J. Farm Sci., 30(4): (503-509) 2017

RESEARCH PAPER

Molecular diversity studies in greengram genotypes differing for powdery mildew resistance

MANISH KUMAR AND SUMANGALA BHAT

Department of Biotechnology, College of Agriculture Dharwad
University of Agricultural Sciences, Dharwad - 580 005, Karnataka, India
E-mail: sumangalabhat09@gmail.com

(Received: March, 2017 ; Accepted: December, 2017)

Abstract: Greengram is one of the most important pulse crops and important source of vegetable dietary protein across South and South East Asia. It also serves as an important green manuring and cover crop for enriching soil fertility due to its high atmospheric nitrogen fixation. Greengram suffers from several serious diseases, of which yellow mosaic virus, powdery mildew and *Cercospora* leaf spot cause considerable yield losses. Powdery mildew caused by *Erysiphe polygoni* can potentially reduce greengram yield by more than 40% if there is no prevention or even cause death of the plants if it occurs at the seedling stage. Therefore, initial efforts have been made to identify the resistant sources and to study the polymorphisms between resistant and susceptible genotypes at molecular level. Six parental (*Vigna trilobata, Vigna umbellata,* SML668, Chinamung, DGGV2 and Selection4) and 54 F_3 families were sown in field at MARS, Dharwad in *kharif* 2012. The percentage of leaf covered by the disease spores was scored visually. The percent was then translated into disease rating scale and calculated percent disease index (PDI) and it ranged from 0 per cent for *Vigna trilobata* and *Vigna umbellata* to 95.06% for Selecion4. Of the six parental genotypes DGGV2, China mung and Selection4 were found highly susceptible to powdery mildew with disease incidence of 95.06 per cent. Four cultivated (SML668, Chinamung, DGGV2, Selection4) genotypes along with two wild relatives (*Vigna trilobata* and *Vigna umbellata*) of greengram were used to study polymorphism at molecular level using sixty RAPD and thirty three SSR primers. Out of 60 RAPD and 33 SSR primers, 13 RAPD and 10 SSR primers were polymorphic. The value of similarity indices ranged from 0.165 to 0.632 for RAPD and 0.160 to 0.60 for SSR primers. The similarity between wild and cultivated varieties varied from 17.32 percent to 30.43 per cent and between wild 54.62 per cent and among cultivated varieties similarity was up to 63.29 per cent. The polymorphic information content (PIC) refers to the value of a marker for detecting polymorphism within a population and it depends on the number of detectable alleles and the distribution of their frequency and equivalent to gene diversity. The PIC value ranged from 0.45 to 0.67**.**

Key words: Diversity analysis, Greengram, Polymorphism, Primers

Introduction

Greengram (*Vigna radiata (*L.) Wilczek 2n=22) is the ancient and well known pulse crop of Asia. It is widely grown crop due to its early maturity, drought tolerance and ability to fix atmospheric nitrogen. Greengram is also known as mungbeen, green bean, mash bean, golden gram and green soy is an important source of dietary protein across Asia. It is originated from India and Central Asia. It is the third most important pulse crop grown in India on an area of 3.55 million hectare with total production of 1.81 million tones (Soren *et al.,* 2012). The importance of greengram in Indian economy is mainly due to its valuable and easily digestable protein (24%), fat (1.3%), calcium (124 mg), phosphorus (326 mg), iron (7.3 mg) and vitamin-B and rich in lysine, an essential amino acid. It also serves as an important green manuring and cover crop for enriching soil fertility due to its high atmospheric nitrogen fixation capacity through rhizobium root nodulation. Greengram suffers from several serious diseases, of which yellow mosaic virus, powdery mildew and *Cercospora* leaf spot cause considerable yield losses. The powdery mildew caused by *Erysiphe polygoni* can potentially reduce greengram yield by more than 40% if there is no prevention or even cause death of the plants if it occurs at the seedling stage screened six genotypes of greengram grown under control and uncontrol conditions against powdery mildew during *kharif,* 2004 and reported 30 per cent yield loss. Although chemical control is effective but they are not economical. Breeding for improved host resistance is expected to be the most effective, efficient and environmentally friendly method of control. So, breeding effort is on to develop resistant varieties. Since environmental factors can influence the occurrence of disease severity, the progress in conventional breeding is hampered. Hence, indirect selection by means of molecular markers linked to resistant genes would be very useful in increasing the accuracy and efficiency of selection for resistance against this disease. Previous studies on inheritance of powdery mildew resistance reported both monogenic and polygenic gene effects. Also quantitative trait loci governing powdery mildew resistance and the molecular markers linked to powderymildew resistance have been reported (Chaitieng *et al.,* 2002; Humphry *et al.,* 2003; Kajonphol *et al.,* 2010; Kasettranan *et al.,* 2010 and Chankaew *et al.,* 2013). Therefore, initial efforts have been made to identify the resistant sources and to study the polymorphisms between resistant and susceptible genotypes at molecular level.

Material and methods

Six parental (*Vigna trilobata, Vigna umbellata,* SML668, Chinamung, DGGV2 and Selection4) and 54 F_3 families were sown in field at Main Agricultural Research Station, Dharwad

in *kharif* 2012. Augmented design was followed for the experiment with susceptible and resistant checks at regular intervals. The incidence of powdery mildew disease on the leaves of different genotypes of greengram was scored by using 0 to 9 scale given by Mayee and Datar (1986), and the percent disease index (PDI) was calculated using the formula

$$PDI = \frac{\text{Sum of individual rating}}{\text{Total number of leaves observed} \times \text{maximum grade}} \times 100$$

For molecular diversity studies genomic DNA was isolated following protolcol given by Agbagwa *et al.* (2012) with minor modification. The extracted genomic DNA was run on 0.8 per cent agarose gel and stained with ethidium bromide (Sambrook *et al.,* 1989). The gel was visualized under trans-illuminator UV light and documented (Syngene, USA). The purity and concentration of genomic DNA was quantified at 260 nm and 280 nm by using nanodrop Spectrophotometer (Nanodrop ND1000 spectrophotometer, USA).

Random amplified polymorohic DNA and SSR reactions carried out in 20 µl volumes containing 100ng template DNA, 0.3 U/µl of Taq DNA polymerase, 2 mM dNTP, 5 pmol/µl primer in 10X reaction buffer. Eppendorf Master Cycler pro was used to run the PCR programme and it was programmed for an initial denaturation of 94°C for 4 min followed by 35 cycles of denaturation at 94°C for 1min, annealing for 1 min at 35°C, extension at 72°C for 1 min, and the final extension was done at 72°C for 10 min. The amplified products were separated on 0.8 per cent agarose gel for RAPD primers and 4 per cent agarose gel for SSR primers for visualizing the bands. Ten µl of amplified products from each sample was mixed properly with 2 µl of loading dye and then poured in the well. A 100 bp (Banglore Genie Pvt. Ltd, Banglore) ladder was used as molecular weight marker. Electrophoresis was done at 50 V for initial 30 minutes and then voltage was increased to 70 for 2 hours. The gel image was documented using gel documentation system (UVitec, Cambridge, England). The amplified fragments were scored as '1' for the presence and '0' for absence of a band generating the 0 and 1 matrix for RAPD and SSR primers. The NTSYS.PC (Numerical Taxonomy System Applied Biostatistics, Setauket, New York) computer program was used for data analysis. The data (band presence (1) or absence (0)) were introduced in the form of a binary matrix and a pair wise similarity matrix was constructed using the Jaccard's coefficient. Dendrogram was drawn by following unweighted pair-group method with arithmetic average (UPGMA).

Results and discussion

The percent disease index (PDI) ranged from 0 to 95.06 per cent. The two wild relatives of greengram *Vigna trilobata*

Table 1. List of genotypes with their percent disease index (PDI)

Sl. No.	Genotype	PDI	Sl. No.	Genotype	PDI
1	DGGV2	95.06	31	CM x V. trilobata-6	45.68
2	Chinamung	95.06	32	CM x V. trilobata-7	77.78
3	Selection4	95.06	33	CM x V. trilobata-8	55.56
4	Vigna triloebata	0	34	CM x V. trilobata-9	45.68
5	Vigna umbellata	0	35	Sel4 x V. trilobata-1	77.78
6	SML668	11.11	36	Sel4 x V. trilobata-2	65.43
7	Sel4 x SML668-1	40.74	37	Sel4 x V. trilobata-3	77.78
8	Sel4 x SML668-2	33.33	38	Sel4 x V. trilobata-4	77.78
9	Sel4 x SML668-3	62.96	39	Sel4 x V. trilobata-5	60.49
10	Sel4 x SML668-4	58.02	40	Sel4 x V. trilobata-6	55.56
11	Sel4 x SML668-5	75.31	41	Sel4 x V. trilobata-7	58.02
12	Sel4 x SML668-6	77.78	42	Sel4 x V. trilobata-8	77.78
13	Sel4 x SML668-7	85.19	43	Sel4 x V. trilobata-9	67.90
14	Sel4 x SML668-8	70.37	44	Sel4 x V. trilobata-10	77.78
15	Sel4 x SML668-9	85.19	45	SML668 x V. trilobata-1	92.59
16	Sel4 x SML668-10	60.49	46	SML668 x V. trilobata-2	82.72
17	Sel4 x SML668-11	67.90	47	SML668 x V. trilobata-3	58.02
18	Sel4 x SML668-12	77.78	48	SML668 x V. trilobata-4	85.19
19	Sel4 x SML668-13	70.37	49	SML668 x V. trilobata-5	70.37
20	Sel4 x SML668-14	60.49	50	SML668 x V. trilobata-6	65.43
21	Sel4 x SML668-15	67.90	51	SML668 x V. trilobata-7	58.02
22	Sel4 x SML668-16	62.96	52	SML668 x V. trilobata-8	87.67
23	Sel4 x SML668-17	85.19	53	SML668 x V. trilobata-9	67.90
24	Sel4 x SML668-18	92.59	54	SML668 x V. trilobata-10	67.90
25	Sel4 x SML668-19	77.78	55	SML668 x V. trilobata-11	72.84
26	CM x V. trilobata-1	77.78	56	SML668 x V. trilobata-12	77.78
27	CM x V. trilobata-2	77.78	57	SML668 x V. trilobata-13	80.25
28	CM x V. trilobata-3	75.31	58	SML668 x V. trilobata-14	75.31
29	CM x V. trilobata-4	85.19	59	SML668 x V. trilobata-15	87.65
30	CM x V. trilobata-5	48.15	60	SML668 x V. trilobata-16	85.19

and *Vigna umbellata* showed 0 PDI and considered as immune to powdery mildew. The cultivated greengram varieties Chinamung, DGGV2 and Selection 4 showed 95.06 PDI and considered highly susceptible. PDI for each genotype is shown in Table 1. Sujatha *et al. (*2011) also reported highly susceptible reaction of China mung to powdery mildew. SML668, a variety of greengram grown in Punjab, supposed to be resistant to powdery mildew was found to be moderately susceptible in this study. It is known fact that genotypes do not perform similar across location. Further, Akhatar *et al.* (2014) also reported the susceptibility of SML 668 to powdery mildew at Birsa Agriculture University farm, Ranchi. Peerajade *et al.* (2004) and Divya (2012) reported susceptibility of these genotypes to powdery mildew. Among the 54 F_3 families screened the PDI ranged from 33.33 per cent to 92.59 per cent and most of families were found to be highly susceptible (HS) under field conditions as shown in Table 2.

Sixty RAPD and thirty three SSR primers were screened among six different genotypes of gren gram. Out of 60 RAPD primers, 13 primers (Table 3) showed polymorphism and out of 33 SSR primers, 10 SSR primers (Table 4) showed polymorphism among powdery mildew susceptible and resistant genotypes of greengram. The binary data scored from the RAPD profiles (Plate 1) and SSR profile (Plate 2) were analysed separately. The data scored from the RAPD profiles were subjected to UPGMA cluster analysis to establish the relationship among the 6 genotypes of greengram. The value of Jaccard's distance varied from 0.165 to 0.632. The similarty between wild (*V. trilobata* and *V. umbellata*) and cultivated types (SML668, Chinamung, DGGV2, Selection 4) varied from 17.32 per cent to 30.43 per cent. The similarity between *V. trilobata* and *V. umbellata* was about 54.62 per cent and among cultivated types it was up to 63.29 per cent. The narrow genetic base among cultivated mungbeen accessions was reported previously by several workers (Datta *et al.,* 2012, Gupta *et al.,* 2013, Kaur *et al.,* 2017). Investigation by Lakhanpaul *et al.* (2000) among 32 accessions of mungbean demonstrated high level of genetic similarity among the accessions. The similarity among *Vigna trilobata* and *Vigna umbellata* with Selection 4, SML668, Chinamung and DGGV2 were low. *Vigna trilobata* and *Vigna umbellata* are immune to powdery mildew and fall in one group and SML668, Selection4, Chinamung and DGGV2 were susceptible and formed a separate group. The dendrogram resulting from the unweighted pair-group method with

Table 2. Grouping of genotypes according to scale given by Mayee and Datar (1986)

Sl. No.	Genotype	Disease reaction	Sl. No.	Genotype	Disease reaction
1	DGGV2	HS	31	CM x V. trilobata-6	S
2	Chinamung	HS	32	CM x V. trilobata-7	HS
3	Selection4	HS	33	CM x V. trilobata-8	HS
4	Vigna trilobata	I	34	CM x V. trilobata-9	S
5	Vigna umbellate	I	35	Sel4 x V. trilobata-1	HS
6	SML668	MS	36	Sel4 x V. trilobata-2	HS
7	Sel4 x SML668-1	S	37	Sel4 x V. trilobata-3	HS
8	Sel4 x SML668-2	HS	38	Sel4 x V. trilobata-4	HS
9	Sel4 x SML668-3	HS	39	Sel4 x V. trilobata-5	HS
10	Sel4 x SML668-4	HS	40	Sel4 X V. trilobata-6	HS
11	Sel4 x SML668-5	HS	41	Sel4 x V. trilobata-7	HS
12	Sel4 x SML668-6	HS	42	Sel4 x V. trilobata-8	HS
13	Sel4 x SML668-7	HS	43	Sel4 x V. trilobata-9	HS
14	Sel4 x SML668-8	HS	44	Sel4 x V. trilobata-10	HS
15	Sel4 x SML668-9	HS	45	SML668 x V. trilobata-1	HS
16	Sel4 x SML668-10	HS	46	SML668 x V. trilobata-2	HS
17	Sel4 x SML668-11	HS	47	SML668 x V. trilobata-3	HS
18	Sel4 x SML668-12	HS	48	SML668 x V. trilobata-4	HS
19	Sel4 x SML668-13	HS	49	SML668 x V. trilobata-5	HS
20	Sel4 x SML668-14	HS	50	SML668 x V. trilobata-6	HS
21	Sel4 x SML668-15	HS	51	SML668 x V. trilobata-7	HS
22	Sel4 x SML668-16	HS	52	SML668 x V. trilobata-8	HS
23	Sel4 x SML668-17	HS	53	SML668 x V. trilobata-9	HS
24	Sel4 x SML668-18	HS	54	SML668 x V. trilobata-10	HS
25	Sel4 x SML668-19	HS	55	SML668 x V. trilobata-11	HS
26	CM x V. trilobata-1	HS	56	SML668 x V. trilobata-12	HS
27	CM x V. trilobata-2	HS	57	SML668 x V. trilobata-13	HS
28	CM x V. trilobata-3	HS	58	SML668 x V. trilobata-14	HS
29	CM x V. trilobata-4	HS	59	SML668 x V. trilobata-15	HS
30	CM x V. trilobata-5	S	60	SML668 x V. trilobata-16	HS

HS- Highly susceptible MS- Moderately susceptible
I- Immune S- Susceptible
CM- China mung Sel4- Selection4

arithmetic average (UPGMA) cluster analysis was constructed (Fig. 1). Singh *et al.* (2014) studied diversity among 35 genotypes and reported that genotypes were grouped into 4 major clusters with most genotypes reflecting relationships according to the species distribution. Gupta *et al.* (2014) developed SSR markers and studied their transferability to related species. Cluster analysis based on similarity co-efficient separated the cultivated and the wild genotypes into separate groups.

The SSR markers were scored visually for their presence (1) or absence (0). To measure the informativeness of the markers, the polymorphism information content (PIC) for each SSR locus was calculated according to the formula (Weir, 1996)

$$PIC = 1 - \acute{O}p_i^2$$

Where, i is the total number of bands detected for a SSR marker, and pi is the frequency of the ith band. Polymorphism information content (PIC) for each SSR primers depicted in Table 5. A dendrogram was constructed based on Jaccard's similarity coefficient with unweighted pair group method and arithmetic average using the NTSYS software (Fig. 2). The value of Jaccard's distance varied from 0.160 to 0.60. A high similarity of 60 per cent was observed between the Selection4 and SML668. The dendrogram obtained considering SSR

Table 3. List of Polymorphic RAPD primers

Sl. No.	Primer	Primer sequence (5'- 3')
1	OPAD01	CAAAGGGCGG
2	OPX1	CTGGGCACGA
3	OPX7	GAGCGAGGCT
4	OPB07	GGTGACGCAG
5	M11	GTCCACTGTG
6	AA7	CTACGCTCAC
7	M05	GGGAACGTGT
8	OPD11	AGCGCCATTG
9	AA04	AATCGGGCTG
10	AA14	AACGGGCCAA
11	M13	GGTGGTCAAG
12	OPAG14	CTCTCGGCGA
13	OPE16	GGTGACTGTG

Table 4. List of polymorphic SSR primers with annealing temperature

Sl No.	SSR primer	Forward Primer sequence (5'- 3')	Reverse primer Sequence (5'-3')	Annealing Temp. (°C)
1	X62	TGGGCTACCAACTTTTCCTC	TGAGCGACATCTTCAACACG	58
2	X65	CAACATTTCAACCTTGGGACAG	ATCAACTGAGGAGCATCATCGA	58
3	X49	GGCAGAATCGTZCAAGTG	GTCAGATTCTCGCTTGCATG	58
4	X88	GCTCTGTCAGTTCCCACTAC	GGTCCTGAACCCAGATGAAC	58
5	X46	AATTGCTCTCGAACCAGCTC	GGTGTACAAGTGTGTGCAAG	58
6	X21	AAACATACCCCTGGCAGTTCC	TTCTGACCTAAGAAAGAGCCTGG	60.5
7	X34	CGGAAGAAGAACGCAGAGTG	GCATCAACAAGGACTTCTGC	60.5
8	MB17	CTTGCTTGCGAGGATGAC	TCCAGTGCAGCAGATTGA	57
9	AB27	ACTGGATGAGGGTTTAGTGCG	CTGTCTTGTCTTGTGGGTTCGTTC	60.5
10	AB50	TCCCACTTCTCCATTACCTCCAC	GAGATTATCTTCTGGGCAGCAAGG	60.5

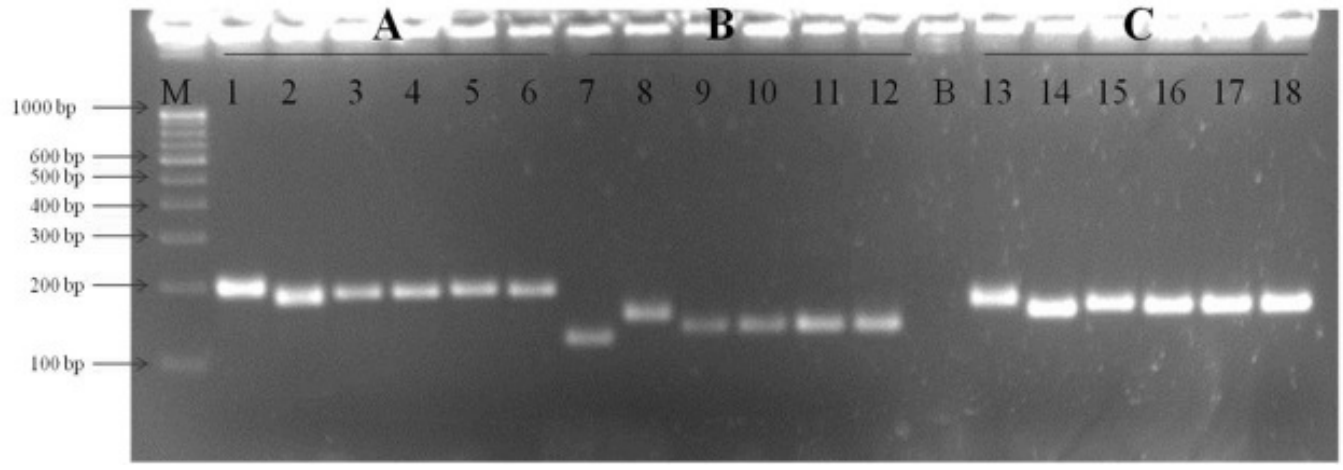

Plate 2. SSR polymorphism of six genotypes

Legend-

M- 100 bp ladder
(A) Primer- X62
(B) Primer- X65 and
(C) Primer- X49
Lane 1, 7 and 13-V. trilobata,
Lane 2, 8 and 14- V. umbellata,
Lane 3, 9 and 15- SML668,
Lane 4,10 and 16– Chinamung,
Lane 5, 11 and 17- DGGV2,
Lane 6, 12 and 18- Selection4.

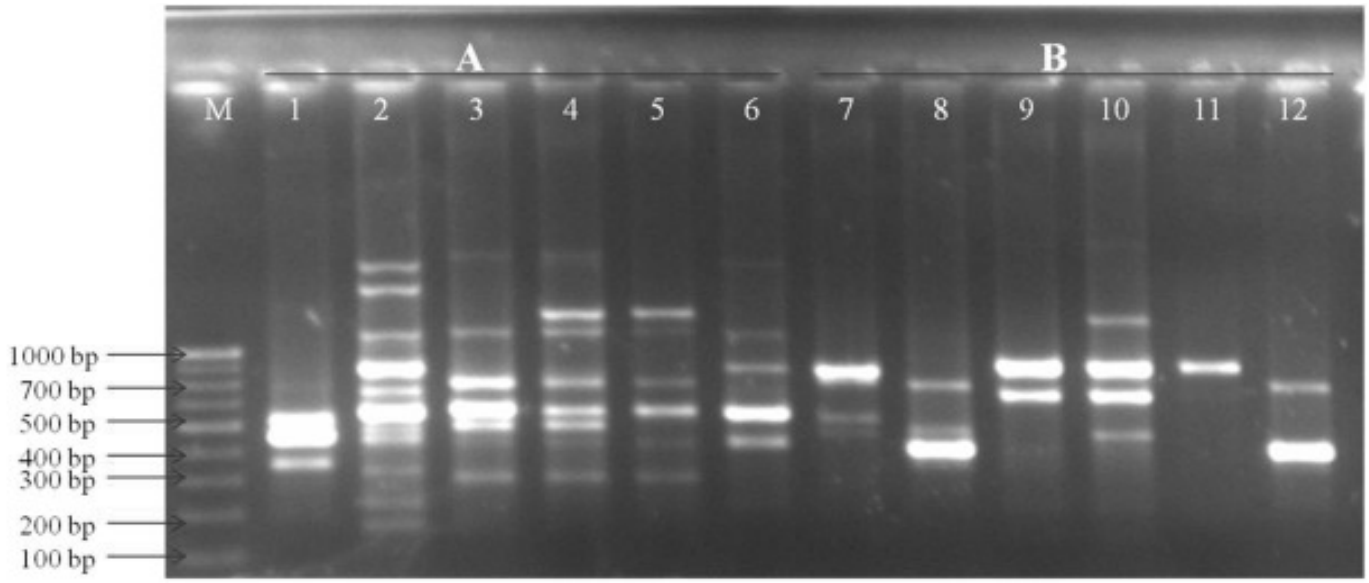

Plate 1. RAPD Plate polymorphism between six genotypes

Legend-

(A) Primer- OPB07,
Lane 1 and 7-V. trilobata,
Lane 3 and 9- SML668,
Lane 5 and 11- DGGV2,
M- 1 kb ladder,
(B) Primer- M11,
Lane 2 and 8- V. umbellata,
Lane 4 and 10 – Chinamung,
Lane 6 and 12- Selection4

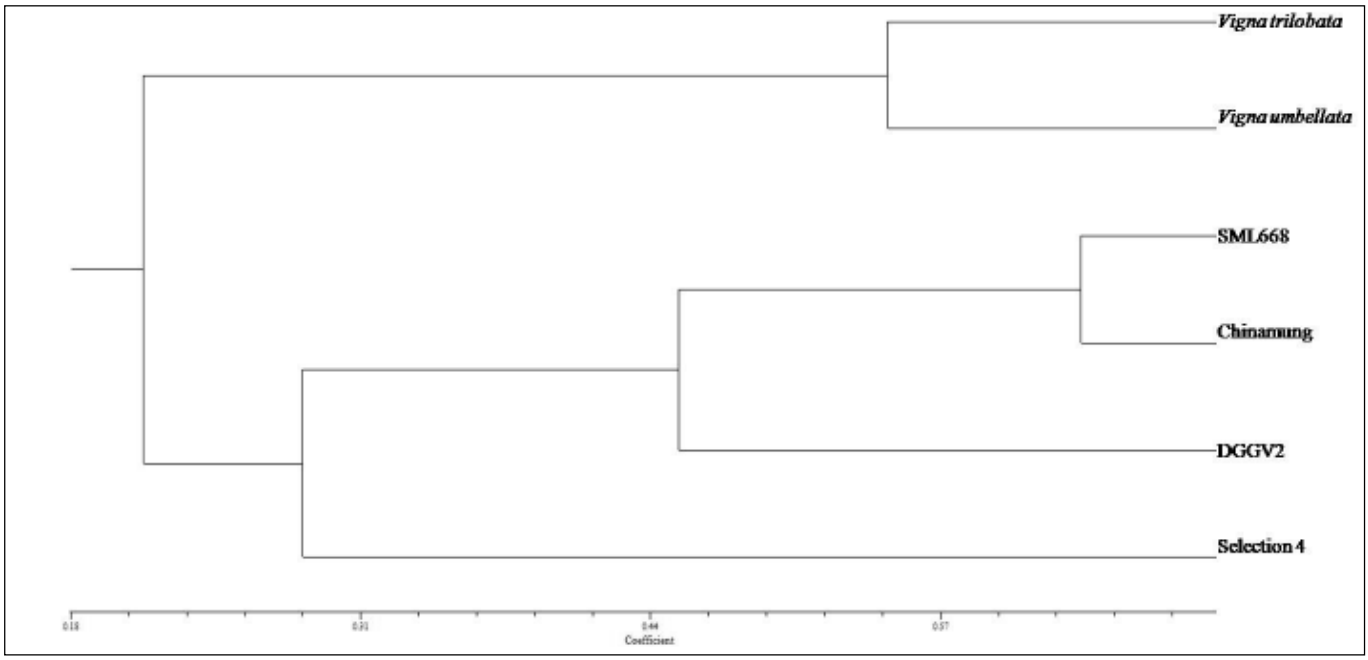

Fig. 1. Dendrogram generated using UPGMA analysis showing relationships among green gram genotypes obtained by RAPD data

based diversity analysis is almost same as the dendrogram obtained through RAPD analysis. The grouping based on molecular data can be correlated with the grouping made based on the disease score. However, some change in the order of closeness among four cultivated genotypes was observed. This variation might be due to intrinsic property of marker systems and also variation present among cultivated genotypes. The polymorphic information content (PIC) was calculated for each polymorphic SSR primers. PIC refers to the value of a marker for detecting polymorphism within a population and it depends on the number of detectable alleles and the distribution of their frequency and equivalent to gene

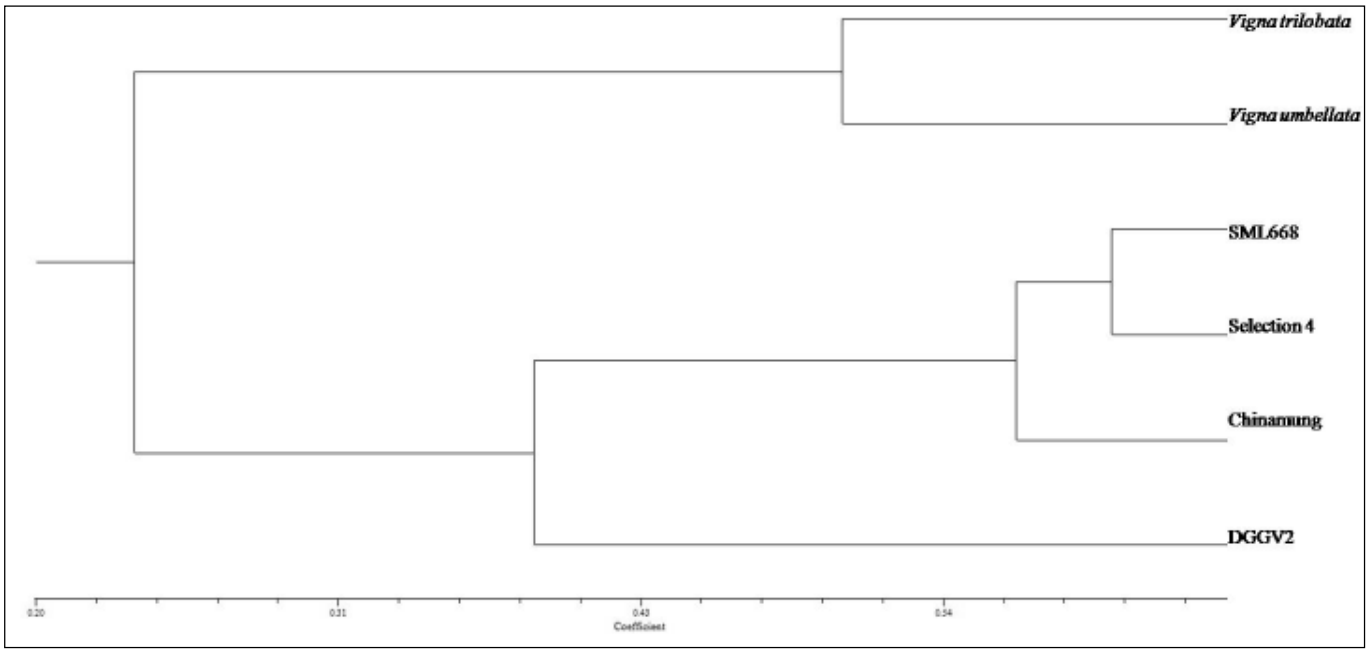

Fig. 2. Dendrogram generated using UPGMA analysis showing relationships among green gram genotypes obtained by SSR data

Table 5. PIC for polymorphic SSR primers

SSR primer	Forward Primer (5'- 3')	Reverse primer (5'-3')	PIC Value
X62	TGGGCTACCAACTTTTCCTC	TGAGCGACATCTTCAACACG	0.5
X65	CAACATTTCAACCTTGGGACAG	ATCAACTGAGGAGCATCATCGA	0.5
X49	GGCAGAATCGTZCAAGTG	GTCAGATTCTCGCTTGCATG	0.5
X88	GCTCTGTCAGTTCCCACTAC	GGTCCTGAACCCAGATGAAC	0.5
X46	AATTGCTCTCGAACCAGCTC	GGTGTACAAGTGTGTGCAAG	0.67
X21	AAACATACCCCTGGCAGTTCC	TTCTGACCTAAGAAAGAGCCTGG	0.50
X34	CGGAAGAAGAACGCAGAGTG	GCATCAACAAGGACTTCTGC	0.45
M17	CTTGCTTGCGAGGATGAC	TCCAGTGCAGCAGATTGA	0.45
AB27	ACTGGATGAGGGTTTAGTGCG	CTGTCTTGTCTTGTGGGTTCGTTC	0.50
AB50	TCCCACTTCTCCATTACCTCCAC	GAGATTATCTTCTGGGCAGCAAGG	0.61

diversity. The PIC value ranged from 0.45 to 0.67 which was in close agreement with earlier reported by Gupta and Gopalakrishna (2010) using adzukibean derived SSR markers in blackgram and Palaniappan *et al.* (2012) while studying genetic diversity in greengram. In the present study both RAPD and SSR primers have shown almost same result for diversity study among six different genotypes of greengram.

In conclusion, the present study revealed that there is a maximum similarity within the cultivated genotypes but low level similarity with the wild type genotypes. The *V. trilobata* shown maximum similarity with DDGV2 (30%) by RAPD and SSR studies. In case of *V. umbellata* the maximum similarity was observed with the SML668 (31%) cultivated genotype. It indicates the wild genotype which is high level of similarity with cultivated genotype can be used for greengram crop improvement programme.

References

Agbagwa, I. O., Datta, S., Patil, P.G., Singh, P. and Nadarajan, N., 2012, A protocol for high quality genomic DNA extraction from legumes. *Genet. Mol. Res.,* 11 (4): 4632-4639.

Akhtar, J., Lal, H. C., Kumar, Y., Singh, P. K., Ghosh, J., Khan, Z. and Gautam, N. K., 2014, Multiple disease resistance in greengram and blackgram germplasm and management through chemicals under rain-fed conditions. *Legume Res.,* 37 (1): 101-109.

Chaitieng, B., Kaga A., Han O. K., Wang X. W., Wongkaew S., Laosuwan P., Tomooka, N. and Vaughan, D., 2002, Mapping new sources of resistance to powdery mildew in mungbean. *Plant Breed.,* 121: 521-525.

Chankaew, S., Somta, P., Isemura, T., Tomooka, N., Kaga, A., Vaughan, D. A. and Srinives, P., 2013, Quantitative trait locus mapping reveals conservation of major and minor loci powdery mildew resistance in four sources of resistance in mungbean (*Vigna radiata* L. Wilczek). *Mol. Breed.,* 32: 121-130.

Dakshayani, R. and Mummighatti, U. V., 2004, Effect of powdery mildew on yield and yield components in greengram. *Karnataka J. Agric. Sci.*, 17 (3): 448-450.

Datta, S., Gangwar, S., Kumar, S., Gupta, S., Rai, R., Kashyap M., Singh, P., Chaturvedi, S., Singh, B. and Nadarajan, N., (2012), Genetic diversity in selected Indian Mungbean [*Vigna radiata* (L.) Wilczek] cultivars using RAPD markers. *Am. J. Plant Sci.,* 3: 1085-1091.

Divya, J. U., 2012, Epidemiology and management of powdery mildew of greengram caused by *Erysiphe polygoni. M. Sc. (Agri) Thesis,* Univ. Agric. Sci., Dharwad, Karnataka (India).

Gupta S. K., Bansal, R. and Gopalkrishnan, T., (2014), Development and characterization of genic SSR markers for mungbean (*Vigna radiata* (L.) Wilczek). *Euphytica,* 195: 245- 258.

Gupta S. K., Bansal, R., Vaidya, U. G. and Gopalkrishnan, T., (2013), Assessment of genetic diversity at molecular level in mungbean (*Vigna radiata* (L.) Wilczek). *J. Food Legumes*, 26 (3&4):19-24.

Gupta, S. K. and Gopalakrishna, T., 2010, Development of unigene-derived SSR markers in cowpea (*Vigna unguiculata*) and their transferability to other *Vigna* species. *Genome,* 53 (7): 508-523.

Hartman, G. L., Wang, T. C. and Kim, D., 1993, Field evaluation of mungbean for resistance to cercospora leaf spot and powdery mildew. *Int. J. Pest Manage.,* 39(4): 418-421.

Humphry, M. E., Magner, T., McIntyre, C. L., Aitken, E. A. and Liu, C. L., 2003, Identification of major locus conferring resistance to powdery mildew (*Erysiphe polygoni* D. C.) in mungbean [*Vigna radiata* (L.) Wiczek] by QTL analysis. *Genome,* 46(5): 738-744.

Kaur, G., Joshi, A. and Jain, D., (2017), Genetic diversity in mungbean (*Vigna radiata* (L.) Wilczek) genotypes. *Brazilian Archives of Biology and Technology,* 59 e 160613.

Kajonphol, T., Sangsiri, C., P., Toojinda, T. and Srinives, P., 2010, SSR map construction and quantitative trait loci (QTL) identification of major agronomic traits in mungbean [*Vigna radiata* (L.) Wilczek]. *SABRAO J. Breed. Genet*., 44(1): 71-86.

Kasettranan, W., Somta, P. and Srinives, P., 2010, Mapping of quantitative trait loci controlling powdery mildew resistance in mungbean [*Vigna radiata* (L.) Wilczek]. *J. Crop. Sci. Biotechnol.,* 13 (3): 155-161.

Lakhanpaul, S., Chadha, S. and Bhat, K. V., 2000, Random amplified polymorphic DNA (RAPD) analysis in Indian mungbean (*Vigna radiata* (L.) Wilczek) cultivars. *Genetica,* 109: 227 – 234.

Mayee, C. D. and Datar, V. V., 1986, Phytopathometry, Tech. Bull 1 (Special Bulletin 3). *Marathwada, Agri. Univ. Press Parabhani.* pp. 218.

Palaniappan, J. and Murugaiah, S., 2012, Genetic diversity as assessed by morphological and microsatellite markers in greengram (*Vigna radiata* L.). *African J. Biotechnol.,* 11 (84): 15091-15097.

Peerjade, D. A., Ravi, R. L. and Rao, M. S. L., 2004, Screening of local mung bean collections for powdery mildew and yellow mosaic virus resistant. *M. Sc. (Agri.) Thesis,* Univ. Agric. Sci., Dharwad, Karnataka (India).

Sambrook, J., Fritsch, E. F. and Maniatis, T., 1989, Molecular Cloning: a Laboratory Manual, 2nd edn. Cold Spring Harbor Laboratory Press, New York.

Singh, A., Dikshit, H. K., Jain, N., Singh, D. and Yadav, R.N., (2014), Efficiency of SSR, ISSR and RAPD markers in molecular characterization of mungbean and other vigna species. *Indian J. Biotechnol., 13*:

Soren, K.R., Patil, P. G., Das, A., Bohra, A., Datta, S., Chaturvedi, S. K. and Nadarajan, N., 2012, Advances in Pulses Genomic Research. *Ind. Ins. of Pul Res.,* Kanpur.

Sujatha, K., Kajjidoni, S.T., Patil, P.V. and Somashekhar, G., 2011, Heterosis for productivity related traits involving diverse parents for powdery mildew reaction in mungbean. *J. Food Leg.,* 24 (2): 101-105.

Weir, B. S., 1996, Genetic data analysis II. ed. Sunderland, Massachusetts, Sinauer associates, p.377.

Kasettranan, W., Somta, P. and Srinives, P., 2010, Mapping of quantitative trait loci controlling powdery mildew resistance in mungbean (*Vigna radiata* L. Wilczek). *J. Crop. Sci. Biotechnol.,* 13 (3): 155-161.

Khajudparn, P., Wongkaew, S. and Thipyapong, P., 2007, Mungbean powdery mildew resistance identification of genes for resistance to powdery mildew in mungbean. *African Crop Sci. Conf. Proc.,* 8: 743-745.