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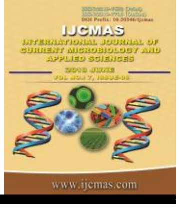

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Evaluation of Genetic Variability and Identification of Micronutrients Rich Recombinant Inbred Lines in Mungbean [*Vigna radiata* (L.) Wilczek]

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ABSTRACT

An investigation was carried out to assess the genetic variability among 70 mungbean Recombinant Inbred Lines (RILs) grown in untreated and treated ($ZnSO_4$ and $FeSO_4$) environments and to identify high micronutrient (Zn and Fe) content RILs. Significant variability among the genotypes was observed for all the characters *i.e.*, days to 50 % flowering and maturity, plant height, number of branches, number of pods, number of seeds per pod, 100-seed weight, seed and biological yield per plot, reaction to MYMV, zinc and iron content in seeds except harvest index. High estimates of GCV, heritability and genetic advance in both the environments for number of branches, seed and biological yield, reaction to MYMV, zinc and iron content in seeds suggested that these characters are controlled by additive gene action. Low GCV coupled with low heritability and low genetic advance observed for number of seeds per pod and harvest index indicated presence of non-additive gene action and high GxE interaction. About 43 % of the RILs responded positively to zinc, 71 % to iron and 29 % to both zinc and iron application. RILs having high zinc and iron contents responded less to their application as compared to those having low to moderate zinc and iron content. RIL-1, 11, 34, 37, 59, 61 and 70 were found to have significantly higher zinc content, RIL-1, 3, 7, 34, and 38 had significantly higher iron and RIL-25, 27, 54, 58, 60 and 67 had higher seed yield.

Keywords

Genetic variability, Micronutrients, Mungbean, Recombinant Inbred Lines

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Introduction

Vigna radiata (L.) Wilczek, commonly known as mungbean or greengram, is the fourth most important pulse crop of India after chick pea, pigeon pea and black gram and is grown throughout the year in all the three crop seasons in different regions of India. The annual production of mungbean is about 1.5 million tonnes from an area of about 3.02 million hectares in India (Anonymous, 2016). Being a short duration pulse crop it fits well in many cropping systems including the intensive rice-wheat cropping system of Indo-Gangetic plains of India.

It can also be grown as intercrop with sugarcane, pigeon pea, castor, maize, poplar and in orchards. It is an excellent source of dietary protein of low flatulence which complements the staple wheat and rice diet in Asia and it represents a cheap source of carbohydrates, folate and iron besides high-quality protein.

Micronutrient malnutrition is recognized as a massive and rapidly growing public health issue especially among the poor people. Micronutrient malnutrition causes several diseases and the affected people are more prone to infection by other diseases resulting in further deterioration in quality of life. Zinc and iron are among the most important micronutrients which are required to maintain metabolic regulation and organ function. Zinc acts as a stabilizer of the structures of cellular membranes and components and plays a major role in gene expression.

Its deficiency in humans reduces growth, sexual maturity and immune defence system. Iron is needed for the synthesis of oxygen transport proteins haemoglobin and myoglobin, for the formation of haeme enzymes and other iron containing enzymes responsible for energy production, immune defence and thyroid function and its deficiency in the body causes anaemia. Both zinc and iron are, therefore, essential to human well-being (Singh *et al.*, 2013).

Increasing the amount of micronutrients in plant foods for human consumption is a challenge which is important particularly for a country like India where the population mostly consumes vegetarian food. Study of inheritance of these traits through estimation of different genetic parameters could be helpful in formulation of an effective breeding programme. Considerable amount of knowledge has been generated during last two decades with regards to yield and component traits; however, very few studies have been conducted to access these parameters for micronutrients content in mungbean seed. Therefore, the present investigation was undertaken with an objective to assess the genetic parameters like variability, heritability, genetic advance and seed micronutrients (Zn & Fe) content in Recombinant Inbred Lines (RILs) of mungbean.

Materials and Methods

This field experiment was carried out in *kharif* 2015 at the Pulses Research Area of Department of Genetics and Plant Breeding and zinc and iron content in soil and seed samples were estimated in the laboratory of Department of Soil Sciences, Chaudhary Charan Singh Haryana Agricultural University, Hisar. The experimental material for the present investigation comprised of 70 mungbean RILs in F_6 generation, their two parents (ML 776 and MH 2-15) and three popular cultivated varieties of mungbean as yield checks (MH 1-25, MH 421 and MH 318). Mungbean genotype ML 776 is having high zinc and iron content in seeds and MH 2-15 is a high yielding MYMV resistant released variety of mungbean which has low zinc and iron content (Taunk *et al.*, 2012). The experiment was conducted in two sets, untreated one with Recommended Doses of Fertilizer (RDF) only and treated one with RDF + 25 kg/ha $ZnSO_4$ as basal dose and 0.5% solution of $FeSO_4$ as foliar spray at flowering stage. Both the sets of the experiment were laid out in Randomized Block Design (RBD) with three replications. All the genotypes were grown in a plot size of 2 rows × 2 m. The row to row distance was kept 30 cm and plant to plant distance 10 cm.

The observations were recorded as the means from five randomly selected plants from each genotype in each replication for plant height, number of branches per plant, number of pods per plant and number of seeds per pod. Other traits *viz*., days to 50% flowering, days to maturity, 100-seed weight, seed yield per plot, biological yield per plot, harvest index, reaction to Mungbean Yellow Mosaic Virus (MYMV) were determined on plot basis. For recording incidence of MYMV, 1 to 9 ratings scale was used (Anonymous, 2016). Atomic Absorption Spectrophotometer (AAS) analysis of Benton-Jones (1989) based on nitric/perchloric acid digestion was followed to estimate the zinc and iron concentration in mungbean seeds of all the genotypes.

The data collected for each quantitative trait mentioned above were subjected to analysis of variance. The phenotypic and genotypic variances and their coefficients were estimated according to the methods suggested by Burton and Devane (1953), whereas estimation of heritability and estimation of expected genetic advance were computed using the formula suggested by Hanson *et al.,* (1956) and Johnson *et al.,* (1955), respectively.

Results and Discussion

The knowledge of nature and extent of genetic variability for various characters are essential in planning the breeding strategies. Analysis of variance of both the environments indicated that mean squares due to genotypes were significant for all the characters studied *viz.* days to 50 % flowering, days to maturity, plant height, number of branches per plant, number of pods per plant, number of seeds per pod, 100-seed weight, seed yield per plot, biological yield per plot, reaction to MYMV, zinc and iron content in seeds except harvest index revealing thereby presence of considerable amount of variability among the RILs of mungbean for all the characters. Similar findings were reported by Khedar *et al.,* (2006), Arshad *et al.,* (2009), Tabasum *et al.,* (2010), Rathor *et al.,* (2015) and Om Vir and Singh (2016) in mungbean for various traits.

All these characters were compared for their performance under untreated and treated environments using Independent t-test (at $p < 0.05$ significance) which revealed that plant height, number of branches per plant, 100-seed weight, seed yield per plot, biological yield per plot, zinc and iron content in seeds were the traits which were affected by the application of zinc and iron. Our findings are in partial confirmation of the previous studies of Samreen *et al.,* (2013) and Singh *et al.,* (2013).

Phenotypic (PCV) and genotypic (GCV) coefficients of variation were worked out for all the traits studied to make further assessment of heritable and non-heritable components in the total variability observed. The GCV and PCV were categorized as low (<10 %), moderate (11-20 %) and high (>20 %) as suggested by Shivasubramanian and Menon (1973). Narrow to medium differences between the genotypic and phenotypic coefficients of variation for different traits under both the environments (Table 1) indicated low to moderate influence of environment in the expression of these traits. The range of PCV for different traits was observed from 4.59 (days to maturity) to 54.52 % (iron content) and from 4.46 (days to maturity) to 39.09 % (reaction to MYMV) under untreated and treated environments, respectively, whereas the range of GCV was 3.91 (days to maturity) to 45.58 % (iron content) and from 3.67 (days to maturity) to 35.04 % (reaction to MYMV) under untreated and treated environments, respectively. The presence of wide range of PCV and GCV revealed the larger extent of phenotypic and genetic variability.

High PCV was observed for iron content, reaction to MYMV, zinc content, seed yield per plot, biological yield per plot, number of pods per plant and number of branches per plant, whereas harvest index, number of seeds per pod and plant height exhibited moderate PCV and 100-seed weight, days to 50 % flowering and days to maturity exhibited low PCV under both untreated and treated environments. High magnitude of GCV was observed for iron content, reaction to MYMV, zinc content, seed yield per plot, biological yield per plot, number of branches per plant under both untreated and treated environments and number of pods per plant only under untreated environment, however, characters *viz*. harvest index, plant height and number of seeds per pod depicted moderate GCV and 100-seed weight, days to 50 % flowering and days to maturity had low GCV under both untreated and treated environments. These observations are relatable to the findings of Gadakh *et al.*, (2013), Jyothsna and Anuradha (2013) and Niharika *et al.*, (2014). Characters like days to 50 % flowering, days to maturity, plant height, number of seeds per pod, 100-seed weight and harvest index have shown low to moderate GCV and PCV which signifies lesser likelihood of much improvement through selection in these traits. These results are in agreement with the findings of Khedar *et al.*, (2006) and Rathor *et al.*, (2015) but show disagreement to the findings of Dhananjay *et al.*, (2009) and Tiwari *et al.*, (2014).

Heritability (broad sense) and genetic advance estimates for different traits were computed to find out the extent of heritable nature of variability and expected genetic gain under selection. Heritability estimates were grouped as low (<30 %), moderate (30-60 %) and high (>60 %) and genetic advance as per cent of mean as low (<10 %), moderate (10-20 %) and high (> 20 %) as suggested by Johnson *et al.*, (1955). Heritability (broad sense) under untreated environment ranged from 40.55 to 83.23 % and under treated environment 48.53 to 81.40 %. Genetic advance as per cent of mean ranged from 6.88 to 65.53 % and 6.22 to 64.70 % under untreated and treated environments, respectively.

High heritability estimates coupled with high genetic advance were recorded for plant height, number of branches per plant, number of pods per plant, seed and biological yield per plot, reaction to MYMV, zinc and iron content in seeds. The high heritability and genetic advance estimates are pleasant indication for the success in selection. This indicated the predominance of additive gene action in the expression of all the listed traits and these traits are likely to respond effectively to phenotypic selection (Johnson *et al.*, 1955). Cognate results were obtained by Arshad *et al.,* (2009), Suresh *et al.,* (2010), Reddy *et al.*, (2011), Prakash and Shekhawat (2012), Gadakh *et al.*, (2013), Singh *et al.*, (2014) and Kumar and Katiyar (2015) but different results were obtained by Begum *et al.*, (2012) and Niharika *et al.*, (2014). Low to moderate heritability and genetic advance estimates were observed for number of seeds per pod and harvest index which indicated that these characters are influenced by environment and can be improved by rigorous selections in large segregating populations, especially early generations. Tabasum *et al.,* (2010) and Begum *et al.*, (2012) obtained analogous results for these characters while, Gadakh *et al.,* (2013), Jyothsna and Anuradha (2013) and Tiwari *et al.*, (2014) obtained dissimilar ones.

Genotypic coefficient of variation together with heritability estimates would give the best indication of the amount of gain due to selection. Number of branches per plant, seed and biological yield per plot, reaction to MYMV, zinc and iron content in seeds had high GCV and corresponding heritability and genetic advance. This conveys that these characters are genetically controlled by additive gene action and can be improved through selection. Arshad *et al.,* (2009), Gadakh *et al.*, (2013) and Raturi *et al.*, (2015) found similar results while Dhananjay *et al.*, (2009) and Tabasum *et al.,* (2010) reported conflicting results for these traits. Number of seeds per pod and harvest index exhibited low GCV and low heritability coupled with low genetic advance. This grants a point that these characters are governed by non-additive gene action and there is high genotype and environment interaction.

Table.1 Estimates for mean, range, GCV, PCV, heritability and genetic advance for different characters in mungbean RILs under untreated and treated environments

Characters	Mean		Range		GCV (%)		PCV (%)		Broad sense heritability (%)		Genetic advance as % of mean	
	UT	**T**	**UT**	**T**	**UT**	**T**	**UT**	**T**	**UT**	**T**	**UT**	**T**
Days to 50 % flowering	35.0	34.7	30.3-40.3	30.3-40.0	7.00	6.53	7.75	7.35	81.66	78.90	13.03	11.96
Days to maturity	58.9	58.3	53.7-62.3	53-61.3	3.91	3.67	4.59	4.46	72.87	67.70	6.88	6.22
Plant height (cm)	40.6	42.2	28.4-49.5	28.6-52.4	11.55	11.27	13.13	12.64	77.46	79.50	20.94	20.71
No. of branches per plant	3.0	3.2	1.8-4.4	2.0-4.8	21.09	22.74	24.13	25.21	76.44	81.40	37.99	42.26
Number of pods per plant	10.9	11.1	6-15.7	6-15	20.86	19.74	25.96	22.32	64.61	78.20	34.55	35.96
Number of seeds per pod	7.5	7.6	5.7-10	6-10.3	11.08	11.57	15.33	15.21	52.26	57.80	16.49	18.13
100-seed weight (g)	3.50	3.66	3.08-4.63	3.15-4.66	8.78	8.44	9.62	9.45	83.23	79.90	16.49	15.55
Seed yield per plot (g)	83.9	90.8	46.5-138.3	48.6-146.7	23.85	23.79	29.74	28.02	64.35	72.15	39.20	38.94
Biological yield per plot (g)	501	539	269-798	293-827	22.37	23.22	26.99	28.96	68.74	64.29	38.19	30.75
Harvest index (%)	16.8	16.92	13.64-21.39	14.48-22.30	11.90	13.43	18.69	19.28	40.55	48.53	9.89	13.43
Reaction to MYMV	3.9	4.1	1-8	2-8	32.64	35.04	37.57	39.09	75.52	80.30	58.44	64.70
Zinc content (mg/kg seed)	21.68	25.69	9.8-45.4	11.8-65.4	31.29	32.27	36.13	36.66	75.02	77.50	48.36	51.51
Iron content (mg/kg seed)	63.65	85.82	13.8-168.9	25.3-178.4	45.58	31.90	54.52	38.33	69.87	69.27	65.53	45.52

UT- Untreated environment; T- Treated environment

Table.2 Assortment of mungbean RILs, parents and checks based on micronutrients content and their response to Zn and Fe application

Micro-nutrient	Category	Response	Genotypes	Total genotypes	Total RILs	Proportion of RILs (%)
Zinc	Low to moderate content in seeds (<30 mg/kg seed)	Positive	RIL-2, 3, 4, 5, 6, 8, 10, 12, 16, 18, 19, 20, 21, 22, 24, 30, 31, 32, 35, 38, 45, 48, 49, 52, 54, 55, 60, 62, 63, MH 2-15, MH 1-25, MH 421, MH 318	63 RILs 1 Parent 3 Checks	29	46
		Neutral	RIL-7, 9, 13, 14, 15, 17, 23, 25, 26, 27, 28, 29, 33, 36, 39, 40, 41, 42, 43, 44, 46, 47, 50, 51, 53, 56, 57, 58, 64, 65, 66, 67, 68, 69		34	54
	High content in seeds (>30 mg/kg seed)	Positive	RIL-1, ML 776	7 RILs 1 Parent	1	14
		Neutral	RIL-11, 34, 37, 59, 61, 70		6	86
Iron	Low to moderate content in seeds (<100 mg/kg seed)	Positive	RIL-2, 9, 11, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 30, 31, 35, 36, 37, 39, 43, 44, 45, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, MH 2-15, MH 1-25, MH 421, MH 318	56 RILs 1 Parent 3 Checks	42	75
		Neutral	RIL-8, 12, 28, 29, 40, 41, 42, 47, 49, 51, 52, 53, 54, 55		14	25
	High content in seeds (>100 mg/kg seed)	Positive	RIL-3, 4, 5, 7, 10, 32, 33, 34, ML 776	14 RILs 1 Parent	8	57
		Neutral	RIL-1, 6, 38, 46, 48, 50		6	43
Zinc and Iron		Positive	RIL-2, 3, 4, 5, 10, 16, 18, 19, 20, 21, 22, 24, 30, 31, 32, 35, 45, 60, 62, 63, ML 776, MH 2-15, MH 1-25, MH 421, MH 318	20 RILs 2 Parents 3 Checks	20	21
		Neutral	RIL-28, 29, 40, 41, 42, 46, 47, 50, 51, 53	10 RILs	10	14

Similar results were reported by Om Vir and Singh (2016) while Gadakh *et al.*, (2013) and Jyothsna and Anuradha (2013) obtained contradictory results. High heritability coupled with high genetic advance but low to moderate GCV estimates were obtained for plant height and number of pods per plant. This unfolded that the above listed traits have lesser variability even then they can be improved through selection. This result is aided by the findings of Dhananjay *et al.*, (2009) and Om Vir and Singh (2016). Further, days to 50 % flowering, days to maturity and 100-seed weight had high heritability coupled with low genetic advance and low to moderate GCV which were also observed by Om Vir and Singh (2016).

From the above discussions, it can be concluded that number of branches per plant, number of pods per plant and biological yield per plot should be kept in mind while selection is to be practiced for high seed yield. Besides, plant height should also be considered while performing selection for high seed yield. However, some differences are obvious in this respect which may be due to the facts that heritability may differ from crop to crop, cross to cross, location to location, year to year and even in the same year from method to method of heritability estimation.

Among the 70 RILs, some were more responsive to zinc and iron application, some were less and other didn't respond at all (Table 2). About 43 % RILs responded positively to zinc application and about 71 % to iron application and exhibited more than 20 % increase in their content in seeds under treated environment. Only 29 % RILs responded to the application of both the micronutrients (Zn and Fe). There were only 10 RILs (about 14 %) which didn't show any response to either of the micronutrient application. RILs having high zinc (>30 mg/kg seed) and high iron (>100 mg/kg seed) contents responded less to the zinc and iron applications as compared to the RILs having low to moderate zinc (<30 mg/kg seed) and iron (<100 mg/kg seed) content. Only about 14 % of the high zinc content RILs responded positively to zinc application, whereas there were 46 % of low to moderate zinc content RILs which responded positively to zinc application. Similarly, among the high iron content RILs 57 % responded positively to iron application, whereas 75 % of the low to moderate iron content RILs responded positively to iron application. Both the parents and all the checks responded positively to zinc and iron application except for parent ML 776 which didn't respond to only iron application.

Maximum zinc content (45.4 mg/kg seed) was found in RIL-61 followed by RIL-1 (40.5 mg/kg seed), RIL-11 (36.4 mg/kg seed), RIL-37 (32.9 mg/kg seed) and RIL-59 (32.3 mg/kg seed) and maximum iron content (168.9 mg/kg seed) in RIL-1 followed by RIL-3 (125 mg/kg seed), RIL-38 (122 mg/kg seed), RIL-37 (121 mg/kg seed) and RIL-7 (118 mg/kg seed) under untreated environment. Response to zinc application was observed maximum in RIL-1 (61 %) among the high zinc content RILs. Likewise, response to iron application was highest in RIL-5 (64 %) among the high iron content RILs. High seed yield per plot was bagged from RIL-25 (125.7 g), RIL-54 (126.3 g), RIL-58 (126.7 g), RIL-60 (123.9 g) and RIL-67 (138.3 g). RIL-1 was observed to have high seed yield (100.5 g per plot) along with high zinc (40.5 mg/kg seed) and iron (168.9 mg/kg seed) content in seeds.

The present study, intended to examine the genotypic variation in yield traits and micronutrients content in mungbean RILs and identification of superior RILs, will be of great use and will serve as a stepping stone in formulation and effective execution of future breeding programmes on biofortification in mungbean. To initiate a systematic breeding programme and to develop mungbean varieties with combination of high seed yield and micronutrients (Zn and Fe) content, the above specified RILs for different traits should be used in crossing programmes.

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