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Cross Legume Species/Genera Transferability of SSR Markers and their Utility in Assessing Polymorphism among Advanced Breeding Lines in Dolichos Bean (*Lablab purpureus* L.)

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A B S T R A C T

The use of transferable cross-species/genera SSR markers is an alternative strategy to ensure availability of markers in genomic resources-limited crops such as dolichos bean. A total of 275 cross legume species/genera SSR markers were examined for their transferability to dolichos bean. 126 of 275 cross legume species/genera SSR markers (45.81%) were found transferable to dolichos bean. Transferability varied with cross legume species/genera with 83.33% of *Medicago truncatula*, 78.57% of greengram, 77.77% of chickpea, 53.33% of soybean and 33.33% of cowpea SSR markers being transferable to dolichos bean. The extent transferability of SSR markers based on simple di-/tri-nucleotide repeat motifs was higher than those based on penta-/tetra-/complex nucleotide repeat motifs. Of the 126 transferable SSR markers, 64 produced amplification in all the 14 advanced breeding lines with an average of 2.42 alleles per SSR locus. Six of the 64 SSR markers were most informative with four allelic variants.

Introduction

Dolichos bean (*Lablab purpureus* L Sweet) var. Lignosus is one of the most ancient legume crops known for its food (Ayyangar and Nambiar, 1935) and fodder (Magoon *et al.*, 1974) values. In India, it is grown as a rain-fed crop for fresh pods containing immature grains for use as a vegetable and as a split dhal to a limited extent. The fresh pods are the harvestable economic product in dolichos bean. It is predominantly a self-pollinated crop (Ayyangar and Nambiar, 1935) with 2n=2x=22 chromosomes (She and Jiang, 2015) with a genome size of 367 Mbp (Iwata *et al.*, 2013). Handling of segregating generations derived from crosses involving deliberately selected parents followed by pedigree selection is the most widely used breeding method for genetic improvement of fresh pod yield and its component traits in dolichos bean (Keerthi *et al.*, 2016).

Classical quantitative genetics-based breeding dolichos bean for pod productivity *per se* traits has been met with limited success, as

these traits are controlled by a large number of genes with complex inheritance and significant cross-over genotype-by-environment interaction. Therefore, phenotype-based selection of individuals for these traits has been less effective. DNA markers which are crop stage non-specific, environmental neutral, easily assayable and simply inherited are increasingly being used as surrogates of difficult-to-breed productivity *per se* traits (the activity popularly known as marker-assisted selection) to augment the pace and efficiency of breeding crop plants. However, identification of markers closely linked to genomic regions which explain substantial portion of phenotypic expression of productivity *per se* traits [the process is called as quantitative trait loci (QTL) mapping] is the pre-requisite for their use as surrogates. 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).

The use of DNA markers in general and SSR markers in particular in dolichos bean breeding is still in its infancy due to their non-availability. There are no reported attempts of developing genomic/expressed sequence tags (EST) SSR markers in dolichos bean. *De novo* development of SSR markers is expensive, laborious and labour intensive (Powell *et al.,* 1996). Nevertheless, the use of transferable cross-species/genera SSR markers in crops where they are not available is an alternative strategy to ensure availability of markers in genomic resources-limited crops such as dolichos bean. Examination of transferability of cross legume species/genera SSR markers in dolichos bean is a prerequisite for their use as surrogates of difficult-to-breed traits. The discovery of high degree of genome synteny among fabaceae members such as soybean, cowpea, mung bean, common bean and alfalfa (Humphry *et al.,* 2002; Choumane *et al.,* 2004) offers opportunity to transfer SSR markers from these crops to genomic resources-limited crops like dolichos bean, a member of fabaceae. However, such studies are limited in dolichos bean. The only reported attempt to examine transferability of cross legume species/genera SSR markers to dolichos bean is from soybean (Yao *et al.,* 2012). The objectives of the present study are to (1) explore transferability of SSR markers from a few other cross legume species/genera such as cowpea, *Medicago truncatula*, greengram and chickpea including soybean to dolichos bean and (2) assess the utility of transferable SSR markersto detect and characterize polymorphism at SSR loci among dolichos bean advanced breeding lines (ABL) differing for pod yield and its component traits (Keerthi *et al.,* 2016).

Materials and Methods

A pair of dolichos bean genotypes (HA 4 and Kadlavare) differing in pod yield and its component traits were used for examining the transferability of cross legume species/genera SSR markers. While HA 4 is a highly popular high yielding photoperiod insensitive determinate bred variety, kadalavare is a high yielding photoperiod sensitive indeterminate landrace variety (Vaijayanthi *et al.,* 2016). The total genomic DNA was extracted from 20 days old seedlings of these two varieties using the Cetyl Trimethyl Ammonium Bromide method (Doyle and Doyle, 1987). The quality and quantity of extracted genomic DNA was checked using 0.8% agarose gel by comparing with uncut lambda DNA.

A total of 275 cross legume species/genera EST and genomic SSR markers which included 150 from cowpea

(http://cowpeagenomics.med.virginia.edu/CGKB/), 90 from soybean (Peakell *et al.*, 1998; Gupta and Prasad, 2009) (http://www.soybase.org/BARCSOYSSR/index.php), 12 from *Medicago truncatula* (Gupta *et al.*, 2012), 14 from greengram (Choudhary *et al.*, 2009) and 9 from chickpea (Bostein *et al.*, 1980) were used to amplify genomic DNA extracted from the selected pair of dolichos bean varieties.

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), 0.3µl *Taq* polymerase (1 unit per µl), 1.0 µl 10X TE buffer, 0.5 µl 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 95ºC (hot start), 0.30 min at 95ºC (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 40 cycles. The PCR products were loaded on two *per cent* 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 dolichos bean

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, *per cent* transferability was calculated as (Number of markers amplified/Total number of markers) × 100. A total of 126 cross legume species/genera SSR markers were transferable to dolichos bean. *Per cent* 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, conditional probability 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.

Assessment of genetic diversity at transferable SSR marker loci

The material for this study consisted of 14 advanced breeding lines (ABL) differing in pod yield and its component traits (Keerthi *et al.*, 2016). The genomic DNA was extracted from these 14 ABL using the Cetyl Trimethyl Ammonium Bromide method (Doyle and Doyle, 1987) and quantified using 0.8% agarose gel. The 14 ABL were genotyped using 126 transferable SSR markers. The transferable SSR markers' priming regions of 14 ABL were amplified following the procedure already described. The PCR products were loaded on four *per cent* 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. Of the 126 transferable SSR markers, only 64 produced amplification in all the 14 ABL.

Scoring of transferable SSR marker assay data

The amplicons of 64 transferable SSR

priming regions of genomic DNA at reported product size range (the amplicons in the same row) were scored as '1' for presence of amplicon and '0' for absence of amplicon for each of the transferable SSR marker locus. The variation in amplicon intensity was not taken into consideration to avoid ambiguity in scoring.

Statistical analysis of SSR marker data

The following population genetic parameters were estimated based on binary data of 64 transferable SSR markers which amplified in all the 14 ABL (supplementary 1).

Polymorphic SSR loci and major and minor allele frequencies

The *per cent* polymorphic loci were calculated as kp/k, where, 'kp' is the number of polymorphic loci and 'k' is the total number of SSR loci. The major alleles are those whose frequencies are ≤0.95. Rare alleles are those whose frequencies are ≤ 0.05.

Polymorphic information content (PIC)

The PIC, proposed as a measure of in formativeness of a genetic marker was estimated (Botstein *et al.,* 1980) as

$$PIC = 1 - \sum_{i=1}^{k} P_i^2 - \sum_{i=1}^{k-1} \sum_{j=i+1}^{k} 2P_i^2 P_j^2$$

Where p_i is the frequency of the i^{th} allele, p_j is the frequency of the j^{th} allele and 'k' is the number of alleles at transferable SSR loci.

Nei's average gene diversity (H_e)

The expected gene diversity for a transferable SSR locus locus was estimated (Nei, 1978) $ash_i = 1 - p_i^2 - q_i^2$

Where, p = frequency of i^{th} allele and q_j = frequency of j^{th} allele

The average gene diversity (H_e) across transferable SSR loci was estimated as

$$\hat{H}_e = \sum_{i=1}^{k} (1 - p_i^2 - q_i^2)$$

Where 'k' is number of SSR loci

As sample size (N) is small in the present study, an unbiased estimate of H_e was estimated as

$$\hat{H}_e = \frac{2N}{(2N-1)} \sum_{i=1}^{k} (1 - p_i^2 - q_i^2)$$

Where, N=number of individuals (Nei, 1978).

Average number of alleles per SSR locus (*n*)

It was estimated by counting the number of detected alleles per SSR locus and averaging over 'k' SSR loci (Hartl and Clark, 1997) as indicated below.

$$n = (1/k) \sum_{i=1}^{k} n_i \ ,$$

Where, n_i is the total number of detected alleles per SSR locus, and 'k' is the number of SSR loci.

Effective number of alleles (A_e)

A_e, the effective number of alleles was calculated by inverting the measure of homozygosis at a locus.

$$A_e = 1/(1-h) = 1 \Big/ \sum_{i=1}^{k} p_i^2 \ ,$$

Where, p_i is the frequency of the i^{th} allele at a

SSR locus and $h = 1 - \Sigma p_i^2$ is the estimate of heterozygosis at a SSR locus.

Results and Discussion

Transferability of SSR markers

One hundred and twenty six cross legume species/genera SSR markers (45.81 %) were found transferable to dolichos bean as they amplified SSR priming genomic regions of the two genetically diverse varieties. Transferability varied with cross legume species/genera with 83.33% of *Medicago truncatula*, 78.57% of greengram, 77.77% of chickpea, 53.33% of soybean and 33.33% of cowpea SSR markers being transferable to dolichos bean (Table 1; Fig. 1). The conditional probability that given cross species/genera SSR markers transferable to dolichos bean was higher from cowpea (closely followed by soybean) than those from other legume species/genera (Table 1; Fig. 2). Several researchers have also reported transferability of SSR markers among legume species/genera. For example (Chandra, 2011) has reported transferability of *Medicago truncatula* EST-SSR markers to forage legumes. (Gupta *et al.,* 2012) have reported 92 *per cent* transferability of greengram SSR markers, 91 *per cent* of adjuki bean SSR markers and 86 *per cent* of cowpea SSR markers to black gram. (Datta *et al.,* 2013) reported highest transferability of SSR markers from common bean followed by lentil, field pea and chickpea to pigeonpea. (Agbagwa *et al.,* 2015) reported successful transferability of SSR markers from *Vigna savi* to pigeonpea. The transferability of cDNA SSR markers from *Vicia sativa* sub sp. *sativa* to *V. ervilia* and *V. sativa* sub sp. *nigra* ranged from 33% to 82%, respectively with an average of 52% (Raveendar *et al.,* 2015).

The extent of transferability of SSR markers based on simple di-/tri-nucleotide repeat motifs was higher than those based on penta-/ tetra-/complex nucleotide repeat motifs (Table 2; Figs. 3 and 4). The present study provided evidence for transferability of cross legume species/genera SSR markers with high transferability success from cowpea and soybean to dolichos bean. The study suggested that it is preferable to choose and use simple di-/tri-nucleotide repeat motifs-based SSR markers for studies aimed at transferability of crops legume species/genera SSR markers to dolichos bean. The success of transferability of SSR markers developed in one species/genera to other species/genera could be attributed to high degree of DNA sequence conservation and stability of primer binding sites flanking SSR regions during evolution of legume species/genera (Decrooq *et al.,* 2003; Zucchi *et al.,* 2003). Among the legumes, a high level of genome conservation between cowpea and mung bean, mung bean and common bean and mung bean and dolichos bean has been (Humphry *et al.,* 2002).

The present study help enrich the available SSR markers in dolichos bean. The 64 transferable SSR markers which produced successful amplification were used to assess polymorphism among ABL differing in pod yield and its component traits (Keerthi *et al.,* 2016).

Transferable SSR markers assay-based polymorphism among ABL

Population genetic parameters

The 64 transferable SSR markers used in the present study were polymorphic with an average of 2.42 alleles per SSR locus while four SSR markers (M 11, M 55 and M 55) were monomorphic (least informative) (Table 3). Six of the 64 SSR markers M 4, M 6, M 9, M 21, M 22 and M 39 were most informative with four allelic variants.

Fig.1 Per cent transferability of cross legume crop species/genera SSR markers to dolichos bean

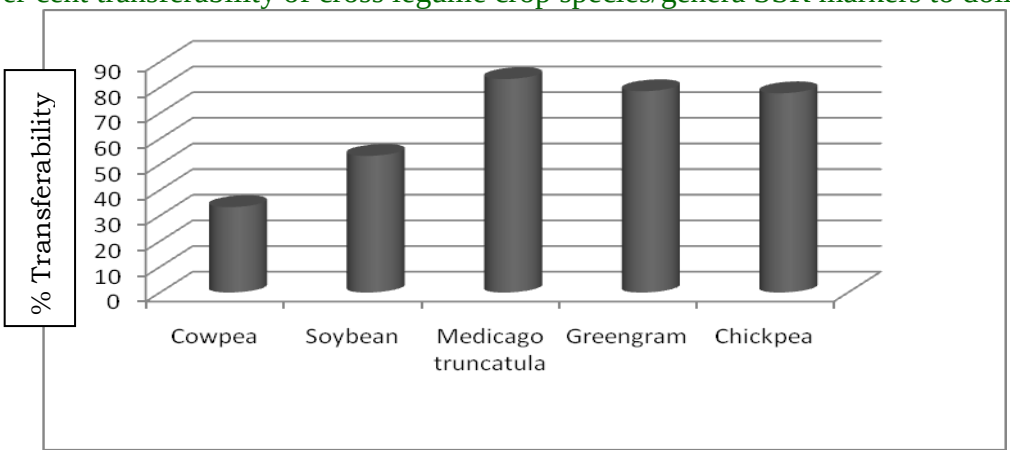

Fig.2 Conditional probability that a given cross legume species/genera SSR marker transferable to dolichos bean is from a particular crop

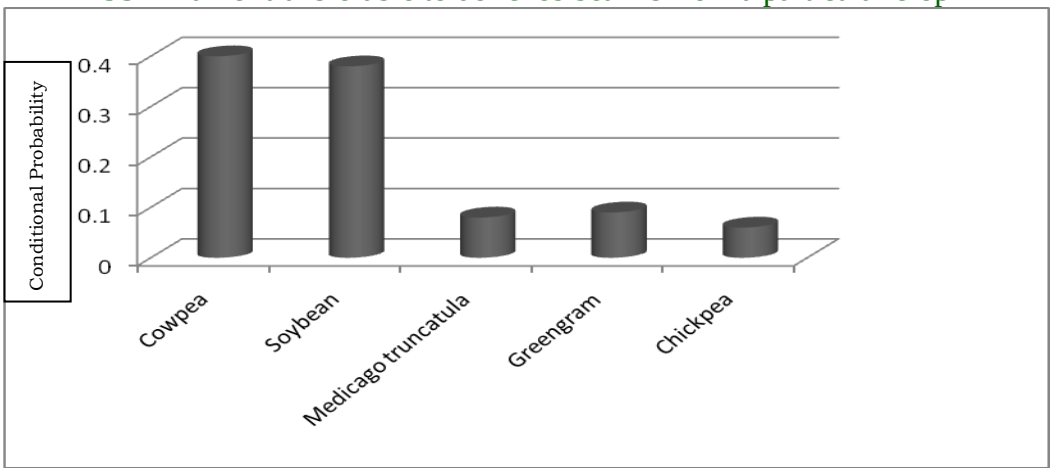

Fig.3 Per cent transferability of cross legume species/genera SSR markers (classified by length of repeat motifs) to dolichos bean

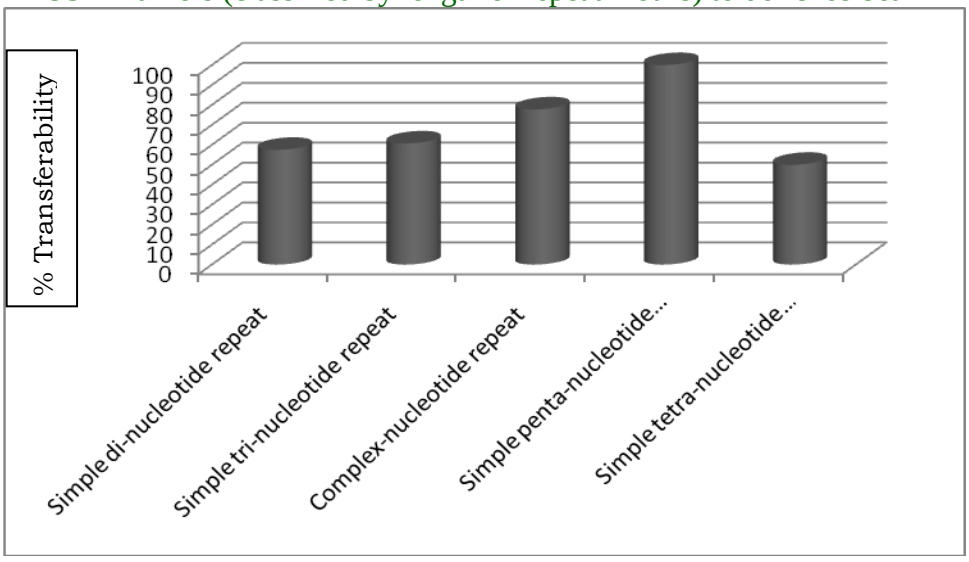

Fig.4 Conditional probability that a given cross legume species/genera SSR markers (classified by length of repeat motifs) transferable to dolichos bean

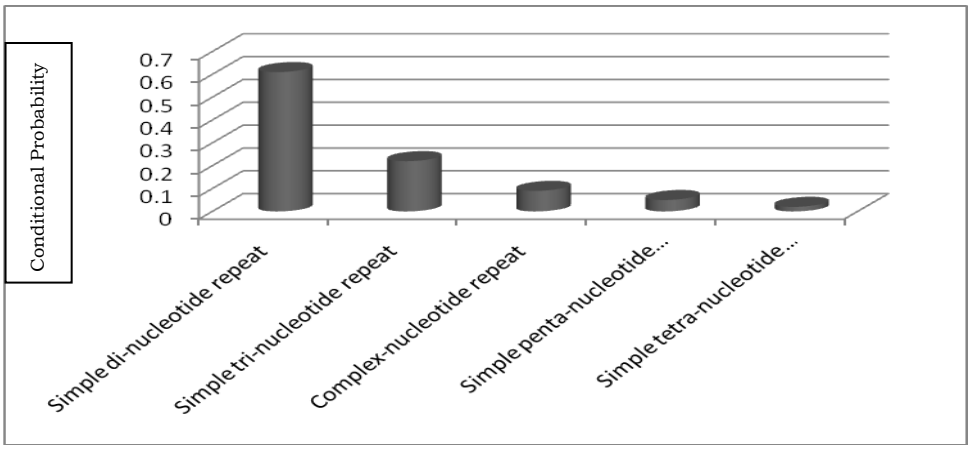

Fig.5 Cladogram depicting classification of 14 dolichos bean advanced breeding lines based on Transferable cross legume species/genera SSR marker allele frequencies

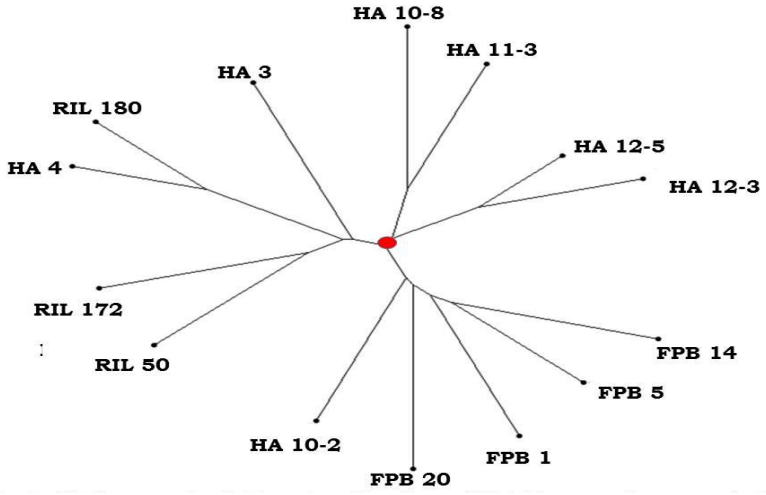

Table.1 Per cent transferability of cross legume species/genera SSR markers to dolichos bean

Crop	Total number of SSR markers used	Number of SSR markers amplified	% Transferability	Conditional probability that a given cross legume species/genera SSR markers transferable to dolichos bean is from a particular crop
Cowpea	150	50	33.33	0.40
Soybean	90	48	53.33	0.38
Medicago truncatula	12	10	83.33	0.08
Greengram	14	11	78.57	0.09
Chickpea	09	07	77.77	0.06
Total	**275**	**126**	**45.81**	

Table.2 Per cent transferability of cross legume species/genera SSR markers classified by length of repeat motifs to dolichos bean

Length of repeat motifs of cross legume species/genera SSR markers	Total number of SSR markers used	Number of SSR markers amplified	% Transferability	Conditional probability that a given transferable cross legume species/genera SSR marker is based on a particular motif
Simple di-nucleotide repeat	80	46	57.50	0.61
Simple tri-nucleotide repeat	28	17	60.71	0.22
Complex-nucleotide repeat	09	07	77.77	0.09
Simple penta-nucleotide repeat	04	04	100.00	0.05
Simple tetra-nucleotide repeat	04	02	50.00	0.02

Table.3 Estimates of parameters specifying transferable cross legume species/genera SSR marker alleles-based diversity among advanced breeding lines of dolichos bean

SSR Locus	Na*	Maj*	Min*	Ne*	H*	PIC	SSR Locus	Na*	Maj*	Min*	Ne*	H*	PIC
M01	3.00	0.50	0.10	2.38	0.58	0.65	M33	2.00	0.63	0.37	1.88	0.47	0.57
M02	2.00	0.70	0.30	1.72	0.42	0.55	M34	2.00	0.60	0.40	1.92	0.48	0.58
M03	3.00	0.45	0.09	2.37	0.58	0.63	M35	3.00	0.43	0.21	2.80	0.64	0.57
M04	4.00	0.71	0.07	1.85	0.46	0.43	M36	3.00	0.64	0.09	2.05	0.51	0.60
M05	2.00	0.63	0.36	1.86	0.46	0.55	M37	2.00	0.67	0.33	1.80	0.44	0.50
M06	4.00	0.61	0.07	2.25	0.56	0.57	M38	3.00	0.67	0.08	1.95	0.49	0.55
M07	2.00	0.70	0.30	1.72	0.42	0.55	M39	4.00	0.60	0.10	2.38	0.58	0.66
M08	3.00	0.71	0.07	1.78	0.44	0.39	M40	2.00	0.64	0.36	1.86	0.46	0.55
M09	4.00	0.50	0.07	2.58	0.61	0.54	M41	3.00	0.66	0.08	1.95	0.49	0.55
M10	2.00	0.56	0.44	1.98	0.49	0.59	M42	2.00	0.71	0.29	1.69	0.41	0.32
M11	1.00	1.00	0.00	1.00	0.00	0.12	M43	2.00	0.64	0.36	1.85	0.46	0.35
M12	3.00	0.66	0.08	1.95	0.49	0.55	M44	2.00	0.57	0.43	1.96	0.49	0.37
M13	2.00	0.78	0.22	1.51	0.34	0.28	M45	2.00	0.57	0.43	1.96	0.49	0.37
M14	3.00	0.64	0.28	2.00	0.50	0.43	M46	3.00	0.54	0.07	2.25	0.56	0.54
M15	2.00	0.60	0.40	1.92	0.48	0.58	M47	3.00	0.71	0.07	1.78	0.44	0.39
M16	2.00	0.78	0.22	1.51	0.34	0.28	M48	2.00	0.82	0.18	1.42	0.30	0.46
M17	2.00	0.57	0.43	1.96	0.49	0.37	M49	2.00	0.67	0.33	1.80	0.44	0.57
M18	2.00	0.82	0.18	1.42	0.30	0.46	M50	2.00	0.91	0.09	1.20	0.17	0.39
M19	2.00	0.42	0.58	1.95	0.49	0.52	M51	2.00	0.70	0.30	1.72	0.42	0.55
M20	2.00	0.6	0.4	1.92	0.48	0.58	M52	2.00	0.71	0.29	1.69	0.41	0.32
M21	4.00	0.33	0.08	3.43	0.71	0.73	M53	2.00	0.64	0.36	1.85	0.46	0.35
M22	4.00	0.41	0.08	3.13	0.68	0.70	M54	2.00	0.86	0.14	1.32	0.24	0.21
M23	2.00	0.50	0.5	2.00	0.50	0.53	M55	1.00	1.00	0.00	1.00	0.00	0.12
M24	3.00	0.50	0.21	2.65	0.62	0.55	M56	2.00	0.86	0.14	1.32	0.24	0.21
M25	2.00	0.83	0.17	1.38	0.28	0.41	M57	1.00	1.00	0.00	1.00	0.00	0.00
M26	3.00	0.50	0.21	2.65	0.62	0.55	M58	2.00	0.67	0.33	1.80	0.44	0.50
M27	2.00	0.50	0.50	2.00	0.50	0.53	M59	3.00	0.64	0.14	2.09	0.52	0.46
M28	3.00	0.75	0.12	1.68	0.41	0.55	M60	3.00	0.64	0.14	2.09	0.52	0.46
M29	2.00	0.50	0.50	2.00	0.50	0.38	M61	3.00	0.50	0.25	2.67	0.63	0.65
M30	2.00	0.70	0.30	1.72	0.42	0.55	M62	3.00	0.50	0.21	2.65	0.62	0.55
M31	2.00	0.80	0.20	1.47	0.32	0.50	M63	2.00	0.67	0.33	1.80	0.44	0.50
M32	2.00	0.86	0.14	1.32	0.24	0.21	M64	2.00	0.64	0.36	1.85	0.46	0.35
							Mean	2.42			1.91	0.44	0.47

* Na = Observed number of alleles; * Ne = Effective number of alleles; * H = Nei's gene diversity; Maj = Major allele frequency; Min = Minor allele frequency; PIC = Polymorphic information content

Supplementary.1 SSR primers used for cross-amplification in dolichos bean

No.	Primer	Repeat motif	Forward primer sequence	Reverse primer sequence	Crop
M1	RAI9	*	CATGGAGATGAAGCTACAGATGGTG	GGTGCTTGCTCAACTAGATCTC	Cowpea
M2	AG2	*	ACGACGTTGTAAAAGTTTCACACACA TTACTCTC	CATTAAGTTCCCATTACCTCGG CGATTGAGTGATTG	Cowpea
M3	Y101	*	GTTTTGCTGACTAGAATGTTATTTTAC	GATTGTAGCTCCTTAGGAAG	Cowpea
M4	AG9	*	ACGACGTTGTAAAAGGTGGTAACAAT TGGAGGGG	CATTAAGTTCCCATTAGCTTGCA ACTTCATTCACAG	Cowpea
M5	Y15	*	GTCAGGCCACAACTACAAAG	CAGTCAACTCTTATTATCTATGACC	Cowpea
M6	Y67	*	GAGAGTTGGATGGCAGATTTAAAAG	GCTTTAGAGGTGCAATCTTGGC	Cowpea
M7	CP267,CP268	*	TCATGAGTTTCCACACACCAA	CCTTCGTATGTATATGTGGCTACTG	Cowpea
M8	MA87	*	GACTACATCCAGCATTTGCACGAGC	GATGTTAGCAGTGATGATCTTCAGC	Cowpea
M9	Y20	*	CTTCCGAGTTTTCCTTTATATAC	GGAATAGGTAGCAGAATAACATTTAG	Cowpea
M10	MA53	*	GCAGAAGCGAAATCTCCTAGAAAGC	GAAAACAATGAACAAGGTGAGGTTC	Cowpea
M11	CP97,CP98	*	TGCAATATAAAAACACTCTCGGATT	ATTTTGTGGCGACCTTTGAC	Cowpea
M12	CP337,CP338	*	GGTGTCAACACCGTTGGAG	TGCAAGCCATTAGAGAATGACA	Cowpea
M13	EX24	*	CGCTCCTCGCTGGCAAAAG	CCTTCCCTACAGTGATATTTCCC	Cowpea
M14	EX34	*	GCTCACCTACGTGTGTTCGATC	GCAAGTGGATGTGGTGATCTC	Cowpea
M15	MS134	*	GAACTTGATAGGATCCTAGA	TTCTGGTATGCACTGAGGGA	Cowpea
M16	EX 12	*	CATGCGCCGTCAAAGAATACTG	GCATTCTTGATGTGTCTCTTACCTG	Cowpea
M17	EX39	*	CAAGAGTCATTCGGCTCCTT	GCTGCACCGTTTTCCGAAAT	Cowpea
M18	MA83	*	GAAGATACCAAGATGCCCCTAAAAC	GTATATGTTAGCTAGCCACGTATGA	Cowpea
M19	L-1	*	GAATTCAACTCTCGTTTCTTCCG	GCATATATTTATATACACATACAAAC	Cowpea
M20	BSOYSSR_01_0646	(AT)20	GGGCAAAATTTCATTTCCCT	TGAGTTGGAATTTATTTGGATGAA	Soybean
M21	BSOYSSR_01_1061	(TA)32	GACTTGTGGTGTTGTTCTTCAA	TGAGCCAGCGTTAATCAAAA	Soybean
M22	BSOYSSR_01_1484	(TC)18	CTTCTCTCAGCACCCTCCAC	AACCCTTCTTCCACTTCCGT	Soybean
M23	BSOYSSR_04_0414	(CT)17	CCATTCTACAATCATGCCCC	AGAAGCTGGCTAAGATGGCA	Soybean
M24	BSOYSSR_04_1136	(AT)21	GAAATGACAATAATGCCGGG	TTCCATTCAAAGCAGAAGCA	Soybean
M25	BSOYSSR_05_0194	(AT)28	CGTGATTTCCAATGTGCCTA	GCCACAACTAGCAAACACGA	Soybean
M26	BSOYSSR_05_1070	(AT)18	TGGGTAGGTTTTTCAGCAATG	GCAAAGGGACCCAAAGGTAT	Soybean
M27	BSOYSSR_06_0026	(AG)18	AATTTGTAAGCAGCATGGCA	CTCTCCGTTCGTCACTCTCC	Soybean
M28	BSOYSSR_09_1284	(CT)18	AGCGATGCAATTATTCCTGG	CATTTCCCATGTTTGGCTCT	Soybean
M29	BSOYSSR_14_1271	(CT)16	AAGGAAGGAAAACCCATGCT	GGGACCACAGCGTTGAATTA	Soybean
M30	BSOYSSR_18_1580	(TA)19	TGCCAAGAAACTATAGGCCG	TGAGTTTCCCTGGTAGTGTGG	Soybean
M31	BSOYSSR_18_1942	(TC)19	CTCCTCATGCTTGGCAAAAT	ATGAGAACGCTGAAAAGGGA	Soybean
M32	BSOYSSR_20_1319	(TA)23	GCGTAGAGCTGGGTTAGAGTAGTTA	GCGTAGAGCTGGGTTAGAGTAGTTA	Soybean

No.	Primer	Repeat motif	Forward primer sequence	Reverse primer sequence	Crop
M33	BARCSOYSSR_20_1325	(CT)18	GGAAGGAGGAAGGAACGAAG	AGAAAGAGCATTCGGGTGAA	Soybean
M34	BARCSOYSSR_20_1327	(CT)19	TCATCTGCAGCACTGATTGA	TGGCTCCATAAGCACAAGAA	Soybean
M35	Satt526F	(ATT)9	GCGGCAAATTCTAATGACTG	GTCGGAGTTCTCAGTC	Soybean
M36	Satt591F	(ATT)17	GCGCGACCTTAATGATA	GCGCCCAAAGCTTAAA	Soybean
M37	Satt534F	(ATT)8	CTCCTCCTGCGCAACAACAATA	GGGGGATCTAGGCCATGAC	Soybean
M38	Satt547F	(ATT)10	GCGCTATCCGATCCATATGTG	TGATTTCGCTAGGTAAAATCA	Soybean
M39	Sat_369F	(AT)28	GCGAATGGGGATAAACAATA GAACAAGA	GCGCAGTGGCTTCACAT	Soybean
M40	Satt632F	(ATT)17	GGGCTATGAAGGGAATGGAAAGGA	CCCATATTGAAGATTTG	Soybean
M41	Satt233F	(ATT)16	AAGCATACTCGTCGTAAC	GCGGTGCAAAGATA	Soybean
M42	Satt538F	(ATT)12	GCAGGCTTATCTTAAGACAAGT	GGGGCGATAAACTAG	Soybean
M43	Gm000742F	(TC)5	CTTCACAGAGAGAGGTGCCC	CTATTGGGTGGAAGGGTTGA	Soybean
M44	Gm001168F	(Tc)16	TGTGGTCCGATTGTTTGCTA	ACACCAAGCTCGAAAACCAC	Soybean
M45	Gm001362F	(AG)5	ATCCACCGGTGTTGTGGTAT	GGTGGATCAAATGGTTGGAC	Soybean
M46	Gm000659F	(GA)9	GATCATGGGCCAGCTTAAAA	AAACTGCTATGGGACCTCGT	Soybean
M47	Gm000664F	(TG)7	GGTGCTGTTCGTGCTGTTAC	ACCGTCACAAAGCAAAAAGG	Soybean
M48	Mt_ESSR02F	(CTTAT)4	GCATGCATTTTGTTGACCAC	GCCACCCAATAATCCAATGT	Medicago
M49	Mt_ESSR06F	(CAT)5	AACTTCTGCGCAACGCTTAT	CGGAGAGCGTGAAAGAAGAG	Medicago
M50	Mt_ESSR39F	(AAAG)5	AAATTTTCGGATGCGATGAG	TTAAAACACAACCGACACGC	Medicago
M51	VrSSR03F	(TGG)5	AAGTTTTTGGTTGACCGCAG	CCCTTGCATAGACAGGTGGT	Vigna sps
M52	VrSSR04F	(CT)5	CTGATTCAGCCTCAGGTTCC	CACCGCTAAGATGCTCACAA	Vigna sps
M53	VrSSR12F	(AT)6-(AG)8	TCCCTCTCCCACCTTCTTCT	GCTAGAGGGATGCTTCACCA	Vigna sps
M54	VrSSR13F	(TG)7	TTGATACGGCCACTTTCTCC	CCATCAACGGTTTTTACGCT	Vigna sps
M55	VrSSR15F	(AT)8	CATGACCGAGAAGACAAGCA	CCACAACAAATCCAAGAGCA	Vigna sps
M56	VrSSR16F	(TGTA)4	TCTCCATCCCCATCTTCATC	GGAGAGATCTGCGACCTTTG	Vigna sps
M57	VrSSR17F	(TGTT)5	AACTTCGTCCTGCGCTTAAA	AGCATGACCACACCAATCAA	Vigna sps
M58	VrSSR19F	(TA)5	AAATGTTCGTGGAATCCTGC	TTTCTTGTCCCTGAGTTCCAA	Vigna sps
M59	AG50B F	*	ATAAATTGGAAGATGTGTTGGC	TACTGATGTGGATTCTCCCAA	Soybean
M60	AG93 F	*	TCCATGCATGTATACTCCACC	TCATATGCCACAGGTTTTGTT	Soybean
M61	CESSRDB13F	(AT)5	ATCTGGGAGCTTGTGAGTTA	TTGTATCTCCTTCAGATGGC	Chickpea
M62	CESSRDB18F	(TA)5CA(TG)2 T(TG)3	TGCAAATAAAGCCTTCAAGT	GAAAGTGGGAAAATGCAATA	Chickpea
M63	CESSRDB33F	(GA)T(GA)TT (GA)5	GCTGCACAAAAAGTACATGA	ATCCATCGAAACACCAATAG	Chickpea
M64	CESSRDB56F	(ATG)4	TGTCTGGAACAACAAGTGAG	GCCAATCAGATTTCCTCTTA	Chickpea

* Repeat motif not available

These six markers along with other 64 SSR markers were polymorphic with major allele frequency of ≤ 0.95. The two tetra-allelic SSR markers (M 21 and M 22) and two tri-allelic markers (M 35 and M 61) exhibited greater ability to discriminate 14 ABL as reflected by higher estimates of Nei's genetic diversity and PIC. These tri- and tetra allelic SSR markers were based on simple perfect di-/tri-nucleotide repeat motifs. The higher discriminating ability of tri- and tetra allelic SSR markers is expected as those markers with simple short perfect repeat motifs have higher mutation rates than those with long repeat motifs and hence exhibit greater allelic variability (Kelkar *et al.,* 2008). Transferable SSR markers from *Vigna savi* revealed a total of 32 alleles in the range of 90-600 bp among 20 cultivars of cultivated species and ten genotypes of five wild species of pigeonpea (Agbagwa *et al.,* 2015). In the present study, the estimates of PIC of 64 transferable SSR markers ranged from 0.12 to 0.66 among ABL of dolichos bean. The polymorphism of cross transferable SSR markers among legume crops has been amply demonstrated. The estimates of PIC of transferable SSR markers from *Vigna savi* to cultivated and wild relatives of *Cajanus adams* ranged from 0.24 to 0.69 with an average of 0.47 (Agbagwa *et al.,* 2015). Datta *et al.,* (2013) reported a fairly high estimates of PIC (ranging from 0.5 to 0.6) of cross transferable SSR markers from common bean, lentil, field pea and chickpea to pigeonpea. The reported significant differences in quantitative traits means of 14 ABL (Keerthi *et al.,* 2016) were amply reflected at transferable SSR marker allelic diversity in the present study. The number of alleles needed to provide same heterozygosis if all the alleles are equally frequent (Hortl and Clark, 1997) as quantified by effective number of alleles (A_e) were obviously more at tetra allelic SSR marker loci than thoseat tri- and bi-allelic markers with a mean of 1.91 alleles (Table 3).

Grouping 14 ABL based SSR maker alleles

The SSR marker alleles-based grouping of 14 ABL into different clusters (Fig. 5) indicated the efficiency of SSR markers to differentiate phenotypically diverse ABL. Thus, higher degree of differences at SSR loci complemented those controlling the quantitative traits. Cross transferable *Vigna savi* SSR markers differentiated 20 cultivars *of Cajanus cajan* from 10 genotypes belonging to five wild relatives at 67% similarity (Agbagwa *et al.,* 2015).

Thus, the present study suggested preferential use of SSR markers from cowpea and soybean and those based on simple di-/tri-nucleotide repeat motifs for studies designed to examine transferability of cross legume species/genera SSR markers to dolichos bean. The study also indicated the utility of transferable cross legume SSR markers for detection and characterization of polymorphism among ABL differing for pod yield and its component traits.

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