Genetic variability for iron and zinc as well as antinutrients affecting bioavailability in black gram (*Vigna mungo* (L.) Hepper)

Jagdish Singh¹ · Rajani Kanaujia¹ · A. K. Srivastava² · G. P. Dixit² · N. P. Singh³

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Abstract The mineral content of pulses is generally high, but the bioavailability is poor due to the presence of phytate and polyphenols which inhibits Fe absorption. In the present study, the genetic variability and heritability for seed Fe and Zn content was studied. The effect of genotypes was significant for all the quality traits indicating presence of enough variability among the blackgram genotypes for the traits. The Fe content in 26 blackgram genotypes ranged from 71.02 to 100.20 ppm, whereas Zn content ranged from 18.93 to 60.58 ppm. Maximum Fe as well as Zn was recorded in genotype SHEKHAR 2 (100.2 and 60.58 ppm respectively). The Phytic acid and polyphenol content among genotypes varied significantly and it ranged from 0.06–0.37% to 5.88–9.03 mg/g, respectively. High phytic acid content was recorded in black gram genotypes COBG 653, Nodai Urd, NP 03 and PKG U 03, whereas high polyphenol content was recorded in PU 31, IPU 99-200, PDU 1 and YAKUBPUR 2. Blackgram genotype COBG 653 had high phytic acid but low polyphenol content. The genotype × year interaction was significant for all the traits under study which indicates differential reaction to the expression of quality characters over years. Fe content in blackgram genotypes showed significant positive phenotypic correlation with Zn content while at genotypic level in addition to Zn, it showed

positive correlation with phytic acid and polyphenol content as well. This indicates that although the traits are genotypically correlated, the expression is masked by the environmental influence. This is further exhibited from low heritability estimates for phytic acid and polyphenol content among the genotypes.

Keywords Blackgram · Iron · Zinc · Polyphenols · Phytic acid · Bioavailability · Micronutrient malnutrition

Introduction

Blackgram (*Vigna mungo* (L.) Hepper), locally known as *urdbean* originated from central Asia and India from where it was domesticated. It is now grown and consumed in many tropical areas of Asia, Africa and Madagascar. It is an important pulse crop of India, cultivated annually on about 3.24 million ha with annual production of 1.96 million tonnes and productivity of about 604 kg/ha. Blackgram seeds are rich in protein (24–26% DM), starch (35% DM), crude fiber (5–6% DM) and minerals (Rajaguru and Ravindran 1985). There are reports that Fe absorption from meals based on legumes was found to be low, ranging from 0.8 to 1.9% (Lynch et al. 1984). Among inhibitors, phytic acid (PA), tannins and dietary fiber are most potent, while ascorbic acid is known to promote iron absorption (Sandberg 2002). A dose-dependent inhibition of Fe, Zn and Ca absorption by phytate has been demonstrated in humans (Fredlund et al. 2002). Inositol pentaphosphate has also been identified as an inhibitor of Fe and Zn absorption (Sandberg et al. 1999). Certain Fe-binding polyphenols are potent inhibitors of non-haem iron absorption (Hurrell et al. 1999). The amount of Fe-binding phenolic galloyl groups in foods roughly corresponds to the degree of inhibition of

✉ Jagdish Singh
jagdish1959@gmail.com

¹ Division of Basic Sciences, ICAR-Indian Institute of Pulses Research, Kanpur, UP 208 024, India

² Division of Crop Improvement, ICAR-Indian Institute of Pulses Research, Kanpur, UP 208 024, India

³ ICAR-Indian Institute of Pulses Research, Kanpur, UP 208 024, India

Fe absorption (Brune et al. 1989). This phenomenon can contribute to human mineral deficiency, particularly with respect to iron and zinc (Erdman 1981). Deficiency of Fe and perhaps Zn, is highly prevalent in developing countries particularly in vulnerable groups such as women of fertile age, infants and adolescents (Reddy and Sanders 1990). Developing micronutrient-dense staple food crops through conventional plant breeding and genetic selection is a relatively new approach known as bio fortification (Bouis et al. 2011). Several studies have reported the existence of genetic variability with regards to Iron (Fe) and Zinc content (Zn) in most of the legume crops. Because these traits are most likely quantitative, it is expected that the environment would have a substantial effect and the genotype $\times$ environment interaction should be important. Some studies have reported a correlation between Fe and Zn content in common bean (Blair et al. 2009), however, some other studies found no correlation (Ribeiro et al. 2008). Therefore, the objectives of the current study were to identify the genetic variability and heritability for Fe, Zn, phytic acid and polyphenol in blackgram genotypes, to assess the genotype $\times$ environment interaction and study the stability for these traits over years to guide the selection of potential parents for breeding programs; and analyse the genetic correlation between the two minerals as well as with anti-nutrients viz., phytic acid and poly phenols which directly affect the bioavailability of these micronutrients.

Materials and methods

Materials

The material for the study comprised of seeds of 26 diverse genotypes of blackgram (*Vigna mungo* (L.) Hepper) which were sown in a randomized complete block design (RCBD) with three replications in rainy/kharif season at research farm of ICAR-Indian Institute of Pulses Research, Kanpur for 2 years i.e. 2014 and 2015. All required agronomic practices and plant protection measures against pests and diseases to raise a successful crop were followed. Seeds were harvested at maturity and subsamples (100 g) of dry blackgram seeds (10–12% moisture) were collected from each of the three replicated field plots of each genotype and thoroughly homogenized to obtain one composite sample for each genotype for each year. Three samples were drawn from the composite samples for quality trait analysis.

Fe and Zn extraction and quantification

Fe and Zn content of the blackgram genotypes were estimated by atomic absorption spectrometry (AOAC 1990). Dried seed sample of each genotype was ground to fine

powder and 0.5 g of each sample was weighed using a Teflon vessel, and then a mixture of concentrated HNO_3 and HClO_4 (10:4) was added. The digestion vessel was closed and heated in the microwave oven. The obtained solutions were allowed to cool at room temperature and then filtered by Whatman No. 1 (110 mm pores size) filter paper into a 50 mL volumetric flask and made the volume using double distilled deionized water. These extracts were used for determination of Fe and Zn utilizing atomic absorption spectrophotometer (GBC Avanta M, GBC Scientific Equipment, Melbourne, Australia) with an air-acetylene flame. The estimated concentrations of minerals were expressed in ppm.

Phytic acid (PA) quantification

Phytic acid was estimated as phosphorus released by phytase and alkaline phosphatase using the standard assay procedure of K-PHYT (© Megazyme International Ireland 2014). This method involves acid extraction of inositol phosphates followed by treatment with a phytase that is specific for phytic acid (IP6) and the lower *myo*-inositol phosphate forms (i.e. IP2, IP3, IP4, IP5). Subsequent treatment with alkaline phosphatase ensures the release of the final phosphate from *myo*-inositol phosphate (IP1) which is relatively resistant to the action of phytase. The total phosphate released is measured using a modified colorimetric method and given as grams of phosphorus per 100 g of sample. Pi is quantified as phosphorus from a calibration curve generated using standards of known phosphorus concentration. Finally the phytic acid concentration is calculated assuming that the amount of phosphorus measured is exclusively released from phytic acid and that this comprises 28.2% of phytic acid.

$$\text{Phytic acid [g/100g]} = \frac{\text{phosphorus [g/100g]}}{0.282}$$

Total phenol

Total phenols were determined according to Folin–Ciocalteu procedure (Singleton and Rossi 1965) with slight modifications. Generally polar solvents are frequently used for recovering polyphenols from plant matrices and the most suitable solvents are aqueous mixtures containing ethanol, methanol, acetone and ethyl acetate (Quy et al. 2014). In the present study maximum recovery of the phenolic compounds was obtained when the sample (2 g) was homogenized in 80% aqueous ethanol at room temperature and centrifuged in cold at 10,000 g for 15 min. To ensure the complete extraction of the phenolic compounds, the residue was re-extracted twice with 80% ethanol and supernatants were pooled and evaporated to dryness in a

water bath at temperature of 40 °C. Residue was dissolved in 5 mL of distilled water. One-hundred microlitre of this extract was diluted to 3 mL with water and 0.5 mL of Folin–Ciocalteu reagent was added. After 3 min, 2 mL of 20% of sodium carbonate was added and the contents were mixed thoroughly. The colour was developed and absorbance measured at 650 nm in a double beam UV–visible spectrometer after 60 min using gallic acid as a standard. The results are expressed as mg gallic acid/100 g.

Statistical analysis

The data was subjected to analysis of variance (ANOVA) for testing the significance of variation due to variety, years and their interaction for four seed quality traits following Gomez and Gomez (1984). Mean values were calculated and compared using *t* test at 5% level of significance. Genetic parameters (variance components, genotypic and phenotypic coefficients of variations and heritability) were estimated according to Singh and Chaudhari (1977). The phenotypic and genotypic correlations were calculated based on the phenotypic and genotypic variance and covariance values estimated through analysis of variance and covariance, respectively for traits under study.

Results and discussion

The results of evaluation of 26 blackgram genotypes in two rainy/*kharif* seasons (2014 and 2015) indicated that the effect due to genotypes was significant ($P \leq 0.01$) for all the quality traits indicating presence of enough variability among the genotypes for the traits under study (Table 1). The Fe content ranged from 71.02 to 100.20 ppm (Table 2). The average iron content for blackgram genotypes was 88.36 ppm. Maximum Fe content was recorded in genotype SHEKHAR 2 (100.2 ppm) followed by YAKUBPUR EARLY (98.99 ppm), MASH-114 (97.91 ppm), PU-31 (97.54 ppm) and PDU-1 (94.79 ppm). Minimum Fe content was recorded in genotype IPU 99-123

(71.02 ppm) followed by UH-85-3 (78.63 ppm). In general the Fe content of the genotypes remained stable over the years and did not varied significantly from one year to other. The Zn content among genotypes ranged from 18.93 to 60.58 ppm with an average value of 39.6 ppm. Maximum Zn content was found in genotype SHEKHAR 2 (60.58 ppm), whereas minimum Zn content was observed in IPU 99-209 (18.93 ppm). Blackgram genotypes SHEKHAR 2, PDU-1, PU-31 had high iron (94.79–100.2 ppm) as well as high zinc content (50.25–60.58 ppm). Earlier studies on mineral contents of legumes indicated that the levels of Fe and Zn in legumes generally range between 18.8–82.4 $\mu\text{g Fe g}^{-1}$ and 32.6–70.2 $\mu\text{g Zn g}^{-1}$ and lentil has the highest iron (122 $\mu\text{g g}^{-1}$), and zinc content of 48 $\mu\text{g g}^{-1}$ (Cabrera et al. 2003). In a recent report (Jajda et al. 2015), the levels of zinc and iron was analyzed in 27 samples of blackgram using atomic absorption spectroscopy (AAS) and inductively coupled plasma mass spectroscopy (ICP-MS). Iron content was found to vary from 88.8–221.9 ppm by ICP-MS to 86.1–212.5 ppm by AAS with an average value of 142.2 ppm by ICP-MS and 143.5 ppm by AAS. Zinc content was found to vary from 32.9 to 50.0 ppm analyzed by ICP-MS and 34.1–54.9 ppm by AAS with an average value of 43.1 ppm by ICP-MS and 43.7 ppm by AAS. The average values for Fe (142.2 ppm) as reported by Jajda et al. (2015) is higher than the values found in the present study for Fe (88.36 ppm), however the average values for Zn (43.1 ppm) reported in the same study is very close to average values (39.6 ppm) found in the present study. The phytic acid content among genotypes varied significantly ($P \leq 0.01$; Table 1). The effect due to years as well as the genotype $\times$ year interaction was also significant. An earlier study (Suneja et al. 2011) also reported significant variation for phytic acid among these genotypes. In our study we found that the mean phytic acid content during 2014 was high (0.32%) as compared to 2015 (0.1%). During 2014, maximum phytic acid content (0.63%) was recorded in Nodai Urd, and PKG U 03, whereas minimum phytic acid content was recorded in IPU-99-200 (0.06%) and IPU-7-3 (0.09%). During 2015, maximum value for phytic acid (0.22–0.24%) was recorded

Table 1 ANOVA for quality traits in 26 genotypes of blackgram

Source	Mean sum of square				
	<i>df</i>	Fe	Zn	Phytic acid	Polyphenols
Replications	2	0.45	2.40	0.00	1.38**
Genotype	25	255.66**	864.57**	0.05**	3.22**
Year	1	2.80	32.89**	1.97**	197.66**
Genotype $\times$ year	25	13.06**	8.84**	0.05**	4.95**
Error	102	3.24	2.43	0.00	0.10

** Significant at $P = 0.01$

Table 2 Mean values for quality traits in 26 genotypes of black gram

Genotypes	Fe (ppm)#			Zn (ppm)			Phytic acid (%)			Polyphenols (mg/100 g)		
	2014	2015	Mean	2014	2015	Mean	2014	2015	Mean	2014	2015	Mean
IPU-99-200	83.50 ^h	91.10 ^{efg}	87.30	45.91 ^{de}	49.10 ^d	47.51	0.06 ^o	0.11 ^d	0.08	7.63 ^b	9.06 ^{cd}	8.35
COBG-653	93.89 ^{cd}	90.56 ^{efgh}	92.23	38.45 ^h	40.54 ^f	39.49	0.54 ^c	0.21 ^b	0.37	4.37 ⁿ	7.67 ^{fg}	6.02
IPU-2-43	83.21 ^h	89.47 ^{fgh}	86.34	33.44 ^{jk}	31.51 ^g	32.48	0.29 ^{gh}	0.16 ^c	0.22	5.57 ^h	7.67 ^{fg}	6.62
SHEKHAR2	99.53 ^a	100.86 ^a	100.20	59.64 ^a	61.51 ^a	60.58	0.43 ^d	0.11 ^d	0.27	5.17 ^{ijkl}	8.60 ^{cdef}	6.88
YAKUBPUR2	84.82 ^{gh}	88.16 ^{hij}	86.49	50.56 ^c	55.54 ^b	53.05	0.13 ⁿ	0.06 ^{efg}	0.10	4.97 ^{kl}	10.33 ^b	7.65
SPS32	90.94 ^{de}	88.55 ^{ghi}	89.75	46.73 ^d	50.77 ^{cd}	48.75	0.53 ^c	0.03 ^h	0.28	5.00 ^{jkl}	8.67 ^{cdef}	6.83
NP03	86.28 ^{gh}	83.93 ^{jk}	85.11	34.32 ^{ij}	38.31 ^f	36.32	0.59 ^b	0.07 ^{ef}	0.33	6.23 ^{ef}	8.17 ^{ef}	7.20
IPU 94-11	90.39 ^{ef}	90.38 ^{fgh}	90.39	29.43 ^l	27.14 ^h	28.28	0.24 ^{ij}	0.05 ^{fgh}	0.15	5.37 ^{hij}	7.43 ^{gh}	6.40
IPU-7-3	87.31 ^g	89.77 ^{fgh}	88.54	33.19 ^{jk}	31.21 ^g	32.20	0.09 ^o	0.04 ^{gh}	0.06	5.50 ^{hi}	9.20 ^c	7.35
NODAI URD	86.36 ^{gh}	88.65 ^{fghi}	87.50	55.05 ^b	52.06 ^c	53.55	0.63 ^a	0.05 ^{fgh}	0.34	4.57 ^{mn}	9.07 ^{cd}	6.82
PDU-1	96.20 ^{bc}	93.31 ^{de}	94.79	56.52 ^b	59.35 ^a	57.94	0.20 ^{kl}	0.04 ^{gh}	0.12	6.30 ^{def}	10.03 ^b	8.17
IPU-96-12	86.80 ^g	86.37 ^{hij}	86.59	36.11 ^{hi}	32.25 ^g	34.18	0.55 ^c	0.05 ^{fgh}	0.30	5.27 ^{hijk}	8.67 ^{cdef}	6.97
PKG U 03	84.40 ^{gh}	82.41 ^{kl}	83.41	31.45 ^{kl}	32.97 ^g	32.21	0.63 ^a	0.04 ^{gh}	0.33	5.27 ^{hijk}	8.10 ^{ef}	6.68
SPS-39	85.31 ^{gh}	82.44 ^{kl}	83.87	30.27 ^l	32.44 ^g	31.36	0.21 ^{jkl}	0.06 ^{efg}	0.13	4.80 ^{lm}	8.50 ^{def}	6.65
IPU-99-123	72.33 ^j	69.71 ⁿ	71.02	34.51 ^{ij}	32.60 ^g	33.56	0.17 ^{lm}	0.04 ^{gh}	0.11	5.27 ^{hijk}	6.50 ^{ij}	5.88
PU-31	97.82 ^{ab}	97.26 ^b	97.54	50.13 ^c	50.37 ^{cd}	50.25	0.15 ^{mn}	0.24 ^a	0.20	6.83 ^{cd}	11.23 ^a	9.03
UH-85-3	78.38 ⁱ	78.87 ^m	78.63	43.58 ^{efg}	48.25 ^d	45.92	0.22 ^{jk}	0.12 ^d	0.17	6.00 ^g	8.81 ^{cde}	7.41
GP-215	83.48 ^h	85.30 ^j	84.39	22.43 ^m	21.67 ^{ijk}	22.05	0.18 ^{klm}	0.05 ^{fgh}	0.11	6.63 ^{cd}	7.40 ^{gh}	7.02
RTM-1	94.35 ^c	94.17 ^{cd}	94.26	22.40 ^m	22.90 ^{ijk}	22.65	0.34 ^e	0.17 ^c	0.26	5.20 ^{hijk}	6.70 ^{ij}	5.95
BLB-67-2	87.51 ^{fg}	88.47 ^{fghi}	87.99	43.22 ^{fg}	44.75 ^e	43.98	0.31 ^{efg}	0.11 ^d	0.21	6.13 ^{ef}	8.10 ^f	7.12
IPU-96-7	87.37 ^{fg}	82.44 ^k	84.91	45.74 ^{def}	48.32 ^d	47.03	0.27 ^{hi}	0.04 ^{gh}	0.16	6.00 ^g	7.03 ^{hi}	6.52
IPU-94-2	87.17 ^g	85.57 ^{ij}	86.37	44.50 ^{efg}	44.45 ^e	44.48	0.22 ^{jk}	0.22 ^{ab}	0.22	5.37 ^{hij}	6.80 ^{hij}	6.08
YAKUBPUR EARLY	99.61 ^a	98.37 ^{ab}	98.99	42.13 ^g	43.20 ^e	42.66	0.30 ^{fgh}	0.06 ^{ef}	0.18	6.43 ^{de}	7.01 ^{hi}	6.72
MASH-114	99.19 ^{ab}	96.62 ^{bc}	97.91	22.41 ^m	24.72 ^{hi}	23.57	0.33 ^{ef}	0.03 ^h	0.18	6.13 ^{ef}	8.25 ^{def}	7.19
MS-131	91.17 ^{de}	90.49 ^{fgh}	90.83	21.00 ^{mn}	20.43 ^{jk}	20.72	0.41 ^d	0.24 ^a	0.32	8.30 ^a	5.63 ^k	6.97
IPU-99-209	83.54 ^h	80.76 ^{lm}	82.15	18.59 ⁿ	19.26 ^k	18.93	0.33 ^{ef}	0.07 ^{ef}	0.20	8.10 ^a	6.30 ^j	7.20
Mean	88.50	88.23		38.14	39.06		0.32	0.10		5.86	8.11	
SE(±)	1.11	0.98		0.91	0.9		0.01	0.01		0.14	0.22	
C.D. (0.05) (individual years)	3.15	2.78		2.59	2.57		0.03	0.02		0.39	0.63	
C.D.(0.05) (over years)												
Genotype			2.06 ^{**}			1.78 ^{**}			0.02 ^{**}			0.37 ^{**}
Year			NS			0.49 ^{**}			0.01 ^{**}			0.10 ^{**}
Year × genotype			2.91 ^{**}			2.52 ^{**}			0.03 ^{**}			0.52 ^{**}
C.V.(%)			2.04			4.03			7.93			4.60

Values in bold are mean for two years

NS = Non significant

** Significant at $P = 0.01$ # Means followed by the same letter in a column do not differ significantly at $P = 0.05$

in genotypes PU-31, MS-31 and IPU 94-2, while most of the other genotypes showed phytic acid content in low range (0.03–0.11%). The mean of 2 years for phytic acid content in blackgram genotypes ranged from 0.06 to 0.37% (Table 2). On the basis of mean of 2 years, maximum phytic acid content was found in COBG 653, Nodai Urd, NP 03 and PKG U 03. Minimum Phytic acid content was

observed in IPU-7-3 (0.06%) followed by IPU-99-200 (0.08%), YAKUBPUR 2 (0.1%), IPU-99-123 and GP-215 (0.11%). The mean values for phytic acid as observed in the present study for 26 genotypes in the year 2014 (0.32%), is very close to the phytic acid values (0.33–0.41%) reported for *Vigna mungo* genotypes in an earlier study (Sorin et al. 2010), however it is lower than

the values reported in other studies (Duhan et al. 1989; Chitra et al. 1995), where the phytic acid content of various blackgram cultivars was higher and showed a narrow variation (0.64–0.66 and 1.29–1.54% respectively) amongst the blackgram genotypes. The variability in phytic acid content of other grain legumes was also studied and it was reported that the phytic acid content of blackgram (13.7 mg/g) was higher than pigeon pea (12.7 mg/g), mung bean (12.0 mg/g) and chickpea (9.6 mg/g). In general, legumes contain varying amounts of polyphenols and generally the amounts are higher in the coloured seeds (Salunkhe et al. 1982). The mean values of 2 years for polyphenol content in blackgram genotypes ranged from 5.88 to 9.03 mg/g (Table 2). The maximum polyphenol content was found in PU 31 (9.03 mg/g) whereas, minimum polyphenol content was recorded in IPU-99-123 (5.88 mg/g). The mean phenol content recorded during 2014 was low (5.86 mg/g) as compared to 2015 (8.11 mg/g). The values for phenol content as recorded in this study are very comparable to the values reported in earlier study (Duhan et al. 1989). However, it is lower than the values reported in another study (Muhammad et al. 2014) (75–86 mg GAE/g). The total phenolic content of blackgram genotypes as reported in the present study is also within the range of phenolic content reported for other legumes like lentil (12 mg/g), chickpea (2.2 mg/g), soybean (2.3 mg/g), yellow peas (2.5 mg/g) and green peas (1.2 mg/g; Han and Baik 2008). Another report (Xu and Chang 2007) also shows that lentil has the highest phenolic content (6.56 mg gallic acid equivalents/g), whereas, in chickpea the value ranges from 0.92 to 1.68 mg gallic acid equivalents/g. Further we have also noticed that blackgram genotype IPU-99-123, which showed minimum polyphenol content (5.88 mg/g) also had low phytic acid content (0.11%), whereas, genotype IPU 99-200 and Yakubpur 2 had low phytic acid content (0.08–0.1% respectively) but had high polyphenol content (8.35 and 7.65 mg/g respectively).

Several studies have demonstrated the negative effect of phytate on Zn and Fe absorption. A recent study on pearl millet showed that Fe was chelated by phytates and insoluble fibers, whereas Zn was almost exclusively chelated by phytates. Similarly, in the case of higher tannin content, the chelating effect of tannin was higher than that of phytates (Lestienne et al. 2005). Studies using in vitro model of iron bioavailability have also shown that even after reduction of the phytate content of staple foods such as sorghum and millets, iron bioavailability remains low in the presence of polyphenolic compounds (Udayasekhara and Deosthale 1988).

In the present study, the Fe content of the genotypes remained stable over the years and did not vary significantly from one year to other indicating high level of

consistency across the two environments for Fe content, whereas, the genotype $\times$ Year interaction was significant for other traits (Table 1) indicating that the blackgram varieties under study show differential reaction to the expression of those quality characters over years. The effect due to years was significant for Zn content, phytic acid and polyphenols indicating that these components vary over years.

The genotype $\times$ year interaction was significant for Zn, phytic acid and polyphenol contents indicating that the blackgram varieties under study show differential reaction to the expression of these quality characters over years. Significant effect of growth, phenology and variation in temperature, relative humidity and rainfall have been reported earlier on grain Fe and Zn in wheat (Zhang et al. 2010). In the present study, the experiments were sown in different fields of the research farm during both the years where the fertility status might have differed and also year 2015 was comparatively dry (Central India and East and Northeast India received below normal rainfall (85 and 87% of its LPA respectively—Annual Climate Summary, 2015, IMD Pune) as compared to 2014, and the low soil moisture as well as different fertility status might be the reasons for variation in the quality traits.

The phenotypic coefficients of variability were higher than corresponding genotypic coefficients of variability for all the traits which clearly indicated the effect of environment upon quality traits (Table 3). Highest estimate of phenotypic coefficients of variability was observed for phytic acid (57.58) followed by Zn (31.19), polyphenols (14.45) and Fe (7.51) content. Highest estimate of genotypic coefficients of variability was observed for Zinc (31.05) followed by phytic acid (29.18), polyphenols (9.53) and Fe (7.33) content which indicates presence of exploitable genetic variability for these traits. Heritability and genetic advance as per cent of mean provides a clear picture of the scope for improvement in various quality traits through selection. The heritability (h^2) was very high for Fe (0.95) and Zn (0.99) content indicating that a major part of variation observed in the character was due to genotypic effect. Recently high broad-sense heritability (h^2_{bs}) for grain Fe (65–71.2%) and Zn (65–80%) as well as narrow-sense heritability (h^2_{ns}) for grain Fe (80%) and Zn (77%) has been reported in pearl millet (Gupta et al. 2009; Velu et al. 2007), which also indicates that substantial portion of total variation for Fe/Zn is due to genetic effects. Low estimates of heritability (h^2) for polyphenol (0.44) and Phytic acid (0.26) content indicated large environmental influence on these traits. Thus due to presence of large variability coupled with high heritability, high genetic advance is expected for zinc content (66.66%). Moderate genetic advance is expected for phytic acid content (30.47%) with low heritability. Lower genetic advance is

Table 3 Genetic components for quality traits in blackgram genotypes

Traits	Mean	Range	Coefficient of variation		Heritability (broad sense)	Expected genetic advance as % of mean
			Phenotypic (PCV)	Genotypic (GCV)		
Fe (ppm)	88.37	71.02–100.20	7.51	7.33	0.95	14.72
Zn (ppm)	38.61	18.93–60.58	31.19	31.05	0.99	63.66
Phytic acid (%)	0.17	0.06–0.32	57.58	29.18	0.26	30.47
Polyphenols (mg/g)	7.32	6.03–9.57	14.45	9.53	0.44	12.96

Table 4 Estimates of genotypic (r_G) and phenotypic (r_P) correlation coefficient among quality traits in blackgram genotypes

Characters	Zn (ppm)	Phytic acid (%)	Polyphenols (mg/g)
Fe (ppm)			
r_G	0.234*	0.363**	0.330**
r_P	0.226*	0.146	0.203
Zn (ppm)			
r_G		−0.049	0.624**
r_P		−0.006	0.416**
Phytic acid			
r_G			−0.041
r_P			−0.438**

** Significant at $P = 0.01$; * Significant at $P = 0.05$

expected for Fe (14.72) content with high heritability and polyphenol content (12.96%) with low heritability.

The genotypic and phenotypic correlation coefficients for the four major traits in blackgram are presented in Table 4. Fe content in blackgram genotypes showed significant positive genotypic ($r_G = 0.234$) and phenotypic correlation ($r_P = 0.226$) with Zn content. However, at genotypic level in addition to Zn, iron showed positive correlation with phytic acid ($r_G = 0.363$) and polyphenol content ($r_G = 0.330$) as well. This indicates that although the traits are genotypically correlated, the expression for phytic acid as well as polyphenols is masked by the environmental influence. In an earlier study (29), the Fe and Zn contents were reported to be positively and highly significantly correlated ($r = 0.49$ – 0.71 ; $P < 0.01$) in pearl millet germplasm. Positive correlations between these two minerals have also been reported in major cereals like pearl millet (Bürger et al. 2014), maize (Long et al. 2004), sorghum (Reddy et al. 2005), rice (Kabir et al. 2003) and wheat (Gomez-Becerra et al. 2010). These results imply that simultaneous selection for high levels of both Fe and Zn contents are possible in those crops. Further in the present study, zinc content showed negative correlation ($r_G = -0.049$ and $r_P = -0.006$) with phytic acid and highly significant positive correlation with polyphenol content ($r_G = 0.624$ and $r_P = 0.416$) at both phenotypic

and genotypic level (Table 3). The phytic acid also showed negative correlation ($r_G = -0.041$ and $r_P = -0.438$) with polyphenol content, which clearly implies that with an increase in phytic acid content, there is a corresponding decrease in the polyphenol content.

Identification of promising genotypes

Superior accessions of black gram for each of the four traits have been identified (Table 5). The genotype PDU-1 possessed high level of Fe and Zn with low level of phytic acid while IPU 94-11 possessed high level of Fe with low level of phytic acid as well as polyphenols. Genotype IPU-96-7 had high level of Zn and low level of phytic acid and polyphenols. SHEKHAR 2, PU-31 and SPS32 possessed high level of Fe and Zn. YAKUBPUR EARLY, RTM-1 and COBG-653 had higher level of Fe while YAKUBPUR2, IPU-99-200, UH-85-3 and IPU-94-2 had higher level of Zinc in addition to low levels of anti-nutritional factors. In the present study we have considered the blackgram genotypes with high level of Fe & Zn and low level of phytic acid and polyphenols as superior genotypes, however, more recent evidence also suggests that phytic acid exhibits antioxidant activity and protects DNA damage (Phillippy 2003), whereas the phenolic compounds besides reducing protein digestibility (Chung et al. 1998) and mineral bioavailability (Sandberg 2002), also exhibit antioxidant properties (Murphy and Hendrich 2002). It has also been reported that pulses with the highest total phenolic content exert the highest antioxidant capacity (Xu and Chang 2007).

Conclusion

The genetic variability and heritability for seed Fe and Zn content was studied. The effect of genotypes was significant for all the quality traits indicating presence of enough variability among the blackgram genotypes for the studied traits. The Genotype x Year interaction was significant for all the traits under study which indicates differential

Table 5 Promising blackgram genotypes identified for quality traits

Trait	Blackgram genotypes
High Fe and Zn	SHEKHAR 2, PU-31,SPS32
High Fe and low polyphenol	YAKUBPUR EARLY, RTM-1, COBG-653
High Zn and low phytic acid	YAKUBPUR2, IPU-99-200, UH-85-3
High Zn and low polyphenols	IPU-94-2
Low Phytic acid and polyphenols	IPU-99-123, SPS-39
High Fe and Zn and low phytic acid	PDU-1
High Fe and Low phytic acid and polyphenol	IPU 94-11
High Zn and Low phytic acid and polyphenols	IPU-96-7

reaction to the expression of quality characters over years. Fe content in blackgram genotypes showed significant positive phenotypic correlation with Zn content while at genotypic level in addition to Zn, it showed positive correlation with phytic acid and polyphenol content as well. This indicates that although the traits are genotypically correlated, the expression is masked by the environmental influence. This is further exhibited from low heritability estimates for phytic acid and polyphenol content among the genotypes. Besides this the superior accessions of black gram for each of the four traits having high Fe and Zn as well as low level of phytic acid and polyphenols have been identified. These compounds were earlier considered as antinutritional factors, however, more recent evidence, provides potential information of their antioxidant activity and their impact on health.

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