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MORPHOMETRIC CHARACTERIZATION AND EVOLUTIONARY RELATIONSHIPS AMONG THE WILD *VIGNA* SPECIES OF THE WESTERN GHATS

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Abstract

The present study was focused on collection of wild *Vigna* species from diverse localities of the Western Ghats of southern India. It has been comprehensively observed that highest variableness expressed in peduncle length while lowest in seed length. The outcome of Pearson correlation analysis exhibits significant variations in vegetative, floral and reproductive traits among the wild *Vigna* species. The dendrogram analysis clearly distinguished the Group-I and Group-II of the sub-cluster-I of *V. silvestris* (*SlvUdp*, *SlvRnp*, *SlvBgv*, *SlvSrk* and *SlvKar*) and further Group-II classified as IIA and IIB of *V. sublobata* (*SubBlr* and *SubHan*) and (*SubKsd*, *SubSrk* and *SubPvt*) are very closely relatives respectively, whereas *V. bournaea* of Group-III accessions (*BorMkf*, *BorUdp*, *BorBgv*, *BorKun* and *BorBsg*) has been exhibited distant relatives of the sub-cluster-II. Hence, the cluster analysis revealed the evolutionary relationships among wild species of *Vigna*.

***Key words* :** Correlation coefficient, Dendrogram, Morphometric analysis, Southern India *Vigna*, Western Ghats.

Introduction

The Western Ghats of India is one of the 34 biodiversity hot spots constitute the diverse two-hundred globally most important eco regions around the world (Olson and Dinerstien, 1998; Aravind *et al.,* 2007). The Western Ghats biogeographic region running parallel to the west coast of south Gujarat of Tapti river to Kanyakumari of peninsular India (Covers states of Gujarat, Maharashtra, Goa, Karnataka, Kerala and Tamil Nadu). According to Nair and Daniel (1986) around 4000 species of flowering flora mainly angiosperms have more than 1500 species and are endemic to various locations of the Western Ghats. The Southern Western Ghats is one of the regional hot spots for various endemic plants and provides a useful resource for forest products (Nayar, 1996). Hence the Southern Western Ghats is recognized as one of the primary sources for unique distribution of endemic wild *Vigna* species.

The family of Leguminosae has been classified into three subfamilies as Papilionoideae, Caesalpinioideae and Mimosoideae. The subfamily Papilionoideae further categorized into more than 476 and 13,860 of genera and species respectively (Wojciechowski *et al.,* 2004). The genus *Vigna* belongs to the subfamily Papilionoideae and tribe Phaseoleae of family Fabaceae, more than hundred to two hundred species are reported around the world (Fery, 2002; Thulin *et al.,* 2004; Delgado-Salinas *et al.,* 2011; Takahashi *et al.,* 2016, Aditya, 2018; Ali *et al.,* 2018). Whereas sixteen to seventeen species recorded (Verdcourt, 1970; Marechal *et al.,* 1978; Tateishi, 1996; Bisht *et al.,* 2005) in the Asian subcontinent. According to Babu et al. (1985) more than twenty three species were observed as cultivated and wild forms in India.

The malnutrition is one of the major issues around the world; there are 867 million chronically undernourished people in the world (FAO, 2013) recent data (FAO, 2018) noted that about 800 million of human population is suffering from critical hunger around the globe. The Indian subcontinent is going through the most critical situation for the same in South Asia and India, the majority of the human population chiefly relying on legumes which well known for their protein rich supplements (Singh and Singh,

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1992; Siddhuraju and Becker, 2001). The genus *Vigna* has been used as economically and nutritionally very vital pulse crop extensively afforded proteins, nutrients and minerals, supplied significant level of folate, potassium, iron, magnesium and cholesterol free from low quantity fats (Andersan *et al.,* 1984; Grusak, 2002). Furthermore, it exhibits considerable quantity of chief source for biotic, abiotic stress resistance during breeding programmes. The important primary and secondary metabolites from *Vigna* species known to reduce various heart related risks of cardiovascular and coronary heart disease (Ahuja and Singh, 1977; Ignacimuthu and Babu, 1987; Jacob *et al.,* 2015; Shweta *et al.,* 2017).

Hence more focus is given towards conservation and sustainable utilization of this essential food crop in developing nations of Asia, Africa and Latin America. The Food and Agriculture Organization Statistics (FAO, 2013) documented that, in the world India is the topmost country an area covered about 26,574 thousand hectares (ha) and produced 17, 236 thousand tonnes in 2010 pulses production. The area of 29 million hectares was supplied about 23 million tons of *Vigna* crop around the world (FAOSTAT, 2013). By the consumer preference of *Vigna* species particularly cowpea around the global production increases to 7.0 from 1.3 million tons from 1981 to 2013 (Gonçalves *et al.,* 2016).

The morphometric evaluation may be used to quantify an attribute of evolutionary importance of the traditional approach towards characterization provides quality advantages and cost-effective approaches. According to Douglas and Soltis (2003), it is necessary to incorporate the evolutionary studies among the various related crop species to reveal their phylogenetic relationships and it is used as the important criteria for the crop improvement programmes to reassign the suitable character. Hence, systematic investigation of wild *Vigna* species revealed the significant range of genetic diversity based on the morphological characters. The dendrogram analysis was afforded the most appropriate method for characterization and grouping. It is necessary to incorporate such methodology in exploring wild *Vigna* species for future crop enhancement and utilization.

Materials and Methods

The wild *Vigna* species of the legume family constitutes unique accessions at various locations of the Western Ghats. In the present investigation wild *Vigna* accessions were collected at southern parts of the Western Ghats, predominantly in Karnataka and Kerala. Further, all the accessions were identified and confirmed by referring standard Flora (Hooker, 1872-1897; Cecil and Dan, 1976; Gamble, 1984). The field visit was conducted during the period of October to December (2015-2016). An overall fifteen accessions of three *Vigna* species such as *Vigna bournaea* Gamble, *Vigna radiata* var. *sublobata* (Roxb.)Verdc. and *V. mungo* var. *silvestris* Lukoti, Marchal & Otoul (Fig. 1 and Table 1) surveyed and their seeds were collected.

The collected seeds were grown in experimental greenhouse at Department of studies in Botany, Karnatak University, Dharwad in India for further observations. The morphological characters were quantified with the help of descriptors of (IBPGR) the International Board for Plant Genetic Resources (1983) and (IPGRI) International Plant Genetic Resources Institute (2006) and were recorded. Figure 1 illustrates preliminary observations of *Vigna* species collected during the field survey. After the successful growth at experimental field morphological evaluation was performed mainly focusing on agronomic characters such as vegetative characters of (PHT)-Plant height (m), (TLL)-Terminal leaflet length (cm), (TLW)-Terminal leaflet width (cm) and (PEL)-Peduncle length (cm). In addition, the floral traits (NFR)-Number of flower per raceme and reproductive traits such as (PDP)-Number of pods per peduncle, (PDL)-Pod length (cm), (SDL)-Seed length (mm), (SDW)-Seed width (mm), (HSW)-Hundred seed weight (g), (NSP)-Number of seeds per pod, (NLP)-Number of locules per pod and (SSP)-Seed set percentage were analyzed.

The geographical locations of collected wild *Vigna* accessions were mentioned in Figure 2. Mean values of various traits were recorded (Table 1). Furthermore, Table 2 illustrates the range, minimum, maximum, mean, standard error, variance and standard deviation for thirteen morphological characters of fifteen accessions of three species of wild *Vigna*. The Pearson correlation coefficient analysis was carried out for correlation between various traits (Table 3). Fig. 3 depicts study of evolutionary relationships among the various wild *Vigna* species and the dendrogram analysis was carried out and table 4 illustrates grouping patterns of various accessions. All the statistical analysis was carried out using IBM SPSS statistical software (2011).

Results and Discussion

The geographical distribution of wild *Vigna* species

The different wild *Vigna* species in Western Ghats of Southern India surveyed and collected at various regions of Karnataka and Kerala states, the *V. sublobata* accessions were collected and respective accession codes were given such as *SubKsd* at Kallanchira of the Kasaragod district in Kerala and

Fig. 1: *V. bournaea* (A1-Habit, A2- pods with flower and A3-seeds), *V. sublobata* (B1-Habit, B2-pods with flowers and B3-seeds) and *V. silvestris* (C1-Habit, C2-pods and C3-seeds).

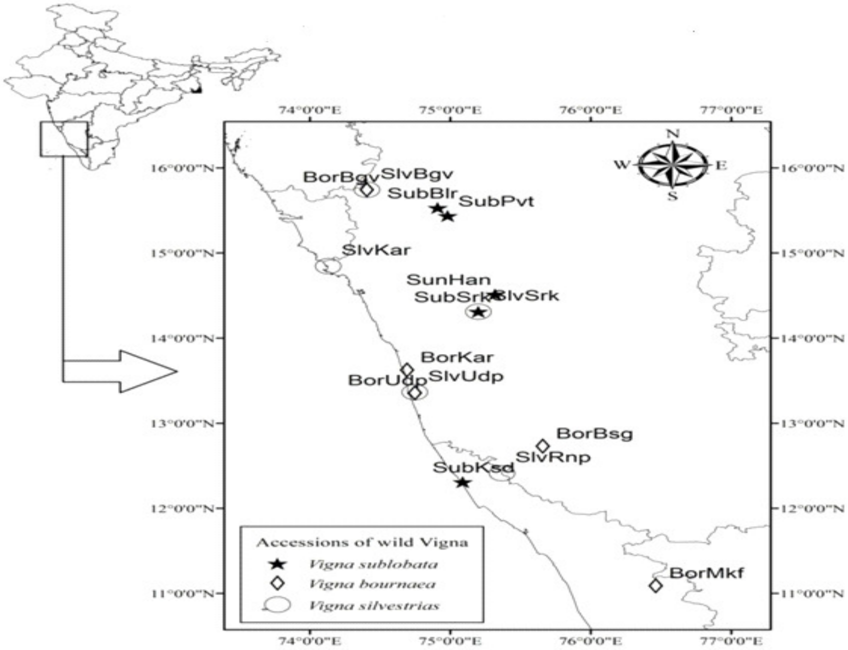

Fig. 2: The distribution of fifteen accessions of wild *Vigna* species in Western Ghats of Southern India.

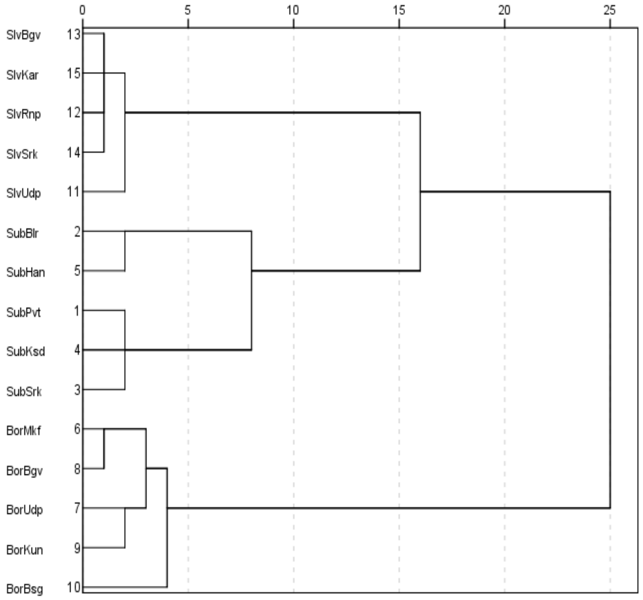

Fig. 3: Dendrogram cluster analysis using average linkage (Between Groups) and method of Euclidean distance among the fifteen accessions of wild *Vigna* species.

SubBlr at Belur village of Dharwad, *SubSrk* at Holekatte village of Shiralkoppa in Shimoga, *SubPvt* at Pavate nagar of Dharwad and *SubHan* at Tumarikoppa near to Hansbavi at Haveri of Karnataka. Furthermore, similar codes were given to *V. bournaea* species as *BorMkf* collected at Mukkali forest of Silent Valley of Pallacaud districts of the Kerala, *BorUdp* at Doddana Gudde in Udupi, *BorBgv* at Uchavade in Belagaum, *BorKun* at Kunadapura and *BorBsg* at Bisaleghat of the Hasana district are found specifically in Karnataka. Additionally, *V. silvestris* accessions such as *SlvRnp*, *SlvUdp*, *SlvBgv*, *SlvSrk* and *SlvKar* were collected at the grassland of Ranipuram forest in Kasaragod district of Kerala, Ambagilu of Udupi, Uchavade of Belgaum, Holekatte of Shiralkoppa in Shimoga and Sadashivagad of Karwar districts in Karnataka respectively. Hence the most important species of different wild *Vigna* accessions were surveyed and clearly depicted in Figure 2.

Descriptive analysis for the various morphological characters in wild *Vigna* species

All the wild *Vigna* accessions were studied for morphometric analysis, considerable highest variability expressed in various quantitative traits (Table 3). The highest variance (18.22) was observed in peduncle length of their standard deviation (4.27) and range (9.33) from minimum to maximum *viz.,* 8.47 to 17.80 values are respectively. Followed by number of locules per pod has the next level higher variance (5.15), their standard deviation (2.27) and ranges (5.77) from minimum to maximum values are 7.40 to 13.17 respectively. Followed by that, next higher level variance (4.95) in the trait of the number of seeds per of pod of their standard deviation (2.22) and ranges (5.70) from minimum to maximum such as 7.20 to 12.90 respectively.

The lowest variability is seen in primarily as the traits of seed length 0.03 is lowest variance and maximum, minimum, range and standard deviation are 3.03, 2.43, 0.60 and 0.17 respectively. Followed by second level lower variance (0.04) observed in the trait of plant height, their standard deviation (0.21) and ranges (0.60) from minimum to maximum *viz.,* 1.75 to 2.35 respectively. The third level lowest variance (0.04) in the trait of the seed width, their standard deviation (0.21) and ranges (0.74) from minimum to maximum such as 1.93 to 2.67 respectively depicted (Table 2). Hence the quantitative traits considerably showed the low and high-level variability and this is a major key for the morphometric analysis and describes the taxonomic resemblance of the different species for genetic diversity among the genus of *Vigna* accessions.

Pearson correlation coefficient analysis

The Pearson correlation analysis was illustrated the variations in seed set percentage character and recorded as positively significantly correlated with plant height (0.587*) and positively correlated with the traits such as leaflet length (0.415), leaflet width (0.351), peduncle length (0.512), seed length (0.198), seed width (0.432), seeds per pod (0.123) and locules per pod (0.037). The negatively significantly correlated with the characters such flowers per raceme (-0.669**), pods per peduncle (-0.640*) and pod length (-0.714**). And negatively correlated with the trait of seed weight (-0.082). As well as the trait of number of locules per pod was observed as positively significantly correlated with the characters such as plant height (0.623*), leaflet width (0.810**), peduncle length (0.677**), seed length (0.526*), seeds per pod (0.996**) and positively correlated with leaflet length (0.118), flowers per raceme (0.411), pods per peduncle (0.334), pod length (0.226) and seed width (0.252). And negatively significantly correlated with seed weight (-0.604*).

Further, the number of seeds per pod character was depicted as positively significantly correlated with the traits such as plant height (0.670**), leaflet width (0.835**), peduncle length (0.718**), seed length (0.540*) and positively correlated with the characters such as leaflet length (0.153), flowers per raceme (0.348), pods per peduncle (0.274), pod length (0.162) and seed width (0.289). While negatively significantly correlated with the trait of seed weight (-0.607*). Consequently the character of seed weight was revealed positively correlated with

Table 1: The quantitative mean values of thirteen traits of wild *Vigna* accessions.

ACC	PHT	TLL	TLW	PEL	NFR	PDP	PDL	SDL	SDW	HSW	NSP	NLP	SSP
SubPvt	1.83	7.83	7.50	9.33	10.11	8.60	6.30	2.53	1.97	1.38	10.80	11.50	93.91
SubBlr	1.95	7.33	7.27	8.67	10.52	8.16	6.20	2.97	2.32	2.13	11.50	11.80	97.46
SubSrk	1.85	8.20	7.30	8.87	11.20	8.80	6.27	2.85	2.33	2.20	11.17	11.90	93.87
SubKsd	1.90	8.50	7.60	9.20	10.80	8.15	6.33	2.67	2.13	1.83	10.50	11.20	93.75
SubHan	2.05	8.30	7.20	9.07	10.17	8.17	6.13	2.51	1.93	1.65	11.33	11.60	97.67
BorMkf	2.30	8.80	8.61	17.33	8.50	7.67	3.87	3.03	2.67	1.83	12.60	12.83	98.21
BorUdp	2.20	8.73	8.67	17.53	7.40	6.80	3.85	2.91	2.41	1.73	12.90	13.17	97.95
BorBgv	2.35	8.33	8.71	17.67	8.62	7.90	4.20	2.83	2.53	1.62	12.50	12.80	97.66
BorKun	2.30	9.40	9.13	17.80	7.33	6.90	3.93	2.73	2.43	1.53	12.70	12.83	98.99
BorBsg	2.29	9.67	8.82	17.73	8.70	7.80	4.11	2.76	2.41	1.60	11.50	11.80	97.46
SlvUdp	1.92	8.18	6.87	8.71	7.50	7.12	4.22	2.77	2.43	2.23	7.60	7.90	96.20
SlvRnp	1.75	8.67	6.80	8.85	7.30	6.83	4.03	2.68	2.34	2.20	7.20	7.40	97.30
SlvBgv	1.80	8.40	6.93	8.93	7.90	7.67	4.30	2.71	2.38	2.33	7.40	7.60	97.37
SlvSrk	1.90	8.50	6.67	8.47	7.60	6.80	4.13	2.57	2.15	1.93	7.60	7.80	97.44
SlvKar	2.10	8.57	6.92	8.67	7.80	7.68	4.27	2.43	2.10	1.87	7.30	7.50	97.33

ACC-Accessions, Plant height (PHT), Terminal leaflet length (TLL), Terminal leaflet width (TLW), Peduncle length (PEL), Number of flower per raceme (NFR), Number of pods per peduncle (PDP), Pod length (PDL), Seed length (SDL), Seed width (SDW), Hundred seed weight (HSW), Number of seeds per pod (NSP), Number of locules per pod (NLP), Seed set percentage (SSP).

Table 2: Descrpitive values of range, minimum, maximum, mean, standard error, variance and standard deviation of wild *Vigna* species among fifteen accessions.

Traits	Variance	Standard Deviation	Standard Error	Maximum	Minimum	Mean	Range
Peduncle length (cm)	18.22	4.27	1.10	17.80	8.47	11.79	9.33
Number of locules per pod	5.15	2.27	0.59	13.17	7.40	10.64	5.77
Number of seeds per pod	4.95	2.22	0.57	12.90	7.20	10.31	5.70
Seed length (mm)	0.03	0.17	0.04	3.03	2.43	2.73	0.60
Plant height (m)	0.04	0.21	0.05	2.35	1.75	2.03	0.60
Seed width (mm)	0.04	0.21	0.05	2.67	1.93	2.30	0.74
Pod length (cm)	1.12	1.06	0.27	6.33	3.85	4.81	2.48
Terminal leaflet length (cm)	0.32	0.56	0.15	9.67	7.33	8.49	2.34
Terminal leaflet width (cm)	0.75	0.87	0.22	9.13	6.67	7.67	2.46
Number of flower per raceme	1.98	1.41	0.36	11.20	7.30	8.76	3.90
Number of pods per peduncle	0.43	0.66	0.17	8.80	6.80	7.67	2.00
Hundred seed weight (g)	0.09	0.29	0.08	2.33	1.38	1.87	0.95
Seed set percentage	2.74	1.65	0.43	98.99	93.75	96.84	5.24

the characters such as seed length (0.226) and seed width (0.227). While negatively significantly correlated with the traits such as plant height (-0.592*), leaflet width (-0.638*) and peduncle length (-0.551*). While negatively correlated with the characters such as leaflet length (-0.330), flowers per raceme (-0.079), pods per peduncle (-0.120) and pod length (-0.039). Followed by, the trait of the seed width was observed as positively significantly correlated with the characters such as leaflet width (0.536*), peduncle length (0.646**), seed length (0.821**) and positively correlated with the traits such as plant height (0.485) and terminal leaflet length (0.338). While negatively significantly correlated with pod length (-0.618*) and negatively correlated with the characters of flowers per raceme (-0.394) and pods per peduncle (-0.335).

Later, the seed length character was observed as positively correlated with the traits such as plant height (0.334), leaflet width (0.484), peduncle length (0.500) and flowers per raceme (0.055). Whereas negatively correlated with the traits such as leaflet length (-0.022), pods per peduncle (-0.028) and pod length (-0.160). Followed by the pod length trait was observed as positively significantly correlated with the characters such as

Table 3: The Pearson correlation coeffcient analysis for fifteen accessions of wild *Vigna*.

Traits	PHT	TLL	TLW	PEL	NFR	PDP	PDL	SDL	SDW	HSW	NSP	NLP	SSP
PHT	1												
TLL	.552*	1											
TLW	.858**	.573*	1										
PEL	.893**	.642**	.961**	1									
NFR	-.245	-.498	-.103	-.316	1								
PDP	-.167	-.459	-.077	-.260	.905**	1							
PDL	-.461	-.630*	-.314	-.537*	.935**	.815**	1						
SDL	.334	-.022	.484	.500	.055	-.028	-.160	1					
SDW	.485	.338	.536*	.646**	-.394	-.335	-.618*	.821**	1				
HSW	-.592*	-.330	-.638*	-.551*	-.079	-.120	-.039	.226	.227	1			
NSP	.670**	.153	.835**	.718**	.348	.274	.162	.540*	.289	-.607*	1		
NLP	.623*	.118	.810**	.677**	.411	.334	.226	.526*	.252	-.604*	.996**	1	
SSP	.587*	.415	.351	.512	-.669**	-.640*	-.714**	.198	.432	-.082	.123	.037	1

* Correlation is significant at the 0.05% level and ** Correlation is significant at the 0.01% level of probability.

Table 4: Characteristics of cluster analysis among fifteen accessions of wild *Vigna* species.

Group lines	Sub-cluster	Percent of Sub-cluster lines	Accession Code
I	5	33.33	*SlvUdp, SlvRnp, SlvBgv, SlvSrk, SlvKar*
IIA	2	13.33	*SubBlr, SubHan*
IIB	3	20.00	*SubKsd, SubSrk, SubPvt*
III	5	33.33	*BorMkf, BorUdp, BorBgv, BorKun, BorBsg*

flowers per raceme (0.935**) and pods per peduncle (0.815**). Whereas negatively significantly correlated with the traits such as leaflet length (-0.630*) and peduncle length (-0.537*). While negatively correlated with the some characters such as plant height (-0.461) and terminal leaflet width (-0.314).

Furthermore, the trait pods per peduncle was taken to assessment as positively significantly correlated with the character of flowers per raceme (0.905**). Whereas negatively correlated with the traits such as plant height (-0.167), leaflet length (-0.459), leaflet width (-0.077) and peduncle length (-0.260). The number of flowers per raceme traits was observed as positively correlated with plant height (-0.245), leaflet length (-0.498), leaflet width (-0.103) and peduncle length (-0.316). Hence present investigation has shown the considerable extensive variations among different characters of three species of the wild *Vigna* of fifteen accessions depicted (Table 3).

Cluster analysis among the fifteen accessions of wild *Vigna* species

The cluster of dendrogram structure classified at 25 percent level, mainly into two sub-clusters such as sub-cluster I and II. And sub-cluster-I, further classified at nearly 16 percent level into Group-I and Group-II. Furthermore, sub-cluster-I has been categorized as group-I about nearly at 2 percent level point made include five lines shows 33.33 percentage of *V. silvestris* (*SlvUdp*, *SlvRnp*, *SlvBgv*, *SlvSrk* and *SlvKar*). Whereas the group-II further classified at nearly about 8 percent level categorized as group IIA and IIB respectively, the group-IIA has been considered as two lines exhibits about 13.33 percentage of *V. sublobata* accessions (*SubBlr* and *SubHan*) and group-IIB has been considered as three lines shows about 20.00 percentage of *V. sublobata* accessions (*SubKsd*, *SubSrk* and *SubPvt*) and both groups have made at 2 percent level respectively.

Then, to depict the main sub-cluster-II further categorized into Group-III at 4 percent level of the dendrogram, include five lines exhibits about 33.33 percentage of *V. bournaea* (*BorMkf*, *BorUdp*, *BorBgv*, *BorKun* and *BorBsg*). Hence the above dendrogram inspection clearly describes the Group-I and Group-II of the sub-cluster-I accessions of *V. silvestris* and *V. sublobata* shows nearly closely relatives and these findings were also agreed with the Bisht *et al.*, (2005), while *V. bournaea* of Group-III have been exhibited distantly relatives among the three wild *Vigna* species. The results of dendrogram analysis illustrated very evidently the evolutionary relationships among the wild *Vigna* species of fifteen accessions (Fig. 3 and Table 4).

Conclusion

The present investigation of wild *Vigna* collected from the Western Ghats of southern India exhibit highest variability expressed in their quantitative traits particularly the maximum variance in peduncle length and followed by lowest variability in traits of seed length. Hence descriptive values of different traits of low and high-level variability are the general key parameters for morphometric analysis and describe the taxonomic resemblance of the various species among genus *Vigna*. The results of the Pearson correlation coefficient for different quantitative traits have clearly showed remarkable variations. Finally, the dendrogram analysis clearly distinguishes the Group-I and Group-II of the sub-cluster-I accessions of *V. silvestris* and *V. sublobata* was observed as close relatives, while *V. bournaea* of Group-III have been exhibited as distant relatives. Hence, cluster analysis was clearly revealed the evolutionary relationships among the species of wild *Vigna*.

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