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Variability Studies in Iron and Zinc as Well as Protein Concentration in Blackgram [*Vigna mungo* (L.) Hepper] Genotypes for Improving Nutritional Quality

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ABSTRACT

Background: Micronutrients significantly impact agricultural production and are necessary for human health. Therefore, a systematic programme on identification of sources with high concentration of iron and zinc is important for overcoming the problem of micronutrient malnutrition. Therefore, present study was undertaken with the objectives (1) to find out iron and zinc concentrations and (2) to identify stable urdbean genotypes. This will be further helpful to set desirable level of iron and zinc concentrations in the upcoming urdbean varieties.

Methods: The present study was conducted to evaluate 30 urdbean genotypes for variability studies of iron, zinc and protein content. The experiment was laid out in the randomized complete block design and replicated thrice at the experimental farm of Punjab Agricultural University, Regional Research Station, Gurdaspur, Punjab (India) during *spring* 2019 and 2020.

Result: Analysis of variance showed that effect due to genotypes was significant for iron, zinc and protein content as well Iron and Zinc content ranged from (28.75 ppm to 90.75 ppm) and (44.75 ppm to 60.0 ppm) respectively, while protein content ranged from (18.7 to 25.13)%. The genotype PDU1 and OBG35 possessed high level of all three nutrients *viz*., Fe, Zn and protein. Fe content showed significant positive correlation with Zn content suggesting that both the nutrient can be improved simultaneously. Micronutrient dense genotypes identified can be used as donar to develop nutrient enriched varieties either through conventional breeding or by using biotechnological tool like marker assisted selection.

Key words: Correlation, DMRT, Genotypes, Iron, Protein, Zinc.

INTRODUCTION

Pulses are the most important food crops grown globally due to high protein content and other nutritional components. Among the major pulses, urdbean (*Vigna mungo* L. Hepper) is India's third most popular pulse crop after mungbean and chickpea in last 15 years in terms of production (Gaur, 2021). It is mainly grown in India, Thailand, Australia, as well as other parts of Asia and the South Pacific (Poehlman, 1991). In India, the urdbean occupies an acreage of 4.14 million hectares with a production of 2.23 mt and the productivity level is 538 kg/ha (Anonymous, 2020-21) while, in Punjab state of India, it is being cultivated on 2.0 thousand hectares with a production of 1.2 thousand tonnes (Anonymous, 2020-21). Urdbean is also known as blackgram, is a rich source of nutrients having 25% protein, which is nearly three times that of cereals, 1.3% fat, 60% carbohydrate (Das *et al*., 2021) and important minerals as well as vitamins (Ghafoor *et al*., 2001) making it balanced vegetarian diet when supplemented with cereals. Being a short duration crop, it is suitable for intercropping with different crops as well as it fits well in crop rotation with cereals, such as wheat or rice (Sakila and Pandiyan, 2018).

Nutritional deficiencies of some minerals in humans can lead to stunted growth and development in children, decrease resistance against diseases and increased mortality rates. Micronutrient malnutrition has received increased attention in recent decades at a global level and efforts have been made to combat them by various strategies such as increased food production, through supplementation, fortification of food and biofortification. Biofortification can be defined as increasing the bioavailable micronutrient content of food crops through genetic selection *via* plant breeding (Welch and Graham, 2004). Food crops rich in nutrients could address deficiencies of micronutrients and thus provide a sustainable solution to global health issues

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(Welch, 2002). Combating malnutrition is the most important challenges among the various global health issues of the 21st century. The World Health Organization (WHO) estimates that globally, more than 2 billion people suffer from micronutrient malnutrition, also known as "hidden hunger" (Ritchie and Roser, 2017). Researchers have highlighted the need for 22 minerals for human well-being (White and Broadley, 2009) however, 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).

Being a very popular food legume crop, it acts as one of the cheapest source of proteins and minerals in vegetarian diet. Therefore, blackgram can help to solve the problem of malnutrition among poor people, who cannot afford costly foods of animal origin. Variability is required in the germplasm to achieve success in the biofortification. Many studies have shown variation in the concentration of minerals in the crops like common bean, peas, chickpeas, lentils *etc.* (Haq *et al*., 2007; Thavarajah *et al*., 2010). In the recent years, only two study has been conducted to estimate iron and zinc concentration in urdbean. In one of the study comprising of 26 urdbean genotypes, iron concentration ranged from 71 to 100 mg/kg and zinc concentrations ranged from 19 to 61 mg/kg (Singh *et al*., 2017) while in other study comprising of 83 genotypes, iron concentration ranged from 19-235 mg/kg (mean 117 mg/kg) and 16-255 mg/kg (mean 91 mg/kg) among tested genotypes at the first and second locations, respectively. For zinc concentration it ranged from 5-134 mg/kg (mean 44 mg/kg) at first location, while at second location it was between 12-59 mg/kg (mean 29 mg/kg) (Gupta *et al*., 2020)

Therefore, a systematic programme on identification of sources with high concentration of iron and zinc is important for overcoming the problem of micronutrient malnutrition. Present study was undertaken with a diverse panel of 30 urdbean genotypes with the objectives (1) to find out iron and zinc concentrations and (2) to identify stable urdbean genotypes. This will be further helpful to set desirable level of iron and zinc concentrations in the upcoming urdbean varieties.

MATERIALS AND METHODS

Thirty urdbean genotypes have been taken in the present study were sown in the randomized complete block design (RCBD) with three replications at the experimental farm of Punjab Agricultural University, Regional Research Station, Gurdaspur, Punjab (India) during *spring* 2019 and 2020. Located at 32.02°N and 75.24°E, Gurdaspur is characterized by high rainfall, high humidity and have clay loom soil. Standard package and practices of the region were followed for raising healthy crop free from insect-pests and diseases. Each genotype was grown in two rows of 2 m row length and row to row spacing was 22.5 cm. Plant to plant distance in each row was 10 cm. Plants were harvested on attaining the physiological maturity. Randomly selected five plants were threshed individually. Seeds threshed from the five plants were used for analysis of iron, zinc and protein content. Seeds of each genotype were washed with distilled water and then dried in hot air oven. Dried seeds were grounded using sterile pestle mortar to obtain fine quality flour. Between samples pestle mortar was changed. Subsamples (100 g) of each genotype were prepared form five plants to make one composite sample for all the three replications for each year. One sample was drawn from the composite sample for quality trait analysis.

Fe and Zn extraction and quantification

Total Fe and Zn concentrations were obtained by wet-acid digestion pf dried samples with diacid mixture of HNO_3 and $HClO_4$ in the ratio of 4:1 according to the procedure described by (Piper, 1966). The digested and filtered solution was then analysed by using atomic absorption spectrophotometry (AAS), method given by (Page *et al*., 1982). Total N content was determined by micro Kjeldahl method (Westerman, 1990). The estimated concentrations of minerals were expressed in ppm and protein in percentage. The experiment was conducted in the micronutrient laboratory of Department of Soil Science, PAU, Ludhiana.

Statistical analysis

The pooled data were analysed using standard analysis of variance (ANOVA) and comparison among treatment means was made by Duncan's multiple range test (DMRT) using R software.

RESULTS AND DISCUSSION

More than 2 billion people in the world are affected by the deficiency of key micronutrients such as Fe and Zn (Huang *et al*., 2020) but being nutrionally rich, pulses provide around 18-28% protein also offer many minerals required essentially by the human beings. Researchers are focussing on crop biofortification programs which require fast, accurate and cost effective methods. The pre-requisite for initiating a breeding program to develop micronutrient rich genotypes is to screen the available germplasm and to identify the source of genetic variation for the target trait.

Iron and zinc contents in grains depend on the micronutrient uptake and translocation efficiency from root to grains (Velu *et al*., 2014). The results pertaining to 30 black gram genotypes evaluated during two *spring* seasons (2019 and 2020) indicated that the effect due to genotypes was significant ($P \leq 0.01$) for the nutrient traits studied indicating the presence of sufficient variability among the genotypes under study (Table 1). In the present investigation (Table 2 and Fig 1), Fe content ranged from 28.75 ppm to 90.75 ppm. The mean iron content in the urdbean genotypes was 72.82 ppm with a CD value of 8.53 ppm. Maximum Fe content was recorded in genotype KPU-11-39 (90.75 ppm) followed by KUG715 (87.5 ppm), KUG511 (84.5 ppm), OBG35 (84.0 ppm) and Mash218 (83.0 ppm) while, minimum

Table 1: Pooled ANOVA of Fe, Zn and Protein for two years.

Source	DF	Mean sum of square		
		Fe	Zn	Protein (%)
Replications	2	29.23	73.81	0.14
Genotype	29	553.13*	74.70*	7.79*
Year	1	1702.53*	826.88*	0.03
Yearx genotype	29	531.15*	46.29	1.38
Error	58	37.13	25.83	1.34
General mean		72.82	51.34	22.14
CV (%)		8.53	7.11	1.62

*Significant at $P \leq 0.01$.

Table 2: Duncan multiple range test (DMRT) of iron (Fe) and zinc (Zn) contents over two consecutive years (*spring* 2019 and 2020) in 30 urdbean genotypes.

Variety	Fe (ppm)	Zn (ppm)	Protein (%)
IPU2-43	73.75^{defghi}	58.75^{abc}	20.50^{jk}
IPU-94-1	64.00^{ij}	$52.75^{abcdefg}$	23.60^{abc}
IPU-94-2	81.75^{abcde}	$53.25^{abcdefg}$	19.85^{kl}
KPU-11-39	90.75^{a}	49.25^{defg}	18.7l
KPU-405	75.50^{cdefgh}	59.25^{ab}	$21.83^{cdefghij}$
KUG253	$80.75^{abcdefg}$	46.75^{efg}	23.60^{abc}
KUG384	71.25^{fghi}	50.25^{cdefg}	$21.55^{defghijk}$
KUG386	64.00^{ij}	49.25^{defg}	$22.67^{bcdefghi}$
KUG511	84.50^{abc}	49.50^{defg}	20.77^{gijk}
KUG662	67.25^{hij}	50.75^{bcdefg}	21.20^{fghijk}
KUG715	87.50^{ab}	$52.00^{abcdefg}$	20.63^{jk}
KUG718	63.50^{ij}	45.00^{g}	23.23^{bcde}
KUG719	67.50^{hij}	59.25^{ab}	23.52^{abcd}
Mash1008	76.00^{cdefgh}	$53.00^{abcdefg}$	$21.67^{cdefghijk}$
Mash114	82.50^{abcd}	49.00^{defg}	21.13^{fghijk}
Mash218	83.00^{abcd}	54.25^{abcdef}	22.75^{bcdefg}
Mash338	68.75^{hij}	56.25^{abcd}	22.75^{bcdefg}
Mash391	68.25^{hij}	54.75^{abcdef}	23.90^{ab}
Mash479	81.25^{abcdef}	$52.00^{abcdefg}$	$21.45^{efghijk}$
NDU-2-13-1	59.25^{j}	46.00^{fg}	21.02^{fghijk}
OBG35	84.00^{abcd}	60.00^{a}	23.85^{ab}
OBG36	71.75^{efghi}	47.75^{defg}	22.75^{bcdefg}
PantU-10-32	74.25^{cdefgh}	44.75^{g}	$22.67^{bcdefghi}$
PantU19	67.75^{hij}	49.25^{defg}	$22.15^{bcdefghij}$
PantU31	77.00^{cdefgh}	48.75^{defg}	23.25^{bcde}
Pathankot local	28.75^{k}	47.25^{efg}	$22.45^{bcdefghij}$
PDU1	80.00^{bcdefg}	55.00^{abcde}	25.13^{a}
PU-10-08	70.75^{ghi}	49.25^{defg}	22.83^{bcdef}
Shekhar2	79.75^{bcdefg}	50.25^{cdefg}	$22.33^{bcdefghij}$
T9	60.00^{j}	46.75^{efg}	20.58^{jk}

*Means with same letter are not significantly different ($P \leq 0.05$).

Fe content was found in genotype Pathankot local (28.75 ppm), followed by NDU-2-13-1(59.25 ppm) and T9 (60.0 ppm). The Zn content ranged from 44.75 ppm to 60.0 ppm. The mean zinc content in the urdbean genotypes was 51.34 ppm with a CD value of 7.11 ppm. Maximum Zn content was recorded in genotype OBG35 (60.0 ppm) followed by KPU405 and KUG719 (59.25 ppm), IPU2-43 (58.75 ppm) and Mash338 (56.25 ppm) while, minimum Zn content was found in genotype PantU10-32 (44.75 ppm), followed by KUG718 (45.0 ppm) and NDU-2-13-1(46.0 ppm). Pooled analysis of variance showed that effect due to year was significant while year × genotype was significant only for Fe. This suggest that Fe content was not stable and varied significantly in the genotypes. In contrast to this, (Singh *et al*., 2017) reported that Fe content was stable over the years and did not varied significantly from one year to other indicating here that there is a difference in soil properties of the test locations as reported also by (Shrestha *et al*. 2018 and Gupta *et al*., 2020). The protein content ranged from (18.7 to 25.13%). The mean protein content in the urdbean genotypes was 22.14% with a CD value of 1.62%. Maximum protein content was recorded in genotype PDU1(25.13%) followed by Mash391 (23.9%), OBG 35 (23.85%), KUG253 and IPU-94-1 (23.6%) and PantU31 (23.25%). Minimum protein content was found in genotype KPU-11-39(18.71%) followed by IPU-94-2 (19.85%) and IPU-2-43(20.5%). A considerable amount of variation for protein content was found and similar results in mungbean was reported by (Singh *et al*. 2016) where protein content ranged from 21.1% to 30.0% with a mean of 24.9 ± 0.2, Significantly positive correlation was observed between Fe and Zn (+0.268) among 30 genotypes of urdbean indicating the possibility of simultaneous selection for both the nutrients. However, nonsignificant but positive correlation was observed between Zn and protein content (+0.163). Singh *et al*. (2016) also reported similar results. Non significantly negative correlation was observed between Fe and protein content (-0.199) exist in the materials under study. This result is in accordance with findings of (Mahajan *et al*., 2015).

Superior genotypes of blackgram for each of the three traits have been identified (Table 3). The genotype PDU1 and OBG35 possessed high level of Fe, Zn and Protein while Mash 218 possessed high level of Fe and Zn, KUG253 possessed high level of Fe and protein and IPU-94-1, Mash391, Mash338 and KUG719 possessed high level of Zn and protein. These common genotypes is being selected among top 10 ranked genotypes for individual traits. Genotypes like KPU-11-39, KUG715, KUG511, OBG35,

Table 3: Promising urdbean genotypes identified for quality traits.

Traits	Blackgram genotypes
High Fe, Zn and protein	PDU1, OBG35
High Fe and Zn	PDU1, Mash218,OBG35
High Fe and protein	PDU1, KUG253,OBG35
High Zn and protein	IPU-94-1, Mash391, PDU1, Mash338, KUG719, OBG35
High Fe	KPU-11-39, KUG715, KUG511, OBG35, Mash218
High Zn	OBG35, KUG719, KPU405, IPU-2-43, Mash338
High protein	PDU1, Mash391, OBG35, KUG253, IPU-94-1
Low Fe	Pathankot local, NDU-2-13-1, T9
Low zinc	PantU-10-32, KUG718, NDU-2-13-1
Low protein	KPU-11-39, IPU-94-2, IPU-2-43

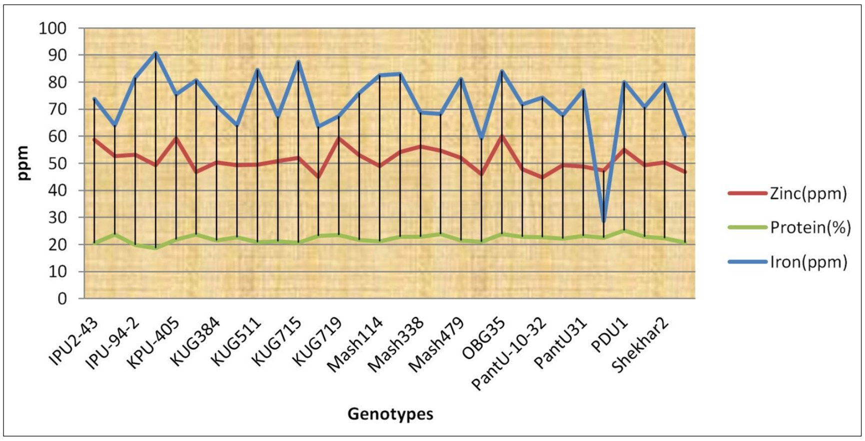

Fig 1: Iron, zinc and protein content in 30 genotypes of urdbean.

Mash218 possessed high level of Fe, while OBG35, KUG719, KPU405, IPU-2-43, Mash338 possessed high level of Zn and PDU1, Mash391, OBG35, KUG253, IPU-94-1 possessed high level of protein selected from top five ranked genotypes from the present study. To develop mapping populations genotypes possessing low level of any traits is equally important. Genotypes like Pathankot local, NDU-2-13-1, T9 possessed low level of Fe, while PantU-10-32, KUG718, NDU-2-13-1 possessed low level of Zn and KPU-11-39, IPU-94-2, IPU-2-43 possessed low level of protein.

CONCLUSION

Iron and zinc are needed for a healthy development of mankind and urdbean can play an important role in the food and nutritional security of millions, particularly in developing countries. Therefore, identification of sources with high concentration of Fe, Zn and protein is important. Literature shows comparable ranges of mineral concentration in most leguminous crop seeds like in common bean, peas, chickpeas, lentils *etc*. From the present study, it may be concluded that wide range of variability for iron (28.75 ppm to 90.75 ppm), zinc (44.75 ppm to 60.0 ppm) and protein (18.7 to 25.13)% were observed between different genotypes. The genotype × year interaction was significant only for Fe under study which indicates differential reaction to the expression of Fe over years. Fe content in blackgram genotypes showed significant positive phenotypic correlation with Zn content. The genotypes PDU1 and OBG35 possessed high level of Fe, Zn and Protein (80.0 ppm, 55.0 ppm and 25.13%) and (84 ppm, 60.0 ppm 23.85%) respectively. Mash218 possessed high level of Fe and Zn (83.0ppm and 54.25ppm) while KUG253 possessed high level of Fe and Protein (80.75 and 23.60%). The genotypes which possessed high level of Zn and protein are as follows: IPU-94-1 (52.75 ppm and 23.60%), Mash391 (54.75 ppm and 23.90%), Mash338 (56.25 ppm and 22.75%) and KUG719 (59.25 ppm and 23.52%). These results provide a useful foundation for the development of new urdbean cultivars that have high mineral content and could be used to develop more nutritious varieties of urdbean and reduce mineral element deficiencies.

Conflict of interest: None.

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