Electronic Journal of Plant Breeding, 1(4): 1148-1152 (July 2010)

Research Article

Mapping of Mungbean Yellow Mosaic India Virus (MYMIV) and powdery mildew resistant gene in black gram [*Vigna mungo*(L.) Hepper]

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Abstract :
Black gram, one of the important species of Asian *Vigna* group of grain legumes, is widely grown in South Asia and is an important source of dietary protein. The two main biological constraints particularly Mungbean Yellow Mosaic India Virus(MYMIV) and powdery mildew pose a major threat to black gram production in India. Several reports on mapping mungbean yellow mosaic virus disease and powdery mildew resistant genes on black gram using parental lines suitable for countries viz. Australia and Japan are available. However, to achieve precision in plant breeding, it is important that mapping of traits are done using parental lines which are best suited for the target area/country. Microsatellite markers facilitate effective screening of mapping population and marker assisted selection for target traits such as disease resistance in many crops. Linkage mapping for identification of genes conferring resistance to these target traits in the crop is underway. The parents selected for MYMIV mapping population are DPU 88-31 as resistant source and AKU 9904 as susceptible one. For establishment of powdery mildew mapping population RBU 38 was used as resistant and DPU 88-31 as the susceptible one. Parental polymorphism was assessed using 363 SSR and 24 RGH markers. Efforts are being made to identify the markers tightly linked to the genes responsible for resistance which will be useful for marker assisted breeding for developing MYMIV and powdery mildew resistant cultivars in black gram.

Key words:
Mapping- marker assisted selection-MYMIV-powdery mildew-molecular markers-EST-SSR

Introduction

The *Vigna* is one of the most important genus in the Fabaceae and contains 100 to 150 species mainly found in Africa and Asia. Among these species, Mungbean [*Vigna radiata* (L.) Wilczek] and urdbean [*V.mungo*(L.)Hepper] are the major pulse crops of Indian subcontinent (Datta and Gupta 2009). Black gram is a self-pollinating diploid(2n=2x=22) annual crop with a small genome size estimated to be 0.56pg/1C(574Mbp) (Gupta et al. 2008). The two major biotic stresses such as Mungbean Yellow Mosaic India Virus (MYMIV) (Mayo 2005) and powdery mildew accounts for the low harvest index of the present day urdbean cultivars. Begmovirus transmitted through the white fly, *Bemisia tabaci* Genn. (Honda et al. 1983). It causes significant yield loss for many legume seeds, not only, *Vigna mungo*, but also in *V. radiata* and *Glycine max* throughout the South-Asian countries. Depending on the severity of the disease the yield penalty may reach up to cent percent (Basak et al. 2004). Powdery mildew(PM), caused by the pathogen *Erysiphe polygoni* DC, is one the most destructive diseases in urdbean.

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Severe infection by the fungus occurs in the cool, dry months where the yield losses owing to PM have been estimated to be around 20-40%(Reddy et al. 1994). Genetic control of resistance to MYMIV in urdbean has been investigated using different methods. There are conflicting reports about the genetics of resistance to MYMIV, claiming both resistance and susceptibility to be dominant. In blackgram, resistance was found to be monogenic dominant(Kaushal and Singh 1988). The digenic recessive nature of resistance was reported by (Singh et al. 1998). Monogenic recessive control of MYMIV resistance has also been reported(Reddy and Singh 1995). It has been reported to be governed by a single dominant gene in DPU 88-31 along with few other MYMIV resistant cultivars of urdbean (Gupta et al. 2005). Kaushal and Singh 1989 studied the genetics of resistance to powdery mildew in blackgram and reported that it is being governed by a single recessive gene. The development of varieties by incorporation of MYMIV and powdery mildew resistant genes into commercially acceptable cultivars is an effective strategy to control the disease. Identification of resistant lines through conventional breeding is time consuming and requires evaluation at 'hot spot' area (Selvi et al. 2006). The disease incidence is seasonal and cannot

be created as and when desired by artificial means. Similary powdery mildew is an obligate parasite, therefore, disease resistance can be identified when the disease is present in the field or greenhouse(Chaitieng et al. 2002). Inoculation can be performed using infected plants and dusting the spores onto the target plants, but environmental factors can influence the severity of the infection(Thakur and Agarwal 1995). Backcrossing is the main breeding method used to transfer resistance against disease. Progress in urdbean breeding is hampered because direct selection after inoculation is difficult to assay if the infection is weak in case of powdery mildew (Chaitieng et al 2002). In such circumstances, indirect selection using molecular markers linked to MYMIV and powdery mildew resistant genes would facilitate precision plant breeding and high-throughput marker assisted selection(MAS) of resistant genotypes. The construction of linkage maps using molecular markers is of fundamental importance for the efficient exploration of a plant genome and to dissect quantitave traits into individual Mendelian factors to increase the breeding efficiency. Simple sequence repeat (SSR) markers comprises the core marker system of the PCR-based molecular markers and are widely used for DNA fingerprinting, genetic mapping, MAS, and studies of genetic diversity and population genetics (Zeitkiewicz et al. 1994). The availability of the molecular maps facilitates marker-assisted selection, map-based cloning, and mapping of quantitative trait loci(QTL) of agronomic importance in many crop plants(Gupta et al. 2008). SSR markers because of their ubiquitous presence in the genome, highly polymorphic nature, and codominant inheritance are the markers of choice for mapping the genes linked to the target trait(Gupta et al. 2008).Two genetic linkage maps have been constructed in black gram(Chaitieng et al. 2006 and Gupta et al.2008) and compared with that of azuki bean map(Han et al. 2005).The comparison revealed high level of conservation of the marker loci between the two genomes. Chaitieng et al. 2006 reported the first genetic linkage map of urdbean with 148 marker loci assigned to the 11 linkage groups, which correspond to the haploid chromosome number.Subsequently another linkage map was constructed with 428 markers which spanned a total distance of 865.1cM with an average marker density of 2cM(Gupta et al.2008). The current study aims at exploiting the colinear relationship of the mapped marker loci between black gram and azuki bean to assess the polymorphism in parents for both MYMIV and powdery mildew mapping population.

Materials and methods

The present investigation was carried out at Indian Institute of Pulses Research, Kanpur. A genotype DPU 88-31 was used as resistant parent for MYMIV and AKU 9904 as susceptible one. Similarly for development of mapping population for powdery mildew, RBU 38 was used as resistant and DPU 88-31 as susceptible one. The disease reaction for MYMIV and powdery mildew was evaluated phenotypically by following methods:

Evaluation for MYMIV reaction

The infector row method consisting of sowing two test rows alternating with spreader rows of susceptible variety was adopted. The test materials were scored after 80% of the plants in spreader rows showed MYMIV incidence. The rating scale suggested by (Singh *et al.* 1988) is adopted.

Disease assay for powdery mildew

The susceptible varieties were planted around and between the plots of the parents and the F_1 progenies as a source of powdery mildew inoculums. Individual of parents and F_1 were scored for powdery mildew response at 55 days after the germination using the scoring system described by Young *et al.* (1993) as follows:1, no visible growth; 2, 0-25% foliage area covered by fungus; 3, 26-50% foliage covered; 4, 51-75% foliage covered; and 5, 76-100% foliage covered.

Development of mapping population

The MYMIV resistant parent DPU 88-31 was crossed with susceptible parent AKU 9904 during Kharif, 2008. Similarly RBU 38 resistant source of powdery mildew was crossed with the susceptible parent DPU 88-31.The crossed seeds were sown during Kharif, 2009 and true hybrids have been identified using the SSR markers. The true F_1s have been selected to develop F_2 mapping population for evaluation of MYMIV and powdery mildew reaction.

DNA extraction

DNA from parents and 12 F_1 individuals were extracted following the method of (Doyle and Doyle 1987) with slight modification. The DNA concentration in the samples was adjusted to 25 ng μl^{-1} for SSR analysis by comparing with known concentrations of standard Lambda DNA on 0.8% agarose gel. Simple sequence repeat amplification reactions were carried out in 20 µl volumes containing 25ng template DNA, 0.6 U of Taq DNA polymerase, 0.2mM dNTP, 20 picomoles primer(each of forward and reverse) in 1X reaction buffer that contained 10mM Tris-HCl (pH 8.3), 50mM KCl, 2.5mM $MgCl_2$, 0.01% gelatin. Amplification was performed in PTC-200(MJ Research, USA) thermocycler and it was programmed for an initial denaturation of 94°C for 3 min followed by 35 cycles of 94°C for 1min, annealing for 1min, 72°C for 2 min, and the final extension was done at 72°C for 7

min. The amplified products were loaded on 3% agarose gels and separated in 1X TAE buffer at 50 V. The gels were visualized under UV after staining with ethidium bromide(0.5µg/ml) and were documented using Bio Rad Gel Doc XR 2.0.

SSR amplification analysis

363 SSR primer pairs published for azuki bean(Han et al. 2005 and Chaitieng et al. 2006), mungbean (Somta et al.2009, Kumar et al. 2002a and Kumar et al. 2002b), cowpea(Lie *et al*. 2001), common bean(Gaiten-Solis et al. 2002 and Blair et al.2003) and 24 *Vigna* specific resistant gene homologs(RGH) were synthesized from (IDT, USA). Data generated by the SSR markers during parental polymorphism studies was recorded in binary fashion and scoring was based on presence and absence of bands.

Results

Three sixty three SSR primer pairs derived from azuki bean (*Vigna angularis*), mungbean (*Vigna radiata*), common bean(*Phaseolus vulgaris*), cowpea (*Vigna unguiculata*) and 24 Vigna- specific resistant gene homologs were tested for their ability to support amplification in black gram. Of the 387 SSR primer pairs, 279 showed cross species amplification and 7 SSR primer pairs showed polymorphism (Table 1). Five primer pairs from azuki bean (CEDG139, CEDG008, CEDG268, CEDG022, CEDG198) and two from mungbean (DMB-SSR182 and DMB-SSR186) were found to be polymorphic for both the parents of MYMIV and powdery mildew mapping population. Representative polymorphism revealed by CEDG022 along with five other azuki bean microsatellite primer pairs(CEDG215, CEDG247, CEDG173, CEDG273, CEDG015) found to be monomorphic.

Discussion

In this paper we have reported the amplification profile of the mapped microsatellite loci obtained by comparative genetic linkage mapping of blackgram (Gupta et al. 2008), azuki bean (Han et al. 2005), and black gram (Chaitieng et al. 2006). Among these markers CEDG139 was reported to be found on linkage group 4 and CEDG268 on linkage group 5 of the maps published in azuki bean by Han et al. 2005 as well as in black gram by Chaitieng et al. 2006 and Gupta et al. 2008, CEDG008 mapped on linkage group 5 of azuki bean by Han et al.2005 as well as in black gram by Chaitieng et al. 2006 indicating their tight linkage in the respective maps. The colinear relationship of the mapped marker loci was explored and these markers were found to be polymorphic among the parents used for both the target traits. Interestingly CEDG022 and CEDG198 present on linkage group 9 and 10 respectively (Han et al. 2005) despite being not conserved across the black gram genome, revealed polymorphism between the parents for MYMIV and powdery mildew mapping population. Although mapping of a major locus conferring to powdery mildew resistance in mungbean has been reported (Humphry *et al.* 2003) yet there is an urgent need to identify markers linked to the target trait in urdbean. Genomic research in blackgram is lagging behind other legume crops such as soybean, cowpea, and common bean, or even their relative but less important azuki bean. A major obstacle is due to lack of high resolution genetic markers. The linkage mapping in black gram can be made possible using genetic markers developed from other related legumes, and this should continue until complete sequenced database in black gram is established. BAC libraries have been constructed for many species. However, there are no reports of a BAC library for *Vigna* species except only one on mungbean(Miyagi et al. 2004). Efforts are needed to develop expressed sequence tag(EST) libraries which offer important information for species that have not been sequenced and are a central source of gene-based markers and single nucleotide polymorphism(SNP) or indel polymorphisms(Galeano et al. 2009). EST –based markers are valuable because they represent sequences that are transcribed and therefore can potentially be associated with phenotypic differences. Furthermore, EST based markers are often highly conserved between species allowing the construction of transcript maps and synteny comparison between the genomes. In conclusion efforts are needed to generate expressed sequence tag (EST) databases for the development of genic microsatellite markers which will accelerate the advancement of genomic resources and will overcome the paucity of the polymorphic markers in black gram and the polymorphic markers identified will be explored by bulk segregant analysis for mapping of MYMIV and powdery mildew resistant genes using F_2 populations. This will assist in precision plant breeding in black gram to develop superior varieties thereby achieving a substantial impact on crop improvement by MAS resulting in sustainable agriculture.

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Table1 : Azuki bean and mungbean SSRs found to be polymorphic between parents for MYMIV as well as powdery mildew

Primer	Sequence 5' -3'	Motif	AKU9904 Size(bp)	DPU88-31 Size(bp)	RBU38 Size(bp)	Alleles	T_a(°C)
CEDG139	CAAACTTCCGATCGAAAGCGCTTG GTTTCTCCTCAATCTCAAGCTCCG AGGCGAGGTTTCGTTTCAAG	(AG)19	230	200	220	1	58
CEDG008	GCCCATATTTTTACGCCCAC CATCTCCCTGAAACTTGTG	(AG)26	140	110	120	1	53
CEDG268	GCTATCAATCGAGTGCAG AGGAATGTGAGATTTG	(AG)16	135	149	137	1	48
CEDG022	AATCGCTTCAAGGTCAAGCC	(AG)27	184	200	183	1	46
CEDG198	CAAGGAAGATGGAGAGAATC	(AG)30	173	190	176	1	48
DMB-SSR182	CCTTCTAAGAACAGTGACATG TAGAGCCTTCTGGTTTTTCACA	(TGA)3..(CT)3…(TCC)4	360,539	338,510	355,527	2	54
DMB-SSR186	AGGAGGAGGATTTTGATGATGA GAGAGAGAAGGAGAGGGAGA ATTCTTTCTCCACCACAATG	(GGT)4…(GGT)3…(GGT)4	248	270	250	1	52

AKU 9904 : Susceptible parent for MYMIV mapping population, **DPU 88-31**:Resistant parent for MYMIV mapping population.
RBU 38: Resistant parent for powdery mildew mapping population, **DPU 88-31**: Susceptible parent for powdery mapping population