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ARTICLE

Genotype-by-Environment Interaction Affects the Essential Mineral Composition of Peanut (*Arachis hypogaea* L.) Kernels

Kim-Yen Phan-Thien,[†] Graeme C. Wright,[‡] and N. Alice Lee*,[†]

[†]Food Science and Technology, School of Chemical Engineering, University of New South Wales, Kensington, NSW 2052, Australia, and [‡]Peanut Company of Australia, P.O. Box 26, Kingaroy, QLD 4610, Australia

The concentrations of 15 essential minerals (B, Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P, Se, and Zn) in kernels of nine diverse peanut genotypes, which were cultivated in five distinct growing environments, were analyzed by inductively coupled plasma-optical emission spectroscopy (ICP-OES) and -mass spectrometry (ICP-MS). The effects of genotype, environment, and genotype-by-environment (G × E) interactions were significant ($P < 0.05$) for all elements excluding Cr. Genetic control of mineral composition was demonstrated by large ($P < 0.05$) genotypic differences in Ca, Mo, K, Na, and P contents, and clustering of some genotypes in environment-centered principal components analysis (PCA) along axes comprising both macro (Ca, Mg, P, and K)- and microelements (Co, Cu, Fe, Mn, and Zn). Mo and Na concentrations were strongly influenced ($P < 0.05$) by the growing environment, with very high levels measured in samples from Bundaberg. The results confirm that that there is breeding potential for several important minerals in peanuts, although significant G × E interactions will complicate the response to selection. From a practical viewpoint, combining genetic improvement with agronomic management may be a useful strategy to consistently achieve desirable mineral concentrations in peanut kernels.

KEYWORDS: Peanut; *Arachis hypogaea*; essential minerals; trace elements; mineral composition; genotype-by-environment interaction; plant breeding; agronomic management; inductively coupled plasma-optical emission spectroscopy; inductively coupled plasma-mass spectrometry

INTRODUCTION

Functional foods, which promote human health beyond providing essential nutrition, are a major growth area for food industry and food research. Global sales of functional foods were projected to grow from US$75 billion in 2007 to $109 billion in 2010, a trend that saw two-thirds of Americans venturing to buy more fortified foods and 69% pursuing a preventative lifestyle, according to 2008 market research (*1*). The same survey found that 49% of adults made a strong effort to consume more Ca, 25% more K, and 24% more Fe. Publications regarding the consumption trends of other countries are more scarce; nevertheless, representative population surveys indicating steady increases in the use of vitamin/mineral supplements suggest that self-management of health is also gaining importance in Australia (*2*) and Europe (e.g., refs *3*−*5*). Essential minerals, including B, Ca, Cl, Co, Cr, Cu, F, I, Fe, Mg, Mn, Mo, K, Na, Ni, P, Se, S, and Zn, are necessary for normal growth, reproduction, and health, and their essentiality means that they are strictly speaking not functional foods. However, several essential minerals are of particular interest to consumers and the food industry, and are treated as functional food traits because of their expected preventative or therapeutic effects on chronic diseases.

*Corresponding author. Tel: +61 (2) 93854363. Fax: +61 (2) 93855966. E-mail: alice.lee@unsw.edu.au.

For example, Ca consumption is linked to the prevention of osteoporosis and has been identified as a major area of opportunity for functional food innovations (*6*). Fe deficiency is the most prevalent nutritional deficiency worldwide and is a leading risk factor for disability and death. In developed countries, Fe deficiency anemia is much less prevalent than in developing countries but still accounts for more than two-thirds of anemia cases in general practice, according to a survey conducted in Norway (*7*). Prevention of Fe deficiency is most crucial for infants, children, adolescents, pregnant women, and underprivileged communities who may have inadequate diets (*8*). High K in the diet can lower blood pressure and reduce cardiovascular disease mortality, as well as delay the progression of renal disease and aid in the management of kidney stone disease (*9*). High Mg intake is also encouraged for hypertensive patients, although there is conflicting evidence regarding the direct relationship between Mg dose and blood pressure (*10*). Recent insights into the functions of selenoproteins in the body, in particular those with antioxidant and cancer-protective properties, has generated attention regarding the potential health benefits of Se supplementation (*11*).

Interest in the mineral composition of plant foods has thus far been restricted to fortification rather than biofortification, or the genetic manipulation of mineral content through plant breeding. Few crop breeding programs have focused on mineral composition, with the notable exception of a large CGIAR program

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Table 1. Description of Peanut Genotypes

genotype	growing season	botanical type	source program	registration	genetic relatedness[b]
D147-p3-115	full-season	ssp. *hypogaea* var. *hypogaea*	QDPI&F[a]	breeding line	sister-line to Sutherland
D175-3-p17-3	ultra-early	ssp. *fastigiata* var. *vulgaris*	QDPI&F	breeding line	unrelated
D192-p397-1	ultra-early	ssp. *fastigiata* var. *vulgaris*	QDPI&F	breeding line	unrelated
D193-p3-6	ultra-early	ssp. *fastigiata* var. *vulgaris*	QDPI&F	breeding line	sister-line to D193-p3-8
D193-p3-8	ultra-early	ssp. *fastigiata* var. *vulgaris*	QDPI&F	breeding line	sister-line to D193-p3-6
Middleton	full-season	ssp. *hypogaea* var. *hypogaea*	QDPI&F	PBR: *Plant Varieties Journal* **2003**, *16* (3)	unrelated
Page	full-season	ssp. *hypogaea* var. *hypogaea*	University of Florida	PBR: *Plant Varieties Journal* **2009**, *22* (3)	unrelated
PCA213	full-season	ssp. *hypogaea* var. *hypogaea*	private breeder, USA	breeding line	unrelated
Sutherland	full-season	ssp. *hypogaea* var. *hypogaea*	QDPI&F	PBR: *Plant Varieties Journal* **2002**, *20* (2)	sister-line to D147-p3-115

[a] QDPI&F refers to the State of Queensland Department of Primary Industries and Fisheries. [b] Sister lines are derived from the same F2 plant.

Table 2. ICP-MS and ICP-OES Detector Settings, Calibration Range, and Instrument Detection Limits (IDL)[a]

ICP-MS						ICP-OES				
mode	element	isotope	calibration range (lower limit)	calibration range (upper limit)	IDL[b]	element	wavelength (nm)	calibration range (lower limit)	calibration range (upper limit)	IDL[b]
DRC	Co	59	0.1	10	0.001	Ca	422.673	0.1	100	0.013
	Cr	52	0.1	10	0.001	Mg	279.077	0.1	100	0.095
	Cu	63	0.1	100	0.003	Mn	257.610	0.01	10	0.027
	Fe	56	1	200	0.010	P	213.617	0.1	100	0.001
	Ni	58	0.1	10	0.005	K	766.490	0.1	200	0.021
	Se	82	0.1	10	0.027	Na	589.592	0.05	10	0.049
	Zn	66	0.1	100	0.013					
Standard	B	11	0.1	100	0.285					
	Mo	98	0.1	10	0.004					

[a] Concentrations are in μg/L and mg/L for ICP-MS and ICP-OES, respectively. [b] IDL were calculated as thrice the standard deviation of 10 calibration blank measurements.

aiming to increase bioavailable Fe, Zn, and carotenoids in a number of staple food crops (*12*). This is likely to change as breeding criteria accommodate demands for new forms of commodity quality, as agricultural and food industries strive to increase competitiveness through product differentiation, niche-marketing, and by value-adding.

From a humanitarian perspective, biofortification of staple food products is seen as one of the key strategies for alleviating micronutrient malnutrition afflicting poor communities, alongside the more traditional interventions of supplementation, fortification, and dietary diversification (*13*). Improved varieties are readily adopted by small-scale farmers because no changes in agronomic practice or extraordinary capital investments are required. Micronutrient-enriched seeds have the added advantage of boosting productivity in nutrient-deficient soils, which are typically cultivated by the rural poor of the developing world (*12*).

In this research, we analyzed the essential mineral composition of nine diverse peanut lines from the Australian breeding program grown in 2008 in five distinct environments and investigated the stability of the peanut mineral phenotypes by standard analysis of variance (ANOVA) and principal components analysis (PCA). Our objectives were to establish the significance of genotype, environment, and genotype-by-environment (G × E) interaction on mineral composition, investigate the breeding potential for mineral content, and identify any genotypes that contained outstanding levels of any elements of interest.

MATERIALS AND METHODS

Materials. Samples comprised nine peanut genotypes (**Table 1**) from the breeding program collaboratively run by the Peanut Company of Australia (PCA), the Grains Research and Development Corporation (GRDC), and the Queensland Department of Employment, Economic Development and Innovation (DEEDI). These represented diverse phenotypes in terms of growing season (full-season, 140 days, or ultra-early, 100−110 days), lipid composition, resistance to soil-borne and foliar disease, and yield characteristics. Each genotype was grown in triplicate/quadruplicate randomly allocated field plots in five distinct growing environments under nonlimiting (nutrient- and water-replete) conditions. The trial locations were Bundaberg in the coastal Burnett region of south Queensland, Taabinga in south Burnett, and Kairi in the Atherton Tableland region of north Queensland. Peanuts at Kairi were subject to differing conditions of stress: controlled (low disease pressure) conditions, soil-borne disease pressure (mainly *Cylindrocladium parasiticum* Crous, Wingfield & Alfenas), and foliar disease pressure (mainly *Cercosporidium personatum* (Berk. & Curt) Deighton, *Cercospora arachidicola* Hori, and *Puccinia arachidis* Speg.). These trials were designated as Kairi, Kairi CBR, and Kairi FDR, respectively. Sound, mature, jumbo and size one (i.e., > 11.9 mm in kernel diameter for full-season and > 9.5 mm for ultra-early) peanut kernels were sent to the University of New South Wales after harvesting and preprocessing in accordance with commercial practice. Preprocessing consisted of drying, shelling, and grading. Raw kernels were stored in vacuum-sealed polyethylene bags in a cool room (< 10 °C) or freezer prior to analysis.

All chemicals and solvents used were of analytical reagent grade. Water was ultrapure (Millipore Corporation). Wheat flour (Standard Reference Material 1567a) and peanut butter (Standard Reference Material 2387) certified reference materials (CRM) were obtained from the US National Institute of Standards and Technology.

Mineralization. Approximately 10 g of kernels was ground in liquid nitrogen with a mortar and pestle after manual removal of the testa until a relatively homogeneous particle size was achieved. A portion of the sample was set aside for gravimetric determination of moisture content. Closed acid digestion was performed in a Milestone Ethos Plus Labstation or Milestone Ethos 1 Advanced Microwave system (Milestone Inc.). The digestion mixture consisted of 0.4 g of sample, 5 mL of concentrated nitric acid (purified by sub-boiling distillation), 2 mL of hydrogen peroxide, and 3 mL of water. The microwave program was 20 min at 200 °C with ramp times dependent on the operating capacity of the microwave used. Digestates were unloaded after internal temperatures had declined to less than 50 °C, diluted to 30 mL with water, and stored at room temperature until the time of analysis.

Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). Analyses of B, Co, Cr, Cu, Fe, Mo, Ni, Se, and Zn concentrations were performed on a PerkinElmer ELAN DRC II ICP-MS in both standard and dynamic reaction cell (DRC) modes of the instrument, using ammonia

as the reaction gas. Only results from the best detection setting for each analyte are treated in this article (**Table 2**). Extracts were diluted 1:9 in 2% nitric acid and quantitated against calibrations of a multielement standard series (Choice Analytical Pty Ltd.) by ELAN software version 3.4 (PerkinElmer SCIEX, 1994−2007).

Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES). Analyses of Ca, Fe, K, Mg, Mn, Na, and P content were performed on PerkinElmer Optima 7300 DV ICP-OES (**Table 2**). Samples were quantitated against calibrations of a multielement standard series (Choice Analytical Pty Ltd.) using WinLab32 for ICP software version 4.0.0.0305 (PerkinElmer, Inc., 2008).

Quality Controls. Each digestion batch included a reagent blank (containing reagent but no sample) to assess batch-to-batch fluctuations in the background measurement. A calibration blank (2% nitric acid) and check standard solutions were regularly measured during the ICP analyses in order to assess signal drift. Measurement of rhodium (ICP-MS) or yttrium (ICP-OES) internal standards in the carrier solution allowed for correction for fluctuations in sample uptake and nebulization. Wheat flour and peanut butter CRM were regularly analyzed to validate the accuracy and precision of the analytical procedures.

Data Analysis. Sample variance was analyzed as a full factorial model by univariate ANOVA in SPSS Statistics 17.0 (SPSS Inc., IBM), while between-level comparisons of genotypes and environments were made using Tukey's honestly significant difference (HSD) test. Multivariate relationships between peanut genotypes, growing environments, and mineral composition were explored by PCA of autoscaled (i.e., grand mean-centered) and environment-scaled (i.e., environment mean-centered) data in The Unscrambler, version 9.8 (CAMO Software AS, 1986−2008).

RESULTS

Method Validation. Accuracy of the experimental method was evidenced by close correspondence between the certified and experimentally derived elemental concentrations in the wheat flour and peanut butter CRM (unpublished data). Measurements of all elements were within 10% of certified concentrations, excluding Na in the wheat flour CRM. A high level of repeatability was achieved, with the relative standard deviation (RSD) of CRM analyses over the course of the experiment remaining at 3−6% for most elements and 10−11% for Mo, Se, and Na in the wheat flour CRM. The moisture contents of all peanut samples were similar at $6.2 \pm 0.4\%$ (i.e., 7% RSD); therefore, comparison between samples did not require conversion to a dry-weight basis.

ANOVA of Genotype and Environment Effects. Genotype and environment main effects were significant ($P < 0.05$) for all elements, with the exception of Cr. Most elements were also subject ($P < 0.05$) to G × E interaction. Tukey's HSD pairwise comparisons indicated that there were statistical differences between genotypes (**Table 3**) and between growing environments (**Table 4**).

PCA of Genotype-by-Environment Interaction. Autoscaled PCA indicated that the data had little underlying structure, as many principal components (PC) were required to explain the variance (i.e., 10 PC to explain 90% of variance). Genotypes were poorly described, with only D192-p397-1 and D193-p3-8 clustering in the upper right quadrant of the scores plot (**Figure 1**), mainly due to positive correlations with Mg, P, and Zn content. Some clustering of growing environments could be observed along the initial three PC, although these accounted for only 47% of variance. Kairi and Kairi FDR formed a group along the PC2 axis, while samples from Bundaberg grouped in the lower right quadrant of the scores plot (**Figure 2**). Kairi CBR and Taabinga scored similarly on PC1 but could be distinguished along PC3, which correlated mainly with Fe and K content (**Figure 3**).

Removal of environmental main effects by block-scaling meant that the subsequent PCA modeled only genotype and G × E effects. Data could not be simplified, i.e., the number of PC required to explain the data remained high, but genotype clusters that were not apparent in the autoscaled PCA could now be

Table 3. Mean Elemental Composition (μg/g) of Peanut Genotypes[a]

genotype	N	B		Ca		Co		Cr		Cu		Fe		K		Mg		Mn		Mo		Na		Ni		P		Se		Zn	
D147-p3-115	17	15.2	b	535	cde	0.101	a	0.025	a	5.97	ef	13.4	c	7782	a	1497	cd	22.6	a	0.212	b	33.9	b	7.23	ab	3442	c	0.081	ab	25.3	b
D175-3-p17-3	17	22.2	a	721	a	0.079	bc	0.023	a	7.06	bc	17.3	a	6404	d	1751	ab	18.7	cd	0.418	ab	11.9	c	4.39	bc	3566	c	0.104	a	26.2	b
D192-p397-1	17	19.9	ab	471	e	0.075	bc	0.024	a	5.82	ef	17.9	a	7087	bc	1762	ab	22.3	a	0.352	b	11.4	c	4.67	abc	4150	a	0.102	ab	29.8	a
D193-p3-6	17	17.5	ab	528	cde	0.062	c	0.012	a	5.29	f	16.2	ab	6848	c	1707	b	20.8	abc	0.359	b	13.1	c	5.27	abc	3886	b	0.107	a	25.9	b
D193-p3-8	17	20.3	ab	490	de	0.062	c	0.018	a	6.26	de	17.0	a	7215	b	1845	a	19.1	bcd	0.357	b	16.3	c	5.68	abc	4212	a	0.108	a	27.0	b
Middleton	17	18.3	ab	625	b	0.078	bc	0.018	a	8.65	a	14.8	bc	7339	b	1697	b	16.7	d	0.698	a	15.7	c	4.15	c	3488	c	0.091	ab	26.4	b
Page	17	15.3	b	593	bc	0.091	ab	0.019	a	7.39	bc	15.0	bc	5948	e	1592	c	20.3	abc	0.281	b	14.4	c	6.98	abc	3465	c	0.076	ab	25.3	b
PCA213	17	16.9	ab	554	cd	0.099	a	0.016	a	7.56	b	16.4	ab	6202	de	1572	cd	21.4	abc	0.246	b	15.1	c	7.45	a	3439	c	0.072	ab	25.2	b
Sutherland	20	15.7	b	518	de	0.093	ab	0.018	a	6.73	cd	14.7	bc	7795	a	1478	d	21.9	ab	0.721	a	42.4	a	5.86	abc	3553	c	0.061	b	27.7	ab

[a] Genotypes marked with different lower-case letters had significantly different concentrations ($P < 0.05$) of that element according to Tukey's HSD test.

Table 4. Mean Elemental Composition (μg/g) of Peanuts Grown at Each Trial Location[a]

environment	N	B		Ca		Co		Cr		Cu		Fe		K		Mg		Mn		Mo		Na		Ni		P		Se		Zn	
Bundaberg	30	19.4	ab	568	ab	0.077	b	0.016	a	7.67	a	15.6	bc	6819	c	1770	a	19.7	c	1.248	a	62.2	a	2.13	c	3781	b	0.059	c	29.7	a
Kairi	27	15.0	c	582	a	0.077	b	0.017	a	6.63	b	15.6	bc	7100	b	1627	b	23.1	a	0.188	bc	11.9	b	8.08	a	3658	bc	0.134	a	27.6	b
Kairi CBR	36	16.1	bc	528	b	0.083	b	0.018	a	5.91	c	14.4	c	6830	c	1511	c	20.1	bc	0.141	c	11.1	b	3.59	c	3442	d	0.099	b	22.7	c
Kairi FDR	36	18.2	abc	556	ab	0.076	b	0.027	a	7.72	a	16.5	ab	7574	a	1636	b	21.6	ab	0.374	b	3.6	c	5.97	b	3946	a	0.111	ab	27.1	b
Taabinga	27	21.0	a	570	ab	0.102	a	0.016	a	5.68	c	17.4	a	6412	d	1756	a	17.7	d	0.114	c	13.9	b	9.98	a	3589	cd	0.033	c	26.3	b

[a] Trial locations marked with different lower-case letters had significantly different ($P < 0.05$) concentrations of that element according to Tukey's HSD test.

observed. Ultra-early genotypes were clearly distinguished from full-season lines along the PC1 axis, which corresponded primarily with higher Fe, Mg, and P concentrations in the former compared to the latter (**Figure 4**). D175-3-p17-3 and Middleton were distinguished on PC2, by a combination of high Ca and relatively low Mn and Zn contents. Meanwhile, PC3 separated the remaining full-season genotypes into two groups: D147-p3-115 and Sutherland with high PC3 scores (i.e., higher K and lower Co and Cu levels), and Page and PCA213 with low PC3 scores (**Figure 5**). Thus, both high-concentration elements, such as Ca, K, Mg, and P, and trace elements, such as Co, Cu, Fe, Mn, and Zn, were important descriptors of genotypic difference.

DISCUSSION

Quality Controls and Method Validation. Several quality controls were implemented to maximize analytical accuracy and precision. These included correction of background contamination from the digestate matrix by subtraction of a reagent blank that was processed in each digestion batch; control of matrix interferences by internal standardization with rhodium or yttrium; and monitoring of calibration stability by regular measurement of a calibration blank (2% nitric acid) and check standards. The reliability of experimental results was validated by subjecting wheat flour and peanut butter CRM to mineralization and analytical conditions that were identical to those of the peanut samples. Both CRM were used for validation because peanut butter was the matrix most similar to raw kernels, whereas certified concentrations of more analytes were available for the wheat flour CRM. Experimentally derived concentrations of all elements for which reference values were available were within 10% of certified concentrations. The exception was Na in the wheat flour CRM, which deviated by 26% from the wheat flour reference value. Na recovery was nevertheless regarded as acceptable because the typical Na concentration of the peanut samples was several times higher than the peanut CRM, and recovery from the peanut butter CRM deviated only 2% from the certified value. Results were repeatable, with the RSD of analyses performed over the course of the experiment reaching a maximum of 10−11% for Mo, Na, and Se analyses, and remaining at just 3−6% for other certified elements.

Overview of ANOVA and PCA Results. ANOVA confirmed that genotype, environment, and G × E effects were significant ($P < 0.05$) for all elements excluding Cr. Pairwise comparisons allowed us to identify genotypes that had relatively high concentrations of specific elements (**Table 3**). Most notably, D175-3-p17-3 contained outstanding concentrations of Ca (721 μg/g) compared to the other genotypes tested and relatively high levels of Fe (17.3 μg/g). Similar Fe content was found in D192-p397-1, D193-p3-6, and D193-p3-8 kernels. D147-p3-115 and Sutherland had very high levels of K (7782 and 7795 μg/g) and Na (33.9 and 42.4 μg/g); Middleton and Sutherland were high in Mo (0.698 and 0.721 μg/g); and high P (4150 and 4212 μg/g) was measured in D192-p397-1 and D193-p3-8 kernels. Samples from Bundaberg contained very high levels of Mo (1.248 μg/g) and Na (62.2 μg/g). The former may be attributed to the practice of Mo application as soil and/or foliar treatment during crop growth. High Na was probably due to the use of irrigation water sourced from underground bores that tend to become increasingly saline toward the end of the season in the absence of rainfall. Otherwise, environmental effects lacked prominence, despite the presence of statistically significant differences according to Tukey's pairwise comparisons (**Table 4**).

PCA demonstrated the complexity of the genotype, environment, and variate relationships to a greater depth than ANOVA. Genetic contribution to seed mineral composition was masked by

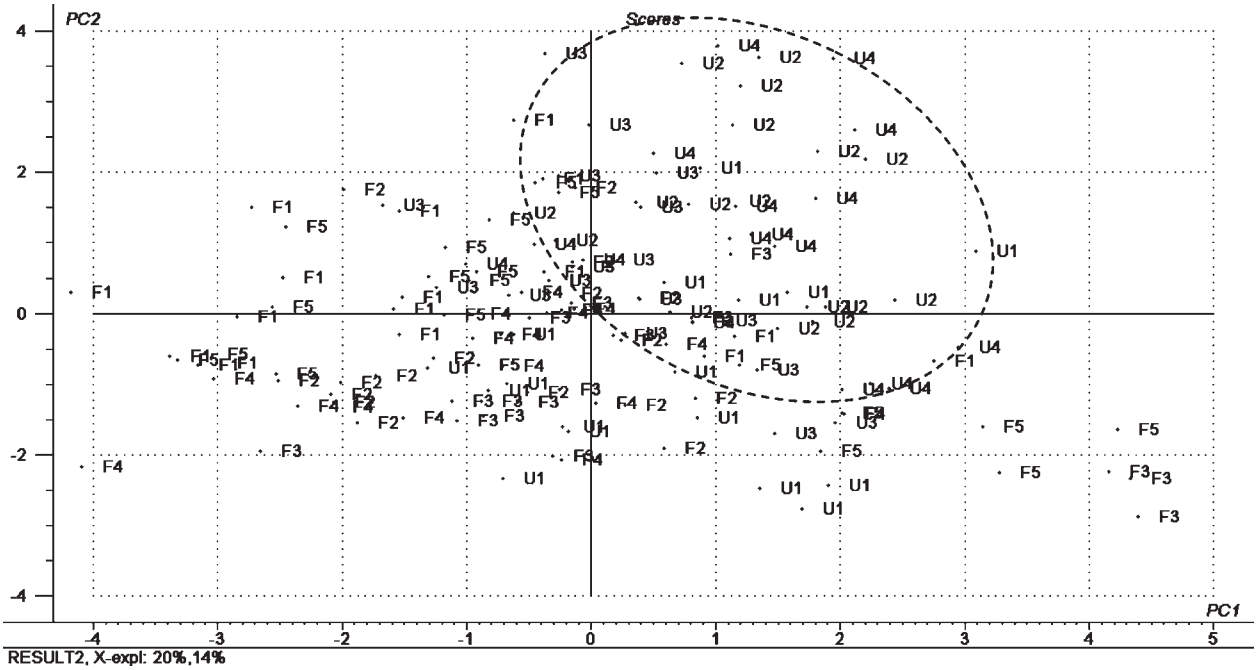

Figure 1. Scores plot of PC1 vs PC2 for autoscaled data, marked by the genotype (F1, D147-p3-115; F2, PCA213; F3, Middleton; F4, Page; F5, Sutherland; U1, D175-3-p17-3; U2, D192-p397-1; U3, D193-p3-6; and U4, D193-p3-8).

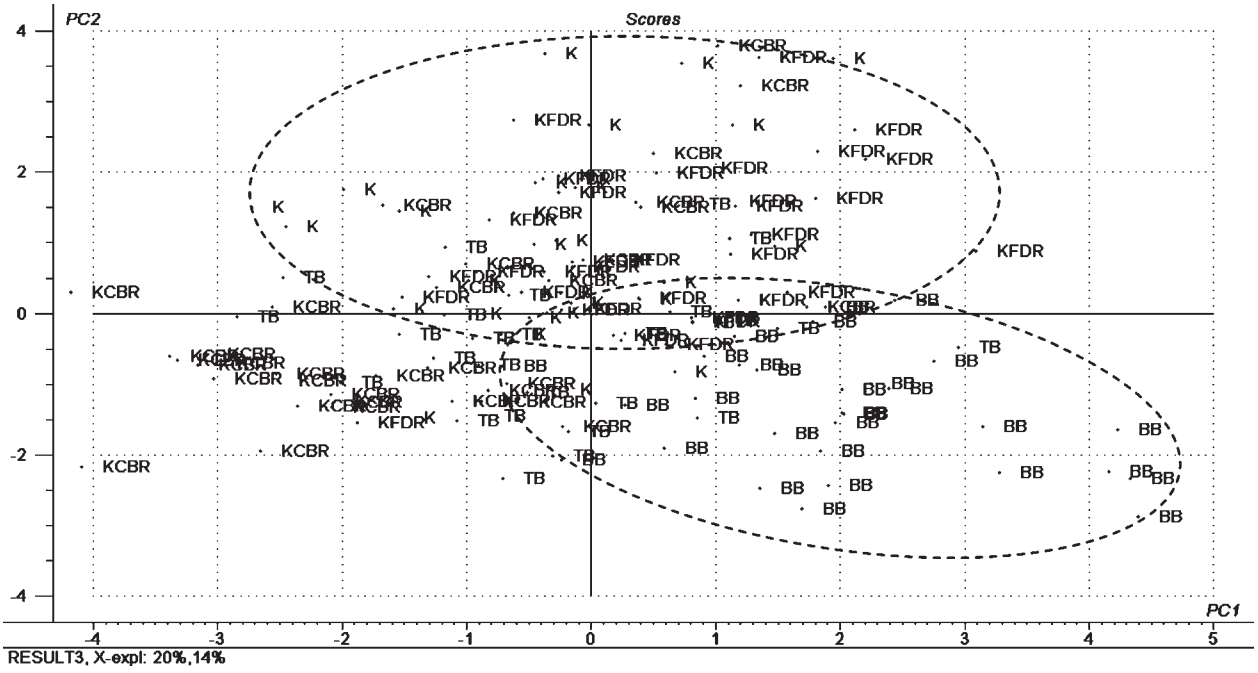

Figure 2. Scores plot of PC1 vs PC2 for autoscaled data, marked by the environment (BB, Bundaberg; K, Kairi; KCBR, Kairi CBR; KFDR, Kairi FDR; and TB, Taabinga).

environmental effects, such that poor clustering of genotypes resulted from PCA of autoscaled data. Distinct genotype groupings were revealed by environment-scaled PCA, even though data remained complex, and many PC were required to explain the variation (i.e., 10 PC to explain 90% of variance). Most prominently, ultra-early genotypes were distinct from full-season genotypes along PC1 mainly because of generally higher Fe, Mg, and P concentrations (**Figure 4**). Subsequent PC gave further separation of genotypes. In particular, D175-9-p17-3 and Middleton were distinguished by high Ca and relatively low Mn and Zn contents; D147-p3-115 and Sutherland had relatively high K and low Co and Cu levels; while Page and PCA213 had relatively low K and high Co and Cu contents (**Figure 5**). It should be emphasized that these indicate only genotypic trends since each PC explained only a small portion of data variance. Nevertheless, the separation of ultra-early and full-season genotypes along PC1 (Fe, Mg, and P) and clustering of the sister-lines D147-p3-115 and Sutherland (K, Co, and Cu), provide enough suggestion of genetic control to warrant further investigation of G × E and trait segregation.

Implications of G × E Interaction for Breeding. Peanuts are bred conventionally in Australia; therefore, new genetic combinations can arise only from the natural processes of sexual recombination

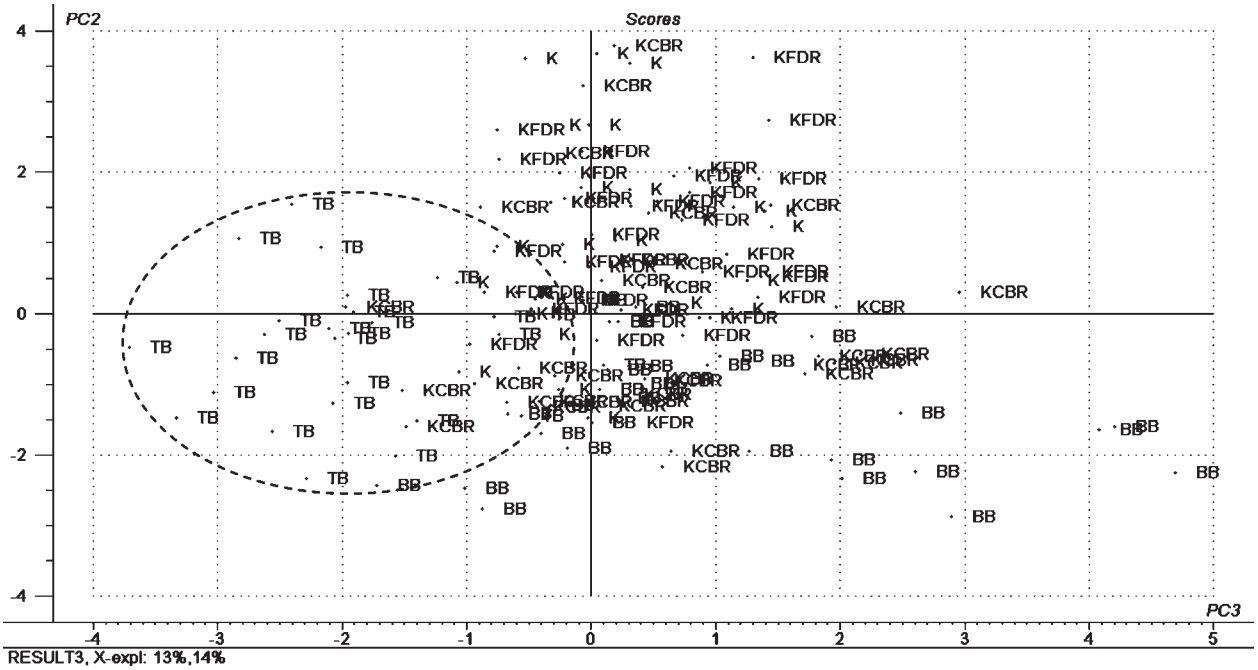

Figure 3. Scores plot of PC3 vs PC2 for autoscaled data, marked by the environment (BB, Bundaberg; K, Kairi; KCBR, Kairi CBR; KFDR, Kairi FDR; and TB, Taabinga).

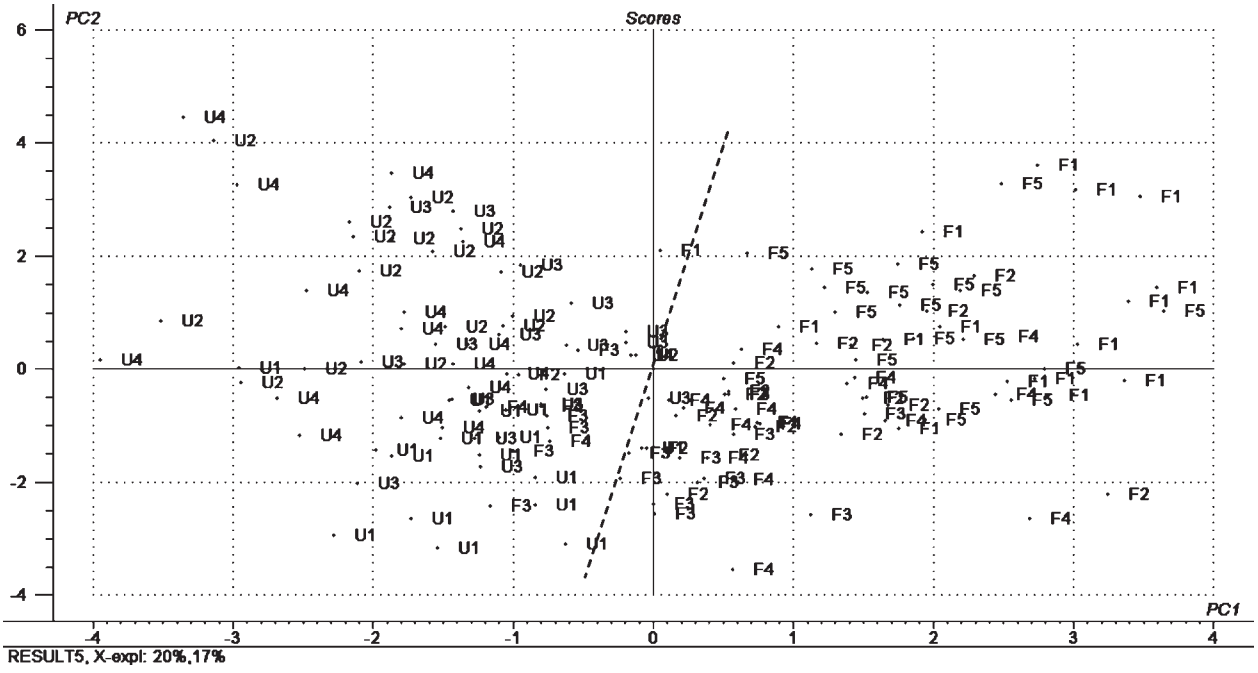

Figure 4. Scores plot of PC1 vs PC2 for environment-scaled data, marked by the genotype (F1, D147-p3-115; F2, PCA213; F3, Middleton; F4, Page; F5, Sutherland; U1, D175-3-p17-3; U2, D192-p397-1; U3, D193-p3-6; and U4, D193-p3-8).

and segregation that occur in a heterozygous breeding population. Variation is broadly considered to have three components, i.e., genetic/genotypic, environmental, and genotype-by-environment (G × E) interaction. G × E interaction introduces uncertainty into the selection process and therefore influences the estimation of trait heritability and response to selection.

There is no consensus on the best way to manage G × E interaction in plant breeding programs. This is a major research topic in agricultural genetics, and a range of predominantly statistical strategies of characterizing G × E interaction and selecting genotypes are proposed in the literature (*14*). Partitioning of total variation into genotype, environment, interaction, and error components is widely practised using standard ANOVA or alternatives that emphasize the extent to which the interaction is due to heterogeneous environmental variance in different genotypes or heterogeneous genetic variance in different environments (*15*). The latter type of G × E interaction most impedes genotype selection because it can lead to reranking in different environments.

When G × E interaction has a stronger influence than the genetic correlations between environments, it becomes an indirect selection for adaption to distinct environments. In view of this, pattern analysis methods have been developed for the classification

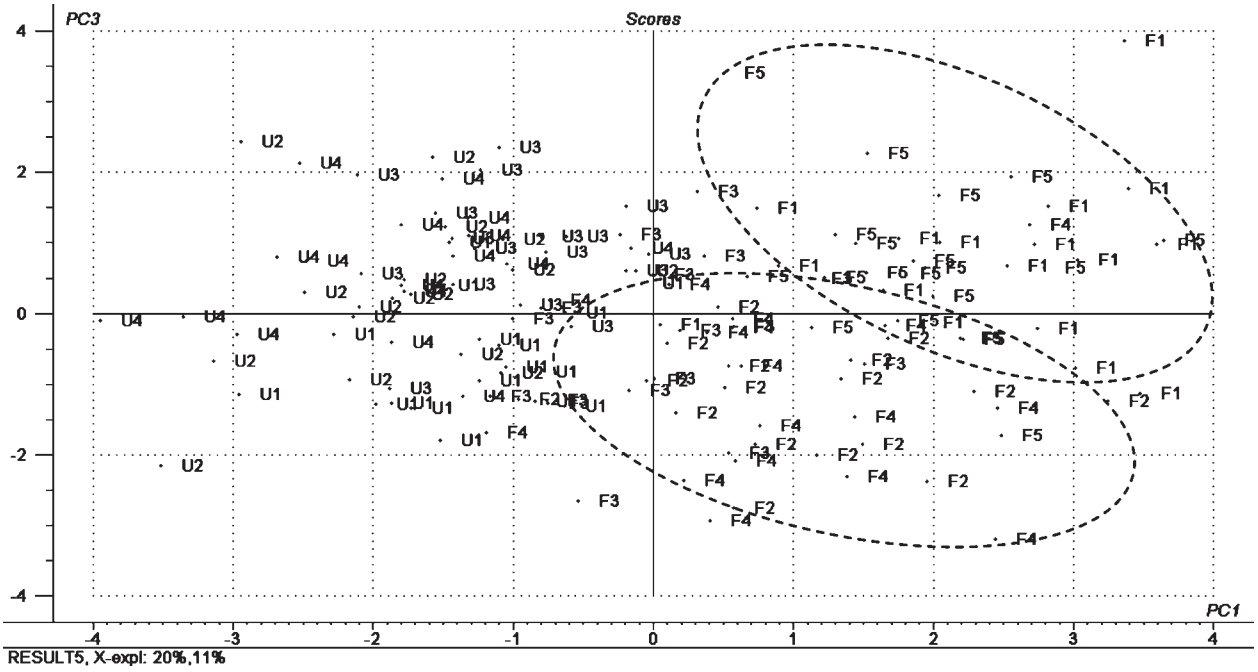

Figure 5. Scores plot of PC1 vs PC3 for environment-scaled data, marked by the genotype (F1, D147-p3-115; F2, PCA213; F3, Middleton; F4, Page; F5, Sutherland; U1, D175-3-p17-3; U2, D192-p397-1; U3, D193-p3-6; and U4, D193-p3-8).

and ordination of environments, particularly in large multienvironment trials, so that these megaenvironments are analyzed rather than individual and possibly highly disparate individual environments (*14*). The foremost methods are variants of PCA known as the Additive Main effects and Multiplicative Interaction model (AMMI) and Genotype main effects and G × E interaction model (GGE), both of which have strong proponents (*16*, *17*). In this study, in addition to performing standard ANOVA, we have followed the GGE approach by environment-scaling data for analysis in order to reveal patterns in the genetic and G × E components of variation. Some clustering of genotypes occurred around both macro- (Ca, Mg, P, and K) and microelements (Co, Cu, Fe, Mn, and Zn), but larger-scale data analysis covering a greater number of genotypes, environments, and seasons is required to fully characterize the G × E interaction affecting mineral accumulation in peanut kernels and to determine whether it can be partitioned into megaenvironments.

Breeding Potential for Mineral Composition. The nine genotypes in this study were selected for the diversity of their mineral content according to our earlier screening of 56 peanut lines from the Australian breeding program (unpublished data). The stability of genetic differences in several elements of interest were confirmed by significant ($P < 0.05$) genotype effects according to ANOVA (**Table 2**). In particular, D175-3-p17-3 kernels contained consistently high concentrations of Ca; Middleton and Sutherland had high Mo levels; D147-p3-115 and Sutherland had very high K and Na levels; while D192-p397-1 and D193-p3-8 were high in P.

Ca is distinct from other major cations in that it enters the peanut seed mainly by direct diffusion from the soil and is largely phloem-immobile, meaning that there is little downward redistribution after transpiration-driven uptake through the xylem (*18*). Genotypes that are less susceptible to pops syndrome caused by Ca deficiency demonstrate improved translocation of Ca from the pod pericarp to the seed, which may be due to differing seed/shell contact, sink signaling by phytohormones such as auxins, or shift in the balance between the assimilate demands of vegetative growth and pods (*19*). A field trial of gypsum ($CaSO_4$) applications to Virginia peanuts found that the smaller-seeded cultivar (73 g/100 seed) had a lower Ca requirement than the larger-seeded cultivar (101 g/100 seed) (*20*), supporting the postulate that greater surface-to-mass ratio should favor Ca accumulation in seed in view of its phloem immobility. However, studies of Ca concentrations in F_1-F_3 progenies of a chickpea cross indicated that Ca content was determined by genetic factors other than weight genes (*21*), while Branch and Gaines (*22*) found no association between Ca concentration and seed size in peanuts. It is clear that there is a strong genetic component to Ca accumulation, and controlled studies to better characterize Ca physiology in peanuts could yield specific genetic or biochemical markers for breeding targets that could aid selection. Traits that lead to high Ca uptake and accumulation in seeds could reduce the need for soil amelioration as well as improve peanut nutritional quality.

Mo is essential for biological nitrogen fixation (BNF) as a cofactor of the most common class of nitrogenase, the enzyme catalyzing the reduction of atmospheric nitrogen (N_2) into ammonium (NH_4^+) in nitrogen-fixing (diazotrophic) bacteria (*23*). For crops that form symbiotic relationships with diazotrophic rhizobacteria, including legumes and several mainly graminaceous nonlegumes, maximizing BNF is a far more efficient and inexpensive method of delivering N requirements than artificial N fertilization. Mo deficiency often limits BNF in the field, especially where intensive cultivation has led to depletion of soil micronutrients, and Mo supplementation through fertilizer mixtures, Mo frits, seed coatings, and foliar sprays have been used to sustain yields (*24*). Breeding for high Mo would be valuable from both nutritional and agronomic perspectives, and the relatively high Mo accumulation in Middleton and Sutherland lines is worthy of further investigation.

The sister lines, D147-p3-115 and Sutherland, had K and Na concentrations that were significantly ($P < 0.05$) higher than those of other genotypes (**Table 2**). The genotypic differences in Na content were especially large, with D147-p3-115 and Sutherland kernels having on average 2- to 3-fold more Na than the other lines, regardless of the growing environment. This result

is interesting when we consider that saline soil is known to inhibit Ca and K uptake, while promoting Fe, Mn, P, and Na accumulation (*25*), and that Ca, Mg, and K have similar affinities for carrier sites across plant cellular membranes such that uptake of these cations is typically competitive (*26*). D147-p3-115 and Sutherland demonstrated the ability to sustain very high uptake of both K and Na, although their Ca and Mg concentrations were among the lowest. Although salinity is not a priority of the Australian peanut breeding program, an approach for manipulating mineral composition may lie in strategies used for breeding salinity tolerance. It should be noted that the high-Na D147-p3-115 and Sutherland lines contained an average of 33.9 and 42.4 μg/g (i.e., 0.85 and 1.1 mg/25 g serving), respectively, which is far below the adequate daily intake of 460−920 mg recommended for an adult (*27*), and would not be nutritionally detrimental.

Agronomic Manipulation of Mineral Composition. The statistical differences between the Mo contents of Middleton and Sutherland and other genotypes suggest a strong genetic component for Mo concentration. However, the prominent response to Mo supplementation irrespective of genotype, which resulted in 3- to 10-fold more Mo in Bundaberg kernels compared to that in other locations (**Table 3**), suggests that agronomic manipulation may be of greater practical significance. Research on several legumes indicate that the G × E interactions affecting seed Mo content and yield responses to Mo fertilization are varied. Plants grown from soybean seed that were highly Mo-enriched by Mo fertilization in the previous season sustained a high level of BNF and produced enhanced yields that were not improved by further fertilization, regardless of increases in seed Mo content (*24*). However, Mo requirements to achieve optimal BNF and yields in bean plants grown from high- and low-Mo seed varied with cultivar, such that the cultivar dominated the response to fertilization regardless of the Mo concentrations of the seed (*28*). Our data confirmed that the Mo content of peanut kernels is strongly affected by genotype, growing environment, and G × E interaction. Further experiments could determine the extent to which the agronomic/cropping history contributes to Mo in seed, compared to Mo fertilization during the current growing season. It is feasible that a combination of genetic and agronomic manipulation could be used to consistently achieve optimal levels of Mo in peanut seed, for maximum BNF, productivity, and postharvest nutritional quality.

The interaction of genotype and endogenous/exogenous Mo is further complicated by the soil chemical ecology. The structural similarity of molybdate (MoO_4^{2-}), sulfate (SO_4^{2-}), and phosphate (HPO_4^{2-} or $H_2PO_4^-$) anions suggests that nonspecific uptake will be competitive. This premise is supported by the finding that single superphosphate, which contains $CaSO_4$, failed to improve yields of Nigerian peanuts despite overcoming the S and P deficiencies typical of the region. In contrast, (sulfate-free) triple superphosphate enhanced yields and Mo uptake, particularly in P- and Mo-deficient soils (*29*). S is thought to depress Mo uptake by lowering soil pH when it undergoes microbial oxidation, which leads to decreased Mo availability and an acidified rhizosphere that favors the uptake of cations over anions, as well as competing directly with molybdate for the uptake into the roots (*29*). Failure to take such considerations into account could be responsible for the apparently inconsistent responses reported by different studies of Mo fertilization in the literature.

The prospect of combining genetic improvement with agronomic management is worth exploring for other elements of interest. In general, response to fertilization has been evaluated in terms of yield outcomes rather than effects on mineral concentrations. Productivity is obviously directly related to plant nutrition, but it may be desirable to boost the mineral content of peanut kernels beyond the yield benefits. In the peanut gypsum trial discussed earlier (*20*), gypsum recommendations were assessed on yield, grade, and seed germination responses. Treatments, however, would also have affected Ca and Mo concentrations in seed and indirectly influenced the uptake of other minerals. The typical K rates were found to be suitable as they had no effect on pod yield or market grade. Yet K fertilization is known to promote proton release, thereby reducing soil pH and enhancing cation uptake, although Fe acquisition may remain impaired by high soil $CaCO_3$ (*30*). Rhizosphere acidification has been shown to enhance Ca, Mg, Mn, and Zn uptake in wheat and chickpea (*31*). Several articles have reported significant genotypic variability in the Se content of wheat, rye, and soybean seed, as well as the edible parts of some vegetables (*32*), although differences may be small compared to the background variation. Scope for agronomic enrichment of Se content is evidenced by at least one study showing that Se in winter wheat grain increased by 0.016−0.029 μg/g, or 10-fold, following Se fertilization at 10 trial sites over 2 years (*33*). We found that Se concentration in peanut kernels was significantly influenced by genotype, environment, and G × E interaction, although absolute differences were small, as in these previous studies. Again, it seems likely that genetic improvement could be coupled with agronomic management to achieve consistently high mineral content.

Further Work. There are several avenues of research that would support the incorporation of mineral composition into the breeding program. First, the reported sample set is limited to nine genotypes grown in five environments in a single season. Enlarging the data set to include more genotypes and environments harvested over at least several seasons would greatly improve the characterization of the G × E interaction and allow more explorative statistical analyses. The breeding lines that we have tested, while genetically diverse, have already been subjected to selection pressures centering on program priorities of yield, disease, and kernel quality criteria. Our current accessions display a promising level of genotypic variation, but breeding efforts would benefit from the assessment of the genetic variability within the international gene bank, especially germplasm adapted to extremes in soil quality. Having said this, several tested lines accumulated high concentrations of Ca (D175-3-p17-3), Mo (Middleton and Sutherland), K, and Na (D147-p3-115 and Sutherland). It would be worth examining the heritability of these traits, as well as the effect of combining genetic and agronomic improvement. Some of the basic physiology regarding the interactions of soil nutrients and factors affecting mineral accumulation remain poorly understood, and elucidation of these topics through controlled studies would illuminate efforts to manipulate mineral composition, both through breeding and soil management.

On a different note, breeding can be regarded as one of the first links in a peanut supply chain that ends with the consumers. The need to contend in an increasingly competitive global marketplace is the driving force for the Australian breeding program, pushing continual improvements in productivity and kernel quality, although outcomes are expected to eventually benefit agriculture and public health more generally. A key question for the peanut industry, then, is how much of which elements are desirable? It is straightforward to compare the mineral content of a typical serving of peanuts with recommended dietary mineral intakes but rather more challenging to evaluate the health impacts and consumer acceptance of a mineral-rich snack food. The issue is further complicated by the varying bioavailability of different species of minerals (*34*), which may be adversely affected by antinutrients such as phytate−mineral complexes (*35*), and may itself be subject to G × E interaction (*36*). Staple food products

may be subject to mandatory or voluntary fortification in the interests of public health; the enrichment of snack foods, however, is essentially market-driven. In this respect, research into consumer perceptions of enriched peanut products, awareness of health benefits, and willingness-to-pay is as important as the scientific investigation, to ensure that breeding targets are in accord with the marketing opportunities for potential new peanut varieties. Snack foods make a large contribution to daily nutrition, especially of children, and healthier food options are attractive as a form of preventative healthcare, from both public health and food marketing perspectives.

Conclusions. A multienvironment study of mineral composition in nine diverse peanut genotypes was conducted. Genotype, environment, and G × E interaction were all found to have significant effects on mineral composition according to standard ANOVA. Several of our current peanut accessions accumulated high concentrations of specific minerals in their kernels, i.e., D175-3-p17-3 had high Ca; Middleton and Sutherland had high Mo; D147-p3-115 and Sutherland had high K and Na; while D192-p397-1 and D193-p3-8 had high P content. Clustering of some genotypes in environment-centered PCA confirmed that there was substantial genetic control of both micro- and macroelement concentrations in peanut kernels, and expansion of the data set is warranted to explore these relationships in greater depth. In addition to the genetic components of mineral composition, several elements were strongly influenced by the growing environment, especially Mo and Na. It is feasible that a strategy combining genetic improvement and agronomic management could be designed to ensure that optimal concentrations of minerals are consistently achieved, bringing benefits during the growing season as well as enhancing postharvest nutritional quality.

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