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ORIGINAL ARTICLE

Genetic diversity and population genetic structure analysis of an extensive collection of wild and cultivated *Vigna* accessions

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Abstract

Vigna is a large, pan-tropic and highly variable group of the legumes family which is known for its > 10 cultivated species having significant commercial value for their nutritious grains and multifarious uses. The wild *vignas* are considered a reservoir of numerous useful traits which can be deployed for introgression of resistance to biotic and abiotic stresses, seed quality and enhanced survival capability in extreme environments. Nonetheless, for their effective utilization through introgression breeding information on their genetic diversity, population structure and crossability is imperative. Keeping this in view, the present experiment was undertaken with 119 accessions including 99 wild *Vigna* accessions belonging to 19 species and 18 cultivated genotypes of *Vigna* and 2 of *Phaseolus*. Total 102 polymorphic SSRs were deployed to characterize the material at molecular level which produced 1758 alleles. The genotypes were grouped into four major clusters which were further sub-divided in nine sub-clusters. Interestingly, all cultivated species shared a single cluster while no such similarities were observed for the wild accessions as these were distributed in different groups of sub-clusters. The co-dominant allelic data of 114 accessions were then utilized for obtaining status of the accessions and their hybrid forms. The model-based population structure analysis categorized 114 accessions of *Vigna* into 6 genetically distinct sub-populations ($K = 6$) following admixture-model based simulation with varying levels of admixture. 91 (79.82%) accessions resembled their hierarchy and 23 (20.18%) accessions were observed as the admixture forms. Maximum number of accessions (25) were grouped in sub-population (SP) 6 and the least accessions were grouped in SP3 and SP5 (11 each). The population genetic structure, therefore, supported genetic diversity analysis and provided an insight into the genetic lineage of these species which will help in effective use of germplasm for development of cultivars following selective prebreeding activities.

Keywords Asiatic *Vigna* · Wild accessions · Genetic diversity · Population genetic structure

Introduction

Vigna is an important genus of flowering plants in the legumes family which has a pan-tropic distribution. This genus comprises > 200 species (Pratap et al. 2014a) encompassed in five sub-genera (*Ceratropis*, *Haydonia*, *Lasiospron*, *Plectrotropis* and *Vigna*) (Takahashi et al. 2016) including ten domesticated species having significant agronomic potential. Among these, seven species belonging to the sub-genus *Ceratotropis* are also known as the Asiatic *Vigna* (Takahashi et al. 2016; Pratap et al. 2015) and include the versatile crops viz., mung bean (*V. radiata*), black gram (*V. mungo* (L.) Hepper), moth bean (*V. aconiotifolia* (Jacq.) Marechal), minni payaru (*V. stipulacea* Kuntze), creole bean (*V. reflexo-pilosa* Hayata), adzuki bean (*V. angularis* (Willd.) Ohwi & Ohashi) and rice bean (*V. umbellata* (Thunb)). These crops are mainly cultivated in South and South-east Asia and Africa (Pratap et al. 2014a, 2021a) and contribute significantly in the food and nutritional security and environmental sustainability. Rice bean (*V. umbellata* (Thumb.) Ohwi & Ohashi), tua pea (*V. glabrescens*) and creole bean (*V. reflexo-pilosa*) have been reported to be domesticated and consumed mostly in South Africa (Pratap et al. 2014a,

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2015; Chankaew et al. 2014) and *V. angularis* (Willd) Ohwi & Ohashi (small red bean or adzuki bean) in East Asia, most probably in Japan (Tomooka 2009). An abundance of their wild and weedy types have been reported to flourish in the Savannah and the forested zones (Harlan 1971; Rawal 1975). At the other hand, *V. trilobata* and *V. stipulacea* have been reported as candidates for neo-domestication for drought tolerance and disease and pest resistance, respectively (Gore et al. 2019; Pratap et al. 2015).

Most of the *Vigna* crops are grown for their nutritious seeds which have a high amount of proteins and micro-nutrients. These crop species, although considered as minor, are important sources of dietary protein and also several micro-elements viz., iron, potassium, zinc, Vitamin A, Vitamin B, folate and thymine in the developing and underdeveloped countries, especially in the predominantly vegetarian diets (Vaz Patto et al. 2015). Many of these species are also valued as forage, cover, and green manure crops in many parts of the world. Nonetheless, while the domesticated and edible *Vigna* are only a few, the non-domesticated or semi-domesticated species are many. A few of these are also found to inhabit rough environments such as non-fertile rocky and mountainous tracts, sandy and salty beaches and uninhabited marshy and swampy lands and, therefore, these are expected to habour many survival-related traits (Pratap et al. 2014b). These species are, therefore, considered as a reservoir of valuable genetic resources for biotic and abiotic stress tolerance and seed quality traits (Douglas et al. 2020; Pratap et al. 2020, 2021b; Nair et al. 2019). Furthermore, many wild species are highly tolerant to extreme environmental conditions including drought and water logging (Bisht et al. 2005; Tomooka et al. 2014), high-salinity (Yoshida et al. 2016), heat and cold stress (HanumanthaRao et al. 2016), and acidic or alkaline soils (Soares et al. 2014) while others have resistance to bruchids (Kaewwongwal et al. 2017), cercospora leaf spot (Singh et al. 2017; Chankaew et al. 2013), and yellow mosaic disease (for a review, please see Singh et al. 2020). Many wild species also serve as a potential source for superior agronomic traits (Kajonphol et al. 2012; Aidbhavi et al. 2021), and photo-thermo insensitivity (Pratap et al. 2014b; Basu et al. 2019). Cross-compatibility studies and identification of tolerant and causative genes facilitating conventional genetics and breeding towards the new breeding concepts such as 'neo-domestication' and 'reverse-breeding' are imperative to harness the desirable traits of wild species for crop improvement (Palmgren et al. 2015). Further, to use wild species in crop improvement programmes through pre-breeding, precise information on their genetic architecture, population structure and relationship with other *Vigna* species are important. Morphological evaluation is highly environment-dependent, especially in *Vigna* crops and, therefore, may give variable results across different environments. Hence classifying the *Vigna* species using highly abundant molecular markers such as multi-allelic SSRs is indeed necessary and has been used in classifying wild species of many crops earlier (Wang et al. 2008; Gwag et al. 2010; Pratap et al. 2015; Sarr et al. 2020). Due to high polymorphism, multiple allelism, reproducibility, co-dominant nature and user-friendliness, the SSR markers are preferred genetic markers to recognize diversity in microsatellite variation (Weber and May 1989). Further, microsatellites or simple sequence repeats (SSRs) are widely distributed across plant genomes and have high sensitivity to detect polymorphisms (Parker et al. 1998). As a result, these have been abundantly deployed in discerning genetic diversity, phylogeny studies and population genetic structure analysis (Sarr et al. 2020; Kempf et al. 2016; Gwag et al. 2010; Pratap et al. 2015) in *Vigna* crops. Besides, SSR markers have been tremendously useful in successful marker-assisted breeding in food legumes (Varshney et al. 2014; Pratap et al. 2017). Several workers successfully utilized the SSR markers in developing mungbean maps (Chankaew et al. 2014; Isemura et al. 2012; Kitsanachandee et al. 2013), which indicated availability of reliable markers for marker-assisted selection and identification of QTLs for desired traits. The present investigation aimed to evaluate the genetic diversity among different accessions of a comprehensive set of Asiatic *Vigna* species, study their population genetic structure and interpret their inter-relationship for devising an effective pre-breeding programme.

Materials and methods

Plant materials

The present study was conducted on a panel of 119 diverse *Vigna* accessions (acc.) including 99 wild *Vigna* accessions belonging to 19 different species, 9 released cultivars of mungbean, 4 of blackgram, 2 cultivated accessions each of *Phaseolus vulgaris*, *V. unguiculata* ssp. *sequipedalis* and *V. umbellata* and 1 accession of *V. unguiculata*. The wild accessions were collected from diversity rich hotspots of Western Ghats, Himalayan region, Central pleateau, and North-Eastern regions of India (Table 1). All the accessions were grown in cemented pots of 1 m diameter during *Kharif* (monsoon) and Spring/Summer season of 2017–2018 and 2018–2019 at the Main Research Farm, ICAR-Indian Institute of Pulses Research, Kanpur. The recommended package of practices for growing *Vigna* crops in the region was followed to raise healthy plants. To counter staggered/reduced germination in wild accessions of *Vigna* due to their hard and waxy seed coat, seed scarification was done following Pratap et al. (2015).

Table 1 Information on different *Vigna* accessions and their grouping based on Neighbour Joining and Bayesian model analysis

S. No.	Accession/collection No.	Corresponding no. in the dendrogram*	Species	Sub population/colour code	UNJ clustering	Place of collection
1	LRM/13-38	96	*V. aconitifolia*	Admixture	D-II	Trichi, Tamil Nadu
2	TMV-1	113	*V. aconotifilia*	Admixture	A-III	Not known
3	IC251372	16	*V. glabrescence*	Admixture	A-III	Madhya Pradesh
4	*V. khandalensis*	114	*V. khandalensis*	Admixture	A-III	Not known
5	IC210575	8	*V. pilosa*	Admixture	B-I	Thrissur, Kerala
6	IC251431	36	*V. radiata var. radiata*	Admixture	B-II	Thrissur, Kerala
7	IC251419	29	*V. radiata var. setulosa*	Admixture	B-I	Thrissur, Kerala
8	IC251423	30	*V. radiata var. setulosa*	Admixture	B-I	Thrissur, Kerala
9	IC253920	51	*V. radiata var. sublobata*	Admixture	B-II	Himalayan region
10	IC277014	56	*V. silvestris*	Admixture	B-I	Western ghats
11	IC277021	57	*V. silvestris*	Admixture	B-I	Western ghats
12	IC277031	58	*V. silvestris*	Admixture	B-I	Western ghats
13	IC277039	60	*V. silvestris*	Admixture	B-I	Thrissur, Kerala
14	LRM/13-32	91	*V. trilobata*	Admixture	C-I	Trichy, Tamil Nadu
15	JAP/10-9	86	*V. trilobata*	Admixture	D-I	Western ghats
16	IC331436	62	*V. trilobata*	Admixture	D-II	South eastern region
17	LRM/13-24	88	*V. trilobata*	Admixture	D-II	Namakkal, Tamil Nadu
18	JAP/10-51	83	*V. trinervia*	Admixture	D-II	Kerala, Western ghats
19	IC247407	12	*V. trinervia* var. *bourneae*	Admixture	B-I	Thrissur, Kerala
20	IC210563	7	*V. trinervia var. bourneae*	Admixture	B-I	Thrissur, Kerala
21	JAP/10-47	81	*V. trinervia* var. *bourneae*	Admixture	B-I	Pathanamthitta, Kerala
22	IC251446	49	*V. umbellata*	Admixture	D-I	North- east region
23	IC251447	50	*V. umbellata*	Admixture	D-I	Thrissur, Kerala
24	Mung Seed-1	101	*V. radiata*	Blue (SP3)	A-III	Not known
25	Trichy local-1	115	*V. stipulaceae*	Blue (SP3)	A-III	Thrissur, Kerala
26	Trichy local-2	116	*V. stipulaceae*	Blue (SP3)	A-III	Thrissur, Kerala
27	*V. trilobata*	117	*V. trilobata*	Blue (SP3)	A-III	Not known
28	Kumur local	118	*V. trilobata*	Blue (SP3)	A-III	Thrissur, Kerala
29	Trichy local	119	*V. trilobata*	Blue (SP3)	A-III	Thrissur, Kerala
30	IC197812	5	*V. umbellata*	Blue (SP3)	A-III	Not known
31	IC528878	70	*V. umbellata*	Blue (SP3)	A-III	Not known
32	*V. umbellata*	120	*V. umbellata*	Blue (SP3)	A-III	Not known
33	NSB007	102	*V. ungiculata*	Blue (SP3)	A-III	Himalayan region
34	TCR279	111	*V. ungiculata*	Blue (SP3)	A-III	Thrissur, Kerala
35	IC203864	6	*V. dalzelliana*	Green (SP2)	C-I	Thrissur, Kerala
36	IC247408	13	*V. dalzelliana*	Green (SP2)	C-I	Thrissur, Kerala
37	IC331615	67	*V. dalzelliana*	Green (SP2)	C-I	Solan, Himalayan region
38	IC210576	9	*V. pilosa*	Green (SP2)	B-I	Western ghats
39	IC210580	10	*V. pilosa*	Green (SP2)	C-I	Western ghats
40	IC251435	40	*V. trilobata*	Green (SP2)	C-I	Thrissur, Kerala
41	IC251436	41	*V. trilobata*	Green (SP2)	C-I	Thrissur, Kerala
42	IC251438	42	*V. trilobata*	Green (SP2)	C-I	Thrissur, Kerala
43	LRM/13-34	93	*V. trilobata*	Green (SP2)	C-I	Trichy, Tamil Nadu

Table 1 (continued)

S. No.	Accession/collection No.	Corresponding no. in the dendrogram*	Species	Sub population/colour code	UNJ clustering	Place of collection
44	LRM/13-43	97	*V. trilobata*	Green (SP2)	C-I	Karur, Tamil Nadu
45	RBL-50	109	*V. umbellata*	Green (SP2)	C-I	Pantnagar, Uttarakhand
46	RBL-6	110	*V. umbellata*	Green (SP2)	C-I	Pantnagar, Uttarakhand
47	IC248326	14	*V. vexiliata*	Green (SP2)	C-I	Not known
48	IC248343	15	*V. vexiliata*	Green (SP2)	C-I	Not known
49	LRM/13-11	87	*V. aconitifolia*	Pink (SP5)	C-II	Dharmapuri, Tamil Nadu
50	LRM/13-26	89	*V. aconitifolia*	Pink (SP5)	C-II	Namakkal, Tamil Nadu
51	LRM/13-33	92	*V. aconitifolia*	Pink (SP5)	C-II	Trichy, Tamil Nadu
52	LRM/13-37	95	*V. aconitifolia*	Pink (SP5)	C-II	Trichy, Tamil Nadu
53	LRM/13-44	98	*V. aconitifolia*	Pink (SP5)	C-II	Karur, Tamil Nadu
54	IC331454	65	*V. trilobata*	Pink (SP5)	C-II	Central plateau
55	IC331456	66	*V. trilobata*	Pink (SP5)	C-II	Central plateau
56	JAP/10-7B	85	*V. trilobata*	Pink (SP5)	C-II	Western ghats
57	IC251440	44	*V. umbellata*	Pink (SP5)	C-II	Thrissur, Kerala
58	IC251441	45	*V. umbellata*	Pink (SP5)	C-II	Thrissur, Kerala
59	IC298665	61	*V. ungiculata*	Pink (SP5)	C-II	Thrissur, Kerala
60	LRM/13-36	94	*V. aconitifolia*	Red (SP1)	D-II	Trichi, Tamil Nadu
61	IC251376	17	*V. hainiana*	Red (SP1)	D-II	MP, Central plateau
62	IC251381	18	*V. hainiana*	Red (SP1)	D-II	MP, Central plateau
63	IC331448	63	*V. hainiana*	Red (SP1)	D-II	Odisha, South-Eastern region
64	IC331450	64	*V. hainiana*	Red (SP1)	D-II	Odisha, South-Eastern region
65	IC276983	55	*V. trilobata*	Red (SP1)	D-II	Madhya Pradesh
66	IC349701	69	*V. trilobata*	Red (SP1)	D-II	Western ghats
67	JAP/10-5	85	*V. trilobata*	Red (SP1)	D-II	Western ghats
68	JAP/10-7A	84	*V. trilobata*	Red (SP1)	D-II	Western ghats
69	LRM/13-30	90	*V. trilobata*	Red (SP1)	D-II	Trichi, Tamil Nadu
70	IC251439	43	*V. umbellata*	Red (SP1)	D-I	North- east region
71	IC251442	46	*V. umbellata*	Red (SP1)	D-I	North- east region
72	IC251444	47	*V. umbellata*	Red (SP1)	D-I	Thrissur, Kerala
73	IC251445	48	*V. umbellata*	Red (SP1)	D-I	Thrissur, Kerala
74	PRR-2007-2	105	*V. umbellata*	Red (SP1)	D-I	Himalayan region
75	PRR-2008-2	106	*V. umbellata*	Red (SP1)	D-I	Himalayan region
76	RB-5-1	108	*V. umbellata*	Red (SP1)	D-I	North- east region
77	IC251383	19	*V. mungo* var. *mungo*	Sky Blue (SP6)	B-II	Thrissur, Kerala
78	IC251385	20	*V. mungo* var. *mungo*	Sky Blue (SP6)	B-II	Thrissur, Kerala
79	IC251386	21	*V. mungo* var. *mungo*	Sky Blue (SP6)	B-II	Thrissur, Kerala
80	IC251387	22	*V. mungo* var. *mungo*	Sky Blue (SP6)	B-II	North- East Region
81	IC251390	23	*V. mungo* var. *mungo*	Sky Blue (SP6)	B-II	Thrissur, Kerala
82	IC251393	24	*V. mungo* var. *mungo*	Sky Blue (SP6)	B-II	Thrissur, Kerala
83	IC251394	25	*V. mungo* var. *mungo*	Sky Blue (SP6)	B-II	Thrissur, Kerala
84	IC251396	26	*V. mungo* var. *mungo*	Sky Blue (SP6)	B-II	Thrissur, Kerala
85	IC251397	27	*V. mungo* var. *mungo*	Sky Blue (SP6)	B-II	Thrissur, Kerala
86	IC251432	37	*V. radiata*	Sky Blue (SP6)	B-II	Thrissur, Kerala
87	IC251433	38	*V. radiata*	Sky Blue (SP6)	B-II	Thrissur, Kerala
88	IC251434	39	*V. radiata*	Sky Blue (SP6)	B-II	Thrissur, Kerala
89	IC260725	54	*V. radiata*	Sky Blue (SP6)	B-II	Thrissur, Kerala

Table 1 (continued)

S. No.	Accession/collection No.	Corresponding no. in the dendrogram*	Species	Sub population/colour code	UNJ clustering	Place of collection
90	IC571775	71	*V. radiata*	Sky Blue (SP6)	B-II	Not known
91	IC251424	31	*V. radiata* var. *radiata*	Sky Blue (SP6)	B-II	Not known
92	IC251425	32	*V. radiata* var. *radiata*	Sky Blue (SP6)	B-II	Thrissur, Kerala
93	IC251426A	33	*V. radiata* var. *radiata*	Sky Blue (SP6)	B-II	Thrissur, Kerala
94	IC251426B	34	*V. radiata* var. *radiata*	Sky Blue (SP6)	B-II	Thrissur, Kerala
95	IC251427	35	*V. radiata* var. *radiata*	Sky Blue (SP6)	B-II	Thrissur, Kerala
96	IC247406	11	*V. radiata* var. *sub-lobata*	Sky Blue (SP6)	B-II	Thrissur, Kerala
97	IC251416	28	*V. radiata* var. *sub-lobata*	Sky Blue (SP6)	B-II	Thrissur, Kerala
98	IC253924	52	*V. radiata* var. *sub-lobata*	Sky Blue (SP6)	B-II	Thrissur, Kerala
99	IC256158	53	*V. radiata* var. *sub-lobata*	Sky Blue (SP6)	B-II	Thrissur, Kerala
100	IC349699	68	*V. radiata* var. *sub-lobata*	Sky Blue (SP6)	B-II	Thrissur, Kerala
101	IC277036	59	*V. silvestris*	Sky Blue (SP6)	B-II	Thrissur, Kerala
102	IPM302-2	76	*V. radiata*	Yellow (SP4)	A-II	IIPR- Kanpur
103	IPM02-3	72	*V. radiata*	Yellow (SP4)	A-II	IIPR- Kanpur
104	IPM2-14	74	*V. radiata*	Yellow (SP4)	A-II	IIPR- Kanpur
105	IPM205-7	73	*V. radiata*	Yellow (SP4)	A-II	IIPR- Kanpur
106	IPM2K-14-9	75	*V. radiata*	Yellow (SP4)	A-II	IIPR- Kanpur
107	IPM410-3	77	*V. radiata*	Yellow (SP4)	A-II	IIPR- Kanpur
108	IPM99-125	78	V. radiata	Yellow (SP4)	A-II	IIPR- Kanpur
109	IPU07-3	79	*V. mungo*	Yellow (SP4)	A-II	IIPR- Kanpur
110	IPU2-43	80	*V. mungo*	Yellow (SP4)	A-II	IIPR- Kanpur
111	MH421	100	V. radiata	Yellow (SP4)	A-II	IIPR- Kanpur
112	Pant U-39	103	*V. mungo*	Yellow (SP4)	A-II	IIPR- Kanpur
113	PDM-139	104	*V. radiata*	Yellow (SP4)	A-II	IIPR- Kanpur
114	VBG-04-008	121	*V. mungo*	Yellow (SP4)	A-II	IIPR- Kanpur
115	Amber	3	*P. vulgaris*	–	A-I	IIPR- Kanpur
116	Yard long bean	99	*P. vulgaris*	–	A-I	Palampur, Himachal Pradesh
117	Palm yard bean	107	Cultivated	–	A-I	Palampur, Himachal Pradesh
118	Utkarsh	112	Cultivated	–	A-I	IIPR Kanpur
119	Goa Cowpea 3	4	*V. ungiculata*	–	A-III	Goa, Central Plateau

*These numbers correspond to the accessions given in Fig. 1

Microsatellite analysis

Total genomic DNA was extracted from fresh young leaves of one plant per accession at early vegetative stage (within 10–12 days of sowing) following the CTAB method (Doyle and Doyle 1990) with minor modifications (Pratap et al. 2015). The quality of extracted DNA was analysed on 0.8% agarose gel. The quantity of DNA was determined using a Nanodrop spectrophotometer ND 1000 (Nanodrop Technologies, DE, USA). Finally, the DNA of each sample was normalized to a concentration of 20–30 ng/µl for Polymerase Chain Reaction (PCR) analysis. Initially, 384 microsatellite markers from different *Vigna* backgrounds viz., cowpea (Li et al. 2001), adzuki bean (Wang et al. 2004), mungbean (Kumar et al. 2002a, b; Somta et al. 2009) and common bean (Gaitan-Solis et al. 2002; Blair et al. 2003) were used to identify polymorphic markers on a panel of 20 diverse *Vigna* genotypes. Out of these, 300 SSR primers showed amplification and 102 primer pairs revealed allele polymorphism (Supplementary Table 1). These 102 polymorphic SSRs were used for genotyping of 119 *Vigna* accessions.

The PCR amplification was carried out using a 96 well Tetrad thermocycler in a reaction volume of 20 µl containing 50–60 ng template DNA, 10 mM dNTPs, 0.6 U of *Taq* DNA polymerase (Fermentas, Mumbai), 10X *Taq* buffer A (Fermentas, Mumbai) with $MgCl_2$, and 5 pmol each of forward and reverse primers (ILS, India). PCR amplifications were performed at an initial denaturation for 5 min at 95 °C, followed by 35 cycles of denaturation for 15 s at 95°C, primer-specific annealing for 15 s at 45–55 °C, and extension at 68–72 °C for 1 min and the final extension at 72 °C for 10 min. The PCR products were separated by horizontal gel electrophoresis on 3% agarose gel in 1X TAE buffer for 3–4 h at 80–100 Volt and stained with ethidium bromide. The gels were documented using gel documentation system (Uvitech, Cambridge). Alleles were recorded on all genotypes according to their fragment sizes (in base pairs). Rare alleles were validated by repeated microsatellite analysis.

Genotypic diversity analysis

The allelic data of 102 polymorphic SSRs were subjected to statistical analysis using GenAlEx version 6.51b2 to calculate the total number of alleles (Na), effective alleles (Ne), private alleles, Shannon information Index (I), observed heterozygosity (Ho), expected heterozygosity/genetic diversity (He), genetic differentiation indices, Pairwise population Nei genetic identity and AMOVA (analysis of molecular variance). The polymorphic information content (PIC) of each marker was calculated using the formula $PIC = 1 - \sum(P_{ij})^2$ where P_{ij} denotes the frequency of *i*th allele of a *j*th locus summed across all alleles revealed by *j*th locus primer in a set of 119 genotypes (Botstein et al. 1980).

To establish the ancestry relationship of the ecological and reproductive characteristics of the species on their genetic diversity, genotypic data of 102 SSR markers on 119 genotypes of 19 *Vigna* species were used to generate genetic distance (GD) following distance based unweighted neighbour joining (UNJ) tree using Darwin V5.4. The co-dominant allelic data of each species were run at 30,000 bootstrap to draw the phylogenetic tree. Later the phylogeny was used as the robust signal for explaining the genetic diversity of the wild relatives and to predict evolutionary history.

Population structure analysis

To determine the genetic structure and define the number of clusters (gene pools), model-based cluster analysis was done using the software STRUCTURE, version 2.3.4 (Pritchard et al. 2000). The number of presumed population (*K*) was set from 2 to 10 and the program was run with ten independent runs for each cluster (*K*) following admixture model and correlated allele frequencies. The program was run with 30,000 burn-in-period and 100,000 Markov Chain Monte Carlo iterations. The optimum number of sub-populations (*k*) was determined using Structure Harvester web v0.6.94 (Earl and vonHoldt 2012) with structure output files based on the adhoc criterion (Delta *K*) proposed by Evanno et al. (2005).

Principal coordinates analysis (PCoA)

The principal coordinate analysis (PCoA) was performed with six sub-populations along with one admixture population identified from structure analysis to find and plot the major patterns within a multivariate dataset genotyped with many SSR loci. Distance matrix was calculated following 'Distance' option and the outcome matrix was used as an input for PCoA analysis following Distance-Standardised method available in GenAlEx 6.5 tool.

Results

Allelic diversity

A total of 102 polymorphic SSRs were used to characterize 119 wild and cultivated accessions belonging to 19 *Vigna* species (Table 1). Most of the primer pairs amplified with varying allele sizes between 130 and 285 bp in wild accessions, 150 and 250 bp in cultivated *Vigna* species, 170 and 240 bp in *Phaseolus* and 130–225 bp in the large beans (Supplementary Fig. 1). All the 102 SSR markers showed different degrees of polymorphism at each locus producing a total of 1758 alleles, as the number of different alleles at each locus (Na) varied from 9 (BMD-6 and BMD-50) to 31 (CP00226) with an average of 17 alleles per locus. Maximum of 13 loci produced 15 alleles per locus followed by 9 loci each producing 16 and 17 alleles per locus. The polymorphic information content (PIC) value of SSRs ranged between 0.78 and 0.93 with an average of 0.882 (Pl see supplementary information). The maximum PIC value of 0.93 was recorded for the SSRs CEDG096A, CP00226 and BM212 followed by 0.92 for the markers PvM03, J01263, BMD-13, SSR-IAC-188, VR022 and X34. The lowest PIC value of 0.77 was recorded for SSRs BMD-6 and VR024. Among the 102 markers used, 100 SSR markers (98.04%) showed high discriminating power in all *Vigna* species i.e. a high PIC value of > 0.80. The number of effective alleles varied from 4 to 16 (CEDG096A). The Shannon's information index varied from 1.683 to 3.093 and the fixation index value ranged from 0.806 to 1.0. Total 47 SSR loci revealed the fixation index value of 1.0. Heterozogosity was observed in 54 SSR loci and the observed heterozygosity ranged from 0.08 to 0.168 (CEDG176). The expected heterozygosity varied between 0.775 and 0.94.

Cluster-based genetic diversity

The molecular data generated through SSR profiling of 119 accessions at 102 loci were used to study genetic inter-relationship between the different *Vigna* accessions. The Unrooted neighbour joining (UNJ) clearly separated these 119 genotypes into four major clusters (cluster A–D) (Fig. 1). Among these, Cluster B was the largest with 38 (31.93%) accessions followed by cluster A with 32 (26.89%) and cluster C with 25 (21%) accessions. Cluster D was the smallest one represented by 24 (20.16%) accessions. Cluster A could be further divided into three sub-clusters viz., AI, AII and AIII with 4, 13, and 15 accessions, respectively. Sub-cluster AIII comprised of *V. trilobata* (3 acc.), *V. stipulaceae* (2 acc.), *V. unguiculata* (3 acc.), *V. umbellata* (3 acc.) and *V. radiata* (1 acc.) which are locally cultivated and *V. glabrescence*, *V. aconitifolia* and *V. khandalensis* (1 acc. each) whose cultivation status is not known. Interestingly, sub-cluster AII accommodated all released cultivars of mungbean and urdbean, irrespective of the place where they were bred while all the 4 outliers (2 cultivars each of *P. vulgaris* and *V. unguiculata* ssp. *sesquipedalis*) were grouped in sub-cluster AI.

The clusters B, C and D were further divided into 2 sub-clusters each. Sub-cluster BI comprised of 11 accessions belonging to *V. silvestris* (4 acc.), *V. trinervia* var. *bourneae* (3 acc.), and *V. radiata* var. *setulosa* and *V. pilosa* (2 acc. each). Sub-cluster BII accommodated 27 accessions including 9 of *V. mungo*, 5 of *V. radiata*, 6 of *V. radiata* var. *radiata*, 6 of *V. radiata* var. *sublobata* and 1 accession of *V. silvestris.* Sub-cluster CI comprised of 14 accessions including 6 of *V. trilobata,* 3 of *V. dalzelliana,* 2 each of *V. umbellata* and *V. vexillata* and 1 accession of *V. pilosa*. Likewise, sub-cluster CII consisted of 11 accessions belonging to *V. aconitifolia* (5 acc.), *V. unguiculata* (1 acc.), *V. umbellata* (2 acc.) and *V. trilobata* (3 acc.). Sub-cluster DI consisted of 10 accessions belonging to *V. umbellata* (9 acc.) and *V. trilobata* (1 acc.). Sub-cluster DII consisted of 14 accessions including 7 of *V. trilobata*, 4 of *V. hainiana*, 2 of *V. aconitifolia* and 1 accession of *V. trinervia*. All accessions of *V. hainiana* grouped together in cluster DII.

Interestingly, all the wild accessions belonging to *V. radiata*, *V. radiata* var. *radiata*, *V. sublobata*, *V. mungo*,

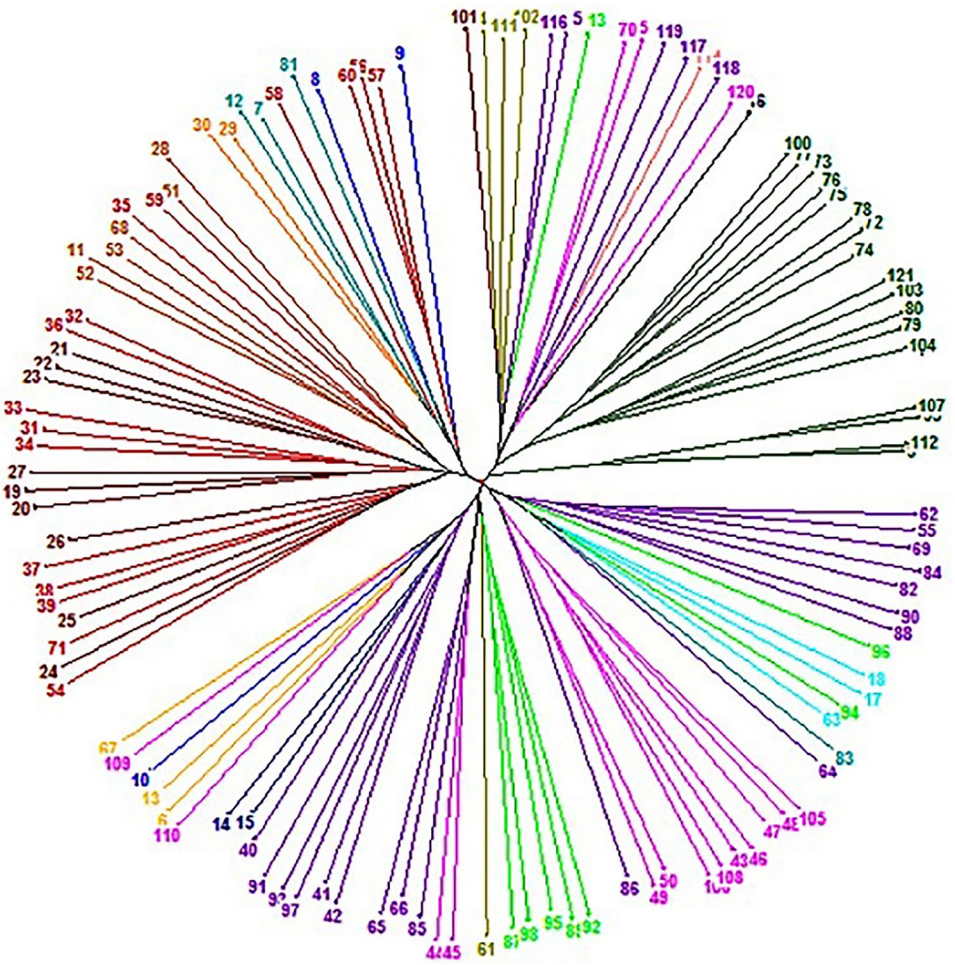

Fig. 1 Cluster-based diversity depicting the grouping of 119 accessions of 19 *Vigna* and 1 *Phaseolus* species. The numbers depict different *Vigna* accessions as decribed in Table 1

V. silvestris, *V. setulosa*, *V. pilosa* and *V. trinervia* var. *bourneae* were grouped in cluster B except that one accession each of *V. pilosa* (IC210580) and *V. trinervia* (JAP/10-51) shared cluster C and D, respectively. The accessions of *V. umbellata*, *V. aconitifolia,* and *V. trilobata* were highly variable and were found to be distributed in all the three major clusters namely, A, C and D.

Population genetic structure

Cluster diversity study singularized two accessions each of *P. vulgaris* and *V. unguiculata* ssp. *sesquipedalis* in a separate sub cluster (AIII). Furthermore, one accession of *V. unguiculata* (Goa Cowpea 3) in AIII sub-cluster was recorded to have comparatively longer pods, bold seeds and higher 100-seed weight as compared to all other accessions of different *Vigna* species under study. These five accessions stood apart at molecular as well as morphological level and were considered as outliers. Therefore, these were removed from the panel for the further study. To determine accurate and reliable population genetic structure, a model-based population structure analysis was used to detect the ancestral and hybrid forms within 114 accessions of *Vigna*. All 114 accessions were categorized into 6 genetically distinct sub-populations ($K=6$) (Fig. 3) following admixture-model based simulation (Fig. 2) with varying levels of admixture. In total, 91 (79.82%) accessions resembled their hierarchy and 23 (20.18%) accessions were observed as the admixture forms. Maximum number of accessions (25) were grouped

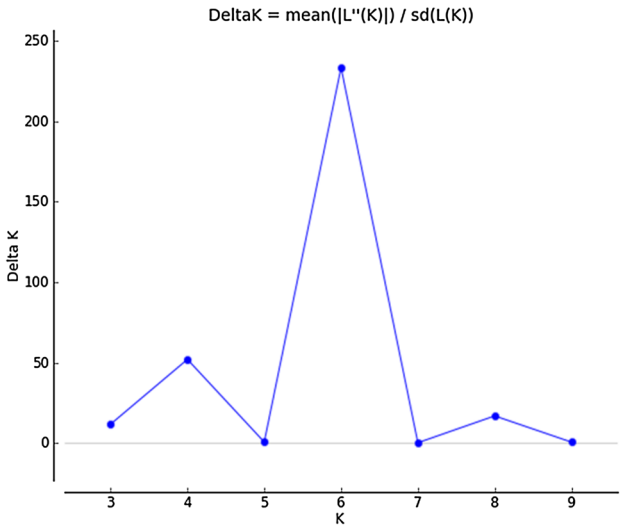

Fig. 3 Bar plot depicting population peak among original population of 114 wild and cultivated *Vigna* accessions

in sub-population (SP) 6 and the minimum number of accessions were grouped in SP3 and SP5 (11 each).

SP1 comprised of 17 (14.91%) accessions including 7 of *V. umbellata*, 5 of *V. trilobata,* 4 of *V. hainiana*, and 1 accession of *V. aconitifolia*. These 17 accessions were altogether clustered in the major cluster D and the remaining 7 accessions of the major cluster D were grouped in the admixture class. Sub population 3 (SP3) comprised of 11 (9.65%) accessions which belonged to *V. umbellata* (3 acc.),

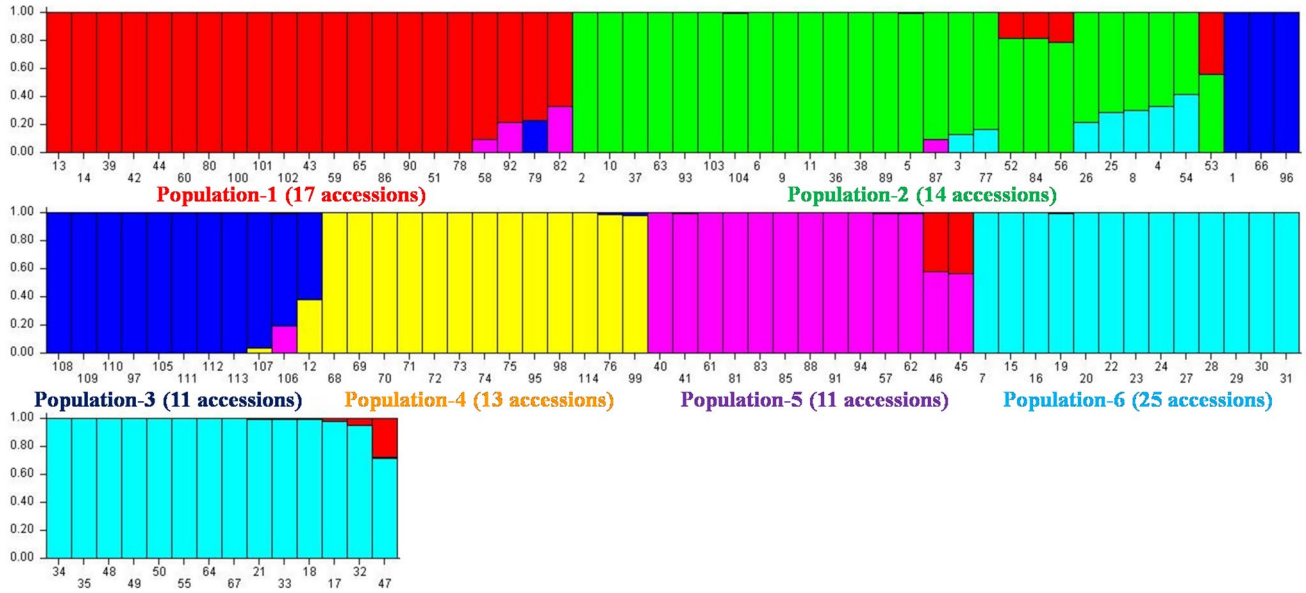

Fig. 2 Model-based clustering for each of the 114 *Vigna* accessions examined based on 102 SSR markers. Each individual bar represents an accession. The different colour bars represent different genetic groups (P1–P6, respectively), and mixture of colours in a bar represents admixture

V. trilobata (3 acc.), *V. stipulaceae* (2 acc.), *V. unguiculata* (2 acc.) and *V. radiata* (1 acc.). Most of the semi-cultivated accessions belonging to *V. radiata* (Mung Seed 1), *V. stipulaceae* (Trichy Local-1 and Trichy Local-2), and *V. trilobata* (Trichy Local and Kumar Local) were grouped in SP3 in the population structure analysis and clustered in AIII as per UNJ tree. Further, the model based ancestry synteny corresponded well with the cluster diversity in case of cultivated *Vigna* accessions which comprised of released cultivars of mungbean and blackgram and these were assigned to SP 4 without any admixture (Table 2). Interestingly, all these genotypes clustered only in sub-cluster AII, falling in Cluster A specifically as per UNJ tree.

The SP2 comprised of 14 (12.29%) accessions belonging to *V. trilobata* (5 acc.), *V. dalzelliana* (3 acc.), and 2 accessions each of *V. pilosa, V. umbellata* and *V. vexillata.* Similarly, SP5 consisted of 11 (9.65%) accessions including 5 of *V. aconitifolia*, 3 of *V. trilobata*, 2 of *V. umbellata*, and 1 of *V. unguiculata*. 13 accessions of SP2 and 11 accessions of SP5 represented cluster CI and CII in UNJ tree, respectively. The exception was only one accession, IC210576, of *V. pilosa* which was grouped in SP2 and categorised in cluster C of UNJ tree. On contrary, LRM/13-32 of *V. trilobata* belonging to cluster C as identified from UNJ tree was grouped in admixture class in the model-based study. Interestingly, 10 out of 11 accessions grouped in cluster BI (except IC210576 of *V. pilosa)* were grouped in the admixture class. The 25 accessions including 9 accessions of *V. mungo*, 5 accessions each of *V. radiata*, *V. radiata* var. *radiata*, and *V. sublobata*, and 1 accession of *V. silvestris* shared single sub-population (SP6). Importantly, these genotypes represent the primary and secondary gene pool (GP 1 and II) of *Vigna* species and clustered majorly in BII as identified from UNJ tree. The accession IC253920 of *V. radiata* var. *sublobata* and IC251431 of *V. radiata* var. *radiata* clustered in BII were grouped as admixture class in model-based analysis.

The accessions which recorded likelihood thresholds lower than 0.70 were considered as admixture forms. A total of 23 (19.33%) accessions including 4 accessions each of *V. trilobata* and *V. silvestris*, 3 of *V. trinervia* var. *baourneae*, 2 each of *V. umbellata*, *V. radiata* var. *setulosa*, *V. aconitifolia*, and 1 each of *V. pilosa*, *V. sublobata*, *V. trinervia*, *V. radiata* var. *radiata*, *V. glabrescence* and *V. khandalensis* were representing two or more ancestories of different sub-populations and hence were considered as the admixture class. All these accessions with an admixture status were clustered in AIII (3 acc.), BI (10 acc.), BII (2 acc.), CI (1 acc.), DI (3 acc.) and DII (4 acc.).

Additionally, the *Vigna* species viz., *V. umbellata* (16 acc.), and *V. trilobata* (20 acc.) were observed as highly variable and these were distributed in 4 different sup-populations

Table 2 Representation of *Vigna* accessions in each sub-population identified from STRUCTURE analysis

Species	SP1	SP2	SP3	SP4	SP5	SP6	Admixture	Total accessions
V. aconitifolia	1 (12.5%)	0	0	0	5 (62.5)	0	2 (25%)	8
V. glabrescence	0	0	0	0	0	0	1 (100%)	1
V. khandalensis	0	0	0	0	0	0	1 (100%)	1
V. mungo	0	0	0	0	0	9 (100%)	0	9
V. radiata var. *sublobata*	0	0	0	0	0	5 (83.3%)	1 (16.6%)	6
V. silvestris	0	0	0	0	0	1 (20%)	4 (80%)	5
V. trilobata	5 (25%)	5 (25%)	3 (15%)	0	3 (15%)	0	4 (20%)	20
V. trinervia	0	0	0	0	0	0	4 (100%)	4
V. umbellata	7 (43.75%)	2 (12.5%)	3 (18.75%)	0	2 (12.5%)	0	2 (12.5%)	16
V. vexillata	0	2 (100%)	0	0	0	0	0	2
V. dalzelliana	0	3 (100%)	0	0	0	0	0	3
V. hainiana	4 (100%)	0	0	0	0	0	0	4
V. pilosa	0	2 (66.6%)	0	0	0	0	1 (33.3%)	3
V. radiata var. *radiata*	0	0	0	0	0	5 (83.3%)	1 (16.6%)	6
V. radiata var. *setulosa*	0	0	0	0	0	0	2 (100%)	2
V. stipulaceae	0	0	2 (100%)	0	0	0	0	2
V. unguiculata	0	0	2 (66.6%)	0	1 (33.3%)	0	0	3
Cultivated *Vigna*	0	0	0	13 (100%)	0	0	0	13
V. radiata	0	0	1 (16.6%)	0	0	5 (83.3%)	0	6
Total	17	14	11	13	11	25	23	114

namely SP1, 2, 3 and 5 as well as in the admixture class (Table 2). The same holds true in cluster analysis where these clustered in A, C and D of UNJ tree. All accessions of *V. hainiana*, 43.75% of *V. umbellata*, 25% of *V. trilobata* and 12.5% accessions of *V. aconitifolia* grouped in SP1.

Genetic diversity within structured *Vigna* populations

The number of alleles in six populations along with the admixture class varied from 4.9 (SP4) to 9.8 (admixture class). The mean expected heterozygosity (mHe) was quite high in each population which varied from 0.67 (SP4) to 0.825 (admixture group). The mean Shannon's information index value varied from 1.34 (SP) to 2 (admixture group). All loci revealed polymorphism in accessions belonging to all populations except in SP4 where one locus (GMES0211) did not reveal polymorphism across the accessions (Table 3). All 114 accessions recorded private alleles which varied from 1 (IC331456 at locus CEDG036; IC251431 at locus CP00226; IC277021 at locus VR011) to 16 (Pant U39 at loci PV-ag005, BMD-55, X49, VR016, VR048, CEDG291, CEDG220, CEDC139, CEDG185, CEDC033, DMBSSR001, CEDG225, CEDG073, VM27, BM212 and BM149) (Supplementary Table 2,). Population-wise, the maximum number of private alleles were observed for SP6 (76), followed by SP4 (75), SP3 (71), SP1 (67), SP5 (48) and SP2 (44) while the admixture population group recorded 80 private alleles (Supplementary Table 3). The Pair-wise population Nei genetic identity value ranged from 0.26 (SP4 vs SP5) to 0.624 (SP1 vs SP7). All the 6 populations alongwith the admixture class recorded mostly low (0.36; SP4) to moderate (0.62; SP1) similarity values (Table 4).

The genetic differentiation indices between populations (Fst) were observed as moderate to high which varied from 0.048 (between SP6 and SP7) to 0.132 (between SP4 vs SP5). Accessions of SP4 had high differentiation indices with rest of the populations including the admixture class. Similarly, admixture class had moderate Fst values (0.046 to 0.071) between rest of the populations (Table 5). An analysis of the molecular variance (AMOVA) was performed using raw data for genetic differentiations (Table 6). The overall genetic variation was divided among population (9%), among individuals within populations (88%), and within individuals (2%). The diversity within individuals of a population was greater than the diversity between the

Table 3 Comparison of mean genetic diversity statistics across population using 102 SSRs

Obs	SP1	SP2	SP3	SP4	SP5	SP6	SP7
N	17	14	11	13	11	25	23
mNa	7.0490196	6.2843137	6.0882353	4.9215686	5.7352941	7.362745098	9.81372549
mNe	4.9140983	4.6305775	4.6634707	3.5515935	4.5106019	4.887059715	6.204441147
mI	1.7014563	1.6192267	1.6105996	1.3394608	1.560906	1.7102658	1.999665197
mHo	0.0155709	0.0287115	0.0347594	0.0113122	0.0231729	0.018823529	0.019607843
mHe	0.7708461	0.7588535	0.7576973	0.673715	0.7510128	0.774047059	0.825049112
muHe	0.794205	0.7869592	0.7937781	0.7006637	0.7867753	0.789843938	0.843383537
mF	0.9806248	0.9629535	0.9559716	0.9834491	0.9702773	0.976332187	0.976362093
%p	100	100	100	99.02	100	100	100

N Number of individuals in each population, *mNa* mean no. of different alleles, *mNe* mean No. of effective alleles = 1/(Sum pi^2), *mI* mean Shannon's Information Index = − 1* Sum (pi * Ln (pi)), *mHo* mean observed Heterozygosity = No. of Hets/*N*, *mHe* mean expected Heterozygosity = 1 − Sum pi^2, *muHe* mean unbiased expected Heterozygosity = (2*N*/(2*N* − 1))*He, *mF* mean Fixation Index = (He − Ho)/He = 1 − (Ho/He), where pi is the frequency of the *i*th allele for the population and Sum pi^2 is the sum of the squared population allele frequencies, %p percent polymorphic loci

Table 4 Pair-wise population Nei genetic identity

SPI	SP2	SP3	SP4	SP5	SP6	SP7	Population
1.000							**SP1**
0.458	1.000						**SP2**
0.398	0.355	1.000					**SP3**
0.300	0.291	0.346	1.000				**SP4**
0.436	0.425	0.346	0.260	1.000			**SP5**
0.432	0.480	0.337	0.306	0.404	1.000		**SP6**
0.624	0.579	0.433	0.358	0.512	0.610	1.000	**SP7**

Table 5 Pair-wise Fst values between populations

SPI	SP2	SP3	SP4	SP5	SP6	SP7	Population
0.000							**SP1**
0.077	0.000						**SP2**
0.086	0.094	0.000					**SP3**
0.122	0.126	0.118	0.000				**SP4**
0.082	0.086	0.097	0.132	0.000			**SP5**
0.078	0.074	0.093	0.120	0.085	0.000		**SP6**
0.046	0.054	0.071	0.101	0.063	0.048	0.000	**SP7**

Table 6 Analysis of molecular variance (AMOVA) of *Vigna* accessions

Source of variation	*d.f.*	Sum of squares	Estimated variation	Variation %	*F* statistics*
Among Populations	6	1318.737	4.299	9	0.094
Among Individuals	107	8794.794	40.566	88	0.975
Within Individuals	114	121.000	1.061	2	0.977
Total	227	10,234.531	45.927	100	

d.f. degrees of freedom

*$P < 0.001$

populations. The observed Fst value was 0.094, suggesting moderate differentiation of *Vigna* sub-populations.

On the basis of principal coordinates analysis (PCoA), it was observed that Population 1 comprising of the accessions of *V. umbellata*, *V. trilobata V. hainiana*, and *V. aconitifolia* clustered in two groups. The population 2 comprising of the accessions belonging to *V. trilobata, V. dalzelliana, V. pilosa, V. umbellata* and *V. vexillata* were scattered as population 1. Similarly, the accessions *V. aconitifolia*, *V. trilobata*, *V. umbellata*, and *V. unguiculata* belonging to population five were also scattered. On contrary, the population three comprising of accessions belonging to *V. umbellata, V. trilobata, V. stipulaceae, V. unguiculata* and *V. radiata* were clustered together except Mung seed-1 and TCR279. Similarly the released varieties of *Vigna* representing population four were uniquely clustered without any admixture as also observed in structure analysis. Population six having the accessions from primary and secondary gene-pool of *Vigna* viz., *V. mungo, V. radiata*, *V. radiata* var. *radiata*, *V. sublobata* and *V. silvestris* were clustered together except IC251426A, IC251425, IC251387 and IC251390. As expected, accessions belonging to admixture groups were scattered all around (Supplementary Fig. 2).

Discussion

The genus *Vigna* is large, genetically variable and pan-tropic in distribution having a considerable agronomic and environmental significance. Many of the *Vigna* species viz., mungbean (*V. radiata*), urdbean (*V. mungo*), moth bean (*V. aconitifolia*) and ricebean (*V. umbellata*) have their centre of origin as well as diversity in India (de Candolle 1884; Vavilov 1926; Zukovskij 1962; Smartt 1985; Pratap and Kumar 2011) and their wild and cultivated forms are found variably distributed across the Himalayan region, central plateau, western ghats and the north-eastern hill regions. Nonetheless, the Asiatic *Vignas are* considered to be recently evolved and hence differentiation of taxa and sub-specific classification using morphological marker is limited and more complex (Baudoin and Marechal 1988). Therefore, classifying this species using highly abundant molecular markers such as multi-allelic SSRs is indeed necessary and has been used to some extent in classifying wild species earlier (Sarr et al. 2020; Kempf et al. 2016; Wang et al. 2008; Gwag et al. 2010; Pratap et al. 2015).

Assessment of genetic diversity helps in identifying the appropriate donors in a pre-breeding activities and breeding programme. The 102 SSRs used in this study revealed high degree of polymorphism by amplifying 1758 alleles from 119 accessions belonging to 19 *Vigna* species with the number of alleles varying between 9 and 31 and the effective number of alleles varying from 4.50 to 15.66. Number of alleles per locus detected in this study is comparatively higher than the earlier reports. Pratap et al. (2015) reported 4–16 alleles per locus in a set of 53 Asiatic *Vigna* accessions including 41 wild accessions belonging to 13 species and 12 commercial cultivars of mungbean, blackgram and rice bean using 53 SSRs. In other similar studies, 6–20 alleles with an average of 13.65 alleles per locus in 422 wild and cultivated genotypes of zombi pea (*V. vexillata* (L.) A. Rich) (Dachapak et al. 2017), 2 to 15 alleles with an average of 6.2 alleles

per marker locus in 737 samples of cowpea (*V. unguiculata* (L.) Walp.) (Sarr et al. 2020), and 8–26 alleles per locus in 127 genotypes of mungbean (Singh et al. 2020) have been reported. A higher number of alleles per locus detected in the present study could be primarily attributed to use of a large number of highly diverse accessions of 19 *Vigna* species. These accessions have been collected from diversity rich endemic hot spots of India which have been reported to be centre of origin/diversity for many of the *Vigna* species (Sangiri et al. 2007; de Candolle 1884; Vavilov 1926; Zukovskij 1962). Additionally this could also be attributed to the deployment of a high number of polymorphic SSRs than the earlier studies (102 SSRs in the present study against 20–53 SSRs in earlier studies). Higher estimates of polymorphism (PIC: 0.78–0.93), Shannon information index (1.683–3.93), number of effective alleles (4–16) and value of expected heterozygosity (0.775–0.94) as compared to the previous studies (Wang et al. 2008; Dachapak et al. 2017; Singh et al. 2020; Pratap et al. 2015; Sarr et al. 2020) also indicates high genetic diversity among the accessions studied. This finding also suggests high usefulness of the material under study for generating additional genetic variability in different *Vigna* crops. A few marker loci (CEDG176, BMD-26, X87 and VR011) revealed higher heterozygosity and, therefore, could be deployed in studying the hybridity of wild relatives. Therefore, it is evident that the SSRs used in this experiment have a great potential for germplasm characterisation and identifying trait-linked markers that could be eventually utilised in marker-assisted breeding programme.

Cluster diversity

All the cultivated *Vigna* accessions clustered in a single sub cluster-AII, whereas the outliers such as Amber and Utkarsh belonging to the genus *Phaseolus,* and 2 accessions of the large beans (*V. unguiculata* ssp. *sesquipedalis*) separated in sub cluster-AI. At morphological level also these accessions were observed to have comparatively longer pods, bold seeds and high 100-seed weight as compared to all other accessions under study. *Phaseolus* is highly diverse from *Vigna* species although it shares high genetic similarity with *V. unguiculata,* justifying their clubbing together in sub cluster AI. Nonetheless, *P. vulgaris* is related with the *Vigna* species in the context of having the same chromosome number with almost similar genome sizes. Vasconcelos et al. (2015) reported numerous breaks of macrosynteny between *P. vulgaris* and *V. unguiculata* which could be due to larger sequence divergence between them. *V. glabrescence* and *V. khandalensis* are not in cultivation although they clustered with the semi-cultivated group in AIII sub-cluster. Earlier, *V. glabrescence* had been reported to be highly cross compatible with mungbean and urd bean (Dana 1968; Krishnan and De 1968; Chen et al. 1989) which indicates that it is genetically very similar to the cultivated species. *V. khandalensis* and *V. stipulacea,* belonging to the section Aconitifoliae are closely related to each other and cross compatible with mungbean (Takahashi et al. 2016; Chavan et al. 1966).

The progenitors of mungbean and urdbean namely *V. radiata* var. *sublobata* and *V. mungo* var. *silvestris*, characterized in Gene pool I, also clustered together in cluster BII as expected along with *V. mungo* and *V. radiata.* The accessions of *V. silvestris* and *V. radiata* var. *setulosa* belonging to secondary gene pool of mungbean and urdbean, respectively clustered with *V. pilosa* (2 acc.), and *V. trinervia* var. *bourneae* (3 acc.) in Sub-cluster BI. In this subcluster, all accessions belonged to diploid *Vigna* species except *V. pilosa* which is a tetraploid ($2n = 4x = 44$) (Chankaew et al. 2014).

Sub-cluster CI represented the accessions belonging to secondary (*V. trilobata*) and tertiary gene-pools (*V. dalzelliana, V. umbellata,* and *V. vexillata*) along with one accession of *V. pilosa*. The geographical distribution of *V. dalzelliana* (O. Kuntze) Verdcourt was limited to India, especially the Andaman Islands, and Sri Lanka (John et al. 2009; Tomooka et al. 2002) and it could be one of the ancestral species of the section Angulares. *V. vexillata* (L.) A. Rich, is reported to be widely distributed in pantropical regions, including Africa, Asia, Oceania, and America while *V. vexillata* and Cowpea (*V. unguiculata*) were observed to be relatively closer at the molecular level (Sonnante et al. 1996) and also cross-compatible to produce an interspecific hybrid (Gomathinayagam et al. 1998). Sub-cluster CII had an array of accessions belonging to the secondary (*V. aconitifolia* and *V. trilobata*) and tertiary (*V. umbellata*) gene-pools along with accessions of unknown gene pool (*V. unguiculata*). Most of the accessions representing major cluster-II were collected from peninsular region (Western Ghats) of India.

The sub-cluster DI consisted 90% of the accessions belonging to the tertiary gene-pool. Sub-cluster DII consisted of 9 accessions representing secondary gene pool (7 of *V. trilobata*, and 2 of *V. aconitifolia)* along with 4 accessions of *V. hainiana* and 1 of *V. trinervia*. Pratap et al. (2015) reported that two accessions of *V. hainiana* (IC251381, IC251376) clustered alongwith *V. umbellata* and *V. glabrescens* while the remaining two accessions (IC331448; IC331450) clustered with *V. trilobata*. This could be due to less number of polymorphic markers employed by them in a smaller panel of genotypes. The accessions of *V. umbellata, V. aconitifolia,* and *V. trilobata* were highly variable and distributed in all the three major clusters namely A, C and D. Among these, mothbean (*V. aconitifolia*) is considered as one of the most primitive *Vigna* crop in respect of its evolution (Smartt 1985) and its wild ancestor primarily occur in south-eastern India (Arora et al. 1984). It is reported to

be cross compatible with mungbean (Pandiyan et al. 2010) and serves as a source for drought and heat tolerance in the subgenus *Ceratotropis*.

The observed clustering pattern of the accessions belonging to different *Vigna* species clearly showed their closeness with the corresponding gene-pools. Nonetheless a few accessions from diverse *Vigna* species also clustered together indicating that there could be a possibility of taxonomic misclassification of a few accessions based only on phenotypic observations which might have led to their assignment to an incorrect species. This could also have occurred during extensive germplasm exchange. It may also be possible that some of these *Vigna* species might have been genetically close to other species leading to spontaneous hybridization and subsequent exchange of genetic materials to produce new species through the process of natural selection without compromising much with their original identity. Moreover, since most of the *Vigna* species are diploid in nature that could be the cause for less genetic divergence among species of interspecific hybrids than the allopolyploid hybrid species (Chapman and Burke 2007). Species that co-occur in the same geographical location have a better chance for interspecific gene flow (Abbott et al. 2008). Moreover ecotypic variation is also one of the important steps in speciation where some degree of reproductive isolation is maintained for the transition of ecotypes to species. Therefore, cross-compatibility studies and a combination of phenotype- and genotype-based classification of the wild accessions must be resorted to establish the closeness of these species with their corresponding clusters.

Population genetic structure

Model-based clustering is tremendously useful to visualise the genetic ancestry and assigning individuals to a defined population in plants, animals as well as humans (Pritchard et al. 2000). In the present study, Bayesian algorithm with ADMIXTURE model divided the 114 *Vigna* accessions including 13 released cultivars into 6 genetically distinct sub-populations with a few admixtures. The results obtained from both DARWIN and STRUCTURE indicated that the grouping of all accessions did not strictly follow their geographical distribution as well as their species lineage. This could be attributed to several reasons including species misclassification, spontaneous hybridization and mutation among the accessions. Spontaneous hybridization may lead to development of new forms hitherto not found in nature resulting in the process of speciation. Likewise, inter-specific outcrossing, although difficult to notice and characterize, is also expected to occur in plants naturally and even a single backcross may develop plants which are morphologically similar to the species with which they were backcrossed (Anderson 1948).

All accessions of *V. radiata* and *V. mungo* and their progenitors viz., *V. sublobata* and *V. silvestris* were categorized together in one group (SP 6 and sub-cluster BII). Similar findings have been reported earlier by Chandel and Laster (1991), Dana and Karmakar (1990), Kumar et al. (2004) and Pandiyan et al. (2010) based upon morphological observations. More interestingly, all the released cultivars of mungbean and blackgram developed at quite distinct geographical locations of Kanpur, Pantnagar, Hisar (north India) and Vamban (south India) were assigned to the same sub-cluster AII in UNJ tree and likewise in SP 4, as also reported in earlier studies (Singh et al. 2020; Pratap et al. 2015). This indicates the inheritance of similar genetic sequences from common lineage/parenatge across these cultivars. Their grouping in the same population group may also be attributed to involvement of common ancestors in their past history (Pratap et al. 2015).

V. hainiana was grouped distinctively with *V. umbellata* and *V. trilobata* despite showing phenotypic similarity with wild types of *V. mungo* and *V. radiata* and also being close to *V. radiata* var. *sublobata* and *V. mungo* var. *silvestris* with respect to pod characteristics (Arora et al. 1973; Chandel et al. 1984; Bisht et al. 2005). However, *V. hainiana* is morphologically more primitive than *V. mungo* var. *silvestris* and *V. radiata* var. *sublobata* with comparatively small flowers and very small seeds and, therefore, could play a significant role in phylogeny and evolutionary studies particularly in the *mungo-radiata* complex (Bisht et al. 2005). Other *Vigna* species such as *V. umbellata*, *V. dalzelliana*, V. *mungo* var. *silvestris, V. radiata* var. *sublobata* and *V. setulosa* were also observed to be closely related to each other and exhibited sympatric distribution (Bisht et al. 2005).

Accessions of *V. umbellata* and *V. trilobata* were highly scattered and grouped in sub populations 1, 2 and 3 alongwith the accessions of *V. hainiana, V. aconitifolia* (LRM/13-36), *V. dalzelliana, V. pilosa, V. vexillata*, *V. stipulaceae* and *V. unguiculata*. The wild types of *V. umbellata* have been observed to be widely distributed than *V. dalzelliana*, a closely related species. Bisht et al. (2005) reported two distinct overlapping groups of *V. umbellata* and *V. dalzelliana* accessions which is also evident from molecular grouping of *V. umbellata* accessions. Likewise, they also documented more widespread distribution and highly diverse nature of *V. trilobata* accessions collected from diverse agro-ecologies of India at phenotypic level. This is more evident from clustering of accessions at molecular level in the present study. A large admixture group (23 accessions) which shared two or more ancestries indicates the possibility of historical inter- and intra-specific gene transfer and is in agreement with previous studies (Pratap et al. 2015; Sarr et al. 2020). The only accession of *V. glabrescense* was grouped in the admixture group as also in the earlier study (Pratap et al. 2015). It could be corroborated with the earlier observations of Dana

(1964) and Bisht et al. (2005) that *V. glabrescens* is probably an amphidiploid combining the genomes of *V. radiata* and *V. umbellata.* However, Goel et al. (2001) reported that *V. glabrescens* is a derivative from *V. umbellata* and *V. angularis* based on the variation observed in rDNA sequences. The accessions of *V. umbellata* and *V. trilobata* used in this study were collected from several diverse locations including the Himalayan region, Western Ghats, Southern Eastern India, North-East region and Central pleateu. Going into the history, it could be suggested that probably their seeds were dispersed historically by monks, merchants and villagers because of their movement from one place to another. In due course of time, adaptation and acclimatization in new environment might have happened accompanied by inter- and intra-specific recombination and natural selection. This might also have happened due to genetic drift, domestication, mutation and background selection (Sangiri et al. 2007).

Genetic diversity in structured *Vigna* populations

The mean expected heterozygosity (mHe) was observed to be quite high in each sub-population which varied from 0.67 to 0.825 and was higher than the observed heterozygosity. These estimates were higher than the previous reports in *V. unguiculata, V. radiata* and other Asiatic *Vigna* species (Sarr et al. 2020; Badiane et al. 2012; Pratap et al. 2015). This could be attributed to the fact that India is the centre of origin as well as diversity for many of the *Vigna* species.

AMOVA showed higher genetic diversity (88.33%) among individuals within the populations and it could be primarily due to representation of diverse *Vigna* species in each population. On the other hand, the low genetic diversity between the populations could be due to the distribution of the similar *Vigna* species in each population.

The genetic differentiation indices observed between populations were moderate to high and the SP4 was observed to have high differentiation indices with rest of the populations. Noticeably, this sub-population consists mainly of released cultivars of mungbean and urdbean and these cultivars were also distinctively clustered in sub-cluster AII. The highest genetic differentiation indices of 0.132 were observed between SP4 and SP5 followed by 0.12 between SP 4 and SP 6. The SP5 consisted of accessions belonging to *V. aconitifolia, V. trilobata, V. umbellata* and *V. ungiculata* and these accessions/species are quite diverse from released cultivars of mungbean and urdbean representing SP4. Similarly SP6 comprised of *V. mungo* var. *mungo, V. radiata* var. *radiata* and their respective wild ancestors. The low genetic differentiation indices (0.077) between SP1 and SP2 could be due to the fact that both the populations shared majority of accessions belonging to *V. trilobata* and *V. umbellata*. Similarly the low to moderate genetic identity observed between different populations was attributed to the representation of accessions belonging to either released cultivars (SP4) or close wild relatives of each species (SP6).

Conclusion

In summary, the 102 SSR markers used in this study successfully detected a high amount of genetic variability in 119 wild and cultivated accessions belonging to 19 different species of *Vigna.* Keeping in view that a large set of wild *Vigna* accessions was deployed in this study, the genotypic phylogenetic data provided an insight into the diversity status and interrelationship of different species which will be of immense use in their utilization in introgression breeding and the subsequent improvement of the cultivated types. This study also suggested that India is most likely the primary centre of origin and centre of diversity of many of the *Vigna* species. Simultaneously, this study also underlined the importance to generate more genotypic as well as phenotypic data to clearly distinguish different *Vigna* species and unleash their genetic potential for improvement of cultivated types.

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Author contributions GK and AP conceptualized, planned and executed the experiment. GK, SPS and AP analyzed the data and interpreted the results. GK, YS, BP and PS undertook field and laboratory experiments, LM provided seeds of some of the accessions. SG, GRP, NPS provided suggestions for improvement of the experiments and the manuscript. All authors read and approved the manuscript.

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Declarations

Conflict of interest The authors declare no conflict of interest.

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