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Effect of seasonal variation on micronutrient content in chickpea (*Cicer arietinum* L.) and identification of accessions having high iron and zinc

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ABSTRACT

Iron (Fe) and zinc (Zn) are two important mineral micronutrients, whose deficiency is prevalent globally. Chickpea being a good source of protein is an important grain legume and is one of the most consumed pulse crop worldwide. It is grown in over 50 countries, with India being its largest producer and consumer. With an aim to assess genetic variation for iron and zinc content as well as to study the environmental effect on the content of these two minerals in the chickpea seeds in this work, 83 accessions including 52 germplasm lines with origins from 21 chickpea-cultivating regions of the world and 31 cultivars developed and adapted for different agro climatic zones in India were analysed for iron and zinc content. The accessions were grown in two cultivation seasons (*rabi* 2012-2013 and *rabi* 2013-2014).Overall, wide ranges for both Fe (2.55-9.63 mg/100g) and Zn (2.58-8.15 mg/100g) content were observed. *Desi* biotypes were found to have higher zinc content than *kabuli* types in both the years. Despite the environmental influence on iron and zinc content, genotypes and cultivars having consistently high Fe and/or Zn in both the growing seasons were identified. Genetic parameters including high heritability of the trait in the present study suggest that the cultivars JGG1, RSG 888 and RSG 44 having high Fe and Zn in both the seasons can directly be used in breeding programs for genetic biofortification-one of the most sustainable and cost-effective solutions that may provide nutritious and affordable food.

Keywords: Biofortification, Cultivars, Chickpea, Iron, Zinc, Mineral malnutrition

Vitamin and mineral micronutrients malnutrition is collectively referred to as hidden hunger, which affects more than two billion people globally, particularly women and children (Muthayya *et al*. 2013). Iron (Fe) and Zinc (Zn) are two important micronutrients that act as cofactors for several proteins including heamoglobin, enzymes, various transcription factors and are required for human growth and development (McCall *et al.* 2000 and Abbaspour *et al.* 2014). Iron deficiency leads to iron-deficient anemia (IDA) affecting physical and mental development as well as learning capacity (Abbaspour *et al*. 2014). Zinc deficiencies are suspected to be equally severe leading to retarded growth, skeletal abnormalities, delayed wound healing, increased abortion risk and diarrhoea (Wessells and Brown 2012, Rehman *et al*. 2016). Chickpea (*Cicer arietinum* L.), the third most important pulse crop based on production and second most important in terms of consumption worldwide, is grown over 50 countries covering 13.7 million hectare area and production about 13.3 million tonnes (Food and Agriculture Organization of the United Nations, 2013, Upadhayaya *et al.* 2016). It is a good source of dietary proteins, carbohydrates and fibres and therefore, nutritionally important particularly for the vegetarian population (Jukanti *et al.* 2012). This makes chickpea a good target crop for genetic biofortification *i.e*. breeding for genetically enhancing the nutrient quality of food crops (Bouis *et al*. 2011). Efforts are underway to quantify the diversity in iron and zinc content in chickpea and to identify the genetic factors contributing towards there traits (Diapari *et al.* 2014 and Upadhyaya *et al.* 2016). Large collections of germplasm are available for chickpea (Upadhyaya *et al.* 2013) that needs to be characterized for their better utilization in breeding programs. So far, the chickpea germplasm screened for mineral micronutrients consisted of predominantly *Kabuli* genotypes (Diapari *et al.* 2014, Upadhyaya *et al.* 2016). Whereas, the *desi* biotypes are more widely used in a decorticated and split form directly as a part of daily diet or in making chickpea flour. Moreover, there is also a need to characterize the cultivars adapted to particular agro climatic zones for the mineral micronutrient contents so that they could be better popularized in the community for cultivation and consumption. Therefore, the present study was undertaken to analyse iron and zinc content in the *desi* biotypes as well as the native Indian cultivars for their effective utilization in breeding programs for genetic biofortification. Iron and zinc content in seeds was measured for two growing seasons for analysing environmental effect on mineral content.

MATERIAL AND METHODS

Eighty-three accessions of chickpea (*Cicer arietinum* L.), including *desi* (66) and *kabuli* (17) types, were used in this study. The accessions included 52 germplasm lines originating from 21 different chickpea growing regions worldwide and 31 native Indian cultivars notified and released for commercial cultivation in India. To control for variation due to spatial effects, the genotypes were grown in randomized complete block design (RCBD) in two

replications at the Experimental and Gamma Field Facility, Bhabha Atomic Research Centre, Trombay, Mumbai (19^0 03" N, 72^0 93" E) during the post rainy (*Rabi*) crop season of 2012/13 and 2013/14. The crop was raised in 2-m rows with 30 cm x 10 cm distance between rows and between plants, respectively. Standard agricultural practices were followed to raise the crop.

Dried seeds were pooled from minimum five single plant harvests, ground into a fine powder and was passed through a fine mesh to obtain homogenous mixture. The sample was processed for iron and zinc estimation. Briefly, 10 ml of acid mixture of HNO_3: $HClO_4$ (5:1, v/v) was added to 100mg of the seed powder and kept overnight for cold digestion, followed by digestion at 120°C till a completely clear and colourless digest was obtained. The cooled digested samples were diluted to 50ml with deionized water. Iron and zinc content was estimated in the diluted samples using GBC 932 B+ Atomic Absorption Spectrophotometer (GBC, Melbourne, Australia) using air-acetylene flame and the concentrations were expressed as mg per 100g dry weight of seed powder.

The replicated data was collected for two seasons, Analysis of Variance (ANOVA) was performed using general linear model (GLM) in Mini Tabver 17 statistical software and genotypic (σ^2G), phenotypic (σ^2P) and error (σ^2E) variances were calculated. Genotypic Coefficient of variation (GCV%) and phenotypic coefficient of variation (PCV%) was calculated as described earlier using standard formulae (Badigannavar *et al.* 2016). Broad sense heritability (H^2) was calculated as the ratio of σ^2G and total phenotypic variance ($\sigma^2P=\sigma^2G+\sigma^2E$) (Badigannavar *et al.* 2016). Genetic advance were calculated as GA (%) = K x σP x H^2x100, where K (selection differential at 5%) = 2.06, σP= phenotypic standard deviation and H^2= broad sense heritability. Genetic advance over mean (GAM) was calculated as percentage of genetic advance over the mean. Student's *t*-test was performed in Microsoft Excel 2007.

RESULTS AND DISCUSSION

Out of the 83 accessions that were analysed for Fe and Zn content, 52 were germplasm accessions originating from 21 diverse geographical locations worldwide. Rest 31 were cultivar developed and released for commercial cultivation in India. The accessions consisted of 66 *desi* and 17 *kabuli* biotypes. The Fe and Zn contents were measured in the harvests of two successive growing seasons (2012/13 and 2013/14).The ANOVA indicated significant genotypic differences for both Fe and Zn content in seeds (Table 1). Both Fe and Zn contents were influenced by the environment and showed significant genotype x year interaction (Table 1). Over the two years' (2012/13 and 2013/14) combined data, Fe content ranged 2.55-9.63 mg/100g, while the Zn content ranged 2.58-8.15 mg/100g. The

Table 1. Mean squared values from the analysis of variance (ANOVA) of iron and zinc concentrations in seeds of chickpea accessions

Source	DF	Zn	Fe
Genotype	82	6.9009***	10.146***
Year	1	78.746***	38.325***
Genotype X Year	82	3.8307***	2.668***
error	166	0.9359	1.382
CV %		18.02	17.99
R^2(%)		86.06	82.60

***Significance at P< 0.001

year-wise distribution of iron and zinc among the accessions is shown in Table 2a and Table 2b. The mean Fe and Zn content were comparable between cultivars and germplasm accessions for the respective years. However, for both Fe and Zn, the range was wider for the germplasm lines than for the popular varieties. Overall, the average Zn content of *desi* biotypes (2012/13: 6.07±1.59 mg/100g; 2013/14: 5.08±1.54 mg/100g) was significantly higher than that of the *kabuli* biotypes (2012/13: 5.04±1.44 mg/100g; 2013/14: 4.13±1.97 mg/100g) in the respective years (p values: 2012/13: 0.017; 2013/14: 0.036). No significant difference in the average Fe content between *desi* and *kabuli* genotypes was observed in the respective years (2012/13: *desi*: 6.97±1.67 mg/100g, *kabuli*: 6.51±1.90mg/100g, p value: 0.332; 2013/14: *desi*: 6.20±1.87 mg/100g, *kabuli*: 6.15±1.88mg/100g, p value: 0.916). The range of Zn and Fe content was slightly more in those of germplasm lines that are not of Indian origin than the germplasm and cultivars of Indian origin. However, the mean Zn and Fe content did not vary between the two seasons in non-Indian germplasm

Table 2a. Year-wise distribution of range and mean[a] iron and zinc in chickpea germplasm lines and popular cultivars

Trait	Germplasm (n=52)		Cultivars (n=31)	
	2012/13	2013/14	2012/13	2013/14
Iron (mg/100g)	1.93-9.61 (6.74±1.94)	2.17-9.65 (6.21±2.17)	3.25-9.25 (7.1±1.24)	3.53-8.10 (6.17±1.22)
Zinc (mg/100g)	2.8-8.44 (5.5±1.49)	1.70-8.92 (4.98±1.74)	3.71-9.21 (6.45±1.64)	2.6-7.9 (4.73±1.55)

a. Mean± standard deviation is given in brackets below the range

Table 2b. Year-wise distribution of range and mean[a] iron and zinc in chickpea germplasm lines of Indian origin and popular cultivars and those of non Indian origin.

	Zn Content (mg/100g)		Fe content (mg/100g)	
	2012/13	2013/14	2012/13	2013/14
Indiangermplasm + cultivars (n=50)	3.19-9.21 (6.03±1.67)	2.60-8.05 (4.89±1.51)	2.95-9.25 (6.95±1.53)	2.17-9.21 (6.13±1.68)
Non-Indian germplasm (n=33)	2.80-8.44 (5.59±1.50)	1.70-8.92 (4.87±1.90)	1.93-9.61 (6.76±1.98)	2.77-9.65 (6.25±2.13)

a. Mean± standard deviation is given in brackets below the range

(p values: zinc 0.092; iron: 0.352), while it varied significantly for the Indian germplasm and the cultivars over two growing seasons (p values: zinc: 0.001; iron: 0.01) (Table 2b). This may be due to possible differences in Fe and Zn uptake, distribution and/or regulation in these germplasm. There are limited studies on the evaluation of chickpea germplasm, particularly the *desi* biotypes, for mineral micronutrient content. A set of 94 accessions (consisting of 23 *desi* and 71 *kabuli* biotypes) grown in Canada over two different years showed Fe and Zn content 3.81- 8.64 mg/100g and 2.52-6.23 mg/100g, respectively (Diapari *et al.* 2014). Furthermore, the Fe and Zn content of chickpea cultivars grown in USA ranged from 4.6-6.7 mg/100g and 3.7-7.4 mg/100g, respectively (Thavarajah and Thavarajah, 2012). In contrast, in the present study, the Indian cultivars showed a higher Fe and Zn content (3.39-8.54 mg/100g and 3.38-8.15 mg/100g, respectively). Recently, a set of 92 accessions (consisting of 39 *desi* and 53 *kabuli* biotypes) grown in India were also analysed for Fe and Zn content and were found to contain 4.02-9.10 mg/100g and 2.68-6.18 mg/100g of iron and zinc, respectively (Upadhyaya *et al.* 2016). Therefore, compared to previous reported data, a higher amount of zinc has been reported in the present study.

Although there was a significant environmental effect on both the traits, 9 genotypes (ICC 4418, IC 269010, ICC 2507, ICC 9586, ICC 8058, ICC 3776, ICC 2242, ICC 3325 and ICC 2263) showed high zinc (> 6 mg/100g) in both 2012/13 and 2013/14 (Table 3) of these, ICC 2507, ICC 9586, ICC 2242 had more than 7 mg/100g of zinc. Among the 52 accessions, 15 (ICC 7184, ICC4814, ICC 8740, ICC 4418, ICC 12928, ICC 8350, ICC 12155, ICC 2720, IC 268936, ICC 1923, ICC 1052, ICC 13441, IC 269010, ICC 14815, ICC 8151) genotypes showed high iron content (>7 mg/100g) over the years (Table 4). Accessions ICC 4418 and IC 269010 had high iron and zinc content over the years. Among the cultivars, 7 genotypes namely Vaibhav, JG 12, Vihar, RSG 888, JG 130, RSG 44, JGG 1 showed more than 7 mg/100g iron content and JAKI 9218, ICCV 37 showed more than 8 mg/100g iron content JGG 1, RSG 44, RSG 888 also contained more has 7 mg/100g of zinc over the years. The genetic parameters to estimate the variability among accessions and to determine the genetic and environmental effects on traits were calculated (Table 5). The genotypic coefficient of variation (GCV%) for iron and zinc content was 37.09 and 32.16, respectively. The phenotypic coefficient of variation (PCV%) was 34.47 and 34.59, respectively. Heritability for both the traits was high at 0.86. The low magnitude of difference between genetic coefficient of variation (GCV%) and phenotypic coefficient of variation (PCV%) indicates a higher genetic influence (Tuhina-Khatun *et al.* 2015). This in combination with high heritability and genetic advance suggests additive gene action and possibility of effective and efficient selection of these traits in early generations (Al-Tabbal and Al- Fraihat 2012). Therefore, the genotypes identified in the present study will be useful in breeding programs for genetic biofortification of chickpea.

Table 3. Germplasm and cultivars having high zinc content in both the growing seasons

Genotypes	Zinc content (2012/13) (mg/100g)	Zinc content (2013/14) (mg/100g)
	Germplasm	
ICC 2242	6.98	8.05
ICC 2263	6.23	7.38
ICC 2507	7.43	7.45
ICC 3776	6.94	8.48
ICC 4418	8.44	6.03
ICC 8058	6.91	8.92
ICC 9586	7.24	7.55
ICC 14098	6.21	6.68
ICC 14669	6.46	6.70
IC 269010	7.58	6.70
	Cultivars	
RSG44	8.39	7.90
RSG888	8.48	7.43
JG315	7.72	6.70
JGG1	8.16	7.20
JG218	6.91	6.60

Table 4. Germplasm and cultivars having high iron content in both the growing seasons

Genotypes	Iron content (2012/13) (mg/100g)	Iron content (2013/14) (mg/100g)
	Germplasm	
ICC 1052	8.27	9.10
ICC 1923	8.32	8.50
ICC 2720	8.53	8.76
ICC 4418	8.82	8.55
ICC 4814	9.16	9.10
ICC 7184	9.61	9.65
ICC 8151	7.25	7.69
ICC 8350	8.76	9.21
ICC 8740	8.90	8.79
ICC 12155	8.70	7.95
ICC 12928	8.80	9.20
ICC 13441	7.91	9.18
ICC 14815	7.31	9.12
IC 268936	8.50	7.76
IC 269010	7.37	7.84
	Cultivars	
JAKI 9218	8.60	8.10
RSG44	9.25	7.64
RSG888	7.10	7.55
VAIBHAV	7.70	7.00
ICCV37	8.20	8.10
JG130	7.40	7.60
JGG1	7.40	7.65
VIHAR	8.70	7.20
JG12	7.40	7.20

To tackle the problem of mineral malnutrition, genetic strategies the maximum potential as a sustainable, inexpensive and effective solution (White and Broadley

Table 5. Two years combined mean, range, and genetic variability components for iron and zinc content in chickpea accessions

Trait	Range (mg/100g)	Mean (mg/100g)	GCV (%)	PCV (%)	H^2	GA	GAM (%)
Iron	2.55-9.63	6.53	37.09	34.47	0.86	3.99	61.07
Zinc	2.58-8.15	5.37	32.16	34.59	0.86	3.29	61.27

GCV=Genetic Coefficient of Variation, PCV=Phenotypic Coefficient of Variation, H^2=Broad sense Heritability, GA=Genetic Advance, GAM= Genetic advance over mean

2011, Haas *et al.* 2016). In a controlled trial, consumption of iron biofortified beans (86 mg Fe/kg) as compared to non-fortified control beans (50 mg Fe/kg) significantly improved the iron status among women in Rwandan (Haas *et al.* 2016). So far, most of the genetic biofortification efforts aimed at cereals like wheat, rice and maize and legumes like beans. In countries like India, Brazil and China, where a smaller amount of several staple crops is consumed rather than a single staple crop to derive nutrition, biofortification of a wide array of food crops is needed (Saltzman *et al.* 2013). So far there are limited studies on biofortification of chickpea. The aim of biofortification is to incorporate the micronutrient-dense traits in those varieties that already have high yield and disease resistance and are preferred for consumption and agronomy (Bouis *et al.* 2013). In this study, in addition to the germplasm accessions, chickpea cultivars having both high iron and zinc have been identified that can be directly used in the breeding programs to expedite the breeding programs for chickpea biofortification.

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