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Genetic diversity in Indian bean (*Lablab purpureus*) accessions as revealed by quantitative traits and cross-species transferable SSR markers

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ABSTRACT

Genetic diversity in 143 Indian bean [*Lablab purpureus* (L.) Sweet] accessions from India was assessed using simple sequence repeat (SSR) markers. A total of 134 SSR markers developed from common bean, cowpea, mung bean, moth bean and Faba bean were used to assess their transferability to Indian bean. Quantitative traits as well as SSR marker data was used to analyse the genetic diversity analysis in 143 Indian bean accessions from different agro-ecological zones of India. Of the 134 SSR primers, 55 (41.0%) were found to be transferable since they showed reproducible polymorphic amplification products in Indian bean. The SSR primers derived from common bean fail to amplify any product in Indian bean. The results indicated that there is a potential for transferring SSR markers within legumes. The identification of a set of transferable SSR markers would enable the application of the SSR technology in Indian bean molecular research. To our knowledge, this is the first ever attempt to evaluate cross-species microsatellite (SSR) markers for a possible dissection of genetic diversity in Indian bean.

Key words: Cross-species marker transferability, Diversity, Indian bean, Polymorphism, Quantitative traits, Simple sequence repeats (SSRs)

Indian bean [*Lablab purpureus* (L.) Sweet, syn. *Dolichos lablab* L.] is a multi-purpose crop of Indian origin that has been widely used for soil improvement, soil protection and weed control (Deka and Sarkar 1983). Indian bean can also be grown as a component crop, in mixed farming systems and is used as food and fodder as well (Wood 1983). Indian bean is considered to be a good source of minerals and vitamins (Basu *et al*. 2002) and antioxidants (Bradley 1999). Continued selections of new cultivars over extended periods have drastically reduced the genetic diversity which jeopardizes the crop improvement programmes. Such narrowing of genetic diversity could be counter balanced by introgression of novel genes from diverse germplasm (Miflin 2000). Unlike improved varieties, the germplasm collections maintained at national active germplasm sites or landraces maintained by farmers are endowed with tremendous genetic variability, as they are not subjected to selection over a long period. Therefore, it has become essential to analyse genetic diversity in *Lablab* and allied genera and develop tools/methodologies to introduce varieties for yield and quality improvement. Assessment of genetic diversity based on phenotype suffers with limitations, since most of the morphological traits are greatly influenced by environmental factors and the development stage of the plant. Molecular markers, in contrast to quantitative traits can reveal the differences among different accessions at DNA level, thus providing a more direct, reliable and efficient tool for germplasm characterization and management. DNA markers are useful tools which in recent years have greatly enhanced the genetic analysis of crop plants (Varshney *et al*. 2002). Among a variety of marker systems, nuclear microsatellites or simple sequence repeats (SSRs) are tandem repeat units of 1-6 bp present throughout the genome have widely been recognized as powerful and informative genetic markers in both animals and plants systems (Jarne and Lagoda 1996). Cross species amplification of SSR loci was reported in a number of crops, thus it might be possible to facilitate more widespread use of SSRs in plants by using SSR markers from across species, leading to saving of time and economic resources.

The genetic diversity studies in *L. purpureus* have been primarily based on random amplified polymorphic DNA (RAPD) (Kumar *et al*. 2008, Rai *et al*. 2010, 2011, Biswas

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et al. 2012) and amplified fragment length polymorphism (AFLP) markers (Maass 2006) no SSRs have been exploited for studies in Indian bean. Thus in the present study 134 SSR markers (40 from *Vigna unguiculata*, 7 from *V. mungo*, 20 from *V. aconitifolia*, 13 from *Phaseolus vulgaris*, and 54 from *Vicea fabae*) were exploited to analyse genetic diversity in 149 accessions of *L. purpureus*. Besides, 10 quantitative characters were also studies to have a clearer picture of the genetic diversity in Indian bean accessions. To our knowledge, this is the first ever attempt to evaluate cross-species microsatellite (SSR) markers for a possible dissection of genetic diversity in Indian bean.

MATERIALS AND METHODS

The plant genetic material for this investigation comprised of 43 genotypes of Indian bean and their field evaluation was conducted at research farm of Indian Institute of Vegetable Research, Varanasi, India for two consecutive years, i.e. 2011-12 and 2012-13. Seeds were sown on the raised bed with row spacing 2 m and seed to seed spacing of 1 m. The experiment was led out in a complete randomized block design (CRBD) with three replications; each replication had 15 plants. The recommended fertilizer dose and cultural practices along with plant protection measures were followed to raise a good crop. Five plants were randomly chosen and data was recorded on ten quantitative traits; viz. (i) days to first flower, (ii) days to first picking, (iii) number of pods/plant, (iv) pod length (cm), (v) pod width (cm), (vi) number of seeds/pod, (vii) seed length(cm), (viii) seed width(cm), (ix) 100 seed weight (g) and (x) fresh pod yield/plant (kg).

To record the data, pods were harvested at edible maturity. The pod length, pod width, seed length, and seed width were measured with the help of meter scale and Vernier calliper. Two years pooled data for quantitative traits was analysed for CRBD (Gomez and Gomez 1984). The effect of different scales of measurement for different quantitative traits were minimized by standardizing the data for each trait separately prior to cluster analysis using the STAND module of NTSYS-pc ver. 2.02 software (Rohlf 1998). Pair-wise distance matrix was used as an input for analysis of clusters. UPGMA-based clustering was done using SAHN module of NTSys- pc.

Genomic DNA from each accession was extracted from young leaves collected from five random plants. The CTAB (Cetyl trimethyl ammonium bromide) protocol with minor modifications was used for this purpose (Doyle and Doyle 1987). The quantity of the isolated DNA was determined using DNA Quant 200 fluorimeter and the quality was ascertained by running in 0.8% agarose gel.

The SSR amplification was carried out according to the protocol of Rai *et al.* (2015) with minor modifications. A total of 134 SSR primers were used for amplification from 149 accessions (Table 3). The PCR amplification was carried out in 25 µl reaction mixture, containing 15 ng genomic DNA, 2.5 mM $MgCl_2$, 10x assay buffer, 100 mM dNTPs, 0.6 U of *Taq* DNA polymerase, and 0.2M primer, and the amplification was performed in a Thermal Cyler. The reaction programme consisted of one cycle of initial denaturation at 94°C for 1 min, 40 cycles each of denaturation at 94°C for 1 min annealing at 55-60°C for 1 min and elongation for 1 min at 72°C and final extension was allowed for 10 min at 72°C for 10 min. The amplified products were separated by electrophoresis in 2.5% agarose gel prepared in TAE buffer [40.0 mM Tris-base, 16.6 M acetic acid, 0.5 M EDTA (pH 8.0)], and visualised in a gel documentation system.

Each accession was scored manually for the presence (1) or absence (0) of a particular amplification product assuming that each product represents a unique locus. Bands with negligible intensity were not taken into consideration, and only clear, unambiguous amplicons were scored, and their sizes were determined using100 bp DNA ladder. The binary (1/0) data matrix was used to calculate Jaccard's similarity coefficient (Jaccord 1908). Cluster analysis was carried out among the genotypes based on Jaccard's similarity coefficients using UPGMA[27] and SAHN-clustering algorithm provided in NTSYS-pc ver. 2.02e software.

RESULTS AND DISCUSSION

Phenotyping of accessions

Mean of the 10 quantitative traits of all 143 accessions of *L. purpureus* is given in Table 1. The data of all the 143 accessions are provided in the supplementary file. The accession VRSEM-3 recorded the earliest (67 days) flower appearance, whereas VRSEM-867 was recorded to be late (137 days). The accession, VRSEM-3 was the earliest (92 days) for first fruit picking, while VRSEM 867 was late (170 days). The number of pods per plant which principally contributes to the overall yield was recorded to be highly varied and it ranged between 814.7 (VRSEM-913) and 34 (VRSEM-708). The genetic pool of Indian bean used in this study also showed a great variability in pod length and width; accession VRSEM-1000 recorded maximum pod length (14.13 cm) while VRSEM-709 recorded the maximum pod width (3.9 cm). The number of seeds/pod varied between 6.0 (VRSEM-927, VRSEM-709) and 3 (VRSEM-863, VRSEM-839, VRSEM-714), whereas the maximum (42 g) and the minimum (13 g) 100-seed weight was recorded in the accessions VRSEM-101 and VRSEM-787, respectively. Pod yield/plant being the most significant trait was recorded to be the maximum (3.7 kg) in VRSEM-913 and the minimum in VRSEM-810 (0.4 kg). It would be of immense use for the establishment of proprietary rights and the determination of cultivar purity. Although morphological markers have been used to study the relationship among common bean genotypes (Baswana *et al*. 1980a, 1980b, Biju *et al*. 2001), but in the case of Indian bean, the information on such relationship studies is limited. Previously, in case of *L. purpureus*, RAPD marker was used to study genetic relationship among 40 genotypes and reported a high level of genetic variation (Liu *et al*. 2001).

Table 1 Mean of the 10 quantitative characters of 143 *L. purpureus* lines

Genotype	Days to first flowering	Days to first picking	Number of pods/ plant	Pod length (cm)	Pod width (cm)	Number of seeds/ pod	Seed length (cm)	Seed width (cm)	100 seed weight (gm)	Yield/ plant (kg)
VRSEM-860	95.00	121.33	231.67	13.70	2.68	4.67	1.23	0.76	33.33	2.40
VRSEM-186	94.67	145.00	234.67	8.60	3.81	4.67	1.85	0.73	35.33	2.37
VRSEM-894	108.00	141.67	164.00	13.50	1.45	4.33	1.36	0.90	41.33	1.93
VRSEM-5	117.00	139.33	126.67	8.84	1.23	4.67	1.24	0.65	25.67	0.57
VRSEM-11	91.00	135.67	155.67	10.13	1.54	5.00	1.06	0.72	25.67	1.29
VRSEM-954	106.00	136.00	165.33	10.20	1.13	4.00	1.25	0.78	26.67	0.68
VRSEM-757	119.00	136.00	175.00	11.40	0.55	4.00	1.20	0.84	25.00	1.74
VRSEM-744	121.00	151.33	200.67	8.57	2.33	4.67	1.26	0.87	32.00	1.41
VRSEM-8	105.00	132.33	507.33	10.73	1.80	4.00	1.25	0.83	34.00	3.29
VRSEM-897	135.00	165.00	119.33	9.50	3.20	5.00	1.15	0.85	33.33	0.92
VRSEM-887	107.00	137.00	453.33	8.43	2.74	4.00	1.19	0.82	33.67	2.73
VRSEM-810	122.00	152.33	58.33	11.20	2.04	5.67	1.13	0.74	25.00	0.47
VRSEM-37	106.00	132.67	391.33	9.50	2.26	4.33	1.05	0.74	32.00	1.93
Ankur Gouldi	121.00	136.00	174.67	10.95	2.53	6.00	1.15	0.76	32.00	1.08
VRSEM-709	118.00	129.00	54.33	13.11	3.90	6.00	1.23	0.87	37.00	0.76
VRSEM-708	101.00	142.00	131.00	11.20	2.32	5.00	1.23	0.82	35.00	1.43
VRSEM-801	103.00	130.00	208.00	10.66	1.59	5.00	1.08	0.81	35.00	1.86
VRSEM-786	110.00	139.00	344.33	7.18	1.33	3.67	0.84	0.92	37.33	1.69
VRSEM-904	90.00	109.33	277.67	7.47	1.34	4.00	0.91	0.75	32.67	1.43
VRSEM-799	105.33	133.33	187.33	12.57	1.30	4.33	1.12	0.80	32.33	1.30
Gomachi Green	99.00	121.00	343.00	8.50	1.25	3.67	1.16	0.83	35.33	1.75
VRSEM-902	102.00	132.00	183.00	6.71	1.25	4.00	0.83	0.74	36.00	0.73
VRSEM-808	110.00	141.67	487.33	10.43	0.95	3.67	1.36	0.70	35.33	2.25
VRSEM-869	109.67	144.00	679.33	9.37	1.33	4.00	1.14	0.81	31.00	1.67
VRSEM-783	110.00	134.00	291.00	11.25	3.59	5.00	1.19	0.84	37.00	3.50
VRSEM-898	107.33	143.67	185.33	8.63	0.81	3.67	1.05	0.68	29.00	0.69
VRSEM-921	120.33	141.33	114.67	9.80	2.67	5.33	1.07	0.74	34.33	1.15
VRSEM-778	105.33	125.33	628.33	8.75	2.23	4.00	1.10	0.82	39.33	3.08
VRSEM-861	108.00	125.00	503.33	9.43	2.33	4.00	1.15	0.84	42.00	2.50
VRSEM-766	121	162	75	9.46	2.35	5	1.1	0.7	30	0.78
VRSEM-751	113	140	208	7.3	2.11	5	1	0.79	32	1.25
VRSEM-940	108	132	163	8.78	2.32	5	1.1	0.82	30	0.9
VRSEM-742	97	135	374	11.5	2.8	5	1.22	0.83	32	0.68
SDL-791	101.00	142.00	131.00	11.20	2.32	5.00	1.23	0.82	35.00	1.43
VRSEM-944	118	143	119	11.8	3.8	4	1.14	0.8	31	1.66
VRSEM-50	111	134	210	7	1.13	4	0.88	0.72	40	1.18
RP-08-26	101.00	142.00	131.00	11.20	2.32	5.00	1.23	0.82	35.00	1.43
VRSEM-2	113	140	500	10.12	1.92	4	1.24	0.9	28	2.25
VRSEM-1	101.00	142.00	131.00	11.20	2.32	5.00	1.23	0.82	35.00	1.43
VRSEM-913	121	141	814	6.6	2.56	4	1.1	0.82	22	3.67
VRSEM-201	104	124	500	9.5	2.3	4	1.14	0.84	40	2
VRSEM-501	110	134	291	11.25	3.59	5	1.19	0.84	37	3.5
VRSEM-1000	122	140	108	14.13	1.64	5	1.2	0.88	41	0.65
VRSEM-6	104	130	295	12.81	2.1	5	1.26	0.89	40	2.36
VRSEM-893	109	116	155	5.93	2.01	4	1	0.8	25	0.93
VRSEM-855	106	139	294	4.8	1.63	4	1	0.78	20	1.03
VRSEM-883	106	141	226	5.65	1.94	4	1.2	0.79	21	1.33
VRSEM-13	107	144	184	12.1	1.23	5	1.21	0.8	26	0.92
VRSEM-97	115	136	336	11.9	1.1	5	1.2	0.85	32	2.1
VRSEM-847	107	136	127	5.75	1.76	4	1.09	0.75	24	0.77
VRSEM-704	105	132	93	11.3	2.5	4	1.17	0.87	37	0.8

(Continued)

Table 1 *(Continued)*

Genotype	Days to first flowering	Days to first picking	Number of pods/ plant	Pod length (cm)	Pod width (cm)	Number of seeds/ pod	Seed length (cm)	Seed width (cm)	100 seed weight (gm)	Yield/ plant (kg)
VRSEM-763	106	139	233	10.7	0.96	5	1.18	0.82	24	1.17
VRSEM-111	106	135	158	13.5	2.3	5	0.99	0.84	37	1.8
VRSEM-938	116	140	141	8.91	2.23	5	1	0.75	31	1.03
VRSEM-109	97	119	342	8.8	1.28	4	1.13	0.81	36	1.71
VRSEM-730	106	153	238	9.2	3.5	5	1.19	0.8	30	2.38
VRSEM-101	105	125	630	8.78	2.1	4	1.1	0.8	42	3.15
VRSEM-805	128	139	293	10	2.3	4	1.12	0.91	16	1.16
VRSEM-784	121	148	68	8.78	2.29	5	1.11	0.75	29	0.48
VRSEM-798	106	128	357	12.25	2.36	5	1.14	0.79	19	2.86
VRSEM- 736	127	129	150	7.8	2.8	3	1.1	0.81	32	1.72
VRSEM- 749	132	138	350	9.4	2.3	4	1.05	0.82	30	2.1
VRSEM- 3	67	92	145	11.81	3	5	1.27	0.93	34	1.72
VRSEM- 764	107	138	278	11.1	2.83	5	1.22	0.92	36	3.01
VRSEM- 14	109	132	474	11.2	2.62	4	1.38	0.97	38	1.7
VRSEM- 815	97	146	672	6.76	1.6	4	1.2	0.7	18	3.7
VRSEM- 741	105	134	127	9.2	1.84	4	1.12	0.82	30	0.7
VRSEM- 767	130	159	438	8.82	2.19	5	0.94	0.72	17	2.63
VRSEM- 890	118	138	400	8.47	2.49	5	1.1	0.81	15	3.00
VRSEM- 888	117	139	127	5.9	2.12	4	0.98	0.63	13	0.77
VRSEM- 847	97	136	127	5.75	1.76	4	1.09	0.75	24	0.89
VRSEM- 850	97	138	168	6.89	2	4	1.04	0.68	17	0.71
VRSEM- 937	116	138	128	8.83	1.23	5	1.3	0.69	26	0.58
VRSEM- 789	124	149	85	10.19	2.98	4	1.14	0.79	19	0.65
VRSEM- 831	97	137	140	7.67	2.52	4	1.4	0.8	31	0.70
VRSEM- 836	90	130	211	5.68	2.23	4	1.59	0.72	16	1.27
VRSEM- 922	130	148	283	7.99	2.55	4	1.12	0.87	27	1.13
VRSEM- 941	83	138	250	8.16	1.56	5	1.19	0.79	27	1
VRSEM- 833	108	142	133	6.72	2.1	4	1.02	0.75	19	0.8
VRSEM- 880	121	138	266	5.68	1.94	5	1.08	0.76	18	0.93
VRSEM- 938	116	140	141	8.91	2.23	5	1	0.75	31	1.03
VRSEM- 909	106	134	260	8.25	1.27	5	1.1	0.81	19	1.3
VRSEM- 830	107	136	133	7.08	2.49	4	1	0.72	23	0.93
VRSEM- 797	106	136	455	8.6	2.75	4	1.21	0.84	33	2.73
VRSEM- 940	108	132	163	8.78	2.32	5	1.1	0.82	30	0.9
VRSEM- 865	97	128	412	6.8	2.18	4	1.1	0.72	16	2.06
VRSEM- 739	120	152	100	10.9	1.1	4	0.97	0.77	32	0.6
VRSEM- 730	106	153	238	9.2	3.5	5	1.19	0.8	30	2.38
VRSEM- 932	107	138	145	9.2	3	5	1.24	0.77	30	1.16
VRSEM- 944	118	143	119	11.8	3.8	4	1.14	0.8	31	1.66
VRSEM- 751	113	140	208	7.3	2.11	5	1	0.79	32	1.25
SDl- 791	121	151	58	11.22	2.08	6	1.1	0.75	24	0.44
VRSEM- 859	111	145	500	5.24	1.39	3	0.89	0.7	17	1
VRSEM- 924	98	138	366	12.6	1.65	5	1.17	0.72	32	2.2
VRSEM- 731	128	149	214	8.13	2.9	4	1.23	0.92	25	1.82
VRSEM- 742	97	135	374	11.5	2.8	5	1.22	0.83	32	0.68
VRSEM- 50	111	134	210	7	1.13	4	0.88	0.72	40	1.18
VRSEM- 913	121	141	814	6.6	2.56	4	1.1	0.82	22	3.67
VRSEM- 739	120	152	100	10.9	1.1	4	0.97	0.77	32	0.6
VRSEM- 816	106	138	130	9.6	1.68	5	1.19	0.78	27	0.65
VRSEM- 884	127	148	106	6.12	1.7	4	0.87	0.72	17	0.53
VRSEM- 917	108	135	66	8.13	1.14	5	1.2	0.66	18	0.46

(Continued)

Table 1 *(Concluded)*

Genotype	Days to first flowering	Days to first picking	Number of pods/ plant	Pod length (cm)	Pod width (cm)	Number of seeds/ pod	Seed length (cm)	Seed width (cm)	100 seed weight (gm)	Yield/ plant (kg)
VRSEM- 714	90	133	111	7.8	2.8	3	1.02	0.82	35	0.5
VRSEM- 852	90	136	121	6.95	2.55	4	1.14	0.78	23	0.67
VRSEM- 887	120	160	72	9.63	2.16	4	1.23	0.88	30	0.43
VRSEM- 746	96	110	91	13.9	2.7	5	1.19	0.75	30	1.18
VRSEM- 867	137	170	174	5.59	1.31	4	0.86	0.53	14	0.57
VRSEM- 111	106	135	158	13.5	2.3	5	0.99	0.84	37	1.8
VRSEM- 702	130	149	258	11.6	2	4	1.19	0.86	29	1.55
VRSEM- 942	106	138	377	9.72	1.35	5	1.07	0.68	20	2.27
VRSEM- 812	108	138	248	7.38	7.33	4	1.16	0.7	25	1.27
VRSEM- 787	115	136	166	10.98	2.48	5	0.84	0.66	13	1.86
VRSEM- 768	76	117	196	9.59	2.63	5	1.2	0.75	29	1.95
VRSEM- 895	97	145	88	7.87	1.98	5	1.13	0.79	35	0.53
VRSEM- 839	116	141	407	5.54	2.33	3	0.94	0.65	14	1.63
VRSEM- 916	87	116	388	8.66	2.03	5	1.04	0.7	18	2.33
VRSEM- 734	82	128	385	10.6	2.7	5	1.28	0.82	30	3.17
VRSEM- 953	91	136	157	10.1	1.56	5	1.05	0.72	24	1.26
VRSEM- 710	113	137	200	8.8	0.82	5	1.15	0.78	30	0.9
VRSEM- 863	105	136	125	5.87	2.01	3	0.99	0.76	17	0.5
VRSEM- 51	79	95	272	7.8	1.5	4	1.04	0.87	32	1.36
VRSEM- 886	106	130	380	6.18	2.33	4	1.01	0.68	20	1.9
VRSEM- 824	108	142	116	6.04	1.52	4	0.93	0.75	21	0.47
VRSEM- 843	106	138	400	8.47	2.49	5	1.18	0.81	35	3
VRSEM- 861	116	140	377	7.23	2.37	4	0.9	0.66	20	2.27
VRSEM- 927	117	138	295	11.18	1.56	6	1.08	0.8	29	2.21
VRSEM- 830	107	136	133	7.08	2.49	4	1	0.72	23	0.93
VRSEM- 769	118	160	103	9.5	2.3	4	1.04	0.73	29	0.83
VRSEM- 821	108	133	121	11.02	2.21	4	1.04	0.72	24	0.67
VRSEM- 899	107	146	74	6.02	1.63	4	0.92	0.71	15	0.37
VRSEM- 765	133	151	82	9.7	3.2	4	1.2	0.91	33	0.83
VRSEM- 801	134	151	155	11.24	2.26	5	1	0.78	22	0.93
VRSEM- 117	90	109	283	7.8	1.38	4	0.93	0.79	32	1.42
VRSEM- 800	106	136	305	10.1	3.82	4	1.04	0.73	27	3.36
VRSEM- 891	105	143	240	9.5	1.44	4	0.89	0.66	15	1.2
VRSEM- 881	107	136	78	10.66	1.46	5	1.16	0.86	30	0.77
VRSEM- 722	106	140	128	6.36	1.72	3	0.93	0.75	28	0.64
VRSEM- 708	121	150	34	11.12	2.8	5	1.28	0.95	30	0.24
VRSEM- 869	105	138	142	7.77	2.6	5	1.12	0.75	25	1
VRSEM- 857	114	145	244	5.97	2.88	4	1.1	0.88	20	1.47
VRSEM- 854	118	142	186	4.9	1.98	4	1	0.68	31	0.47
VRSEM- 735	97	144	450	10.3	2.5	4	1.21	0.84	32	2.7
VRSEM- 900	105	138	156	10.59	3.51	5	1.22	0.85	37	1.57

Analysis of cross-species transferability of SSR markers to Indian bean

Due to the availability of large EST datasets and advanced bioinformatics tools, it has become possible to systematically search for SSRs from the EST sequences. Since these SSRs are derived from ESTs, corresponding to the transcribed component of a gene unit, they have been shown to possess a high potential for inter-speciûc transferability (Thiel *et al*. 2003, Corderio *et al*. 2001). Genomic SSRs markers were extensively used for molecular breeding, genetic diversity analysis and for studying genetic relationships. However, its unavailability in the wild species of *L. purpureus* and allied genera has severely affected the use of these species in marker-assisted intro-gression programmes. The cross-species amplification of SSR marker was recorded as positive, only when sharp band (s) in the size range 80-700 bp were reproduced in at least two replicates of PCR reactions. In this way, a total of 134 SSRs markers reported from studies with cowpea, mungbean, mothbean, common bean and faba bean were used to search

orthologous match in *L. purpureus*. Among the 134 SSRs markers, 50 SSRs (41.0%) showed cross-transferability to all of the 143 accessions used in this study (Table 2). Of 50 SSR primers, 21 (42%) primers were monomorphic, while 29 (58%) of the cross-transferable primers produced polymorphic products and distinguished the studied accessions. Out of the total 134 primers from different genera, 84 (58.9%) failed to amplify any DNA sample from the present study. The monomorphic nature of the SSR markers in related genera revealed low genetic diversity and high level of conservation of these loci and their flanking regions. The possibility of a higher degree of conservation across the genera and potentially among species of the same genera was reported previously (Kresovich *et al*. 1995, Dayanandan *et al*. 1997). Lower level of polymorphism may also be possible due to 'ascertainment bias' whereby a microsatellite chosen to be longer in the source species is likely to be shorter in the new target species (Ellegren *et al*. 1995). It has been suggested that such ascertainment operates in part via a restriction in microsatellite length, such that occasional deletions or internal point mutation that leads to shorter and less polymorphic loci in a different species (Vowles and Amos 2006).

Among the 50 cross-transferable SSRs markers which were obtained in the study, (16) 40% of cowpea, (5) 71.4% of mung bean, (10) 50% of moth bean, and 19 (35%) of faba bean amplified in Indian bean. Notably, all of the 13 SSRs from common bean failed to amplify any product (0% transferability) in the Indian bean indicating probably no potential of marker cross-transferability from common bean to Indian bean. This study indicates medium- to high-level cross-transferability of genomic SSRs markers among *Lablab* and allied genera. The maximum (71.4%) cross-transferability was exhibited by mung bean, followed by markers from moth bean (50%) and cowpea (40%). The SSRs from faba bean exhibited the least (35%) cross-transferability.

Polymorphic information content of the 29 SSR primer pairs, which showed polymorphic products across the accessions ranged between 0.09 and 0.94 (Table 3). Such

Table 3 Description of 29 SSR primers used in present study; their sequence, polymorphic products and polymorphic information content (PIC) value of each primer pair

Primer	Sequence (5'……3')	Annealing temperature (^{0}C)	Band size (bp)	Number of products amplified	Number of polymorphic products	PIC value
VM1	F-cacccgtgattgcttgttgR-gtcccctccctcccactg	59.75	500	2	2	0.80
VM3	F-gagccgggttcaataggtaR-gagccagggcagaggtagt	58.85	300	1	1	0.40
VM4	F-agtaaatcacccgcacgatcgR-aggggaaatggagaggat	56.75	100	2	2	0.19
VM8	F-tgggatgctgcaaagacacR-gaaaaccgatgccaaatag	54.5	200	4	3	0.09
VM18	F-agccgtgcacgaatgatR-tggcctctacaacaacactct	55.3	250	2	2	0.67
VM19	F-tattcatgcgtgacactaR-tcgtggcacccctatc	53.3	400	2	2	0.69
VM21	F-tagcaactgtctaagcctcaR-ccaacttaaccatcactcac	55.3	150	2	2	1.06
VM24	F-tcaacaacacctaggagccaaR-atcgtgacctagtgcccacc	59.6	300	2	2	1.00
VM31	F-cgctcttcgttgatggttatgR-gaaaaagggaggaacaagcacaac	59.4	250	2	2	0.74
VM34	F-agctcccctaacctgaatR-taacccaataataagacacat	51.9	700	2	1	0.74
VM37	F-tgtccgcgttctataaatcagcR-cgaggatgaagtaacagatgatc	58.6	300	2	1	0.20
VM38	F-aatgggaaaagaaagggaagcR-tcgtggcatgcagtgtcag	57.3	200	5	4	0.88
CEDG008	F-aggcgaggtttcgtttcaagR-gcccatatttttacgcccac	57.3	200	3	3	0.60
CEDG198	F-caaggaagatggagagaatcR-ccttctaagaacagtgacatg	55.6	200	2	2	0.07
DMB-SSR182	F-tagagccttctggtttttcacaR-aggaggaggattttgatgatga	56.5	250	3	3	0.96
DMB-SSR186	F-gagagagaaggagagggagaR-attctttctccaccacaatg	56.3	100	2	2	0.39
AGB1	F-catgcagaggaagcagagtgR-gagcgtcgtcgtttcgat	57.7	200	2	2	0.43
AGB8	F-caccgggagtggctgaca R-gtttggggcggagttcga	58.35	300	4	4	0.68
AGB9	F-atccgtagagaggtgaacggR-atgagtgcagtttggtgcag	57.3	500	3	3	1.04
82	F-aggggtccaaggggttaaatR-acagggaagcatcaacaatg	56.3	400	2	2	1.06
88	F-ggttttgaatagaaatgcaaR-aagatgtgtcaatattgtttt	48.5	300	4	4	1.08
92	F-caagcttgttgagagccaaaR-gaacgaggctcacgaaaata	5.35	500	4	4	0.94
110	F-agcccatggttcaaatgcaaR-gcagtcatgccactgctta	54.3	150	2	2	0.53
111	F-gaatggaccggttctggattR-ccctaatcccttcactaataca	56.5	250	4	4	0.80
112	F-gatgttgttggtgttgtttaR-caattaggagcaaaatcaga	51.2	200-800	4	4	0.43
122	F-gagaggcggttatgttgttaR-gatctctctctctctct	54.5	700	3	3	0.39
126	F-agttgaggttttgaacccaaR-aggaggcctggttgtttta	53.5	500	2	2	0.56
127	F-gatgtttatgggaaaatctgatR-tccttgacaaaaaaacaaaattg	51.5	250-700	2	2	0.29
129	F-ttgaacccagccgccgctgaR-aggggacgctcattttgttg	60.5	300	3	3	0.69

Table 2 Details about cross-species transferability of 134 SSR loci from different legume crop species

Crop	Number of primers tested	Primers that yielded monomorphic product	Primers that yielded polymorphic product*	Primer that did not amplify any product
Vigna unguiculata	40	4	12 (30)	24
V. mungo	7	1	4 (57)	2
V. aconitifolia	20	7	3 (15)	10
Phaseolus vulgaris	13	0	0 (0)	13
Vicia fabae	54	9	10 (18.5)	35

*Figures in parenthesis denote the per cent of cross species primers producing polymorphic products.

high PIC values were also reported by Fatokun and co-workers after studying 48 wild accessions of cowpea (0.29 to 0.87) (Fatokun *et al.* 2008), and Li and associates after studying cultivated cowpea genotypes (0.02 to 0.73) (Li *et al.* 2001). In the present study, a total of 98 alleles were detected with an average of 3 alleles per locus (Table 3); marker DAT detected the highest (6) number of alleles while, VM38 had two alleles each. Fatokun and co-workers in their study detected alleles ranging from 4 to 13 with an average of 7.5 alleles per primer (Fatokun *et al*. 2008), whereas Li and associates reported 2 to 7 alleles in cowpea breeding lines (Li *et al*. 2001). Similarly, 5 to 11 loci were reported in the case of cowpea lines from Benin reported (Zannou *et al*. 2008).

Genetic diversity analysis based on SSR markers

The chance of successful cross-species SSR amplification is inversely related to the evolutionary distance between the two accessions of a given species; thus as a consequence, the chance of successful cross-species amplification outside a genus is much lower than that reported within a genus. Previously, a 3-13% cross-species amplification among genera of *Fabaceae* family was reported, which is much lower compared to 65% within the genus *Glycine* (Peakall *et al*. 1998). Genetic similarities measured through analysis of data on the SSR markers from the 143 accessions of *D. lablab* revealed varying degrees of genetic relatedness. Jaccard's similarity coefficient ranged from 0.50 to 0.85. Although, cross-transferability value (16.2%) obtained in this study is marginally better than those previously reported for other plant families (Peakall *et al*. 1998, Kuleng *et al*. 2004), it would still not be enough to practically supply a critical mass of DNA markers for research in Indian bean. The success of cross-species amplification rate could be improved by using SSRs based on expressed sequence tags (EST-SSRs) rather than using genomic SSR (g-SSRs), since EST-SSRs come from transcribed regions of the genome, and are likely to be conserved across a broader taxonomic range. In fact, it has been reported that EST-SSRs were 3 times more transferable across species of the genus *Helianthus* than g-SSRs (Pashley *et al*. 2006).

This study highlights a reliable and efficient way of obtaining genomic SSRs markers for wild relatives of *L. purpureus*. Overall, this study indicates medium to high level of cross-transferability of g-SSRs markers among *Lablab* and allied genera. These approaches, together with the development of specific markers, e.g. SSRs, In/dels, EST-SSRs for Indian bean will contribute to increase the Indian bean genomic resources which will expedite Indian bean molecular breeding in years to come. In sum, the availability of a large number of ESTs for vegetable legumes provides ample opportunity for the systematic development of molecular markers, in particular SSRs. Because these SSRs are a part of genes, they can be potentially transferred to related species. The highest similarity coefficient (0.80) was observed in genotypes VRSEM-50 and VRSEM-111, revealing them to be the most diverse accessions. Overall, the accessions VRSEM-887, VRSEM-869 and VRSEM-783 were placed on one side of the dendrogram while the accessions, VRSEM-67, VRSEM-14 and VRSEM-815 were places on opposite side, indicating these accessions to hold maximum potential for their utilization as diverse parents. The dendrogram also revealed that 2 accessions, viz. VRSEM-730 and VRSEM-805 are very similar with similarity coefficient of 0.08, which was also the least similarity coefficient. In this study, the larger range of similarity values for cultivars revealed by microsatellite markers provides greater confidence for the assessments of genetic diversity and relationships, which can be used in future breeding programs.

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REFERENCES

Basu A K, Samantha S K and Sasmala S K. 2002. Genetic analysis for some seed parameters in *Lablab* bean. *Vegetable Science* **29**: 17–9.

Baswana K S, Pandita M L, Dhankar B S and Pratap P S. 1980a. Genetic variability and heritability studies on Indian bean (*Dolichos lablab* var. lignosus L.). *Haryana Journal of Horticulture Science* **9**: 52–5.

Baswana K S, Pandita M L, Pratap P S and Dhanker B S. 1980b. Genetic divergence for yield and its components in Indian bean. *Haryana Journal of Horticulture Science* **9**: 184–7.

Biju M G, Prasanna K P and Rajan S. 2001. Genetic divergence in Indian bean. *Vegetable Science* **28**: 163–4.

Biswas M S, Zakaria M and Rahman M M 2012. Assessments of genetic diversity in country bean (*Lablab purpureus* L.) using RAPD marker against photo-insensitivity. *Journal of Plant Development* **19**: 65–71.

Bradley M J. 1999. Legume genetic resources with novel value added industrial and pharmaceutical use. (In) *Perspective on New Crops and New Uses*, pp 196–201 Janick J (Ed). ASHS Press, Alexandria.

Cordeiro G M, Casu R, McIntyre C L, Manners J M and Henry R J. 2001. Microsatellite markers from sugarcane (*Saccharum* spp.) ESTs cross transferable to *Erianthus* and sorghum. *Plant Science* **160**: 1 115–23.

Cregan P B, Mudge J, Fickus E W, Marek L F and Danesh D. 1999. Targeted isolation of simple sequence repeat markers through the use of bacterial artificial chromosomes. *Theoretical Applied Genetics* **98**: 9–928.

Dayanandan S, Bawa K S and Kesseli R. 1997. Conservation of microsatellites among tropical trees (Leguminoseae). *American Journal of Botany* **84**: 1 658–63.

Deka R K and Sarkar C R. 1983. Nutrient composition and anti-nutritional factors of *Dolichos lablab* L. seeds. *Food Chemistry* **38:** 239–46.

Doyle J J and Doyle J L. 1987. A rapid DNA isolation procedure from small quantity of fresh leaf material. *Phytochemical Bulletin* **119**: 11–5.

Ellegren H, Primmer C R and Sheldon B C. 1995. Microsatellite evolution: Directionality or bias. *Nature Genetics* **11**: 360–2.

Fatokun C A, Ogunkanmi A, Ogundipe O T and Ng N Q. 2008. Genetic diversity in wild relatives of cowpea (*Vigna unguiculata*) as revealed by simple sequence repeats (SSR) markers. *Journal of Food Agricultural Environment* **6**: 263–8.

Gomez K A and Gomez A A. 1984. *Statistical Procedures for Agricultural Research*. John Wiley and Sons, New York, USA.

Gupta P K and Varshney R K. 2000. The development and use of microsatellite markers for genetics and plant breeding with emphasis on bread wheat. *Euphytica* **113**: 163–85.

Jaccard P. 1908. Etude comparative de la distribution florale dans une portion des Alpes et des Jura. *Bulletin del la Societe Vaudoise des Sciences Naturelles* **37**: 547–79.

Jarne P and Lagoda P J L. 1996. Microsatellites, from molecules to populations and back. *Trends in Ecology and Evolution* **11**: 424–9.

Kresovich S, Szewc-McFadden A K, Bliek S M and McFerson J R. 1995. Abundance and characterization of simple sequence repeats (SSRs) isolated from a size-fractionated library of *Brassica napus* L. (rapeseed). *Theoretical Applied Genetics* **91**: 206–11.

Kumar V, Sharma S, Kero S, Sharma S, Sharma K, Kumar M and Bhat KV. 2008. Assessment of genetic diversity in common bean (*Phaseolus vulgaris* L.) germplasm using amplified fragment length polymorphism (AFLP). *Scientia Horticulture* **116**: 138–43.

Li C D, Fatokun C A, Ubi B, Singh B B and Scoles G J. 2001. Determining genetic similarities and relationships among cowpea breeding lines and cultivars by microsatellite markers. *Crop Science* **41**: 189–97.

Liu C J. 1996. 1996. Genetic diversity and relationship among *Lablab purpureus* genotypes evaluated using RAPD as marker. *Euphytica* **90**: 115–9.

Maass B L. 2006. Changes in seed morphology, dormancy and germination from wild to cultivated Indian bean germplasm (*Lablab purpureus*: Papilionoideae). *Genetics Resources Crop Evolution* **53**: 1 127–35.

Miflin B. 2000. Crop improvement in the 21st century. *Journal of Experimental Botany* **51**: 1–8.

Pashley C H, Ellis J R, McCauley D E and Burke J M. 2006. EST databases as a source for molecular markers: Lessons from *Helianthus*. *Journal of Heredity* **97**: 381–8.

Peakall R, Gilmore S, Keys W, Morgante M and Rafalski A. 1998. Cross-species amplification of soybean (*Glycine max*) simple sequence repeats (SSRs) within the genus and other legume genera: implications for the transferability of SSRs in plants. *Molecular Biology Evolution* **15**: 1 275–87.

Rai N, Kumar A, Singh P K, Singh M, Datta D and Rai M. 2010. Genetic relationship among Indian bean (*Lablab purpureus*) genotypes cultivars from different races based on quantitative traits and random amplified polymorphic DNA marker. *African Journal of Biotechnology* **9**: 137–44.

Rai N, Rai K K, Venkataravanappa V and Saha S. 2015. Molecular approach coupled with biochemical attributes to elucidate the presence of DYMV in leaf samples of *Lablab perpureus*. L genotypes. *Applied Biochemistry Biotechnology* DOI 10.1007/ s12010-015-1915.

Rai N, Singh P K, Rai A C, Rai V P and Singh M 2011. Genetic diversity in Indian bean (*Lablab purpureus*) germplasm based on morphological traits and RAPD markers. *Indian Journal of Agricultural Sciences* **81**: 801–6.

Rohlf F J. 1998. NTSys-pc numerical taxonomy and multivariate analysis system. vVer. 2.02 Exeter Publications, Setauket: New York.

Thiel T, Michalek W, Varshney R K and Graner A. 2003. Exploiting EST databases for the development of cDNA-derived microsatellite markers in barley (*Hordeum vulgare* L.). *Theoretical Applied Genetics* **106**: 411–22.

Varshney R K, Thiel T, Stein N, Langridge P and Graner A. 2002. *In silico* analysis on frequency and distribution of microsatellites in ESTs of some cereal species. *Cell Molecular Biology Letter* **7**: 537–46.

Vowles E J and Amos W. 2006. Quantifying ascertainment bias and species-specific length differences in human and chimpanzee microsatellites using genome sequences. *Molecular Biology and Evolution* **23**: 598–607.

Wood I M. 1983. Lablab bean (*Lablab purpureus*) for grain and forage production in the Ord River irrigation area. *Australian Journal of Experimental Agriculture and Animal Husbandry* **23**: 162–71.

Zannou A, Kossou D K, Ahancheedee A, Zoundjihekpon J and Agbicodo E. 2008. Genetic variability of cultivated cowpea in Benin assessed by random amplified polymorphic DNA. *African Journal of Biotechnology* **7**: 4 407–1