Genetic Evaluation of Micronutrient Traits in Diploid Potato from a Base Population of Andean Landrace Cultivars

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ABSTRACT

Micronutrient malnutrition is a global health problem. An improved understanding of the genetic variation of important micronutrient traits within a potato breeding population will help devise breeding strategies for the biofortification of this important food staple. The dataset consisted of 556 individuals from 17 full-sib diploid families grown in 2006 in Huanuco, Peru, and 1329 individuals from 32 full-sib families grown in 2009 in Ayacucho, Peru. Genetic parameters were estimated using univariate and multivariate individual Bayesian models for micronutrient tuber content including Fe and Zn. Genetic variance was additive and heritability estimates were moderate (0.36 to 0.57) and inflated if the common environment of full-sibs was not taken into account. Posterior modes of genetic correlation estimates between minerals, when analyzed on a dry-weight basis, were all positive (0.04 to 0.72) and between minerals and tuber dry matter were negative (–0.14 to –0.38). On a fresh-weight basis, genetic correlations between minerals and tuber dry matter were small but positive (0.05 to 0.18). The implications and challenges for selective breeding to enhance micronutrient content in potato tubers are discussed.

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Abbreviations: AA, ascorbic acid; BLUP, best linear unbiased predictors; CIP, International Potato Centre; DIAG, diagonal matrix; DIC, deviance information criterion; EAR, estimated average requirement; GEI, genotype-by-environment interaction; ICP, inductively coupled plasma–optical emission spectrophotometry; MCMC, Markov chain Monte Carlo; MET, multienvironment trials; MVN, multivariate normal; RCBD, randomized complete block design; REML, restricted maximum likelihood; US, unstructured matrix.

Improving the health benefits of major food staples by enhancing micronutrient content of essential vitamins and minerals in the edible portions has become an important target for plant breeders in recent years (Bouis and Welch, 2010; Graham et al., 1999; Gregorio, 2002; Nestel et al., 2006; Pfeiffer and McClafferty, 2007a; Sands et al., 2009). Biofortification is the genetic improvement of the nutritional value of food crops through conventional plant breeding or biotechnology. It is supported by predictive cost-benefit analysis as an effective approach to help reduce micronutrient deficiencies (Nestel et al., 2006) and has been endorsed as a priority development goal by the Copenhagen Consensus, an international think-tank on global poverty (Horton et al., 2009). Global micronutrient deficiencies do not

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tend to receive the same attention (from the media or otherwise) as calorific malnutrition and are a problem in poorer communities in particular, especially for women, infants, and children. Affected communities may often have an adequate supply of carbohydrate and protein, but lack some vitamins and minerals that are essential for healthy body function. The effects of micronutrient deficiencies are not always immediately apparent, and therefore are often described as a "hidden hunger" (Stein et al., 2005). Iron deficiency alone is estimated to impact on 2.7 billion people globally (Hirschi, 2009), and the effects are reported to include impaired physical activity, impaired cognitive development, and both maternal and infant mortality. Zinc deficiency is also a widespread global problem and can lead to infant and child respiratory infection, diarrhea, stunting, and mortality (Stein, 2010).

Reducing micronutrient malnutrition is likely to lead to an improvement in public health and in economic outcomes at a local scale and beyond, as well as an improved quality of life for individuals (Stein et al., 2005). The importance of potato as a food staple in poorer regions of Asia, sub-Saharan Africa, and Latin America, combined with evidence for genetic variability for mineral concentrations in a favorable food matrix, have made biofortification a new potential breeding target at the International Potato Centre (CIP; Bonierbale et al., 2007). Although agronomic and postharvest practices can affect nutritional content (Hirschi, 2009; Rengel et al., 1999), the variation in micronutrient levels in many food crops is considered to have an exploitable genetic component (Graham et al., 1999; Gregorio, 2002). Knowledge on the level and type of genetic variation present in crop gene pools is required to help determine an appropriate breeding strategy.

A crop breeding program requires estimates of variance components, not only to obtain genetic parameters to help define a breeding strategy, but also to predict breeding values to identify superior parents and breeding lines for variety development. Linear mixed models provide an improved representation of the underlying random and error components, that is, the ability to model different (co)variance structures when pedigree information is used and analysis is further extended to multiple traits and multienvironment (MET) trials (Oakey et al., 2007). Pedigree information is exploited via the relationship matrix **A** (Henderson, 1976), accounting for the expected additive genetic relationships between all individuals in the pedigree. Exploiting these relationships, a breeding value can be fitted for all members of the pedigree, even those without trait records, hence such models are named *individual* or *individual plant* models (but more commonly referred to as *animal models*) as opposed to the more traditional family-based approaches. Combining information on the individual and all relatives in a selection program greatly increases the accuracy of selection, (Lynch and Walsh, 1998). Variance components are required for the estimation of the BLUPs (best linear unbiased predictors) of breeding values. In practice, the true variance components are unknown but are estimated from the data either by likelihood approaches (usually by REML, restricted maximum likelihood; Patterson and Thompson, 1971) or from Bayesian inference (e.g., Sorensen and Gianola, 2002). Bayesian inference using Markov chain Monte Carlo (MCMC) sampling methods (often via the Gibbs sampler) is attractive for variance component and variance ratio estimation, as the posterior distribution provides the credible interval as a realistic measure of uncertainty around the point estimate (Waldmann and Ericsson, 2006). Prior information can also be included in Bayesian inference if available from previous studies, such as evaluation of previous breeding generations. Bayesian methods have remained out of reach for most plant breeders because of the apparent lack of user-friendly software to apply individual models to crop data. This may partly explain the limited number of crop breeding programs reporting the use of these approaches for genetic evaluation.

Previous studies have demonstrated genetic diversity in Andean potato germplasm for micronutrient traits. Andre et al. (2007) found significant diversity in the tuber content of Fe, Zn, Ca, vitamin C, carotenoids, and phenolics from a sample of 74 genotypes of a CIP core collection, which was made up of eight taxonomic groups from the *Solanum tuberosum* species. Burgos et al. (2007) identified genotype variability in Fe and Zn concentrations for landrace cultivars from several taxa of *Solanum*. Derived from a base population of diploid landrace accessions, the breeding population of the present study was initiated in 2004 at CIP in Lima, Peru, in coordination with the HarvestPlus Biofortification Challenge Program (Pfeiffer and McClafferty, 2007a). The CIP aims to enhance the micronutrient content of potato tubers at the diploid level and use this material in a prebreeding strategy before introduction as parental material into tetraploid breeding populations. The objective of this study was to estimate variance components and genetic parameters of important micronutrient traits from a breeding population based on landrace genotypes using data collected from tuber progeny field tests. This will assist in the recommendation of selection procedures and the development of a breeding strategy for biofortification. The study also illustrates that Bayesian procedures using the MCMC to fit the individual model are now more accessible to plant breeders for the routine estimation of variance components, genetic parameters, and breeding values.

MATERIALS AND METHODS

Plant Material

Three diploid cultivar groups of *Solanum tuberosum*, namely *stenotomum*, *goniocalyx*, and *phureja*, made up the parental base

Table 1. Base parents (G_0) of the first generation (G_1).

Female	Group†	Cultivar name
702736	*Stn*	Puca Micnush
703280	*Gon*	unknown
703312	*Stn*	Morada Taruna
703317	*Stn*	Chingos
702815	*Stn*	Morar Nayra Mari
703291	*Phu*	Rosca
703825	*Gon*	China Runtush
704393	*Gon*	Maria Cruz
701165	*Stn*	Calhua Rosada
703168‡	*Gon*	Puca Pishgush
703352	*Gon*	Cashpadana Amarilla
703421§	*Stn*	Poluya
703831	*Gon*	Pampuna
703831	*Gon*	Pampuna
700313	*Stn*	Cuchipa Ismaynin
703197	*Stn*	Yana Sucre
704481	*Gon*	Amarilla
Male	**Group†**	**Cultivar name**
703287	*Stn*	Cceccorani
703421	*Stn*	Poluya
703825	*Gon*	China Runtush
704218	*Phu*	Yema de Huevo

† Stn: Stenotomum; Gon: Goniocalyx; Phu: Phureja.

‡ No progeny measured for mineral content.

§ No progeny measured for vitamin C content.

population. For the first generation (G_1), a sample of cultivars of the three species from the study of Burgos et al. (2007) was identified as base parents (G_0; Table 1) and crossed following a nested mating design, that is, each of a group of males mated to a subset of females. Seventeen full-sib families and four half-sib families from four males and 16 females were generated; 703825 ('China Runtush') and 703421 ('Poluya') were both female and male parents. The first generation (G_1) was grown in 2006 in Huanuco, Peru, at an altitude of 3800 m. Tuber families, consisting of three tubers (clones) per genotype, were grown within full-sib family groups with three replications of each family in a randomized complete-block design (RCBD). Planting distances were 0.3 m between plants and 0.9 m between rows. At harvest, tuber samples of 12 genotypes, if possible, were taken at random from each replicate within each family for micronutrient analysis.

All analyses were conducted on peeled tubers. Mineral content was determined by inductively coupled plasma–optical emission spectrophotometry (ICP) at Waite Analytical Services in Australia. For further details of tuber sample preparation and analytical methods for mineral determination, see Burgos et al. (2007). Micronutrients analyzed included Fe, Zn, Ca, and vitamin C. Aluminum was used as an indicator of contamination of samples with soil or dust, as it is often found in higher levels in the soil and lower levels in grains and tubers (Pfeiffer and McClafferty, 2007b). Ascorbic acid (AA; vitamin C) concentrations were evaluated by the spectrophotometric method of Egoville et al. (1988). The method is based on the ability of AA to reduce dye 2,6-dichloroindophenol. Concentrations are expressed in mg/100 g, fresh weight. The dry matter content of the individual samples was determined on the basis of differences in weight before and after oven drying at 100°C and used to estimate the concentration in mg/100 g, dry weight. In G_1, there were 556 observations, which included 487 for mineral content and 527 for vitamin C and dry matter content. Family sizes analyzed ranged from 23 to 36 genotypes.

Parent selection for the second generation (G_2) was based on the phenotypic values of individuals from the G_1 trials for higher Fe, Zn, and other desirable agronomic characteristics. Over 40 potential parents were initially chosen, but natural attrition (due to male or female parent sterility, for example) resulted in a final crossing scheme made up of eight female parents and eight male parents intercrossed in a factorial mating design, that is, each female member of the group was mated to each male member using two sets of four females × four males generating 32 full-sib families. For G_2, seedlings from the factorial crosses were transplanted into the field in Huancayo, Peru (2007–2008), using a RCBD with four replicates and 30 plants per replicate. At harvest, a set of tuber families from across the complete trial were retained and planted as a RCBD in Ayacucho, Peru (2008–2009). Three plants (clones) per genotype were planted in each plot within full-sib family groups with three replicate groups per family. At harvest, tuber samples from clones of each three-plant plot were pooled and analyzed for the micronutrient content of peeled tubers by ICP and for dry matter content, as previously described. In total, there were 1329 progeny records analyzed for Fe, Zn, Ca, and dry matter content, with family size ranging from 19 to 74 genotypes. There was no data collected for vitamin C from the G_2 Ayacucho breeding trial.

Data Analysis

A Bayesian approach based on an individual model was used to estimate variance components, heritabilities, and genetic correlations for various micronutrient traits in potato. The general form of the full univariate model was:

$$\mathbf{Y} = \mathbf{Xb} + \mathbf{Z}_1\mathbf{a} + \mathbf{Z}_2\mathbf{c} + \mathbf{Z}_3\mathbf{f} + \mathbf{e}$$

where $\mathbf{Y}$ is a vector of observations on the trait under study, and $\mathbf{X}$, $\mathbf{Z}_1$, $\mathbf{Z}_2$, and $\mathbf{Z}_3$ are known incidence matrices. In a traditional generalized linear model, the vector of replicate effects $\mathbf{b}$ may be considered as fixed effects, but in the Bayesian analysis, $\mathbf{b}$ was fitted with a prior of zero mean and large variance. The vector of random additive genetic effects of individual genotypes, $\mathbf{a}$, has the distribution assumed to be multivariate normal (MVN), with the parameters $(0, \sigma_a^2, \mathbf{A})$, $\mathbf{c}$ is a vector of common environmental effects with the distribution assumed to be MVN, with the parameters $(0, \sigma_c^2, \mathbf{I}_c)$, $\mathbf{f}$ is a vector of family effects with the distribution assumed to be MVN, with the parameters $(0, \sigma_f^2, \mathbf{I}_f)$, and $\mathbf{e}$ is the vector of errors distributed MVN with parameters $(0, \sigma_e^2, \mathbf{I}_e)$, $\mathbf{I}_c$, $\mathbf{I}_f$, and $\mathbf{I}_e$ represent identity matrices of size equal to the number of common environments, families, and plants, respectively. The subscripted σ^2 is the variance of each of the random effects (σ_a^2, σ_c^2, σ_e^2 additive genetic, common environment, and error variance, respectively). $\mathbf{A}$, the numerator relationship matrix, describes the additive genetic relationships among individual genotypes and was generated

from the pedigree. In matrix format, the random effects from the general form of the univariate model are defined by:

$$\begin{bmatrix}\mathbf{a}\\ \mathbf{c}\\ \mathbf{f}\\ \mathbf{e}\end{bmatrix} \sim \mathbf{N}\left(\begin{bmatrix}\mathbf{0}\\ \mathbf{0}\\ \mathbf{0}\\ \mathbf{0}\end{bmatrix}\begin{bmatrix}\mathbf{A}\sigma_a^2 & \mathbf{0} & \mathbf{0} & \mathbf{0}\\ \mathbf{0} & \mathbf{I}\sigma_c^2 & \mathbf{0} & \mathbf{0}\\ \mathbf{0} & \mathbf{0} & \mathbf{I}\sigma_f^2 & \mathbf{0}\\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{I}\sigma_e^2\end{bmatrix}\right)$$

Data were log-transformed for both univariate and multivariate analyses. Weak priors were assumed for variance components in G_1 that followed an inverse χ^2 distribution with 1 df, $\sigma^2 \sim Inv - \chi^2(1,\phi)$, where ϕ is a scale parameter which apportioned the prior variance equally between the variance components. Trait variances and covariances estimated from the multivariate analysis in G_1 were used as priors for the analysis of G_2 data.

In a factorial design (G_2), the full-sib family component of variance (the male × female interaction) is expected to estimate 1/4 of the dominance effect (Bernardo, 2002). The σ_a^2 and σ_f^2 variance components have the following genetic expectations under the linear mixed model:

$$\sigma_a^2 = 4\sigma_{GCA}^2 = V_A + 1/4V_{AA} + 1/16V_{AAA} + \ldots$$

$$\sigma_f^2 = \sigma_{SCA}^2 = 1/4V_D + 1/8V_{AA} + 1/8V_{AD} + 1/16V_{DD} + \ldots$$

where V_A is the additive genetic variance; V_D is the dominance genetic variance; and V_{AA}, V_{DD}, and V_{AD} are the epistatic genetic variances due to interactions of additive effects, dominance effects, and additive and dominance effects at two loci; σ_{GCA}^2 is the variance due to the general combining ability of the parents; and σ_{SCA}^2 is the variance due to the specific combining ability of the crosses. For this study, epistatic genetic effects were assumed negligible, and σ_f^2 was not estimated from data of the nested design (G_1) due to the relatively small number of full-sib progenies measured. In general, heritability (narrow-sense) was obtained from:

$$h^2 = \frac{V_A}{V_P} = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_c^2 + \sigma_f^2 + \sigma_e^2},$$

where V_P is the phenotypic variance with common environment and family (G_2 only) components of variance included where appropriate, as indicated by deviance information criterion (DIC) tests (Spiegelhalter et al., 2002), for both univariate and multivariate analyses.

The common environment ratio (c^2) of full sibs was:

$$c^2 = \frac{V_c}{V_P} = \frac{\sigma_c^2}{\sigma_a^2 + \sigma_c^2 + \sigma_f^2 + \sigma_e^2},$$

where V_C is the common environmental effect of full-sibs.

Heritability in the broad-sense was obtained from:

$$H^2 = \frac{\sigma_a^2 + 4\sigma_f^2}{\sigma_a^2 + \sigma_f^2 + \sigma_c^2 + \sigma_e^2}.$$

All models were fitted using MCMC methods implemented in R (R Development Core Team, 2012) using MCMCglmm (Hadfield, 2010). For univariate analyses, 80,000 iterations were used, storing every 34th sample after an initial burn-in of 12,000. Posterior modes of variance components, and narrow-sense heritabilities from a univariate model in G_1 for all traits were reported. The model for the univariate analyses in G_1 included additive and common environment effects but ignored any full-sib family effect.

Univariate models were further extended to accommodate multivariate analyses, which included Fe, Zn, Ca, vitamin C, and dry matter in G_1 and Fe, Zn, Ca, and dry matter in G_2. For multivariate analyses in both G_1 and G_2, iteration number was increased to 250,000, storing every 95th sample after an initial burn-in of 60,000. Different (co)variance structures for the random effects were fitted, as outlined in Table 4, where DIAG (diagonal) fitted different trait variances and zero covariances between each pair of traits and US (unstructured) fitted both different traits variances and covariances between each pair of traits:

$$\mathbf{DIAG} = \begin{bmatrix}\boldsymbol{\sigma}_{t_1}^2 & \mathbf{0} & \cdots & \mathbf{0}\\ \mathbf{0} & \boldsymbol{\sigma}_{t_2}^2 & \cdots & \mathbf{0}\\ \vdots & \vdots & \ddots & \vdots\\ \mathbf{0} & \mathbf{0} & \cdots & \boldsymbol{\sigma}_{t_n}^2\end{bmatrix}$$

$$\mathbf{US} = \begin{bmatrix}\boldsymbol{\sigma}_{t_1}^2 & \boldsymbol{\sigma}_{t_1,t_2}^2 & \cdots & \boldsymbol{\sigma}_{t_1,t_a}^2\\ \boldsymbol{\sigma}_{t_2,t_1}^2 & \boldsymbol{\sigma}_{t_2}^2 & \cdots & \boldsymbol{\sigma}_{t_2,t_n}^2\\ \vdots & \vdots & \ddots & \vdots\\ \boldsymbol{\sigma}_{t_n,t_1}^2 & \boldsymbol{\sigma}_{t_n,t_2}^2 & \cdots & \boldsymbol{\sigma}_{t_n}^2\end{bmatrix}$$

so that $\boldsymbol{\sigma}_{t_n}^2$ is the variance for trait n and $\boldsymbol{\sigma}_{t_{n_1},t_{n_2}}^2$ represents the covariance between two traits, n_1 and n_2. Models were tested using the DIC; Models 1 to 5 and Models 1 to 8 were tested for G_1 and G_2 data, respectively. A summary of the genetic parameters was provided by the mode and 95% credible interval of the posterior distributions.

RESULTS

Table 2 summarizes the mineral and vitamin data for G_1 and G_2 on a dry-weight basis. Coefficients of phenotypic variation (CV%) for micronutrients were highest for Ca followed by vitamin C. Variation for Fe and Zn were similar in both G_1 and G_2. Mean and CV% for tuber percentage dry matter content (Table 2) were also similar in both G_1 and G_2.

Posterior modes of heritabilities for micronutrient traits from univariate analyses were moderate, as shown in Table 3 (with the 95% credible interval of the posterior

Table 2. Summary of phenotypic micronutrient data (mg kg^{-1} dry wt.) and dry matter content (DM, %) in the first and second generations (G_1 and G_2, respectively).

Cycle	Trait	*n*	Minimum	Maximum	Mean	Standard deviation	CV%†
G_1	Fe	487	9.5	37.3	19.0	3.9	20.4
	Zn	487	7.2	27.5	15.8	3.0	18.7
	Ca	487	40.5	780.0	163.9	87.2	53.2
	vitamin C	527	140.4	918.7	399.5	117.9	29.5
	DM	487	15.5	36.4	26.4	2.9	11.0
G_2	Fe	1329	7.0	42.5	21.0	5.0	23.7
	Zn	1329	2.8	38.9	15.4	3.4	22.4
	Ca	1329	52.3	689.7	171.9	71.0	41.3
	DM	1326	12.6	35.0	26.0	3.3	12.1

† Coefficient of variation as the standard deviation expressed as a percentage of the mean.

Table 3. Posterior modes for variance components and heritabilities (h^2) from univariate analyses of first generation (G_1) data.

	Variance components				h^2
	σ^2_a	σ^2_c	σ^2_e	c^2†	[95% credible interval]
Iron	0.034	0.015	0.024	0.21	0.45 [0.30 0.65]
Zinc	0.028	0.013	0.019	0.22	0.42 [0.32 0.63]
Calcium	0.109	0.031	0.133	0.15	0.36 [0.18 0.70]
Vitamin C	0.043	0.035	0.033	0.32	0.38 [0.23 0.62]
Dry matter	0.014	0.011	0.008	0.35	0.41 [0.31 0.54]

† c^2, common environment ratio of full sibs.

Table 4 (Co)variance structures and model deviance information criterion (DIC) for the multivariate analyses of G_2 data where DIAG (diagonal matrix) has a zero covariance structure and US is an unstructured covariance between the response variables.

Model	Individual	Common environment	Family	Error	DIC†	
1	DIAG	–	–	DIAG	2008	2001
2	DIAG	DIAG	–	DIAG	1470	1472
3	US	–	–	US	781	781
4	US	DIAG	–	US	0	4
5	US	US	–	US	14	12
6	US	DIAG	DIAG	US	38	43
7	US	US	DIAG	US	32	36
8	US	US	US	US	31	32

†Difference in DIC (2 runs) from Model 4 (set to zero).

distributions in parentheses). Estimates for all traits were inflated when the common environment of full-sibs was not taken into account (results not tabulated), such that heritabilities for Fe, Zn, and vitamin C were 0.67 [0.46, 0.79], 0.70 [0.52, 0.81], and 0.76 [0.55, 0.88], respectively.

For the multivariate analyses of G_2, eight different models were fitted (Table 4). Models 1 and 2 were equivalent to running univariate analyses for each trait, as all traits are assumed independent with zero covariances and heterogeneous variances. Based on the DIC, Model 4 was the best-fitting model; a multivariate model with unstructured (co)variance matrices for both individual (genotype) and residual error, and common environment effects with heterogeneous variances and zero covariances between response variables (traits). The inclusion of a full-sib family effect did not improve the model fit. Although Model 4 was the preferred model, the broad-sense heritabilities may be of interest and are therefore presented, with estimates (from Model 6) of 0.57 [0.43, 0.72], 0.55 [0.38, 0.69], and 0.59 [0.46, 0.74] for Fe, Zn, and Ca, respectively. Model 4 was also the best-fitting model in the multivariate analysis of G_1 (results not shown), although the family effect (Models 6–8) was not tested.

Posterior modes of narrow-sense heritabilities, as shown in Table 5, were moderate. From G_1 to G_2, estimates increased for Fe (marginal increase; Fig. 1b), Ca, and dry matter, and slightly decreased for Zn (Fig. 1c), but were relatively stable given that trials were over two different sites and years. Analysis of G_2 data was repeated using weaker priors of variance components, reducing the degree of belief. In comparing the two runs, the MCMC trace output appeared to be reasonably stable, with heritability estimates [credible intervals] of 0.44 [0.25, 0.67] for Fe, 0.30 [0.17, 0.58] for Zn, 0.60 [0.37, 0.76] for Ca, and 0.27 [0.15, 0.41] for dry matter.

Posterior modes of the genetic correlations between Fe and Zn were positive in both G_1 and G_2 (Table 5, Fig. 1d). Genetic correlations between Ca and Fe/Zn were close to zero and shifted to become more positive from G_1 to G_2, and correlations between the mineral traits and vitamin C in G_1 were effectively zero. Between the mineral traits and dry matter content, correlations were negative in both G_1 and G_2 (Table 5, Fig. 1e, 1f). In comparison, genetic parameters estimated on a fresh-weight basis were similar in general, with the exception of the genetic correlations between the minerals and dry matter content which were positive (G_2 results shown in Table 5).

DISCUSSION

Genetic Variation and Heritabilities

From a breeding perspective, it is acknowledged that the success of biofortification will be determined by the type

Table 5. Posterior modes for heritability (diagonal, boldface) and additive genetic correlations (below diagonal) for Fe, Zn, Ca, vitamin C, and tuber dry matter content from a multivariate analysis of G_1 and G_2 data (Model 4) estimated on a dry-weight or fresh-weight basis.

Trait	Iron	Zinc	Calcium	Vitamin C	Dry matter
G_1, dry wt. basis					
Iron	**0.41** [0.29 0.50]				
Zinc	0.45 [0.32 0.64]	**0.38** [0.27 0.47]			
Calcium	0.04 [–0.23 0.34]	0.12 [–0.15 0.39]	**0.42** [0.29 0.64]		
Vitamin C	–0.01 [–0.18 0.29]	0.10 [–0.15 0.30]	0.05 [–0.27 0.33]	**0.38** [0.28 0.50]	
Dry matter	–0.23 [–0.42 –0.06]	–0.24 [–0.41 –0.07]	–0.19 [–0.36 0.07]	–0.06 [–0.28 0.10]	**0.32** [0.22 0.38]
G_2, dry wt. basis					
Iron	**0.43** [0.28 0.65]				
Zinc	0.72 [0.42 0.88]	**0.36** [0.17 0.54]			
Calcium	0.35 [–0.04 0.61]	0.57 [0.18 0.76]	**0.57** [0.37 0.71]		
Dry matter	–0.34 [–0.61 0.08]	–0.38 [–0.66 0.10]	–0.14 [–0.49 0.20]		**0.42** [0.25 0.57]
G_2, fresh wt. basis					
Iron	**0.45** [0.27 0.59]				
Zinc	0.61 [0.33 0.84]	**0.26** [0.14 0.42]			
Calcium	0.07 [–0.32 0.52]	0.45 [–0.02 0.77]	**0.51** [0.31 0.80]		
Dry matter	0.18 [–0.13 0.36]	0.14 [–0.13 0.38]	0.05 [–0.23 0.27]		**0.52** [0.41 0.60]

of genetic control and amount of genetic variation, the relationships between the target micronutrients with other important agronomic and quality traits, and genotype stability for target micronutrients across different environments. In the present study, the additive genetic variance (V_A) was estimated directly making use of the additive genetic relationships via the **A** matrix, and the dominance variance (V_D) from the estimate of the full-sib family effect (σ_f^2), with the expectation of $1/4V_D$. This study supports the hypothesis that tuber micronutrient content is under genetic control, and in the population studied, this control appeared to be additive. There was insufficient data to reliably detect any real nonadditive genetic component. Exploiting the additive gene effects present in this diploid population will result in the genetic improvement of important micronutrients in potato tubers. Furthermore, the magnitudes of narrow-sense heritability estimates point towards individual rather than family-based selection as a selection strategy for important micronutrients. The moderate heritabilities also suggest that the level of within-family variation is such that superior individuals will potentially be identified from within a number of families. Graham et al. (1999) suggested that the mechanisms controlling the uptake, transport, and loading of micronutrients are likely to be additive, indicating that emphasis should be placed on an approach of population improvement from recurrent selection. Parents of the G_2 progeny were selected from individuals of G_1 that had higher Fe and/or Zn, but selections also included genotypes with desirable agronomic features. Truncation selection is expected to reduce additive genetic variance due to Bulmer's gametic-phase disequilibrium (Falconer and Mackay, 1996). No inference can be made on this effect with these data, of course, because of the large sampling errors involved, but it should be noted that selection in this case was not strictly truncated, and the selection differential would have been reduced because many preferred crosses did not result in progeny. There is limited information available in the published literature about the genetic control of micronutrient content in staple crops, including potato, using data from designed crossing trials. In studies on the variation in potato clones (commercial tetraploid breeding lines and cultivars), Brown et al. (2010, 2011) estimated broad-sense heritabilities and reported significant genetic variation and genotype-by-environment interaction (GEI) for tuber Fe content, but a scarcity of exploitable variation for Zn tuber content in two out of three trial locations. A study by Haynes et al. (2012) on *S. tuberosum* × (*S. phureja-S. stenotomum*) 4×–2× clones found significant levels of genetic variation for a number of micronutrients and a GEI for Zn. In an assessment of 23 potato genotypes for Zn content after applications of foliar Zn fertilizers over 4 yr, White et al. (2012) identified significant genotype differences and environmental effects, but no evidence of GEI. Burgos et al. (2007) found significant environmental effects and GEI over two highland locations for Fe and Zn in native Andean diploid accessions, a number of which formed the basis of the breeding population in the present study. The GEI in this case was due largely to a rescaling of genotypes suggesting heterogeneity of genetic variance, which is of less concern than if there had been a significant reranking of genotypes.

Although not apparent in the present study, significant changes in heritability estimates for tuber mineral content across trials and/or years may be expected, given that various environmental components affecting crop mineral availability have been reported, such as the physical and chemical properties of soil (e.g., White and Zasoski, 1999; Po et al., 2010). Low heritabilities due to large within-trial

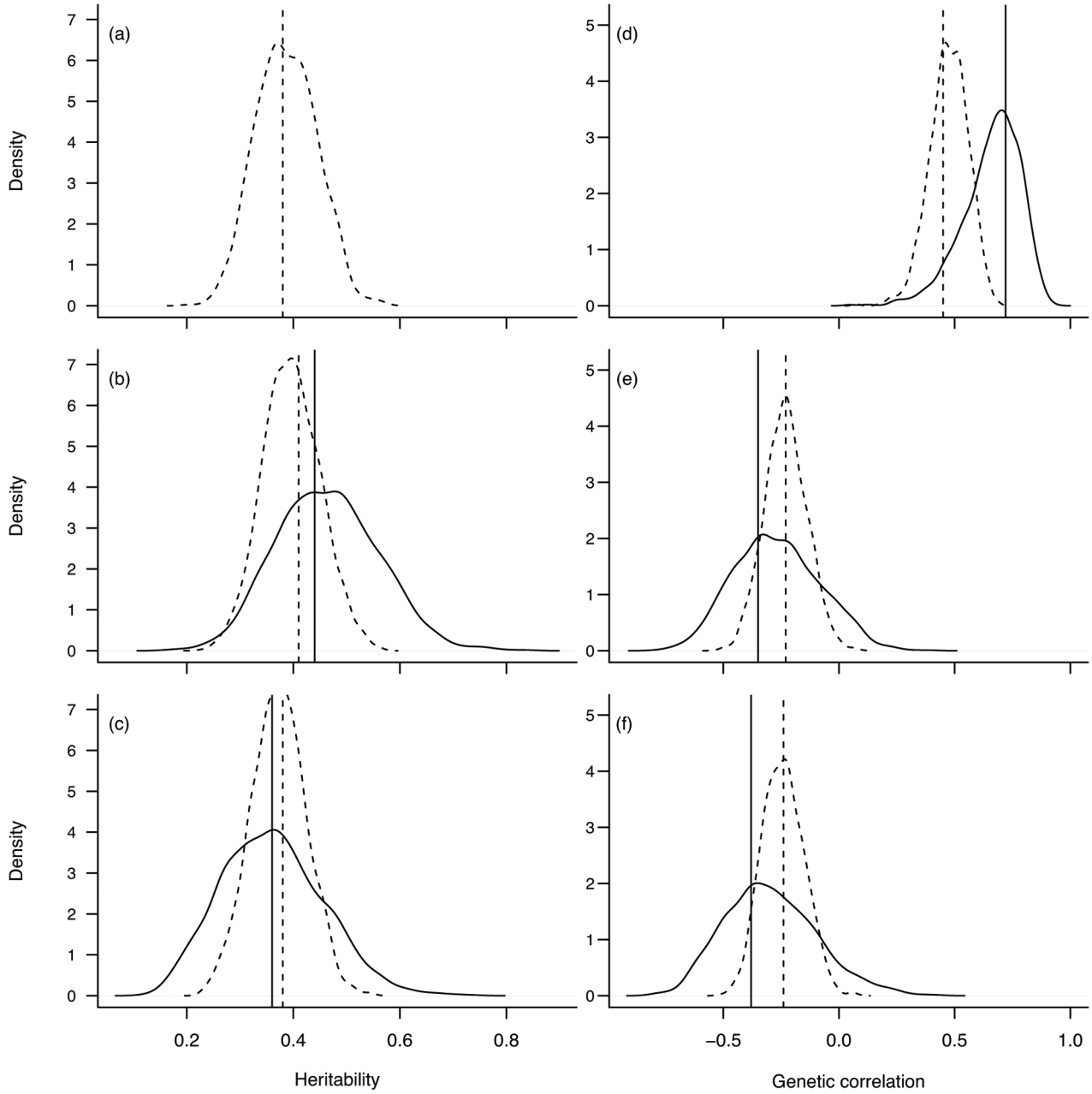

Figure 1. Marginal posterior distributions for narrow-sense heritability of: (a) vitamin C in Generation 1 (G_1); (b) Fe in G_1 and G_2; (c) Zn in G_1 and G_2. Marginal posterior distributions for additive genetic correlation between: (d) Fe and Zn in G_1 and G_2; (e) Fe and dry matter content in G_1 and G_2; (f) Zn and dry matter in G_1 and G_2; where the broken line is G_1 and the solid line is G_2, and the vertical lines indicate the posterior modes of the distributions.

error variances will compromise the selection for micronutrient traits in the early stages of a selection program. Accumulation of Zn (and, to a lesser extent, Fe) in edible portions of food crops is reported to be particularly sensitive to environmental variables (Pfeiffer and McClafferty, 2007b), which suggests that more effective strategies to identify genotypes with high stable mineral expression across environments may be required. Soil Zn deficiency is common in many crop growing regions (Cakmak, 2008; White and Zasoski, 1999), and its availability and accumulation in the edible portions of crops is therefore likely to be a complex function of soil mineral status and interactions with other environment, agronomic, and management factors (e.g., Po et al., 2010; White and Zasoski, 1999; White et al., 2009). In durum wheat (*Triticum aestivum* L.), for example, there is evidence that the N status affects Fe and Zn accumulation in grain (Kutman et al., 2011) and N availability itself will be dependent on soil N status and condition, among other interacting factors. In potato tubers, Zn assimilation in tubers has been linked with the co-transport of N (White et al., 2012), but in long-term field studies, Šrek et al. (2010) found no differences in the Fe and Zn content of tubers under different rates of N, K, and P field treatments. Further research on the extent and type of GEIs of

key micronutrients in potato would give some indication of the requirements for MET testing and the benefits of developing a marker-assisted selection strategy.

In the RCBD, replication of full-sib families should go some way at least to remove confounding that occurs between the common environment effect and dominance effects. Failing to account for nonadditive and nongenetic effects can inflate heritabilities and reduce the precision of breeding value estimates. The grouping of families is common in early-stage trials and is often preferred by the breeder for practical reasons, for example, visual evaluation of groups of individuals with known parental combinations. A common environment effect was fitted in the analysis which appeared to account for some bias in the additive variance estimates; ignoring the common environment of full-sibs increased heritability by 0.22 for Fe and 0.28 for Zn in G_1. Improving heritability estimates may require the dispersal of family groups which will also help to remove confounding between common environment and dominance effects (random allocation of individuals rather than families), more replication (as a trade-off with selection intensity), as well as a greater understanding and control of the nongenetic factors, such as soil heterogeneity within a trial. Local field variation on a scale that may not be accounted for in the trial design by blocking would be perhaps better accommodated by also including spatial effects within the mixed model framework (Gilmour et al., 1997; Piepho et al., 2008).

Difficulties in recovering useful genetic variation from unadapted wild relatives or landraces are a barrier for its use in many crop species. Andean landrace potatoes are a valuable source of germplasm for potato breeding and an important part of the diet for rural populations in Peru living in the high Andes (Burgos et al., 2007). Biofortification with Fe and Zn is therefore likely to benefit poorer communities at risk of micronutrient malnutrition in this region (Burgos et al., 2007). While diploid landrace potatoes are not adapted outside of the highland tropics, population improvement by recurrent selection at the diploid level may increase micronutrient trait values and at the same time improve tuber shape. Taking advantage of variability for tuber dormancy and selecting for functional levels of unreduced gametes will enable transfer of gains obtained at the diploid level to more widely-adapted tetraploid populations and the development of varieties suited for new environments and wider scale deployment. The method to increase ploidy level to the tetraploid via the 4×–2× first division restitution mechanism is well established in potato (Ortiz et al., 1991), but the effects on the genetic control of micronutrient traits in the 4× genetic background will need to be determined.

Genetic Correlations

For the improvement of staple crops, breeding programs will seek to simultaneously improve important micronutrients such as Fe and Zn without detriment to yield and quality. In the present study, positive genetic correlations were found between Fe and Zn, indicating that evaluation and selection for one will result in concomitant increase in the other. Repeated analysis using weaker priors of variance components resulted in a posterior mode of the genetic correlation between Fe and Zn of 0.80 [0.40 0.91]. Datasets from several centers of the Consultative Group on International Agricultural Research (CGIAR) have demonstrated genetic variation and positive correlations between Fe and Zn across different genotypes of the range 0.44 to 0.61 for a number of crops including potato, maize (*Zea mays* L.), lentil (*Lens culinaris* Medik.), wheat, and yam (*Dioscorea* spp.; Gregorio, 2002; Pfeiffer and McClafferty, 2007b), but these studies gave no indication of the type of genetic control in these crops. In the present study, negative genetic correlations were found between dry matter and Fe, Zn, Ca, and vitamin C (genetic correlation close to zero for vitamin C in G_1) when analyzed on a dry-weight basis. In contrast, these genetic correlation estimates were positive (but small) when analyzed on a fresh-weight basis. Although no data were available to investigate further, a possible explanation may be due to the greater concentration of some minerals at the surface layers of tubers, as is reported for minerals such as Fe and Ca (Subramanian et al., 2011). The higher surface area–volume ratio of small tubers or a dilution effect as tubers increase in size may result in the relationship between minerals and dry matter on a fresh-weight basis being confounded by tuber size (with smaller tubers tending to have a higher dry matter concentration than large tubers). A negative genetic correlation between minerals and dry matter content is not particularly helpful for breeders, as a higher dry matter content is often associated with favorable sensory and cooking characteristics in potato. Consumer acceptance of new and improved cultivars has proved difficult when failing to match the preferred traditional types for certain characteristics. For example, breeding sweet potato (*Ipomoea batatas* var. *batatas*) for high carotene concentration to combat vitamin A deficiency has encountered market resistance in Uganda, where the preference is for white roots. An education program has been necessary to increase consumer acceptance for orange roots in the region (Thomas zum Felde, CIP, personal communication, 2012). Relatively low levels of Ca are found in potato tubers, and therefore potatoes are unlikely to provide a useful source of the macronutrient given the amount required in the human diet on a daily basis (Brown et al., 2012). However, as Ca deficiency in potato tubers is reported to be related to the increased incidence of several physiological disorders (Palta, 1996), a better understanding of its genetic control and relationship with Fe and Zn is likely to be of interest with regard to the possible consequences of long-term recurrent selection to enhance tuber micronutrient content. Further studies are

required to understand the relationships between micronutrient content and agronomic and end-use quality characteristics in potato.

The micronutrient content of tubers reported in this study indicates that this genetic material will provide a useful source of dietary Fe and Zn, acting as a suitable base for further improvement. For example, the maximum mineral content in the G_1 progeny generation (on a fresh-weight basis) was 10.4 mg kg^{-1} for Fe and 6.7 mg kg^{-1} for Zn (data not shown). A household average consumption of 253 g ae^{-1} d^{-1} (grams per adult male equivalent per day) in Peru (Rose et al., 2009) will therefore provide 56% of the estimated average requirement (EAR) of Fe for children aged 4 to 8 yr and 28% for female adults aged 19 to 30, based on dietary reference intakes (http://fnic.nal.usda.gov/dietary-guidance/dietary-reference-intakes/dri-tables, verified 6 May 2014). Similarly, consumption will provide 37% of the EAR of Zn for children aged 4 to 8 yr and 22% for female adults aged 19 to 30. However, these estimates make a number of assumptions, and actual values will depend on various factors such as micronutrient bioavailability. The relationship of target micronutrients with promoters and inhibitors may affect bioavailability on consumption (Welch and Graham, 2004). Pfeiffer and McClafferty (2007b) suggested that strategies to breed micronutrient-dense crops should consider indirect selection for bioavailability and reduced postharvest and cooking losses, as well as direct selection for increased concentration. Vitamin C has been shown to act as a promoter that enhances the bioavailability of Fe and Zn. From multivariate analysis in the present study, genetic correlations between Fe and vitamin C, and between Zn and vitamin C were essentially zero, given the posterior modes and credible intervals estimated from these data.

Bayesian Analysis of Genetic Parameters

The implementation of a MCMC procedure to fit the individual model in this study using the R package MCMCglmm (Hadfield, 2010) demonstrates that Bayesian approaches, which can be applied to both Gaussian and non-Gaussian traits, are now more readily accessible to plant breeders. Other available software such as WinBUGS (Lunn et al., 2000) and MTGSAM (Van Tassell and Van Vleck, 1996) have been used for tree and crop breeding data to estimate quantitative genetic parameters, for example, Waldmann et al. (2008) in Scots Pine; and Gonçalves-Vidigal et al. (2008) in common bean. Waldmann (2009) presented the case for WinBUGS as an evaluation tool for nonspecialists but Hadfield's MCMCglmm-R is arguably more approachable for many plant breeders as it shares similar syntax with the popular plant breeding and trial evaluation software ASReml-R (Butler et al., 2009), a point noted by Apiolaza et al. (2011). That said, it should not be treated as a black box. The appropriate choice of priors is perhaps more important for the analysis of plant breeding data as small datasets are more typical than in animal or forest tree breeding studies, and priors may therefore have a greater influence on the posterior distribution; in this context, poor prior choice will not be overwhelmed by the data. A cautionary approach is therefore required in this instance, as priors can sometimes and unwittingly lead to incorrect inferences for the posterior modes due to the Markov Chains becoming trapped at a local maximum. In the present study, alternative priors were tested, following the recommendations of Gelman (2006), using the JAGS program (Plummer, 2003) within R (package rjags). This included a uniform prior on the variance, standard deviation, and heritability as noninformative priors. Although not shown, results compared favorably with those obtained using the inverse gamma distribution, with small equal parameters as the prior distribution for the variances which are, by default, those used in the MCMCglmm package. It seems reasonable that estimates from a previous generation (G_{n-1}) should be an appropriate choice of priors for the following generation (G_n), which was the approach taken in the present study. Blasco (2001) and Waldmann and Ericsson (2006) reviewed the advantages and disadvantages of REML and Bayesian based methods, as well as the choice of priors, when applied to the individual animal model.

CONCLUSIONS

Additive genetic effects were important for the micronutrient traits examined in this study, with no detection of significant nonadditive effects. Genetic correlations between Fe and Zn were strong and positive. An improvement strategy employing recurrent cycles of selection may therefore optimize genetic gains in this population for micronutrients Fe and Zn that are important targets for the biofortification of potato tubers. The genetic correlations between micronutrients (Fe, Zn, Ca) and vitamin C were close to zero, and genetic correlations between micronutrients (Fe, Zn, Ca) and tuber dry matter, an important sensory and processing character, were negative when analyzed on a dry-weight basis, and small but positive when analyzed on a fresh-weight basis. Trial design to remove the common environment of siblings and to better account for potential local-scale field heterogeneity of mineral availability should be considered. With publically-available software such as MCMCglmm for R (Hadfield, 2010), Bayesian procedures to fit the individual model are now more accessible for plant breeders to estimate variance components and genetic parameters. As well as breeding issues, it is generally acknowledged that the success of biofortification in potato and other crop species will also depend on nongenetic factors such as mineral bioavailability, palatability, and the acceptance of new cultivars over traditional types.

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