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CROPS AND SOILS RESEARCH PAPER Iron and zinc concentrations in peanut (Arachis hypogaea L.) seeds and their relationship with other nutritional and yield parameters

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SUMMARY

Biofortification (delivery of micronutrients via micronutrient-dense crops) can be achieved through plant breeding and offers a cost-effective and sustainable approach to fighting micronutrient malnutrition. The present study was conducted to facilitate the initiation of a breeding programme to improve the concentration of iron (Fe) and zinc (Zn) in peanut (Arachis hypogaea L.) seeds. The experiment was conducted with 64 diverse peanut genotypes for 2 years in eight different environments at the International Crops Research Institute for the Semi-Arid Tropics, Patancheru, India to assess the genetic variation for Fe and Zn concentrations in peanut seeds and their heritability and correlations with other traits. Significant differences were observed among the genotypes and environments for Fe (33-68 mg/kg), Zn (44-95 mg/kg), protein (150-310 mg/g) and oil (410-610 mg/g) concentration in seeds and their heritability was high, thus indicating the possibility of improving them through breeding. As seen in other plants, a significant positive association between concentrations of Fe and Zn was observed. Trade-offs between pod yield and Fe and Zn concentrations were not observed and the same was also true for oil content. Besides being high yielding, genotypes ICGV 06099 (57 mg/kg Fe and 81 mg/kg Zn) and ICGV 06040 (56 mg/kg Fe and 80 mg/kg Zn) had stable performance for Fe and Zn concentrations across environments. These are the ideal choices for use as parents in a breeding programme and in developing mapping populations.

INTRODUCTION

Micronutrient deficiencies affect a large segment of the population in the developing world (WHO 2002). Iron (Fe) and zinc (Zn) are receiving increasing attention globally as their deficiency is widespread, particularly in developing countries. Iron deficiency primarily affects women and children. The consequences of malnutrition are varied and far-reaching. In infants and young children, undernutrition and growth retardation are associated with reduced physical activity, lowered resistance to infection, impaired intellectual development and cognitive abilities, and increased morbidity and mortality. Despite the large-scale intervention programmes, Fe-deficiency anaemia remains the most widely prevalent nutritional problem in the world.

Plant foods remain the major source of minerals and vitamins for the poor in developing countries since animal products, which are rich in micronutrients, are beyond their reach. Enhancing the Fe and Zn concentrations of plant foods that are consumed daily may prove to be an effective and convenient way of overcoming deficiencies of these micronutrients in human diets (Bouis 1996; Grusak & DellaPenna 1999; House et al. 2002). However, bioavailability of Fe and Zn from plant foods is low (Gibson 1994; Sandberg 2002) due to the presence of anti-nutrient factors such as phytates and polyphenols (Engle-Stone et al. 2005). It is possible to enhance the bioavailability by addition of erythorbic acid or ascorbic acid in processed foods (Fidler et al. 2004) and through fermentation of food,

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which activates endogenous phytases (Hemalatha et al. 2007).

Significant genetic variation in the seed concentrations of Fe and Zn of various crops is reported in the literature - rice, Oryza sativa L. (Sarla et al. 2012); cassava, Manihot esculenta Crantz (Maziya-Dixon et al. 2000); common wheat, Triticum aestivum L. (Morgounov et al. 2007); maize, Zea mays L. (Maziya-Dixon et al. 2000); sorghum, Sorghum bicolor (L.) Moench (Ashok Kumar et al. 2009; Reddy et al. 2010); pearl millet, Pennisetum glaucum (L.) R. Br. (Velu et al. 2011); common beans, Phaseolus vulgaris L. (Beebe et al. 2000; House et al. 2002; Blair et al. 2009); chickpea, Cicer arietinum L. (Thavarajah & Thavarajah 2012); lentil, Lens culinaris Medic. (Thavarajah et al. 2010); field peas, Pisum sativum L. (Amarakoon et al. 2012); soybean, Glycine max (L.) Merr. (Raboy et al. 1984); and peanut, Arachis hypogaea L. (Lal & Singh 2007; Asibuo et al. 2008; Singh et al. 2011; Upadhyaya et al. 2012a). Furthermore, simultaneous improvement of both Fe and Zn concentrations in seeds is possible, since a strong positive association is reported between them in peanut (Upadhyaya et al. 2012a), common bean (Gregorio 2002; House et al. 2002), lotus (Lotus japonicus (Regel) K. Larsen) (Klein & Grusak 2009), sorghum (Ashok Kumar et al. 2009; Reddy et al. 2010), wild emmer wheat (Triticum turgidum ssp. dicoccoides (Körn.) Thell.) (Peleg et al. 2008), common wheat (Morgounov et al. 2007) and maize (Maziya-Dixon et al. 2000). A similar correlation in cassava was weak (Maziya-Dixon et al. 2000). Accumulation of Fe and Zn in plants is also highly influenced by environmental factors. Temperature during the seed-filling stage influences accumulation of seed Fe and Zn in common beans (Thavarajah et al. 2010). Similarly, temperature and soil mineral content of Fe and Zn are reported to influence grain Fe and Zn concentrations in wheat (Joshi et al. 2010; Singh et al. 2011).

The nature of inheritance and presence of quantitative trait loci (QTL) for Fe and Zn seed concentrations in various crops have been reported in the literature. In cereals, additive and dominant gene effects, environmental effects and reciprocal effects (Gregorio 2002) and a few QTL with considerable additive \times additive epistatic interactions (Lu et al. 2008) in rice, a preponderance of additive gene action in pearl millet (Velu et al. 2011), and strong genotype by environment (G \times E) interaction and very low broad sense heritability in wheat (Joshi et al. 2010) have been documented. In inter-specific crosses of common

bean, the inheritance of Fe concentration in seed is reported to be quantitative (Guzmán-Maldonado *et al.* 2003) and that of Zn to be simple (Cichy *et al.* 2005). Blair *et al.* (2009) reported five QTL for Fe and six for Zn concentration that were clustered on the upper half of linkage group B11, explaining up to 0·48 of phenotypic variance and suggesting the presence of an important locus useful for marker-assisted selection in bean.

Peanut is considered highly nutritious for humans and is considered a high-energy food as it contains 480-500 mg/g high-quality edible oil, 260-280 mg/g easily digestible protein and 200 mg/g carbohydrates (Jambunathan 1991). It is also a rich source of vitamins E, K and B complex. Of the 20 minerals necessary for normal body growth and maintenance, seven, including Fe and Zn, are present in peanut. It is also rich in dietary fibre and is rated as a low sodium food. Developing countries, where micronutrient deficiencies are widespread, contribute c. 0.98 of the world's peanut-growing area and 0.96 of global peanut production (FAO 2011). More than 100 countries grow the crop. Thus, peanut can contribute significantly towards reduction of protein-energy and micronutrient malnutrition. There are only a few studies on genetic variation in Fe and Zn concentrations in peanut seed (Lal & Singh 2007; Asibuo et al. 2008; Singh et al. 2011; Upadhyaya et al. 2012a). Except for the study of Upadhyaya et al. (2012a) on a mini core collection of peanut germplasm in India, the other studies are preliminary in nature and of limited value. Improving concentrations of Fe and Zn in peanut seed through breeding requires a good knowledge of the extent of genetic variation, stable genotypes that maintain superior performance across environments and knowledge of trait inheritance. The present study was conducted to (i) estimate the extent of variation for Fe and Zn concentrations along with oil and protein concentrations in peanut seed from selected germplasm and advanced breeding lines, (ii) determine the heritability of these traits, (iii) find correlations among these traits with yield parameters and (iv) identify genotypes with stable performance for Fe and Zn concentrations across environments that can be used as parents in breeding programmes and to develop mapping populations.

MATERIALS AND METHODS

Sixty-four groundnut genotypes consisting of germplasm accessions and popular varieties originating from India, Africa and South America, and advanced breeding lines and inter-specific derivatives developed at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) were included in the current study. These genotypes belonged to three botanical varieties – A. hypogaea subsp. hypogaea var. hypogaea (Virginia type), A. hypogaea subsp. fastigiata var. vulgaris (Spanish type) and A. hypogaea subsp. fastigiata var. fastigiata (Valencia type). Each of these botanical types has different plant, pod and seed characteristics (Krapovickas & Gregory 1994). The experiment was conducted in eight environments in Alfisols (Alfisol-Patancheru Soil Series; Udic Rhodustolf) fields at ICRISAT, Patancheru, India (17°31′N, 78°16′E, 545 m a.s.l.) during four cropping seasons (rainy seasons of 2009 and 2010, and postrainy seasons of 2009/10 and 2010/11). It was laid out in an 8 × 8 Alpha Lattice design with two replications. The plot size in the 2009 rainy season consisted of two 4 m rows on ridges 60 cm apart. In the other three seasons, it was four 4 m rows 30 cm apart grown on a broad bed and furrow system. The plant to plant distance within a row in all seasons was 10 cm. Standard agronomic management practices was followed in each season: 60 kg phosphorus pentoxide (P2O5) as a basal application, seed treatment with mancozeb (2 g/kg seed) and imidachloprid (2 ml/kg seed), preemergence application of pendimethalin (1 kg active ingredient/ha), irrigation soon after planting, and subsequently as and when needed, or as per the requirement of the treatment, gypsum (400 kg/ha) at peak flowering and protection against insect pests and diseases.

In the rainy seasons of 2009 and 2010, two sets of experiments were grown in the same field, with one receiving irrigation as and when required (supplementary irrigation) and the other solely under rainfed conditions (barring one irrigation soon after sowing). Similarly, in the post-rainy seasons of 2009/10 and 2010/11, two sets of experiments were grown in the same field with one receiving full irrigation and the other under managed moisture stress 60 days after sowing (DAS) until harvest. The moisture stress was created by skipping alternate irrigations from 60 DAS. Thus, the genotypes were evaluated in eight different environments for Fe, Zn, oil and protein concentrations in the seeds, pod yield, shelled weight from 100 g of pods and 100-seed weight.

Soil analysis to estimate the Fe and Zn status of the experimental block was conducted in all eight

environments. In each block of a replication, two soil samples were collected from a depth of 15 cm before sowing using an auger. Subsequently, all 16 samples in one replication (eight blocks in a replication) were bulked, thoroughly mixed and foreign materials such as roots, stones, pebbles and gravel were removed. After this, soil was quartered by spreading it in a circle, dividing the sample into four equal parts and discarding the opposing quarters. Quartering was repeated to obtain a final soil sample of 100-150 g, which was used to estimate micronutrient status in the soil. The samples were analysed at the Charles Renard Analytical Laboratory (CRAL) at ICRISAT, Patancheru. The quality of analysis was assured by regularly monitoring and analysing the standard samples received from the International Plant and Soil Analytical Exchange Laboratory, Wageningen Evaluating Programmes for Analytical Laboratories (WEPAL), located in the Netherlands (http://www. intranet.icrisat.org/gtaes/Services/laboratories.htm).

Protocol for estimation of iron and zinc concentrations in groundnut seeds

Iron and Zn concentrations were estimated following the protocol given by Sahrawat *et al.* (2002). The protocol involved tri-acid digestion of ground seed samples and estimation of Fe and Zn concentrations in the aliquot by atomic absorption spectrometer (AAS Varian SpectrAA-20, Varian Techtron Pty. Limited, Mulgrave, Victoria, Australia). The standards for Fe and Zn were obtained from *M/s* Merck, Germany. Along with standards, internal control standards were also used for every batch of 20 samples. The detection limits were 0·2 mg/kg for Fe and 0·1 mg/kg for Zn. The Fe and Zn concentrations are expressed as defatted meal weight basis. Adequate precautions were taken to avoid any possible contamination of Fe and Zn at all the stages of analysis.

Protocol for determination of oil and protein contents

The oil content was determined by a nuclear magnetic resonance (NMR) spectrometer. High correlation (r=0·97) between the estimates of Soxhlet and NMR methods was reported by Jambunathan *et al.* (1985). The protein content was determined using a Technicon Autoanalyser (Pulse Instrumentation Ltd, Saskatoon, Canada) (Singh & Jambunathan 1980).

Statistical analysis

For each trait, pooled analysis of variance over eight environments (combination of 2 years, two seasons and two stress conditions) using the SAS GLM procedure (SAS Inst. 2002-08, SAS V 9.2) was performed considering environments, replications, blocks and genotypes as fixed effects. Before pooling data over environments, the Bartlett χ^2 test was used to test homogeneity of error variance of all environments. Since heterogeneity among the environment variances was confirmed for all traits, data were appropriately transformed and pooled analysis was carried out. Adjusted means are the least-square means. Contrast analysis was done to compare (1) rainy season rainfed (RSRF) environment (mean of 2009 and 2010 RSRF environments) v. post-rainy season irrigated (PRSIR) environment (mean of 2009/10 and 2010/11 PRSIR environments), (2) RSRF environment v. post-rainy season moisture stressed (PRSS) environment (mean of 2009/10 and 2010/11 post-rainy season imposed moisture stressed environment), (3) PRSS environment v. PRSIR environment and (4) RSRF environment v. rainy season supplemental irrigation (RSSIR) environment (2009 and 2010 rainy season with irrigation as and when needed environments).

Since the G×E effect was significant, a genotype and genotype×environment (GGE) biplot (Yan & Tinker 2006) was drawn to study the performance of genotypes based on mean value and stability. A standard biplot is the scatter plot that graphically displays both the row factor and column factors of a two-way table data. A biplot graphically displays a matrix with application to principal component analysis (PCA) (Kroonenberg 1995). In order to generate a biplot, a two-way table representing two factors was subjected to singular value decomposition. The singular value decomposition of a matrix $X = (x_{ij})_{vxs}$ is given by

$$x_{ij} = \sum_{k=1}^{r} u_{ik} \lambda_k v_{kj}$$

where (u_{ik}) is the element of the matrix U_{vxs} characterizing rows, λ_k is the singular value of a diagonal matrix L_{sxs} , v_{kj} is the element of the matrix V_{sxs} characterizing the columns and r represents the rank of matrix $X \le \min(v,s)$. PC scores for row and column factors were calculated after singular value partitioning of $(x_{ij})_{vxs}$ (Yan 2002). A biplot was obtained using the first two components and the proportion of variation explained by them was calculated.

The fixed effect two-way model for analysing multienvironments genotype trials was:

$$E(Y_{ij}) = \mu + g_i + e_j + (ge)_{ij}$$

where μ is the grand mean, g_i and e_j are the genotype and environmental main effects, respectively, and $(ge)_{ij}$ is the G × E effect. The sites regression model is given by Crossa & Cornelius (1997) and Yan & Kang (2003):

$$E(Y_{ij}) = \mu + e_j + \sum_{n=1}^{r} \zeta_{in}^* \eta_{jn}^*$$

where r is the number of PCs required to approximate the original data, ξ_{in}^* and η_{jn}^* are the ith genotype and the jth environmental scores for PCn, respectively. In the site regression method, PCA is applied on residuals of an additive model with environment as the only main effect. Therefore, the residual term $\sum_{n=1}^r \xi_{in}^* \eta_{jn}^*$ contains the variation due to G and G×E. A two-dimensional biplot (Gabriel 1971; Parsad et al. 2007) derived from the above two-way table of residuals is called a GGE biplot (G plus G×E) (Yan et al. 2000). A GGE biplot graphically depicts the genotypic main effect (G) and the G×E effect contained in the multi-environment trials: they have been found very useful in understanding G×E, mega-environment identification and genotype recommendation.

Correlation coefficients among different traits over environments were calculated by the Pearson method using the SAS CORR procedure (SAS Institute 2008, SAS V9.2). The broad sense heritability (H^2) over environments was calculated as the ratio of genetic variance (V_G) to total phenotypic variance (V_P) (genotypic and environmental variances) given by the equation, $H^2 = H^2 = V_C/V_P$ (Fehr 1991):

$$V_{\rm p} = \sigma_g^2 + (\sigma_{ge}^2/e) + (\sigma_e^2/re)$$

where σ_g^2 is the variance for genotype, σ_{ge}^2 the variance for G×E, σ_e^2 the error variance, e the number of environments and r the number of replications.

Heritability estimates were computed using SAS Version 9.2 (SAS Institute 2008, SAS V 9.2).

RESULTS

Iron and zinc status of soil in experimental plots

The Fe concentration in the soil of the experimental plots varied from 7.2 to $32.8 \, \text{mg/kg}$ and that of Zn from 2.47 to $9.75 \, \text{mg/kg}$ across the eight experiments. In all the experiments, the Fe and Zn concentrations of the soil were above critical limits (Fe $2.0 \, \text{mg/kg}$ and

Table 1. Combined analysis of variance for different traits included in the study on 64 peanut genotypes under different environments at ICRISAT, Patancheru, during the 2009–11 cropping season

				Mean sum o	Mean sum of squares and their significance	significance		
Source of variation	D.F.	Fe	Zn	PC	OC	SW	ΡΥ	HSW
Genotypes	63	8 (P<0·01)	18 (P<0·01)	19 (P<0·01)	29 (P<0·01)	7 (P<0.01)	11 (P<0.01)	24 (P<0·01)
Environments	_	683 (P<0·01)	626 (P < 0.01)	2680 (P<0·01)	9654 (P<0·01)	1863 (P<0·01)	144 (P<0·01)	379 (P < 0.01)
Rainy season rainfed (RSRF) v. post-rainy		5 (P < 0.05)	7 (P < 0.01)	1 (NS)	22255 (P<0·01)	290 (P < 0.01)	48 (<i>P</i> <0·01)	13 (<i>P</i> <0.01)
season irrigated (PRSIR)								
RSRF v. post-rainy season stress (PRSS)		7 (P < 0.01)	2374 (P < 0.01)	5125 (P<0·01)	15393 (P<0·01)	3646 (P < 0.01)	26 (P < 0.01)	0 (NS)
PRSS v. PRSIR		25 (P < 0.01)	2124 (P < 0.01)	5248 (P<0·01)	631 (P < 0.01)	1879 (P < 0.01)	144 (P < 0.01)	13 $(P < 0.01)$
RSRF v. rainy season supplemental		306 (P < 0.01)	3 (NS)	1111 (P<0·01)	1719 (P < 0.01)	159 (P < 0.01)	270 (P < 0.01)	124 (P < 0.01)
irrigation (RSSIR)								
REP (environment)	8	4 (P < 0.01)	11 $(P < 0.01)$	6 (P < 0.01)	4 (P < 0.01)	2 (P < 0.05)	4 (P < 0.01)	3 (P < 0.01)
Block (environment×replication)	112	2 (P < 0.01)	2 (P < 0.01)	2 (P < 0.01)	1 (P < 0.05)	1 (NS)	1 (NS)	1 (NS)
Environments × genotypes	441	2 (P < 0.01)	2 (P < 0.01)	4 (P < 0.01)	2 (P < 0.01)	2 (P < 0.01)	2 (<i>P</i> <0·01)	2 (P < 0.01)

degrees of freedom; NS, not significant; Fe, iron concentration (mg/kg); Zn, zinc concentration (mg/kg); PC, protein concentration (mg/g); OC, oil concentration (mg/g); SW, shelled weight per 100 g of pods; PY, pod yield (kg/ha); HSW, 100-seed weight (g) Zn 0.75 mg/kg, Olsen's method) (Muhr et al. 1965). The soil pH of the experimental plots was between 7.1 and 7.9.

Combined analysis of variance

The combined analysis of variance showed significant differences among genotypes and environments and their interactions for Fe and Zn concentrations and other traits included in the study (Table 1). The environments are also compared based on the performance of genotypes for Fe and Zn concentrations and other traits (Table 1). Except for protein concentration in the RSRF v. PRSIR comparison, 100-seed weight in RSRF v. PRSS and Zn concentration in RSRF v. RSSIR, the difference in performance for all the traits was significant (P < 0.05 or 0.01) in the aforementioned three comparisons and PRSS v. PRSIR. The mean performance of genotypes for nutritional and agronomic traits for individual environment over 2 years and mean over eight environments are given in Tables 2–6, respectively. The mean Fe concentration of genotypes over eight environments ranged from 42.8 to 58.2 mg/kg and that of Zn concentration between 55·3 and 81·0 mg/kg (Table 2).

The range and mean of various traits under study for each environment and over environments are summarized in Table 7. The environmental mean for various traits across environments was between 46 and 53 mg/kg for Fe concentration, 56 and 81 mg/kg for Zn concentration, 480 and 530 mg/g for oil concentration, 200 and 280 mg/g for protein concentration, 57 and 67 g for shelled weight, 1·75 and 6·16 t/ha for pod yield and 37 and 55 g for 100-seed weight.

Heritability

The broad-sense heritability over eight environments was 0.81 for Fe concentration, 0.92 for Zn concentration, 0.81 for protein concentration, 0.92 for oil concentration, 0.70 for shelled weight, 0.82 for pod yield and 0.91 for 100-seed weight (Table 7). The zero values for heritability of Fe and Zn concentrations occurred in the 2010–11 post-rainy season under the irrigated conditions environment, where genotypic differences for these traits were not significant.

Genotypes stable for iron and zinc concentrations across environments

The GGE biplots were drawn only for Fe and Zn concentrations (Figs 1 and 2), the two main traits of

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Table 2. Mean Fe and Zn concentration of peanut genotypes (over 2 years) studied in four environments and the overall mean performance over eight environments

					Fe					Zn		
C.I.			F	RS	Р	RS		F	RS	Р	RS	
SI. No.	Genotype	Growth habit	RF	SIR	IR	S	Mean	RF	SIR	IR	S	Mean
1	ICGV 87128	SB	43.4	51.7	45.5	48.0	47.3	51.1	52.2	66.4	71.8	60.2
2	ICGV 87141	VB	43.5	43.0	44.4	50.7	44.9	49.8	47.6	61.7	68.2	55.7
3	ICGV 86590	SB	46.3	46.0	44.4	51.5	46.3	60.1	57.7	64.8	75.8	64.9
4	ICGV 87123	SB	48.5	50.6	45.6	48.7	48.8	58.7	57.4	67.9	<i>77</i> ·1	65.5
5	ICGV 91114	SB	49.9	49.8	49.3	51.9	50.6	58.3	58.2	76.0	74.3	66.2
6	ICGV 93468	SB	39.4	47.2	44.7	50.7	45.2	48.8	52.1	71.2	70.6	60.7
7	ICGV 89280	SB	43.7	44.2	38.9	44.1	42.8	51.5	52.0	68.8	71·1	60.0
8	ICGV 07356	VB	44.4	43.5	41.6	48.5	43.5	49.1	48.4	61.5	65.8	55.3
9	ICGV 00350	SB	52.5	43.2	45.4	50.5	48.9	61.4	58.2	71.4	76.7	68·1
10	ICGV 00440	VB	43.0	44.1	40.2	48.6	44.4	51.1	53.4	69.3	71.8	61.7
11	ICGV 86143	SB	44.8	49.8	48.0	45.4	47.1	59.9	59.3	70.9	71.1	66.9
12	ICGV 86015	SB	42.6	42.7	42.8	48.0	45.3	54.1	48.9	68.0	69.4	61.7
13	ICGV 86564	VB	49.3	45.7	53.3	52.5	51.4	62.7	55.7	71.6	70.9	66·1
14	ICGV 89322	VB	49.8	50.2	43.7	50.1	49.1	59.6	57.0	68.6	73.2	66.0
15	ICGV SM 90704	VB	44.2	46.2	40.7	53.2	47.0	60.3	58.3	66.4	78.0	66.2
16	ICGV 93437	SB	53.4	42.9	49.2	49.3	48.6	54.3	51.5	68.4	69.9	61.5
17	ICGV 87157	SB	55.4	48.0	46.6	49.0	49.4	63.5	59.7	76.1	72.8	68.4
18	ICGV 05155	VB	52.3	45.8	45.0	52.2	47.9	70.4	76.3	78.4	86.0	77.2
19	ICGV 06040	VB	58.8	59.3	52.5	58.4	56.1	74.3	75.4	76.3	91.3	80.1
20	ICGV 06099	VB	61.0	59.4	53.0	56.1	57.3	78.7	78.0	78.6	85.8	81.0
21	ICGV 06420	SB	50.8	48.6	48.8	55.0	50.7	73.5	72.7	77.7	83.2	76.8
22	ICGV 00323	SB	47.4	45.0	54.7	52.8	49.2	69.9	64.7	73.6	75.0	71.3
23	ICGV 04149	VB	51.6	51.1	52.7	58.0	52.9	68.2	68.8	74.3	80.1	72.4
24	ICGV 06236	SB	53.3	40.4	53.6	58.0	49.9	68.8	63.2	78.7	80.6	72.4
25	ICGV 04068	SB	54.5	47.5	55.1	52.3	52.9	66.5	64.7	74.2	81.4	72.1
26	ICGV 04093	VB	57·6	49.6	47.5	56.7	52·8	77·2	74·0	74.7	86.9	77·6
27	ICGV 07220	SB	50.2	49.6	50·6	50.9	50·1	72.2	70.3	72.8	80.0	74.6
28	ICGV 07247	VB VB	51·1	47.3	52·9	54·0	52·2	75·4	68·2	74·7	84.5	76·4
29	ICGV 97235	VB VB	50·7	46·1	50·7	57·2 47·3	52·0	73·8	71·3	74·5	83.0	75·7
30	ICGV 86699	VB VB	59.8	51.2	51·0		52.5	72.6	67.5	68.4	80.2	72.7
31	ICGV 87846 ICGV 86590	VB CD	46·7	46·1	49.8	55·3	49·9	68·0	60·2	70·8	80.8	69·5
32 33		SB SB	52·0 48·6	43·9	42·1 53·3	51·5	46∙8 51∙9	67·0 59·9	56·4	71·0	78·4	67·8
33	Erget	SB		49·5	33·3 49·6	52·8 58·4			55·4 65·5	70·2 78·3	82·7 86·7	66·5 72·8
	Faizpur 1-5		54·2	58·6			55·5 45·0	66·5				
35 36	Goldin-1 Leafmutant	SB SB	43·4 49·1	46·3 46·4	43·4 51·0	46·6 53·1	45·0 51·1	55·3 56·7	49·3 57·9	67·9 75·1	72·8 79·9	61·2 67·2
37	Mutant 3	SB	56.1	55.1	55.2	52.5	55.9	65.8	61.8	80.2	83.3	71.9
38	Natal Common	SB	60.3	56.7	53.7	54.4	56.8	64.7	63.2	75.3	83.9	71.4
39	TPT 1	SB	47·5	49.3	52.5	51.6	51·0	59.6	55.7	76·8	75.9	65.7
40	Sir of Bizapur	SB	51.1	48.0	51.3	50.0	49.9	63.0	58·1	77.7	76·0	67·3
41	Shantung Kuno 203	SB	46.4	45.5	48.2	50.5	47·6	5 <i>7</i> ·1	54.0	80.4	81.1	68.2
42	U4-47-7	SB	48.8	55.1	53.6	53.5	52.1	60.6	57.9	78·3	82.6	69.0
43	VRR 245	SB	49.2	47·1	53.5	51.3	49.4	61.1	58·2	75·2	73.3	67·5
44	Gajah	SB	48.4	45.1	47·8	50.5	48.2	53.3	57·5	70.0	73·5	64.0
45	Spanish Improved	SB	48.2	48.0	50.5	55·4	50.7	57·3	56·2	77.4	77·1	66.7
46	White flower (SB)	SB	54.5	46.3	44.6	43.0	46.7	53.3	51.8	69.6	71.4	61.8
47	Abuya	VAL	53.4	46.7	48.6	51.3	49.9	68.9	62.4	83.9	79.0	72.9
48	Gangapuri	VAL	51.3	45.0	50.5	53.2	48.9	61.3	57.6	83.5	79.8	69.8
49	Large Leaf	VAL	45.1	41.6	47·5	49.2	46.2	54.9	51.9	64.4	70.9	60.6
50	CS 16	VB	53.3	56.7	50.2	51.5	52.7	67.4	68.7	76.9	82.6	73.0
51	CS 39	VB	60·1	60.4	54.2	60.2	58.2	67.0	70·5	75·4	81.0	73.9
٠.	20 00		50 1	JU 1	5.2	30 2	J	3, 0	. 0 3		5.0	

Table 2. (Cont.)

					Fe					Zn		
C.I.			R	lS.	Р	RS		F	lS.	Pl	RS	
SI. No.	Genotype	Growth habit	RF	SIR	IR	S	Mean	RF	SIR	IR	S	Mean
52	Chitala White	VB	47.2	43.1	47.7	48.9	47.3	56.0	51.4	65.2	68.4	60.7
53	G N L Mutant	VB	44.9	47.1	43.1	48.4	46.5	56.9	56.2	69.9	71.2	63.3
54	K 4-11	VB	45.5	48.3	46.5	51.1	47.8	53.2	54.5	68.3	69.0	61.5
55	Manfred 68	VB	43.7	44.8	46.2	47.9	45.9	55.3	54.9	68.2	75.1	62.6
56	Mukulu Red	VB	51.9	49.0	52.7	57.9	52.1	63.0	59.0	67.2	84.1	67.6
57	Sangdi	VB	46.0	41.8	47.3	45.9	45.2	55.7	51.5	67.2	74.6	62.8
58	TAG 24	SB	48.3	50.6	52.3	51.8	50.2	60.8	58.7	77.3	76.2	67.8
59	JL 24	SB	53.2	55.9	49.6	55.4	52.7	65.9	69.0	83.3	87.3	77.3
60	TMV 2	SB	51.3	4 <i>7</i> ·1	53.6	51.5	51.2	59.0	55.1	73.7	77.0	67.2
61	GPBD 4	SB	57.6	55.9	52.2	55.6	55.5	65.9	65.9	73.5	76.0	70.5
62	ICGV 00351	SB	48.5	40.4	50.1	48.6	46.5	62.7	55.2	72.7	74.5	67.0
63	ICGV 93261	SB	45.3	48.6	48.2	58.2	50.0	63.0	59.5	75.3	77.9	68.7
64	PI 259747	VAL	54.5	46.0	50.2	54.5	50.1	57.6	51.9	74.8	80.0	65.9
	Mean		50.0	48.2	48.8	51.9	49.7	61.9	59.6	72.8	77.4	67.9
	Minimum		39.4	40.4	38.9	43.0	42.8	48.8	47.6	61.5	65.8	55.3
	Maximum		61.0	60.4	55.2	60.2	58.2	78.7	78.0	83.9	91.3	81.0
	CV(%)						9.0					6.6

Fe, iron concentration (mg/kg); Zn, zinc concentration (mg/kg); RS, rainy season; PRS, post-rainy season; RF, rainfed environment; SIR, supplementary irrigation environment; IR, irrigated environment; S, imposed moisture stressed environment; CV, coefficient of variation expresses the variation as a percentage of the mean.

interest in the present study. The genotypes were represented by the numbers 1-64, and the environments were represented by the year/season followed by growing conditions (stress (ST), irrigated (IR), rainfed (RF)) in the figures. The average tester coordinate (ATC) (line with an arrow head in the biplot figures) on the X-axis passes through the biplot origin and represents the average of the environments, which is defined by the average of PC1 and PC2 (first and second PCs) scores over all environments. The genotypes falling on or close to ATC are stable across the environments tested. All the genotypes on the right-hand side of the coordinates on the Y-axis, i.e. perpendicular to ATC, are the ones that perform above average, and the farther they are from the origin the better is their performance. A genotype falling away from the coordinate of the Y-axis and at the same time on or close to ATC is not only a good performer for the trait under consideration, but also stable across the environments. Based on this, the best genotypes that are stable across the environments are identified. The five best and relatively stable genotypes for Fe and Zn concentrations and their performance for other traits studied in different environments are given in Tables 8 and 9, respectively.

Correlation studies

The correlation values of different trait pairs over eight environments are given in Table 10. The Fe concentration showed significant (P<0.001) positive associations with oil and protein concentration and Zn concentration, and significant (P < 0.001) negative associations with shelled weight (r = -0.22). However, with the exception of Zn concentration (r = 0.535; P < 0.001), the magnitude of the associations was low. Similarly, the Zn concentration was significantly (P < 0.001) and positively correlated with pod yield, 100-seed weight, protein concentration, but significantly (P < 0.001) and negatively with shelled weight. Except for protein concentration (r = 0.678; P < 0.001), the magnitudes of association between Zn concentration and the other traits were low. The protein and oil concentrations were significantly and negatively correlated (r=-0.554; P<0.001). The significant (P < 0.001) and positive association between protein concentration and pod yield and 100-seed weight were of low magnitude. The pod yield was significantly positively associated with 100-seed weight (r=0.533; P<0.001) and shelled weight (r=0.342; P<0.001).

Table 3. Mean oil and protein concentration of peanut genotypes (over 2 years) studied in four environments and the overall mean performance over eight environments

					PC					OC		
C.I.			R	RS	Р	RS		F	RS	Р	RS	
SI. No.	Genotype	Growth habit	RF	SIR	IR	S	Mean	RF	SIR	IR	S	Mean
1	ICGV 87128	SB	193	193	247	282	227	513	507	475	472	491
2	ICGV 87141	VB	193	196	244	266	224	489	487	480	471	484
3	ICGV 86590	SB	200	200	253	270	229	506	508	478	484	494
4	ICGV 87123	SB	177	179	259	284	224	551	524	498	486	516
5	ICGV 91114	SB	181	178	271	294	230	538	518	483	458	501
6	ICGV 93468	SB	186	197	248	280	227	504	477	475	459	480
7	ICGV 89280	SB	166	167	237	265	208	518	507	487	482	496
8	ICGV 07356	VB	203	211	249	273	233	509	458	472	463	476
9	ICGV 00350	SB	188	206	239	267	225	577	535	544	514	544
10	ICGV 00440	VB	210	209	253	261	234	486	475	475	486	484
11	ICGV 86143	SB	195	193	228	271	222	540	518	507	480	513
12	ICGV 86015	SB	193	199	254	277	232	500	487	480	471	487
13	ICGV 86564	VB	207	208	239	259	229	529	505	515	502	516
14	ICGV 89322	VB	220	216	261	269	243	500	499	495	485	497
15	ICGV SM 90704	VB	236	229	265	276	253	460	447	455	471	457
16	ICGV 93437	SB	177	169	265	290	226	530	534	465	441	494
17	ICGV 87157	SB	183	180	256	289	226	553	537	489	476	513
18	ICGV 05155	VB	193	190	238	257	220	593	575	549	525	562
19	ICGV 06040	VB	227	230	262	282	250	558	569	542	511	545
20	ICGV 06099	VB	225	225	248	291	248	569	554	524	490	535
21	ICGV 06420	SB	190	191	241	260	221	600	593	552	528	570
22	ICGV 00323	SB	252	255	270	293	268	512	497	490	462	491
23	ICGV 04149	VB	255	251	268	296	268	527	519	500	472	502
24	ICGV 06236	SB	233	259	272	291	264	487	458	458	456	464
25	ICGV 04068	SB	226	229	256	264	242	565	561	540	511	544
26	ICGV 04093	VB CD	217	215	257	282	242	551	551	514	494	530
27	ICGV 07220	SB	242	240	262	281	255	541	541	509	499	524
28	ICGV 07247	VB	237	237	263	287	256	551	538	513	490	525
29	ICGV 07235	VB	242	227	270	287	255	553	546	511	494	529
30	ICGV 86699	VB VB	216	223	245	274	239	530	501	478	465	495
31 32	ICGV 87846	VB SB	221 202	227 196	235 235	274 274	239 226	549	505 502	512 FOE	494 486	513 508
33	ICGV 86590	SB					228	535		505 407		
33	Erget	SB	206	210 180	241 250	262 296	228	552 537	508 553	497 504	494	510 514
3 4 35	Faizpur 1-5 Goldin-1	SB	185 192	183	250	269	222	503	492	487	464 462	514 484
36	Leafmutant	SB	178	189	278	291	234	551	528	481	469	506
37	Mutant 3	SB	180	169	261	303	228	547	538	499	470	513
38	Natal common	SB	190	179	270	286	231	524	521	475	473	496
39	TPT 1	SB	168	165	250	291	218	555	535	494	474	509
40	Sir of Bizapur	SB	174	181	257	288	225	522	498	480	465	488
41	Shantung Kuno 203	SB	174	175	251	271	218	517	510	464	448	480
42	U4-47-7	SB	174	175	256	293	227	539	531	480	457	500
43	VRR 245	SB	172	178	262	277	224	526	513	471	464	490
44	Gajah	SB	159	185	255	288	224	540	532	479	455	499
45	Spanish improved	SB	156	172	258	280	219	530	512	481	474	497
46	White flower (SB)	SB	183	188	242	240	216	569	528	523	509	529
47	Abuya	VAL	200	186	277	299	243	548	544	473	467	502
48	Gangapuri	VAL	178	192	280	292	238	528	516	466	459	488
49	Large leaf	VAL	212	210	253	274	236	453	443	473	470	461
50	CS 16	VAL VB	231	228	252	298	253	533	496	493	466	501
51	CS 39	VB VB	213	218	269	295	248	517	547	509	478	515
٥.	20 33	, 0	-15	210	200	200	210	517	517	505	17.0	515

Table 3. (Cont.)

					PC					OC		
			R	lS.	PI	RS		R	RS.	Pl	RS	
SI. No.	Genotype	Growth habit	RF	SIR	IR	S	Mean	RF	SIR	IR	S	Mean
52	Chitala white	VB	201	195	218	243	215	505	488	484	468	489
53	G N L mutant	VB	214	201	247	262	231	472	511	482	479	490
54	K 4-11	VB	211	206	224	244	222	475	442	500	471	474
55	Manfred 68	VB	199	197	211	264	219	494	486	495	481	488
56	Mukulu red	VB	206	203	242	253	227	525	515	510	497	513
57	Sangdi	VB	197	187	215	259	213	497	495	499	484	493
58	TAG 24	SB	189	187	261	279	229	534	508	479	475	501
59	JL 24	SB	190	192	276	304	239	530	529	473	456	497
60	TMV 2	SB	183	167	272	295	229	529	523	470	460	497
61	GPBD 4	SB	201	196	248	287	232	570	571	526	485	540
62	ICGV 00351	SB	216	209	238	275	234	486	464	490	458	475
63	ICGV 93261	SB	214	216	232	271	233	554	539	550	520	538
64	PI 259747	VAL	188	183	241	290	225	508	478	506	483	493
	Mean		200	200	252	278	233	528	515	495	478	504
	Minimum		156	165	211	240	208	453	442	455	441	457
	Maximum		255	259	280	304	268	600	593	552	528	570
	CV (%)						4.6					2.9

PC, protein concentration (mg/g); OC, oil concentration (mg/g); RS, rainy season; PRS, post-rainy season; RF, rainfed environment; SIR, supplementary irrigation environment; IR, irrigated environment; S, imposed moisture stressed environment; CV, coefficient of variation expresses the variation as a percentage of the mean.

DISCUSSION

Biofortification (delivery of micronutrients via micronutrient-dense crops) offers a cost-effective and sustainable approach for addressing the issue of micronutrient deficiencies in humans (Bouis 1996; Grusak & DellaPenna 1999; House et al. 2002). If there is sufficient genetic variation for the density of micronutrients in edible parts of the crop, biofortification can be achieved through plant breeding (Mayer et al. 2008). Before a breeding programme is initiated to enhance Fe and Zn concentrations in peanut seeds, it is essential to study the extent of variability for these traits and their heritability and association with other important nutritional and yield parameters. Furthermore, genotypes that are rich in these micronutrients and have a stable performance across environments need to be identified for use as sources/parents to develop high-yielding cultivars with high Fe and Zn concentrations.

Iron and Zn concentrations in the soil, or their application to the soil/foliage, influence their concentration in the seeds (Joshi *et al.* 2010; Singh *et al.* 2011). The variation in Fe and Zn concentrations in the soil across environments will affect discrimination of

genotypes for these micronutrients in the seed, as high-Fe and high-Zn genotypes will accumulate more of these nutrients than low-Fe and low-Zn genotypes grown at the same location during the same growing season environments (Gregorio 2002). Therefore, conducting experiments with uniform levels of Fe and Zn in soil is essential to discriminate genotypes based on their Fe and Zn concentrations. In the present study, Fe and Zn are not applied to the experimental materials either as seed treatment or foliar application.

There is significant genetic variability for all the traits, including Fe and Zn concentrations in peanut seed that can be successfully exploited to develop high-Fe and high-Zn high-yielding cultivars. Both genotypes and environments were significantly different for Fe and Zn concentrations and other traits included in the study (Table 1). The G×E interaction was also significant for all the traits, but its magnitude was small. Upadhyaya *et al.* (2012a, b) also recorded similar observations in their studies of peanut. The environment variance in the present study was the largest component in total variation, followed by genotype.

Compared to the RSRF environment, the yield-related traits (pod yield, shelled weight and 100-seed

Table 4. Mean shelled weight per 100 g of pods for peanut genotypes (over 2 years) studied in four environments and the overall mean performance over eight environments

SI. No. Genotype				Shelled	weight per 100	g of pods	
				RS	Р	RS	
	Genotype	Growth habit	RF	SIR	IR	S	Mean
1	ICGV 87128	SB	67.8	63.0	67.8	70.5	63.9
2	ICGV 87141	VB	67.0	65.3	68.9	68.8	63.3
3	ICGV 86590	SB	65.3	64.0	61.7	57.3	60.1
4	ICGV 87123	SB	66.3	67.3	70.0	64.4	66.1
5	ICGV 91114	SB	61.5	61.3	69.4	65.3	64.4
6	ICGV 93468	SB	65.0	62.8	72.0	60.7	64.5
7	ICGV 89280	SB	63.5	62.8	68.8	62.0	61.8
8	ICGV 07356	VB	71.8	66.3	72.7	65·1	67.9
9	ICGV 00350	SB	65.3	64.5	66.8	56.3	63.2
10	ICGV 00440	VB	66.3	64.5	68.7	65.8	64.5
11	ICGV 86143	SB	62.0	59.8	69.8	63.7	64.5
12	ICGV 86015	SB	68.0	66.3	67.8	67.8	67.7
13	ICGV 86564	VB	69.0	66.3	69.0	67.8	66.0
14	ICGV 89322	VB	62.3	66.3	67.6	66.0	62.9
15	ICGV SM 90704	VB	61.8	60.0	65.2	62.0	59.4
16	ICGV 93437	SB	65.3	64.5	66.0	68·1	66.0
17	ICGV 87157	SB	59.5	63.3	63·1	60.8	58·1
18	ICGV 05155	VB	65.5	65.8	70.3	52.6	63.2
19	ICGV 06040	VB	65.3	65.3	64.5	65.8	62.3
20	ICGV 06099	VB	64.3	64.5	66.8	58.8	62.9
21	ICGV 06420	SB	65.3	68.8	71.3	52.1	64.6
22	ICGV 00323	SB	65.5	66.3	72.4	70.1	68.2
23	ICGV 04149	VB	71.8	70.0	71.6	65·1	69.2
24	ICGV 06236	SB	61.3	64.8	66.5	57.3	61.6
25	ICGV 04068	SB	66.8	63.8	67.8	57.1	63.8
26	ICGV 04093	VB	62.5	61.3	63.4	60.0	59.6
27	ICGV 07220	SB	66.3	67.3	71.8	59.9	66.9
28	ICGV 07247	VB	67.8	66.5	70.1	68.3	65.0
29	ICGV 07235	VB	66.0	63.5	63.6	67.5	62.6
30	ICGV 86699	VB	58.5	59.5	59.7	56.5	56.4
31	ICGV 87846	VB	66.5	65.3	62.5	52.3	62.0
32	ICGV 86590	SB	65.5	59.0	68·1	50.9	60.8
33	Erget	SB	57.3	60.5	59.7	53.3	57.1
34	Faizpur 1-5	SB	60.0	65.0	62.0	60.0	61.7
35	Goldin-1	SB	62.0	57.5	67·1	67.0	60.4
36	Leafmutant	SB	59.0	58.8	67·1	64.2	61.9
37	Mutant 3	SB	62.5	60.5	67.8	62·1	63.7
38	Natal common	SB	61.3	57·5	68.7	63.8	60.9
39	TPT 1	SB	67.5	64.0	64.3	62.9	64.5
40	Sir of Bizapur	SB	66.3	64.5	64.8	59.6	63.2
41	Shantung Kuno 203	SB	67.0	68.0	65.2	59.4	65.3
42	U4-47-7	SB	62.5	63.8	64.2	59.8	63.4
43	VRR 245	SB	66.5	62.3	65.6	64.2	65.7
44	Gajah	SB	61.3	64.3	64.0	61.3	61.9
45	Spanish improved	SB	65.0	61.8	68.5	67.5	67·1
46	White flower (SB)	SB	66.5	62.8	67.4	60.7	65·1
47	Abuya	VAL	64.5	64.3	68·1	61.9	65.4
48	Gangapuri	VAL	61.8	58.0	62.3	67.3	62.7
49	Large leaf	VAL	64.5	63.5	69.4	67.5	63.0
50	CS 16	VB	60.0	61.5	65.0	58.5	57.5
51	CS 39	VB	60.5	60.3	66.0	65.0	58.1

Table 4. (Cont.)

				Shelled	weight per 100	g of pods	
			R	2S	Р	RS	
SI. No.	Genotype	Growth habit	RF	SIR	IR	S	Mean
52	Chitala white	VB	65.0	66.3	56.9	57.0	58.8
53	G N L mutant	VB	64.8	62.0	62.4	64.3	60.5
54	K 4-11	VB	62.3	57.5	65.7	58.3	59.1
55	Manfred 68	VB	65.0	63.8	59.6	58.8	58.6
56	Mukulu red	VB	59.5	60.8	64.6	51.8	56.0
57	Sangdi	VB	62.8	61.3	69.4	65.3	62.3
58	TAG 24	SB	58.3	62.5	72.3	67.9	65.4
59	JL 24	SB	57.0	58.0	57.6	61.5	56.4
60	TMV 2	SB	65.0	64.5	59.3	65.4	63.4
61	GPBD 4	SB	62.3	60.8	70.0	63.3	64.8
62	ICGV 00351	SB	64.3	64.3	66.8	61.7	64.4
63	ICGV 93261	SB	65.0	62.5	72.5	66.5	64.3
64	PI 259747	VAL	58.5	56.8	59.4	53.5	53.6
	Mean		63.9	63·1	66.4	62.0	62.7
	Minimum		57.0	56.8	56.9	50.9	53.6
	Maximum		71.8	70.0	72.7	70.5	69.2
	CV (%)						6.5

RS, rainy season; PRS, post-rainy season; RF, rainfed environment; SIR, supplementary irrigation environment; IR, irrigated environment; SIR, imposed moisture stressed environment; CV, coefficient of variation expresses the variation as a percentage of the mean.

weight) and Zn concentration were higher in the PRSIR environment, whereas the reverse was true for Fe concentration and oil concentration. The difference for protein concentration was not significant. When RSRF and post-rainy season with imposed stress environments were compared, all traits except for oil concentration and shelled weight were significantly higher in the latter. Within the post-rainy season environments, Fe, Zn and protein concentrations were significantly higher in the imposed stress environment compared to the irrigated environment, whereas oil concentration and other yield parameters (pod yield, shelled weight and 100-seed weight) were higher in the irrigated environment. In the comparison of the two rainy season environments, Fe concentration, oil content and shelled weight were higher in the rainfed environment, whereas the rest, except for Zn concentration, were higher in the supplemental irrigated environment. In wild emmer wheat, Peleg et al. (2008) also observed significant genotype × irrigation interaction for Fe, Zn and protein concentration without affecting the overall mean of these traits under the two irrigation regimes. Variation in soil Fe and Zn concentrations (Gregorio 2002; Joshi et al. 2010; Singh et al. 2011) and environmental factors such as

temperature during the seed-filling stage (Joshi et al. 2010; Thavarajah et al. 2010; Singh et al. 2011) are reported to influence kernel Fe and Zn concentrations in groundnut.

The Fe concentration in the genotypes over eight environments was up to 58 mg/kg and that of Zn was up to 81 mg/kg (Table 2). The range for these two traits expressed in the ICRISAT mini-core collection of 184 accessions of peanut belonging to both subspecies, A. fastigiata and A. hypogaea, was from 18.3 to 30.8 mg/kg for Fe concentration and from 28.4 to 43.8 mg/kg for Zn concentration (Upadhyaya et al. 2012a). Based on one environment data from 20 peanut varieties belonging to two subspecies in Ghana, Asibuo et al. (2008) reported a range of 2-37 mg/kg for Fe and 0-65 mg/kg for Zn concentration in seed. Kintampo Local was the variety lowest in seed concentration of both Zn and Fe in Ghana. The range of Zn seed concentration in the study on 70 genotypes by Lal & Singh (2007) in India was from 11 to 77 mg/kg and in the study of Singh et al. (2011) on 60 genotypes it was from 32 to 67 mg/kg. Three genotypes, Gangapuri, ICGV 87141 (ICGS 76) and ICGV 87128 (ICGS 44), included in the present study were also studied by Upadhyaya et al. (2012a). The Fe and

Table 5. Mean pod yield of peanut genotypes (over 2 years) studied in four environments and the overall mean performance over eight environments

				Ī	Pod yield (kg/ha	a)	
				RS	Р	RS	
SI. No.	Genotype	Growth habit	RF	SIR	IR	S	Mean
1	ICGV 87128	SB	2564.5	2590.8	5323.0	2419·1	3258.7
2	ICGV 87141	VB	2870.0	2902.4	5310.3	2666.2	3480.3
3	ICGV 86590	SB	2886.3	2680.9	5830.4	2671.3	3478.8
4	ICGV 87123	SB	3091.2	2490.7	5120.6	2941.6	3458.9
5	ICGV 91114	SB	2640.9	2586.7	4969.7	2855.9	3420.8
6	ICGV 93468	SB	3096.8	2839.0	4981.1	3196.7	3544.1
7	ICGV 89280	SB	2366.5	2645.9	6259.9	3208.8	3689.4
8	ICGV 07356	VB	2042.4	2768.7	6122.0	2683.0	3462.1
9	ICGV 00350	SB	2463.0	2406.6	6280.3	2854.6	3473.2
10	ICGV 00440	VB	2886.4	3095·1	6576.5	3949.7	4107.8
11	ICGV 86143	SB	2488.4	3008.5	5531.1	3257.4	3470.7
12	ICGV 86015	SB	2906.5	2712.2	5896.4	3291.2	3687.2
13	ICGV 86564	VB	2852.5	3194.7	5967.0	2595.6	3747.7
14	ICGV 89322	VB	2644.5	2742.4	5195.5	2961.0	3339.4
15	ICGV 59322 ICGV SM 90704	VB VB	2552.1	2396.1	4749.3	2062.9	2947.0
16	ICGV 93437	SB	2550.7	2560.3	4228.8	3262·1	3146.3
17	ICGV 87157	SB	2982.0	2649.1	4522.9	2373.0	3090.9
18	ICGV 07157	VB	3982.2	3797.8	6541.7	3528.5	4430.4
19	ICGV 05133	VB VB	3407.0	3850.6	6140.4	4012.3	4238.9
20	ICGV 06040	VB VB		3259.3	7017·6		4300.2
		SB	3484.8			3548.1	
21	ICGV 06420		3717.7	3315.4	6162.5	3320.9	4211.4
22	ICGV 00323	SB	3026.0	2554.9	3912.7	2578.0	2958.5
23	ICGV 04149	VB	3537.4	3843.0	5699·5	3015.8	4017.8
24	ICGV 06236	SB	2606.8	2496.2	4511.7	2532.4	3019.6
25	ICGV 04068	SB	3278.0	3082.0	6793.3	3490.5	4217.7
26	ICGV 04093	VB	2917.0	3916.8	7078.5	2638.6	4203.2
27	ICGV 07220	SB	3546.5	2792.2	6878.5	4463.3	4404.1
28	ICGV 07247	VB	2979.9	3239.9	6829.1	3378.8	4177.2
29	ICGV 07235	VB	3576.6	2966.7	5987.1	2964.0	4053.5
30	ICGV 86699	VB	3054·1	2723.0	5740.7	2998.0	3667.2
31	ICGV 87846	VB	2919·1	3128.3	5146.0	2515.8	3518.8
32	ICGV 86590	SB	3473.1	2913.0	5703.0	2446.7	3714.4
33	Erget	SB	2575.2	2360.7	4767.8	2552.7	3141.6
34	Faizpur 1-5	SB	2565.7	2373.9	4078-2	2514.0	2969.3
35	Goldin-1	SB	2512.5	2309.0	5908.9	2754.3	3375.9
36	Leafmutant	SB	2274.5	2500.7	2827-2	2331.3	2574.5
37	Mutant 3	SB	2928.0	2540.3	4605.5	2753.6	3407.6
38	Natal common	SB	2096.5	2233.6	3778.7	2803.6	2787.1
39	TPT 1	SB	2277.6	2840.2	3939.9	2536.4	3010.9
40	Sir of Bizapur	SB	2205·1	2470.5	3781.8	2717.5	2895.0
41	Shantung Kuno 203	SB	2345.9	2482.7	4078.8	2932.9	2894.6
42	U4-47-7	SB	2148.6	2648.3	3521.1	2549.9	2660.3
43	VRR 245	SB	2256.7	2386.7	4020.1	2830.3	2735.2
44	Gajah	SB	2332.2	2568·1	4100.5	2872.7	2916.5
45	Spanish improved	SB	2170.0	2270.6	3993·1	3080.0	2936.1
46	White flower (SB)	SB	2241.8	2705.6	5313.2	3699.4	3406.0
47	Abuya	VAL	2824.8	2168.5	3607.6	3070.7	2887.2
48	Gangapuri	VAL	2713·1	2396.3	3848.6	2718.1	2877.3
49	Large leaf	VAL	2474.1	2579.6	4684.3	2201.7	2936.7
50	CS 16	VB	2883.8	2607.4	5031.7	2097.7	3115.7
51	CS 39	VB	2770.4	2552.8	5591.0	2452.4	3220.7

				!	Pod yield (kg/ha	a)	
			R	2S	Р	RS	
SI. No.	Genotype	Growth habit	RF	SIR	IR	S	Mean
52	Chitala white	VB	2191.9	2672.3	3422.4	2789-2	2734.3
53	G N L mutant	VB	2219.4	2875.2	4981.2	3164·1	3385.0
54	K 4-11	VB	2366.0	2979.7	3981.7	3292.9	3088.4
55	Manfred 68	VB	2100.7	2382.9	4030.8	2456.5	2729.4
56	Mukulu red	VB	2760.7	2538.5	5494.4	1702.3	3099.5
57	Sangdi	VB	2148.5	2617.6	5009.7	2770.8	3037.8
58	TAG 24	SB	2478.8	3067.5	3854.3	3099.5	3034.9
59	JL 24	SB	2695.8	2454.1	3642.0	1961.9	2516.8
60	TMV 2	SB	2384.6	2480.6	3807.3	2398.2	2682.4
61	GPBD 4	SB	2333.4	2465·1	4861.5	2802.8	3140.0
62	ICGV 00351	SB	2657.2	2714.1	6370.7	3572.0	3711.1
63	ICGV 93261	SB	2541.5	2867.8	6478.3	3161·1	3698·1
64	PI 259747	VAL	2359.7	2235.0	4837.8	2222.5	2838.6
	Mean		2706.6	2742.1	5082·1	2867.9	3349.7
	Minimum		2042.4	2168.5	2827-2	1702.3	2516.8
	Maximum CV (%)		3982·2	3916·8	7078.5	4463·3	4430·4 16·6

RS, rainy season; PRS, post-rainy season; RF, rainfed environment; SIR, supplementary irrigation environment; IR, irrigated environment; SIR, imposed moisture stressed environment; CV, coefficient of variation expresses the variation as a percentage of the mean.

Zn concentration levels reported by Upadhyaya et al. (2012a) in these genotypes are similar to that observed in the present study when expressed on defatted meal basis. The oil concentration in ground-nut genotypes shows a large environmental influence and procedures of estimating oil concentration can also introduce errors; therefore, it may be desirable to express Fe and Zn concentrations on a defatted meal basis.

The broad-sense heritability for Fe and Zn concentrations was high, indicating possible gains through selection. Similarly, the heritability was high for protein concentration and kernel oil content as well as for yield parameters. In spite of highly significant environmental effects, the heritability values were high due to the low magnitude of $G \times E$ interactions for different traits included in the study. Based on their study of a mini-core collection of peanut, Upadhyaya et al. (2012a) also reported high heritability (>0·70) for Fe and Zn concentrations. However, the estimates of broad-sense heritability in a study are specific to the population used in the study and the environment under which the study is conducted, and can therefore not be generalized for the trait. In other

crops, such as spring wheat, a strong $G \times E$ interaction and very low broad sense heritability for Fe and Zn concentrations have been reported (Joshi *et al.* 2010).

For Fe concentration, the five best and relatively stable genotypes are Mutant 3, Natal Common, ICGV 06040, CS 39 and ICGV 06099 in order of their stability. Among these, Mutant 3 and Natal Common were highly stable followed by ICGV 06040 and CS 39. ICGV 06099, although a high performer for Fe concentration, was relatively less stable than the other four genotypes. Similarly, for Zn concentration, the stable genotypes included ICGV 06040, ICGV 06099, ICGV 06420, ICGV 05155 and ICGV 04093. ICGV 06040 and ICGV 06099 were not only highly stable but also high performers for Zn concentration. When both Fe and Zn were considered together, ICGV 06099 and ICGV 06040 were stable genotypes across environments with high yield and high concentration of both the micronutrients.

The absence of association, or low association, between pod yield and Fe and Zn concentrations and the significant positive association between Fe and Zn concentrations indicated that these traits can be

Table 6. Mean 100-seed weight of peanut genotypes (over 2 years) studied in four environments and the overall mean performance over eight environments

SI. No. Genotype 1 ICGV 87128					HSW		
			F	RS	P	RS	
Sl. No.	Genotype	Growth habit	RF	SIR	IR	S	Mean
1	ICGV 87128	SB	39.9	39.2	52.0	45.3	42.9
2	ICGV 87141	VB	40.6	41.1	56.9	41.1	45.3
3	ICGV 86590	SB	41.4	36.2	54.7	41.6	42.4
4	ICGV 87123	SB	37.5	35.2	49.3	44.7	41.7
5	ICGV 91114	SB	37.4	34.4	50.9	42.0	41.0
6	ICGV 93468	SB	40.0	36.3	54.5	47.7	44.1
7	ICGV 89280	SB	47.8	50.2	72.3	59·1	57.3
8	ICGV 07356	VB	46.5	37.5	62·1	47.4	47.9
9	ICGV 00350	SB	39.1	31.0	47.0	38.1	37.7
10	ICGV 00440	VB	58·1	51.1	75.8	59.6	61.7
11	ICGV 86143	SB	39.2	37.4	47.0	45.7	41.4
12	ICGV 86015	SB	44.9	41.9	63.5	50.8	50.4
13	ICGV 86564	VB	62.5	56.2	73.1	62.3	63.4
14	ICGV 89322	VB	40.2	47.6	67.3	51.5	51.2
15	ICGV SM 90704	VB	39.1	39.1	50.4	43.0	42.9
16	ICGV 93437	SB	32.8	30.2	36.3	33.6	32.8
17	ICGV 87157	SB	40.7	33.8	58.6	43.6	42.6
18	ICGV 05155	VB	40.2	39.8	43.5	36.8	40.1
19	ICGV 06040	VB	50.9	48.8	52.3	43.1	47.4
20	ICGV 06099	VB	52.6	45.4	52.6	41.1	47.6
21	ICGV 06420	SB	36.4	33.0	43.3	37.7	37·1
22	ICGV 00323	SB	35.8	34.2	35.5	31.4	33.3
23	ICGV 00323	VB	42.3	38.4	50·4	40.9	42.6
24	ICGV 06236	SB	37.0	38.3	48.0	38.6	39.6
25	ICGV 04068	SB	45·8	43.6	54.0	43.4	46.4
26	ICGV 04093	VB	41.3	41.4	44.5	35.2	41.9
27	ICGV 07220	SB	46.2	42.1	49.2	41.1	44.5
28	ICGV 07247	VB	48.8	42.3	50.0	39.2	46.0
29	ICGV 07235	VB VB	46.4	39.7	48.0	40.6	44.4
30	ICGV 86699	VB VB	47.3	44.6	50.0	42.7	46.5
31	ICGV 87846	VB VB	47.3	42.6	64.4	47.8	51.3
32	ICGV 86590	SB	44.1	34.5	44.7	37.7	40.7
33	Erget	SB	43.9	38.2	57.0	41.3	44.1
34	Faizpur 1-5	SB	33.2	32.9	39.7	38.2	36.6
35	Goldin-1	SB	44.2	38.3	55·6	45·6	45.0
36	Leafmutant	SB	32.0	29.5	36.8	32.3	32.8
37	Mutant 3	SB	34.5	36·5	44.4	45·9	40.3
38	Natal common	SB	34.4	34·2	40.1	38.0	36.3
39	TPT 1	SB	31.2	32.5	35.8	34.8	33.7
40	Sir of Bizapur	SB	32.6	29.4	35·3	31.4	31.8
41	Shantung Kuno 203	SB	45.6	37.0	54·1	46.7	45.5
42	U4-47-7	SB	30.8	35.0	38.2	33.9	35.7
43	VRR 245	SB	31.8	34.1	34.9	34.8	33.7
44	Gajah	SB	31.5	38.9	42.4	34·0 34·1	37·6
45	Spanish improved	SB	30.8	31.1	40.2	36.1	35.2
	White flower (SB)	SB	38.3				
46 47		VAL	38·3 32·4	37⋅8 36⋅8	54·2 44·4	46·2 37·3	44·4 38·5
	Abuya Gangapuri	VAL VAL	34·3	34·2	37·6		35·8
48	Gangapuri					35·6	
49 50	Large leaf CS 16	VAL VB	46·3 48·5	40·3 41·0	60·2 53·1	50·0	48·4 45·3
						35·6	
51	CS 39	VB	35.9	41.6	62.0	43.5	45.1

Table 6. (Cont.)

					HSW		
			F	RS	P	RS	
Sl. No.	Genotype	Growth habit	RF	SIR	IR	S	Mean
52	Chitala white	VB	44.2	42.8	51.6	34.5	43.6
53	G N L mutant	VB	32.5	34.0	46.7	39.7	38.4
54	K 4-11	VB	35.3	36.6	43.4	32.9	36.9
55	Manfred 68	VB	40.5	40.8	50.7	46.6	45.0
56	Mukulu red	VB	35.5	35.5	60.8	34.9	41.6
57	Sangdi	VB	33.7	34.0	47.8	39.6	38.6
58	TAĞ 24	SB	35.1	35.0	46.6	46.2	42.1
59	JL 24	SB	32.6	34.9	43.3	31.4	35.5
60	TMV 2	SB	30.8	31.7	37.3	33.4	34.3
61	GPBD 4	SB	35.4	30.7	41.8	38.6	37.5
62	ICGV 00351	SB	34.6	38.3	47.5	34.3	39.1
63	ICGV 93261	SB	37.3	34.1	46.5	36.4	39.6
64	PI 259747	VAL	34.7	34.4	43.8	34.7	37.4
	Mean		39.7	37.9	49.6	40.9	42.0
	Minimum		30.8	29.4	34.9	31.4	31.8
	Maximum		62.5	56.2	75.8	62.3	63.4
	CV (%)						11.5

HSW, 100-seed weight (g); RS, rainy season; PRS, post-rainy season; RF, rainfed environment; SIR, supplementary irrigation environment; IR, irrigated environment; S, imposed moisture stressed environment; CV, coefficient of variation expresses the variation as a percentage of the mean.

improved simultaneously. The same holds true for oil concentration, which can also be improved simultaneously with Fe and Zn concentrations. However, an increase in oil concentration will have an adverse impact on protein concentration. Significant positive associations between Fe and Zn concentrations in seed have been reported in several crops (common bean: Gregorio 2002; House et al. 2002; lotus: Klein & Grusak 2009; sorghum: Ashok Kumar et al. 2009; Reddy et al. 2010; wild emmer wheat: Peleg et al. 2008; common wheat: Morgounov et al. 2007 and maize: Maziya-Dixon et al. 2000), including peanut (Upadhyaya et al. 2012a). Interestingly, when a subset of 20 peanut genotypes with high concentrations of Fe and Zn was studied to determine correlations, the correlation coefficient between Fe and Zn concentrations was not significant (data not given). Thus, when attempting to improve both Fe and Zn concentrations, the selection should be based on the estimation of both Fe and Zn concentrations in the seed, because, though a positive association was observed in general, the same did not hold good for high Fe and Zn genotypes. Similarly, no association was observed between Fe and Zn concentrations with oil and protein

concentration, 100-seed weight, pod yield and shelled weight. Thus, all the traits can be improved simultaneously.

No studies on the inheritance of Fe and Zn concentrations in peanut have been reported in the literature, but they are available for other crops as reviewed in the Introduction section of the present paper. In inter-specific crosses of common bean, the inheritance of Fe concentration was suggested to be quantitative (Guzmán-Maldonado et al. 2003), while simple inheritance was suggested for Zn concentration (Cichy et al. 2005). Blair et al. (2009) reported five QTLs for Fe and six QTLs for Zn concentration clustered on the linkage group B11, explaining up to 0.48 of phenotypic variance, suggesting the presence of an important locus useful for marker assisted selection in bean. In rice, where the presence of additive and dominant gene effects, environmental effects and reciprocal effects was reported for Fe concentration, Gregorio (2002) suggested a delay in the selection for high Fe in the later generations while following the bulk breeding method in early generations for selection for agronomic traits: alternatively, he suggested that a single seed descent method could be

Table 7. Variability and heritability of traits included in the study based on the performance of 64 peanut genotypes under different environments at ICRISAT, Patancheru, during the 2009–11 cropping seasons

		Fe			Zn		Р	C		C	C		9	SW		P	Y		Н	ISW	
ENV	R	М	Н	R	М	Н	R	М	Н	R	М	Н	R	М	Н	R	М	Н	R	М	Н
2009 RSSIR	36–68	49	43	45–87	63	86	160–260	200	85	410–610	520	82	51–70	61	54	1428–3230	1789	86	32–56	39	64
2009 RSRF	41-68	53	40	49-90	66	79	150-260	200	86	440-600	530	77	55-71	63	24	1212-3139	1749	79	33-64	41	61
2009/10 PRSIR	33-58	46	81	50-86	68	80	210-300	260	79	450-540	490	90	55-77	66	61	2144-5999	4006	78	30-87	45	90
2009/10 PRSS	39-65	51	62	60-91	73	75	250-300	270	73	450-530	480	83	52-77	67	67	1815-4940	3177	70	30-84	45	91
2010 RSSIR	39-65	48	56	46-74	56	72	160-260	200	83	430-580	510	69	56-74	65	45	2829-4857	3695	22	25-56	37	79
2010 RSRF	37-60	47	70	44-73	58	65	150-250	200	90	440-600	520	80	55-75	65	58	2763-5265	3664	11	27-65	39	81
2010/11 PRSIR	42-60	52	0	70-86	77	0	200-280	250	66	450-560	500	83	55-75	66	55	3229-8639	6159	51	39-74	55	54
2010/11 PRSS	41-61	53	35	67–95	81	62	220-310	280	77	430-530	480	74	52-69	57	72	1440-4559	2559	37	27-55	37	20
Over 8 ENV	33-68	50	81	44-95	68	92	150-310	230	81	410-610	500	92	51-77	64	70	1212-8639	3350	82	25-87	42	91

ENV, environment; RSRF, rainy season rainfed; RSSIR, rainy season supplemental irrigation; PRSIR, post-rainy season irrigated; PRSS, post-rainy season stress; R, range; M, mean; H, heritability in broad sense (%); Fe, iron concentration (mg/kg); Zn, zinc concentration (mg/kg); PC, protein concentration (mg/g); OC, oil concentration (mg/g); SW, shelled weight per 100 g of pods; PY, pod yield (kg/ha); HSW, 100-seed weight (g).

genotypes in Tables 2 and 3).

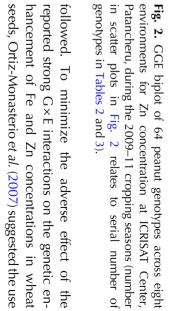
Patancheru, during the 2009–11 cropping seasons (number in scatter plots in Fig. 1 relates to serial number of

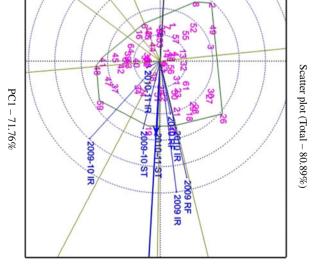
GGE biplot of 64 peanut genotypes across eight

for Fe concentration at ICRISAT

Center,

environments





PC2 - 9.13%

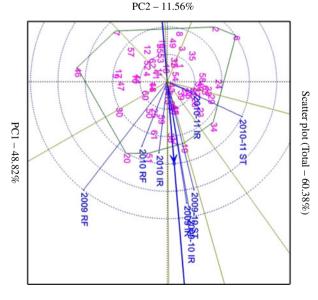


Table 8. The five stable and best-performing peanut genotypes for Fe concentration across eight environments at ICRISAT, Patancheru, during the 2009–11 cropping seasons

	ICGV 06040			ICGV 06099			Mutant 3			Natal common			CS 39			Across 64 genotypes and eight environments		
Trait	Maximu	m Minimu	m Mean	Maximi	um Minimun	n Mean	Maximui	m Minimum	Mean	Maximu	m Minimui	n Mean	Maximu	n Minimum	Mean	Maximum	Minimun	Grand n mean
Fe	65	47	56	69	50	57	61	52	56	64	52	57	68	53	58	69	32	50
Zn	95	65	80	88	72	81	87	55	72	86	62	71	85	62	74	95	43	68
PC	280	230	252	290	220	248	300	170	228	290	170	231	300	200	248	320	150	233
OC	580	510	545	580	470	535	570	470	513	540	460	496	560	470	515	610	420	504
SW	70	36	62	70	44	63	70	56	64	76	43	61	66	28	58	77	24	63
PY	6742	2290	4239	8140	2318	4300	6604	1465	3408	5198	1295	2787	6663	1252	3221	8342	1174	3350
HSW	61	35	47	58	31	48	48	32	40	45	31	36	61	35	45	88	23	42

Fe, iron concentration (mg/kg); Zn, zinc concentration (mg/kg); PC, protein concentration (mg/g); OC, oil concentration (mg/g); SW, shelled weight per 100 g of pods; PY, pod yield (kg/ha); HSW, 100-seed weight (g).

Table 9. The five stable and best-performing peanut genotypes for Zn concentration across eight environments at ICRISAT, Patancheru, during the 2009–11 cropping seasons

	ICGV 05155		IC	ICGV 06040			ICGV 06099		ICGV 06420			ICGV 04093			Across 64 genotypes and eight environments			
Trait	Maximum	Minimum	Mean	Maximum	Minimum	Mean	Maximum	Minimum	Mean	Maximum	Minimum	Mean	Maximum	Minimum	Mean	Maximum	Minimum	Grand mean
Fe	54	40	48	65	47	56	69	50	57	60	43	51	61	44	53	69	32	50
Zn	90	59	77	95	65	80	88	72	81	90	64	77	93	65	78	95	43	68
PC	260	180	220	280	230	252	290	220	248	260	190	221	290	210	242	320	150	233
OC	610	520	562	580	510	545	580	470	535	610	530	570	600	490	530	610	420	504
SW	70	35	63	70	36	62	70	44	63	75	33	65	65	44	60	77	24	63
PY	7841	3002	4430	6742	2290	4239	8140	2318	4300	7020	2940	4211	8342	2328	4203	8342	1174	3350
HSW	48	33	40	61	35	47	58	31	48	46	32	37	48	34	42	88	23	42

Fe, iron concentration (mg/kg); Zn, zinc concentration (mg/kg); PC, protein concentration (mg/g); OC, oil concentration (mg/g); SW, shelled weight per 100 g of pods; PY, pod yield (kg/ha); HSW, 100-seed weight (g).

Table 10. Correlations between different traits studied in 64 peanut genotypes under different environments at ICRISAT, Patancheru, during the 2009–11 cropping seasons

Traits	Fe	Zn	PC	OC	SW	PY
Fe	_					
Zn	0.535 (P < 0.001)	_				
PC	0·166 (<i>P</i> <0·001)	0.678 (P < 0.001)	_			
OC	0.228 (P < 0.001)	-0.042 (NS)	-0.554 (P < 0.001)	_		
SW	-0.225 (P < 0.001)	-0.279 (P < 0.001)	-0.133s (NS)	0·117 (NS)	_	
PY	-0.078 (NS)	0.168 (P < 0.001)	0.208 (P < 0.001)	0·040 (NS)	0.342 (P < 0.001)	_
HSW	-0.089 (NS)	$0.153 \ (P < 0.001)$	0.202 (P < 0.001)	−0.012 (NS)	0.299 (P < 0.001)	$0.533 \ (P < 0.001)$

P-values are given in parenthesis. NS, not significant; Fe, iron concentration (mg/kg); Zn, zinc concentration (mg/kg); PC, protein concentration (mg/g); OC, oil concentration (mg/g); SW, shelled weight per 100 g of pods; PY, pod yield (kg/ha); HSW, 100-seed weight (g).

of a systematic check, alpha lattice design and spatial analyses of segregating and advanced populations, and application of Zn fertilizer (foliar or soil application) to harmonize soil Zn concentration. Although G×E interaction for Fe and Zn concentrations in the peanut seeds in the present study was significant but relatively small, it would be advisable to adopt these strategies in genetic enhancement of these micronutrients in peanut as well. While improving Fe and Zn concentrations in plants, it would be desirable to also pay attention to reducing bio-inhibitors to enhance the bioavailability of Fe and Zn in human diets.

CONCLUSIONS

Significant genetic variability for kernel Fe and Zn concentrations, protein and oil concentration and their high heritability favour genetic enhancement of these traits in peanut. The concentration of Fe is 1.4-fold higher than the normal genotypes and that of Zn 1.5fold higher. Similarly, protein concentration is 1·1-fold higher and oil concentration is 1·2-fold higher than the normal genotypes. Barring associations between protein content with oil content, and shelled weight with Fe and Zn concentrations, which are negative, all other associations are either positive, low or absent. Thus, it is possible to simultaneously improve Fe and Zn concentrations, pod yield and 100-seed weight with either protein or oil concentration. Genotypes, ICGV 06099 and ICGV 06040, have high concentrations of both Fe and Zn in their seeds, and are stable across environments. They are also high yielding. Because of these characteristics, these genotypes make an ideal choice as parents in a breeding programme aiming to improve Fe and Zn concentrations in peanut seeds.

They can also be used in developing mapping populations to identify QTLs associated with high Fe and Zn concentrations in peanut seed.

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