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Short Communication

Cross transferability, diversity analysis and tagging of MYMV resistance in mungbean using RGH markers

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Abstract

Mungbean and urdbean suffer from paucity of genetic markers associated with MYMV disease. As an alternative option RGHs were tested for transferability in 13 and 12 genotypes of mungbean and urdbean, respectively. Fifty-five RGH markers from cowpea, chickpea and *Medicago* exhibited a transferability of 58.2%, which collectively yielded 51 alleles with an average of 1.59 alleles per locus. PIC of individual loci ranged from 0.19 to 0.64. Phylogenetic tree formed two different clusters of mungbean and urdbean with six sub clusters. Two putative markers identified and high level of transferability indicated its importance for marker-assisted breeding.

Key words: Genetic diversity, Mungbean yellow mosaic virus (MYMV), resistance gene homologs (RGHs), transferability, *Vigna*

Mungbean (*Vigna radiata* (L.) Wilczek) and urdbean (*Vigna mungo* (L.) Hepper) are two important short duration grain legume crops and major source of dietary protein in India with an annual production of 2.5-3.0 mt. (Baask et al. 2009). Average yields of the two crops are very low (0.5-1 t ha^{-1}) due to inherently low yield potential of the ruling varieties and their susceptibility to diseases. Susceptibility to mungbean yellow mosaic virus (MYMV) is the most destructive viral disease of mungbean damaging crop from 32 to 78%. Lack of uniform screening technique has hindered development of MYMV resistant varieties therefore, indirect selection using resistantce genes linked molecular markers would be an effective approach. Resistance Gene Homologues (RGHs) that encode protein family NBS-LRR can be efficiently characterized using polymerase chain reaction. Comparative genome mapping in plants has demonstrated conservation in genomes during evolution (Choi et al. 2004). Studies suggest a simple genetic relationship of mungbean with cowpea (Menancio-Hautea et al. 1993), chick pea (Chaudhary et al. 2008) *and Medicago truncatula* (Gutierrez et al. 2005) enabling transfer of markers. Present study was conducted with an aim to develop markers linked to MYMV and check the transferability of RGH markers across mungbean and urdbean to use them in marker assisted breeding.

Plant material and disease screening, designing of RGH markers and DNA extraction, PCR amplification and RGH scoring are detailed here. Thirteen genotypes of mungbean namely, AKM 8803, HUM 1, HUM 12, PM 1, SML 668, KKM4, VGG4, PS 16, SML 134, TARM 2, TAP7, Pusa Baisakhi and Chinamung and 12 of urdbean *viz*., IPU 0243, Uttara, DU 1, TAU1, PDU1, Shekhar 2, T 9, Manikya, Barabanki local, DU2, DU3, LBG 685 were used in the present study. White flies required for inoculating MYMV were starved for 24 h and were fed for 48 h on diseased mungbean plants. Two weeks after sowing 10-20 viruliferous white flies were released on healthy plants. National Center for Biotechnology Information (NCBI) databases were searched for sequences of

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RGH clones and EST's in related *Vigna* species. Twenty four markers were designed for amplifying the repeat regions. Another set of 31 reported RGH markers [1], were also used in the present study. Genomic DNA of two-leaf staged plants was isolated using a modified Cetyl-trimethyl ammonium bromide (CTAB) method (Saghai-Maroof et al. 1984). PCR amplification was carried out in 20 µl reaction volume, non-polymorphic bands on 4% agarose gels were resolved on a denaturing polyacrylamide gel, stained using modified silver staining protocol and scored manually as. "+" / "–" (presence or absence). Frequency of abundant allele, genotype frequency and PIC were estimated using Power Marker version 3.25. Phylogenetic tree was constructed using Neighbor-joining algorithm (DARwin 5.0).

Transferability of 55 markers was examined on 25 genotypes of mungbean and urdbean. Twnety three markers amplified successfully in all 25 genotypes (42%), while 9 amplified in either of the crops. Eleven polymorphic markers collectively yielded 30 alleles, with an average of 2.72 polymorphic alleles per locus (Table 1).

Only 2 markers among 11 polymorphic markers were found specific to MYMV resistance in mungbean. RGH-1TG produced 450 bp and 500 bp amplicon in the resistant and susceptible genotypes respectively (Fig. 1).

Phylogenetic tree formed two separate clusters (a and b) of mungbean and urdbean, which further formed sub clusters '1' to '6'. DU 3, Shekhar 2 and T

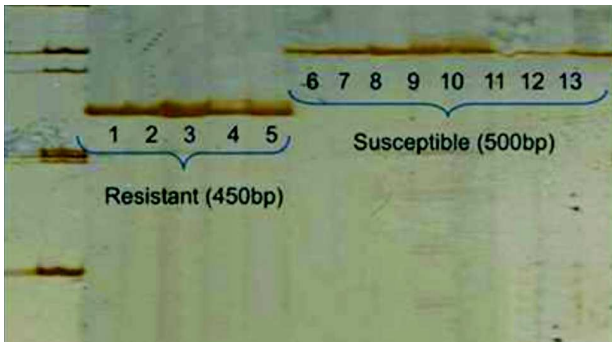

Fig. 1. PCR amplification profile generated by RGH marker RGH-1TG in mungbean as observed in polyacrylamide gel electrophoresis (13 mungbean genotypes: 1. AKM 8803, 2) HUM-1, 3) HUM12, 4 PANT MUNG 1, 5 all variety SML 668, 6 KKM 4, 7 VGG 4, 8 PS-16, 9 SML 134, 10 TARM 2, 11 TAP 7, 12 Pusa Baisakhi and 13 China Mung)

Table 1. Number of alleles, allele frequency, gene diversity, polymorphic information content for twenty five genotypes of mungbean and urdbean

Markers	Frequ-ency of allele count	Abun-dant allele	Allele no.	Trans-fera-bility (%)	Gene diver-sity	PIC
XLRR	0.56	14	4	100	0.59	0.53
Pto kin1	0.61	11	2	72	0.48	0.36
NBS	1.00	25	1	100	0.00	0.00
S2	1.00	25	1	100	0.00	0.00
S1-INV	0.36	9	4	100	0.69	0.64
Wlrk-S	1.00	25	1	100	0.00	0.00
Cre3Ploop	0.48	12	3	100	0.63	0.55
Xa1LR	1.00	25	1	100	0.00	0.00
RGH-1CGa	1.00	25	1	100	0.00	0.00
RGH-1CGb	0.88	22	2	100	0.21	0.19
RGH-1TG	0.68	17	2	100	0.44	0.34
MtB331	1.00	25	1	100	0.00	0.00
MtB99	0.48	12	4	100	0.67	0.61
h2119h6a	1.00	25	1	100	0.00	0.00
h213m22a	0.52	13	2	100	0.50	0.37
VuRS01N7V	1.00	25	1	100	0.00	0.00
VuRS01N9V	0.56	14	2	100	0.49	0.37
VuRS02A2R	1.00	25	1	100	0.00	0.00
VuRS01A03R	1.00	25	1	100	0.00	0.00
VuRS01G04R	1.00	25	1	100	0.00	0.00
VuRS01L21R	1.00	17	1	68	0.00	0.00
VuRS02H07V	1.00	25	1	100	0.00	0.00
VuRS03A23V	0.48	12	3	100	0.63	0.55
VuRS03G17V	1.00	25	1	100	0.00	0.00
VuRS03O19V	1.00	18	1	72	0.00	0.00
VuRS02H06R	1.00	25	1	100	0.00	0.00
S1	1.00	13	1	52	0.00	0.00
CLRR-INV1	1.00	13	1	52	0.00	0.00
Pto-kin1IN	0.75	6	2	32	0.38	0.30
VuRS02F16V	1.00	14	1	56	0.00	0.00
VuRS02L24R	1.00	13	1	52	0.00	0.00
VuRS02B24R	1.00	13	1	52	0.00	0.00
Mean	0.86	18.53	1.59	88	0.18	0.15

9 of urdbean and HUM1, HUM 12 of mungbean exhibited 100% similarity. Resistant genotypes in mungbean formed cluster 5 and susceptible genotypes formed clusters 4 and 6 (Fig. 2).

Detection of 2.72 polymorphic alleles per locus in present study was comparable to earlier findings based an using genomic SSR markers in *Vigna* species including cowpea (4.6 alleles per locus) (Li et al. 2001), urdbean (4.1 alleles per locus) (Chowdhary et al. 2009) and adzuki bean (4 alleles per locus) [3]. Revelation of only 2 markers being associated genetically with MYMV in mungbean, but not in urdbean

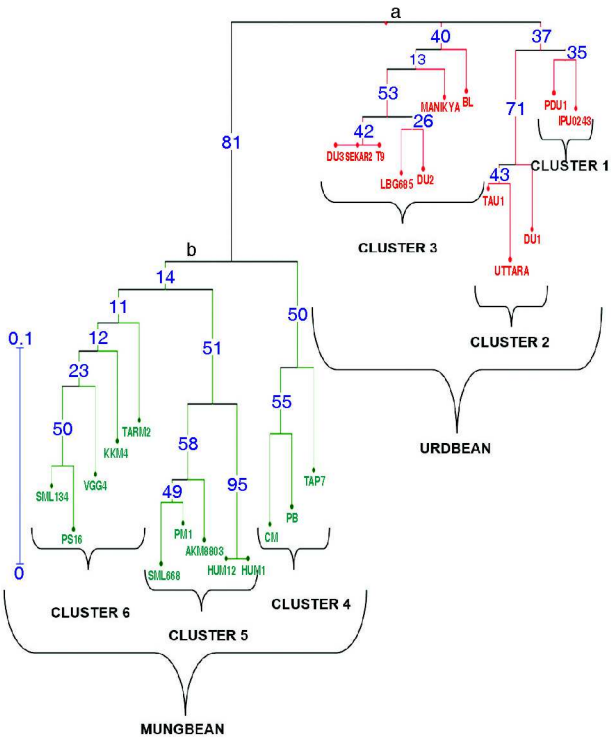

Fig. 2. Neighbor joining Phenogram of 25 genotypes based on genotypic data using DARwin 5.0

could be due to the RGHs are in general not fast evolving and highly diverse as compared to genomic SSR markers (Eujayl et al. 2001; Gutierrez et al. 2005) as they originate from highly conserved proportion of the genome (Varshney et al. 2005) and use of information from *Medicago*, chickpea etc. will limit frequency with which the target genes amplify in *Vigna* as they are distantly related (Datta et al. 2010; Gupta and Gopal Krishna 2010). High level of transferability of markers from cowpea, chickpea and *Medicago,* indicated the conservation of RGH sequences in genus *Vigna* during evolution (Varshney et al. 2005). Deviations from the expected amplicons size in original species may be due to a major genetic rearrangement in target RGH loci or due to the duplication of loci in the genome. High PIC values indicated their usefulness in differentiating the accessions that are closely related. Allele frequency and allele count were directly proportional. Genotypes exhibiting 100% similarity could be traced back to the pedigree where they were developed by wild hybridization. From clustering of the genotypes it is clear that RGHs developed in one species can be used in related species for which sufficient sequence information is not available for marker development. Polymorphic RGH markers identified in this study would be helpful in marker assisted breeding. Putative markers identified in this study *viz*., RGH-1TG and MtB99 could be further validated using mapping populations. The study also indicated that there is high potential for the transfer of RGHs from closely related species, circumventing the laborious cloning and screening procedures involved in characterizing RGHs for mungbean and urdbean.

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