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Genome Analysis of Mungbean [*Vigna radiata* (L.) Wilczek] using Simple Sequence Repeats (SSR) Mapping Data

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ABSTRACT

Background: Agricultural research chiefly focuses on the ways to increase productivity of staple food crops like wheat and rice, but still there are crops where research focus is meagre like nutritionally important mungbean crop grown by marginal farmers in crop rotation systems. Mungbean is leguminous crop which is high in protein content thus it offers health benefits at cheaper rates. The present work emphasizes on finding genetic diversity in mungbean germplasm on the basis of chemical and molecular analysis for micronutrients variation (iron and zinc). The identified molecular markers having linkage with high iron and zinc concentrations in the seeds can prove helpful in expansion of biofortification programme.

Methods: Fifty-one green gram genotypes *viz*. varieties released from CCS Haryana Agricultural University (HAU), Hisar, Punjab Agricultural University (PAU), Ludhiana, Indian Institute of Pulse Research (IIPR), Kanpur and some advanced breeding lines were included in the study. Acid digested samples were used for determination of Fe and Zn by Atomic Absorption Spectrophotometer. Young seedlings leaves were used for isolation of genomic DNA using 2% CTAB (cetyltrimethyl ammonium bromide).

Result: Total of fifty-one mungbean genotypes were tested using fifty simple sequence repeat (SSR) primers. Out of fifty primers screened, 16 primers generated 35 bands. Iron (Fe) and zinc (Zn) in mungbean lines was 36.90 to 107.1 mg/kg and 14.2 to 53.8 mg/kg respectively. The molecular studies based on SSR markers also indicates existence of ample genetic diversity at molecular level.

Key words: Genome, Iron, Micronutrients, Mungbean, SSR, Zinc.

INTRODUCTION

For the developing world especially the vegetarian population of India, mungbean [*Vigna radiata* (L.) Wilczek] is considered as a magical diet to fight against malnutrition. Mungbean is abundant in carbohydrate, protein and micronutrients especially iron content which makes it an economical source of getting nutrition. It is also known as green gram, moong bean or golden gram. It is a notable leguminous crop which falls in sub-genous *Ceratotropis* (2n=2x=22) (Smartt, 1990). It is a warm season crop of short life cycle (approx 60 days) it is well known rotational crop (Singh *et al.,* 2018). It requires minimal inputs thus can perform well in the rainfed as well as in the irrigated land. Other benefits which mungbean offers is it has the ability to fix atmospheric nitrogen thus improves soil conditions and help in increase in the productivity of the successive crop (Kim *et al*. 2015). At a more disaggregated country level, India is the world's largest producer, accounting for 34% of area and 24% of production (Joshi and Rao, 2016).

The protein content in mungbean is quiet high (20%) in comparison to the staple crops alongwith it is a credible source of folate and dietary fibre. Its high digestibility and low flatulence further make mungbean a favourable food for the masses (Lavanya, 2008). Microelement deficiencies especially of iron, zinc, selenium etc are major human growth limiting factors worldwide. These deficiencies play a major role in malnutrition among the global population. One of the significant factors for this deficient condition is non-availability of these trace elements in soil. And if these micronutrients are present in soil then there is lack of these nutrients in edible plant parts like in leaves, seeds or fruits. Keeping all these benefits of SSRs in mind the existing research was planned to evaluate the genetic diversity for micronutrients especially iron and zinc in mungbean genotypes. With the identification of trait linked to molecular marker it will be easy for scheming and executing the subsequent breeding experiments. These identified makers can prove helpful in association and linkage studies. This in turn can be advantageous in expansion of biofortification programme.

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MATERIALS AND METHODS

Plant material

Fifty-one green gram genotypes were used in this study (Appendix I) which the varieties released from CCS Haryana Agricultural University (HAU), Hisar, Punjab Agricultural University (PAU), Ludhiana, Indian Institute of Pulse Research (IIPR), Kanpur and some advanced breeding lines.

Estimation of Fe and Zn contents

Finely powdered healthy seeds were used in chemical analysis for Fe and Zn content. Seeds were surface cleaned and oven dried before grinding. Ground seed sample (1 g) was mixed with 25 mL di-acid mixture (HNO_3:$HClO_4$, 5:1 v/v) and kept overnight. Heat digestion was done till clear white precipitates settled down at the bottom. Crystals were dissolved by diluting in double distilled water. Contents were filtered through Whatman filter paper and filtrate was made 50 mL with double distilled water. This acid digested sample was used for determination of Fe and Zn by Atomic Absorption Spectrophotometer 2380 (Perkin Elmer, USA). The absorbance as recorded at 248 nm and 213 nm for Fe and Zn content, respectively. Data were evaluated against standard curves prepared from standard iron solution of 1000ppm which is diluted to 0 to 100 ppm and zinc solution of 100ppm which is diluted to 0 to 50 ppm.

APPENDIX I: List of fifty-one mungbean (*Vigna radiata*) genotypes.

S. No.	Genotypes	S. No.	Genotypes
A1	GP111	A27	NDMZ2151
A2	MH421	A28	LGG460
A3	Pusa 1431	A29	IPM-02-03-3
A4	MH534	A30	IPM-05-03-6
A5	MH565	A31	IPM-5-3-21
A6	Pusa 1501	A32	IPM6-LS-1
A7	SML 668	A33	IPM-2K-08-2
A8	P 1502	A34	IPM-3072
A9	MH318	A35	EC-399223
A10	Basanti	A36	EC30400
A11	BDYR-1	A37	EC-39407
A12	Ganga-8	A38	EC-393410
A13	2KM138	A39	EC-470094
A14	ADGG13034	A40	EC-581523
A15	M169	A41	EC-581523-B
A16	Muskan	A42	IPM-06-5
A17	Pusa1432	A43	IPM-302-2
A18	IPM9901 8	A44	IPM-02-09-3
A19	Pusa1542	A45	IPM-410-3
A20	Pusa1502	A46	IPM-02-14
A21	Asha	A47	Samrat
A22	ML818	A48	MH-1142
A23	MH98-1	A49	COGG-8
A24	PusaVishal	A50	ML776
A25	SMH-99-1A	A51	Sattya
A26	SonaMung1		

DNA extraction and PCR assay

Young seedlings leaves were used for isolation of genomic DNA using 2% CTAB (cetyltrimethyl ammonium bromide) extraction method (Saghai *et al*., 1984). All DNA samples were given RNase treatment and were further purified. Quality and quantity of DNA was checked by agarose gel electrophoresis (0.8% agarose gel) and UV spectrophotometer. The DNA was normalized to the final concentration of 40ng/µl by comparing with lambda DNA (50 ng/µl). Fifty primers were screened to identify primers that were reproducible and generated the most polymorphic pattern. PCR reactions were carried out in Thermo Cycler (Eppendorf) in 20 µL reaction mixture containing 1U Taq DNA polymerase (MBI Fermentas), 1X- PCR buffer, 0.7 µM primer, 5% DMSO and 200 µM of dNTPs mix. PCR cycles consisted of initial denaturation at 94°C for 3 min, 38 cycles of denaturation at 94°C for 1 min, variable annealing temperature for 1 min, extension at 72 °C for 1 min and a final extension at 72 °C for 5 min. The amplification product (10 µL) was electrophoresed on 1.5% agarose gel in 1Ã- TBE buffer and stained with ethidium bromide. Bands were visualized under UV transilluminator and photographed using BioRad Gel Documentation system. The isolated DNA was used for PCR amplification using fifty SSR primers (Table 2, Appendix II). PCR reaction was carried out in 10 µl reaction mixture containing 25 ng genomic DNA, 1.5 units of Taq DNA polymerase, 0.2 mM of dNTPs, 1.5 mM $MgCl_2$ and 0.2 µM of each primer. The optimized PCR programme was as follows: an initial step of 3 minutes at 9 5°C, followed by 38 cycles of 30 seconds at 94°C, variable annealing temperature for 40 seconds, 30 second at 72°C and a final extension step of 7 minutes at 72°C. PCR products were separated on 2.5% agarose gel electrophoresis or 6% polyacrylamide gel electrophoresis for better resolution.

Data analysis

Clearly resolved bands were scored for presence (1) or absence (0). Molecular weights of the bands were estimated by using 100 bp DNA ladder (MBI Fermentas) as standards. The data set of cultivars and reproducible bands were used to calculate pair-wise similarity coefficient using Jaccard coefficient. The similarity matrix thus generated was subjected to cluster analysis by UPGMA and a dendrogram was generated to study the relatedness of the cultivars. All numerical taxonomic analysis was computed by NTSYS software.

RESULTS AND DISCUSSION

Chemical analysis

The micronutrient in different selected mungbean genotypes content *i.e.* iron ranges from 36.90 to 107.1 mg/ kg and zinc from 14.2 to 53.8 mg/kg (Fig 1, Table 1). The study reported A12 (Ganga-8), A10 (Basanti), A50 (ML-776), A6 (Pusa-1501), A3 (Pusa-1431), A19 (Pusa-1542), A43 (IPM-3022), A45 (IPM-4103), A5 (MH-565), A18 (IPM-99018) and A25 (SMH-99-1A) showing more than 100 mg/kg Fe content. A5

APPENDIX II: List of primers with monomorphic bands and no amplification product.

1	MBSSRG8	AATTGCAGAATCCCGTGAACAAGAGCGTCTTTGCCTGTTT	-monomorphic-
2	MBSSRG9	CGTAATGCGTCCATACCACACCGATGCTCTTTTTCATGGT	-monomorphic-
3	Bng91	AGCCTGAACCGAGAGGAAGTGAGCTCAACATTCCAGAGGC	-no amplification-
4	BMd-27	GGACCCACCATCACCATAACTGGTGGAGGTGGAGATTTGT	-no amplification-
5	BM184	AGT GCT CTA TCA AGA TGT GTG ACA TAA TCA ATG GGT CAC TG	-no amplification-
6	BM185	AAG GAG GTT TCT ACC TAA TTC CAAA GCA GGG ATG TAG TTG C	-no amplification-
7	BM158	CCGAGCACCGTAACTGAATGCCGCTCGCTTACTCACTGTACGC	-no amplification-
8	BM201	TGGTGCTACAGACTTGATGGTGTCACCTCTCTCCTCCAAT	-no amplification-
9	PVBR218	TGT AAA TGG CAG GCA GTG AAATG ACC ACG CAG TGA CAG AG	-no amplification-
10	PVBR233	AGA GAG GGT TGT GGT TGG TGTTA ATC CCG CTT TAC GCA AC	-no amplification-
11	PVBR250	GGT GGA GAG TGG TGG ACA GTCGA AAC CCT ACC ACT TTT TCC	-no amplification-
12	PVBR251	TGA AGT TGC AGC TAG GTT GGGGT TGT GCT TGT GTT GTT GG	-no amplification-
13	PVBR78	AAT TCG TGT CCT CTG TCT GTGACG ACG GAG AGA GAG GTT GA	-no amplification-
14	PVBR213	ACA ATG TAG ACA GCG CAG CAGCT CTT TCT CCT CCC ATC CT	-no amplification-
15	BM199	AAGGAGAATCAGAGAAGCCAAAAGTGAGGAATGGATGTAGCTCAGG	-no amplification-
16	BM161	TGCAAAGGGTTGAAAGTTGAGAGTTCCAATGCACCAGACATTCC	-no amplification-
17	BM157	ACTTAACAAGGAATAGCCACACAGTTAATTGTTTCCAATATCAACCTG	-no amplification-
18	BM199	AAGGAGAATCAGAGAAGCCAAAAGTGAGGAATGGATGTAGCTCAGG	-no amplification—
19	BMd-16	ATGACACCACTGGCCATACAGCACTGCGACATGAGAGAAA	-no amplification-
20	BMd-22	GGTCACTTCCGGAGCATTCCGGGAAATGGAAGTCACAGT	-no amplification-
21	BMd-33	TACGCTGTGATGCATGGTTTCCTGAAAGTGCAGAGGTGGTG	-no amplification-
22	CEDG279	GGTCTTTCTAAGCGGAGCACCTGCCTCTCTACACAAGTGG	-no amplification-
23	CEDG111	TGGAAGTTTCCAAGAGGGTTTTCTCTCACCACCTTTTACCTTCTCA	-no amplification-
24	CEDG248	CAGAACACAAAAGGGTTCTCGGTGGATTCACTCGCTTCC	-no amplification-
25	CEDG118	AACCCAACCAACCCTTGTGGTAAGGCTGGAATCATAATACCGCCTTGT	-no amplification-
26	BMd-12	CATCAACAAGGACAGCCTCAGCAGCTGGCGGG TAAAA CAG	-no amplification-
27	BMd-41	CAGTAAATATTGGCGTGGATGATGAAAGTGCAGAGTGGTGGA	-no amplification-
28	CEDG008	AGGCGAGGTTTCGTTTCAAGGCCCATATTTTTACGCCCAC	-no amplification-
29	BM210	ACC ACT GCA ATC CTC ATC TTT GCCC TCA TCC TCC ATT CTT ATC G	-no amplification-
30	PVBR82	CCC AAA GAG AAT GCA AGG TTGCT TCC CTT TCA ACG ACA TC	-no amplification-
31	PVBR229	TTG GCT TTC TCT TTC CTC TCT CGAT TGA GAT GGA AAG GCT ACA T	-no amplification-
32	PVBR113	TGC ATT CTT CCT CCC ATC TTTTG ATT TGA TTT GAT CAG TGG TG	-no amplification-
33	PVBR215	AAT GGA AAA GGG GGA AGA GAGCA ACA TTG CAG AGA GAC GA	-no amplification-
34	MBSSRG1	AATTGCAGAATCCCGTGAACAAGAGCGTCTTTGCCTGTTT	-monomorphic-

(MH-565) had the highest Fe content (107.1 mg/kg), while A34 (IPM-3072) had the lowest Fe content (36.9 mg/kg). Zinc content also varied in selected mungbean *i.e.* 23.15 to 40.46mg/kg. In genotypes ML-776 (A50), MH-565 (A5), Pusa-1501 (A6), SML-668 (A7), MH-421 (A2) and Basanti (A10) Zn content was reported more than 35mg/kg. In present study reported that genotype ML-776 (A50) had the highest Zn content (53.8 mg/kg) and M169 (A15) genotype had the lowest Zn content (14.2 mg/kg). Both micronutrient Fe and Zn content found higher in A6 (Pusa-1501), A50 (ML-776) and A10 (Basanti) genotypes.

Genome diversity in fifty-one mungbean genotypes were identified using SSR markers (Fig 2). Sixteen primers were found polymorphic out of fifty primers which generated 35 bands (Table 2). The number of band varied between 2 (MBSSRG15) and 4(MBSSRG12), with an average of bands 2.19 per primers. In PCR amplified product size of the band ranged from 180-35bp. By using MBSSRG14 primer a representative SSR profile was obtained (Fig 3).

SSR polymorphism among genotypes

Among the fifty one genotypes polymorphism range was 75-100% and average polymorphism was observed 97.14%. The Jaccard $^{TM}_{S}$ similarity coefficient was reported to be in range from 0.45 to 0.90, in SSR profile analysis, showing the wide range of genetic diversity at molecular level. Highest value of similarity (0.94) was reported in genotypes IPM-02-03-3, IPM-05-03-6, IPM2K-08, 1PM-3072, Asha and SMH-99-1, whereas minimum similarity values of 0.11 was reported in genotypes *i.e.* MH421 and NDM2-215-1.

Cluster analysis

In fifty one genotypes cluster analysis showed the genetic relatedness ranged from 0.45 to 0.90, *i.e.*, 60-89 percent. At an arbitrary cut-off 47% similarity level on a dendrogram, the mungbean genotypes were grouped into two main

Table 1: Iron (Fe) and Zinc (Zn) content in seeds of mungbean (*Vigna radiata*) genotypes.

S. No.	Genotypes	Fe Content (mg/kg)	Zn Content (mg/kg)	S. No.	Genotypes	Fe Content (mg/kg)	Zn Content (mg/kg)
A1	GP111	46.4	38.16	A27	NDMZ2151	83.20	19.23
A2	MH421	70.6	31.28	A28	LGG460	66.60	23.24
A3	Pusa 1431	104.9	41.43	A29	IPM-02-03-3	74.70	27.70
A4	MH534	83.0	35.37	A30	IPM-05-03-6	74.70	18.96
A5	MH565	107.1	39.17	A31	IPM-5-3-21	52.05	18.31
A6	Pusa 1501	106.4	45.37	A32	IPM6-LS-1	65.45	16.24
A7	SML 668	82.2	43.25	A33	IPM-2K-08-2	57.75	16.68
A8	P 1502	91.6	44.22	A34	IPM-3072	36.90	25.54
A9	MH318	96.6	47.72	A35	EC-399223	44.65	18.38
A10	Basanti	102.8	52.01	A36	EC30400	78.70	14.59
A11	BDYR-1	51.45	43.31	A37	EC-39407	66.30	18.64
A12	Ganga-8	105.6	35.40	A38	EC-393410	62.35	25.05
A13	2KM138	51.45	26.96	A39	EC-470094	49.60	20.24
A14	ADGG13034	63.75	20.03	A40	EC-581523	52.10	19.01
A15	M169	56.85	14.25	A41	EC-581523-B	44.90	19.67
A16	Muskan	50.90	43.46	A42	IPM-06-5	94.50	20.81
A17	Pusa1432	64.35	15.39	A43	IPM-302-2	103.15	27.48
A18	IPM9901 8	108.10	41.48	A44	IPM-02-09-3	95.45	23.08
A19	Pusa1542	106.05	36.78	A45	IPM-410-3	99.85	19.80
A20	Pusa1502	84.80	19.21	A46	IPM-02-14	44.55	22.76
A21	Asha	97.55	30.90	A47	Samrat	59.05	13.90
A22	ML818	59.85	23.93	A48	MH-1142	61.85	20.95
A23	MH98-1	52.00	32.42	A49	COGG-8	58.35	18.89
A24	PusaVishal	84.70	29.36	A50	ML776	106.2	53.85
A25	SMH-99-1A	101.45	26.96	A51	Sattya	43.70	25.22
A26	SonaMung1	66.35	18.73				

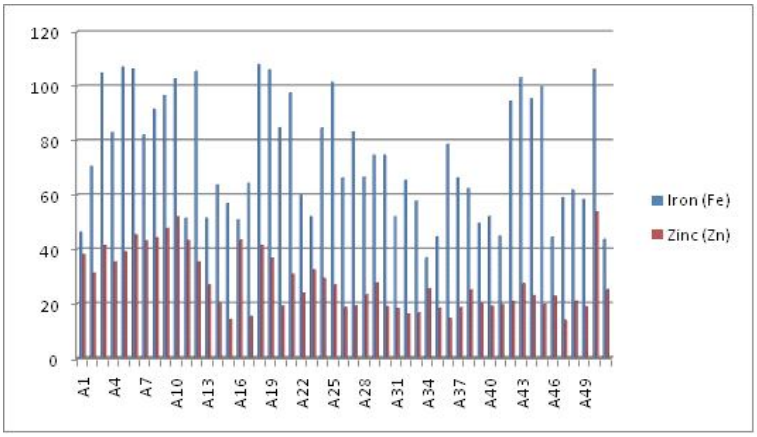

Fig 1: Iron (Fe) and Zinc (Zn) content in mungbean (*Vigna radiata*) genotypes.

groups, cluster I and cluster II (Fig 4). Cluster I and cluster II contains the seventeen and thirty four accessions respectively. At similarity coefficient value of 0.51 these two clusters are sub-clustered as IA, IB, IIA and IIB. The sub-cluster IA and IB consisted of 10 and 7 genotypes respectively. Sub-group I1A and IIB contain 9 and 25 genotypes respectively. These subgroups are further subdivided into sub-groups. The genotypes A31, A32, A35, A36, A23 and A27 in IA, IIA sub-cluster are genetically related. More than 94% genetic similarity is found.

Principal component analysis (PCA)

Total variation of 78.8% showed in SSR data could be interpreted by three main component based on first, second and third *eigen* vectors, which explain for 51.03, 19.64 and 8.13% variations, respectively. The clustering of 51 genotypes are presented in 3-D scaling (Fig 5).

Table 2: SSR primers used for genetic diversity analysis in leaves of fifty-one mungbean genotypes.

Primer	Sequences	Band size (bp)	Total no. of bands	No. of monomorphics band	No. of polymorphic bands	% polymorphism	PIC value
MBSSRG2	GTCGATGACCCAAATCCAAT TGCGTTCAAAGACTCGATG	300-340	3	0	3	100	0.64
MBSSRG3	ATCTGACGAGAGCATGTGGA CTCCCCTTTAGCCACAATCA	270-300	2	0	2	100	0.48
MBSSRG4	GAAGCGCATTCGTACTGACA TACAACCGAAGACACGCAAG	300-320	2	0	2	100	0.47
MBSSRG5	TGATGTGTTCCTCCCGAGTT AACAAGTACCCGTTGCCAAG	300-330	2	0	2	100	0.44
MBSSRG6	ACCTTCAGGCTTCAACAACG CGACGTAGAAACACACGATCA	180-200	2	0	2	100	0.50
MBSSRG7	GTCGATGACCCAAATCCAAT TTGCGTTCAAAGACTCGATG	280-350	2	0	2	100	0.49
MBSSRG10	CGCCTCCTCTCCTCTTCAG CCGATGCTCTTTTTCATGGT	300-350	2	0	2	100	0.43
MBSSRG11	AATTGCAGAATCCCGTGAAC AAGAGCGTCTTTGCCTGTTT	290-310	2	0	2	100	0.49
MBSSRG12	TTGCAGAATCCTGTGAACCA AAGAGCGTCTTTGCCTGTTT	280-350	4	1	3	75	0.73
MBSSRG13	ATCATTGTCGATGCCCAAAC AGGATTCTGCAATTCACACCA	250-380	2	0	2	100	0.50
MBSSRG14	TTGCAGAATCCTGTGAACCA AAGAGCGTCTTTGCCTGTTT	270-310	2	0	2	100	0.48
MBSSRG15	ATCATTGTCGATGCCCAAAC AATTGCAGAATCCCGTGAAC	280-300	2	0	2	100	0.50
Bng95	TGAAAGTGCAGAGTGGTGGA TTGGCGTGGATGATTTATCT	180-200	2	0	2	100	0.45
PVBR269	TCG CCC CAT ATT CAC TTT TC TGG TGT GCA GAA AGT CTG TGA	290-320	2	0	2	100	0.46
BM202	ATG CGA AAG AGG AAC AAT CG CCT TTA CCC ACA CGC CTT C	260-300	2	0	2	100	0.48
BM141	TGA GGA GGA ACA ATG GTG GC CTC ACA AAC CAC AAC GCA CC	280-320	2	0	2	100	0.49
		35	1	34			
		2.1	0.06	2.04	97.14		

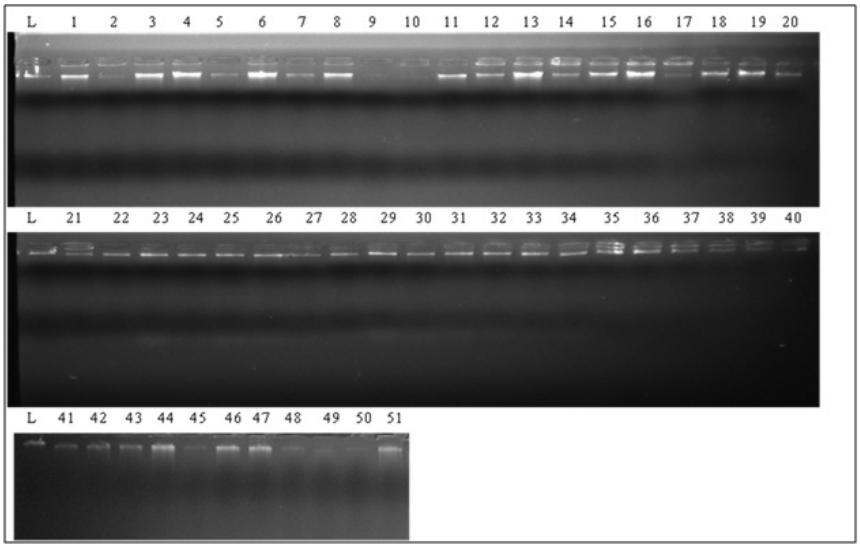

Fig 2: High molecular weight DNA of 51 mungbean genotypes.

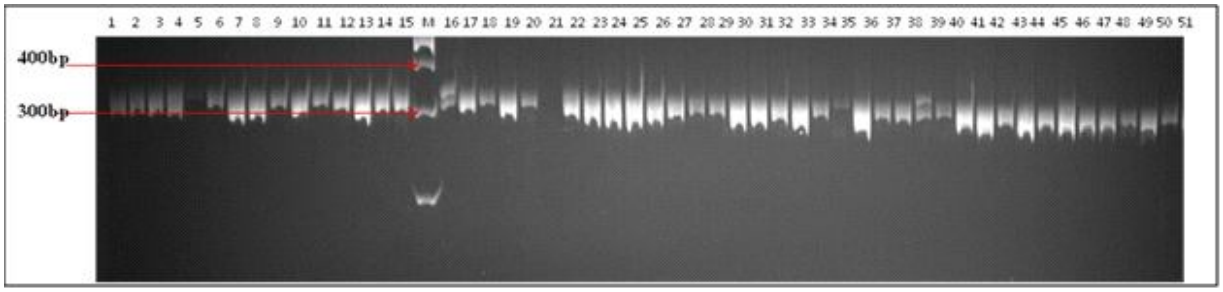

Fig 3: Diversity analysis among fifty-one genotypes using MBSSRG14 marker.

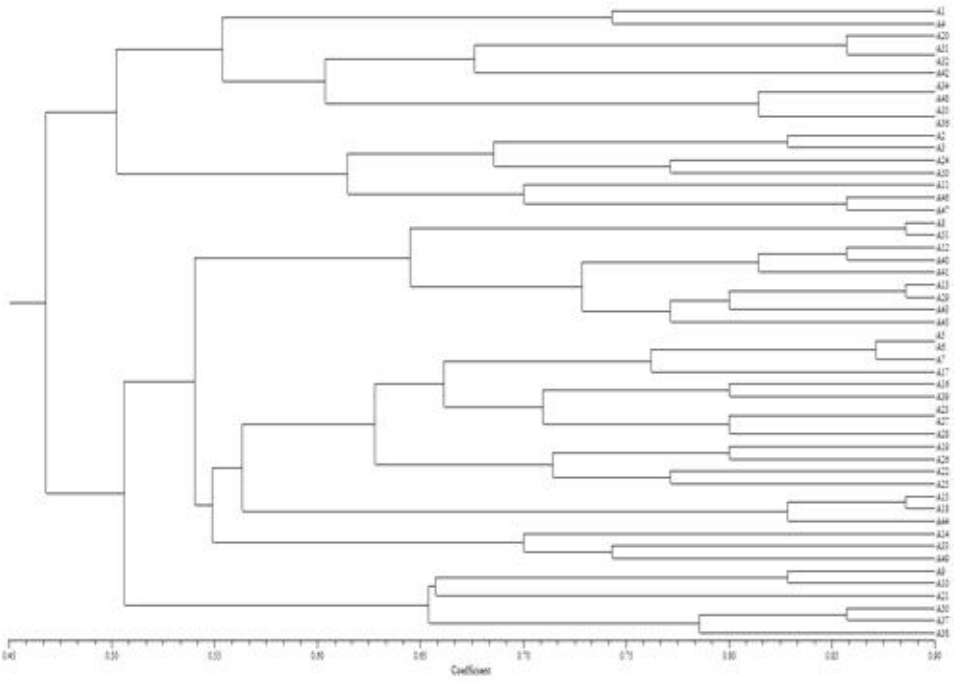

Fig 4: UPGMA dendrogram showing relationship among fifty-one mungbean genotypes based on Jaccard's similarity matrix data using 16 SSR markers.

Majority of clustering showed the same pattern as presented in dendrogram with small variations. A2 and A29 genotypes were distantly placed in both the analyses. Polymorphic information content value range from 0.43 (MBSSRG10) to 0.70 (MBSSRG12). Reasonable diversity of mungbean genotypes may be exploited in breeding program by selecting parents for development of micronutrient improved variety.

A deficiency of micronutrient *i.e.* iron, zinc, iodine, folate and lack of vitamins A in staple food crops leads to malnutrition in human population. It is a major challenge to the agriculture scientist (Jawal-deh *et al*., 2019). Deficiency of micronutrients is effected 2 billion peoples all over the world, especially in developing countries (Stein, 2010; Cakmak *et al*., 2010). Metals deficiencies like iron and zinc are mainly affecting the population of developing countries because they are depending for their daily diet mainly on cereal crops (Kenzhebayeva *et al*., 2019). Micronutrient enrichment of the staple food crops by genetic manipulation is the most promising strategy to combat the malnutrition problem (Tiwari *et al*., 2010). Micronutrient enrichment of the crops can be done by several approaches *i.e.* conventional or molecular breeding (Welch and Graham, 2004), genetic engineering (Pederson *et al*., 2007) and agronomic biofortification (Cakmak, 2008). Due to low, non-recurrent expenditure and higher public acceptability breedind for micronutrient enrichment has been considered as best approach (Nestel *et al*., 2006; Monasterio *et al*., 2007). Genome diversity and phylogenetic relationship in plants can be identified by using different markers. Extensive observations of mature plants are required in traditional methods which are base on morphological traits (Wrigley *et al*., 1987). A vast amount of information and a number of databases are generated by using molecular markers in genome analysis which is used in crop breeding (Joshi *et al*., 1999).

Among fifty four accessions of mungbean Lavanya *et al*. (2008) reported the extent of genetic diversity by using random amplified polymorphic DNA (RAPD) profile. They reported seven primer generated 174 amplification product

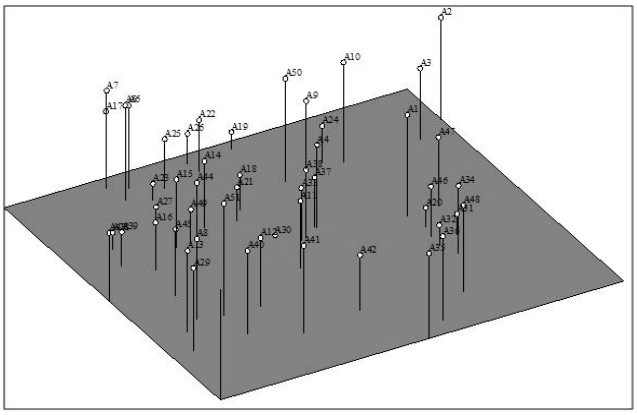

Fig 5: Three dimensional PCA (principal component analysis) scaling of fifty-one genotypes mungbean using similarity matrix data of sixteen SSR markers.

out of 40 primers. The average number of band was observed 24.85 bands per primer. Selvi *et al*. (2006) was also found a RAPD marker associated with mungbean yellow mosaiv virus (MYMV) resistance and suggested that may be useful in selection of MYMV resistant mungbean genotypes. Genetic diversity (83% polymorphism) among mungbean cultivars, wild accessions and landraces by using RAPD and ISSR marker were reported by Chattopadhyay *et al*. (2005). Microsatellite markers were used by Seehalak *et al*., in 2009 to study the polymorphism by using 78 primers within 22 Thai accessions of mungbean and eight polymorphic loci detected 2 to 3 alleles per locus with an average of 2.25.

In our report the different genotypes selected using SSR markers could be potential source of germplasm for mungbean improvement. Our study will work as milestones in identification of micronutrient enrich diverse genotype, which is used to generate a micronutrient (Fe and Zn) improved mungbean variety in breeding programmes.

In our study different high iron genotypes *i.e.* Ganga-8, Basanti, ML-776, Pusa-1431, Pusa-1542, IPM-4103, MH-1565, IPM-99018 and SMH-99-1A were found to be quite different based on similarity coefficient and cluster analysis and they can be used for micronutrient improvement in breeding programmes. Based on similarity coefficient and cluster analysis, high zinc content containing genotypes such as ML-776, MH-1565, pusa-1501, SML-668, MH-421 and Basanti were also found to be quite distinct as these fall in different sub-groups. In present study ML-776 (high Fe and Zn) and Satya (low Fe and Zn) genotypes showed reasonable diversity which are from different sub group that may be exploited or selecting parents for breeding programmes. In previous study genotype ML-776 also reported as high iron and zinc and Satya as low iron and zinc variety based on RAPD and SRAP analysis (Aneja *et al*., 2013).

CONCLUSION

Critical analysis of the results revealed that *V. radiata* (L.) genotypes; Ganga-8, Basanti, ML-776, Pusa-1431, Pusa-1542, IPM-4103, MH-1565, IPM-99018 and SMH-99-1A having high iron content and ML-776, MH-1565, Pusa-1501, SML-668, MH-421 and Basanti having high zinc content can be effectively utilized in crop improvement programmes for biofortification (iron and zinc) in mungbean.

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