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VARIATION IN CLOVE (*SYZYGIUM AROMATICUM*) GERMPLASM IN THE MOLUCCAN ISLANDS

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SUMMARY

One hundred clove trees from a total of 34 wild and cultivated populations, mainly in the north and central Moluccas, were examined for morphological variation. Data were evaluated using cluster analysis, principal co-ordinates analysis and canonical variates analysis. Considerable morphological variation was evident and was largely attributable to between-population sources. There was more variation among wild cloves than among cultivated populations. Several of the cultivated populations were shown to be morphologically distinct. Flower bud characters were most valuable in distinguishing between populations; leaf and flower bud cluster characters were also useful. Permanent germplasm collections are needed to conserve variation.

INTRODUCTION

The clove, *Syzygium aromaticum* (L) MERR. & PERR., has had an unusual and turbulent history in the Moluccas. Indigenous populations in the northern islands, regarded as the true centre of origin of the species, were forcibly eradicated in the 17th century in an attempt to preserve a trade monopoly (TIDBURY, 1949; PURSEGLOVE et al., 1981). The degree of genetic diversity that remains in the area has thus been open to doubt (WIT, 1969), although recent unpublished reports in Indonesia have referred to some distinctive populations in the north Moluccas. To the present day, there appear to have been few introductions of clove germplasm from the Moluccas to other parts of Indonesia or to other countries. Cultivated cloves show little variation and probably derive from a narrow genetic base (PURSEGLOVE, 1968).

In Indonesia three cultivated varieties are popularly recognised (HADIWIJAYA, 1979): Siputih and Sikotok, both reported in West Sumatra by BRINKGRIEVE (1933), and Zanzibar, believed to derive from a few seeds reintroduced from the island of Zanzibar in 1932 (DEINUM, 1949). The Zanzibar variety is the most extensively cultivated and the majority of seeds presently used for new plantings are obtained from only a few mother trees. A wild type, Cengkeh hutan, is also recognised but is of little commercial value.

At present, clove production in several regions of Indonesia, notably Sumatra and West Java, is being severely affected by a systemic bacterial disease known as Sumatra disease (BENNETT et al., 1985), and also by a *Phyllosticta* leaf blight known locally as Cacar daun (KASIM et al., 1980). Long-term control of Sumatra disease in particular, and improvements in other desirable cultural characteristics, are unlikely to be achieved through manipulation of the germplasm resources that are presently available outside the Moluccas. This report describes a survey of wild and cultivated cloves carried out in 1983 to locate and examine sources of germplasm variation in the central and north Moluccas. Multivariate analyses, which are described by SNEATH & SOKAL (1973), were used in an attempt to characterise this variation and to detect interrelationships between populations. It was hoped that such a study would help to rationalise the selection of potentially valuable germplasm in a search for disease resistance and also for characters that may be useful with respect to other aspects of crop improvement.

MATERIALS AND METHODS

One hundred trees from a total of 34 populations were sampled (Table 1). The Tibobo, Tauro, Sibela, Indari, Dokiri, AFO 1, AFO 2 and Air mata populations consisted of cultivated types documented by earlier, unpublished surveys, and which are believed to be growing only in the Moluccas. The three popularly recognised varieties, as identified by local farmers or officials, were sampled wherever they were encountered in the Moluccas and for comparative purposes in Bogor (West Java) and North Sulawesi. Cengkeh hutan trees were sampled in four locations in the Moluccas and also in Bogor. The Cengkeh raja (1–4) populations were thought by local farmers to represent a series of hybrids between Siputih and Cengkeh hutan trees. Two other hybrids and two solitary but distinct individuals (Daun buntal and a dwarf mutant) were also included in the survey. Up to five trees from each population were examined. Where more than one type of tree was present at a single location, trees that appeared dissimilar in gross morphology were sampled as separate populations.

General morphological characters as well as leaf, flower bud cluster and flower bud characters were recorded for every tree where data were available, and are listed in Table 2. Data collected for the majority of characters were of a continuous nature but some binary, ordered multistate and unordered multistate data were also used. Where applicable, data points for individual samples were obtained by taking ten separate readings and deriving a mean value. In cases where such means have been obtained from continuous data, coefficients of variation for individual samples were computed and included in the subsequent analyses as additional diagnostic characters.

Means and ranges were calculated for all characters. Overall coefficients of variation were obtained and analyses of variance carried out for all continuous and ordered multistate data. Coefficients of similarity (GOWER, 1971) were computed for all combinations of pairs of samples. These coefficients were subjected to cluster analysis using the group average link method of SOKAL & MICHENER (1958). Principal coordinates analysis was used to display the variation in two dimensional space (GOWER, 1966). Goodness of fit estimates and character loadings were obtained from canonical variates analysis (RAO & SLATER, 1949).

Table 1. Thirty four clove populations, location details and numbers of sampled trees evaluated in this study.

Population number	Population	Number of trees sampled	Area	Long.	Lat.	Alt. (m)	Island
1	Zanzibar	5	Bogor	106°47'E	6°34'S	240	Java
2	Zanzibar	3	Telaga Kodok	128°11'E	3°36'S	200	Ambon
3	Zanzibar	1	Soasio	127°27'E	0°39'N	60	Tidore
4	Sikotok	3	Bogor	106°47'E	6°34'S	240	Java
5	Sikotok	5	Marikurubu	127°22'E	0°48'N	60	Ternate
6	Sikotok	2	Tibobo	127°29'E	1°06'N	100	Halmahera
7	Sikotok	2	Tauro	127°31'E	0°59'N	250	Halmahera
8	Sikotok	2	Tinoor	124°53'E	1°21'N	500	Sulawesi
9	Siputih	5	Bogor	106°47'E	6°34'S	240	Java
10	Siputih	3	Mamala	128°11'E	3°33'S	50	Ambon
11	Siputih	4	Amahusu	128°07'E	3°44'S	5	Ambon
12	Siputih	2	Haria Gunung	128°38'E	3°36'S	300	Saparua
13	Siputih	2	Senduk	124°53'E	1°21'N	70	Sulawesi
14	Cengkeh raja 1	3	Mamala	128°11'E	3°33'S	50	Ambon
15	Cengkeh raja 2	3	Mamala	128°11'E	3°33'S	50	Ambon
16	Cengkeh raja 3	3	Mamala	128°11'E	3°33'S	50	Ambon
17	Cengkeh raja 4	3	Mamala	128°11'E	3°33'S	50	Ambon
18	Tibobo	5	Tibobo	127°29'E	1°06'N	100	Halmahera
19	Tauro	5	Tauro	127°31'E	0°59'N	250	Halmahera
20	Sibela	6	Sibela	127°31'E	0°43'S	650	Bacan
21	Indari	5	Indari	127°17'E	0°27'S	450	Bacan
22	Dokiri	5	Dokiri	127°24'E	0°38'N	60	Tidore
23	AFO 1	4	Marikurubu	127°22'E	0°48'N	650	Ternate
24	AFO 2	3	Air Tegetege	127°22'E	0°48'N	400	Ternate
25	Air mata	2	Haria Gunung	128°38'E	3°36'S	300	Saparua
26	Daun buntal	1	Mendawong	127°29'E	0°38'S	15	Bacan
27	Dwarf mutant	1	Bogor	106°47'E	6°34'S	240	Java
28	C. hutan × Siputih hybrid	1	Bogor	106°47'E	6°34'S	240	Java
29	Zanzibar × Indari hybrid	1	Soasio	127°27'E	0°39'N	60	Tidore
30	Cengkeh hutan	1	Amahusu	128°07'E	3°44'S	5	Ambon
31	Cengkeh hutan	2	Haria Gunung	128°38'E	3°36'S	300	Saparua
32	Cengkeh hutan	2	Sibela	127°31'E	0°43'S	650	Bacan
33	Cengkeh hutan	2	Indari	127°17'E	0°27'S	4500	Bacan
34	Cengkeh hutan	3	Bogor	106°47'E	6°34'S	240	Java

RESULTS

The populations surveyed showed considerable variation. In general, visual observations were in agreement with some of the characteristics of the three popularly recognised varieties; thus Zanzibar trees were low branching, densely foliated, had dark green mature leaves and reddish young leaves and flower buds; Sikotok trees wee low branching, densely foliated, and had large and broad mature leaves; and Siputih trees did not have a low branching habit, were sparsely foliated and had light green, small and narrow mature leaves. The Sibela population comprised exceptionally tall and slender trees, possibly due to competition from other forest trees cleared only recently. The Indari and Tibobo populations appeared similar to that at Sibela but had reddish

Table 2. Simple statistics for 52 morphological characters evaluated in 100 trees from 34 clove populations.

	Nature of data[1]	Mean	Range	Coefficient of variation (%)	Population variance (P)[6]
General characters					
Height (m)/age (years)	C	0.76	0.10 – 2.33	48.07	<0.001
Cross sectional area of trunk (cm^2)/height (m)	C	42.40	1.64 – 313.96	94.36	<0.001
Trunk division	B	0.47	0(no division) – 1(division)	–	–
Tree conformation	UM	2.80	1(round) – 3(pyrimidal) – 5(cylindrical)	–	–
Branching height	B	0.66	0(not low) – 1(low)	–	–
Branching conformation	OM	3.40	1(vertical) – 5(horizontal)	31.10	<0.01
Leaf abundance	B	0.65	0(sparse) – 1(dense)	–	–
Young leaf colour	OM	3.11	1(green/yellow) – 5(scarlet/red)	39.22	<0.001
Bud clusters in upper part of tree	B	0.85	0(absent) – 1(present)	–	–
Bud clusters in middle part of tree	B	0.83	0(absent) – 1(present)	–	–
Bud blusters in lower part of tree	B	0.73	0(absent) – 1(present)	–	–
Flowering time	OM	2.52	1(June) – 7(December)	42.09	<0.001
Flower bud yield	OM	1.51	1(high) – 3(low)	47.96	<0.001
Flower bud quality	OM	1.35	1(good) – 3(poor)	56.79	<0.001
Leaf characters					
Proximal – distal leaf symmetry[2]	C	1.03	0(symmetrical) – 2(asymmetrical)	55.69	<0.001
Lamina length (mm)[3]	C	127.50	56.50 – 233.00	20.95	<0.001
CV[5] lamina length (%)	C	8.00	4.00 – 15.00	32.11	<0.01
Lamina breadth (mm)[3]	C	50.90	26.40 – 130.30	32.29	<0.001
CV lamina breadth (%)	C	9.88	2.00 – 21.00	34.92	<0.001
Lamina length/breadth[3]	C	2.59	1.72 – 3.38	12.97	<0.01
CV lamina length/breadth (%)	C	8.49	3.00–14.00	30.49	<0.05
Petiole length (mm)[3]	C	20.93	12.20–31.10	14.70	<0.05
Petiole length/lamina length[3]	C	0.17	0.07–0.26	19.99	<0.001
CV petiole length/lamina length (%)	C	12.87	6.00 – 46.00	37.83	<0.001
Mature leaf colour[4]	C	0.53	0(light green) – 1(dark green)	78.52	<0.001
Leaf acumination[2]	C	2.82	1(not acuminate) – 4(highly acuminate)	27.15	<0.001
CV leaf acumination (%)	C	21.40	0.00 – 52.00	52.27	<0.01

Table 2. (Continued).

	Nature of data[1]	Mean	Range	Coefficient of variation (%)	Population variance (P)[6]
Flower bud cluster characters					
Number of peduncles per bud[3]	C	2.33	0.71 – 4.33	32.30	<0.001
CV number of peduncles per bud (%)	C	14.63	0.00 – 44.00	44.30	ns
Length of longest branch (mm)[3]	C	31.38	18.89 – 49.00	23.02	<0.001
CV length of longest branch (%)	C	14.93	0.00 – 36.00	43.59	ns
Number of peduncles in longest branch[3]	C	4.85	3.00 – 7.00	17.16	<0.001
CV number of peduncles in longest branch (%)	C	13.69	0.00 – 47.00	49.97	ns
Peduncle length (mm)[3]	C	6.67	4.25 – 13.58	26.53	<0.001
CV peduncle length (%)	C	16.00	0.00 – 44.00	40.76	ns
Flower bud characters					
Corolla diameter (mm)[3]	C	5.64	4.62 – 7.84	10.83	<0.01
CV corolla diameter (%)	C	5.22	1.00 – 12.00	46.93	<0.001
Calyx tube diameter (mm)[3]	C	4.82	3.78 – 6.22	12.88	<0.001
CV calyx tube diameter (%)	C	5.43	2.00 – 11.00	37.74	ns
Corolla height (mm)[3]	C	4.20	3.10 – 6.26	15.43	<0.001
CV corolla height (%)	C	8.66	1.00 – 21.00	40.00	ns
Calyx tube length (mm)[3]	C	13.23	8.74 – 17.62	14.08	<0.001
CV calyx tube length (%)	C	6.22	2.00 – 14.00	41.61	ns
Corolla diameter/height[3]	C	1.36	1.10 – 1.83	10.55	<0.001
CV corolla diameter/height (%)	C	7.50	3.00 – 22.00	36.75	ns
Calyx tube diameter/length[3]	C	0.37	0.27 – 52	16.22	<0.001
CV calyx tube diameter / length (%)	C	7.16	2.00 – 19.00	46.11	ns
Corolla height/calyx tube length[3]	C	0.32	0.26 – 0.41	12.71	<0.001
CV corolla height/calyx tube length (%)	C	9.94	3.00 – 16.00	31.65	ns
Corolla colour[2]	C	4.43	1(scarlet/red) – 5(green/yellow)	46.23	<0.001
Calyx tube colour[2]	C	4.05	1(scarlet/red) – 5(green/yellow)	24.19	<0.001
Calyx tube shape	UM	1.97	1(tapering) – 2(parellel) – 3(bulge at base) –	–	

[1] C = continuous, B = binary, UM = unordered multistate, OM = ordered multistate.
[2, 3] and [4] Characters for which a single continuous value for each tree has been obtained from a mean of 10 ordered multistate values, 10 continuous values and 10 binary values respectively.
[5] CV = coefficient of variation.
[6] Probability value (P) for F ratio obtained by testing between-population variance against within-population variance. Characters for which $P > 0.05$ are designated by ns.

young leaves and flower buds and the mature leaves were of a lighter green. The population at Tauro also had red young leaves together with a low, drooping branching habit and dark green, highly acuminate mature leaves. Distinctive characteristics of other types included the blunt, asymmetric leaves of the Daun buntal, the very small leaves of the Air mata and the low, broad branching habit of the Dokiri. The Cengkeh hutan and Cengkeh raja 2, 3 and 4 populations were distinguished by their large, asymmetric leaves and low leaf length/breadth scores.

Simple statistics for all characters studied are presented in Table 2, from which it is evident that most of the characters exhibited a high degree of variation. The only characters with coefficients of variation of less than 15% are lamina length/breadth, petiole length, corolla diameter, calyx tube diameter, calyx tube length, corolla diameter/corolla height and corolla height/calyx tube length. Only 10 characters failed to register a significantly greater between- than within-population variance, all of these being measures of variation for a given character within a single tree.

A phenogram resulting from cluster analysis was constructed to the nearest 5% similarity level (Fig. 1). Numbers at the base correspond to population numbers given in Table 1. The analysis produced 10 groups (Groups 1–10) at a similarity level of 70%, three of these (Groups 2, 9 and 10) being single, ungrouped trees. Group 1 was large consisting of 62 individuals. All the Zanzibar trees, all but two of the Sikotok trees and all but four of the Siputih trees were clustered together in this group. The Tibobo, Tauro, Sibela, Indari, AFO 1 and AFO 2 populations also appeared in Group 1. The Dokiri and Air mata populations were both clustered in Group 5. The Cengkeh hutan and Cengkeh raja 2, 3 and 4 trees were clustered in Groups 6, 7, 8, 9 and 10. The analysis further subdivided Group 1 into 13 groups (Groups A–M) at the 80% similarity level. In general these groups corresponded with individual populations; thus Groups C, E and K comprised the entire Tauro, AFO 1 and Indari populations respectively. Most of the Tibobo population was clustered in Group B and most of the Sibela trees in Group J. The Zanzibar trees appeared in Groups A and D, being grouped with AFO 2 in the latter case. Most of the Sikotok and Siputih trees were clustered in Groups F, G and H.

Principal co-ordinates analysis was not particularly successful in accounting for the observed variation; the first 10 co-ordinates captured only 57.42%. Principal co-ordinates 1 and 2 accounted for 11.8% and 9.56% respectively. The values of these co-ordinates were used to produce a scatter diagram (Fig. 2), on to which have been superimposed the 10 groups delineated by cluster analysis at the 70% level. Despite the apparent lack of success in accounting for the observed variation, Fig. 2 shows that the principal co-ordinates analysis was in reasonable agreement with the cluster analysis.

The groups distinguished by cluster analysis at the 70% similarity level (Groups 1–10) were subjected to canonical variates analysis. Unordered multistate data were excluded as were 24 trees which lacked a full complement of data. This resulted in the exclusion of three trees from each of Groups 1, 4 and 5, seven trees from Group 6, one tree from Group 7 and all trees from Groups 8, 9 and 10 (Fig. 1). The first two canonical variates accounted for 52.89% and 22.13% of the variation respectively. The analysis discriminated well between groups, allocating all individuals to their original groups except for eight of the 59 trees in Group 1, which were all reallocated

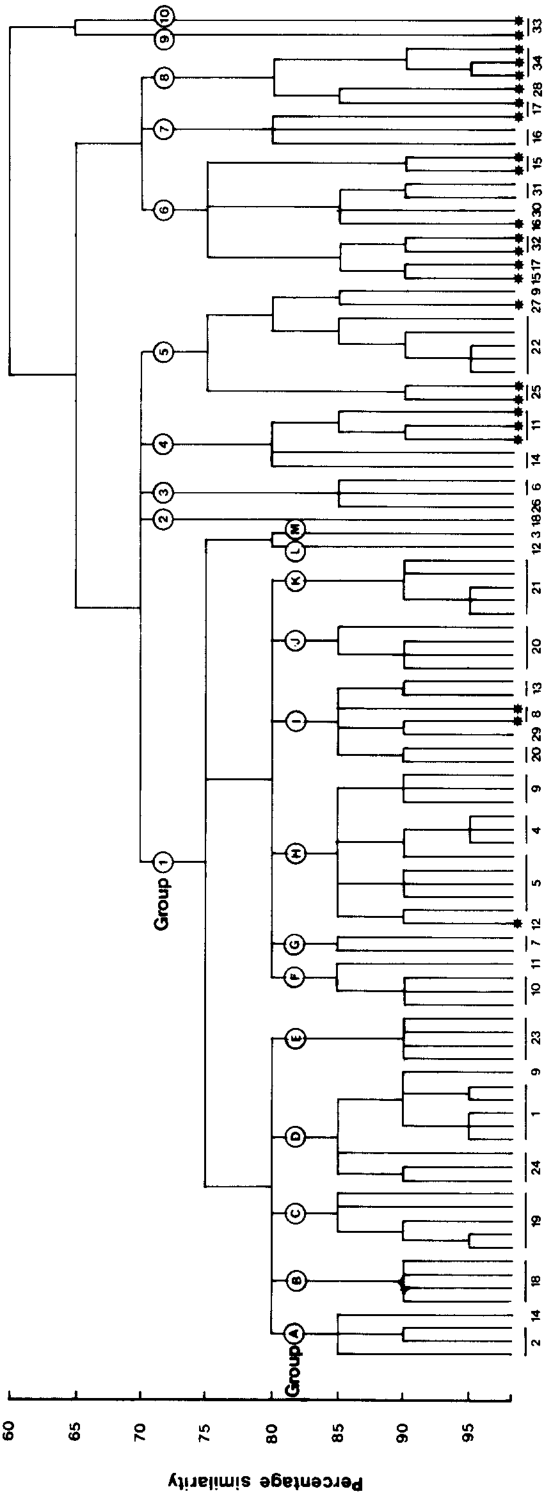

Fig. 1. Phenogram obtained for 100 individuals from 34 clove populations following cluster analysis. Numbers beneath the phenogram correspond to population numbers provided in Table 1. Individuals underscored with an asterisk were excluded from the canonical variates analyses.

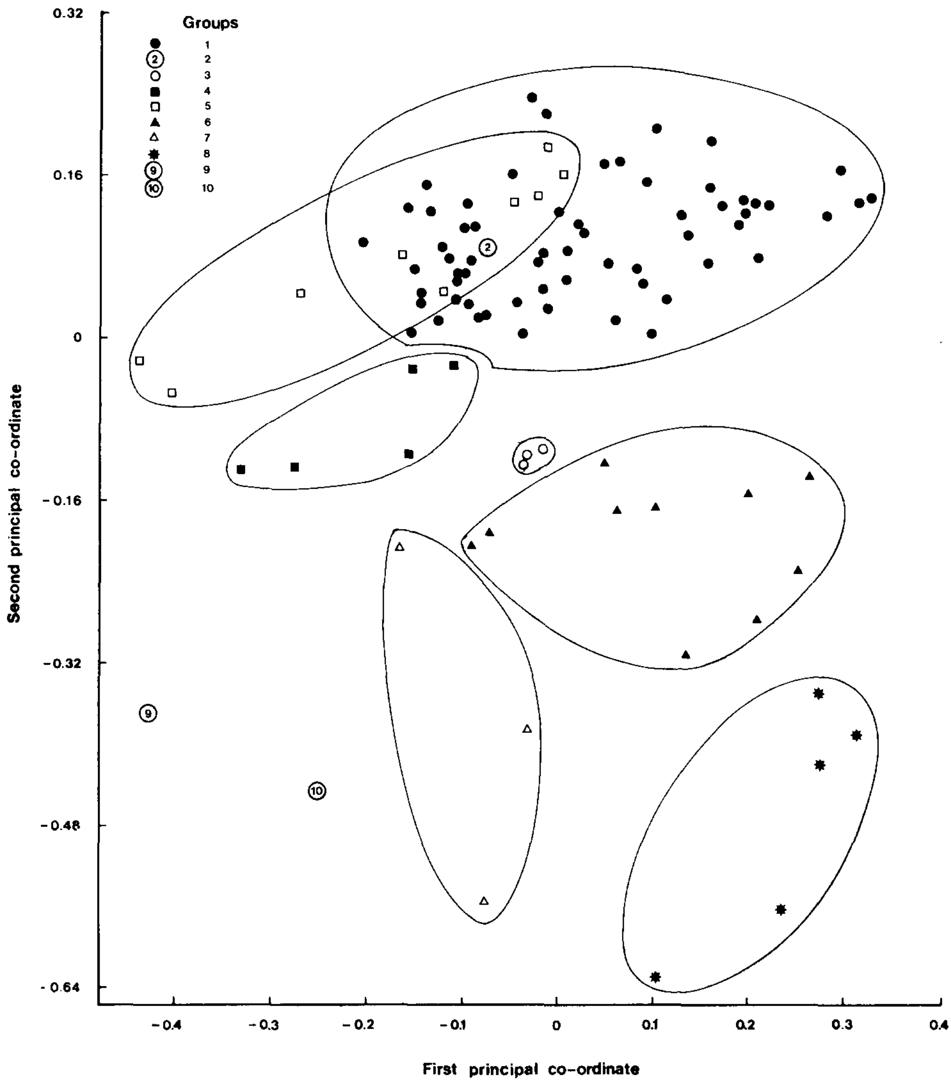

Fig. 2. Scatter diagram for 100 individuals from 34 clove populations following principal co-ordinates analysis. Groups 1–10 correspond to those delineated by the cluster analysis at the 70% similarity level (Fig. 1).

to Group 3. Table 3 lists characters with the largest loadings on canonical variates 1 and 2, indicating that such characters were major contributors to the observed variation and thus most useful in distinguishing between Groups 1–7. These included three leaf characters, three flower bud cluster characters and five flower bud characters.

A separate canonical variates analysis was carried out on the 13 groups (Groups A–M) in Group 1, from which only three trees were excluded because of incomplete data. The first two canonical variates accounted for 69.18% and 15.53% of the variation respectively. Again, discrimination between groups was good with all trees being allocated to their original groups. Characters with the largest loadings on canonical

Table 3. Characters exhibiting largest loadings on canonical variates 1 and 2 following canonical variates analysis on Groups 1–7 (Fig. 1). Percentage variation accounted for by canonical variates is also shown.

	Canonical variates	
	1	2
Leaf characters		
Lamina length	2.86	–9.49
Lamina breadth	–	9.81
Lamina length/breadth	–	7.68
Flower bud cluster characters		
Length of longest branch	–3.03	–
Number of penduncles in longest branch	2.89	–
Peduncle length	2.81	–
Flower bud characters		
Corolla diameter	4.27	–5.92
Calyx tube diameter	–	5.90
Calyx tube length	–6.27	6.84
Calyx tube diameter/length	–	–7.86
Corolla height/calyx tube length	–5.29	11.75
% variation	52.89	22.13

Table 4. Characters exhibiting largest loadings on canonical variates 1 and 2 following canonical variates analysis on Groups A–M (Fig. 1). Percentage variation accounted for by canonical variates is also shown.

	Canonical variates	
	1	2
General characters		
Branching conformation	–12.63	–
Leaf characters		
Lamina length	13.84	5.47
CV lamina length	11.46	–
Petiole length	–	–6.83
Petiole length/lamina length	17.67	8.32
Flower bud cluster characters		
Length of longest branch	–	5.31
Number of peduncles in longest branch	–18.10	–
Flower bud characters		
Calyx tube diameter	–	7.38
Corolla height	14.96	–7.81
Calyx tube length	–19.70	–
Corolla diameter/height	15.22	–
Calyx tube diameter/length	–	–9.44
Corolla height/calyx tube length	–	–9.34
Corolla colour	–10.23	–
Calyx tube colour	10.01	–
% variation	69.18	15.53

variates 1 and 2 are listed in Table 4. These comprised branching conformation, four leaf characters, two flower bud cluster characters and eight flower bud characters.

DISCUSSION

Although environmental influences cannot be discounted, ranges and overall coefficients of variation presented in Table 2 suggest that considerable diversity exists amongst the clove populations included in this survey. Furthermore, most of the variation was shown to be attributable to differences between, rather than within, populations (Table 2). It is probable that the scattered and insular distribution of many of the cultivated populations in the Moluccas, together with the practice of planting seed from local mother trees, has resulted in genetic isolation and contributed to the conservation and pattern of the observed variation.

Through the application of multivariate methods it has been possible to analyse this variation and to examine inter-relationships between populations. Thus cluster analysis at the 70% similarity level placed almost all the cultivated cloves, excepting the Dokiri and Air mata populations (Group 5), into a single group (Group 1), whilst Cengkeh hutan trees were distributed between Groups 6, 8, 9 and 10 (Fig. 1). This indicates that more variation exists between Cengkeh hutan trees than between cultivated populations, a fact well illustrated by the scatter diagram following principal co-ordinates analysis (Fig. 2). It also suggests that the Dokiri and Air mata populations are the most distinctive of the cultivated types.

Groups A–M, delineated by cluster analysis at the 80% similarity level were largely representative of individual, cultivated populations and the validity of these groupings is reinforced by the allocation of all individuals to their original groups following canonical variates analysis. This encourages the view that these populations can be regarded as morphologically distinct and as such, sources of potentially valuable variation. It should be noted however that groups distinguished by cluster analysis were not entirely consistent with populations or varieties subjectively identified in the field. Thus the Zanzibar populations from Telaga Kodok and Bogor appeared in different groups (Groups A and D), and Group H comprised representatives from Sikotok and Siputih populations. It is of particular interest that the Zanzibar populations were clustered with the Tibobo, Tauro, AFO 1 and AFO 2 populations at the 75% similarity level (Fig. 1), pointing to the possible origin of this variety.

In listing characters shown to be most useful in distinguishing between Groups 1–7 (Table 3), canonical variates analysis was in broad agreement with visual observations. Thus, for example, the Cengkeh hutan and Cengkeh raja trees in Groups 6 and 7 had large, broad leaves while those of the Dokiri and Air mata populations (Group 5) were extremely small. Also, the long branches of peduncles and large flower buds of the Cengkeh hutan and Cengkeh raja trees were in marked contrast to the shorter branches and smaller flowers of the populations in Group 1. Visual distinctions between Groups A–M were less obvious. However, several of the characters identified by canonical variates analysis as being useful in this respect (Table 4) have also been noted by previous authors. BRINKGRIEVE (1933) recorded that farmers in West Sumatra recognised differences in branching conformation while HADIWIJAYA (1979), has reported variation in the size and shape of mature leaves. Differences in flower bud cluster characters have been observed by TIDBURY (1949) and variation in the size, shape and colour of flower buds was first defined by Rumphius in 1741 (see WIT, 1969). Both of the canonical variates analyses identified flower bud characters as being of particular diagnostic significance.

These results confirm that considerable variation in potentially valuable clove germplasm remains in the Moluccan islands. Multivariate analyses have been able to characterise part of this variation and to identify morphologically distinct groups. In order to conserve such variation, and to facilitate screening programmes for disease resistance and other agronomically desirable traits it is important that permanent clove germplasm collections are established both within and outside the Moluccas. The findings reported here provide a sound basis for the initiation of such collections and also for a more detailed characterisation and description of clove types. Future surveys should attempt to locate novel populations, paying special attention to the islands of Halmahera and Morotai from where several, unconfirmed reports were received of cloves growing wild in the forests.

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