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Baseline status and effect of genotype, environment and genotype × environment interactions on iron and zinc content in Indian chickpeas (*Cicer arietinum* L.)

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Abstract Genetic biofortification is a cost-effective strategy to address iron (Fe) and zinc (Zn) deficiencies prevalent worldwide. Being a rich and cheap protein source, chickpea, a food legume grown and consumed across the globe, is a good target for biofortification. Nineteen popular commercial cultivars of India were analysed for Fe and Zn content at four locations representing different agro-climatic zones to study the genotypic and genotype × environment interactions on Fe and Zn. Distribution of phytic acid (PA), an important anti-nutrient that chelates and reduces the mineral bioavailability, was also analysed. Influence of other agronomic traits like days to flowering, plant height and 100 seed weight on Fe and Zn content was also studied. All the traits showed significant G and G × E interactions; however, the magnitude of variance of GXE was lesser than that of G alone. Genotype + genotype-by-environment and genotype-by-trait biplots were used to assess the relations between different environments, genotypes and traits. Iron and zinc content showed positive correlation between them indicating a possibility of their co-selection in breeding. A negative correlation between Zn and PA was observed. However, there was very low variability for PA content in the cultivars under study, indicating that moderate PA is naturally selected in these cultivars during breeding. Despite significant GXE interactions, cultivars with high Fe (> 70 μg/g) and Zn content (> 40 μg/g) at three out of four test locations were identified. Such genotypes will

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be useful in breeding programs for enhancing mineral micronutrient content and understanding the molecular mechanisms governing their differential uptake.

Introduction

'Hidden hunger' that includes mineral micronutrient malnutrition, affects more than two billion people worldwide (Muthayya et al. 2013). Micronutrient deficiencies, particularly of iron (Fe) and zinc (Zn), are more prevalent affecting more than 60% and 30%, respectively, of the world population (Amarakoon et al. 2012). Various strategies like food fortification, supplementation, diet diversification, agronomic and genetic biofortification are proposed to counter these deficiencies. Among these, genetic biofortification is the most practical, cost-effective and sustainable solution (Saltzman et al. 2013). Genetic biofortification involves improving the genetic constituents of plants for increased uptake and accumulation of mineral micronutrients in the seeds or other edible portion of the plants. This is achieved either through conventional plant breeding approaches or biotechnological interventions.

Among the crop plants, staple cereals like rice, maize and wheat are the main target crops for genetic biofortification. However, in countries like India and China, where diets are more diverse and do not depend on sole staple crops, there is a need to increase the repertoire of target crops including legumes, which together with cereals are an important constituent of daily diets (Saltzman et al. 2013). Among the legumes, the work on biofortification is mostly limited to beans that are highly consumed in the African continent (Haas et al. 2017).

Chickpea is an internationally important food legume grown in over 50 countries, with cultivation area around 14.5 Mha and production of 14.7 MT (FAOSTAT 2017). India is the highest contributor in terms of area, production as well as consumption (Muehlbauer and Sarker 2017). Chickpea constitutes an important source of protein to people who are vegetarian. Initial studies indicate a wide diversity for iron and zinc content in chickpea germplasm and

cultivars (Diapari et al. 2014; Upadhyaya et al. 2016; Joshi-Saha et al. 2018). To breed for high iron and/or zinc cultivars, it is essential to identify germplasm with high nutrient content and to study the effect of environment on these traits. Therefore, the present work was undertaken to study the status of these two micronutrients in popular cultivars bred for particular agro-climatic zones and the effect of environment and other agronomic characters like days to flowering, plant height and seed size on iron and zinc content with respect to four different environments. In addition, identification of genotypes with consistently high Fe and/or Zn over the locations would be useful in breeding programs.

Materials and methods

Plant material

Nineteen cultivars of chickpea (*Cicer arietinum* L.), including *desi* and *kabuli* types, released for commercial cultivation in different agro-climatic zones of India were used in the present study (Online Resource Table S1, Fig. S1). They were grown at four locations in different agro-climatic zones, viz. Nandyal from southern zone, Junagadh from central zone, IIPR main campus and IIPR new research campus (henceforth termed as IIPR-MC and IIPR-NRC) both from northeast plain zone (Online Resource Table S2, Fig. S1) in a randomized complete block design (RCBD) with three replications during the post rainy (*Rabi*) crop season of 2015–2016. Standard agricultural practices were followed to raise the crop. Data on days to 50% flowering (DF), plant height (PH), 100 seed weight (SW) and yield per plant (Yld) were recorded for each replication.

Sample preparation for biochemical analysis

The seeds of each genotype were harvested separately for each of the 3 replications. The seeds were ground into a fine powder and passed through a fine mesh to obtain homogeneous mixture. This was used to estimate iron (Fe), zinc (Zn) and phytic acid (PA).

Iron and zinc estimation

Five millilitres of acid mixture ($HNO_3/HClO_4$; 5:1, v/v) was added to 200 mg of the seed powder and kept overnight for cold digestion, followed by digestion at 120 °C using a microwave digester (Ethos UP, Milestone, Italy). The cooled digested samples were diluted to 50 ml with deionized water. Iron and zinc content was estimated in these samples using a GBC 932 B + Atomic Absorption Spectrophotometer (GBC, Melbourne, Australia) fitted with an air-acetylene flame. The concentrations were expressed as μg/g (ppm) on a dry-weight basis.

Phytic acid estimation

Phytic acid content was measured according to the previously reported method (Joshi-Saha and Reddy 2015). Briefly, 50 mg of seed powder was mixed with 1 mL of 2.4% HCl and incubated for 16 h. The tubes were centrifuged at 10,000g at 10 °C for 20 min. The extract was transferred to a tube containing 0.1 g NaCl and kept in − 20 °C for 20 min. The mixture was centrifuged at 10,000g at 10 °C for 20 min, and the clear supernatant was collected for the development of a colour reaction using Wade's reagent (0.03% $FeCl_3 \cdot 6H_2O$ + 0.3% sulfosalicylic acid). The absorbance of colour-reaction products was read at 500 nm on a DU 640 spectrophotometer (Beckman Coulter, Fullerton, CA, USA).

Data analysis

Analysis of variance (ANOVA) was performed using general linear model (GLM), followed by Tukey's post hoc test in MiniTab ver 17 statistical software. Genotypic ($\sigma^2 g$), phenotypic ($\sigma^2 p$) and error ($\sigma^2 e$) variances were calculated. Genotypic coefficient of variation (GCV %) and phenotypic coefficient of variation (PCV %) were calculated (Badigannavar et al. 2016). Broad-sense heritability (H^2) at individual environments and across environments was calculated as described earlier (Phuke et al. 2017). Genetic advance was calculated as GA (%) = $K \times \sigma P \times H^2 \times 100$, 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.

Biplot analysis

Genotype + genotype-by-environment (GGE) and genotype-by-trait (GT) biplots were constructed using R software utilizing the "GGEBiplotGUI" package (Yan and Tinker 2006). The GGE biplots were constructed from the first two principal components (PC1 and PC2) that were derived by subjecting mean values to singular-value decomposition (Frutos et al. 2014). For testing the mean performance and stability of an accession, the biplots were drawn using the mean vs stability function with no scaling (scale = 0), tester centred G + GE (centring = 2) with genotype focused (row metric preserving) singular-value partitioning (SVP = 1). For testing the environments, the discriminativeness vs representativeness function was utilized with no scaling (scale = 0), tester centred G + GE (centring = 2) with environment focused (column metric preserving) singular-value partitioning (SVP = 2). The GT biplot method was employed to display the genotype-by-trait two-way data in a biplot for each environment and across environments (Yan and Rajcan 2002).

Results

Genotypic variance, genotype × environment interaction and heritability

The four locations differed from each other with respect to soil type, pH, Fe and Zn content and total crop duration (Online Resource Table S2). Analysis of variance showed significant genotype, location and genotype × location interactions for all the traits under study (Table 1).

Seed Fe, Zn and PA content showed highly significant genotypic variance in both pooled (Table 1) and individual environments, except for Junagadh, Gujarat, where the genotypic variation of Fe content in seeds was insignificant (Table 2). However, the average seed Fe content at Junagadh was similar to IIPR-MC and IIPR-NRC of the northeast plain zone (NEPZ) (Table 3). The genotype × location interactions were also significant for Fe, Zn and PA content (Table 1). However, the genotype × location variance ($\sigma^2 gl$) was lower than the genotypic variance ($\sigma^2 g$) for these traits (Table 4).

Table 1 Analysis of variance for biochemical and agronomic traits using general linear model

Source	df	Mean square due to					
		Zn	Fe	PA	DF	PH	SW
Genotype	18	201.40***	211.77***	10.86***	553.77***	90.96***	455.00***
Location	3	1735.81***	7056.09***	8.09***	8516.60***	2818.77***	255.44***
Replication	2	22.49^{ns}	208.01*	0.76^{ns}	2.98^{ns}	8.14^{ns}	1.55^{ns}
Genotype × Location	54	82.39***	206.42***	4.13***	77.20***	32.33***	16.64***
Error		11.56	59.61	1.24	5.02	8.93	1.08
CV %		9.04	10.69	10.46	3.71	6.86	4.34
R^2 (%)		89.09	82.64	74.67	98.14	89.85	98.38

***, **, * indicate P levels of < 0.001, < 0.01 and < 0.05, respectively

Zn: zinc; Fe: iron; PA: phytic acid; DF: days to 50% flowering; PH: plant height; SW: 100 seed weight

Table 2 Adjusted mean-squared values from analysis of variance for biochemical and agronomic traits at individual locations

Trait	Source	Location			
		Nandyal	Junagadh	IIPR-MC	IIPR-NRC
Zn	Genotype	101.99***	78.84***	67.67***	207.08***
	Error	17.34	7.31	8.916	13.60
	CV %	9.64	8.24	9.19	8.93
	R^2	76.23	84.73	78.69	87.82
Fe	Genotype	489.83***	145.27^{ns}	133.69***	91.02**
	Error	93.72	88.31	34.93	32.38
	CV %	10.77	14.48	8.97	8.39
	R^2	75.12	45.85	66.95	59.81
PA	Genotype	5.86***	4.42**	3.53***	9.17***
	Error	1.48	1.38	0.92	1.02
	CV %	11.60	10.59	9.38	9.71
	R^2	67.66	62.2	71.24	86.62
DF	Genotype	298.92***	139.68***	104.99***	241.789***
	Error	3.72	0.47	8.89	6.89
	CV %	3.59	1.42	3.90	4.20
	R^2	97.44	99.29	84.83	94.32
PH	Genotype	44.04***	53.12**	26.75***	64.05***
	Error	9.36	20.10	4.13	2.07
	CV %	7.69	9.24	4.03	4.04
	R^2	69.03	55.59	75.41	93.61
SW	Genotype	134.76***	101.89***	164.63***	103.64***
	Error	2.06	0.25	1.14	0.91
	CV %	5.65	2.34	4.14	4.11
	R^2	96.87	99.49	98.56	98.17

***, **, * indicate P levels of < 0.001, < 0.01 and < 0.05, respectively

Zn: zinc; Fe: iron; PA: phytic acid; DF: days to 50% flowering; PH: plant height; SW: 100 seed weight

Table 3 Descriptive statistics across locations

Variable	Location	Mean[#]	SD	Coef. Var	Minimum	Maximum
Zn (µg/g)	Nandyal, AP	43.2[a]	6.9	15.9	33.8	54.2
	Junagarh, GJ	32.8[b]	5.6	17.1	25.7	46.8
	IIPR-MC	32.5[b]	5.3	16.3	25.5	41.4
	IIPR-NRC	41.3[a]	8.7	21.1	28.2	60.3
Fe (µg/g)	Nandyal, AP	89.9[a]	15.5	17.2	69.9	125.5
	Junagarh, GJ	64.9[b]	10.4	16.0	53.0	81.3
	IIPR-MC	65.9[b]	8.3	12.6	52.5	84.3
	IIPR-NRC	67.8[b]	7.3	10.7	58.2	78.9
PA (mg/g)	Nandyal, AP	10.5[b]	1.7	16.5	8.7	14.2
	Junagarh, GJ	11.1[a]	1.6	14.0	9.5	15.1
	IIPR-MC	10.2[b]	1.4	13.6	8.2	12.5
	IIPR-NRC	10.4[b]	2.1	20.2	6.2	14.0
DF (days)	Nandyal, AP	53.7[c]	9.9	18.5	36.7	71.0
	Junagarh, GJ	48.5[d]	6.7	13.9	33.7	59.7
	IIPR-MC	76.4[a]	6.3	8.3	62.7	85.0
	IIPR-NRC	62.5[b]	9.1	14.5	48.0	79.0
PH (cm)	Nandyal, AP	39.8[c]	4.5	11.4	35.2	47.3
	Junagarh, GJ	48.5[b]	5.5	11.4	37.1	57.1
	IIPR-MC	50.4[a]	3.4	6.7	45.6	56.7
	IIPR-NRC	35.6[d]	4.7	13.2	27.4	44.2
SW (g)	Nandyal, AP	25.4[a]	6.7	26.3	16.7	38.8
	Junagarh, GJ	21.2[c]	5.7	27.0	11.9	34.4
	IIPR-MC	25.8[a]	7.3	28.4	15.9	42.0
	IIPR-NRC	23.3[b]	5.8	25.0	14.3	33.2

[#]Grouping information using Tukey's pairwise comparisons and 95% confidence

Zn: zinc; Fe: iron; PA: phytic acid; DF: days to 50% flowering; PH: plant height; SW: 100 seed weight

Agronomic traits such as days to flowering, plant height and 100 seed weight also showed significant genotype and genotype × location variation in the overall pooled location data (Table 1) with the magnitude of genotype × location variance $\sigma^2 gl$ lower than the genotypic variance (Table 4). Individual environments were also analysed for the genotypic variances (Table 2) and other genetic parameters

Table 4 Genetic parameters based on overall four locations data

Parameter/traits	Zn	Fe	PA	DF	PH	SW
$\sigma^2 g$ (Vg)	63.28	50.72	3.20	182.91	27.34	151.3
$\sigma^2 gl$ (Vgl)	23.61	48.93	0.96	24.06	7.8	5.18
$\sigma^2 p$ (Vp)	70.15	67.92	3.54	189.34	30.03	152.69
GCV %	21.16	9.86	16.88	22.43	11.99	51.47
PCV %	22.27	11.41	17.76	22.82	12.57	51.70
H^2	0.90	0.75	0.90	0.97	0.91	0.99
GA	15.56	12.68	3.5	27.38	10.28	25.22
GAM	41.39	17.56	33.04	45.41	23.57	105.54

$\sigma^2 g$ (Vg): genotypic variance; $\sigma^2 p$ (Vp): phenotypic variance; $\sigma^2 gl$ (Vgl): variance due to G × E; GCV %: genotypic coefficient of variation; PCV %: phenotypic coefficient of variation; H^2: broad-sense heritability; GA: genetic advance over mean; GAM: genetic advance over mean

Zn: zinc; Fe: iron; PA: phytic acid; DF: days to 50% flowering; PH: plant height; SW: 100 seed weight

Table 5 Genetic parameters in four locations	$\sigma^2 g$	$\sigma^2 p$	GCV %	PCV %	H^2	GA	GAM
Trait: Zn							
Nandyal	28.22	34.00	12.30	13.50	0.83	9.97	23.08
Junagadh	23.84	26.28	14.89	15.63	0.91	9.58	29.21
IIPR-MC	19.58	22.56	13.62	14.61	0.87	8.49	26.14
IIPR-NRC	64.49	69.03	19.44	20.12	0.93	15.99	38.72
Trait: Fe							
Nandyal	132.04	163.28	12.78	14.21	0.81	21.29	23.68
Junagadh	18.99	48.42	6.71	10.72	0.39	5.62	8.66
IIPR-MC	32.92	44.56	8.71	10.13	0.74	10.16	15.42
IIPR- NRC	19.55	30.34	6.52	8.12	0.64	7.31	10.78
Trait: PA							
Nandyal	1.46	1.95	11.50	13.31	0.75	2.15	20.47
Junagadh	1.01	1.47	9.06	10.93	0.69	1.72	15.48
IIPR-MC	0.87	1.18	9.15	10.63	0.74	1.65	16.22
IIPR-NRC	2.72	3.06	15.85	16.82	0.89	3.20	30.79
Trait: DF							
Nandyal	98.40	99.64	18.47	18.59	0.99	20.31	37.82
Junagadh	46.40	46.56	14.05	14.07	0.997	14.01	28.88
IIPR-MC	32.03	35.00	7.41	7.74	0.92	11.15	14.60
IIPR-NRC	78.30	80.60	14.16	14.36	0.97	17.97	28.75
Trait: PH							
Nandyal	11.56	14.68	8.54	9.63	0.79	6.22	15.62
Junagadh	11.01	17.71	6.84	8.68	0.62	5.39	11.11
IIPR-MC	7.54	8.92	5.45	5.92	0.85	5.20	10.32
IIPR-NRC	20.66	21.35	12.77	12.98	0.97	9.21	25.87
Trait: SW							
Nandyal	44.23	44.92	26.18	26.39	0.98	13.60	53.53
Junagadh	33.88	33.96	27.46	27.49	0.998	11.98	56.49
IIPR-MC	54.50	54.88	28.61	28.71	0.99	15.15	58.74
IIPR-NRC	34.24	34.55	25.11	25.23	0.99	12.00	51.51

Zn: zinc; Fe: iron; PA: phytic acid; DF: days to 50% flowering; PH: plant height; SW: 100 seed weight

$\sigma^2 g$ (Vg): genotypic variance; $\sigma^2 p$ (Vp): phenotypic variance; $\sigma^2 gl$ (Vgl): variance due to G × E; GCV %: genotypic coefficient of variation; PCV %: phenotypic coefficient of variation; H^2: broad-sense heritability; GA: genetic advance over mean; GAM: genetic advance over mean

(Table 5). All the agronomic traits showed significant genotypic variations across all locations (Table 2).

Genetic parameters calculated from the pooled data for all the locations indicated similar genetic coefficient of variation (GCV %) and phenotypic coefficient of variation (PCV %) values for the respective traits (Table 4). All the traits showed high heritability (≥ 0.9), except for iron content that showed a moderately high heritability of 0.75 (Table 4). Genetic parameters were also calculated for individual location data for all the traits (Table 5). In all locations, these traits showed moderate-to-high heritability; however, Fe content in Gujarat showed lower $\sigma^2 g$ than $\sigma^2 p$ and very low heritability (Table 5). Among the

biochemical traits, Zn content was more heritable than Fe and PA content, and among the agronomic traits, DF and 100SW were most heritable traits at all locations (Table 5).

Mean performance

The descriptive statistics (mean, standard deviation, coefficient of variation and range) for each location is summarized in Table 3. Since the genotype × location interactions were significant in pooled ANOVA (Table 1), Tukey's test was performed to determine the location differences for each trait.

Table 6 Correlation of iron and zinc content with other traits

| Trait | Zn | | | | | Fe | | | | | PA | | | | |
	L1	L2	L3	L4	Across	L1	L2	L3	L4	Across	L1	L2	L3	L4	Across
Fe	0.17	0.07	0.30*	0.29*	0.40***	–	–	–	–	–	–	–	–	–	–
PA	0.47***	0.35**	0.43**	0.70***	0.38***	0.12	– 0.12	0.37*	0.23	0.03	–	–	–	–	–
DF	0.22	0.38**	– 0.11	0.33*	– 0.01	0.16	– 0.22	– 0.09	0.14	– 0.17*	0.33*	0.27*	– 0.03	0.51***	0.06
PH	0.04	– 0.33*	– 0.10	– 0.11	– 0.49***	0.14	– 0.08	– 0.03	– 0.13	– 0.23***	0.05	– 0.18	0.04	– 0.13	– 0.001
SW	– 0.06	– 0.48***	– 0.07	– 0.15	– 0.09	0.36**	– 0.11	– 0.15	– 0.12	0.14*	– 0.16	– 0.36**	– 0.20	– 0.28	– 0.27***
Y	– 0.15	– 0.54***	– 0.12	– 0.71***	– 0.45***	– 0.1	– 0.005	0.25	– 0.25	– 0.18*	– 0.37*	– 0.40**	– 0.45***	– 0.50***	– 0.35***

L1: Nandyal; L2: Junagadh; L3: IIPR-MC; L4: IIPR-NRC; across: average of four location

Zn: zinc; Fe: iron; PA: phytic acid; DF: days to 50% flowering; PH: plant height; SW: 100 seed weight, Y: yield

***, **, * indicate P levels of < 0.001, < 0.01 and < 0.05, respectively

All four locations differed with respect to all the agronomic traits. Days to flowering was earliest in Junagadh, Gujarat (CZ), while longest at IIPR-MC (NEPZ), which also showed highest plant height (PH) and seed weight (Table 3). Interestingly, at IIPR-NRC, which also comes under NEPZ, these traits were significantly different from that of IIPR-MC, indicating the influence of soil type and other parameters on these characters.

The average zinc content in seeds was found to be high at Nandyal and IIPR-NRC despite differences in geographical location and crop phenology. In contrast, both IIPR-MC and IIPR-NRC despite from similar geographical region (NEPZ) contained significantly different levels of Zn. The average seed Fe content was highest at Nandyal, while other three locations showed similar Fe content. Overall, Nandyal showed significantly higher Zn and Fe content, followed by IIPR-NRC (Table 3). Average seed PA content was similar at all locations; however, at Junagadh, it was slightly more than at other locations.

Correlation between grain iron, zinc content and agronomic traits

Phenotypic correlations among traits were calculated for each location and across locations (Tables 6, 7). Based on pooled data from all the locations, Zn and Fe content showed positive correlation. This positive correlation was also observed in IIPR-MC and IIPR-NRC. Zinc and PA also showed positive correlation in pooled location data as well as individually at all the four locations. In addition, a negative correlation between Zn and PH was observed in overall pooled data. Fe content showed a very weak positive correlation with SW in overall pooled data. In this data set, Fe content showed a negative correlation with DF and PH. Overall, PA showed a negative correlation with SW.

The correlation analysis of yield with other traits indicates that Zn content is negatively correlated with the yield in overall pooled dataset and at two locations (Junagadh and IIPR-NRC), while Fe content showed a weak negative correlation with yield in overall dataset (Table 6). Moreover, PA content showed a negative correlation with yield in overall dataset as well as at all the individual locations (Table 6).

Table 7 Correlations among agronomic traits

Trait	DF					PH					SW				
	L1	L2	L3	L4	Across	L1	L2	L3	4	Across	L1	L2	L3	L4	Across
PH	0.41**	0.07	0.49***	0.10	0.23***	–	–	–	–	–	–	–	–	–	–
SW	− 0.36**	− 0.36**	− 0.18	− 0.31*	− 0.03	0.33*	0.31*	0.15	0.62***	0.19**	–	–	–	–	–
Y	− 0.52***	− 0.30*	0.12	− 0.39**	0.45***	0.05	0.52***	0.22	0.10	0.49***	0.32*	0.61***	0.16	0.03	0.30***

L1: Nandyal; L2: Junagadh; L3: IIPR-MC; L4: IIPR-NRC; across: average of four location

PH: plant height; SW: 100 seed weight; Y: yield

***, **, * indicate P levels of < 0.001, < 0.01 and < 0.05, respectively

GGE biplots

The environment vector view of the GGE biplot based on Fe content is shown in Fig. 1a. This biplot explains 80.2% of total variation of the environment-centred G × E. Junagadh and IIPR-MC were highly correlated showing similar responses for Fe content, while Nandyal was not correlated with these two environments, although it was the most discriminating environment. On the other hand, Nandyal and IIPR-NRC showed slight positive correlation; yet IIPR-NRC was the least discriminating (Fig. 1a). Thus, there were two distinct/discriminative environments, IIPR-MC/Junagadh as one mega-environment and Nandyal, for Fe content and can be useful for selecting specifically adapted genotypes. In contrast, IIPR-NRC is a non-discriminating test environment and is less useful for Fe content. The performance of genotypes can also be evaluated based on GGE biplots.

Cultivars Virat, Vihar and PUSA391 showed high Fe content at Nandyal, while JG11, JAKI9218 and JG315 performed well at IIPR-MC and Junagadh (Fig. 1a). The 'which-won-where' view of a biplot indicates the most responsive genotypes (present on the vertices of the polygon) at some or all locations. Such plot for Fe content (Fig. 1b) indicated that Virat at Nandyal and JG11 at IIPR-MC and Junagadh were the best performers. In mean vs stability view of the GGE biplot, the AEA axis (single arrowed line) indicates the highest mean performance (Fig. 1c). Thus, Virat had the highest mean Fe content followed by JG11 and PUSA391. RSG44 and RSG888 had mean Fe content similar to the grand mean, and ICCV37 had the lowest mean Fe content (Fig. 1c). The other axis points to more variability, i.e. lesser stability in either direction. Thus, Virat, JG11 and PUSA 391 were also less stable genotypes, while JAKI 9218 and JG315 were more stable. RSG44 was most stable and close to the 'ideal' genotype.

GGE biplots based on Zn content explain 80.5% of total variation of the environment-centred GXE. The environment vector view indicated that Junagadh and IIPR-MC were highly correlated (Fig. 2a). IIPR-MC was the most representative environment (smallest angle with the AEA), followed by Junagadh and Nandyal. While IIPR-NRC and Nandyal were the most discriminative environments, the vectors for these locations indicated no correlation between them (Fig. 2a). RSG44, ICCV37, RSG888 and Vihar
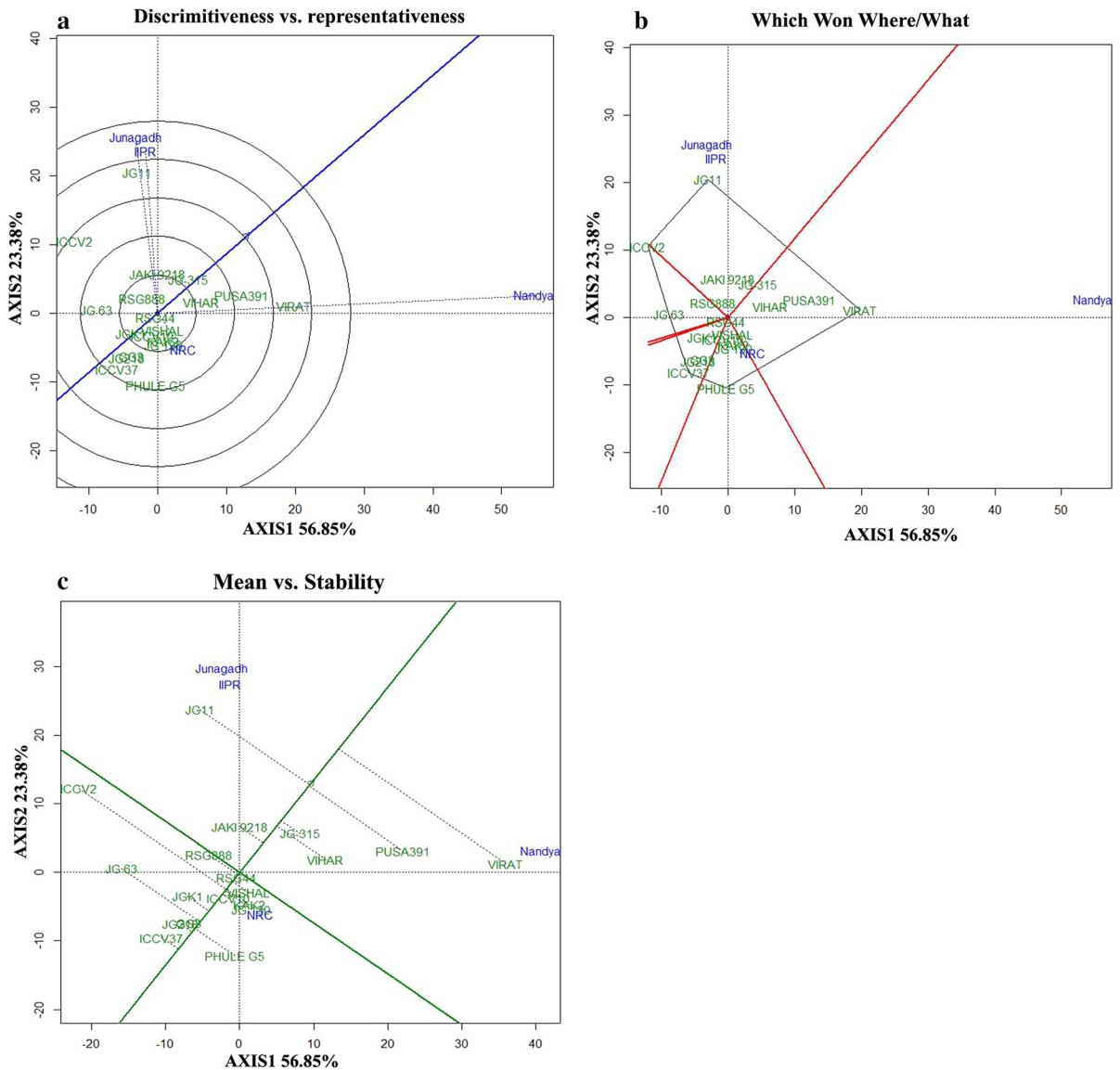
Fig. 1 GGE biplots based on the first and second principle component based on mean iron (Fe) content of 19 chickpea accessions at four locations (Junagadh, Nandyal, IIPR-MC and IIPR-NRC) in India. 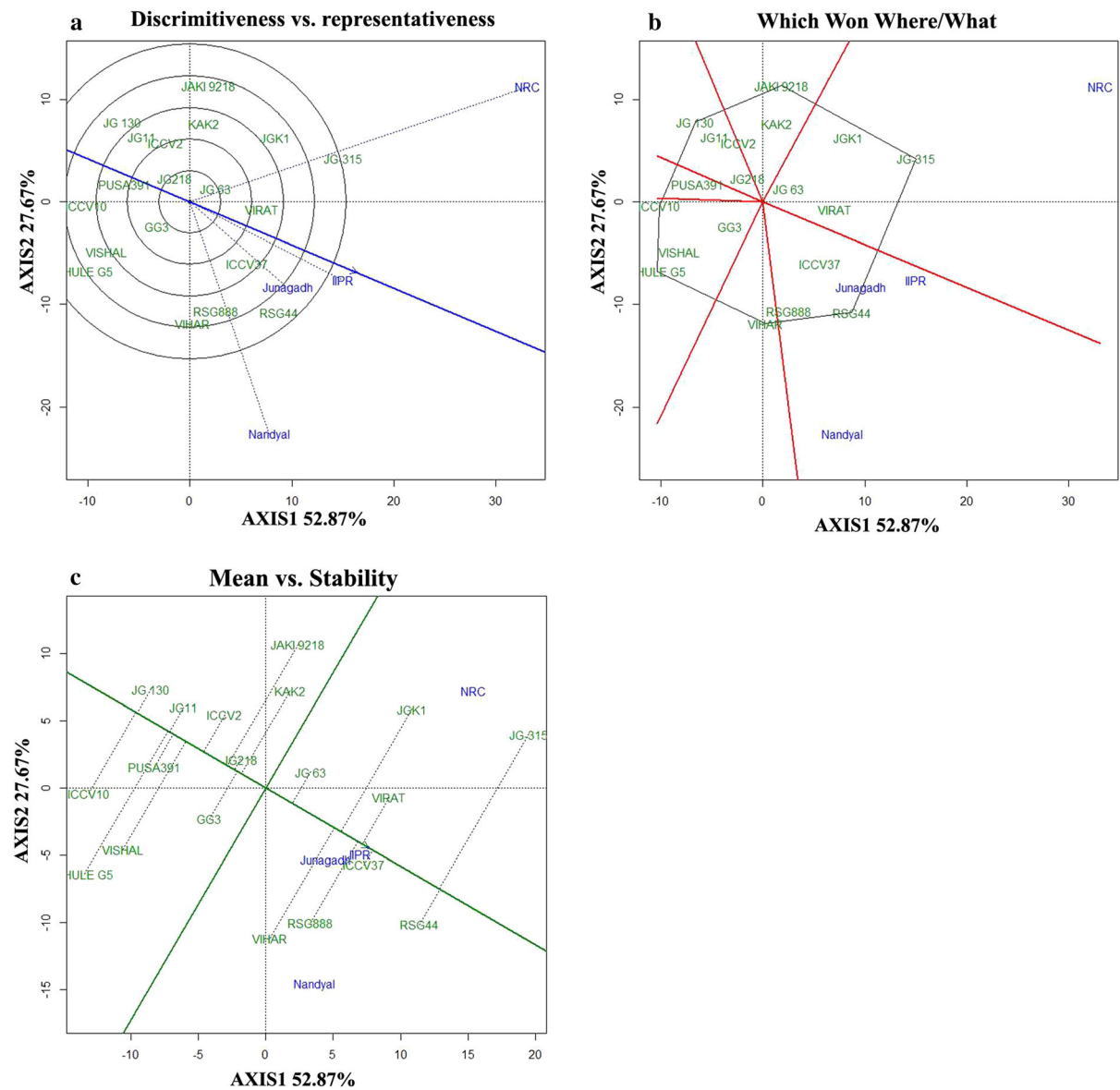**a** Discriminating ability and representativeness of 4 test environments, **b** which-won-where/what biplot of 4 test environments, **c** ranking of 19 chickpea accessions based on both mean iron (Fe) content and stability over 4 environments

performed well at IIPR-MC, Junagadh and Nandyal, while JG315, JGK1 and JG63 performed well at IIPR-NRC. Virat performed well at all the locations (Fig. 2a). The 'which-won-where' view of the biplot indicated JG315 as the winning genotype at IIPR-NRC, while RSG44 at the other three locations (Fig. 2b). Mean vs stability view of the GGE biplot indicated that JG315 had the highest Zn content at IIPR-NRC, followed by Virat and JGK1. RSG44 had high mean Zn content in the other three environments followed by ICCV37, RSG888 and Vihar (Fig. 2c). JG63, ICCV37, Virat and RSG44 were also more stable than the other genotypes.

Genotype-by-trait (GT) biplots

Genotype-by-trait (GT) biplots were created based on combined data for all locations as well as for

Fig. 2 GGE Biplot based on the first and second principle component based on mean zinc (Zn) content of 19 chickpea accessions at four locations (Junagadh, Nandyal, IIPR-MC and IIPR-NRC) in India. **a** Discriminating ability and representativeness of 4 test environments, **b** which-won-where/what biplot of 4 test environments, **c** ranking of 19 chickpea accessions based on both mean zinc (Zn) content and stability over 4 environments

individual locations. In such biplots, the cosine of the angle between the vectors of the two traits approximates the Pearson correlation coefficient between them (angle smaller than 90°: positive correlation, angle more than 90°: negative correlation and an angle of 90° indicates no correlation). Fe and Zn content were positively correlated overall as well as at all the locations except Junagadh (Fig. 3b–e). Zinc and PA

were also positively correlated overall and at individual locations. However, no correlation was observed for Fe and PA (Fig. 3a).

Among the agronomic traits, there was a slight negative correlation between Fe and DF, which was not significant at individual locations (Table 6). No negative correlation was seen between Zn and DF across environments, and a positive correlation was

observed at Junagadh and IIPR-NRC (Table 6). There was positive correlation between Fe content and 100 SW in overall dataset and at Nandyal, whereas a negative correlation between Zn content and SW at Junagadh. Overall, a negative correlation of Fe and Zn content with PH was observed. Phytic acid content was negatively correlated with SW in overall data set (Table 6).

As represented by the vector lengths for each trait, overall DF and SW followed by Fe and Zn content and plant height contributed maximum to the variation (Fig. 3a–e). These traits, except for PA, were also well represented in all the four locations.

The polygon view is useful for comparing the genotypes on the basis of multiple traits and to identify the genotypes that are better in certain aspects, and can be used in breeding programmes (Online Resource Fig. S2a, b, c, d, e). Thus, JG315, RSG44 and RSG888 performed well in terms of both Fe and Zn content.

Overall, RSG888, RSG44, JG315, Virat and Vihar showed high content of both Fe (> 70 μg/g) and Zn (> 40 μg/g). Of these, RSG44 and JG315 showed high Fe and Zn at all locations except Junagadh. In addition, despite G × E interactions, cultivars JG11, JG63, JAKI9218 and ICCV37 contained high Fe (> 70 μg/g) and Zn (> 40 μg/g) at three test locations (Online Resource Table S3). The overall mean vs stability biplot indicated that JG315, RSG44 and RSG888 had higher than average Fe and Zn content with more stability (Fig. 4).

Discussion

Nineteen popular cultivars were assessed for variability in their Fe, Zn and PA content and the influence of environment on these traits. Four environments were chosen: the central zone (CZ), southern zone (SZ) and two locations of the north-east plain zone (NEPZ) of India. Over the four locations, mean Zn and Fe content of cultivars ranged from 32 to 43 μg/g and 65–90 μg/g, respectively. Previously, the range of mean Zn and Fe content from 94 diverse chickpea accessions grown across two locations and two seasons in Canada was reported to be 27–44 μg/g and 42–56 μg/g, respectively (Diapari et al. 2014). In another study analysing 91 chickpea genotypes, the mean Zn and Fe content was 46.2 ± 9.1 and 63.3 ± 13.3 μg/g, respectively (Upadhyaya et al. 2016). Mean Zn and Fe contents of
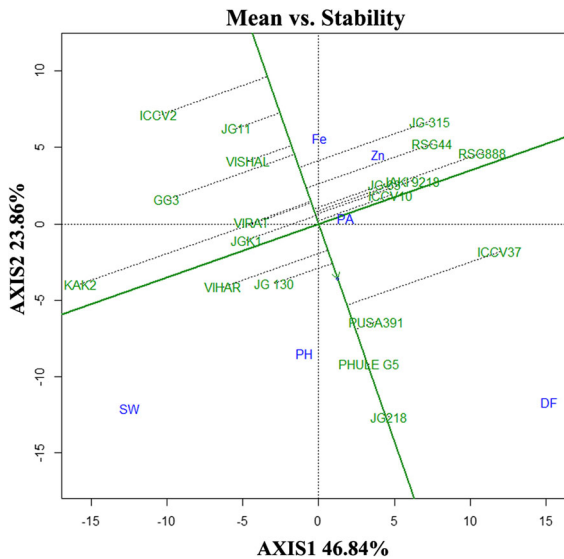
47–65 μg/g and 62–71 μg/g, respectively, were also reported in Indian chickpea cultivars grown at one location for two seasons (Joshi-Saha et al. 2018). Although a strong G × E interaction was observed in the present study, yet the overall contribution towards variation in the data for all the traits was highest for location (environment) effect, followed by genotypes, while contribution of interaction of genotypes with environment (genotype × location) was least (Tables 1, 4). Similar observations were made for 17 groundnut lines evaluated in 130 locations in Thailand over 30 years (Putto et al. 2008). However, for Fe content the variance due to G × E ($\sigma^2 gl$) was closer to the variance due to genotypes ($\sigma^2 g$) (Table 4), indicating that both genotypes and their interactions with environment contribute almost equally to the total variation in Fe. Moreover, the magnitude of G × E interactions for Fe was more than that for Zn. Similar results were also reported for sorghum, where magnitudes of genotype × year × location ($\sigma^2 gyl$) interactions were higher for Fe than those observed for Zn (Phuke et al. 2017).

A positive correlation was observed between Fe and Zn content suggesting that these two micronutrients can be improved simultaneously through an appropriate breeding approach. The correlation was influenced by environment, indicating that both genetic and environmental factors influence these traits. Similar positive correlations were also observed in chickpea cultivars and breeding lines (Tan et al. 2018; Vandemark et al. 2018), sorghum (Phuke et al. 2017), millets (Kanatti et al. 2014), wheat (Velu et al. 2014), rice (Swamy et al. 2016) and maize (Mallikarjuna et al. 2015). This positive correlation could be due to the presence of transporters like zinc-regulated transporters, iron-regulated transporter-like proteins (ZIP) common for both the minerals (Guerinot 2000).

A negative correlation was observed for PA and Zn content but not for PA and Fe content; however, it is interesting to note that although there was significant genotypic variation for PA content in all the locations, the range of average PA across four locations (10.2 ± 1.4–11.1 ± 1.6 mg/g) was very narrow. Over the four locations, the average PA in 19 genotypes ranged from 9.1 to 12.8 mg/g (Online Resource Table S4a), indicating that possibly the cultivars have naturally been selected for low PA during their development. Similar results have been observed in commercially grown pea genotypes in
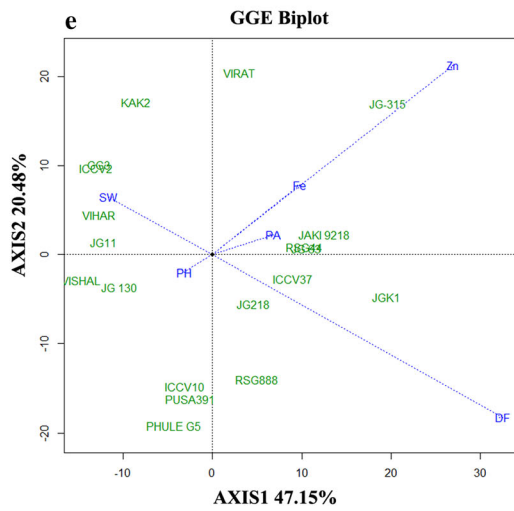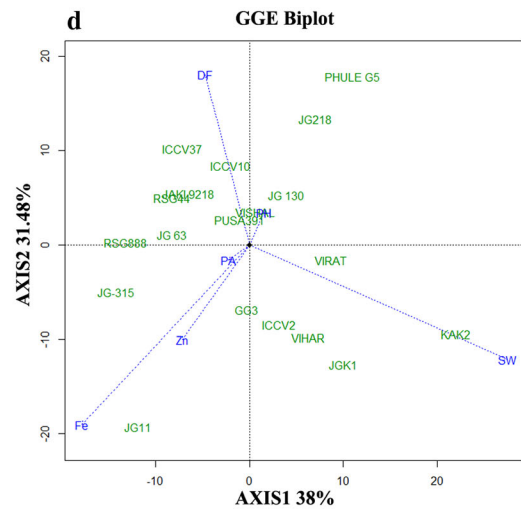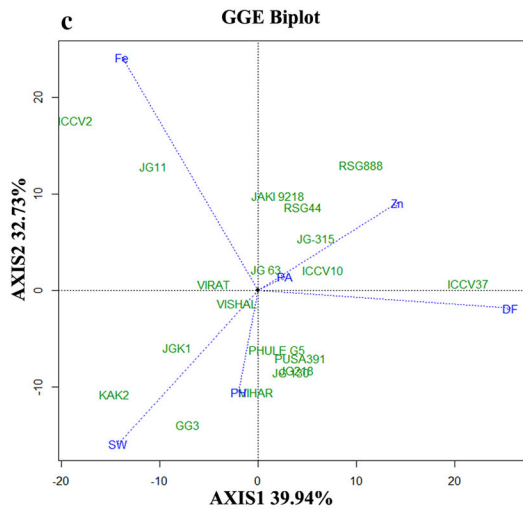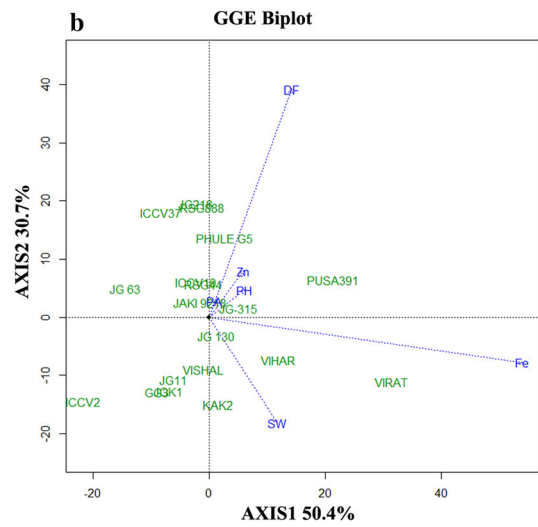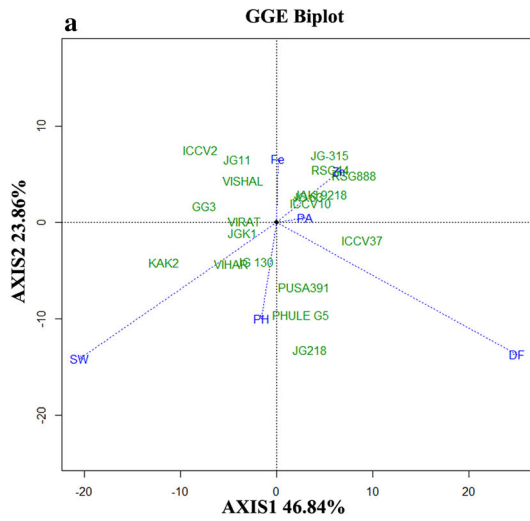
Fig. 4 Genotype-by-trait (GT) biplot based on 1st and 2nd principle component showing ranking of 19 chickpea accessions based on both mean value and stability over four locations (Junagadh, Nandyal, IIPR-MC and IIPR-NRC) in India, for 6 yield and quality traits. Traits: Fe (iron), Zn (zinc), PA (phytic acid), DF (days to 50% flowering), PH (plant height), SW (100 seed weight)

North Dakota (Amarakoon et al. 2012) and lentils (Thavarajah et al. 2009). The PA/Fe molar ratios of more than 10 affect Fe bioavailability in humans (Engle-Stone et al. 2005). In the current study, the average PA/Fe molar ratio (averaged over four locations) ranged from 9.8 to 15, which is similar to that reported in field pea cultivars (9–11.1) (Amarakoon et al. 2012) and common bean (11.7–16.2) (Hu et al. 2006). Genotypes grown in Nandyal showed lower PA/Fe molar ratios (range 7.6–13.4) as compared to other locations (Online Resource Table S4b). Interestingly, genotypes Virat and JG11 that showed high mean Fe also had overall lower PA/Fe molar ratios of 11.5 and 9.8, respectively, suggesting their

suitability for the breeding programs. Overall PA/Zn molar ratios ranged from 17.1 to 41.9. Similar to PA/Fe molar ratio, cultivars grown at Nandyal showed a lower range of PA/Zn molar ratios (range 17.3–27.7) than that of other locations (Online Resource Table S4b). Similar PA/Zn ratios were observed in 10 cultivated chickpeas in Canada (Bueckert et al. 2011). A PA/Zn molar ratio of 15 or more affects the bioavailability of Zn (Ma et al. 2007). Microwave and other traditional methods of cooking reduce the PA levels by 28–41% (Alajaji and El-Adawy 2006). Therefore, the inherent lower PA level in these cultivars and lowering effect of cooking make chickpeas a good dietary Fe and Zn source.

The yield was also evaluated in the present study (Online Resource Table S5a-e). The correlation analysis indicated that Zn content was negatively correlated with the yield in overall dataset and at two locations, while Fe content showed a weak negative correlation with yield in overall dataset. Moreover, PA content showed a negative correlation in overall dataset as well as at all the individual locations. Similar results were observed in chickpea and lentil cultivars and breeding lines grown in the US Pacific Northwest, where a strong negative correlation of Zn with yield was observed (Vandemark et al. 2018). In another study, using 94 diverse accessions of chickpea grown at two locations in Canada, a negative correlation of zinc with yield was observed, while the negative correlation of iron was not significant at all the locations (Diapari et al. 2014). The negative correlation of Zn and yield indicates that in order to breed for genotypes with both high zinc and yield, this negative linkage between the two traits needs to be broken by judiciously choosing the parents with efficient mineral uptake and translocation systems in the hybridization programs. Additionally, induced mutagenesis can also be a useful tool to break such deleterious linkages and to improve traits like nutrient uptake and translocation to seeds. This also highlights the importance of the use of genomic tools, such as marker-assisted selection strategies, to selectively introgress high mineral content into a high-yielding background.

Increased PA content reduces the bioavailability of mineral micronutrients. Interestingly, a negative correlation of grain yield with PA content was also observed in overall dataset and in all the locations. Similar negative correlation of PA content with yield

was observed in commercial durum wheat cultivars (Magallanes-López et al. 2017). This also suggests that increasing the yield can indirectly increase the mineral bioavailability.

The biplots effectively explain the variability, as the first two principle components explain almost 80%, and G + GE combined effect accounts for more than 10% of the total variation (Rakshit et al. 2012). GGE biplot analysis reveals that there can be two mega-environments for testing Fe content: Nandyal and the second comprised of two locations (IIPR-MC and Junagadh). IIPR-NRC was the least discriminating environment. The soil iron content did not vary greatly in these locations except for IIPR-NRC that had low iron content (Online Resource Table S2). Of these, Nandyal was the most discriminative location for Fe content. Cultivars Virat at Nandyal and IIPR-NRC and JG11 at IIPR-MC and Junagadh were the best performers for Fe content. Notably, the average Fe of JG11 at Nandyal location was high (86.5 µg/g), indicating that JG11 could be a suitable donor for high Fe. In contrast, cultivar RSG44 was closest to the ideal genotype and was most stable. Cultivars RSG888, JG315, Vihar and JAKI 9218 were also relatively stable for Fe content. Notably these genotypes had overall higher average Fe (> 70 µg/g) and Zn (> 40 µg/g) contents, except for JAKI 9218 with overall average Zn content of 36.4 µg/g.

Biplots for Zn indicated that Junagadh and IIPR-MC were highly correlated and together with Nandyal can constitute one mega-environment like Fe. The soil Zn content at these three locations was also at par (Online Resource Table S2). Furthermore, IIPR-MC and IIPR-NRC although belonged to the same zone, they differed with respect to seed Zn content. This could be due to different levels of Zn present in the soil of these locations. RSG44 was the best performer at these three locations. The environment like IIPR-MC, which was discriminative as well as representative, will be suitable for selecting generally adapted genotypes. In contrast, Nandyal and IIPR-NRC being more discriminating can be used for selecting locally adapted genotypes. Notably, Nandyal was also the most discriminating location for Fe as well. In terms of cultivar performance with respect to Zn, RSG44, ICCV37, RSG888 and Vihar performed well at IIPR-MC, Junagadh and Nandyal, and JG315, JGK1 and JG63 performed well at IIPR-NRC.

RSG888, RSG44, JG315, Virat and Vihar cultivars showed high average Fe (> 70 µg/g) and Zn (> 40 µg/g) content. RSG44 and JG315 showed high Fe and Zn at all the locations except Junagadh, which was a non-discriminating environment for Fe content. RSG888 and RSG44 are related by pedigree and have previously been identified as source of high Fe and Zn at another location based on two growing season data (Joshi-Saha et al. 2018). Additionally, despite G × E interactions, cultivars JG11, JG63 and JAKI 9218 and cultivar ICCV37 were found to contain high Fe (> 70 µg/g) and high Zn (> 40 µg/g), respectively, in three test locations.

Overall, there was a slight negative correlation between Fe and DF. Negative association of 50% days to flowering and Fe content was reported in sorghum (Phuke et al. 2017) and chickpea (Vandemark et al. 2018). The magnitude of the negative association of DF with Fe was low (− 0.17), suggesting that genotypes with early flowering and high Fe can be selected, which is evident from JG11, an early flowering and maturing cultivar that has higher Fe content across three locations. Overall, slight positive correlation of Fe content with 100 SW was observed. However, a strong negative correlation was observed between Zn content and 100 SW. Similar positive and negative correlations of Fe and Zn content, respectively, with 100 SW were observed in chickpea (Vandemark et al. 2018). Larger seed size in chickpea fetches premium market price; however, in breeding for increased Fe and/or zinc content, it will be imperative to select for optimum seed size. Moreover, it is suggested that in order to meet the higher seed mineral load, breeding for increased uptake and/or translocation of the minerals to seeds is needed to sustain the increased mineral concentration in high-yielding lines.

Compliance with ethical standards

References

Alajaji SA, El-Adawy TA (2006) Nutritional composition of chickpea (*Cicer arietinum* L.) as affected by microwave cooking and other traditional cooking methods. J Food Compost Anal 19:806–812. https://doi.org/10.1016/j.jfca.2006.03.015

Amarakoon D, Thavarajah D, McPhee K, Thavarajah P (2012) Iron-, zinc-, and magnesium-rich field peas (*Pisum sativum* L.) with naturally low phytic acid: a potential solution to global micronutrient. J Food Compost Anal 27:8–13. https://doi.org/10.1016/j.jfca.2012.05.007

Badigannavar A, Girish G, Ramachandran V, Ganapathi TR (2016) Genotypic variation for seed protein and mineral content among post-rainy season- grown sorghum genotypes. Crop J 4:61–67. https://doi.org/10.1016/j.cj.2015.07.002

Bueckert RA, Thavarajah D, Thavarajah P, Pritchard J (2011) Phytic acid and mineral micronutrients in field- grown chickpea (*Cicer arietinum* L.) cultivars from western Canada. Eur Food Res Technol 233:203–212

Diapari M, Sindhu A, Bett K, Deokar A, Warkentin TD, Tar'an B (2014) Genetic diversity and association mapping of iron and zinc concentrations in chickpea (*Cicer arietinum* L.). Genome 57:459–468

Engle-Stone R, Yeung A, Welch RM, Glahn RP (2005) Meat and ascorbic acid can promote Fe availability from Fe-phytate but not from Fe-tannic acid complexes. J Agric Food Chem 53:10276–10284

Food and Agriculture Organization (FAO) (2017) FAO-STAT Statistical Database of the United Nation Food and Agriculture Organization (FAO) Statistical Division. Rome. http://www.fao.org/faostat/en/#data/QC. Accessed 11 Feb 2020

Frutos E, Galindo MP, Leiva V (2014) An interactive biplot implementation in R for modelling genotype-by-environment interaction. Stoch Envrion Res Risk Assess 28:1629–1641

Guerinot ML (2000) The ZIP family of metal transporters. Biochim Biophys Acta 1465:190–198

Haas JD, Luna SV, Lung'aho MG, Ngabo F, Wenger M, Murray-Kolb LE, Beebe S, Gahutu JB, Egli IM (2017) Consuming iron biofortified beans significantly improved iron status in Rwandan women after 18 weeks. J Nutr 146:1586–1592

Hu Y, Cheng Z, Heller LI, Krasnoff SB, Glahn RP, Welch RM (2006) Kaempferol in red and pinto bean seeds (*Phaseolus vulgaris* L.) coats inhibits bioavailability using an in vitro digestion/human CaCO-2 cell model. J Agric Food Chem 54:9254–9261

Joshi-Saha A, Reddy KS (2015) Repeat length variation in 5′UTR of *myo*-inositol monophosphatase gene is related to phytic acid content and contributes to drought tolerance in chickpea (*Cicer arietinum* L.). J Exp Bot 66:5683–5690

Joshi-Saha A, Mehzabin P, Reddy KS, Ramachandran V (2018) Effect of seasonal variation on micronutrient content in chickpea (*Cicer arietinum* L.) and identification of accessions having high iron and zinc. J Food Legumes 3:1–4

Kanatti A, Rai KN, Radhika K, Govindaraj M, Sahrawat KL, Rao AS (2014) Grain iron and zinc density in pearl millet: combining ability, heterosis and association with grain yield and grain size. Springer Plus 3:763

Ma G, Li Y, Jin Y, Zhai F, Kok FJ, Yang X (2007) Phytate intake and molar ratios of phytate to zinc, iron and calcium in the diets of people in China. Eur J Clin Nutr 61:368–374

Magallanes-López AM, Hernandez-Espinosa N, Velu G et al (2017) Variability in iron, zinc and phytic acid content in a worldwide collection of commercial durum wheat cultivars and the effect of reduced irrigation on these traits. Food Chem 237:499–505

Mallikarjuna MG, Thirunavukkarasu N, Hossain F, Bhat JS et al (2015) Stability performance of inductively coupled plasma mass spectrometry-phenotyped kernel minerals concentration and grain yield in maize in different agro-climatic zones. PLoS ONE 10:e0139067. https://doi.org/10.1371/journal.pone.0139067

Muehlbauer FJ, Sarker A (2017) Economic importance of chickpea: production, value and world trade. In: Varshney RK, Thudi M, Muehlbauer FJ (eds) The chickpea genome, compendium of plant genomes. Springer, Berlin, pp 5–12

Muthayya S, Rah JH, Sugimoto JD, Roos FF, Kraemer K, Black RE (2013) The global hidden hunger indices and maps: an advocacy tool for action. PLoS ONE 8:e67860

Phuke RM, Kotla A, Kommineni R, Jabeen F et al (2017) Genetic variability, genotype × environment interaction, correlation, and GGE biplot analysis for grain iron and zinc concentration and other agronomic traits in RIL population of Sorghum (*Sorghum bicolour* L. Moench). Front Plant Sci 8:172

Putto W, Patanothai A, Jogloy S, Hoogenboom G (2008) Determination of mega-environments for peanut breeding using the CSM-CROPGRO- peanut model. Crop Sci 48:973–982

Rakshit S, Ganapathy KN, Gomashe SS, Rathore A et al (2012) GGE biplot analysis to evaluate genotype, environment and their interactions in sorghum multi-location data. Euphytica 185:465–479

Saltzman A, Birol E, Bouis HE, Boy E, De Moura FF, Islam Y, Pfeiffer WH (2013) Biofortification: progress toward a more nourishing future. Glob food Secur 2:9–17. https://doi.org/10.1016/j.gfs.2012.12.003

Swamy BPM, Rahman MA, Inabangan-Asilo MA et al (2016) Advances in breeding for high grain zinc in rice. Rice 9:49

Tan GZH, Das Bhowmik SS, Hoang TML et al (2018) Investigation of baseline iron levels in Australian chickpea and evaluation of a transgenic biofortification approach. Front Plant Sci 9:88

Thavarajah P, Thavarajah D, Vandenberg A (2009) Low phytic acid lentils (*Lens culinaris* L.): a potential solution for increased micronutrient availability. J Agric Food Chem 57:9044–9049

Upadhyaya HD, Bajaj D, Das S et al (2016) Genetic dissection of seed-iron and zinc concentrations in chickpea. Sci Rep 6:24050

Vandemark GJ, Grusak MA, McGee RJ (2018) Mineral concentrations of chickpea and lentil cultivars and breeding lines grown in the U.S. Pacific Northwest. Crop J 6:253–262. https://doi.org/10.1016/j.cj.2017.12.003

Velu G, Ortiz-Monasterio I, Cakmak I, Hao Y, Singh RP (2014) Biofortification strategies to increase grain iron and zinc concentrations in wheat. J Cereal Sci 59:365–372. https://doi.org/10.1016/j.jcs.2013.09.001

Yan W, Rajcan IR (2002) Biplot analysis of test sites and trait relations of soybean in Ontario. Can J Plant Sci 42:11–20

Yan W, Tinker NA (2006) Biplot analysis of MET data: principals and applications. Can J Plant Sci 86:623–645. https://doi.org/10.4141/P05-169