GENETIC DIVERGENCE AND POPULATION DIFFERENTIATION IN *VIGNA SUBLOBATA* (ROXB) BABU AND SHARMA (LEGUMINOSAE-PAPILIONOIDEAE) AND ITS CULTIGENS

SUBODH K. SHARMA, C. R. BABU* and B. M. JOHRI*

Department of Environment, Bikaner House, Shahjahan Road, New Delhi 110011, India.
* Department of Botany, University of Delhi, Delhi 110007, India.

ABSTRACT

Genetic divergence among nine natural populations of *Vigna sublobata* (Roxb.) Babu and Sharma, and two agricultural populations of *V. mungo cv.* T-9 and *V. radiata cv.* R-16 was investigated by multivariate statistical analysis. Six metric characters (scored from plants grown under uniform environmental conditions in the experimental garden) were employed in the analyses. D^2 statistic and Euclidean distance coefficient were used as measures of genetic divergence. Cluster analyses were also carried out. *V. radiata* is distantly related to the populations of *V. sublobata* and *V. mungo*, and shows affinity with one of the populations of *V. sublobata* designated S_6. *V. sublobata* is genetically diverged from *V. mungo* and constitutes a distinct species. The populations of *V. sublobata* show different levels of divergence. It has been suggested that the population S_6 can be used as a bridging genotype in hybridization programmes involving *V. radiata* and *V. mungo*. The results are discussed in the light of relevant literature.

INTRODUCTION

IT is generally agreed among plant breeders that large and diverse gene pools are increasingly required to meet the ever-changing demands, opportunities, and challenges of the future[1-4]. The importance of such gene pools is not only because of their contribution to broaden the genetic base of crop plants, but also because of their role in generating transgressive variation[5,6].

The wild relatives are important gene pools, and constitute an invaluable resource for the genetic manipulation of agricultural crops. Further, the natural populations of wild relatives are endowed with a wider ecological amplitude. Consequently, they provide genetic basis for long-term adaptation and may even contribute to short-term physiological flexibility[7].

Vigna sublobata is the putative progenitor of *V. mungo*, and probably also of *V. radiata*. Taxonomically, these three species form a 'notorious complex'[8]. Studies on the population biology of this complex revealed significant genetic variability in morphological and biochemical traits[9]. The assessment of genetic relatedness among different populations of the complex *mungo-radiata-sublobata* is essential for the selection schemes at the utilization of the genetic variability encountered in natural populations.

Agricultural geneticists have widely used multivariate statistics to assess genetic divergence of genotypes used as parental stocks in plant breeding programmes[5,10-13].

The present paper deals with the genetic divergence and population differentiation in *V. sublobata*, *V. mungo* and *V. radiata*, as evident by D^2 and Euclidean distance coefficient values and cluster analyses.

MATERIALS AND METHODS

Nine different natural populations of *V. sublobata* (designated S_1, S_2, S_3, S_4, S_5, S_6, S_7, S_8 and S_9) were sampled from different ecozones of Western Ghats of Maharashtra range (table 1). These populations, together with plants of *V. mungo cv.* T-9 (VM) and *V. radiata cv.* RS-16 (VR), raised from seeds obtained from the Indian Agricultural Research Institute, New Delhi, were grown under uniform environmental conditions at the experimental garden of the Department of Botany, University of Poona, Poona, India. Complete randomised design (CRD) was used as an experimental design. Six normally distributed metric traits — (a) ratio of stipule length to stipule breadth, (b) number of tubercles (swollen nodes of inflorescence axis bearing flowers) per inflorescence, (c) length of pod, (d) breadth of pod, (e) number of seeds per pod, and (f) ratio of seed length to breadth — were scored when the plants bore ripe fruits.

One-way 'analysis of variance' was carried out for all the six traits to test the statistical significance of

Table 1 *Populations sampled, their sampling sites and ecological features*

Population	Site	Ecological features
S_1	Base of hillock; no forest	Common
S_2	Middle of hillock; barren exposed slopes	Common
S_3	Above the middle of hillock; near water tank	Not common; associated with asteraceous herbs
S_4	Top of hillock; exposed grassy slopes	Abundant; other associates are grasses
S_5	Plateau; roadsides (2 km stone)	Rare; gregarious in gravelly soils
S_6	Plateau; roadsides (3 km stone)	Rare; gregarious
S_7	Behram's plateau; open grassy slopes	Frequent; gregarious; other associates are grasses
S_8	Behram's plateau; open grassy slopes	As in S_7
S_9	Railway track; periphery of forest	Common; along shady, moist areas

The geographical region of S_1 to S_4 was Poona and S_5 to S_9 belong to Khandala-Lonavala region.

variance between populations and to account for the variability within the populations.

Following Sneath and Sokal[14], the D^2 values between pairs of populations in all combinations and the Euclidean distance coefficient values between pairs of populations on overall characters (Q-technique) were calculated. The computation was done on IBM 1620 computer (with the programme available at the Indian Agricultural Research Statistical Institute, New Delhi).

As suggested by Rao[15], Tocher's method was followed for the determination of group constellation based on D^2 matrix. The clustering of populations based on distance matrix was done by one of the arithmetic average clustering methods, the unweighted pair-group method, following Sneath and Sokal[14].

In order to rank the populations analyzed and to equate them with conventional taxonomic categories, the clusters formed were classified into groups (phenons) at two levels of distance coefficient (phenon lines) of the dendrogram.

RESULTS

Table 2 provides the results of one-way analysis of variance for all the six traits scored. For all the traits, the variance between populations was statistically significant at $P < 0.05$.

The D^2 values between pairs of populations in all possible combinations are given in table 3. VR showed highest D^2 values in all combinations, and the mi-

Table 2 *One-way analysis of variance for six phenotypic traits of different populations*

Character	Source of variation	Degrees of freedom	Mean squares (MS)	Variance ratio (F)
Ratio of stipule Length to breadth	Between populations Within populations	10 153	57.2313 1.4307	40.0023**
Number of tubercles per inflorescence	Between populations Within populations	10 153	193.6231 2.6770	72.3280**
Length of pod	Between populations Within populations	10 153	13.7955 0.1719	80.2530**
Breadth of pod	Between populations Within populations	10 153	6.7260 0.0038	127.4700**
Number of seeds per pod	Between populations Within populations	10 153	66.1176 1.4852	44.5176**
Ratio of seed length and breadth	Between populations Within populations	10 153	0.3199 0.0207	15.4500**

** Highly significant ($P < 0.01$).

Table 3 D^2 *values between pairs of populations in all possible combinations*

Population	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	S_9	VM	VR
S_1	—	20.92	11.61	15.83	10.13	63.28	3.05	14.64	8.50	16.64	191.20
S_2		—	3.94	4.78	12.13	26.74	9.15	4.14	10.60	7.49	133.21
S_3			—	6.12	4.85	25.43	4.35	5.10	1.87	6.12	145.04
S_4				—	7.78	41.93	5.79	0.69	9.68	9.70	174.15
S_5					—	33.06	4.07	6.39	2.83	8.78	153.12
S_6						—	44.43	40.46	29.33	23.41	76.89
S_7							—	5.21	3.93	7.23	166.32
S_8								—	8.99	11.02	165.06
S_9									—	7.00	156.72
VM										—	128.85
VR											—

nimum value (76.59) was obtained in combination with S_6 which, in fact, revealed maximum distance from VM. The D^2 value between VM and S_3 population was lowest (6.12). Among *V. sublobata* populations, S_6 showed higher D^2 values with all other populations, whereas lower values were obtained for all combinations involving both S_2 and S_7.

The distance coefficient values in all combinations involving VR were high, but the lowest value (1.65) was observed in combination with VM (table 4). However, a high distance coefficient (1.69) was obtained between VM and S_7.

Altogether 6 clusters were formed as a result of group constellation by Tocher method (based on D^2 values between pairs of populations in all combinations) (figure 1). In general, the clustering pattern reflects the genetic divergence among populations.

Table 5 provides the intra- and inter-cluster average distance values ($D = D^2$). The average distance between S_4 and S_8 of cluster I was minimum, whereas the average intra-cluster distance for cluster IV was maximum (3.07). The average distance between clusters III and VI was highest (13.37) and the minimum value was obtained between II and III clusters.

Figure 2 illustrates the genetic relation between populations based on the distance coefficient values. VM was the first to join with S_7 population at $\Delta = 0.67$ (0.67 phenon line), 6 groups were formed; whereas only 3 groups observed at $\Delta = 1.22$ (1.22 phenon line).

DISCUSSION

Genetically, *V. radiata* is distantly related to both *V. sublobata* and *V. mungo* as evident from D^2 statistical analysis (table 3; figure 1), distance matrix (table 4), and grouping of clusters (figure 2). This strongly justified its treatment as a distinct species—a view also held by other workers[8,16,17]. Recent studies on seed-coat patterns (by SEM) also confirm this view[18]. VR is related to VM as well as to *V. sublobata*

Table 4 *Euclidean distance between pairs of populations in all combinations*

Populations	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	S_9	VM	VR
S_1	—										
S_2	1.91	—									
S_3	1.22	1.76	—								
S_4	1.38	0.61	1.33	—							
S_5	1.21	1.05	0.65	0.98	—						
S_6	2.11	1.58	1.30	1.81	1.28	—					
S_7	0.66	0.71	1.20	0.78	0.95	1.87	—				
S_8	1.36	0.96	0.59	0.95	0.67	1.45	1.13	—			
S_9	1.04	0.69	0.88	0.54	0.64	1.23	0.69	0.98	—		
VM	0.80	0.67	0.92	0.65	0.67	1.69	0.34	0.83	0.61		
VR	2.46	2.30	2.25	2.77	2.48	1.72	2.48	2.53	2.13	1.65	
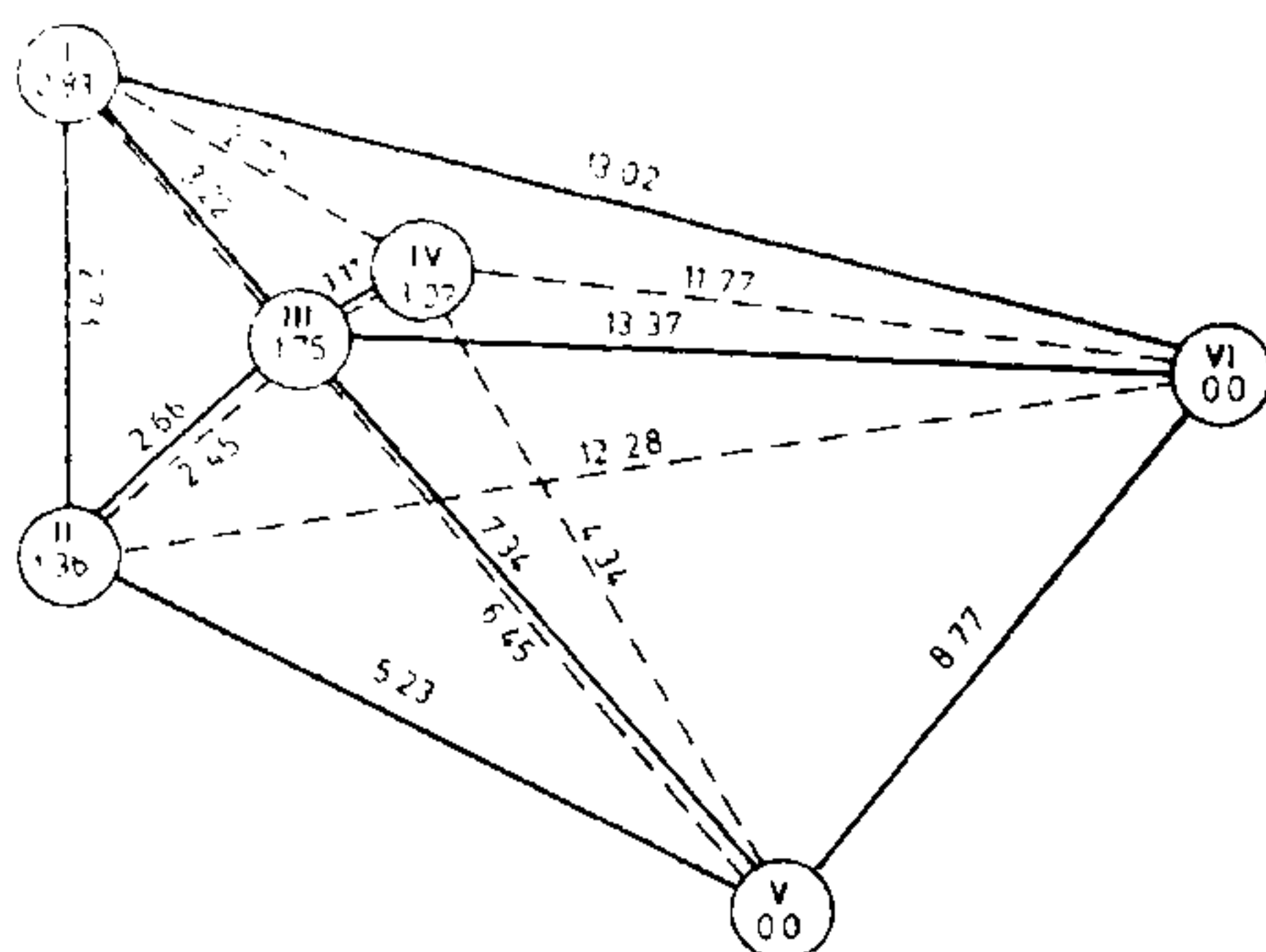
Figure 1. Group constellation diagram (based on cluster analysis of D^2 values) showing different clusters formed and their interrelationships. Values indicated on lines represent genetic divergence between clusters (D values). S_1-S_9 = populations of *Vigna sublobata*; VM = *V. mungo*; VR = *V. radiata*. I—Cluster I (S_4-S_8); II—Cluster II (S_3-S_9); III—Cluster III (S_1-S_7); IV—Cluster IV (S_2-S_5-VM); V—Cluster (S_6); VI—Cluster VI (VR). The composition of cluster is in parenthesis.

because the average distance between cluster VI (VR) and cluster V (S_6) is lowest among all combinations involving VR cluster (figure 1). Such a relationship is also revealed by the fact that VR is joined with the VM and *V. sublobata* group through the group consisting of S_6 only (*see* figure 2). This implies that the population S_6 may be used as a bridging genotype in the

hybridization programme involving VM and VR for generating transgressive variation and/or to introduce other desirable traits from natural populations to cultivars.

VM is genetically less diverged from *V. sublobata* and is closest to populations S_2, S_5 and S_7. This is evident by the inclusion of S_2, S_5 and VM in a single cluster (IV) of D^2 analysis and also by the fact that both VM and S_7 form the first cluster in the cluster analysis based on distance matrix (figures 1, 2). Such genetic relationship between VM and populations of *V. sublobata* is also evident by the low D^2 and distance coefficient values in combination involving VM with S^2; and the formation of one group comprising VM and all populations of *V. sublobata* except S_6 at $\Delta = 1.22$ (see figure 2). These observations suggest that *V. sublobata* is closely related to *V. mungo* as has been suggested by previous studies[16, 19, 20]. However, the high average intracluster distance for cluster IV $(S_2, S_6$ and VM) implies that both *V. sublobata* and VM are genetically differentiated, and the former could be best treated as a distinct species. Our SEM studies[18] on seed-coat patterns and the results[9] on phenotypic variability confirm this view. The taxonomic aspects of this complex are being published elsewhere.

Among populations of *V. sublobata*, S_6 has reached a higher level of genetic divergence than other populations because the D^2 and distance coefficient values in all combinations involving S_6 and the rest of natural populations are high (see tables 3 and 4). The inclusion of S_6 alone in a distinct cluster (cluster V) implies that it is genetically more distant to all other populations than they are to each other. The other natural populations are also genetically differentiated.

Table 5 *Average intra- and inter-cluster distance (D)* = (D^2) *values*

Clusters	I	II	III	IV	V	VI
I (S$_4^*$ and S$_8$)	0.83	2.73	3.22	2.70	6.45	13.02
II (S$_3$)		1.36	2.66	2.45	5.23	12.28
III (S$_1$ and S$_7$)			1.75	3.37	7.34	13.37
IV (S$_2$, S$_5$ and VM)				3.07	4.34	11.77
V (S$_6$)					0.00	8.77
VI (VR)						0.00

*Populations included in the cluster
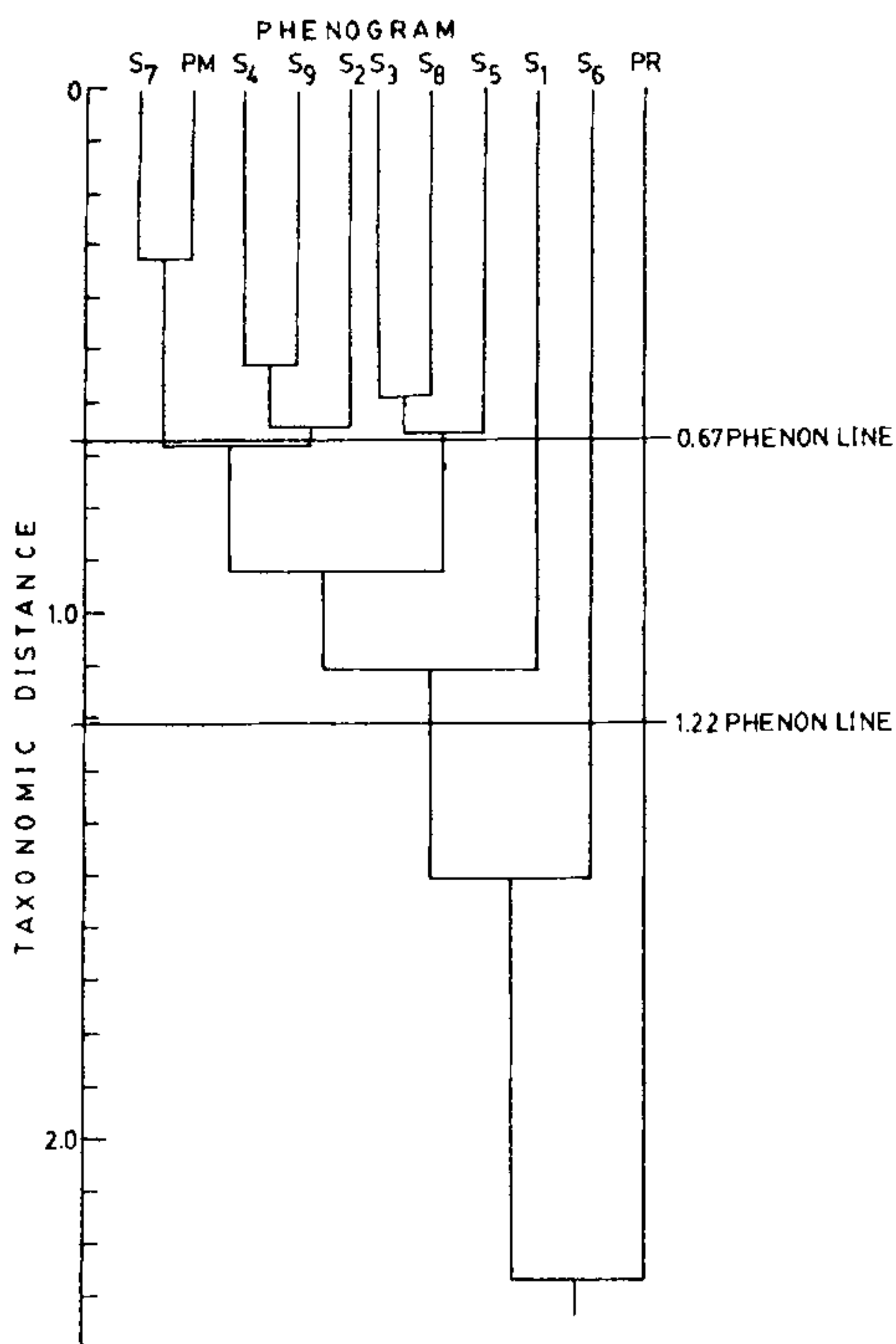
Figure 2. Phenogram (based on cluster analysis of Euclidean distance coefficient values derived from n(6) × t(11) matrix) showing different clusters formed and their interrelationships. Note the formation of six groups at the level $\Delta = 0.67$ (0.67 phenen line) and three groups at the level $\Delta = 1.22$ (1.22 phenen line). S_1–S_9 = populations of *Vigna sublobata*; VM = *V. mungo*; VR = *V. radiata*.

The results obtained from D^2 analysis are in conformity with distance matrix based on Euclidean distance coefficient, except in minor details.

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