Morphometrical studies on black pepper *(Piper nigrum* L.). I. Cluster analysis of black pepper cultivars

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Abstract

Forty four cultivars and seven wild black pepper *(Piper nigrum)* accessions (51 Operational Taxonomic Units) were subjected to single linkage and centroid linkage cluster analysis. The 22 characters employed in the study resolved into two distinct sets. Based on character correlations, six clusters were recognised while certain characters did not show correlation with others. Centroid clustering led to the grouping of the Operational Taxonomic Units into 11 clusters. Among these, four groups had one cultivar, four had two cultivars, one had four cultivars, one had seven cultivars and one had 28 cultivars each. The cultivars that were unique and did not cluster with any other are Karimunda, Panniyur-1, Vadakkan and Kuthiravally. The inclusion of 28 cultivars in one group also shows that a majority of common cultivars resemble one another closely and probably had a common origin. The study is useful in establishing the relative resemblances of major black pepper cultivars grown in Kerala and Karnataka of India.

Key words: black pepper, centroid linkage, cluster analysis, morphometrics, *Piper nigrum,* single linkage.

Abbreviation

OTU: Operational Taxonomic Unit

Introduction

More than 100 cultivars of black pepper *(Piper nigrum* L.) are known to exist in India, the major variability being in Kerala and Karnataka. The cultivars are either named after a place where they are grown or after a person who popularised it in that area, or based on certain morphological traits. As a result the same cultivar is known by different names in different localities and much confusion exists in their identity. Hence, a cluster analysis of major black pepper

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cultivars was carried out during a biosystematic study of the genus *Piper*, with the aim of grouping related cultivars. The present paper reports the results of this study carried out using 44 cultivars and 7 wild collections of *P. nigrum*. The usefulness of cluster analysis in taxonomic studies has been discussed by Sneath & Sokal (1973).

Materials and methods

Fifty one OTUs comprising of 44 cultivars and 7 wild *P. nigrum* collections were included in the study (Table 1). The cultivars were collected from various black pepper growing areas of Kerala and Karnataka and were maintained in the germplasm conservatory of the Indian Institute of Spices Research at Peruvannamuzhi (Kozhikode District, Kerala). The wild collections were collected from forests of Kerala and Karnataka and were also maintained in the germplasm conservatory. Twenty

Table 1. Black pepper cultivars (OTUs) studied for cluster analysis

No.	Cultivar/Accession	No.	Cultivar/Accession
1.	Aimpiriyan	26.	Nedumchola
2.	Arakulam munda	27.	Neyyattinkaramundi
3.	Arimulaku	28.	Ottaplackal (No. 812)
4.	Balancotta	29.	Panniyur-1
5.	Bilimalligesara	30.	Perambramunda
6.	Cheriyakaniakkadan	31.	Perumkodi
7.	Cheppukulamundi	32.	Poonjaranmunda
8.	Cholamundi	33.	Sagar Local
9.	Jeerakamundi	34.	Thevanmundi
10.	Karimunda	35.	Thommankodi
11.	Kaniakkadan	36.	Thulamundi
12.	Karivilanchy	37.	Udakkere
13.	Karimkotta	38.	Uthirancotta
14.	Kalluvally (Pulpally)	39.	Vadakkan
15.	Kalluvally (2)	40.	Valiakaniakkadan
16.	Kallubalancotta	41.	Vattamundi
17.	Kottanadan	42.	Vellanamban
18.	Kuching	43.	Velliyaranmunda
19.	Kuriyalmundi	44.	Vokkalu
20	Kuthiravally	45.	*P. nigrum* (wild) (Acc. 2077)
21.	Kurimalai	46.	*P. nigrum* (wild) (Acc. 2071)
22.	Malamundi	47.	*P. nigrum* (wild) (Acc. 2009)
23.	Mundi	48.	*P. nigrum* (wild) (Acc. 2059)
24.	Narayakkodi	49.	*P. nigrum* (wild) (Acc. 2060)
25.	Neelamundi	50.	*P. nigrum* (wild) (Acc. 2015)
		51.	*P. nigrum* (wild) (Acc. 2062)

two morphological characters were employed in the study (Table 2). One hundred observations were recorded for each character from five clonal plants and the pooled data were used for analysis.

Two cluster analysis techniques were used: 1) The average linkage method or unweighted pair group method using arithmetic averages (UPGMS) for grouping the characters (Hartigan 1981), and 2) The centroid linkage method or unweighted pair group centroid method (UPGCM) for grouping the cultivars (Engleman 1981). The BMDP-81 programme package developed by the Department of Biomathematics, University of California, Los Angeles, USA was used for the analysis. The computer analysis was carried out at the Computer Centre of the Carneigie-Mellon University, Pittsburgh, USA. The programmes employed were BMDP/IM (average linkage) and BMDP/2M (centroid linkage).

Results and discussion

Cluster analysis of characters

The statistics for the 22 characters studied and the intercharacter correlations are given in Tables 3 and 4, respectively. The process of average linkage is given in Table 5 and the results of cluster analysis of characters are depicted in Fig 1. Character correlations revelaed that most of the characters had weak correlations only. The clustering of characters by the average linkage analysis led to the following grouping:

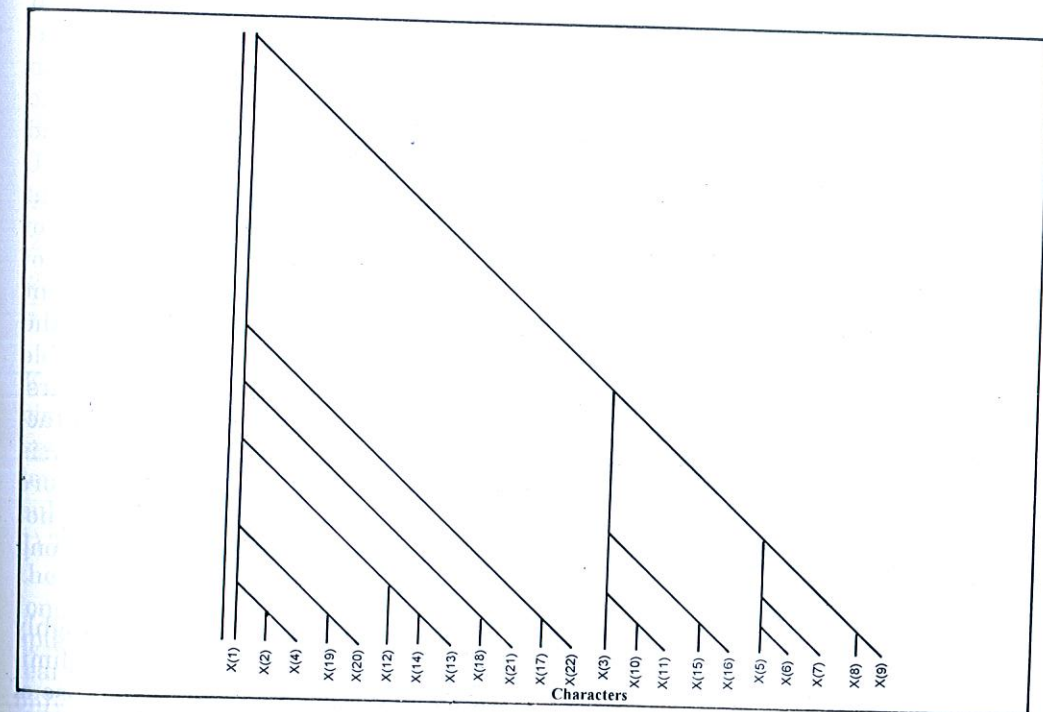1. Leaf length (LL), leaf breadth (LB), leaf size index

Fig. 1. Clustering of characters in black pepper

Table 2. Characters and their states used in the study of black pepper cultivars

No.	Character
1	Leaf length (LL)
2	Leaf breadth (LB)
3	Leaf length/Leaf breadth
4	Leaf size index (LL x LB / 100)
5	Leaf thickness
6	Epidermal thickness (lower)
7	Epidermal thickness (upper)
8	Mesophyll thickness
9	Stomatal frequency per mm^2
10	Guard cell length
11	Guard cell breadth
12	Spike length
13	Peduncle length
14	Leaf length/spike length index
15	Leaf shape: 1) ovate 2) cordate 3) ovate-elliptic 4) ovate-lanceolate
16	Leaf base: 1) round 2) cordate 3) acute
17	Leaf margin: 1) even 2) wavy
18	Leaf shape of orthotropic shoot: 1) ovate 2) cordate
19	Fruit shape: 1) round 2) oblong 3) obovate
20	Fruit size: 1) small 2) medium 3) bold
21	Colour of new shoot: 1) purple 2) whitish green
22	Spike shape: 1) straight 2) curved or twisted

All measurements are in mm

2. Fruit shape, fruit size

3. Spike length (SL), LL/SL, peduncle length

4. LL/LB, guard cell length, guard cell breadth

5. Leaf shape, leaf base

6. Leaf thickness, upper epidermal thickness, lower epidermal thickness

Characters such as leaf shape of orthotropic shoots, colour of emerging shoot, leaf margin, spike shape, mesophyll thickness and stomatal frequency were independent having little correlation with other characters. Characters from 1 to 22 were widely distant from characters 3 to 9 and these two sets of characters were wide apart in the cluster diagram (Fig. 1).

In black pepper, leaf and spike characters are very important diagnostic characters. In the case of leaf length and leaf breadth, there is almost a continuous spectrum of variation ranging from the very small leaves of Nedumchola to the large leaves of Balancotta and Doddale. Chandy *et al.* (1984) and Kanakamony *et al.* (1985) used leaf characters including colour variations of the abaxial side for the classification of cultivars. However, in the present study it was found that the colour of the underside of leaves is not a reliable character for identification of cultivars. Ibrahim *et al.* (1984) used leaf characters in a discriminant function analysis between Malabar and Travancore cultivars. The present study could not establish any such differences among the cultivars.

Fruit shape and fruit size though highly correlated, are less useful in the delimitation of cultivars, except in the case of cultivars having easily recognisable traits such as the oblong fruit shape of

Table 3. Statistics for the variables (characters) studied in black pepper cultivars

Variable No.*	Mean	Standard deviation	Smallest Value	Smallest Case	Largest Value	Largest Case
1	133.4573	21.8560	71.4000	44	191.0000	4
2	76.0710	13.4020	47.5000	26	205.6000	20
3	1.7957	0.3020	1.0900	20	2.7000	39
4	103.0976	29.8856	40.0000	26	181.4500	4
5	0.3644	0.0266	0.3130	10	0.4170	37
6	0.1304	0.0123	0.1060	11	0.1650	38
7	0.1034	0.0117	0.0800	34	0.1440	7
8	0.1318	0.0116	0.1050	47	0.1520	26
9	103.9070	15.7790	61.2000	10	130.4000	39
10	0.0253	0.0013	0.0220	17	0.0280	36
11	0.0912	0.0015	0.0150	43	0.0250	39
12	103.3676	28.0055	33.7000	44	171.6000	20
13	11.0347	3.1258	5.3000	44	21.0000	13
14	1.3925	0.4098	0.6700	20	2.4900	49
15	1.4118	0.8984	1.0000	1	4.0000	47
16	1.3529	0.6877	1.0000	1	3.0000	4
17	1.2157	0.4154	1.0000	1	2.0000	2
18	1.2157	0.4154	1.0000	2	2.0000	1
19	1.3725	0.5621	1.0000	1	3.0000	6
20	1.7451	0.7705	1.0000	1	3.0000	3
21	1.0196	0.1400	1.0000	1	2.0000	29
22	1.0784	0.2715	1.0000	2	2.0000	1

* Refer Table 1 for details of characters

Karivilanchy. Spike characters, especially spike length, exhibit a wide spectrum of variability. Based on spike length-leaf length relationships, the cultivars fall into three distinct groups: 1) Those with spikes much shorter than the leaf, 2) Those with spike much longer than the leaf, and 3) Those with spikes length more or less equal to the leaf length. The first group comprise of only a very few cultivars such as Kuriyalmundi, Kalluvally (Pulpally), Vokkalu and three wild collections (1060, 2071, 2009). The second group comprises of Karimkotta, Kuthiravally, Poonjaranmuda, Thommankodi and Vellanamban. All the other cultivars fall in the third group. It seems that during the process of domestication selection pressure for longer spikes might not have been very strong, indicating that the growers might have looked for many other characters such as profuse and regular yielding habit, bold fruit size, etc. The cultivars in the first group are poor yielders and are not
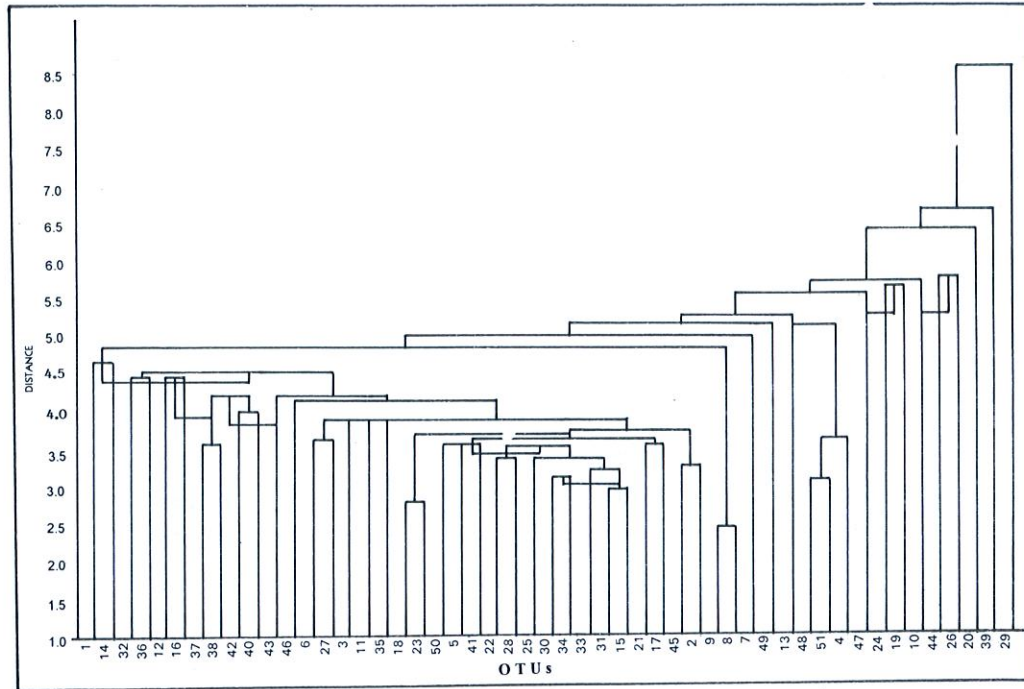
Fig. 2. Dendrogram based on centroid cluster analysis employing 20 characters in black pepper

popular with growers; those in the second group, though good yielders, are less popular because of alternate bearing tendencies. The third group consists of both high and low yielders.

Stebbings (1951) suggested that correlated characters were those which contributed to outstanding biological success and described organisms possessing such characters as having attained adaptive peaks. Successful organisms, therefore, posses complexes of correlated characters and these correlations allow the recognition of distinct taxa. Sporne (1976, 1977) discussed the importance of character correlation in the taxonomy of angiosperms. According to him, positively correlated characters are usually more primitive ones and they are concentrated in the primitive taxa. The relatively weak character correlation observed in black pepper is probably indicative either of its more advanced nature or its more recent origin. A similar study on the *Piper* taxa of Western Ghats (Ravindran *et al.* 1992) revealed a strong correlation among nine characters, while such a situation is not found in *P. nigrum*, indicating probably the more recent origin of black pepper.

Clustering of cultivars

The results of the centroid cluster analysis of OTUs (cultivars) are given in Table 5 and Fig. 2, which gives the order of successive amalgamation of OTUs, the OTUs involved in each amalgamation step, the number of OTUs at each step and the distance among the OTUs. The analysis led to the sorting out of OTUs into the following 11 distinct groups or clusters:

Table 4. Inter character correlations among 22 characters studied in black pepper cultivars

	1*	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1*	1.000																					
2	0.542	1.000																				
3	0.437	-0.344	1.000																			
4	0.968	0.879	0.078	1.000																		
5	-0.016	-0.257	0.286	-0.143	1.000																	
6	0.168	-0.143	0.331	0.029	0.701	1.000																
7	0.092	-0.019	0.105	0.038	0.586	0.538	1.000															
8	-0.249	0.096	-0.068	-0.172	0.567	0.149	0.063	1.000														
9	-0.178	-0.037	-0.033	-0.104	1.145	-0.076	-0.120	0.293	1.000													
10	0.277	-0.055	0.375	0.141	0.192	0.191	0.114	-0.083	-0.004	1.000												
11	0.170	0.018	0.383	0.135	-0.006	0.098	0.067	-0.089	0.007	0.571	1.000											
12	0.330	0.260	0.140	0.308	0.061	0.061	0.001	0.030	-0.096	0.076	0.078	1.000										
13	0.384	0.238	-0.172	0.370	-0.024	0.098	0.028	-0.071	-0.186	0.117	0.148	0.509	1.000									
14	0.141	0.080	0.002	0.149	-0.060	0.009	0.029	-0.128	-0.025	-0.002	-0.007	-0.787	0.295	1.000								
15	0.326	0.086	0.195	0.241	-0.245	0.139	-0.103	-0.295	-0.073	0.176	0.171	-0.029	0.045	0.178	1.000							
16	0.181	-0.026	0.196	0.095	-0.163	-0.039	-0.159	-0.071	-0.201	0.184	0.118	0.048	-0.061	0.013	0.634	1.000						
17	-0.036	-0.103	0.027	-0.096	0.060	-0.069	0.108	-0.101	-0.175	-0.016	-0.086	-0.277	-0.115	0.226	-0.243	-0.062	1.000					
18	0.038	0.263	-0.218	0.156	-0.065	0.025	0.050	0.111	-0.045	-0.053	0.054	0.233	0.126	-0.124	-0.162	0.008	-0.159	1.000				
19	-0.222	-0.278	0.024	-0.275	0.078	-0.061	-0.008	0.161	0.022	0.115	-0.019	-0.280	-0.274	0.069	-0.162	-0.075	0.356	-0.225	1.000			
20	-0.319	-0.281	-0.071	-0.360	-0.008	-0.249	-0.099	0.136	-0.034	-0.195	-0.118	-0.364	-0.454	0.144	-0.019	-0.016	0.113	-0.137	0.464	1.000		
21	0.066	0.280	-0.186	0.209	-0.126	-0.144	-0.213	-0.039	0.136	0.074	-0.015	0.187	0.122	-0.130	0.094	0.134	-0.074	0.270	-0.080	-0.041	1.000	
22	-0.109	-0.071	-0.078	-0.111	0.006	0.038	0.058	-0.021	-0.035	-0.126	-0.078	-0.245	-0.045	0.203	-0.135	-0.044	0.202	0.202	0.057	0.289	-0.041	1.000

* 1-22 denote characters studied (Refer Table 1)

Table 5. Average linkage clustering of characters studied in black pepper cultivars

Variable No.	Outer boundary of cluster	Number of items in cluster	Distance or similarity when cluster formed
1	9	22	9.63
2	4	2	87.86
4	1	3	70.49
19	20	2	46.43
20	1	5	28.92
12	13	3	40.18
14	12	2	78.70
13	1	8	25.63
18	21	2	26.97
21	1	10	15.71
17	22	2	20.17
22	1	2	14.64
3	9	10	12.63
10	11	2	57.05
11	3	3	37.93
15	16	2	63.41
16	3	5	17.32
5	9	5	19.69
6	5	2	70.09
7	5	3	56.17
8	9	2	29.28
9	1	22	9.63

A. OTUs 1 and 14 (Aimpiriyan and Kalluvally (Pulpally)

B. OTUs 32 and 36 (Poonjaranmunda and Thulamundi)

C. OTUs 12, 16, 37, 38, 40, 42 and 43 (Karivilanchy, Kallubalancotta, Uddakere, Uthirancotta, Vellanamban, Valiyakaniakkadan and Velliyaranmunda)

D. OTUs 46, 6, 27, 3, 11, 35, 18, 23, 50, 5, 41, 22, 28, 25, 30, 34, 33, 31, 15, 21, 17, 45, 2, 9, 8, 7, 49, 13 (Wild Coll. 2071, Cheriyakaniakkadan, Neyyattinkaramundi, Arimulaku, Kaniakkadan, Thommankodi, Kuching, Mundi, Wild Coll. 2015, Bilimalligesara, Vattamundi, Malamundi, Ottaplackal, Neelamundi, Perambramunda, Thevanumdi, Sagar local, Perumkodi, Kalluvally (2), Kurimalai, Kottanadan, Wild Coll. 1077, Arakulam munda, Jeerakamundi, Cholamundi, Cheppukulamundi, Wild Coll. 2060 and Karimkotta)

E. OTUs 48, 51, 4 and 47 (Wild Colls. 2059, 2062, Balancotta and Wild Coll. 2009)

F. OTUs 24 and 19 (Narayakkodi and Kuriyalmundi)

G. OTU 10 (Karimunda)

H. OTUs 44 and 26 (Vokkalu and Nedumchola)

I. OTU 20 (Kuthiravally)

J. OTU 39 (Vadakkan)

K. OTU 29 (Panniyur-1)

The OTUs in the same group are to be treated as similar having high coefficients of similarity. The unique cultivars which did not cluster with any others are Karimunda, Kuthirvally, Vadakkan and Panniyur-1. Among these, Panniyur-1 is a hybrid, and Vadakkan, a natural polyploid. Karimunda, the original home of which is the eastern part of the Kottayam District, is the most popular cultivar in Kerala. Kuthiravally is also a popular cultivar in certain areas. The reason for the

Table 6. Centroid linkage of characters studied in black pepper cultivars

No.	Cases	No. of cases	Distance
1.	9,8	2	2.446
2.	23,18	2	2.843
3.	32,15	2	3.028
4.	51,48	2	3.101
5.	34,30	2	3.139
6.	33,15	3	3.286
7.	30,15	5	3.059
8.	45,2	2	3.291
9.	28,22	2	3.392
10.	25,15	6	3.397
11.	22,15	8	3.571
12.	41,5	2	3.591
13.	15,5	10	3.479
14.	38,37	2	3.592
15.	21,17	2	3.610
16.	17,5	12	3.571
17.	27,6	2	3.640
18.	48,4	3	3.671
19.	5,2	14	3.173
20.	50,2	15	3.627
21.	18,2	17	3.699
22.	11,3	2	3.878
23.	35,5	18	3.947
24.	3,2	20	3.887
25.	6,2	22	3.913
26.	42,40	2	4.059
27.	46,2	23	4.182
28.	43,2	24	4.204
29.	40,37	4	4.218
30.	37,2	28	3.857
31.	16,12	2	4.477
32.	12,2	30	3.946
33.	36,32	2	4.509
34.	32,2	32	4.512
35.	14,1	2	4.715
36.	2,1	34	4.467
37.	8,1	36	4.817
38.	7,1	37	4.963
39.	49,1	38	5.210
40.	13,1	39	5.267
41.	4,1	42	5.224
42.	47,1	43	5.630
43.	24,19	2	5.707
44.	19,1	45	5.343
45.	10,1	46	5.728
46.	44,26	2	5.811
47.	26,1	48	5.286
48.	20,1	49	6.445
49.	39,1	50	6.727
50.	20,1	51	8.650

uniqueness of these two cultivars is not very clear - probably they might have also originated as hybrids. Principal component analysis showed that Karimunda gets differentiated from all other cultivars based on leaf anatomical and stomatal characters (Ravindran *et al.*, this issue). Both leaf thickness and stomatal frequency are the lowest in Karimunda. Kuthiravally gets delineated from other cultivars by leaf and spike characters. Chemically, Kuthiravally had 92 per cent similarity with Thommankodi (Ravindran 1990), and also resemble in leaf shape but was separated mainly based on leaf anatomy and spike character. This probably indicates that morphological divergence might have happened more rapidly than chemical divergence. Vadakkan is a natural polyploid with a somatic chromosome number of 2n=78. The most important feature distingushing this cultivar is the very bold frutis. Principal component analysis revealed a very distinct position for this cultivar by virtue of characters such as leaf morphology, leaf anatomy, spike characters and stomatal characters (Ravindran *et al.*, this issue). This cultivar has the broadest stomata and produces the boldest fruits. This did not show high chemical affinity with other cultivars. The analysis led to the clubbing of Aimpiriyan with Kalluvally (Pulpally). Both of them are from the same area (Pulpally) of Wynad District. Chemically they showed 75 per cent similarity (Ravindran 1990). The results indicate that these two cultivars are closely related. Similar is the case of Poonjaranmunda and Thulamundi.

Twenty eight cultivars clustered into a single group (D), and these include Kuching, Mundi, Malamundi, Neelamundi, Perumkodi, Karivilanchi, Kottanadan, etc. The differences among

them for the 22 characters studied are small and they are very difficult to be distinguished when grown together in a field. Computation of intercluster D^2 values showed that cluster D is closely related to clusters A, B and C, indicating that though these cultivars are included in separate groups based on cluster analysis, all of them probably could have derived from a common ancestor, and that the variation among them could have arisen through processes such as reproductive isolation and domestication. These two mechansims are known to be the major forces responsible for variations in the genus *Piper* (Ravindran *et al.* 1990).

Cluster E consists of only one cultivar, Balancotta, along with three wild *P. nigrum* collections. Balancotta is a very distinct cultivar having large leaves, drooping branches and light green fruits. The resemblance of this cultivar with wild *P. nigrum* is shown by the clustering observed. Probably Balancotta could be a straight adoption of a wild *nigrum* type that has not undergone much changes later during the process of domestication.

Cluster F consists of two cultivars, Narayakkodi and Kuriyalmundi. Narayakodi was earlier a favoured cultivar in certain areas of Kottayam District. It is moderate yielder, having good quality and high dry recovery. Kuriyalmundi is a cultivar collected from the Bison Valley of Idukki District. This cultivar has short curved spikes (5-6 cm) and small fruits and the two are easily distinguishable based an spike characters; however, based on overall homology the two cultivars are included in the same group.

The situation is similar in group G consisting of Vokkalu and Nedumchola. Vokkalu is a cultivar from Sagar of Shimoga District of Karnataka, while Nedumchola is a collection from Idukki District of Kerala. Vokkalu has the smallest spike among all the cultivars of black pepper, and each spike (3-4 cm including the peduncle) has only 1-6 berries. Nedumchola has the smallest leaf among all the cultivars studied (8.5 cm x 4.5 cm on an average) and the spkes are also very small (5-6 cm). The vine itself is characteristically small statured.

The cluster analysis study is useful in establishing the relative resemblances of the cultivars. The cluster analysis has shown that 28 out of 51 OTUs clustered in one group, thereby indicating the absence of significant morphological divergence among them. On the other hand, the remaining 23 cultivars fell into 10 groups thereby indicating significant morphological divergence among them. It is very plausible that domestication of black pepper from the forest grown black pepper plants could have started at many centers isolated both spatially and temporally (Ravindran & Babu 1988). The usefulness of cluster analysis in taxonomic studies was earlier shown in *Piper* species (Ravindran, Balakrishnan & Babu 1992) and in *Cinnamomum* species occurring in Kerala (Ravindran *et al.* 1991). The study also indicates the probable origin of black pepper. The present understanding on the species points to a hybrid origin for black pepper, but nothing definite is known about the putative parents. Based on morphological grounds Ravindran (1990) suggested *P. wightii* and *P. galeatum* as the probable parents of *P. nigrum;*

however, this is yet to be confirmed and further studies are required in this direction. It has been pointed out that segregation, chance corssing and accumulation of mutations could have been the forces leading to the divergence of black pepper in which both random mating and active gene flow are absent (Ravindran *et al.* 1990). Most of the present day land races and old cultivars are straight selections from forest grown plants.

A principal component analysis was also carried out to find out the specific characters that led to the resemblances and divergences among the cultivars, the results of which are also presented in this issue.

Acknowledgements

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