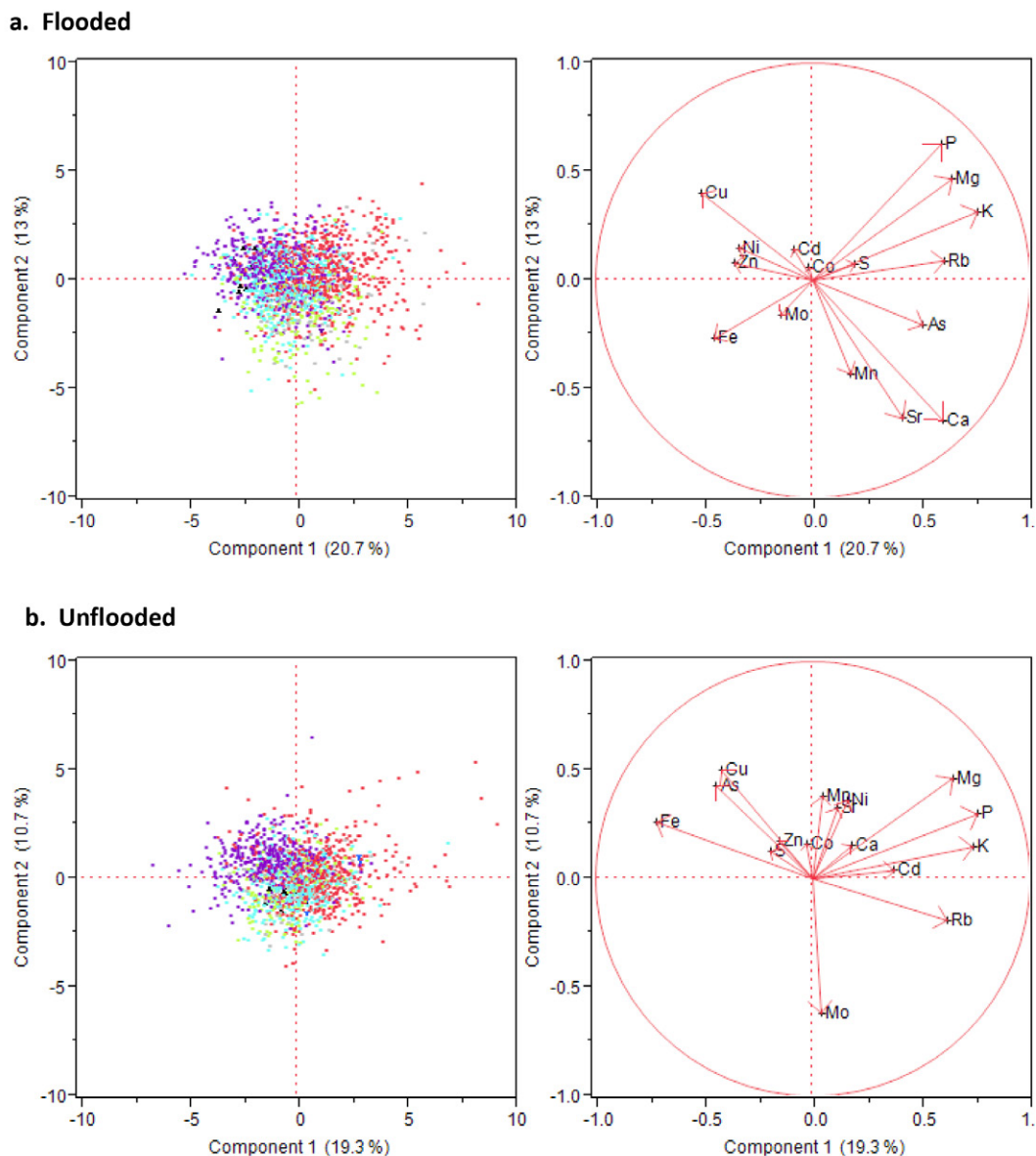


Figure 5. Principal components 1 and 2 (PC1 and PC2) determined from the entire set of 1763 diverse rice (*Oryza sativa* L.) accessions were similar under both (a) flooded and (b) unflooded conditions with the first two components (together explaining approximately 30% of the variance) and identifying ancestral subgroupings among the diverse rice accessions. Red dots indicate *indica* subgroup (IND) accessions, green = *aus* subgroup (AUS), light blue = tropical *japonica* subgroup (TRJ), purple = temperate *japonica* subgroup (TEJ), and dark blue triangles = modern U.S. TRJ accessions. The relatively low percentage of *aromatic* subgroup (ARO) accessions (gray) masks their presence in the figure. When data were analyzed per subgroup (Supplemental Fig. S2), the same elements were found important within each subgroup with P, K, Mg, and Rb consistently together in one of the main two components; Ca, Sr, Cu, Mn, and Mo in the second component; and As and Cu loading in nearly equal proportions between PC1 and PC2, with As reversing sign (positive versus negative loading) in the flooded versus unflooded data.

the DHD correlations (Table 2), with P, K, Mg, and Rb loading positively and Cu and Fe loading negatively onto PC1 under both flooded and unflooded conditions, and As loading positively onto PC1 under flooded conditions, but negatively onto the unflooded PC1. The PC2 elements determined across all 1763 accessions under both flooded and unflooded conditions were Ca, Sr, and Mn. The PC1 explained approximately 20% of the variance among accessions under both flooded and unflooded conditions; PC2 explained an additional 11 to 13%. Together, the PC1 and

PC2 explained approximately 30% of variance, and divided the accessions according to their ancestral subgroups (Fig. 5). The subgroup identifications plus the preponderance of DHD-correlated elements loading on PC1 again raised the question of whether the observed correlations might be driven by nonrandom distribution of grain ionomics alleles among the subgroups. However, when the PCAs were conducted within each individual subgroup (Supplemental Fig. S2), what was most noteworthy was the consistency of the element groupings identified as the first and second

principal components within and across the ancestral subgroups, and regardless of the field watering treatment.

Correlations between Grain Element Concentrations and Grain Shape

Because it is known that some elements are more concentrated in the outer bran surface layers than in the starchy endosperm (Bryant et al., 2005; Liang et al., 2008; Lombi et al., 2009; Hansen et al., 2012), and that the surface to volume ratio is minimized with a round compared to long narrow grain shape, it was anticipated that grain shape might be a significant determinant of the grain ionome. To evaluate relationships between grain element concentrations and grain shape, this study included data on GL, GW, and roundness (GL/GW ratio) collected on the USDA Core Collection in prior studies (Yan et al., 2007). As mentioned earlier, the TEJ subgroup is, on average, shorter and rounder in grain shape than the other subgroups (Fig. 4), and being adapted to geographic regions with colder and shorter growing season, the TEJ also have shorter average DHD than the other rice subgroups (Fig. 2 and 3). This causes a relatively strong positive correlation between DHD and GW and GL/GW when data is analyzed across the 1763 diverse accessions (Table 2), but this association is not due to genetic linkage or physiological cause-and-effect relationship between low DHD and rounder grain shape but, rather, to breeding selections that favored nonrandom distribution of these traits among the ancestral subgroups. In contrast to the correlation seen between DHD and grain shape in Table 2, none of the elements were associated with GL, GW, or GL/GW under flooded conditions, and only Fe was weakly associated with GW under unflooded conditions. The associations between the grain elements and grain shape remained low or insignificant when the data were analyzed by individual subgroups or regions of origin (data not shown). Grain shape was not found to be as significant a determinant of grain ionomes as originally hypothesized, meaning that genes and mechanisms with stronger impact than bran:endosperm ratio alone exist among the rice accessions. The fact that grain shape did not prove to be a major determinant of grain ionomics means that differences in grain shape are not expected to confound future studies designed to identify genetic and molecular mechanisms controlling grain ionomics genes.

Correlations between Elements and Days to Heading

Plants with a longer vegetative phase (later heading date) have a longer window of opportunity to mine mineral elements out of the soil, potentially building reserves in leaf tissues that can be later translocated to grains. It would follow, then, that extending the vegetative phase might be one method for increasing grain concentration of elements

considered mobile within plants. Alternatively, later grain fill periods generally occur under cooler air and root zone temperatures, impacting transpiration rates which in turn affect root uptake rates, and rates of translocation of elements within plants (Quintana et al., 1999; Xiloyannis et al., 2001; Tani and Barrington, 2005). Additionally, soil chemistry and bioavailability of soil elements continue to change over time as the duration of flooding is extended (Somenahally et al., 2011b). With the PCA identifying six of the seven DHD-correlated elements as of primary importance, further evaluation of the associations between the grain element traits and DHD is warranted.

If an association between DHD and grain concentration of an element is due to duration-enhanced uptake and leaf storage of that element, then one might expect the element to increase with DHD over both flooded and unflooded field conditions, and for that element to be considered mobile as opposed to fixed or chemically bound within leaf and stem tissues. The elements P, K, Mg, Mo, S, and Ni are considered remobilized from leaves to newer leaves and/or grain (Raun et al., 1998) and were in abundant supply in the fertilized Texas field plots. As a chemical analog of K, Rb is also considered a remobilized element. The scatter plots of DHD by K and P (Fig. 6) are fairly representative of those for Rb and Mg, respectively. Both K and Rb were significantly correlated with DHD under both flooded and unflooded conditions, while P was significantly correlated with DHD under unflooded conditions. Like the P-DHD flooded correlation ($r = 0.15$), correlations with Mg (both $r = 0.2$) were positive but below the $\alpha = 0.05$ significance threshold. The graphs show a general upward trend, whether or not the correlations were above or below the threshold for statistical significance, as predicted if the increased grain concentrations were due to enhanced uptake of these elements by the plants over a longer vegetative phase followed by remobilization during grain fill. In contrast, Fe (Table 2, Fig. 6) and Cu (Table 2) are negatively associated with DHD under both flooded and unflooded conditions, which would be consistent with these elements being poorly remobilized within plants, making the Fe and Cu stored in vegetative tissues unavailable to the developing grain (Raun et al., 1998).

Arsenic is unique among the elements for having reversed association with DHD between the flooded (positive correlation) and unflooded (negative correlation) fields (Table 2, Fig. 6). Several reviews of current knowledge on the different forms of As in the rhizosphere under flooded and unflooded conditions and differences in their bioavailability based on how they are taken up by plant roots and subsequently transported throughout or sequestered within specific cells or plant tissues have been published (Zhao et al., 2009, 2012; Carey et al., 2010, 2011; Tuli et al., 2010; Moore et al., 2011, 2013; Kuramata et al., 2013). With low grain As being more

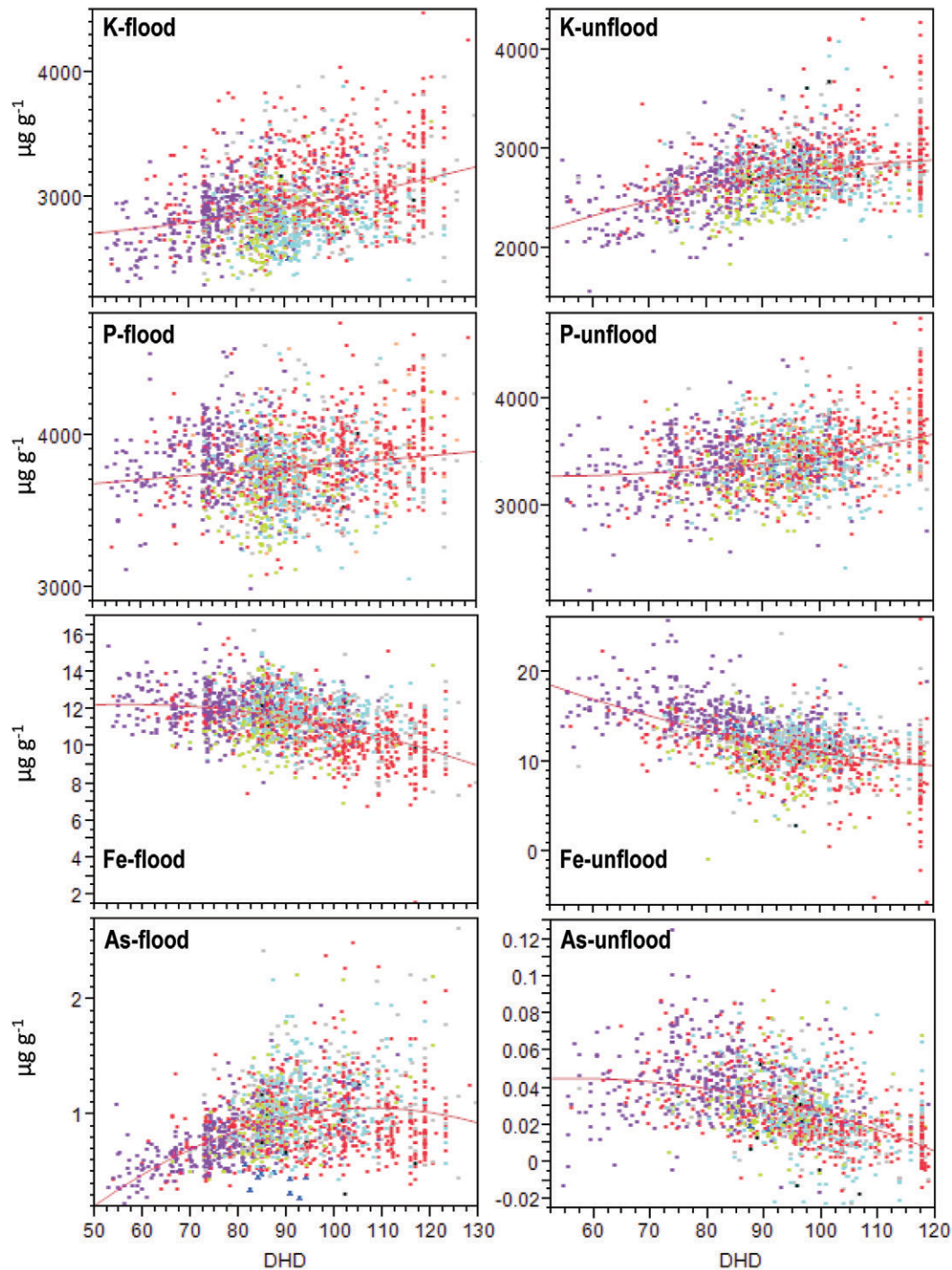


Figure 6. Scatter plots and quadratic means for K, P, Fe, and As, four elements whose grain concentrations were significantly associated with days from planting to heading (DHD) under flooded and/or unflooded field conditions across all 1763 accessions and within four of the five subgroups. Red dots indicate *indica* subgroup (IND) accessions, green = *aus* subgroup (AUS), light blue = tropical *japonica* subgroup (TRJ), purple = temperate *japonica* subgroup (TEJ), gray = *aromatic* subgroup (ARO), and dark blue triangles = modern U.S. TRJ accessions.

common among the TEJ, which are also noted for earlier heading, than among the IND and AUS, which are noted for longer vegetative stages in general, it came as no surprise that the average DHD among the 10 accessions with lowest grain As was less than the average DHD among the accessions with highest grain As (74 ± 19 and 103 ± 14 , respectively). What is new and of particular interest here is

the near-linear relationship between maximum observed grain As and DHD that results in a scatter plot with a narrower range in grain As among early-heading (mostly TEJ) accessions than among the later heading (mostly IND) accessions (Fig. 6). This lends support to reports that As availability in soil solution increases as the length of flooding period increases owing to an increase in the

population of Fe-reducing microbes over time, which in turn results in increased release of reduced As into the soil solution under prolonged flooding (Somenahally et al., 2011a, 2011b). Changes in As availability in soil solution during the growing season would also be consistent with As being the sole element for which ANOVA indicated less variance due to Subgroup than to Geographic Region since region of origin or collection site would be even more directly related to length of growing season and maturity differences than Subgroup.

Straighthead is a physiological disease in rice that is induced with application of As-based herbicide to the resistance screening nursery. It is interesting to note, therefore, that most rice accessions found to be resistant to straighthead disease are also early maturing, with none having greater than 92 DHD (Yan et al., 2004; Agrama and Yan, 2010; Pan et al., 2012).

The wide range in grain As observed among the accessions with a mid to late DHD (87 to 120 DHD, Fig. 6) increases the confidence in identifying lines as “As accumulators” (significantly above the grain As mean per DHD) versus “As excluders” compared with selections made among the lower grain As TEJ accessions with DHD from 50 to 65 d.

The linear association observed for maximum observed grain As concentration when plotted against DHD indicates two things. First, that one method for reducing grain As might be to produce early-maturing rice cultivars, within limits since early flowering has also been associated with reduced rice yield (Wu et al., 1998; Samonte et al., 2006). Second, that if one wants to identify and study differences in the uptake, transport, and/or sequestration mechanisms that ultimately affect grain As concentrations, one should not merely study late high-As lines compared with early low-As lines but should also compare “As accumulators” and “As excluders” of similar DHD to be more certain of comparing accessions with similar exposure to available soil As.

Significance to Understanding and Knowledge in Plant Physiology

Deficiencies of micronutrients such as iron and zinc frequently limit plant growth and crop yields. If the mechanisms of uptake, distribution, and regulation of micronutrients were clearly understood, it might be feasible to breed plants better able to grow in soils now considered marginal and to increase crop biomass on soils now in cultivation. Furthermore, as most people rely on plants as their dietary source of micronutrients, plants that serve as better sources of essential nutrients would improve human health. The patterns of the grain ionome as displayed in the diverse accessions can suggest possible control mechanisms of element concentrations in the plant and identify appropriate genetic material to use in studies delimiting the mechanisms.

The correlations noted between grain elemental concentrations along with other accumulation patterns are of interest because they suggest the possibility of shared mechanisms or genes for root uptake and/or transport or partitioning between rice tissues or into rice grains, as noted previously for the chemical analogs Rb and K. As another example, the correlation between Ca and Mn under flooded conditions may partially reflect not only a shared intracellular transporter (as hypothesized by Fox and Guerinot, 1998), but also movement into the grain only at the early stages of seed formation when the seeds are still supplied by xylem (Oparka and Gates, 1981). In contrast, Mg, P, and K do not share uptake mechanisms, are not tightly chemically coupled with respect to soil solution levels in flooded vs. unflooded soil conditions (De Datta 1987), and were not found correlated in terms of their concentrations in plant vegetative tissues (Baxter et al., 2008; Baxter, 2009), yet they were found highly correlated in grains of this widely diverse set of rice germplasm, whether grown flooded or unflooded, as well as in seeds of *A. thaliana* (Baxter et al., 2008; Vreugdenhil et al., 2004) and maize (Baxter et al., 2012a). In rice, as in many cereal grains, most of the seed P is found in the form of a mixed K–Mg salt of phytic acid in the aleurone layer and germ (Bryant et al., 2005), so one possible explanation of the positive P–Mg–K intercorrelations might be that their levels are all driven by phytate levels in the grain. Vreugdenhil et al. (2004) found colocalization of QTLs for K, Ca, and P with a high phytate locus in *A. thaliana* suggesting that phytate levels were affecting P–K associations. However, in both the present and a prior study (Bryant et al., 2005) a phytateless mutant of rice did not display altered total grain P concentration compared with the parental line, suggesting that something other than, or in addition to, phytate levels in the bran is controlling total grain accumulation of P. Interestingly, Zhang et al. (2014) identified eight QTLs for grain K in a biparental mapping study, four of which were also associated with grain concentration of P, and two of which were correlated with concentrations of K, P, and Mg.

Further study with selected accessions may be able to clarify the factors underlying the strong correlations seen among P, K, and Mg in rice and other seeds. Among the 1763 accessions, rare exceptions to the strong P–K–Mg correlations could be found, such as in Core 311012, which was high in P and K but extremely low in Mg under both flooded and unflooded conditions, and Core 311625, which was high in P and Mg but low in grain K, regardless of flooded or unflooded field conditions. However, no accession had negative correlations between P and both Mg and K. For Core 310241, the P–K correlation was negative, with its grains being concentrated for P and low in K (more so under flooded conditions than unflooded), but with above-average grain Mg, the P–Mg correlation remained positive.

In addition to developing high-yielding, early-maturing lines to limit grain As concentrations as discussed above, another strategy suggested for reducing grain As would be to develop cultivars better suited to unflooded production (Marin et al., 1993; Williams et al., 2007; Xu et al., 2008). Because the flooding of rice fields affects more than just the rice plant, numerous plant traits are required for high yield potential under unflooded conditions. For example, in addition to increased tolerance to water stress, a high-yielding cultivar adapted to aerated production would need to compete well with weeds, be resistant to blast disease and stem borer insects, and, depending on the growth climate, would require increased tolerance to either heat or cold stress during pollination and grain fill. Furthermore, because Cd increases in availability under unflooded conditions, causing an increase in grain Cd in unflooded as compared with flooded rice, it may become necessary to add reduced Cd accumulation to the list of desired traits (Uraguchi and Fujiwara, 2012).

Observations of Particular Relevance to Efforts to Enhance Grain Ionomics of U.S. Rice

Modern U.S. rice cultivars are already, on average, comparatively high in grain Zn under flooded conditions (standard in the U.S.), though there were accessions found among the AUS and IND subgroups with twice this concentration. The U.S. rice cultivars were also notably low in K, S, Ca, Sr, and As, while higher than average for Cu and Cd. It must be remembered, however, that none of the accessions contained grain Cd high enough to be of dietary concern unless they were grown unflooded. The U.S. rices were less exceptional for any of the elements under unflooded conditions. Cross-progeny derived from parents in different ancestral subgroups can suffer from hybrid sterility because of genetic incompatibility; fortunately, other TRJ accessions were found high in K, S, Ca, and Sr that could be incorporated into U.S. breeding programs to raise the grain concentrations of these elements. With the U.S. cultivars already low for grain As among worldwide rices, research to identify novel As-excluding mechanisms would be required to further reduce grain As under the lengthy flood period and highly reduced soil conditions used in the present study. Since grain As varied most widely under moderate to late DHD, it may prove most fruitful to identify novel As-exclusion mechanisms in studies comparing accessions within these DHD groups.

CONCLUSIONS

Though environmental variance was large for some elements (flooded: As, Cd, Co, Ni, Rb; unflooded: Cd, Mn, Ni, Rb), there was significant genetic variance for grain concentration of all 16 elements observed, suggesting that breeders could select for enhanced grain concentration of all 16 elements. The H^2 for the elements ranged from 0.14 to 0.75; the particularly low

H^2 determined for grain Ni under both flooded and unflooded conditions indicate that breeding to alter grain Ni would be particularly confounded and slowed by environmental variance. For each element, germplasm accessions with extreme grain phenotypes were identified to support further genetic and physiological investigations. Shape was not as significant a determinant of grain ionome as originally hypothesized, but an extended vegetative phase (late maturity) was associated with concentrations of several grain elements. Highly similar sets of elements were identified by PCA as being of primary importance in explaining the variance across as well as within subgroups, and in flooded and unflooded conditions.

Seed Availability

Small quantities of seed of any of the 1763 accessions reported here may be obtained from the USDA-ARS Genetic Stocks-Oryza (GSOR) Collection at the USDA-ARS DBNRRRC in Stuttgart, AR, where they are identified by the GSOR numbers provided in Supplementary Table 1. Instructions for requesting seed as well as the element concentration LS means provided here in Supplementary Table 1 can be found at <http://ars.usda.gov/spa/dbnrrc/gsor> (accessed 9 Sept. 2014). We ask that appropriate recognition be given when one or multiple of the accessions and grain ionomics data presented here contribute to future research.

Supplemental Information Available

Two supplemental tables and two supplemental figures are available with the online version of this manuscript.

Supplemental Figure S1. Proportion of each ancestral subgroup represented by the accessions collected from each of the 14 worldwide rice growing regions. The width of the bars per region also indicate the proportion of the 1763 accessions that originated from a country in that region.

Supplemental Figure S2. Principal components 1 and 2 determined across and within ancestral subgroups.

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