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Research Article

Cross-species amplification and genetic variation among blackgram genotypes using SSR markers developed from mungbean DNA sequence scaffolds harbouring putative resistance genes

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Abstract
The cross species amplification of 97 mungbean derived resistance gene-SSR markers were investigated for diversity analysis in a set of 44 blackgram genotypes. A total of 68(70%) SSR markers showed amplification in blackgram. Our of 68 markers, thirty randomly selected markers were used to study the genetic variation among 44 blackgram genotypes varying for yellow mosaic disease (YMD) and powdery mildew disease (PMD) reaction. Thirty SSR primers collectively amplified 90 alleles in blackgram with an average of three alleles/locus. The polymorphic information content (PIC) of the SSR markers ranged from 0 to 0.86 with an average of 0.43. Cluster analysis based on UPGMA neighbour-joining method grouped the 44 genotypes into seven clusters. The genotypes NDU-1 and PU-19 were observed to be highly dissimilar with similarity coefficient of 0.27 in comparison to other genotypes. YMD and PMD resistant and susceptible genotypes could be differentiated by three (MRGSSR 12, MRGSSR 56, MRGSSR 77) and four SSR markers (MRGSSR12, MRGSSR 32, MRGSSR56 and MRGSSR65), respectively. Two of these markers *viz*., MRGSSR12 and MRGSSR56 were mutually effective in differentiating YMD and PMD resistant genotypes. These were located in mungbean scaffolds JJMO01002369 and JJMO01001477 and exhibited homology with TMV resistance protein N and DNA damage-repair/toleration protein DRT100, respectively.

Keywords: Blackgram, Cross species amplification, Resistance genes, Genomic-SSR, Polymerase chain reaction, Diversity analysis.

INTRODUCTION

Blackgram, commonly known as urdbean is grown in India for its protein rich seeds. It is a self-pollinating, annual diploid ($2n = 2x = 22$) crop with a genome size of approximately 574 Mbp (Arumuganathan and Earle, 1991). The major yield-limiting factors in blackgram are various biotic (viruses, fungi, bacterial pathogens, and insects) and abiotic (salinity, drought, etc.) stresses (Souframanien *et al*., 2017). Among the biotic constraints, yellow mosaic virus disease (YMD) transmitted by white fly and powdery mildew disease (PMD) caused by *Erysiphe polygoni* are major threats causing yield losses upto 85% (Varma and Malathi, 2003) and 40-90% (Channaveeresh *et al*., 2014), respectively. Both YMD and PMD resistance in blackgram were reported to be under the control of single recessive gene (Reddy and Singh, 1995; Singh *et al*., 1998; Kaushal and Singh, 1989).The disease screening becomes complicated due to rapid evolution of yellow mosaic

viruses leading to emergence of new strains with wider host range and difficulties in screening breeding population for powdery mildew disease; especially when weather conditions are unfavourable for strong fungal growth and hot spots of natural epidemics are not always available (Channaveeresh *et al*., 2014).Therefore, development of elite cultivars with durable resistance requires pyramiding of resistance genes from several sources. This necessitates development of molecular markers for as many resistance genes as possible for their reliable introgression and marker assisted selection.

During the course of evolution, plants have developed complex defense mechanisms to counteract pathogens (Staskawicz *et al*., 1995) through PAMP (Pathogen associated molecular pattern) Triggered Immunity (PTI) and/or Effector Triggered Immunity (ETI). In ETI, resistance (R) genes products recognize products of avirulence genes of the pathogens (Scofield *et al*., 1996) and evoke defense responses such as hypersensitive reaction, strengthening of the cell wall, phytoalexin production etc. (Dangl *et al*., 1996). These R genes are classified into four structurally distinct classes based on protein domains they encode (Ellis *et al*., 2000). Molecular characterization of these R genes reveal their highly conserved nature among plant species and presence of conserved domains/motifs such as nucleotide binding sites (NBSs), leucine-rich repeats (LRRs), transmembrane domains (TMs) and Toll/interleukin-1 regions (TIR). These domains are known to be involved in the detection of diverse pathogens, including bacteria, viruses, fungi, nematodes, insects and oomycetes (McHale *et al*., 2006). This information has been exploited for exploring resistance gene analogues (RGA) in several crops such as blackgram (Basak *et al*., 2004), common bean (Lopez *et al*., 2003) and peanut (Yuksel *et al*., 2005). Degenerate primers derived from R genes conserved motifs have been used for targeting RGAs and profiling of different cultivars of potato, tomato, barley, and lettuce (Van der Linden *et al*., 2004). Furthermore, some of the RGAs have been transformed into molecular markers such as dCAPS (Derived Cleaved Amplified Polymorphic Sequence) and CAPS (Cleaved Amplified Polymorphic Sequence) to detect the presence of SNPs (Single Nucleotide Polymorphism) and their subsequent mapping on to the mapping populations of faba bean, pea, and chickpea (Palomino *et al*., 2009; Torres *et al*., 2010).

Although, RGH (resistance gene homologues) markers are much more effective than random markers, they rarely correspond to functional genes due to interference of large numbers of pseudo-genes, less expressed RGHs were easily lost when amplified from cDNA due to the interference of highly expressed RGHs in random cloning (Ren *et al*., 2014). EST-derived RGHs can overcome these disadvantages but require EST database which are limited for blackgram. Moreover, some non-resistance genes like the R genes also harbour NBS-LRR (Nucleotide binding site-Leucine rich repeat) motifs and therefore, necessitates further confirmation of target sequence amplified through sequencing or qPCR (Yuksel *et al*., 2005). Thus, there is a need to search for other ways for exploring R genes rather than completely dependent on targeting RGHs through degenerate primers. Blackgram is assumed to be closely related to mungbean because both originated from the Indian subcontinent (Zukovaskij, 1962). This relatedness can be exploited for transferability of molecular markers such as simple sequence repeats (SSRs) from one species to other as reported in several legume crops such as blackgram (Gupta and Gopalakrishna, 2009; Souframanien and Gopalakrishna, 2009)and *Glycine* (Peakall *et al*., 1998). SSRs are the markers of choice because of their ease to use, high reproducibility, hyper variability, locus specificity, and co-dominant nature. Moreover, Studies on disease resistance genes have indicated a high level of polymorphism and presence of SSRs at certain loci (Yu *et al*., 1996). In the present study, 23 putative disease resistance genes identified by Kang *et al*., (2014) from mungbean whole genome shotgun sequencing were exploited for developing genomic resources in blackgram with the following objectives: 1) Developing SSR markers from mungbean scaffolds homologous to resistance genes, 2) Analyzing cross-species amplification of developed SSR markers and 3) Studying genetic variation in blackgram genotypes differing in YMD and PMD reactions.

MATERIALS AND METHODS

A total of 44 blackgram genotypes [EC-168200, PUSA-3, IPU02-43, KU96-3, KU96-7, IPU07-3, DPU88-31, TU94-2, Azad-1, LBG-752, LBG-17, LBG-693, LBG-623, TAU-1, Trombay Wild (TW), LBG-703, LBG-20, T-9, Nayagarh, Pant-U19, PU31, PLU-1, TU-43-1, TU-55-1, NDU-1, TU-67, WBG-17, WBG-57, WBG-13, COBG-653, PLU-710, Sharda mash, EC168058, LBG-685, LBG-709, Sheela, EC168234, EC168242, EC168243, IPU-02-6, IPU-99-247, SPS-30, ANU-11, IPU-99-40] including diverse cultivars, landraces, and one wild accession differing for disease reaction(YMD and PMD) were used in the study. Disease reactions of each of the genotypes were considered based on the Annual report of Mungbean, urdbean, lentil, lathyrus, rajmash and fieldpea (MULLaRP) and published literature (**Table 1**). Total genomic DNAs were extracted from young seedlings using Dellaporta method (Dellaporta *et al*., 1983). The quantity and quality of DNA were checked using Nanodrop ND 1000 spectrophotometer (Thermo Scientific, USA). The working DNA samples were diluted to a standard concentration of 15ng/µl.

The present study was carried out at Nuclear Agriculture & Biotechnology Division, Bhabha Atomic Research Centre, Mumbai, during 2019. A total of 23 putative

Table 1. Blackgram genotypes used in the study with their disease reaction to YMV and powdery mildew disease

S. No.	Genotypes	Reaction to YMV	Reaction to PMD	References
1	EC-168200	R	NA	Gupta *et al*., 2015
2	PUSA-3	R	NA	Gupta *et al*., 2015
3	IPU02-43	R	R	Bandi, 2018; Aktar *et al*., 2014; Gupta *et al*., 2013
4	KU96-3	R	R	Gupta *et al*., 2015
5	KU96-7	R	NA	
6	IPU07-3	R	R	Gupta *et al*., 2015
7	DPU88-31	R	S	Gupta *et al*., 2013
8	TU94-2	R	MR	Bandi, 2018; Gupta *et al*., 2015
9	Azad-1	R	NA	Anonymous, 2022
10	LBG-752	MS	R	Bandi, 2018; Priyanka *et al*., 2018
11	LBG-17	S	R	Bandi, 2018; Srivastava *et al*., 2011
12	LBG-693	S	NA	
13	LBG-623	S	R	Bandi, 2018; Priyanka *et al*., 2018
14	TAU-1	S	S	Gupta *et al*., 2015
15	Trombay Wild	S	NA	Gupta *et al*., 2015
16	LBG-703	S	NA	
17	LBG-20	S	R	Gupta *et al*., 2013; Srivastava *et al*., 2011 Priyanka *et al*., 2018
18	T-9	S	MR	Bandi, 2018; Srivastava *et al*., 2011
19	Nayagarh	R	NA	Gupta *et al*., 2015
20	Pant-U19	R	R	Gupta *et al*., 2015
21	PU31	R	R	Bandi, 2018; Aktar *et al*., 2014
22	PLU-1	R	NA	Gupta *et al*., 2013
23	TU-43-1	R	MR	
24	TU-55-1	R	MR	
25	NDU-1	R	NA	
26	TU-67	S	NA	
27	WBG-17	S	NA	
28	WBG-57	S	S	Basandrai *et al*., 1999
29	WBG-13	S	NA	
30	COBG-653	S	S	Equbal *et al*., 2015
31	PLU-710	S	NA	
32	Sharda mash	S	S	
33	EC168058	S	NA	Gupta *et al*., 2015
34	LBG-685	S	MR	Bandi, 2018
35	LBG-709	S	R	
36	Sheela	NA	NA	
37	EC168234	NA	NA	
38	EC168242	NA	NA	
39	EC168243	NA	NA	
40	IPU-02-6	NA	NA	
41	IPU-99-247	NA	NA	
42	SPS-30	NA	NA	
43	ANU-11	R	R	
44	IPU-99-40	NA	NA	

R: Resistant; S: Susceptible; MR: Moderately resistant; MS: Moderately susceptible; NA: Not available

resistance proteins identified in mungbean (*Vigna radiata* var. *radiata* cultivar:VC1973A) from whole genome shotgun (wgs) sequences (Kang *et al*., 2014) available in NCBI database (Accession: PRJNA243847, ID: 243847) was used in this study. Amino-acid sequence of proteins were downloaded from uniprot (The UniProt Consortium 2017, https://doi.org/10.1093/nar/gkw1099). All 23 proteins were searched for sequence homology with scaffolds assembled from mungbean whole genome sequencing (*Vigna radiata* var. *radiata*, taxid:3916) with the help of tBLASTn algorithm. Significant mungbean scaffolds were searched for SSRs and primer-pairs were designed with the help of websat (http://purl.oclc.org/NET/websat/) online software (Martins *et al*., 2009). Thirty randomly chosen SSR primers were used to study the genetic variation among 44 blackgram genotypes. PCR reactions were carried out in a 25 µl reaction volume in an Eppendorf Master Cycler (Eppendorf, Hamburg, Germany) with following composition: 75 ng of genomic DNA,10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2.5 mM $MgCl_2$, 0.08% Nonidet P40, 0.2 mM dNTPs, 1.5 pmoles of forward and reverse primers, and 0.5 unit of *Taq* DNA polymerase (Fermentas Life Sciences).The amplification conditions were initial denaturation at 94°C for 3 min,5 cycles of: 94°C for 30 s, 56 to 46°C (-1°C each cycle), 72°C for 1 min, and followed by 35 cycles of: 94°C for 30 s, 46°C for 1 min, 72°C for 1 min and ended up with a final extension at 72°C for 7 min. PCR products were resolved on 3% agarose gels in TBE buffer at 80 V and images were captured in a gel documentation system (Syngene, U.K).

Genotyping was done as presence (1) or absence (0) of bands for each allele of the marker regardless of their intensity. Polymorphic information content (PIC) was calculated by the formula of Anderson *et al*. (1993): PIC = $1-\Sigma(P_{ij})^2$, where P_{ij} is the frequency of the jth allele for the ith locus. Genotypic data was analyzed through NTSYS-pc version 2.0 software (Rohlf *et al*., 1998) and dendrogram was generated using Jaccard's similarity coefficient.

RESULTS AND DISCUSSION

A total of 97 primer-pairs were designed for SSRs lying in mungbean scaffolds harbouring R genes, of which 74, 17, 2, and 4 primers were designed targeting di-nucleotides, tri-nucleotides, tetra-nucleotides and penta-nucleotides, respectively. Out of 97 SSR primers, 68 SSR primers (70%) showed amplificationin blackgram. Thirty of the primers showing amplification were randomly selected for genetic variation analysis in 44 blackgram genotypes differing in disease reaction to YMD and PMD. These 30 primers belonging to different scaffolds of mungbean (**Table 2**) harboured TMV resistance protein N, DNA-damage-repair/toleration protein DRT100, probable disease resistance protein At4g33300, protein suppressor of npr1-1, constitutive 1, putative disease resistance protein RGA4, and different putative resistance proteins. All 30 SSR primers collectively amplified 90 alleles in blackgram genotypes with an average of 3 alleles/locus. Twenty-eight of the thirty primers screened were found to be polymorphic with PIC ranging from 0.01 to 0.86 with an average of 0.43 (**Table 2**).The PIC values ranged from 0 to 0.86 and 0.11 to 0.67 for di-nucleotide and tri-nucleotide repeat motifs, respectively. Representative DNA amplification of blackgram genotypes using mungbean sequence derived SSR marker MRGSSR 118 is shown in **Fig.1**.The transferability of mungbean based SSR markers to blackgram was found to be 70% which is high compared to other similar reports such as 50% for cowpea unigene-SSR markers (Souframanien *et al*., 2017) and 68% collectively for azukibean, common bean, cowpea and mungbean (Souframanien and Gopalakrishna, 2009).The extent of transferability of SSR markers depends on evolutionary relationship between the species and conservation of PCR primer binding sites flanking the SSR motifs (Souframanien *et al*., 2017).The cross-species amplification of SSR markers from mungbean indicates that the sequence flanking the SSRs are conserved between mungbean and blackgram. Similarly, microsatellite markers were reported to be transferable in *Phaseolus* (Gaitan-Solis *et al*., 2002) and major pulses (Pandian *et al*., 2000). High transferability rate and less frequent null alleles observed in this study in comparisonto other reports could be due to use of genomic SSR markers which are not associated with problems of disrupted priming sites due to intron splice sites, large introns and additionally markers used in this study were developed from mungbean which is more closely related to blackgram compared to other *Vigna* species (Zukovaskij, 1962).

In the present study, allelic variation at 30 SSR loci with an average PIC of 0.43 which is comparable to genomic SSR markers from other *Vigna* species (Souframanien and Gopalakrishna, 2009) and supports utilization of these resistance genes based genomic-SSR markers in blackgram. MRGSSR12 and MRGSSR110 designed for di-nuleotide repeats $(AT)_{13}$ and $(AT)_7$ were found to be highly polymorphic with PIC values of 0.86 and 0.83, respectively. These highly polymorphic markers were derived from scaffolds homologous with TMV resistance protein N and putative disease resistance protein At4g11170. Di-nucleotides based primers were observed to exhibit high PIC value which is consistent with the earlier reports of such primers derived from cowpea (Souframanien and Gopalakrishna, 2009) and soybean (Hisano *et al*., 2007). Similarly, significance of variable repeat motifs can be comprehended by their positional effect. When present in the coding sequences or regulatory regions they could cause a frame shift, alteration of gene expression, inactivation of gene activity, and/or a change of function, and eventually phenotypic changes (Li *et al*., 2004).

Table 2. Details of the 30 SSR markers developed from mungbeanWGS scaffolds harbouring nucleotide sequences homologous to putative resistance genes and cross-species amplification in blackgram

Marker	Mungbean sequence ID	Repeat motif	Forward primer	Reverse primer	Putative function	No. of alleles	PIC
MRGSSR1	JJMO01000583	(AC)7	ATGTCGTGCATAGTCGTAGGTG	ATTGAAACAGGAGCTTCCAAGA	Protein suppressor of npr1-1, constitutive 1	2	0.01
MRGSSR3	JJMO01000123	(GA)7	ACGGACTCTAGCAAATGGAAAG	ATGGGAACCAAGAAGACAGAAA	Probable disease resistance protein At5g66900	2	0.33
MRGSSR9	JJMO01000647	(TTA)8	CCTTTCTCCCTGTCATCTTCAT	GCCAGAGATTTCCACCTACAAT	TMV resistance protein N	2	0.39
MRGSSR10	JJMO01001488	(ATG)6	GCAACACTTCTGCTTTACATGG	CACTTACATGGCCTGGATTTTA	TMV resistance protein N	3	0.33
MRGSSR12	JJMO01002369	(AT)13	CATTGTTAAAACGTACCAACGG	AAATCGCCTCAAAGTATAGGGAC	TMV resistance protein N	7	0.86
MRGSSR13	JJMO01001492	(ATC)8	AAATACACACACGCACTCACAT	GTTGGGTGAAGGGTAAGCTC	Probable disease resistance protein At4g33300	1	0
MRGSSR20	JJMO01002209	(TA)8	CCCAATCCCCGACTTAAATAAC	AGGCTATGTTTCAGATGCTGCT	Disease resistance RPP13-like protein 4	6	0.75
MRGSSR32	JJMO01002369	(TA)9	GCACCTATGTTGAGATCCATGA	AGAAAGAAAACAGGGCAGACAA	TMV resistance protein N	7	0.81
MRGSSR34	JJMO01002369	(TGA)7	ACACCTTCCTCACCACTCCTTA	GATCAAATACCCCACAAGCACT	TMV resistance protein N	3	0.67
MRGSSR36	JJMO01000583	(AG)7	AAAGGGAAGAGGTAAACGGAAG	AATTGATTGGGTAGGTGAAAGC	Protein suppressor of npr1-1, constitutive 1	2	0.37
MRGSSR39	JJMO01001147	(AT)8	GTAGAAATGCTTCCTCCTTCCA	ATTCTCTCCCGTATTGAAAAGC	Putative disease resistance protein RGA4	2	0.72
MRGSSR45	JJMO01000120	(AT)33	CTTCACATGCTACACTTCAGGG	GCACAATACCAAACCAGATTCA	Translation factor GUF1 homolog, mitochondrial	2	0.26
MRGSSR48	JJMO01001646	(CA)7	GGGGTTGAGATTTGGTGTATGT	TCATTTGTGAGGCTTAGGGTCT	Putative disease resistance RPP13-like protein 1	3	0.7
MRGSSR51	JJMO01000319	(CT)7	CATGATCCATCTTTAGCCATGA	TACCAGCAACTTTAGGCCAACT	Disease resistance protein RPP8	5	0.81
MRGSSR56	JJMO01001477	(ATA)6	GTCCAAACCTTTCAGACTCACC	TTGCAGACACAGCCACTCTAAT	DNA-damage repair / toleration protein DRT100	6	0.32
MRGSSR57	JJMO01000121	(TA)13	TGTTGAGAAATAGGACCCTTGG	ACCCGAAACCCGACTAGAATAA	DNA-damage repair / toleration protein DRT100	2	0.55
MRGSSR65	JJMO01000122	(TAA)17	AATTATGTTGCGTCATCACAGG	TTACTCCAGTTTCCCAAATGCT	Probable disease resistance protein At4g33300	4	0.11
MRGSSR77	JJMO01000040	(CT)7	TTTGGTGACATAGTTTGAGGCA	AAGCAAAGGAAGAATGAGGTTG	Putative disease resistance RPP13-like protein 1	2	0.63
MRGSSR82	JJMO01002047	(TA)32	ATCATTCTTGATGTTGCTGGTG	TTGCAGAGTCAAACACAGAGGT	DNA-damage repair / toleration protein DRT100	1	0
MRGSSR84	JJMO01000772	(AG)9	GAGAGAGAGCCTGTGGAGAGAG	GATGTTACTGGAAATTGCGGAT	DNA-damage repair / toleration protein DRT100	1	0
MRGSSR86	JJMO01000850	(TA)23	TCAACCATCCCAAGTATTTCTG	AAAACACACACACACACACACA	DNA-damage repair / toleration protein DRT100	9	0.54
MRGSSR91	JJMO01000853	(GA)7	CCTTAATCAAATTCCATCTCCG	CTCCAGCTACTATTCCCTCGAA	Putative disease resistance protein RGA3	2	0.5
MRGSSR99	JJMO01000583	(TA)6	TCTATATGATCCTCTGGCTCGC	GAGAAAAGACGAAGGCAAGAAA	Protein suppressor of npr1-1, constitutive 1	2	0.32
MRGSSR101	JJMO01000452	(TG)7	ATCAAGCAGACCCTTGTCTCTC	AAGCCTCTTTGTATAGACCCGTT	Putative disease resistance protein RGA4	2	0.04
MRGSSR102	JJMO01001147	(AT)38	GTTCGTTCTCCCTTTCCTTCTT	ATGGAGGTTAAGGTGTGATTGG	Putative disease resistance protein RGA4	2	0.04
MRGSSR103	JJMO01001477	(AC)8	ACCAACTTCAAAGCCAATGTG	TCTCTTTCCTTGAGCATAAGTGG	DNA-damage repair / toleration protein DRT100	1	0
MRGSSR104	JJMO01000782	(TA)6	GTAAGCCTGCCGTTCATATTTC	TTTTAGTTTGTATGGCGAGTGC	Putative disease resistance protein RGA4	2	0.59
MRGSSR105	JJMO01002368	(GAT)8	AGTTCCACCACCACAGGATTAG	AGTTGGCATCCATCAATAGACA	Putative disease resistance protein RGA4	2	0.55
RGSSR110	JJMO01000129	(AT)7	TTTTCATTCCACCCGTCC	TTTCTTTCTGCACCCAACATAG	Putative disease resistance protein At4g11170	3	0.83
MRGSSR118	JJMO01000124	(TTA)9	GCCAAGACAGATTGGAAGAAAT	AGACCATGAATTTGGAACCAGT	TMV resistance protein N	2	0.52
					Total number of alleles and PIC average	90	0.43

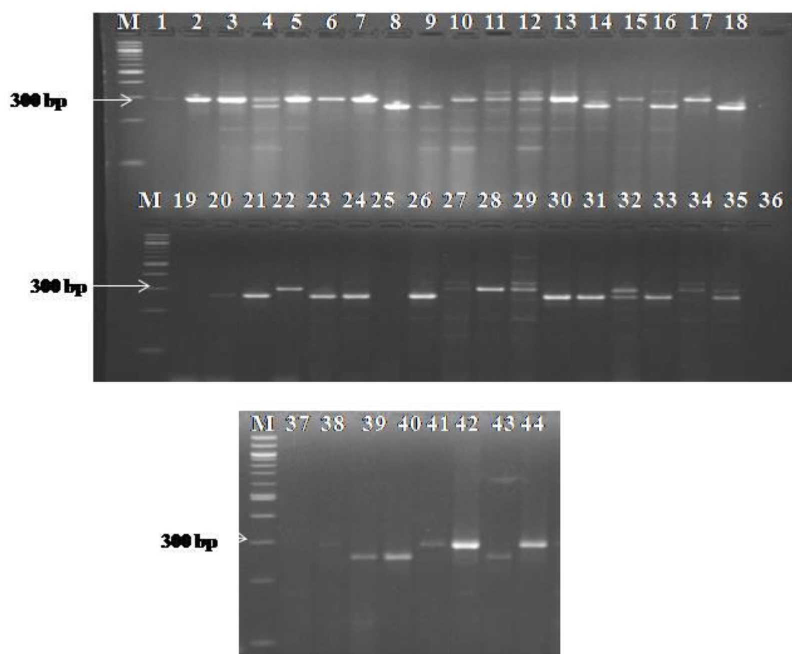

Fig. 1. PCR amplification using MRGSSR118 genomic-SSR marker in 44 blackgram genotypes. Lane M 100 bp marker. λ DNA Eco RI and Hind III double-digest marker. Lanes 1 – 44 blackgram genotypes as listed in materials and methods.

Amplification of 30 SSR primers in 44 blackgram genotypes differing in their disease reactions (YMD and PMD) were analysed. In terms of YMD, 3 SSR markers MRGSSR12, MRGSSR56 and MRGSSR77 were differentially amplified predominantly in YMD resistant genotypes in comparison to susceptible genotypes. These markers MRGSSR12 [$(AT)_{13}$], MRGSSR56 [$(ATA)_6$] and MRGSSR77 [$(CT)_7$] were designed from mungbean scaffolds JJMO01001477, JJMO01002369 and JJMO01000040 exhibiting homology with TMV resistance protein N, DNA damage repair/toleration protein DRT100 and putative disease resistance RPP13-like protein 1, respectively. Similarly, YMD resistant and susceptible genotypes were differentiated using resistance gene analogues derived SSR and ISSR markers, in mungbean and blackgram (Maiti *et al*., 2011; Gupta *et al*., 2015; Souframanien and Gopalakrishna, 2009). While in case of PMD, four markers namely MRGSSR12, MRGSSR 32, MRGSSR56 and MRGSSR65 differentiated resistant (IPU02-43, KU96-3, IPU-07-3, LBG-752, LBG-17, LBG-623, LBG-20, Pant U-19, Pant U-31, LBG-709, ANU-11) and susceptible genotypes (DPU-88-31, TAU-1, WBG-57, COBG-653, Sharda Mash). Two of these markers viz., MRGSSR 32 and MRGSSR 65 showed amplification in five and one resistant genotypes, respectively, out of 11 resistant genotypes and were not amplified in five susceptible genotypes studied.

Therefore, two SSR primers, MRGSSR12 and MRGSSR 56 derived from mungbean scaffolds having homology with putative disease resistance genes were identified in the present study that could differentiate both YMD and PMD resistant and susceptible genotypes. MRGSSR12, designed from mungbean scaffold which shared homology with TMV resistance protein N. TMV resistance protein N is a disease resistance protein having one TIR, one NB-ARC domains and six LRR repeats which guard the plants against pathogens through direct or indirect interaction with avirulence protein and triggers a defense system including the hypersensitive response, which restricts the pathogen's growth and spread (The Uniprot Consortium,https://doi.org/10.1093/nar/gkw1099).

The cross-species amplification of mungbean derived resistance gene-SSR markers were investigated for diversity analysis in the set of 44 blackgram genotypes comprising of 43 cultivars and one wild species. Cluster analysis based on neighbour-joining method grouped the 44 genotypes into seven clusters (**Fig. 2**). Cluster I with 3 sub-clusters (Ia, Ib and Ic) comprised of 14 blackgram genotypes. Cluster Ia comprised of 2 genotypes, EC168200 and LBG-17, which are resistant to YMD and PMD, respectively. Of the five genotypes grouped under cluster 1b the genotypes Pusa-3, LBG 752, and IPU02-043 are resistant to YMD. Three of the seven genotypes constituting cluster Ic are YMD resistant (DPU88-31, IPU07-3 and KU96-7). The YMD resistant cultivars NDU-1 and PU-19 are clustered separately in cluster VII with

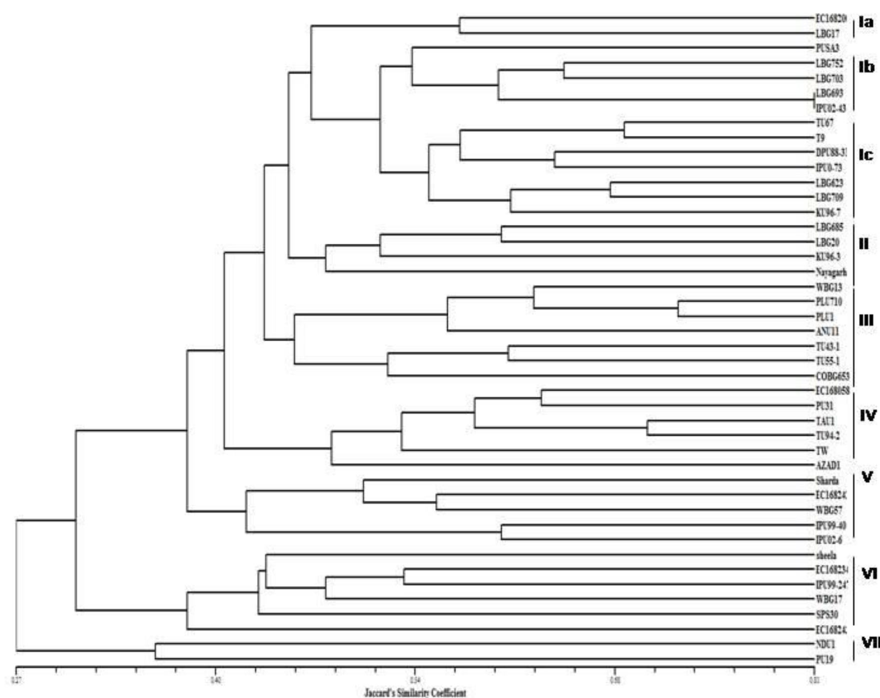

Fig. 2. Dendrogram constructed using Jaccard's similarity coefficient and UPGMA clustering among 44 blackgram genotypes based on mungbean derived SSR markers.

a similarity index value of 0.27. The highest similarity coefficient was observed between LBG693 and IPU02-43 (0.81).

The genetic closeness between some of the cultivars could be explained due to common parents in their pedigree. For example, genotypes IPU07-3 and IPU02-43 although from the different crosses, DPU88-31 x PDU-1 and DPU88-31 x DUR-1, respectively, had one parent in common and were grouped together in cluster I. Moreover, both genotypes were resistant to YMD and PMD. DPU88-31 was grouped with one of its parent T9 in sub-cluster Ic of cluster I. Likewise genotypes Pusa3 and DPU88-31 both are grouped together in cluster I along with their one common parent, T9. In this study, grouping of blackgram genotypes based on disease resistance were observed in cluster I. Similar grouping of genotypes based on disease resistance was reported in blackgram (Souframanien and Gopalakrishna, 2009). Grouping of individuals based on disease reaction observed in this study could be due to presence of resistance gene in the mungbean scaffolds used for designing the markers. For example, TMV resistance protein N, Protein suppressor of npr1-1, constitutive 1, putative late blight resistance protein homolog R1B-8, and putative disease resistance protein At4g11170 exhibited homology within the same scaffold JJMO01000125.Clustering of resistance gene analogues was also reported in several other species such as rice (Monosi *et al*., 2004), tomato (Dickinson *et al*., 1993) and other species (Sheperd and Mayo, 1972).

In the present study, 68 mungbean resistance genes harbouring WGS scaffolds derived genomic-SSR primers showed cross species amplification in blackgram. These transferable genomic-SSR markers would be a valuable resource for blackgram genetic analysis because resistance genes-based SSR marker polymorphism would represent the variation present in the resistance sources of blackgram genotypes. However, SSR primers which differentiated YMD and PMD blackgram genotypes need further confirmation and validation of their association with the resistance trait. These SSR markers derived from resistance proteins homologous sequences could be lying within either the coding sequences, untranslated regions or regulatory regions of resistance genes, and would offer an opportunity to investigate the consequences of SSR polymorphisms on gene functions and regulations associated with disease resistance. These SSR markers would be helpful in the selection of appropriate genotypes in breeding programmes aiming at developing multiple stress tolerant cultivars.

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