Int.J.Curr.Microbiol.App.Sci (2018) *7*(5): 3736-3744

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 7 Number 05 (2018)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2018.705.433

Simple Sequence Repeat (SSR) Marker Assay-Based Genetic Diversity among Dolichos Bean (*Lablab purpureus* L. Sweet) Advanced Breeding Lines Differing for Productivity *per se* Traits

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Keywords

Gene diversity, Polymorphic information content, SSR markers

Article Info

Accepted:
26 March 2018
Available Online:
10 May 2018

A B S T R A C T

Increased and continued use of the diverse genotypes is a prerequisite for developing and diversifying the genetic base of crop cultivars. DNA markers which are crop-stage non-specific environmental neutral, easily assayable and amenable for automation are being used to assess the diversity of germplasm accessions and/or breeding lines. DNA markers also provide information on the population structure, allelic richness, and parameters that specify diversity among the genotypes to help breeders to choose those most appropriate for use in cultivar development. Hence SSR markers were used to assess diversity at marker loci among 16 phenotypically diverse dolichos bean genotypes. In the present study, 52 of 55 SSR-based markers were polymorphic, resulting in 94.55% polymorphism. Amplification of genomic DNA segments complementary to 55 SSR primers resulted in 133 scorable alleles with an average of 2.5 alleles per SSR loci. SSR markers exhibited differential ability to discriminate 16 genotypes as indicated by the estimates of effective multiplex ratio which ranged from 1.89 to 4.73 and marker index ranged from 0.69 to 3.40. The average gene diversity in the present study is more than that reported in dolichos bean. The estimates of Shanon's diversity index complemented those of average gene diversity. These results indicate that these SSR markers are highly informative and could be used to assess genetic diversity among the genotypes. The genotypes, HA 10-8, FPB 15 and RIL 162 share different alleles, FPB 8 and RIL 21 share similar alleles. Hence, the genotypes, HA 10-8, FPB 15 and RIL 162 could be used in crossing programme to derive genotypes with combination of desired traits.

Introduction

Dolichos bean is one of the ancient crops widely distributed in Indian subcontinent, Africa, and Southeast Asia (Smartt, 1985), where it has been used as a grain legume and vegetable for more than 3500 years (Fuller, 2003). Despite its wide distribution in the tropics and range of adaptability and diversity, it remains as an important, but underutilized crop in many of these regions (Engle and Altoveros, 2000), as evidenced from limited area planted to this crop and efforts towards its genetic improvement (Ramesh and Byregowda, 2016). However, it's utility as a vegetable and pulse (Ramesh and Byregowda,

2016), and/or forage crop (Magoon *et al.*, 1974) in tropical regions with humid to semi-arid climates has resulted in re-assessing its potential in tropical farming systems (Pengelly and Lisson, 2003). A wide use of underutilized crop species such as dolichos bean would contribute to temporal and spatial heterogeneity into agricultural production systems and hence sustainable supply of diverse nutritious food (Ebert, 2014). It can contribute to food security and better nutrition, increased income to rural poor, ecosystem stability and cultural diversity associated with local food habits (Ebert, 2014). Enhancement of its economic value through the development of widely adapted high yielding cultivars with broad genetic base is expected to offer competitive edge to dolichos bean to enable its popularity and wider cultivation (Ramesh and Byre Gowda, 2016).

Increased and continued use of the diverse genotypes is a prerequisite for developing and diversifying the genetic base of crop cultivars. DNA markers which are crop-stage non-specific environmental neutral, easily assayable and amenable for automation are being used to assess the diversity of germplasm accessions and/or breeding lines. DNA markers also provide information on the population structure, allelic richness, and parameters that specify diversity among the genotypes to help breeders to choose those most appropriate for use in cultivar development. Of late, DNA marker-based genetic diversity assessment has gained importance due to the speed and quality of data generated.

Of the several DNA-based markers those based on simple sequence repeats (SSR) are now the markers of choice in various applications of plant breeding research as they are co-dominant, multi-allelic, highly polymorphic even between closely related lines, require low quantity of DNA, easily automated for high throughput genotyping, and are highly transferable between populations (Benabdelmouna *et al.*, 2001). The objective of the present investigation is to assess phenotypically diverse dolichos bean genotypes at SSR marker loci.

Materials and Methods

Plant material and experimental design

The material for the study consisted of 16 phenotypically diverse genotypes which include two released varieties (HA 3 and HA 4), six advanced breeding lines (ABL) (HA 11-3, HA 10-8, FPB 3, FPB 8, FPB 15 and FPB 21) and eight recombinant inbred lines (RIL 11, RIL 162, RIL 185, RIL 332, RIL 180, RIL 60, RIL 21 and RIL 25) (Keerthi *et al.*, 2015). Among these, HA 3, HA 4, HA 11-3, HA 10-8, FPB 3, FPB 8, FPB 15, FPB 21, RIL 162, RIL 185, RIL 180 and RIL 60 are high yielding with greater number of branches, racemes plant^{-1} and pods plant^{-1}. The remaining genotypes (RIL 11, RIL 332, RIL 21 and RIL 25) are low yielding with fewer number of branches plant^{-1}, racemes plant^{-1} and pods plant^{-1}. The genotypes are being maintained at All India Coordinated Research Project (AICRP) on pigeonpea, University of Agricultural Sciences (UAS), Bengaluru, India.

SSR marker assay

The total genomic DNA from 16 genotypes was extracted from young leaves using the Cetyl Trimethyl Ammonium Bromide method (Doyle and Doyle, 1987). The quality and quantity of extracted genomic DNA of all the 16 genotypes were checked using 0.8% agarose gel. A total of 55 dolichos bean specific expressed sequence tag (EST)-based SSR markers (Table 1) were used for genotyping 16 genotypes at the Plant Molecular Biological Laboratory, Department

of Genetics and Plant Breeding (GPB), UAS, Bengaluru.

The SSR priming regions of 16 genotypes were amplified using 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 95^0C (hot start), 0.30 min at 95^0C (denaturation), 1 min at 50, 54 and 56^0C (annealing), 1 min at 72^0C (extension), 10 min at 72^0C (final extension) followed by infinite time at 4^0C for holding. The denaturation, annealing and extension step were carried out for 40 cycles. The PCR products were loaded on 4 *per cent* hi-media agrose gel in 1X TAE buffer stained with ethidium bromide and bromophenol blue as loading dye. Amplicons were separated in an electrophoresis unit at 80 V for five hours using 1X TAE buffer.

Scoring of SSR marker data

The different sized amplicons of SSR priming regions of genomic DNA at defined product size range (the amplicons in the same row) were scored as different alleles at each of the SSR marker locus. The variation in amplicon intensity was not taken into consideration to avoid confusion in scoring.

Estimation of population genetic parameters

Various population genetic parameters such as polymorphic SSR loci, polymorphic information content (PIC), Nei's average gene diversity, average number of alleles per locus, effective number of alleles per locus and major and minor allele frequency were estimated using the software, Power Marker V3.25 (Liu and Muse, 2005).

Results and Discussion

SSR marker assay-based polymorphism

In general, expressed sequences are conserved across genotypes within a species. Hence, EST-based SSR markers generally show lower polymorphism compared to those based on genomic SSR markers (Saha *et al.,* 2006). However, in the present study, 52 of 55 EST SSR-based markers were polymorphic, resulting in 94.55% polymorphism which was higher than that based on genomic SSRs (25-50%) in dolichos bean (Zhong *et al.,* 2008; Zhang *et al.,* 2013). A study by Woodhead *et al.,* (2005) indicated that the differentiation of gentypes based on EST-SSRs was comparable to that based on genomic SSRs and AFLPs. Polymorphic information content (PIC) is yet another measure of genetic diversity at a marker locus. The estimates of PIC ranged from 0.34 to 0.68 (Table 1). Higher the PIC values, higher is the amount of information which can be derived from a marker locus. Based on the criteria reported by Botstein *et al.,* (1980), 21 of the 52 polymorphic SSR loci were highly informative (PIC > 0.5) and the remaining 31 SSR markers were reasonably informative (0.5 > PIC > 0.25). Thus, present results indicate large differences at SSR marker loci used in the study.

Allelic richness and discriminating ability of SSR markers

Amplification of genomic DNA segments complementary to 55 SSR primers resulted in 133 scorable alleles with an average of 2.5 alleles per SSR loci. The number of alleles per loci varied from 1 to 5 (Table 2). As many as 18 markers were tri-allelic, four were tetra-allelic and one was penta-allelic (most informative); 29 were biallelic and 3 were mono-allelic (least informative). Major allele frequency ranged from 0.75 (KTD 250) to 0.31 (KTD 241) with an average of 0.56.

Table.1 List of SSR primers used to characterize genotypes in dolichos bean

Sl. No	Primer name	Forward primer (5'-3')	Reverse primer (5'-3')	Annealing Temp
1	KTD120	TGTAGAGTGGGAGTTAGTGTTGT	GAAGTACAAAGACCCTACTCCAG	56
2	KTD129	CTGCATGCGTATAATAGAGAAG	CCTCACACTGTATTACTGAGCTT	56
3	KTD130	CAGAGTATAAAGGAGAGGAGTCAT	CTCACAATTGTTTAGGTGGAAG	56
4	KTD132	GTAACAGTTATAGCTTGCTGTCG	CTTCTCCTAATTCTCCTTCACTTC	56
5	KTD133	GATGAAGGTGAAGAGAGTATGAGT	TAGCAGTGAAGAAGTGAGTGAGTA	56
6	KTD138	GATGAAGAAGGTTGTAGAGTTGTG	CTATCTCACACTTTCCTTACACCT	56
7	KTD140	GTGCCTCATAATTCTCTCTGTC	GCATGAAGTGTTAGCTACAGAA	56
8	KTD144	CTTTCTCCTTCTCTTTCTCACTC	GAAGACGGGTAGTTCCTAGTTAT	56
9	KTD147	TCTGTGAACTAAGCTGAAACAG	GAGGCTCAAAGTAGTAGATGATG	56
10	KTD150	AGACTACAATGTCTTGACACACC	TGTAGTAGTGTGGTGTTAGTTCTG	56
11	KTD159	GGGTTACTAGTAGTGGAAGAAGAA	GGGAGTCAACAATAACCCTAATAC	56
12	KTD162	ACACTGTTTGACTTAGAACTAGCC	GATTGTGGTACTCTCTTCTCAAG	56
13	KTD171	TAGCAGACAGAGTCTGAGATTAAG	CTCGTGTGTAGATTCAGAGTTAAG	56
14	KTD182	AGTGGGATCAACTAATTCTGAC	ACTGGACCAAGTTATCAAACAC	56
15	KTD183	TACCAGAGACTAATTGAACGTG	CTACCTCAGTCTTCGTTCTCTATC	56
16	KTD184	TCATTTCCAAGCTTCTGTAGTC	GAGTCGAAGAGTATGGAGAGAA	56
17	KTD185	ATTCGTGATCAGTGAGTTGTCT	TACTGCTACTCCATACCCTAGAAT	56
18	KTD193	AGAGAGATCCTTGAGAGAAACAC	TGGTCCATACTCAGACTACTAAGA	56
19	KTD195	TGGTTGAATGAGAGAGTAAAGG	GTTTCTTCAAGGTACATGTCTCAC	56
20	KTD199	TTCTTCTCTTCAACTTCACTCC	ACGAAGACAAGGAAGAGAAATC	56
21	KTD200	CTGAACTCACTTCTACCTTCTTCT	AAGACATGAGCATGTAGTGGTA	56
22	KTD201	GAAGCTAAATTCCTCCTCTTCT	GTTTAAGGATTCTTCTGACGAC	56
23	KTD207	GAGGTATCAGACTCATCACATTC	CCTATATTCGACATTCGTTCTC	56
24	KTD209	GTTGTGTCATGCCATAGCTGTAT	CTGATTATCACTCTGAGAAGAGG	56
25	KTD225	GTCTTTCACCATTTCTTCCTC	GTGTACACAGACACACACGATAC	56
26	KTD233	AGGTTGTAAGAGTGAGAAGGAA	GGATAGATAGACCTCAGAAGAAGA	56
27	KTD240	GCTCAATGTGAATGAAACAGAC	GACTCACTCCATTCTCTCTAACTT	56
28	KTD241	GTTAAGCCTTGAGATCTGACAC	CTTCACCTCACTCACAACATT	56
29	KTD245	AAGGAGAGAGTTAAGGTTGTAGAG	AAAAGTGCCACATTCTCTCTC	56
30	KTD249	ACTACCCTATAGTCTCTCTGTGCT	AGAAGATGATCTCAGATTCCAC	56
31	KTD250	GAGGAATCTGAGTTGGAGACTAC	ACTGTCCCTCTCATTCACTTT	56
32	KTD251	GTCTTGAAGAGTTTAGAGACGAGT	CTTAACATCACAACACAACACC	56
33	KTD252	CAGGTAAGTAGGAAGAAACAAGAG	CTTCTCGTGTTCACAAACAAC	56
34	KTD254	TGAGAAGTTAACAGACAGAGAGAG	TCACACTCTCATCAACCCTAC	56
35	KTD262	CGGCTCTATGAATGTAATACTGAG	AAACGGCGAAGAGTTAAGAT	56
36	KTD266	CTTGGTCACCTTTCTCATGT	GTTTCTTGTTTCCTCCAACTAC	56
37	KTD267	GAAACCTCAAATACGAACTCC	CCAGGTAGTGGTAGTAGTAGTAGGA	56
38	KTD279	AGTCTAGTCTACCACCTAAAGCAC	GATAGAGGAGTTGCTGCATTAT	56
39	KTD280	TGGGAGATTTGTCTTGTAGTAGAG	AGTAGAATAGGCAAAGGCAAC	56
40	KTD289	ACACCACATCACACACTTATTC	CTTGCTGACTGTTCTCCATT	56
41	KTD296	CTATCGACCTCCTCTCTACTCTC	AATACTACCAGCCGATTTCCT	56
42	KTD121	CAGTTTAGGAAGAGTACATTGGAG	CTATTGAACACTCCGCCTTATAG	54
43	KTD142	GAGACCTTCTCTTGCTAGTTTCT	ATACTTCACTCCTCCTACTTCAAC	54
44	KTD203	GTGTACCATAGGAGAATGACAA	ATGTTGTAGAGACAGAGAGAGACA	54
45	KTD206	GGAAAGCACTCATTATTCAGAC	ATAAACGTAGTTGCCACTCTCT	54
46	KTD211	TACAGAAGAGAACCGTGTAAGA	CATACAATACAACGTCCACAAC	54
47	KTD255	GAACTGAAAGAGAGGGATGAT	GGGCAGAGAGACAGTAATAATAAG	54
48	KTD261	CTTGAGAAACTCCACCATGT	CGAGGAGAGAAACAGAGATAGAT	54
49	KTD272	AATCTTAACAGGGTCAGAAGC	CTCTCCCTCCCATAACTAACTT	54
50	KTD275	CTCTTGTTCGTACTCTTCCTATTC	CAGATGAGAAGGACCGTTAAT	54
51	KTD116	GATACTGAAAACAGCTCCTTACC	CTCCTCTGAGTCTCTTCATGTTA	50
52	KTD243	ATCGATAGTGCAGAGAAGCTAT	GATCTCCGTCAGGTTAGAAAC	50
53	KTD269	CATCAGAGAGAAACTTGTGTTG	CTCTCACTCTCTTCCTCGTTC	50
54	KTD293	CTTGCCCTTGTAATAGAGGTC	ATCTTCTCCGACTCATTTCC	50
55	KTD131	TTCCCTCCTTCATATAGTTGAC	TTACAGACCTAAGTTCAAGAGG	50

Table.2 Estimates of population genetic parameters based on SSR marker profile of 16 dolichos bean genotypes

Sl. No.	Markers	Number of alleles	Major allele frequency	Minor allele frequency	Effective number of alleles	Nei's gene diversity	Corrected gene diversity	PIC	Shannon's Information index	EMR	MI
1	KTD184	4	0.50	0.06	2.72	0.63	0.65	0.57	1.14	3.78	2.39
2	KTD241	4	0.31	0.13	3.66	0.73	0.75	0.68	1.33	3.78	2.75
3	KTD245	4	0.56	0.07	2.37	0.58	0.60	0.59	1.08	3.78	2.19
4	KTD249	5	0.44	0.06	3.56	0.72	0.74	0.68	1.42	4.73	3.40
5	KTD250	2	0.75	0.20	1.47	0.32	0.33	0.35	0.50	1.89	0.61
6	KTD251	2	0.69	0.27	1.64	0.39	0.40	0.40	0.58	1.89	0.74
7	KTD262	2	0.63	0.33	1.80	0.44	0.46	0.43	0.64	1.89	0.84
8	KTD266	3	0.44	0.19	2.72	0.63	0.65	0.56	1.04	2.84	1.79
9	KTD267	2	0.69	0.27	1.64	0.39	0.40	0.40	0.58	1.89	0.74
10	KTD280	3	0.56	0.06	2.17	0.54	0.56	0.45	0.86	2.84	1.53
11	KTD195	3	0.38	0.20	2.78	0.64	0.66	0.62	1.05	2.84	1.82
12	KTD193	2	0.69	0.31	1.75	0.43	0.44	0.34	0.62	1.89	0.81
13	KTD203	2	0.56	0.44	1.97	0.49	0.51	0.37	0.69	1.89	0.93
14	KTD206	3	0.63	0.13	2.13	0.53	0.55	0.47	0.90	2.84	1.51
15	KTD211	2	0.56	0.44	1.97	0.49	0.51	0.37	0.69	1.89	0.93
16	KTD255	3	0.38	0.27	2.92	0.66	0.68	0.64	1.09	2.84	1.87
17	KTD272	3	0.44	0.07	2.27	0.56	0.58	0.53	0.89	2.84	1.59
18	KTD275	2	0.63	0.38	1.88	0.47	0.48	0.36	0.66	1.89	0.89
19	KTD296	2	0.63	0.38	1.88	0.47	0.48	0.36	0.66	1.89	0.89
20	KTD116	2	0.69	0.31	1.75	0.43	0.44	0.34	0.62	1.89	0.81
21	KTD243	2	0.63	0.38	1.88	0.47	0.48	0.36	0.66	1.89	0.89
22	KTD185	3	0.50	0.19	2.61	0.62	0.64	0.54	1.02	2.84	1.75
23	KTD200	2	0.56	0.44	1.97	0.49	0.51	0.37	0.69	1.89	0.93
24	KTD201	3	0.56	0.13	2.23	0.55	0.57	0.55	0.93	2.84	1.56
25	KTD207	2	0.56	0.44	1.97	0.49	0.51	0.37	0.69	1.89	0.93
26	KTD209	2	0.69	0.27	1.64	0.39	0.40	0.40	0.58	1.89	0.74
27	KTD225	4	0.50	0.07	2.65	0.62	0.64	0.62	1.14	3.78	2.35
28	KTD233	2	0.69	0.21	1.51	0.34	0.35	0.43	0.52	1.89	0.64
29	KTD289	3	0.50	0.25	2.67	0.63	0.65	0.55	1.04	2.84	1.77
30	KTD252	2	0.56	0.44	1.97	0.49	0.51	0.37	0.69	1.89	0.93
31	KTD147	3	0.50	0.13	2.46	0.59	0.61	0.51	0.97	2.84	1.68
32	KTD144	3	0.56	0.19	2.42	0.59	0.60	0.52	0.98	2.84	1.66
33	KTD133	2	0.69	0.31	1.75	0.43	0.44	0.34	0.62	1.89	0.81
34	KTD240	3	0.63	0.13	2.13	0.53	0.55	0.47	0.90	2.84	1.51
35	KTD199	3	0.44	0.19	2.72	0.63	0.65	0.56	1.04	2.84	1.79
36	KTD254	2	0.56	0.44	1.97	0.49	0.51	0.37	0.69	1.89	0.93
37	KTD120	2	0.56	0.44	1.97	0.49	0.51	0.37	0.69	1.89	0.93
38	KTD140	3	0.56	0.13	2.33	0.57	0.59	0.50	0.95	2.84	1.62
39	KTD142	3	0.50	0.13	2.46	0.59	0.61	0.51	0.97	2.84	1.68
40	KTD279	2	0.50	0.50	2.00	0.50	0.52	0.38	0.69	1.89	0.95
41	KTD150	2	0.56	0.40	1.92	0.48	0.50	0.45	0.67	1.89	0.91
42	KTD159	2	0.56	0.40	1.92	0.48	0.50	0.45	0.67	1.89	0.91
43	KTD162	2	0.63	0.38	1.88	0.47	0.48	0.36	0.66	1.89	0.89
44	KTD171	2	0.50	0.47	1.99	0.50	0.51	0.46	0.69	1.89	0.94
45	KTD182	2	0.56	0.44	1.97	0.49	0.51	0.37	0.69	1.89	0.93
46	KTD183	2	0.69	0.31	1.75	0.43	0.44	0.34	0.62	1.89	0.81
47	KTD129	3	0.63	0.13	1.99	0.50	0.51	0.51	0.86	2.84	1.41
48	KTD121	2	0.50	0.50	2.00	0.50	0.52	0.38	0.69	1.89	0.95
49	KTD130	2	0.56	0.44	1.97	0.49	0.51	0.37	0.69	1.89	0.93
50	KTD132	3	0.50	0.13	2.46	0.59	0.61	0.51	0.97	2.84	1.68
51	KTD131	2	0.44	0.50	2.00	0.50	0.52	0.53	0.69	1.89	0.95
52	KTD138	3	0.44	0.21	2.65	0.62	0.64	0.66	1.03	2.84	1.77
	Mean	2.65	0.56	0.27	2.17	0.49	0.51	-	-	-	-

PIC: Polymorphic information content; EMR: Effective multiplex ratio; MI: Marker index

Table.3 Inter genotypic distance between 16 dolichos bean genotypes based on SSR marker profile

Genotypes	HA 3	HA 4	HA 11-3	RIL 11	RIL 185	FPB 8	FPB 15	RIL 332	FPB 3	RIL 162	HA 10-8	FPB 21	RIL 21	RIL 25	RIL 60
HA 4	0.42														
HA 11-3	0.49	0.56													
RIL 11	0.47	0.51	0.38												
RIL 185	0.42	0.47	0.53	0.60											
FPB 8	0.62	0.49	0.51	0.53	0.58										
FPB 15	0.45	0.55	0.56	0.56	0.58	0.60									
RIL 332	0.40	0.55	0.45	0.40	0.44	0.47	0.51								
FPB 3	0.60	0.44	0.49	0.44	0.56	0.67	0.55	0.42							
RIL 162	0.40	0.44	0.38	0.42	0.45	0.49	0.44	0.58	0.45						
HA 10-8	0.36	0.51	0.56	0.42	0.60	0.40	0.53	0.44	0.47	0.40					
FPB 21	0.62	0.58	0.47	0.55	0.58	0.65	0.40	0.53	0.65	0.44	0.40				
RIL 21	0.42	0.38	0.35	0.38	0.36	0.38	0.40	0.42	0.38	0.36	0.40	0.38			
RIL 25	0.35	0.44	0.47	0.36	0.27	0.38	0.38	0.42	0.42	0.42	0.38	0.36	0.38		
RIL 60	0.35	0.35	0.42	0.38	0.42	0.40	0.35	0.38	0.33	0.35	0.42	0.42	0.36	0.42	
RIL 180	0.31	0.40	0.58	0.38	0.51	0.49	0.45	0.42	0.36	0.40	0.42	0.36	0.31	0.36	0.56

The number of alleles needed to provide same heterozygosis if all the alleles are equally frequent (Hartl and Clark, 1997) as quantified by effective number of alleles (Ne) were more for tri-allelic SSR markers than the bi-allelic markers with an average of 2.17 alleles per marker. When allelic frequencies are similar, the estimate of effective number of alleles is close to the observed number of alleles at a locus. Therefore, large differences between observed and the effective number of alleles indicate low frequencies of a few alleles because they are present in only one or a few genotypes. For this reason, estimate of effective number of alleles could be useful in indicating rare alleles (Laurentin, 2009). In the present study, large differences between the estimates of observed and the effective number of alleles indicate relatively low frequency of a few alleles, which could be considered as rare alleles. SSR markers exhibited differential ability to discriminate 16 genotypes as indicated by the estimates of effective multiplex ratio which ranged from 1.89 to 4.73 and marker index ranged from 0.69 to 3.40 (Table 2).

SSR marker-based genetic diversity

Laurentin (2009) opined that conceptually 'gene diversity' is the most appropriate measure of genetic diversity in self-pollinated crops like dolichos bean. It has been amply demonstrated that a minimum of 50 genotypes need to be used for DNA marker-based genetic diversity assessment (Bonin, 2007). In the present study, marginal differences between standard Nei's average gene diversity estimate and unbiased gene diversity (UGD) (Table 2) suggested that 16 dolichos bean genotypes considered for the study is adequate for genetic diversity assessment. Average gene diversity as a measure of variability is more appropriate for inbreeding species such as dolichos bean and is loosely referred as average heterozygosis (Weir, 1996). The average gene diversity was as low as 0.32 at SSR marker locus, KTD 250 and as high as 0.72 at SSR marker locus KTD 241 with an average of 0.52 among 16 genotypes. The average gene diversity in the present study is more than that reported in dolichos bean by Venkatesha *et al.,* (2007) and (Kinmani *et al.,* 2012), by Sarikamis *et al.,* (2009) in common bean and by Gwag *et al.,* (2006) in mung bean.

Shanon's diversity index, being relatively insensitive to bias caused by inability to detect heterozygous individuals (Dawson *et al.,* 1995) is being used to measure population diversity (Bussel, 1999). Shanon's diversity index is being largely used as a measure of diversity in plant genetic resources (Upadhayaya *et al.,* 2007). In the present study, estimates of Shanon's diversity index complemented those of average gene diversity. These results indicate that these SSR markers are highly informative and could be used to assess genetic diversity among the genotypes.

Inter-genotypic differentiation

Most published studies do not explain the choice of coefficient of diversity in relation to the type of marker and or ploidy level and pollination control system of the crop being investigated (Kosman and Leonard, 2005). While investigating the diversity of related genotypes in predominately self-pollinated crops such as dolichos bean where only homozygous genotypes were used in the study, Simple Matching Coefficient (SMC) is the most appropriate diversity measure as it takes care of absence of the amplicons in addition to presence of the amplicons in both the compared genotypes as causes of similarity (Laurentin, 2009). Dissimilarity coefficient among 16 genotypes (Table 3) ranged from 0.65 (between the genotypes HA 10-8 with FPB 15 and RIL 162) to 0.27

(between FPB 8 and RIL 21). These results indicate that while HA 10-8, FPB 15 and RIL 162 share different alleles, FPB 8 and RIL 21 share similar alleles. The genotypes, HA 10-8, FPB 15 and RIL 162 could be used in crossing programme to derive genotypes with combination of desired traits. The strategy of crossing genotypes with markedly distinct DNA marker-based profile has resulted in appearance of higher proportion of >50% of new and useful quantitative trait loci alleles in rice and tomato (Tanksley and McCouch, 1997).

Acknowledgements

Senior author gratefully acknowledges Kirkhouse Trust, UK for providing financial support in the form of fellowship for conducting thesis research for partial fulfilment for the award of PhD degree by University of Agricultural Sciences, Bengaluru, India.

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How to cite this article:

Keerthi, C.M., S. Ramesh, M. Byregowda and Vaijayanthi, P.V. 2018. Simple Sequence Repeat (SSR) Marker Assay-Based Genetic Diversity among Dolichos Bean (*Lablab purpureus* L. Sweet) Advanced Breeding Lines Differing for Productivity *per se* Traits. *Int.J.Curr.Microbiol.App.Sci.* 7(05): 3736-3744. doi: https://doi.org/10.20546/ijcmas.2018.705.433