

Variability and Stability for Kernel Iron and Zinc Contents in the ICRISAT Mini Core Collection of Peanut

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ABSTRACT

Iron and zinc are essential minerals in human and animal nutrition. Low genetic variability has been a major bottleneck in genetic enhancement of the nutritional status of food crops and/or cultivars. Recently, peanut (*Arachis hypogaea* L.) is gaining importance as a food in the world. We assessed the nutritional quality of 184 peanut mini core accessions along with four control cultivars to identify stable genotypes with high kernel Fe and Zn contents and with good agronomic performance for use in crop improvement. Significant genotypic and genotype \times environment interactions were observed for both nutritional traits and all agronomic traits in the entire mini core collection and within each of subsp. *fastigiata* Waldron and subsp. *hypogaea*. Forty-eight accessions with higher Fe content, 43 accessions with high Zn content, and 23 accessions high in both minerals coupled with superior agronomic traits were identified. Among them, ICG 4750, ICG 7963, ICG 14705, and ICG 15419 were highly diverse and stable for either or both nutrients, produced pod yield similar to or greater than the control cultivars, and have high shelling percentage, except ICG 15419, and high 100-seed weight, except ICG 4750. High positive correlation between Fe and Zn contents in peanut kernels will be desirable and useful for breeders as selection for either of the nutrients would be effective for both the nutrients.

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Abbreviations: σ^2_g , variance due to genotype; σ^2_{ge} , variance due to genotype \times environment; b, regression coefficient; S^2_d , deviation from regression.

PEANUT IS WIDELY GROWN WORLDWIDE in more than 100 countries of tropical, subtropical, and warm temperate regions annually on 24 million ha with a total in-shell production of 37.6 million t and productivity of 1.56 t ha⁻¹ (FAO, 2010). It is a rich source of protein, fat, carbohydrates, minerals, and vitamins, all essential to humans as well as to livestock. Although it is essentially an oil crop, it is also a popular snack food with its use in various confectionery and other processed food products in many countries.

Micronutrient malnutrition arising from Fe and Zn deficiencies alone affect over 3 billion people around the world (Upadhyaya et al., 2011). Nearly 500,000 children (<5 yr of age) die annually because of Fe and Zn deficiencies (Black et al., 2008). It is also accompanied by serious physical incapacity, mental impairment and decreased health. Widespread micronutrient malnutrition results in an enormous negative socioeconomic impact at the individual, community, and national levels (Darnton-Hill et al., 2005; Stein, 2010).

Peanuts with high protein and low oil but with improved oil quality and rich in minerals will be the most preferred confectionery products. Efforts at ICRISAT and elsewhere have succeeded in identifying or developing peanut cultivars with variation in seed size, protein, oil content, and oil quality (Upadhyaya et al., 2012),

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but no concerted efforts were made to identify sources of the essential minerals such as Fe and Zn. The genebank at ICRISAT in Patancheru, India, maintains a large global collection (15,445 accessions) of peanut germplasm. However, it is impractical to screen such a large collection for quality traits mainly as it involves huge cost in chemical analysis. Frankel (1984) proposed the development of a reduced subset of germplasm in the form of core collection (10% of entire collection) representing diversity in the entire collection of a species as the ideal resource to identify new sources of variation for use in crop improvement programs. Toward this end, Upadhyaya et al. (2003) developed a core collection of 1704 accessions in peanut and subsequently a mini core (1% of the entire collection) (Upadhyaya et al., 2002) collection of 184 accessions after detailed morpho-agronomic evaluation of the core collection. The mini core collection adequately represented the wide range of variability present in the core as well as in the entire collection. Extensive evaluation of the mini core collection over the years resulted in the identification of diverse accessions tolerant to drought and salinity (Upadhyaya, 2005; Srivastava, 2010), resistance to multiple diseases (Kusuma et al., 2007), and for seed quality traits such as protein, oil, and oleic:linoleic fatty acid ratio (Upadhyaya et al., 2012). However, no systematic efforts were made, except a few studies that reported some variability in kernel Fe and Zn in popular cultivars (Asibuo et al., 2008), breeding lines (Nigam et al., 2010), and market samples (Cabrera et al., 2003), to mine germplasm for variation in grain Fe and Zn concentrations. There is no published report on variability and stability of Fe and Zn in germplasm subsets representing diversity of entire collection in peanut. This investigation was initiated to study variation for kernel Fe and Zn concentrations in the peanut mini core collection, developed from global collection at ICRISAT (Upadhyaya et al., 2002), identify genetically diverse accessions with high kernel Fe and Zn and good agronomic performance, and make these accessions available to researchers worldwide for use in peanut breeding and genomic studies.

MATERIALS AND METHODS

One hundred eighty-four accessions of peanut mini core collection representing two subspecies and six botanical varieties (Upadhyaya et al., 2002) together with four controls (M 13, ICGS 76, ICGS 44, and 'Gangapuri') were evaluated for three seasons (2009 rainy and 2009/2010 and 2010/2011 postrainy seasons) in alpha design with three replications in precision fields on Alfisol (clayey-skeletal, mixed, isohyperthermic family of Udic Rhodustalfs) (Nagabhushana et al., 1987) at Patancheru (17°24' N, 78°12' E, and 536 m altitude), India. The precision fields at ICRISAT have a slope of 0.5%, uniform fertility, and Fe and Zn contents always greater than the critical limits (2.0 mg kg⁻¹ for Fe and 0.75 mg kg⁻¹ for Zn) (K.L. Sahrawat, ICRISAT, personal communication, 2012). The plot size consisted of two rows 4 m in length spaced at 60 cm between rows and 10 cm within row.

All the four controls are released cultivars in India. M13 (Reddy, 1988) and ICGS 76 (also known as ICGV 87141 or PI 546372) (Nigam et al., 1991), which are adapted to rainfed agroecologies, belong to *A. hypogaea* subsp. *hypogaea* var. *hypogaea* while ICGS 44 (also known as ICGV 87128 or PI 537112) (Nigam et al., 1990), adapted to irrigated postrainy season, belongs to subsp. *fastigiata* var. *vulgaris* Harz and Gangapuri (Isleib et al., 1994) belongs to subsp. *fastigiata* var. *fastigiata* (Waldron) Krapov. & W. C. Greg. The seeds were sown at uniform depth and the crop specific agronomic practices and plant protection measures were followed to raise a successful crop. The plots received 60 kg P₂O₅ and 400 kg gypsum ha⁻¹ with 12 irrigations in the postrainy season and six irrigations in the rainy season, totaling about 5 cm of water each time the crop was irrigated. Five representative plants in each plot were tagged randomly to record observations on various qualitative and quantitative traits. Data were recorded on a plot basis for days to 50% flowering, pod yield (kg ha⁻¹), shelling percentage, and seed weight (g per 100 seeds). A 200 g matured pod sample was used to estimate shelling percentage. Sound mature kernels were provided to the Central Analytical Services Laboratory, ICRISAT, Patancheru, for chemical analysis. Care was taken to avoid contamination of seeds with dust, trash, or other foreign material. The seed samples were dried, powdered, and digested using the tri-acid mixture to determine the Fe and Zn contents by atomic absorption spectrophotometry (Sahrawat et al., 2002).

Statistical analysis of the data was done following the residual maximum likelihood method (Patterson and Thompson, 1971) with seasons as fixed and entries as random on GenStat 14.1 (VSN International, 2011). The components of phenotypic variance due to genotype (σ^2_g) for the entire mini core and for subsp. *fastigiata* and subsp. *hypogaea* for individual seasons and across seasons and the variance due to genotype \times environment (σ^2_{ge}) for the entire mini core and subsp. *fastigiata* and subsp. *hypogaea* and their residuals were calculated. The significance among environments was tested using Wald (1943) statistics. Stability analysis was performed (Eberhart and Russell, 1966) to identify stable accessions for kernel Fe and Zn contents across environments. Principal component analysis of data on four agronomic and two nutritional traits on 23 selected entries and control cultivars was performed (Hotelling, 1933). The mean observations for each trait were standardized by subtracting from each observation the mean value of the trait and subsequently dividing by its respective standard deviation (SD). This resulted in standardized values for each trait with zero mean and SD of 1. The standardized values were used to perform principal component analysis using GenStat 14.1. Cluster analysis using the Ward (1963) method was performed with scores of the first five principal components.

RESULTS AND DISCUSSION

Residual maximum likelihood analysis of individual environment data indicated highly significant σ^2_g for pod yield, days to flowering, shelling percentage, 100-seed weight, and kernel Fe and Zn contents. The magnitude of σ^2_g was similar for both nutrients in the rainy season (2009) but the σ^2_g for Zn was two to four times greater in postrainy seasons than σ^2_g for kernel Fe. The pooled analysis of data over three environments also revealed the significance of

Table 1. Estimates of variance for nutritional quality and agronomic traits in the ICRISAT peanut (*A. hypogaea*) mini core collection.

Source of variation	Fe (mg kg ⁻¹)	Zn (mg kg ⁻¹)	Days to 50% flowering	Pod yield (kg ha ⁻¹)	Shelling (%)	100-seed weight (g)
2009 rainy season						
σ^2g^\dagger	8.64**	11.59**	4.37**	448,578**	14.07**	37.11**
σ^2 subsp. <i>fastigiata</i>	7.00**	12.39**	0.99**	461,815**	19.83**	36.86**
σ^2 subsp. <i>hypogaea</i>	8.73**	8.93**	2.48**	415,797**	13.26**	41.40**
2009/2010 postrainy season						
σ^2g	15.67**	31.22**	9.85**	93,499**	20.56**	42.52**
σ^2 subsp. <i>fastigiata</i>	8.09**	17.40**	4.12**	44,060**	8.94**	42.43**
σ^2 subsp. <i>hypogaea</i>	12.02**	20.27**	5.08**	51,262**	11.29**	38.93**
2010/2011 postrainy season						
σ^2g	8.01**	31.47**	14.93**	447,280**	21.15**	51.85**
σ^2 subsp. <i>fastigiata</i>	6.23**	22.47**	11.56**	431,060**	22.78**	41.33**
σ^2 subsp. <i>hypogaea</i>	5.27**	24.11**	3.73**	468,613**	16.15**	53.69**
Pooled						
σ^2g	6.16**	4.57**	7.94**	158,605**	8.37**	57.21**
$\sigma^2g \times$ environment	12.85**	11.88**	1.76*	177,227**	6.78**	18.54**
σ^2 subsp. <i>fastigiata</i>	2.38**	6.29**	3.52**	160,825**	8.94**	51.48**
σ^2 subsp. <i>fastigiata</i> \times environment	4.65**	10.74**	2.35**	149,184**	4.76**	10.45**
σ^2 subsp. <i>hypogaea</i>	5.09**	8.60**	3.08**	139,313**	7.64**	56.46**
σ^2 subsp. <i>hypogaea</i> \times environment	3.34**	9.25**	0.24 NS [‡]	165,338**	6.33**	23.73**
σ^2 error	4.20**	4.90**	2.59**	130,816**	30.82**	23.54**
Environment (Wald statistic [§])	965.2**	1,467.1**	13,198.6**	3,186.9**	511.5*	2,314.3**

*Significant at the 0.05 probability level.

**Significant at the 0.01 probability level.

[†] σ^2g , variance due to genotype.[‡]NS, nonsignificant.[§]Wald, 1943.

σ^2g for all the traits (Table 1), indicating wide genetic variation among the mini core accessions for these traits. The σ^2ge was significant for all traits indicating that the growing environments influenced the performance of the genotypes. The relative proportion of these variances revealed that σ^2ge was twice that of σ^2g for grain Fe and Zn while σ^2g was two- to fourfold greater than σ^2ge for days to flowering and 100-seed weight and both were nearly equal for pod yield and shelling percent (Table 1).

Furthermore, σ^2g within subsp. *fastigiata* (98 accessions) and subsp. *hypogaea* (86 accessions) was highly significant for all the traits indicating the presence of significant variation at subspecies level for these traits in the mini core collection. Lung'aho et al. (2011) for Fe and Pixley et al. (2011) for Fe and Zn also reported highly significant genotypic variation in maize (*Zea mays* L.). The σ^2ge was also highly significant in both subspecies except for days to flowering in subsp. *hypogaea*. The magnitude of σ^2ge compared to σ^2g for both Fe and Zn in subsp. *fastigiata* was higher (twofold) while it was reverse for Fe and equal for Zn in subsp. *hypogaea*. Oikeh et al. (2003) also reported a lower proportion of genetic variance for these traits (12% for Fe and 29% for Zn contents) in maize. The σ^2ge was lower compared to σ^2g for seed weight in both subspecies and for shelling percent in subsp. *fastigiata* while both the σ^2g and σ^2ge were equally important for shelling percent in subsp. *hypogaea* and pod yield in both subspecies.

The σ^2ge was of equal importance for days to flowering in subsp. *fastigiata* and nonsignificant in subsp. *hypogaea* (Table 1). The significance of Wald statistic (Wald, 1943)) indicated that the environments were significantly different.

The Fe content varied from 20.7 to 30.8 mg kg⁻¹ among accessions of subsp. *fastigiata* and from 18.3 to 30.1 mg kg⁻¹ among subsp. *hypogaea* (Supplemental Table S1) while the variation for grain Zn in subsp. *fastigiata* ranged from 30.3 to 43.8 mg kg⁻¹ and from 28.4 to 42.9 mg kg⁻¹ in subsp. *hypogaea*, with both subspecies showing nearly similar range. Asibuo et al. (2008) reported greater variability in 12 cultivars belonging to subsp. *fastigiata* (49 to 63 mg kg⁻¹ for Zn and 26 to 37 mg kg⁻¹ for Fe) and seven cultivars of subsp. *hypogaea* (44 to 65 mg kg⁻¹ for Zn and 20 to 35 mg kg⁻¹ for Fe). Cabrera et al. (2003) reported a range of 20.0 to 26.6 mg kg⁻¹ for Fe and 29.9 to 37.8 mg kg⁻¹ for Zn contents among five commercially branded peanut samples. Nigam et al. (2010) also reported high variability for Fe and Zn among 31 elite peanut breeding lines, with the genotypes ICGV 86143 and X-14-4-B-19-B recording high values for Fe and Zn, respectively.

The mean Fe and Zn contents were higher (12.4 and 14.8%, respectively) in subsp. *fastigiata* than subsp. *hypogaea* and both nutrients were higher in postrainy season (19.5 and 39.2%, respectively) than in the rainy season. Overall, subsp. *fastigiata* had higher Fe (26.2 mg kg⁻¹) and Zn (37.3

Table 2. Performance and stability of top 48 high Fe *A. hypogaea* subsp. *fastigiata* and subsp. *hypogaea* accessions selected from the ICRISAT peanut mini core collection.

Accession	Origin	Fe (mg kg ⁻¹)	b [†]	S ² d [‡]	DF [§]	Pod yield (kg ha ⁻¹)	Shelling (%)	100-seed weight (g)
Subsp. <i>fastigiata</i>								
ICG 15042	Unknown	26.5	0.92	-1.42	26	2041	62.0	42.6
ICG 1274	Indonesia	27.1	1.41	3.24	28	1404	64.9	55.5
ICG 13858	Uganda	27.1	0.98	0.55	27	1484	67.6	41.7
ICG 4911	Malawi	27.2	1.59	2.58	31	1731	66.1	34.9
ICG 12189	Unknown	27.4	1.24	0.54	31	1966	65.1	47.6
ICG 5475	Kenya	27.5	1.32	-0.97	29	1787	68.0	40.2
ICG 14710	Cameroon	27.5	2.25**	0.47	27	1466	68.4	41.4
ICG 5195	Sudan	27.6	1.16	2.39	31	1536	67.6	28.2
ICG 10474	Cuba	27.7	0.94	-1.96	26	1377	62.9	39.3
ICG 10554	Argentina	27.7	1.19	3.73	33	1250	60.7	28.2
ICG 12879	Myanmar	27.8	2.44**	3.08	32	2096	69.9	37.9
ICG 334	China	27.8	1.39	-1.60	29	1667	66.2	45.0
ICG 11687	India	27.8	1.64	-1.62	31	1686	65.8	33.5
ICG 13491	Central African Republic	27.8	2.29**	1.90	31	1833	60.7	43.6
ICG 3343	India	27.9	0.84	1.36	29	2063	65.9	46.5
ICG 10092	Zimbabwe	28.0	0.49	6.90*	26	1839	66.1	48.1
ICG 8517	Bolivia	28.0	1.44	1.38	31	1858	65.3	39.9
ICG 3102	India	28.1	1.13	-1.12	30	2179	67.4	45.7
ICG 4955	India	28.2	2.00**	0.05	28	1787	69.3	45.3
ICG 5221	Argentina	28.2	0.80	3.22	28	2005	62.5	46.6
ICG 11515	China	28.3	1.39	8.85*	30	1811	62.5	46.6
ICG 14127	United Kingdom	28.3	0.70	8.53*	31	1566	67.1	37.3
ICG 9809	Mozambique	28.4	1.89*	2.63	31	1716	69.6	36.3
ICG 9418	Martinique	28.4	1.47	10.50**	31	1258	69.8	40.2
ICG 13603	Indonesia	28.5	0.77	3.31	31	1678	66.6	42.4
ICG 12682	India	28.7	1.98*	-0.89	31	1503	70.4	37.8
ICG 9249	Mauritius	28.7	1.33	-2.06	31	1621	67.0	41.2
ICG 3240	Uganda	28.8	0.70	16.01**	27	1984	66.2	43.6
ICG 11651	China	28.9	1.63	40.89**	31	2110	62.7	52.6
ICG 5494	Malaysia	29.3	1.27	-1.75	31	1768	62.9	45.6
ICG 14118	United Kingdom	29.7	2.61**	15.79**	32	1709	66.9	41.2
ICG 7969	Zimbabwe	30.0	0.91	0.54	31	1699	65.9	40.8
ICG 9507	Philippines	30.2	1.23	12.97**	28	1814	67.2	46.5
ICG 15309	Brazil	30.5	1.37	-0.63	29	1664	66.3	38.0
ICG 442	United States	30.8	0.46	40.44**	30	1349	68.7	35.1
Gangapuri [¶]	India	23.6	1.16	16.50**	26	1767	67.5	44.2
ICGS 44 [¶]	India	23.7	0.52	10.77**	31	2036	67.6	49.7
Subsp. <i>hypogaea</i>								
ICG 6892	United States	25.5	0.42	0.35	39	1842	67.9	42.9
ICG 11322	India	25.5	1.77	3.57	33	2151	65.5	51.1
ICG 15419	Ecuador	25.9	1.12	1.78	28	2512	59.6	58.8
ICG 6913	United States	26.4	0.59	14.28**	42	1546	53.4	51.0
ICG 14482	Nigeria	26.4	1.18	0.51	33	1933	69.0	54.0
ICG 6402	Unknown	26.6	1.31	-1.86	31	1438	64.4	37.4
ICG 5286	Zambia	26.8	1.02	4.74	33	1426	66.0	53.5
ICG 5827	United States	27.2	1.20	1.16	35	1640	64.4	50.1
ICG 9905	Zambia	27.3	0.99	19.59**	35	1561	57.4	58.5
ICG 14705	Cameroon	27.3	1.76	-0.80	30	1921	68.4	46.9
ICG 13982	United States	28.6	1.57	-1.40	32	1694	66.1	43.6
ICG 7963	United States	29.4	0.98	5.37	30	2031	64.3	45.0
ICG 5051	United States	30.1	2.27**	6.17*	33	863	61.0	59.8
ICGS 76 [¶]	India	22.1	0.68	-1.38	34	2231	69.1	55.4
M 13 [¶]	India	19.4	1.04	-0.70	34	2169	69.4	56.7
LSD (5%)		2.22			1.67	365.8	4.86	5.01
CV, %		8.27			5.37	20.04	8.42	11.2

*Significant at the 0.05 probability level.

**Significant at the 0.01 probability level.

[†]b, regression coefficient.

[‡]S²d, deviation from regression, a nonlinear response.

[§]DF, days to 50% flowering.

[¶]Control cultivar.

Table 3. Performance and stability of top 43 high Zn *A. hypogaea* subsp. *fastigiata* and subsp. *hypogaea* accessions selected from the ICRISAT peanut mini core collection.

Accessions	Origin	Zn (mg kg ⁻¹)	b [†]	S ² d [‡]	DF [§]	Pod yield (kg ha ⁻¹)	Shelling (%)	100-seed weight (g)
Subsp. <i>fastigiata</i>								
ICG 3775	Brazil	40.3	1.33	1.12	28	1943	64.7	34.6
ICG 297	United States	40.4	1.35	-1.66	28	1873	62.9	54.0
ICG 7181	India	40.4	1.40	1.87	26	1717	65.4	40.7
ICG 13603	Indonesia	40.4	1.20	20.94**	31	1678	66.6	42.4
ICG 6201	Cuba	40.5	1.26	13.04**	26	1678	64.1	40.4
ICG 14985	Unknown	40.7	1.67	91.52**	28	2225	65.9	54.7
ICG 15042	Unknown	40.7	1.05	20.90**	26	2041	62.0	42.6
ICG 10566	Congo	41.1	0.99	13.14**	26	1536	64.9	46.2
ICG 13858	Uganda	41.1	1.18	20.89**	27	1484	67.6	41.7
ICG 15309	Brazil	41.2	1.42	-0.08	29	1664	66.3	38.0
ICG 14106	United Kingdom	41.2	1.56	12.71**	26	1597	65.8	40.7
ICG 10474	Cuba	41.6	1.08	-1.93	26	1377	62.9	39.3
ICG 14118	United Kingdom	41.8	1.56	1.63	32	1709	66.9	41.2
ICG 1274	Indonesia	41.9	1.31	49.65**	28	1404	64.9	55.5
ICG 14710	Cameroon	42.1	2.19**	19.27**	27	1466	68.4	41.4
ICG 332	Brazil	42.2	0.64	-2.38	26	1694	63.5	54.3
ICG 5221	Argentina	42.2	1.03	32.93**	28	2005	62.5	46.6
ICG 11515	China	42.3	0.80	-0.37	30	1811	62.5	46.6
ICG 10092	Zimbabwe	42.5	0.88	17.95**	26	1839	66.1	48.1
ICG 9249	Mauritius	42.9	1.16	-2.03	31	1621	67.0	41.2
ICG 3681	United States	43.2	1.22	62.34**	26	1561	65.5	44.0
ICG 10890	Peru	43.3	1.17	12.70**	29	1175	69.3	32.2
ICG 11651	China	43.8	1.03	24.28**	31	2110	62.7	52.6
Gangapuri [¶]	India	37.6	2.00**	0.45	26	1767	67.5	44.2
ICGS 44 [¶]	India	33.3	0.80	36.85**	31	2036	67.6	49.7
Subsp. <i>hypogaea</i>								
ICG 11855	Korea	33.5	1.03	-2.41	34	1887	61.5	54.6
ICG 4343	India	33.6	0.90	-0.08	34	2162	65.2	44.0
ICG 6667	United States	33.7	0.72	1.58	34	1344	63.1	52.6
ICG 188	India	33.8	0.98	32.03**	26	1866	64.1	42.1
ICG 5327	United States	34.0	0.87	10.54*	34	1663	61.6	55.6
ICG 4746	Israel	34.0	0.88	-2.38	34	1049	67.2	57.8
ICG 6766	United States	34.1	0.79	-1.18	35	1404	59.8	54.8
ICG 14466	Nigeria	34.2	0.79	10.39*	34	1838	65.1	49.2
ICG 5051	United States	34.2	-0.06**	12.51**	33	863	61.0	59.8
ICG 3053	India	34.4	1.04	2.90	35	1759	66.4	47.0
ICG 12672	Bolivia	35.0	0.96	-2.40	33	1913	61.9	45.5
ICG 11219	Mexico	35.4	0.71	-2.31	34	1609	65.8	56.6
ICG 5662	China	38.8	1.56	-2.42	33	2102	65.8	67.7
ICG 6402	Unknown	38.9	1.94**	-1.84	31	1438	64.4	37.4
ICG 5827	United States	40.0	1.06	-0.39	35	1640	64.4	50.1
ICG 6913	United States	40.1	0.84	96.60**	42	1546	53.4	51.0
ICG 15419	Ecuador	40.2	1.00	10.17*	28	2512	59.6	58.8
ICG 14705	Cameroon	42.1	1.38	-2.38	30	1921	68.4	46.9
ICG 7963	United States	42.2	0.82	9.57*	30	2031	64.3	45.0
ICG 13982	United States	42.9	1.57	14.89**	32	1694	66.1	43.6
ICGS 76 [¶]	India	30.9	0.48	-2.31	34	2231	69.1	55.4
M13 [¶]	India	29.5	1.30	0.68	34	2169	69.4	56.7
LSD (5%)		2.45			1.67	365.8	4.86	5.01
CV, %		6.31			5.37	20.04	8.42	11.24

*Significant at the 0.05 probability level.

**Significant at the 0.01 probability level.

[†]b, regression coefficient.

[‡]S²d, deviation from regression, a nonlinear response.

[§]DF, days to 50% flowering.

[¶]Control cultivar.

mg kg⁻¹) than subsp. *hypogaea* (23.3 mg kg⁻¹ for Fe and 32.5 mg kg⁻¹ for Zn). The Fe (26.3 mg kg⁻¹) and Zn (38.7 mg kg⁻¹) contents were higher in the postrainy season than

in the rainy season (22.0 mg kg⁻¹ for Fe and 27.8 mg kg⁻¹ for Zn). At subspecies levels also, Fe content was higher by 21.9% in subsp. *fastigiata* and 15.6% in subsp. *hypogaea* while

Table 4. Performance and stability of 23 accessions of *A. hypogaea* subsp. *fastigiata* and subsp. *hypogaea* for Fe and Zn contents in the ICRISAT peanut mini core collection.

Accessions	Origin	Fe (mg kg ⁻¹)	b [†]	S ² d [‡]	Zn (mg kg ⁻¹)	b	S ² d	DF [§]	Pod yield (kg ha ⁻¹)	Shelling (%)	100-seed weight (g)
Subsp. <i>fastigiata</i>											
ICG 1274	Indonesia	27.1	1.41	3.24	41.9	1.31	49.65**	28	1404	64.9	55.5
ICG 3102	India	28.1	1.13	-1.12	37.7	1.23	22.19**	30	2179	67.4	45.7
ICG 4750	Paraguay	25.9	1.41	-0.90	37.7	1.43	0.37	29	2042	67.5	37.5
ICG 5221	Argentina	28.2	0.80	3.22	42.2	1.03	32.93**	28	2005	62.5	46.6
ICG 9249	Mauritius	28.7	1.33	-2.06	42.9	1.16	-2.03	31	1621	67.0	41.2
ICG 10092	Zimbabwe	28.0	0.49	6.90*	42.5	0.88	17.95**	26	1839	66.1	48.1
ICG 10474	Cuba	27.7	0.94	-1.96	41.6	1.08	-1.93	26	1377	62.9	39.3
ICG 11515	China	28.3	1.39	8.85**	42.3	0.80	-0.37	30	1811	62.5	46.6
ICG 11651	China	28.9	1.63	40.89**	43.8	1.03	24.28**	31	2110	62.7	52.6
ICG 13603	Indonesia	28.5	0.77	3.31	40.4	1.20	20.94**	31	1678	66.6	42.4
ICG 13858	Uganda	27.1	0.98	0.55	41.1	1.18	20.89**	27	1484	67.6	41.7
ICG 14118	United Kingdom	29.7	2.61**	15.79**	41.8	1.56	1.63	32	1709	66.9	41.2
ICG 14710	Cameroon	27.5	2.25**	0.47	42.1	2.19**	19.27**	27	1466	68.4	41.4
ICG 14985	Unknown	26.1	1.89*	-0.99	40.7	1.67	91.52**	28	2225	65.9	54.7
ICG 15042	Unknown	26.5	0.92	-1.42	40.7	1.05	20.90**	26	2041	62.0	42.6
ICG 15309	Brazil	30.5	1.37	-0.63	41.2	1.42	-0.08	29	1664	66.3	38.0
Gangapuri [¶]	India	23.6	1.16	16.50**	37.6	2.00**	0.45	26	1767	67.5	44.2
ICGS 44 [¶]	India	23.7	0.52	10.77**	33.3	0.80	36.85**	31	2036	67.6	49.7
Subsp. <i>hypogaea</i>											
ICG 5051	United States	30.1	2.27**	6.17*	34.2	0.06**	12.51**	33	863	61.0	59.8
ICG 5827	United States	27.2	1.20	1.16	40.0	1.06	-0.39	35	1640	64.4	50.1
ICG 14705	Cameroon	27.3	1.76	-0.80	42.1	1.38	-2.38	30	1921	68.4	46.9
ICG 6402	Unknown	26.6	1.31	-1.86	38.9	1.94**	-1.84	31	1438	64.4	37.4
ICG 7963	United States	29.4	0.98	5.37	42.2	0.82	9.57*	30	2031	64.3	45.0
ICG 13982	United States	28.6	1.57	-1.40	42.9	1.57	14.89**	32	1694	66.1	43.6
ICG 15419	Ecuador	25.9	1.12	1.78	40.2	1.00	10.17*	28	2512	59.6	58.8
ICGS 76 [¶]	India	22.1	0.68	-1.38	30.9	0.48	-2.31	34	2231	69.1	55.4
M13 [¶]	India	19.4	1.04	-0.70	29.5	1.30	0.68	34	2169	69.4	56.7
LSD (5%)		2.22			2.45			1.67	365.80	4.86	5.01
CV, %		8.27			6.31			5.37	20.04	8.42	11.24
Broad-sense heritability, %		73.49			72.90			90.10	68.27	59.42	86.67

*Significant at the 0.05 probability level.

**Significant at the 0.01 probability level.

[†]b, regression coefficient.

[‡]S²d, deviation from regression, a nonlinear response.

[§]DF, days to 50% flowering.

[¶]Control cultivar.

the Zn content was higher by 45.1% in subsp. *fastigiata* and 31.9% in subsp. *hypogaea* in the postrainy season in the mini core. The trend was similar in the control cultivars also (Supplemental Table S1).

It is not only desirable that cultivars should possess high levels of both macro- and micronutrients but also be productive and adapted to a wide range of environments (Oikeh et al., 2003). Considering the trait value of the best control cultivar in each of the subspecies as a benchmark, we selected accessions (48 for Fe content, 43 for Zn content, and 23 for both nutrients) that were significantly superior to their respective control cultivars. These accessions are presented in Tables 2, 3, and 4 along with their agronomic performance.

For kernel Fe, 35 accessions of subsp. *fastigiata* (26.5 to 30.8 mg kg⁻¹ or 11.8 to 30.0% over best control) and 13 accessions of subsp. *hypogaea* (25.5 to 30.1 mg kg⁻¹ or 15.4 to 36.2% over best control) were significantly superior to their respective control cultivars, ICGS 44 (23.7 mg kg⁻¹) and ICGS 76 (22.1 mg kg⁻¹) (Table 2). ICG 14710, ICG 12879, ICG 13491, ICG 4955, ICG 9809, ICG 12682, and ICG 14118 of subsp. *fastigiata* and ICG 5051 of subsp. *hypogaea* showed high linear response to favorable environments as revealed by the highly significant deviation of their regression (regression coefficient [b]) values (1.89 to 2.61) from unity (Table 2). The deviations from regression (S²d) were statistically nonsignificant from zero for all accessions except ICG 10092, ICG 11515, ICG 14127, ICG 9418, ICG

3240, ICG 11651, ICG 14118, ICG 9507, ICG 442, and controls Gangapuri and ICGS 44 in subsp. *fastigiata* and ICG 6913, ICG 9905, and ICG 5051 (subsp. *hypogaea*), where the S^2d differed highly significantly from zero, indicating that they are environment sensitive and their performance is unpredictable based on regression. Among the most stable accessions, ICG 12189, ICG 3343, ICG 3102, ICG 5221, and ICG 15042 (subsp. *fastigiata*) and ICG 11322, ICG 15419, ICG 14705, and ICG 7963 (subsp. *hypogaea*), respectively, produced pod yield between 1966 and 2179 kg ha⁻¹ and between 1921 and 2512 kg ha⁻¹, which was at par with the best subsp. *fastigiata* control, ICGS 44 (2036 kg ha⁻¹), and the best subsp. *hypogaea* control, ICGS 76 (2231 kg ha⁻¹). Most of these accessions had greater 100-seed weight (>45 g) and shelling percent (>65%). Few of these high kernel Fe accessions in the previous investigation were reported to contain ~29% seed protein (ICG 4911, ICG 13982, and ICG 7963) and relatively high in oleic:linoleic fatty acid ratio (2.7 to 3.0) (ICG 5475, ICG 5221, and ICG 15419) compared to the best control, ICGS 76 (23.8% protein and 2.7 oleic:linoleic fatty acid ratio) (Upadhyaya et al., 2012).

Among the high Zn accessions (Table 3), 23 accessions of subsp. *fastigiata* (40.3 to 43.8 mg kg⁻¹ or 7.2 to 16.5% over best control) and 20 of subsp. *hypogaea* (33.5 to 42.9 mg kg⁻¹ or 8.4 to 38.8%) were significantly superior to their respective control cultivars, Gangapuri (37.6 mg kg⁻¹) and ICGS 76 (30.9 mg kg⁻¹), with most of these showing linear response to environments. ICG 5051 with a near zero regression coefficient value showed better adaptation to poor environments and least response to better environments. The S^2d was statistically nonsignificant from zero for only nine accessions (ICG 3775, ICG 297, ICG 7181, ICG 15309, ICG 10474, ICG 14118, ICG 332, ICG 11515, and ICG 9249) of subsp. *fastigiata* and 12 accessions (ICG 11855, ICG 4343, ICG 6667, ICG 4746, ICG 6766, ICG 3053, ICG 12672, ICG 14705, ICG 11219, ICG 5662, ICG 6402, and ICG 5827) of subsp. *hypogaea*, indicating their stability across environments for Zn content. ICG 14710 was the most unstable with significant S^2d (19.27), indicating that its performance is unpredictable in all environments. Among the most stable accessions, ICG 3775, ICG 297, and ICG 11515 (subsp. *fastigiata*) and ICG 4343, ICG 12672, ICG 14705, and ICG 5662 (subsp. *hypogaea*) yielded (1811–1943 and 1913–2162 kg ha⁻¹, respectively) at par with respective best controls (2036 kg ha⁻¹ for ICGS 44 and 2231 kg ha⁻¹ for ICGS 76), with most of these having higher 100-seed weight (>44 g) and shelling percentage (>65). Two high Zn (>40 mg kg⁻¹) accessions of subsp. *hypogaea*, ICG 15419 (2512 kg ha⁻¹) and ICG 7963 (2031 kg ha⁻¹), were high yielding and responded linearly to environments for Zn. Additionally, among these stable high Zn accessions, ICG 4766 was reported to have high oil content (49.5%) and ICG 6766 and ICG 15419 have been reported to be rich in oleic acid content (60.1 to 61.1%) and high oleic:linoleic

acid ratio (3.0 to 3.1), compared to the best control, ICGS 76 (58.3% oleic acid and 2.7 oleic:linoleic fatty acid ratio) (Upadhyaya et al., 2012).

A set of 23 accessions (16 of subsp. *fastigiata* and 7 of subsp. *hypogaea*), which had both high Fe (≥ 25.9 mg kg⁻¹) and Zn (≥ 34.2 mg kg⁻¹) content, were also identified (Table 4). Among them, four accessions (ICG 4750, ICG 9249, ICG 10474, and ICG 15309) of subsp. *fastigiata* and two accessions (ICG 5827 and ICG 14705) of subsp. *hypogaea* were highly stable for both nutrients across environments. However, ICG 9249, ICG 10474, ICG 15309, and ICG 5827 produced significantly lower pod yield (1377 to 1664 kg ha⁻¹) than the best controls. The remaining two accessions, ICG 4750 and ICG 14705, produced pod yield (1921 to 2042 kg ha⁻¹) at par with the controls with high shelling percentage (>65) and high seed weight (>45 g) (except ICG 4750). ICG 14705, ICG 4750, and ICG 7963 were also reported to have high oil content (>46%), ICG 4750 and ICG 7963 were reported to have high protein (>29%), and ICG 15419 was reported to have high oleic:linoleic fatty acid ratio (3.0) (Upadhyaya et al., 2012).

Trait correlation analysis revealed that Fe and Zn contents were significantly and positively correlated in the entire mini core (0.714) (values above the diagonal in Table 5) and among the select set of 23 accessions (0.746) (values below the diagonal in Table 5), as also revealed in other crops (Pixley et al., 2011; Lung'aho et al., 2011; Dwivedi et al., 2012). As both traits showed high broad-sense heritability (73%; Table 4), simultaneous selection for improving Fe and Zn is a distinct possibility by selecting for either of the nutrients. Significant negative correlations were observed between Fe content and days to flowering (−0.536) in the entire mini core but not in the selected set (−0.162), which confirmed the fact that correlations vary with the sample size and the genotypes in the sample. Likewise, Zn content also showed highly significant negative correlation (−0.750) with days to flowering in the entire mini core but a medium negative correlation (−0.482) among the selected set of lines. As correlations between these two nutrients and agronomic traits were of low magnitude, selection for Fe and Zn rich, high yielding, early maturing peanut cultivars with high 100-seed weight should be effective.

The cluster analysis of the 23 selected accessions (Fig. 1), based on the first five principal components, which explained approximately 80% of the variation, indicated that the high yielding and high nutrient lines (ICG 4750, ICG 7963, ICG 14705, and ICG 15419) are also highly diverse, as they represent different and distinct clusters. Therefore, they could be very useful parental lines for breeding high yielding Fe and Zn rich cultivars. Interestingly, all the improved control cultivars grouped into one cluster while the control Gangapuri, from India, occupied a different cluster. Of the 22 selected accessions on which information on biological status is available in the ICRISAT databases,

Table 5. Coefficient correlations of entire mini core (above the diagonal) and selected 23 accessions and four control cultivars (below the diagonal).

Traits	Fe (mg kg ⁻¹)	Zn (mg kg ⁻¹)	Days to 50% flowering	Pod yield (kg ha ⁻¹)	Shelling (%)	100-seed weight (g)
Fe, mg kg ⁻¹	1	0.714*	-0.536*	0.050	0.189*	-0.176*
Zn, mg kg ⁻¹	0.746*	1	-0.750*	0.094	0.161*	-0.103
Days to 50% flowering	-0.162	-0.482*	1	-0.166*	-0.204*	0.208*
Pod yield, kg ha ⁻¹	0.468*	-0.201	0.038	1	0.103	0.250*
Shelling, %	0.415*	-0.330	0.141	0.111	1	-0.315*
100-seed weight, g	-0.347	-0.418*	0.352	0.262	-0.242	1

*Significant at the 0.05 probability level.

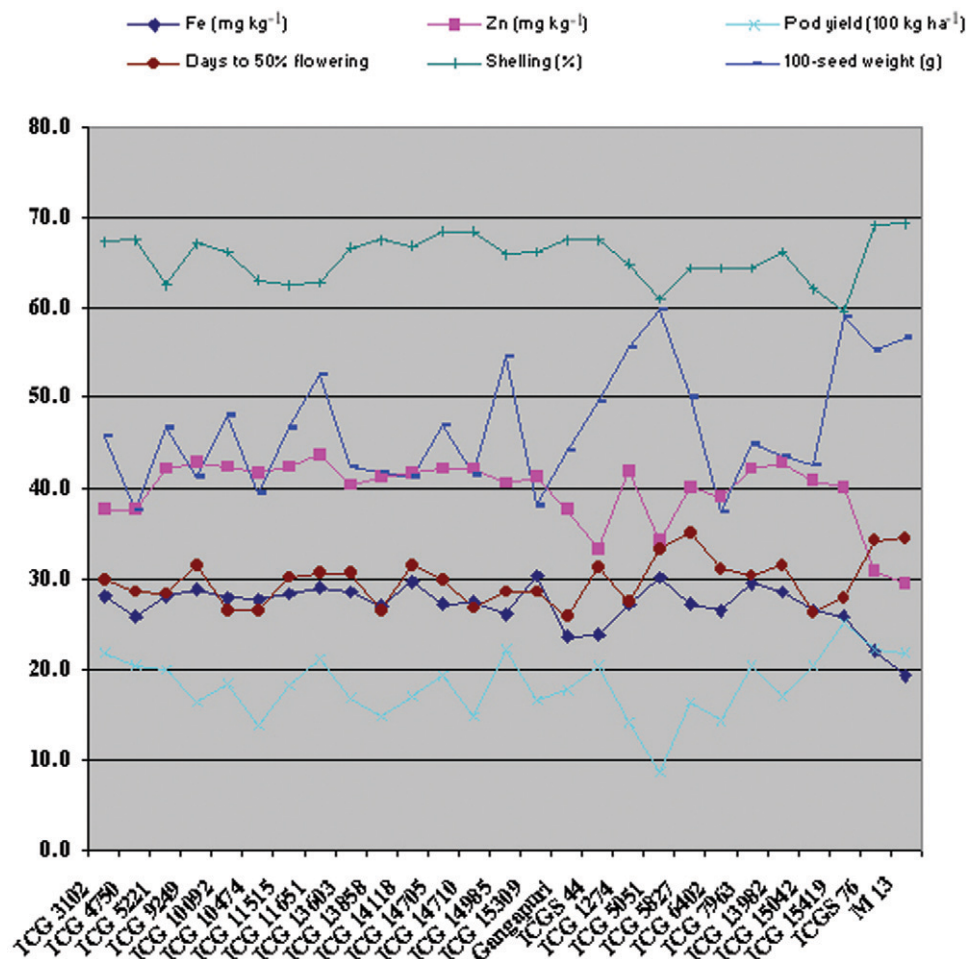


Figure 1. Graphical representation of performance of superior accessions selected for high Fe, Zn, and combined nutritional traits in the mini core collection of peanut.

14 are landraces and eight breeding lines but without information on their pedigree. The delineation of the 23 selected accessions into different clusters (Fig. 2) was based on agronomic and nutritional traits.

A number of high nutrient rich lines have also been reported possessing other desirable agronomic traits. For example, ICG 5827 for resistance to drought and salinity (ICRISAT, 2009; Srivastava, 2010), ICG 14985 for resistance to drought and aflatoxin (*Aspergillus flavus*) (ICRISAT, 2009), ICG 11515 for resistance to rust and high transpiration efficiency (Kusuma et al., 2007; ICRISAT, 2009), ICG 4750 for resistance to intermittent drought and seed invasion by *A. flavus* (aflatoxin contamination)

and high protein (29.7%) (Jiang et al., 2010; Upadhyaya et al., 2012; Hamidou et al., 2011), ICG 15419 for resistance to late leaf spot and for high oleic acid (61.1%) (Ajay, 2006; Upadhyaya et al., 2012), ICG 14710 for high linoleic acid content and bacterial wilt resistance (ICRISAT, 2009), ICG 6402 for late leaf spot, early leaf spot, and rust (ICRISAT, 2009), ICG 7963 for resistance to late leaf spot and *A. flavus*, ICG 11651, ICG 14705, and ICG 14118 for sound mature kernels (Yugandhar, 2005), ICG 11651 for resistance to peanut bud necrosis (Khaleed, 2008), and ICG 10092 for cold (12°C) tolerance at germination (Upadhyaya et al., 2001). The agronomic desirability of these high Fe and Zn lines makes them valuable for use in

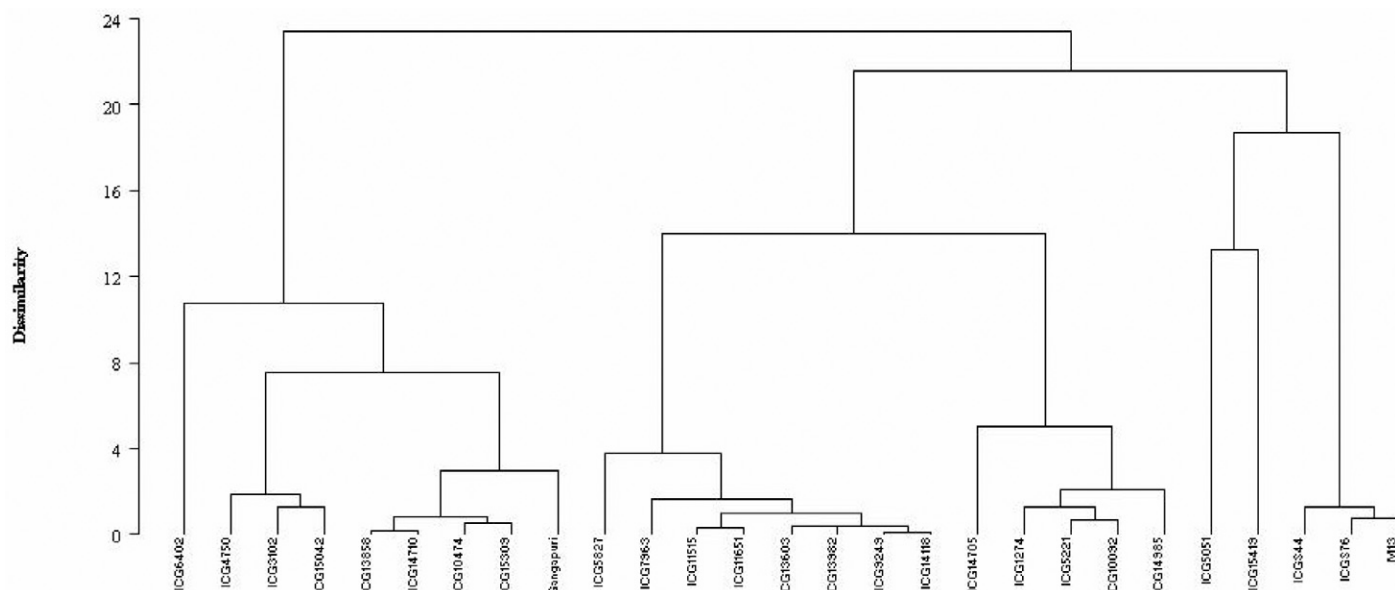


Figure 2. Dendrogram of the selected 23 superior peanut mini core accessions and four control cultivars based on scores of first five principal components (80.4% variation).

peanut crop improvement programs for improving these nutrients without any setbacks to agronomic performance. Germplasm collections at ICRISAT are available following the terms and conditions of the Standard Material Transfer Agreement of the International Treaty on Plant Genetic Resources for Food and Agriculture.

CONCLUSION

Among the 184 accessions of peanut mini core collection evaluated for Fe and Zn content and agronomic performance, 48 were rich in Fe, 43 in Zn, and 23 in both nutrients. Several accessions with high Fe and/or Zn contents and good agronomic performance were identified. Of the 23 accessions, ICG 4750, ICG 14705, ICG 7963, and ICG 15419 were highly diverse and stable for either or both nutrients across environments and had high pod yield (1921 to 2512 kg ha⁻¹), high shelling percentage, except ICG 15419, and high 100-seed weight, except ICG 4750. The accessions identified in this study would be used in developing Fe and Zn rich peanut cultivars with superior agronomic performance.

Supplemental Information Available

Supplemental material is available at <http://www.crops.org/publications/cs>.

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