Sequence related amplified polymorphism (SRAP) analysis for genetic diversity and micronutrient content among gene pools in mungbean [*Vigna radiata* (L.) Wilczek]

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Abstract *Vigna radiata* (L.) Wilczek, commonly called mungbean is an important pulse crop. Commercial cultivars contain low levels of iron and zinc and it is important to assess genetic variability in the available germplasm for improving micronutrient content in commercial cultivars. The present study was undertaken to study molecular diversity using Sequence-related amplified polymorphism (SRAP) among 21 *Vigna radiata* genotypes. Twenty nine SRAP primer combinations produced a total of 121 amplified bands which were polymorphic with an average of 4.65 bands per primer. The size of amplified bands ranged from 70 bp to 3,000 bp and 6 out of 29 SRAP primers were most useful in fingerprinting *Vigna radiata* genotypes under study. The similarity coefficients between different genotypes ranged from 0.45 to 0.96 with an average similarity value of 0.71. At an arbitrary cut-off at 60 % similarity level on a dendrogram, the *Vigna radiata* accessions were categorized into two major clusters. ML1108 and 2KM115 were found to be genetically similar. SMH99-1A and ML776 showed high iron and zinc content while Satya was poor in iron as well as zinc content. Mapping population involving ML776 and Satya could be used for tagging gene(s) for micronutrient content. The results indicated that SRAP markers were efficient for identification of *Vigna radiata* genotypes and assessment of the genetic relationships among them.

Keywords Mungbean · Iron and zinc content · Genetic variability · SRAP

Introduction

Vigna radiata (L.) Wilczek, commonly called as mungbean or greengram, is a well known pulse crop belonging to the subgenus *Ceratotropis*, family *Leguminaceae*, with diploid chromosome number ($2n=22$) (Smartt 1990). The annual world production area of mungbean is about 5.5 million hectare (Weinberger 2003) of which about 90 % is in Asia (Lambrides and Godwin 2007). India is the biggest producer of mungbean where about 2.99 million ha are cultivated (Singh and Ahlawat 2005). Mungbeans, mainly cultivated in India, China, Thailand, Philippines, Indonesia, Myanmar and Bangladesh, grown widely as human food but can be used as a green manure crop and as forage for livestock. Mungbeans contain 20 % protein, also a good source of folate and dietary fibre, its food has easy digestibility and low proportions of flatulence factors which also add to its value among the pulse crops (Lavanya et al. 2008).

Micronutrient malnutrition (Hidden Hunger), a condition that develops when the body does not get the optimum amount of vitamins, minerals and other micronutrients which are essential to maintain metabolic regulation and organ function (Welch and Graham 1999), afflicts more than 40 % of the world population. Crop biofortification with micronutrients is one of the focus areas in crop improvement. The micronutrients (iron and zinc) are essential elements needed in small amounts for adequate human nutrition. 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 defense and

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thyroid function, its deficiency in the body causes anaemia. 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 defense system (Frossard et al. 2000).

The assessment of genetic diversity is mandatory to produce improved crop cultivars and molecular markers, heritable entities that are associated with economically important traits, can be used by plant breeders as selection tools (Darvasi and Soller 1994) as these can detect differences in DNA sequence and are less ambiguous than phenotypic markers. An exhaustive characterization of the available mungbean germplasm is needed to identify the useful genetic diversity (Lavanya et al. 2008). Li and Quiros (2001) developed Sequence-related amplified polymorphism (SRAP) markers which are 17 or 18 nucleotides long, contain core sequences (13 to 14 bases long), where the first 10 or 11 bases starting at the 5' end, are non specific "filler" sequences, followed by the sequence CCGG in the forward primer and AATT in the reverse primer. They used two step amplification involving first five cycles at 35 °C followed by 35 cycles at 50 °C annealing temperature. The first low annealing temperature takes care of effective primer binding in cases of partial matches in the target DNA.

SRAP markers are more powerful for revealing genetic diversity among closely related cultivars than SSR, ISSR, or RAPD markers (Budak et al. 2004) and can be used for linkage map construction (Yeboah et al. 2007), genomic and cDNA fingerprinting, gene tagging (Li and Quiros 2001), genetic diversity analysis (Li et al. 2009), map-based cloning (Zhang et al. 2010), hybrid identification (Mishra et al. 2011) and sex determination (Zhou et al. 2011). In our study, SRAP markers were used for the first time to analyse genetic diversity in important cultivars and advanced breeding lines of mungbean varying in micronutrient (Fe and Zn) content.

Materials and methods

Plant material

Twenty one genotypes of mungbean *Vigna radiata* (L.) Wilczek (Table 1) included varieties released from Chaudhary Charan Singh Haryana Agricultural University (Hisar), Punjab Agricultural University (Ludhiana), Indian Institute of Pulses Research (Kanpur) and some advanced breeding lines. Plants were grown in pots under greenhouse conditions using standard agronomic practices.

DNA isolation

Total genomic DNA was isolated from young and healthy leaf tissue of 3–4 week old plants using the CTAB method (Saghai-Marooof et al. 1984). All the DNA samples were subjected to RNase treatment and further purified. The quality and quantity of different DNA samples was estimated using UV absorption spectrophotometer (Biophotometer, Eppendorf) and by running the DNA samples on 0.8 % agarose gel in 1× TBE buffer using λ DNA as control. DNA samples were stored at –20 °C until use.

SRAP analysis

PCR was performed in a 20 μ l reaction volume containing 10 × PCR buffer (containing 25 mM MgCl₂), 200 μ M of dNTPs, 0.6 μ M of primers (forward and reverse), 1U Taq DNA Polymerase and 25 ng DNA. Twenty nine SRAP primer combinations (Tables 2 and 3) were screened by PCR. PCR conditions included initial denaturation at 94 °C for 4 min and 10 cycles of denaturation at 94 °C for 1 min, primer annealing at 35 °C for 1 min, and primer extension at 72 °C for 1 min, followed by 30 cycles of denaturation at 94 °C for 1 min, primer annealing at 50 °C for 1 min, and

Table 1 List of *Vigna radiata* (L.) Wilczek genotypes used for the present study

Sr. no.	Genotype	Origin	Sr. no.	Genotype	Origin
1.	Ganga8	IIPR, Kanpur	12.	MH98-1	CCSHAU, Hisar
2.	SMH99-1A	CCSHAU, Hisar	13.	Muskan	CCSHAU, Hisar
3.	Asha	CCSHAU, Hisar	14.	2KM115	IIPR, Kanpur
4.	MH125	CCSHAU, Hisar	15.	LM10	PAU, Ludhiana
5.	ML776	PAU, Ludhiana	16.	ML803	PAU, Ludhiana
6.	ML735	PAU, Ludhiana	17.	BG39	Germplasm line from Bangladesh
7.	2KM138	IIPR, Kanpur	18.	Satya	CCSHAU, Hisar
8.	MH421	CCSHAU, Hisar	19.	ML759	PAU, Ludhiana
9.	ML1108	PAU, Ludhiana	20.	2KM151	Test line in coordinated trials
10.	PDM91-249	IIPR, Kanpur	21.	GP181	Germplasm line
11.	ML839	PAU, Ludhiana			

Table 2 List of SRAP primer combinations used in the study

S. no.	SRAP primer	Forward primer	Reverse primer
1.	me1em1	TGA GTC CAA ACC GGA TA	GAC TGC GTA CGA ATT AAT
2.	me1em2	TGA GTC CAA ACC GGA TA	GAC TGC GTA CGA ATT TGC
3.	me1em3	TGA GTC CAA ACC GGA TA	GAC TGC GTA CGA ATT GAC
4.	me1em4	TGA GTC CAA ACC GGA TA	GAC TGC GTA CGA ATT TGA
5.	me1em5	TGA GTC CAA ACC GGA TA	GAC TGC GTA CGA ATT AAC
6.	me1em6	TGA GTC CAA ACC GGA TA	GAC TGC GTA CGA ATT GCA
7.	me1em7	TGA GTC CAA ACC GGA TA	GAC TGC GTA CGA ATT CAA
8.	me2em1	TGA GTC CAA ACC GGA GC	GAC TGC GTA CGA ATT AAT
9.	me2em2	TGA GTC CAA ACC GGA GC	GAC TGC GTA CGA ATT TGC
10.	me2em3	TGA GTC CAA ACC GGA GC	GAC TGC GTA CGA ATT GAC
11.	me2em4	TGA GTC CAA ACC GGA GC	GAC TGC GTA CGA ATT TGA
12.	me2em6	TGA GTC CAA ACC GGA GC	GAC TGC GTA CGA ATT GCA
13.	me2em7	TGA GTC CAA ACC GGA GC	GAC TGC GTA CGA ATT CAA
14.	me2em12	TGA GTC CAA ACC GGA GC	GAC TGC GTA CGA ATT GTC
15.	me2em13	TGA GTC CAA ACC GGA GC	GAC TGC GTA CGA ATT GGT
16.	me3em1	TGA GTC CAA ACC GGA AT	GAC TGC GTA CGA ATT AAT
17.	me3em2	TGA GTC CAA ACC GGA AT	GAC TGC GTA CGA ATT TGC
18.	me3em3	TGA GTC CAA ACC GGA AT	GAC TGC GTA CGA ATT GAC
19.	me3em4	TGA GTC CAA ACC GGA AT	GAC TGC GTA CGA ATT TGA
20.	me4em1	TGA GTC CAA ACC GGA CC	GAC TGC GTA CGA ATT AAT
21.	me4em2	TGA GTC CAA ACC GGA CC	GAC TGC GTA CGA ATT TGC
22.	me4em3	TGA GTC CAA ACC GGA CC	GAC TGC GTA CGA ATT GAC
23.	me4em4	TGA GTC CAA ACC GGA CC	GAC TGC GTA CGA ATT TGA
24.	me4em13	TGA GTC CAA ACC GGA CC	GAC TGC GTA CGA ATT GGT
25.	me4em15	TGA GTC CAA ACC GGA CC	GAC TGC GTA CGA ATT CTG
26.	me5em13	TGA GTC CAA ACC GGA AG	GAC TGC GTA CGA ATT GGT
27.	me6em1	TGA GTC CAA ACC GGA CA	GAC TGC GTA CGA ATT AAT
28.	me7em1	TGA GTC CAA ACC GGA CC	GAC TGC GTA CGA ATT AAT
29.	me8em1	TGA GTC CAA ACC GGA GC	GAC TGC GTA CGA ATT AAT

primer extension at 72 °C for 1 min. The amplification was completed with a 5 min final extension at 72 °C. Amplified products were stored at −20 °C till further use.

The amplification products were separated by electrophoresis on 2.5 % agarose gel with 1× TBE buffer and stained with ethidium bromide (EtBr). Electrophoresis was carried out at 60 V and 40 mA and PCR amplified products were visualized under BioRad Gel Documentation system.

Data analysis

Clear and well marked bands were coded in a binary form by denoting ‘0’ and ‘1’ intended for absence and presence of bands, respectively in each genotype and the data was used as input for further analysis. Molecular weights of the bands were estimated by using 100 bp DNA ladder (MBI Fermentas) as standard. Cluster analysis (UPGMA) and dendrogram construction

were performed with NTSYS-PC (Exeter Software, New York) version 2.0 (Rohlf 1998).

Chemical analysis for iron and zinc content

Seeds were surface cleaned and oven dried before grinding. Ground seed sample (1 g) was mixed with 25 ml diacid mixture [HNO₃: HClO₄, 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 millipore distilled water and the contents were filtered through Whatman No. 42 filter paper. The volume of the filtrate was made to 50 ml with millipore distilled water. This filtrate was used for the determination of iron and zinc content by Atomic Absorption Spectrophotometer 2380, Perkin Elmer (USA). The analysis was done at 248 nm and 213 nm for iron and zinc content respectively. The micronutrient content was assayed in two crop seasons.

Table 3 DNA polymorphism generated using 29 combinations of SRAP primers in 21 mungbean genotypes

Sr. no.	Primer combination	Band size (bp)	Total no. of bands	No. of ^a bands	No. of ^b bands	Polymorphism (%age)
1.	Me1em1	70–1000	2	0	2	100
2.	Me1em2	70–3000	10	0	10	100
3.	Me1em3	650	1	0	1	100
4.	Me1em4	70–3000	12	0	12	100
5.	Me1em5	1700	1	0	1	100
6.	Me1em6	70	1	0	1	100
7.	Me1em7	70–250	2	0	2	100
8.	Me2em1	300–400	2	0	2	100
9.	Me2em2	70–950	3	0	3	100
10.	Me2em3	70	1	0	1	100
11.	Me2em4	70–2000	9	0	9	100
12.	Me2em6	No amplification				
13.	Me2em7	70–1850	12	0	12	100
14.	Me2em12	70–550	3	0	3	100
15.	Me2em13	250–1700	6	0	6	100
16.	Me3em1	No amplification				
17.	Me3em2	250	1	0	1	100
18.	Me3em3	70–3000	9	0	9	100
19.	Me3em4	70–2000	4	0	4	100
20.	Me4em1	70–2000	4	0	4	100
21.	Me4em2	70	1	0	1	100
22.	Me4em3	70–2000	8	0	8	100
23.	Me4em4	70–1200	8	0	8	100
24.	Me4em13	70	1	0	1	100
25.	Me4em15	70–2500	8	0	8	100
26.	Me5em13	70	1	0	1	100
27.	Me6em1	No amplification				
28.	Me7em1	200–600	3	0	3	100
29.	Me8em1	200–3000	8	0	8	100
Total		70–3000	121		121	

^a Monomorphic^b Polymorphic

Results

Micronutrient content in Mungbean genotypes

The selected genotypes showed variability for iron and zinc contents (Tables 4 and 5) and analysis of variance showed this variation is highly significant across genotypes at 1 %. ML776 was found to have maximum iron content (100.97 mg/Kg) as well as high zinc content. Lowest iron content was found in ML803 (29.95 mg/Kg). Highest zinc content was found in MH421 (35.70 mg/Kg) and lowest zinc content was found in 2KM151 (20.13 mg/Kg). The values of iron and zinc content of different genotypes were observed to be significantly positively correlated with each other ($r=0.434^*$). The different genotypes used in the study were categorized as high iron

(with iron content above 90 mg/Kg), low iron (with iron content below 50 mg/Kg), high zinc (with zinc content above 35 mg/Kg), low zinc (with zinc content below 25 mg/Kg), high iron- high zinc and low iron- low zinc genotypes (Table 5). ML776 and SMH99-1A, only two genotypes out of twenty genotypes studied, were categorized as high iron and high zinc genotypes while only one genotype (Satya) was low iron and low zinc genotype.

SRAP analysis

Twenty nine SRAP primer pairs were used, out of which 26 sets of primers produced clear amplification patterns and showed polymorphisms in all the genotypes. Table 3 shows the data statistics of polymorphism obtained from 26 primer

Table 4 Iron and zinc contents in selected mungbean genotypes

Sr. no.	Genotype	Iron content (mg/Kg)	Zinc content (mg/Kg)
1.	Ganga 8	91.42±0.39	26.51±0.88
2.	SMH99-1A	93.15±1.27	35.45±0.90
3.	Asha	90.00±2.05	26.22±1.53
4.	MH125	95.33±2.29	33.58±1.96
5.	ML776	100.97±2.76	34.68±0.93
6.	ML735	68.71±1.65	32.10±0.67
7.	2KM138	67.85±1.37	28.40±1.51
8.	MH421	90.70±2.51	35.70±1.37
9.	ML1108	70.74±0.79	34.08±1.23
10.	PDM91-249	58.99±2.92	31.38±2.97
11.	ML839	48.81±1.03	34.57±1.47
12.	MH98-1	44.10±2.53	31.83±2.55
13.	Muskan	41.19±2.36	27.81±1.05
14.	2KM115	44.02±2.24	27.55±1.62
15.	LM10	46.45±1.98	33.27±0.94
16.	ML803	29.95±1.12	26.38±0.62
17.	BG39	49.43±2.89	21.63±3.27
18.	Satya	38.63±0.69	20.92±3.79
19.	ML759	52.93±1.59	22.88±1.56
20.	2KM151	66.33±1.73	20.13±2.13
21.	GP181	42.50±1.54	25.78±1.32
Analysis of variance for iron and Zinc content			
Source of variation	DF	Mean sum of squares	
		Iron	Zinc
Treatment	20	1,484.879 ^a	76.292 ^a
Error	42	11.27	10.15
Total	62		

^aHighly significant for iron and zinc

pairs. A total of 121 polymorphic bands were observed, ranging from 1 to 12 bands per primer combination, with an average of 4.65 bands per primer set. The size of amplification products ranged from 70–3,000 bp. All the primer combinations displayed 100 % polymorphism. A representative SRAP profile obtained with the primer combinations me2em12 and me2em13 is given in Fig. 1.

Cluster tree analysis

Dendrogram was constructed by UPGMA (unweighted pair group method of arithmetic averages) subprogram of NTSYS-PC programme using similarity matrix generated from SRAP data for 21 genotypes. The dendrogram constructed depicted the genetic relationships among genotypes (Fig. 2).

Table 5 High and low iron genotypes on the basis of chemical analysis

High iron genotypes (mg/Kg)	Low iron genotypes (mg/Kg)
ML776 (100.97±2.77)	ML803 (29.95±1.12)
MH125 (95.33±2.30)	Satya (38.63±0.68)
SMH99-1A (93.15±1.27)	Muskan (41.19±2.36)
Ganga 8 (91.42±0.39)	GP181 (42.50±1.53)
High zinc genotypes (mg/Kg)	Low zinc genotypes (mg/Kg)
MH421 (35.70±1.371)	2KM151 (20.13±2.13)
SMH99-1A (35.45±0.89)	Satya (20.91±3.79)
ML776 (34.68±0.93)	BG39 (21.63±3.27)
ML839 (34.57±1.47)	ML759 (22.88±1.56)
High iron, high zinc genotypes	Low iron, low zinc genotype
ML776	Satya
SMH99-1A	

Fig. 1 a–b Electrophoretic pattern of PCR amplified fragments of selected mungbean genotypes using me2em12 (a) and me2em13 (b) primers [100 bp ladder (M), Negative control (C), Ganga8 (a), SMH99-1A (b), Asha (c), MH125 (d), ML776 (e), ML735 (f), 2KM138 (g), MH421 (h), ML1108 (i), PDM91-249 (j), ML839 (k), MH98-1 (l), Muskan (m), 2KM115 (n), LM10 (o), ML803 (p), BG39 (q), Satya (r), ML759 (s), 2KM151 (t), GP181 (u)]

At an arbitrary cut-off at 60 % similarity level on a dendrogram, the mungbean accessions were clustered into two major groups. One of the groups consisted of genotypes SMH99-1A and Asha and the other group consisted of rest of the genotypes. Cluster analysis showed that genetic relatedness of the 16 genotypes ranged from 0.60 to 0.98 i.e. 60–98 %. The genotypes ML1108 and 2KM115 were found to be genetically similar. Their genetic similarity was more than 98 %. The genotypes ML776 (i.e. both high iron and high zinc) and Satya (i.e. both low iron and low zinc) differed not only in their micronutrient content but were also genetically apart in the dendrogram as they fall in different clusters based on genetic similarity using SRAP markers.

Fig. 2 UPGMA dendrogram for the 21 genotypes of mungbean based on SRAP analysis using Jaccard's similarity coefficient

Principal component analysis (PCA)

Additionally, the relationships among mungbean genotypes were further examined by 2-D and 3-D scatter plots of different genotypes derived by the Principal Component Analysis based on Jaccard's similarity coefficient. SRAP data showed that 87.52 % of the total variation could be explained by the three principal components based on the first, second and third Eigen vectors which account for 75.98, 7.93 and 3.61 % variation respectively. The groupings of the 21 genotypes are shown in the 2-D (Fig. 3) scaling. It was evident from this analysis that majority of the groupings followed the same pattern as depicted in the

Fig. 3 Two dimensional PCA Scaling of 21 mungbean genotypes using SRAP markers (a: Ganga8; b: SMH99-1A; c: Asha; d: MH125; e: ML776; f: ML735; g: 2KM138; h: MH421; i: ML1108; j: PDM91-249; k: ML839; l: MH98-1; m: Muskan; n: 2KM115; o: LM10; p: ML803; q: BG39; r: Satya; s: ML759; t: 2KM151; u: GP181)

dendrogram with minor differences. ML776 and Satya occupied unique and distant positions in both the analyses.

Putative molecular marker for differentiating iron dense genotypes

On the basis of micronutrient content, we divided the genotypes into two major groups, one group was rich in iron and zinc content and the other group was iron and zinc deficient. A critical evaluation of analysis of banding pattern revealed that me2em7 primer amplified a unique band (700 bp size) which was present only in iron dense genotypes i.e. Ganga8, SMH99-1A, MH125 and ML776 (above 90 mg/Kg) and absent in genotypes having low iron content i.e. BG39 and ML759 (less than 50 mg/Kg) (Fig. 4)

Discussion

More than half of the world's population suffers from iron and zinc deficiency (World Health Organization 2002), popularly called as hidden hunger. Biofortification through genetic

manipulation of staple food crops is the most promising strategy to alleviate micronutrient deficiency (Tiwari et al. 2010). There are several approaches to biofortify crops, including agronomic biofortification (Cakmak 2008), genetic engineering (Brinch-Pederson et al. 2007) and conventional or molecular breeding (Welch and Graham 2004). Among these approaches, breeding for micronutrient enhancement has been considered as the best strategy due to low and non-recurrent expenditure and higher public acceptability (Nestel et al. 2006; Ortiz-Monasterio et al. 2007). HarvestPlus is a challenge program of Consultative Group on International Agricultural Research (CGIAR) which aims at increasing the micronutrient contents of staple foods to reduce micronutrient malnutrition. The food crops emphasized for biofortification by HarvestPlus are wheat, maize, rice, sweet potato, common beans and cassava. The Department of Biotechnology, Government of India, has taken up biofortification in rice for iron enrichment and wheat for zinc enrichment by marker-assisted selection. Mungbean is an important and essential source of dietary protein particularly in the predominantly vegetarian population of Indian subcontinent and constitute a significant part of Asian diet (Lakhanpaul et al. 2000). No major efforts have been taken up for iron and zinc biofortification in mungbean using conventional or marker assisted breeding worldwide. However, biofortification in mungbean should be taken up and assessment of the genetic diversity is an important first step in this direction.

Several markers can be used to identify and assess the genetic diversity and phylogenetic relationships in plant genetic resources. The traditional method based on morphological traits requires extensive observation of mature plants but these are ambiguous markers because of environmental influences (Wrigley et al. 1987). Genome analysis based on molecular markers has generated a vast amount of information and a

Fig. 4 Putative molecular marker (me2em7) for differentiating micro-nutrient (iron and zinc) dense genotypes [100 bp ladder (M), Negative control (C), Ganga8 (a), SMH99-1A (b), MH125 (c), ML776 (d), BG39 (e), ML759 (f)]

number of databases are being generated for use in crop breeding (Joshi et al. 1999). However the choice of markers to be employed in genome analysis is very crucial. SRAP marker system is a new, simple and efficient marker system and has been found immensely useful in several crops. Being PCR-based marker, SRAP markers have additional advantages, including requirement for relatively a small amount of template genomic DNA and good levels of polymorphism in many plant species. Especially, these types of DNA markers can be detected without any prior knowledge of the genome sequence. The merits of SRAP over other marker systems are its simplicity and reliability, reasonable throughput rate, targets ORFs in genome, numerous co-dominant and clear high-intensity bands rarely overlapping, not crop-specific, easy isolation of bands for sequencing, cost-effective. Any of the forward primers can be combined with any of the reverse primers, so many primer combinations are possible, reducing the cost of PCR, very important in the developing countries (Aneja et al. 2012).

In past, Lavanya et al. (2008) used RAPD profiles to identify the extent of diversity among 54 accessions of mung bean. Out of the 40 primers screened, seven primers generated 174 amplification products with an average of 24.85 bands per primer. A random amplified polymorphic DNA (RAPD) marker was found to be associated with mungbean yellow mosaic virus (MYMV) resistance (Selvi et al. 2006) and suggested to be useful for the selection of MYMV resistant genotypes. Chattopadhyay et al. (2005) used RAPD and ISSR markers to assess the genetic diversity among selected germplasm of mungbean [*Vigna radiata* (L.) Wilczek] comprising varieties, landraces and wild accessions reporting high polymorphism (83 %). Seehalak et al. (2009) used microsatellite markers to study polymorphism among 22 cultivated Thai accessions of mungbean using 78 primers and eight polymorphic loci detected two to three alleles per locus with an average of 2.25. There are no SSR marker analysis reports in Indian mungbean germplasm.

Since SRAP was reported in 2001 by Li and Quiros, it has emerged as a new and useful PCR marker technique for germplasm characterization, cultivar identification, molecular mapping and gene cloning in crop plants in recent years (Aneja et al. 2012). We found that these markers could detect 4.65 alleles per locus making SRAP analysis very useful in assessing mungbean diversity as these were highly polymorphic (100 %). We found a 700 bp amplified product using me2em7 primer combination in iron dense genotypes (Fig. 4). This SRAP marker can be validated in other micronutrient dense genotypes by screening a large number of germplasm. Further it can be converted into a SCAR marker for its use in marker-assisted selection in breeding iron dense mungbean genotypes (Aneja 2010). SRAP emerges as highly productive technique in crops where genome sequence is not available particularly in case of under-privileged crops such as mungbean for marker assisted breeding and related applications.

The iron and zinc contents vary in different crops. Previous studies on other legume crops indicated iron content in the range of 41–132 mg/Kg in lentils and 44–135 mg/Kg in chickpea (Sarker et al. 2009). The iron content in selected mungbean genotypes ranged from 46.31 mg/kg to 106.15 mg/kg and the zinc content varied from 23.31 mg/kg to 40.46 mg/kg (Table 4) in our study. In common bean, iron content variation was in the range of 40–84.6 ppm and zinc content in the range of 17.7–42.4 ppm with significant correlations between trials, between methods and between minerals (upto $r=0.715$). In our study, the value of correlation coefficient (r) was found to be 0.434. The inheritance of iron and zinc accumulation in common bean was shown to be predominantly quantitative with some common QTL found for both minerals (Blair et al. 2009). Co-localizing QTL was most notable on linkage group B11 for seed iron and zinc. It was suggested that seed iron and zinc accumulation is controlled multigenically or oligogenically, with some common genes affecting the accumulation of both minerals in the genetic background of the population analysed.

There is no information available on the nature and number of genes controlling iron and zinc content in mungbean. Our preliminary studies on assessment of genetic diversity for micronutrient content reveals that ML776 and Satya fall in high iron and zinc and low iron and zinc group respectively based on the micronutrient content. Taunk et al. (2012) also reported ML776 as high iron, high zinc variety and genetically distant from Satya which is low iron, low zinc variety based on RAPD analysis. We also found that ML776 and Satya are genetically distant and fall apart in the dendrogram based on SRAP analysis. For developing a mapping population and tagging gene(s) for micronutrient content, these two genotypes can serve as ideal parents since these are contrasting and genetically apart. These can also be used for developing Recombinant Inbred Lines (RILs) for linkage mapping and gene tagging for micronutrient content. Based on SRAP analysis, we could not group the genotypes on the basis of micronutrient content because the high/low micronutrient content genotypes were not clubbed together.

The discovery of QTL for mineral content can open the way for marker-assisted selection to breed new commercial varieties of mungbean with higher micronutrient concentration. For marker-assisted selection, a molecular linkage map is required. Mungbean F9 recombinant inbred lines (RILs) of TC1966 X Pagasa 7 was used in developing linkage map using amplified fragment length polymorphism (AFLP) markers (Sholihin and Hautea 2002). Another linkage map was constructed for mungbean consisting of 255 restriction fragment length polymorphism (RFLP) loci using a recombinant inbred population of 80 individuals (Humphry et al. 2002). This map was compared to a previously published map of lablab using a common set of 65 RFLP probes and marker order between mungbean and lablab was found to be highly conserved but no map contained enough markers to

condense into 11 putative linkage groups. There is large scope for saturating the existing linkage map of mungbean. SRAP, a highly polymorphic marker system offers great potential for improving the existing linkage map and association mapping as these markers have been used in different crops for these applications (Aneja et al. 2012).

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