

Identification of Cross Legume Species / Genera SSR Markers Polymorphic to Parents of Recombinant Inbred Lines Derived from Two Bi-parental Crosses in Dolichos Bean (*Lablab purpureus* L. Sweet).

H. R. UDAY KUMAR, M. BYRE GOWDA AND S. RAMESH

Department of Genetics and Plant Breeding, College of Agriculture, UAS, GKVK, Bengaluru-560 065

ABSTRACT

One hundred cross legume species / genera SSR markers (65 from soyabean, 12 from medicago, 14 from greengram and 9 from chickpea) were used to identify those polymorphic to parents of recombinant inbred lines (RILs) derived from two bi-parental crosses in dolichos bean, a genomic resources limited crop. Of these, 43 SSR markers (43%) amplified and eighteen of them (41.86%) were polymorphic between the parents of RILs. Among the 18 cross legume polymorphic SSR markers, those based on, di-nucleotide repeat motifs exhibited highest polymorphism (55.55%), followed by tetra- (16.66%), complex- (16.66%) and tri-nucleotide repeat motifs (11.11%). The identified polymorphic cross legume SSR markers are suggested for use in various applications in dolichos bean breeding research.

DOLICHOS bean (*Lablab purpureus* L. Sweet) with chromosome number $2n=22$, commonly known as hyacinth bean, field bean, Indian bean, sem, lubia bean, butter bean, and Egyptian kidney bean (Shivashankar and Kulkarni, 1989). It is considered as poor man's bean and is one of the major sources of dietary protein to the people who depend on vegetarian diet in southern parts of Karnataka, Tamil Nadu and Andhra Pradesh. It is mainly grown as a rainfed crop for its fresh immature beans for use as a vegetable (Shivashankar and Kulkarni, 1989). Fresh pods containing immature beans are economic product in dolichos bean.

Sustainable production of dolichos bean requires development of high fresh pod yielding varieties with a range of maturity that matches diverse production environments. However, phenotype based selection for fresh pod yield and its component traits is rather less effective owing to their complex inheritance and significant cross over $G \times E$ interaction. 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 fresh pod yield and its component traits. However, identification of DNA markers polymorphic to parents of the mapping populations which are required to identify those linked to genomic regions controlling fresh pod

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-allelic and co-dominant inheritance, possibility of multiplexing and amenability for automation (Powell *et al.*, 1996). However, SSR markers specific to dolichos bean are limited.

Nevertheless, the use of transferable cross legume species / genera SSR markers in crops where they are not available is an alternate strategy to ensure availability of markers. The discovery of high degree of genome synteny among fabaceae members such as soybean, cowpea, mung bean, common bean and alfalafa (Humphry *et al.*, 2002) offers opportunity to transfer SSR markers from these crops to dolichos bean, a member of fabaceae. However, such studies are limited in dolichos bean. The only reported attempt to examine transferability and polymorphism of cross legume species / genera SSR markers to dolichos bean is from soybean (Yao *et al.*, 2012). The present study was carried out with an objective to identify the cross legume species / genera SSR markers polymorphic between the parents of RILs derived from two bi-parental crosses in dolichos bean.

One hundred cross legume species / genera SSR markers which included 65 from soybean (Peakall *et al.*, 1998; Yao *et al.*, 2012: <http://www.soybase.org/BARCSOYSSR.edu/CGKB/>), 12 from *Medicago trunculata* (Gupta and Prasad 2009), 14 from greengram (Gupta *et al.*, 2012) and 9 from chickpea (Choudhary *et al.*, 2009) were used for the study. Among these 100 markers, those based on dinucleotide repeats constituted 72 per cent, while, those based on tri-, tetra-, penta- and complex nucleotide repeat motifs constituted 10 per cent, 4 per cent, 4 per cent and 10 per cent, respectively (Fig. 1). These markers were used to identify those polymorphic between the parents of RILs derived from HA 4 × CPI 31113 and HA 4 × CPI 60125. While, HA 4 is a popular photo period insensitive determinate variety, CPI 31113 and CPI 60125 are photo period sensitive and indeterminate germplasm accessions. HA 4 differs from CPI 31113 and CPI 60125 for fresh pod yield and its component traits such as number of racemes, raceme length, fresh pods raceme⁻¹, and fresh pods plant⁻¹.

The DNA was extracted from the parents by using CTAB method and quantified. The cross legume species / genera SSR markers were tested for amplification at annealing temperatures of 53°C and 56°C. The amplicons were separated on 2 per cent agarose gel to examine the SSR marker amplification in dolichos bean. The appearance of well separated unambiguous amplicons is considered as successful amplification of cross legume SSR markers. The SSR markers which successfully amplified their complementary priming regions of the parents were

used to identify those polymorphic between parents (HA4, CPI 31113 and CPI 60125). The amplicons were separated on 6 per cent horizontal polyacrylamide gels (hPAGE) and visualized by post electrophoresis ethidium bromide (0.1µg/ml) staining.

Forty three of 100 cross legume SSR markers were found amplified in parents of RILs. 57.14 per cent of 14 markers from green gram, 44.61 per cent of 65 markers from soybean, 40 per cent of nine markers from chickpea and 16.66 per cent of 12 markers from *Medicago trunculata* were amplified (Table I). Of the 43 amplified SSR markers, those based on di-nucleotide repeat motifs showed highest amplification *percent* (72.09%) followed by those based on complex- (13.95%), tri- (6.97%) and tetra-nucleotide repeat motifs (6.97%) (Fig. 1).

Eighteen of 43 cross legume species/genera SSR markers were polymorphic (41.86%) between the

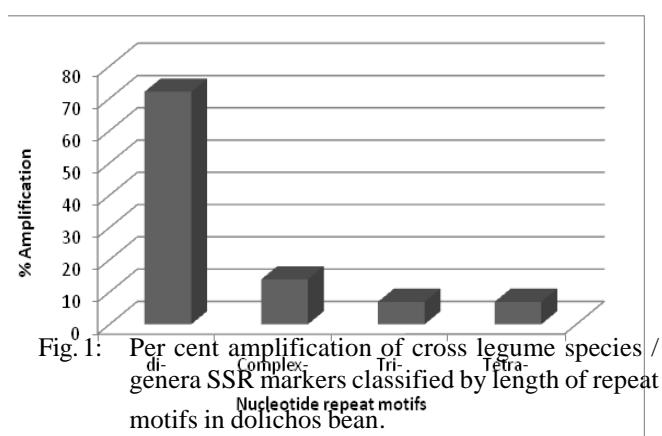


TABLE I

Crop and repeat motifs based distribution of amplified cross legume species / genera SSR markers

Crop	Total number of markers used	Number of markers amplified	% amplification	Length of repeat motifs			
				di-	Tri-	Tetra-	Complex-
Soybean	65	29	44.61	26	-	-	03
Medicago trunculata	12	02	16.66	-	01	01	-
Green gram	14	08	57.14	04	01	02	01
Chickpea	9	04	16.66	01	01	-	02
Total	100	43	43	31	03	03	06

parents of RILs derived from two bi-parental crosses in dolichos bean. Of the eighteen polymorphic markers, those based on di-nucleotide repeat motifs showed highest polymorphism (55.55%), followed by tetra- (16.66%), complex- (16.66%) and tri- (11.11%) nucleotide repeat motifs (Table II and Fig. 2). Yu *et al.*, (2000) also reported higher discriminative ability of dinucleotide repeat motifs-based markers than those based on tri-, tetra- and complex repeat motifs. SSR markers transferable from *Medicago* were used for polymorphism survey in *Trifolium repense* L. and a genetic map was constructed (Demdoum *et al.*, 2012). Gupta *et al.* (2013) reported 69 per cent amplification of cross legume SSR markers and 16

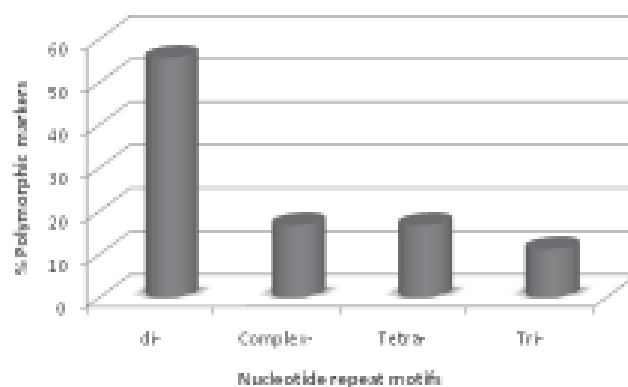


Fig. 2: Per cent polymorphic cross legume species / genera SSR markers classified by length of repeat motifs in dolichos bean.

TABLE II

Number of polymorphic SSR markers between parents of RILs derived from two bi-parental crosses in dolichos bean

Crop	Total number of polymorphic markers	Length of repeat motifs			
		di-	Tri-	Tetra-	Complex-
Soybean	12	10	-	-	02
Medicago trunculata	1	-	-	01	-
Green gram	3	-	01	02	-
Chickpea	2	-	01	-	01
Total	18	10	02	03	03

per cent of them were polymorphic among a set of genotypes in blackgram. The present study showed possibility of using cross legume species / genera SSR markers in dolichos bean for genotyping the two RIL populations for gene mapping and subsequently for markers assisted breeding after validation.

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(Received : May, 2016 Accepted : June, 2016)