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Research Article

CROSS LEGUME SPECIES/GENERA TRANSFERABILITY OF SSR MARKERS POLYMORPHIC TO PARENTS OF F_2 MAPPING POPULATION IN BLACKGRAM (*VIGNA MUNGO* L. HEPPER)

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Abstract: Three hundred and ninety-five cross legume species/ genera SSR markers (65 from soyabean, 12 from medicago, 9 from chickpea, 105 from adzukibran, 70 from cowpea and 134 from mungbean) were used to identify those polymorphic to parents of F_2 mapping population *viz*., TAU-1 and LBG-17 in blackgram, a genomic resource limited crop. Of these 395 SSR markers, 315 SSR markers (79.50%) amplified and 71 of them (22.62%) were polymorphic between the parents of F_2 population. Among the 71 cross legume polymorphic SSR markers, those based on, di-nucleotide repeat motifs exhibited highest polymorphism (67.60 %), followed by tri- (18.30 %), complex-(9.85 %) and tetra-nucleotide repeat motifs (4.22 %). The identified polymorphism cross legume SSR markers is suggested for use in various applications in blackgram breeding research. The present study showed possibility of using cross legume species/ genera SSR markers in blackgram for genotyping the F_2 mapping population for gene mapping and subsequently for marker assisted selection.

Keywords: *Vigna mungo, Blackgram, Transferability, Simple Sequence Repeat, Repeat Motifs, F_2 population*

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Introduction

Blackgram [*Vigna mungo* L. Hepper] is one of the most important grain legumes with easily digestible protein and low flatulence content. It is a self-pollinated diploid (2n=2x=22) crop with a small genome size estimated to be 0.56 pg/1C (574Mbp) [1]. It is widely cultivated in India, Burma, and Thailand regions of Asia. It is an ancient and well known leguminous crop due to its nutritional quality and the suitability to the cropping system. It is rich in easily digestible protein (24%), contains vitamin B1 (0.42mg), B2 (0.37), Niacin (2.0 mg), iron (8.7 mg), calcium (185 mg), P (345 mg) and Fat (1.2%) per 100 g dry seed. It is mainly used as dal and in preparation for some special dishes like the sprouted urdbean which is very popular in Japan and is highly valued for digestibility and freedom from the flatulence effect. Since it serves as a cheaper source of protein for the poor, it is rightly called the poor man's meat and the plant can also be used as green manure. Blackgram crop is a mini fertilizer factory as it restores soil fertility by fixing atmospheric nitrogen and thus producing nitrogen equivalent of around 22 hectare^{-1}[2]. To increase production of black gram, there is need of developing high yielding varieties which requires a systematic breeding approach to be adopted. However, phenotypic based selection for seed yield and its component traits is rather less effective owing to their complex inheritance and significant cross over G × E interactions. The DNA markers are proven to be powerful surrogates for such difficult to select traits. Marker assisted selection (MAS) requires a priori identification of DNA markers closely linked to genomic regions controlling seed yield and its component traits. However, identification of DNA markers polymorphic to parents of the mapping population which are required to identify those linked to genomic regions controlling seed yield and its component traits is a pre requisite for implementing MAS. Of the several marker systems, those based on simple sequence repeats (SSR) are the primary choice of crop breeders owing to their hyper variability, higher reproducibility, mono-locus multi-allilic, and co-dominant inheritance, possibility of multiplexing and amenability for automation [3].

However, SSR markers specific to blackgram are limited. Nevertheless, the use of transferable cross legume species/ genera SSR markers in crops where they are not available is an alternative strategy to ensure availability of markers. The discovery of high degree of genome syntany among Fabaceae members such as soyabean, mung bean, adzuki bean and cowpea [4] offers opportunity to transfer SSR markers from these crops to blackgarm, a member of Fabaceae. However, such studies are limited in blackgram. The only reported attempt to examine transferability and polymorphism of cross legume species/ genera SSR markers to blackgram is from adzukibean and mungbean the present study was carried out with an objective to identify the cross legume species/ genera SSR markers polymorphic between the parents of F_2 mapping population in blackgram.

Material and Methods

A pair of blackgram genotypes (TAU-1 and LBG-17) differing inseed yield and its component traits were used for examining the transferability of cross legume species/genera SSR markers. While TAU-1 is a highly popular high yielding powdery mildew susceptible variety, LBG-17 is a low yielding powdery mildew resistant variety [5]. The total genomic DNA was extracted from 20 days old seedlings of these two varieties using the Cetyl Trimethyl Ammonium Bromide method [6]. The quality and quantity of extracted genomic DNA was checked using 0.8% agarose gel by comparing with uncut lambda DNA.

A total of 395 cross legume species/genera genomic SSR markers which included 65 from soybean [7], 12 from *Medicago trunculata* [8], 134 from greengram [9], 105 from adzuki bean [10], 70 from cowpea (http://cowpeagenomics.med.virginia.edu/CGKB/), and 9 from chickpea [11] were used to amplify genomic DNA extracted from the selected pair of black gram varieties.

Table-1 *Crop and repeat motifs-based distribution of amplified SSR markers of cross legume species/ genera in blackgram*

Crop	Total number of markers used	Number of markers amplified	% Transferability	Repeat motifs			
				Di	Tri	Tetra	Complex
Medicago trunculata	12	4	33.33	-	2	2	-
Chickpea	9	4	44.44	-	3	-	1
Soybean	65	40	61.54	25	8	4	3
Adzuki bean	105	89	84.76	51	21	11	6
Cowpea	70	48	68.57	21	7	4	16
Green gram	134	129	96.27	69	37	7	16
Total	395	314	79.50	166	78	28	42

SSR marker assay

The SSR priming regions of the two varieties were amplified using polymerase chain reaction (PCR) with *Taq* DNA polymerase. PCR mixtures contained approximately 2.0 µl of DNA (30ng per µl), 0.15µl *Taq* polymerase (1 unit per µl), 1.0 µl 10X TE buffer, 1.0µl DNTPs (2mM) and 1.0 µl each of forward and reverse primers (1 µM) in a total of 10 µl solution. The PCR cycle consisted of 5 min at 94ºC (hot start), 0.30 min at 94ºC (denaturation), 1 min at different annealing temperature, 1 min at 72ºC (extension), 10 min at 72ºC (final extension) followed by infinite time at 4ºC for holding. The denaturation, annealing and extension step were carried out for 35 cycles. The PCR products were loaded on two percent sigma agrose gel in 1X TAE buffer stained with ethidium bromide and bromophenol blue as loading dye. Amplicons were size separated using electrophoresis unit at 80 V for five hours using 1X TAE buffer.

Criterion to assess transferability of SSR markers to blackgram

Those SSR markers which successfully amplified SSR priming regions of DNA of the two genotypes and produced single and specific bands at reported expected product size range were considered as transferable SSR markers. Based on this criteria, percent transferability was calculated as (Number of markers amplified/Total number of markers) × 100. A total of 71 cross legume species/genera SSR markers were transferable to blackgram. Percent transferability of cross legume SSR markers classified by length (di/tri/tetra/penta/complex-nucleotides) repeat motifs was estimated. To take into account variable number of SSR markers from different crops and with different lengths of repeat motifs that a given transferable SSR marker was based on a particular legume crop/length of repeat motif was estimated as the ratio of number of transferable markers based on a particular legume crop/length of repeat motif to the total number of transferable markers.

Results and Discussion

Three hundred and ninety-five cross legume species/ genera SSR markers were used for the study. Among these 395 SSR markers, those based on di-nucleotide repeats constituted 73 percent while those based on tri-, tetra-, penta- and complex nucleotide repeat motifs constituted 10 percent, 3 percent, 4 percent and 10 percent respectively [Fig-1]. These markers were used to identify those polymorphic between TAU-1 and LBG-17. Three-fifteen of 395 cross legume species/genera SSR markers were found amplified in parents of F_2 mapping population. 33.33 percent of 12 markers from *Medicago trunculata*, 44.44 percent of 9 markers from chickpea, 61.54 percent of 65 markers from soybean, 84.76 percent of 105 markers from adzuki bean, 68.57 percent of 70 markers from cowpea and 96.27 percent of 134 markers from greengram could successfully amplify blackgram genomic regions [Table-1]. Of the 315 amplified SSR markers, those based on di-nucleotide repeat motifs showed highest amplification percent (52.86 %) followed by those based on complex-(13.33 %), tri- (4.12 %) and tetra-nucleotide repeat motifs (8.90 %) [Fig-1]. Seventy-one of 315 cross legume species/genera SSR markers were polymorphic (22.54 %) between the parents of F_2 mapping population. Of the 71 polymorphic markers, those based on di-nucleotide repeat motifs showed highest polymorphism (67.60 %), followed by tri- (18.30), complex- (9.85 %) and tetra- nucleotide repeat motifs (4.22 %) [Table-2] and [Fig-2]. Yu *et al.* (2000) [12] also reported higher discriminating ability of dinucleotide repeat motifs-based SSR markers than those based on tri-, tetra- and complex repeat motifs. Several researchers have also reported transferability of SSR markers among legume species/genera. For example Chandra, (2011) [13] has reported transferability of *Medicago truncatula* EST-SSR markers to forage legumes. Gupta *et al.*, (2012) have reported 92 % transferability of greengram SSR markers, 91% of adjuki bean SSR markers and 86% of cowpea SSR markers to black gram [14]. In the present study, total of 71 (transferable cross legume species/genera SSR markers) blackgram specific SSR markers, the transferable cross species / genera SSR markers served as an alternate strategy to enrich the available SSR markers to blackgram.

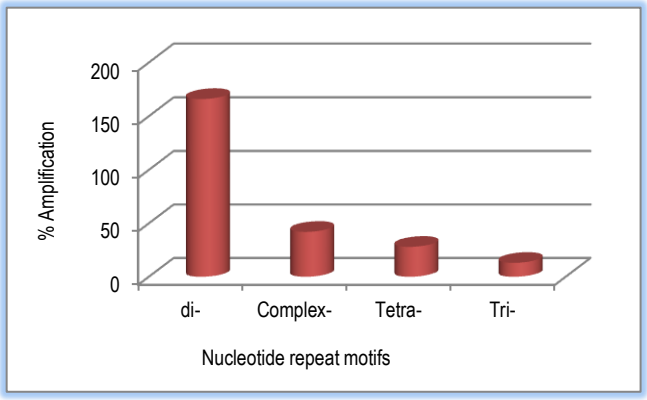

Fig-1 Percent amplification of cross legume species/ genera SSR markers classified by length of repeat motifs in blackgram

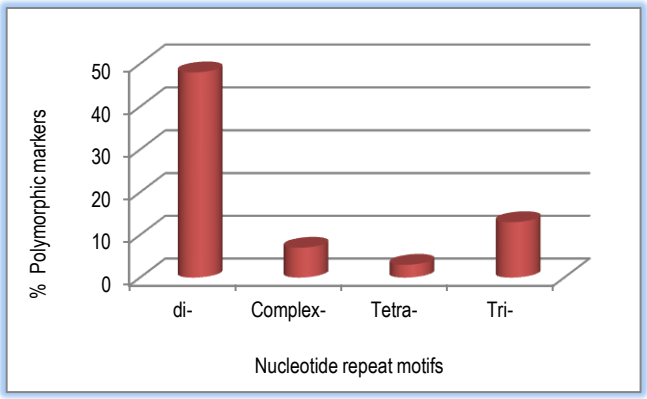

Fig-2 Percent polymorphic cross legume species/genera SSR markers classified by length of repeat motifs in blackgram

Table-2 *Number of polymorphic SSR markers between parents of F_2 derived from bi-parental crosses in blackgram*

Crop	Total number of polymorphic markers	Repeat motifs			
		Di	Tri	Tetra	Complex
Medicago trunculata	1	-	-	1	-
Chickpea	3	-	2	-	1
Soybean	7	5	1	-	1
Adzuki bean	17	14	2	1	-
Cowpea	9	9	-	-	-
Green gram	34	20	8	1	5
Total	71	48	13	3	7

Conclusion

Blackgram (*V. mungo* L. Hepper) is an important food legume species. A major challenge for marker assisted breeding in this species is the lack of sufficient DNA markers.

A set of 395 simple sequence repeat (SSR) markers developed from six other legume species *viz*. Soybean, *Medicago trunculata*, greengram, adzuki bean, cowpea and chickpea were evaluated for their transferability on two black gram varieties. In total 314(79.50%) SSR markers could produce amplicons and these were defined as transferable. Considerable transferability was observed with marker derived from related species belonging to genus Vigna such as Mungbean (96.27%), adzukibean (84.76%), cowpea (68.57%), soyabeam (61.54%), chickpea (44.44%)and Only 33.33% markers derived from *Medicago trunculata* were transferable to blackgram. Thus, the present study suggested preferential use of SSR markers from mungbean, cowpea, soybean, adzukibean and chickpea those based on simple di-/trinucleotide repeat motifs for studies designed to examine transferability of cross legume species/genera SSR markers to blackgram. The present study showed possibility of using cross legume species/ genera SSR markers in blackgram for genotyping the F_2 mapping population for gene mapping and subsequently for marker assisted selection.

Application of research: The identified polymorphic cross legume species/ genera SSR markers are suggested for use in various applications in blackgram breeding research.

Research Category: Genetics and Plant Breeding

Abbreviations: SSR- Simple Sequence Repeats, MAS-Marker assisted selection

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University: University of Agricultural Sciences, Bengaluru, 560065, India
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Study area / Sample Collection: MAS Lab, Department of Genetics and Plant Breeding, UAS, GKVK, Bengaluru.

Cultivar / Variety / Breed name: Blackgram [*Vigna mungo* L. Hepper]

Conflict of Interest: None declared

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.
Ethical Committee Approval Number: Nil

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