



ISSN: 0976-3031

Available Online at <http://www.recentscientific.com>

International Journal of Recent Scientific Research
Vol. 6, Issue, 6, pp.4866-4872, June, 2015

**International Journal
of Recent Scientific
Research**

RESEARCH ARTICLE

GENETIC DIVERSITY IN OIL PALM GERmplasm AS SHOWN BY HIERARCHICAL CLUSTERING METHODS

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ARTICLE INFO

Article History:

Received 14th, May, 2015

Received in revised form 23th,
May, 2015

Accepted 13th, June, 2015

Published online 28th, June, 2015

Key words:

Oil palm, germplasm,
genetic diversity, cluster
analysis

ABSTRACT

Multivariate statistical tools like cluster analysis have proved useful in characterizing and studying genetic diversity of germplasm resources. Thus, this study was aimed at classifying the diversity pattern in oil palm germplasm using two hierarchical clustering methods (single linkage clustering method and Ward's method). 595 oil palm genotypes grouped into 44 accessions were morphologically characterized for yield traits, bunch quality traits, morphological and physiological traits and fatty acid traits. The two clustering methods classified the accessions into eight groups and differ slightly in the assigning of the accessions into groups. The oil palm germplasm with different characteristics were identified and the genetic distance within and between the groups was estimated.

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INTRODUCTION

Genetic diversity is the variation in the genetic constitution of individuals within or among species and it is the cornerstone of the ability of organisms to adjust to changes in their environment through natural selection. Crop genetic diversity is not only a raw material for industrial agriculture but is of utmost importance to food security and sustainable agriculture as it enables farmers to adapt crops suited to their own site specific ecological needs and cultural traditions (Singh *et al.*, 2008). According to Kumar and Singh (2006), exploitation of heterosis has been the major aim in cross-pollinated crops for which hybrid development or population improvement are commonly adapted procedures. In view of this, genetic variability is requisite to attain genetic benefits in a breeding program.

Characterization of genetic variability and an estimate of the genetic relationship among varieties are crucial to any breeding program. Moreover, getting accurate estimates of genetic diversity levels among germplasm sources may enhance efficiency of breeding efforts to improve crop species (Singh *et al.*, 2008). Plant breeding deals with high-yielding genotypes

and parental selection is the first step in any plant breeding program and this can be done using different selection methods based on the parent performance. Multivariate analysis is one of these methods that can be used in the selection program (Singh *et al.*, 2008). Cluster analysis is a multivariate statistical technique that has emerged as one of the leading methods of multivariate analysis due to its usefulness (Kettenring, 2006). It was originally developed for biological classification and its main aim in the analysis of data from plant breeding trials is to group the varieties into several homogenous groups such that those varieties within a group have a similar response pattern across the locations (Kroonenberg *et al.*, 1995). The resultant from cluster analysis are usually pictured in the form of hierarchical trees; also called a dendrogram (Siracli *et al.*, 2013). Cluster analysis has been well utilized in classifying different germplasm resources like

MATERIALS AND METHODS

Plant Breeding Materials

Oil palm germplasm prospected in 1973 by the Malaysian Agricultural Research and Development Institute (MARDI)

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and the Nigerian Institute for Oil Palm Research (NIFOR) at forty five different locations in Nigeria were used for the purpose of this research. For characterization and evaluation, data on yield traits (1982-1986), bunch quality traits (1982-1987), morphological and physiological traits (1986) and fatty acid traits (1981-1997) were recorded. The data as gotten from the Malaysian Palm Oil Board (MPOB) were arranged according to the collection sites (total of forty-four populations as a population was missing from the data) , averaged and analyzed for simple descriptive statistics (mean, minimum, maximum, range, standard deviation, variance, coefficient of variation) using the Statistical Analysis System (SAS). The means of each trait were also standardized using computer software “Microsoft Excel 7” for windows to give equal weight to all traits before clustering was applied.

Cluster Analysis

The standardized data were subjected to two hierarchical clustering analysis methods; single linkage clustering analysis (SLCA) and minimum variance method of WARD (1963) with the aid of “THE UNSCRAMBLER[®]X” software (CAMO software version 10.1). The theory behind clustering is an expected positive relationship between the variables Euclidean distance and the similarity of the observations. As a result, cluster analysis is driven between minimizing the Euclidean distance of observations within a cluster, and maximizing the Euclidean distance between clusters (Vural & Karasu, 2007). The single linkage (Sneath, 1957) which is an agglomerative hierarchical method merges groups based on the minimum distance between objects in two groups; therefore the distance between Clusters R and Q is defined by:

$$d_s(R, Q) = \min_{i \in R, j \in Q} d(i, j) \quad (1)$$

where, $d(i, j)$ is the distance between the i^{th} and j^{th} objects. The ward’s method optimizes an objective function; that is, it minimizes the sum of squares within groups and maximizes the sum of squares between groups. Ward’s method is similar to the linkage methods in that it begins with N clusters, each containing one object, it differs in that it does not use cluster distances to group objects. Instead, the total within-cluster sum of squares (SSE) is computed to determine the next two groups merged at each step of the algorithm. The error sum of squares (SSE) is defined (for multivariate data) as:

$$SSE = \sum_{i=1}^k \sum_{j=1}^{n_i} (y_{ij} - \bar{y})^2 \quad (2)$$

where y_{ij} is the j^{th} object in the i^{th} Cluster and n_i is the number of objects in the i^{th} Cluster.

RESULTS

Single Linkage Clustering (SLCA)

The oil palm germplasm based on all traits were classified into eight groups using single linkage cluster algorithm. The dendrogram resulting from the analysis is presented in Figure

1. Cluster 1, 2, 3, 4, 5 and 6 were singleton with one population each (2.27 % each) which includes NGA 02, NGA 19, NGA 12, NGA 25, NGA 34 and NGA 1 respectively. Cluster 7 contains 34 populations (77.27 %) namely: NGA 03, NGA 04, NGA 05, NGA 06, NGA 07, NGA 08, NGA 09, NGA 10, NGA 11, NGA 13, NGA 14, NGA 15, NGA 16, NGA 17, NGA 18, NGA 20, NGA 21, NGA 22, NGA 23, NGA 26, NGA 27, NGA 28, NGA 29, NGA 30, NGA 31, NGA 32, NGA 33, NGA 35, NGA 36, NGA 37, NGA 38, NGA 43, NGA 44 and NGA 45. The last cluster, Cluster 8 contains only four populations (9.09 %) namely: NGA 39, NGA 40, NGA 41 and NGA 42.

The mean values of different clusters for all the variables are presented in Table 1. Cluster 1 showed high mean values for MNW, SF, IV and C18:1. Cluster 2 had high mean values for M/F and C18:0. Cluster 3 also showed high mean values for FFb, BNO, MFW, OWM, OB, OY, KY, TEP, LL, LN, BDM, TDM, BI, *e, f*, NAR and C16:0. Cluster 4 had high mean values for ABW, FB, PCS, HT and VDM. Cluster 5 also had high mean values for C12:0, C14:0 and C18:2. Cluster 6 showed high mean values for ODM, RL, LW, LA, LAI, DIAM, LAR and *f* while Cluster 8 had high mean values for K/F, K/B, FP and HT.

Ward Hierarchical Clustering (WHCA)

The oil palm germplasm based on traits were also classified into eight groups using Ward’s hierarchical algorithm as shown in Figure 2. Cluster 1 contains a single population (2.27 %) and includes NGA 12. Cluster 2 contains two populations (4.55 %) namely: NGA 02 and NGA 19. There were eleven populations in Cluster 3 (25 %) namely: NGA 09, NGA 10, NGA 11, NGA 25, NGA 27, NGA 28, NGA 30, NGA 31, NGA 32, NGA 33, and NGA 37. Cluster 4 contains five populations (11.36 %) namely: NGA 13, NGA 14, NGA 43, NGA 44, and NGA 45. Cluster 5 also contains eleven populations (25 %) which include NGA 05, NGA 06, NGA 07, NGA 08, NGA 20, NGA 21, NGA 22, NGA 23, NGA 35, NGA 36, and NGA 38. Seven populations were in Cluster 6 (15.91 %) namely: NGA 15, NGA 16, NGA 17, NGA 18, NGA 26, NGA 29, and NGA 34. Cluster 7 contains four populations (9.09 %) namely; NGA 39, NGA 40, NGA 41 and NGA 42. Cluster 8 contains three populations (6.82 %) and includes NGA 01, NGA 03 and NGA 04.

The mean values of different clusters for all the variables are given in Table 2. Cluster 1 showed highest mean values for FFb, BNO, PB, MF, OWM, OB, OY, KY, TEP, LN, BDM, TDM, BI, *e*, NAR, C12:0 and C16:0. Cluster 2 had high mean values for IV, C18:0 and C18:1. Cluster 3 also had high mean values for MFW, MNW and FB. Cluster 4 had high mean value for C16:1. Cluster 5 showed high mean values for ABW, PCS, LL, LW, HT, LA, LAI, VDM and *f*. Cluster 6 also showed high mean value for ODM while Cluster 7 on the other hand showed high mean values for KF, SF, KB, FP, C14:0 and C18:2. The last group, Cluster 8 showed high mean values for RL, DIAM, LAR and *f*.

Table 1 Means for 8 clusters as generated by single linkage clustering method

Traits	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6	Cluster 7	Cluster 8
FFB	136.62	153.07	190.29	151.94	153.64	145.25	156.80	102.07
BNO	13.79	12.57	17.23	12.71	13.72	15.34	13.18	15.33
ABW	10.29	12.48	11.22	12.81	11.65	9.84	12.45	7.02
MFW	8.91	7.47	9.69	9.63	8.68	8.77	9.67	6.68
MNW	5.29	3.78	4.96	5.11	4.62	4.94	5.24	4.08
PB	1.96	3.45	5.08	2.32	2.76	2.55	2.39	2.58
MF	40.71	49.33	48.55	46.97	46.72	43.62	45.79	38.86
KF	12.81	11.67	12.24	11.97	13.16	12.10	12.31	15.58
SF	46.48	39.00	39.22	41.06	40.12	44.27	41.90	45.56
ODM	72.32	73.83	75.07	74.86	74.24	75.28	74.63	73.26
OWM	45.14	48.58	50.57	49.26	48.51	50.05	48.78	48.56
FB	65.34	66.03	65.44	67.08	65.70	63.68	66.99	66.42
OB	12.08	15.80	16.13	15.54	14.94	13.87	14.99	12.53
KB	8.10	7.29	7.41	7.74	8.25	7.41	7.95	9.93
OY	16.43	23.86	30.21	23.71	23.11	20.24	23.59	12.89
KY	10.96	10.97	13.86	11.81	12.70	10.91	12.45	10.22
TEP	23.01	30.44	38.52	30.80	30.73	26.79	31.06	19.03
FP	28.20	25.98	25.64	28.36	27.09	28.42	27.42	30.67
PCS	14.47	14.96	15.69	16.58	14.39	15.83	16.25	10.39
RL	4.34	4.67	4.67	4.53	4.53	4.76	4.67	3.57
LL	79.43	84.25	85.88	82.87	81.71	83.46	84.99	68.86
LW	4.31	4.36	4.24	4.44	4.15	4.56	4.40	3.85
LN	154.86	157.34	162.64	158.94	154.67	160.38	159.41	147.71
HT	1.05	1.10	1.02	1.33	0.98	1.04	1.27	1.33
LA	6.08	6.69	6.78	6.71	5.98	6.96	6.85	4.52
LAI	3.60	3.96	4.02	3.97	3.54	4.12	4.05	2.68
DIAM	0.71	0.66	0.67	0.70	0.64	0.75	0.68	0.64
LAR	20.62	22.21	21.86	19.97	21.25	22.22	20.95	19.65
BDM	10.66	12.42	15.22	12.07	12.05	11.39	12.42	8.10
VDM	8.35	7.81	7.96	9.61	7.73	9.09	9.04	7.10
TDM	19.01	20.23	23.18	21.67	19.78	20.48	21.46	15.20
BI	0.56	0.61	0.65	0.56	0.60	0.56	0.58	0.53
E	0.79	0.81	0.92	0.86	0.82	0.80	0.85	0.75
F	0.77	0.80	0.82	0.81	0.77	0.82	0.81	0.66
NAR	10.35	10.06	11.27	10.71	10.86	9.69	10.41	11.18
IV	57.49	57.15	50.82	54.30	54.59	54.70	54.15	54.14
C12:0	0.00	0.00	1.00	0.73	2.10	0.01	0.77	0.99
C14:0	0.40	0.40	1.35	1.05	1.80	1.02	1.22	1.39
C16:0	33.10	32.80	42.35	38.24	36.80	39.42	38.85	38.79
C16:1	0.00	0.00	0.00	0.01	0.00	0.10	0.07	0.03
C18:0	8.60	9.60	5.75	6.16	6.90	6.58	6.23	5.94
C18:1	46.90	45.30	38.55	42.65	39.10	41.00	41.20	41.09
C18:2	9.90	10.50	10.20	0.00	12.10	10.97	10.57	10.69

* Figures in bold indicate maximum value

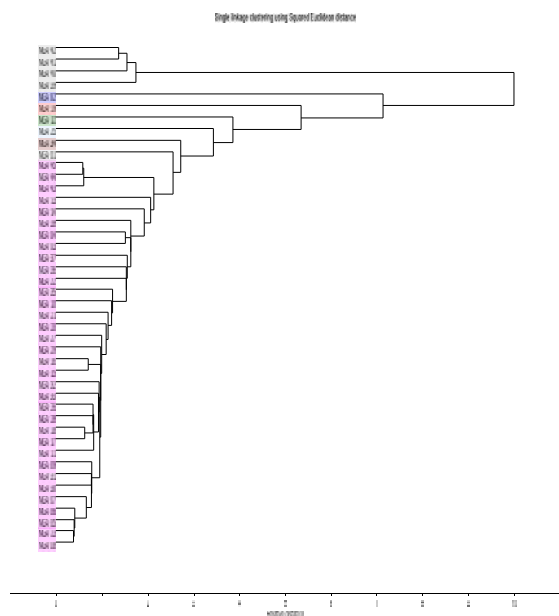


Figure 1 Dendrogram of 44 oil palm accessions based on 43 characters as generated by single linkage method

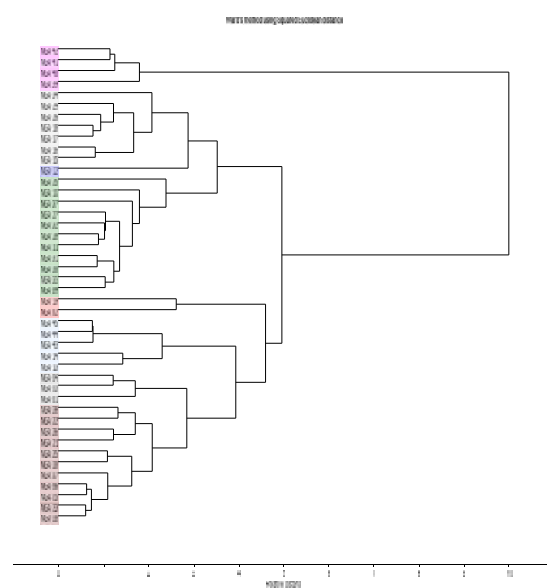


Figure 2 Dendrogram of 44 oil palm accessions based on 43 characters as generated by Ward's method

Table 2 Means for 8 clusters as generated by Ward's method

Traits	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6	Cluster 7	Cluster 8
FFB	190.29	144.85	157.06	160.31	151.50	159.63	102.07	145.86
BNO	17.23	13.18	13.19	13.98	12.08	13.64	15.33	14.32
ABW	11.22	11.39	12.45	11.98	13.15	12.16	7.02	10.62
MFW	9.69	8.19	10.24	9.02	9.71	9.26	6.68	9.00
MNW	4.96	4.54	5.46	5.03	5.37	4.82	4.08	5.16
PB	5.08	2.71	2.26	2.24	2.22	2.81	2.58	1.82
MF	48.55	45.02	46.72	44.27	44.74	47.96	38.86	42.65
KF	12.24	12.24	11.61	13.90	12.50	12.08	15.58	12.10
SF	39.22	42.74	41.68	41.83	42.76	39.96	45.56	45.25
ODM	75.07	73.08	75.22	73.51	74.39	75.02	73.26	74.24
OWM	50.57	46.86	49.15	47.50	48.63	49.17	48.56	48.69
FB	65.44	65.69	67.48	67.14	66.82	66.69	66.42	65.45
OB	16.13	13.94	15.52	14.13	14.54	15.75	12.53	13.57
KB	7.41	7.69	7.57	9.02	8.06	7.71	9.93	7.71
OY	30.21	20.15	24.48	22.70	22.04	25.37	12.89	19.84
KY	13.86	10.96	11.90	14.39	12.19	12.35	10.22	11.36
TEP	38.52	26.73	31.63	31.33	29.36	32.78	19.03	26.66
FP	25.64	27.09	28.26	25.67	27.69	27.29	30.67	27.76
PCS	15.69	14.71	16.33	15.78	17.29	15.04	10.39	15.91
RL	4.67	4.51	4.60	4.74	4.70	4.58	3.57	4.78
LL	85.88	81.84	85.14	82.52	86.50	82.85	68.86	85.15
LW	4.24	4.34	4.35	4.43	4.47	4.29	3.85	4.63
LN	162.64	156.10	157.94	160.40	160.72	157.82	147.71	159.36
HT	1.02	1.07	1.35	1.02	1.38	1.16	1.33	1.18
LA	6.78	6.38	6.72	6.73	7.13	6.43	4.52	7.18
LAI	4.02	3.78	3.98	3.98	4.22	3.81	2.68	4.25
DIAM	0.67	0.69	0.68	0.68	0.70	0.66	0.64	0.73
LAR	21.86	21.42	20.40	21.50	20.42	21.34	19.65	22.32
BDM	15.22	11.54	12.42	12.66	11.94	12.67	8.10	11.70
VDM	7.96	8.08	9.38	8.07	9.75	8.27	7.10	9.04
TDM	23.18	19.62	21.80	20.73	21.69	20.94	15.20	20.74
BI	0.65	0.58	0.57	0.61	0.55	0.60	0.53	0.56
E	0.92	0.80	0.87	0.82	0.84	0.85	0.75	0.80
F	0.82	0.79	0.81	0.81	0.83	0.79	0.66	0.83
NAR	11.27	10.21	10.76	10.22	10.11	10.78	11.18	9.58
IV	50.82	57.32	54.33	53.72	54.62	53.97	54.14	54.50
C12:0	1.00	0.00	0.83	0.80	0.68	0.94	0.99	0.41
C14:0	1.35	0.40	1.19	1.27	1.17	1.30	1.39	1.14
C16:0	42.35	32.95	38.28	39.29	38.51	38.94	38.79	39.15
C16:1	0.00	0.00	0.05	0.09	0.08	0.05	0.03	0.11
C18:0	5.75	9.10	6.34	6.39	6.18	6.26	5.94	6.14
C18:1	38.55	46.10	41.72	40.33	41.71	40.58	41.09	41.34
C18:2	10.20	10.20	9.56	10.68	10.57	10.83	10.69	10.64

* Figures in bold indicate maximum value

Table 3 Proximity matrix of Euclidean distance using single linkage method

Case	Single Linkage Proximity Matrix							
	cluster1	cluster2	cluster3	cluster4	cluster5	cluster6	cluster7	cluster8
cluster1	0.000	.579	1.286	.618	.636	.402	.607	.967
cluster2	.579	0.000	.866	.406	.288	.456	.309	1.391
cluster3	1.286	.866	0.000	.887	.733	1.037	.739	2.068
cluster4	.618	.406	.887	0.000	.396	.441	.324	1.341
cluster5	.636	.288	.733	.396	0.000	.402	.159 ^b	1.393
cluster6	.402	.456	1.037	.441	.402	0.000	.357	1.103
cluster7	.607	.309	.739	.324	.159	.357	0.000	1.395
cluster8	.967	1.391	2.068 ^a	1.341	1.393	1.103	1.395	0.000

^{a,b} indicates highest and least distance respectively

Table 4 Proximity matrix of squared Euclidean distance using Ward's method

Case	Ward Linkage Proximity Matrix							
	cluster1	cluster2	cluster3	cluster4	cluster5	cluster6	cluster7	cluster8
cluster1	0.000	1.107	.534	.435	.812	.397	4.276	1.062
cluster2	1.107	0.000	.156	.217	.097	.227	1.365	.092
cluster3	.534	.156	0.000	.052	.047	.023	2.007	.140
cluster4	.435	.217	.052	0.000	.091	.041	2.138	.178
cluster5	.812	.097	.047	.091	0.000	.108	1.629	.045
cluster6	.397	.227	.023	.041	.108	0.000	2.212	.222
cluster7	4.276 ^a	1.365	2.007	2.138	1.629	2.212	0.000	1.276
cluster8	1.062	.092	.140	.178	.045 ^b	.222	1.276	0.000

^{a,b} indicate highest and least distance respectively

Genetic Distance

The least genetic distance among all the oil palm germplasm was between NGA 08 and NGA 23 (2.272) as shown in the proximity matrix of Euclidean distance (Table not shown due to size) while the highest genetic distance was between NGA 12 and NGA 41 (19.326), followed by NGA 12 and NGA 42 (19.302). The highest inter cluster distance as indicated by the Euclidean distance of single linkage method (Table 3) was between cluster 3 and cluster 8 while the least was between cluster 5 and cluster 7. Furthermore, as shown in the squared Euclidean distance of Ward's method (Table 4), the highest genetic inter cluster distance was between cluster 1 and cluster 7 while the least was between cluster 5 and cluster 8.

DISCUSSION

Samples will be grouped in terms of their nearness or similarity (Hossain *et al.*, 2011). The distribution pattern of all the oil palm germplasm into eight groups revealed the existence of significant genetic diversity among the accessions. Based on the number of clusters selected during analysis, there is no standard procedure or best criterion to determine the exact number of clusters needed in grouping data (Singh *et al.*, 2008; Hair *et al.*, 1995). The number of clusters selected was based on the PC model used in previous studies by the author. Furthermore, two clustering methods were used because a single clustering method might not be always optimal and efficient in revealing genetic associations (Mohammadi & Prasanna, 2003). According to Beebe *et al.* (1998), it is not possible to say what linkage method works best as it depends on the shape of the clusters, therefore, using more than one method is recommended if the aim is to learn as much as possible from the data.

Single linkage clustering analysis (SLCA) was selected from the agglomerative linkage types because it has been reported to be a better technique in that it adequately provides a clearer and more informative display of relative positions of the genotypes (Aremu *et al.*, 2007; Aliyu & Fawole, 2001). Ward's method was also used because it has generally been considered to be very efficient and the most common approach to doing hierarchical clustering analysis (Siracli *et al.*, 2013; Ferreira & Hitchcock, 2009; Kumar & Singh, 2006). As it can be seen from the results, clustering of oil palm germplasm by both SLCA and WHCA differ in some ways in assigning populations to groups. Also from the results of both methods, NGA 12 was placed in a separate group while NGA 39, NGA 40, NGA 41 and NGA 42 were placed in the same groups. NGA 12 had highest values for yield and yield components except in fatty acid content while NGA 39, NGA 40, NGA 41 and NGA 42 had the lowest values for yield traits and its component. This finding corroborates the research of Rajanaidu and Rao (1987).

Furthermore, when selecting genotypes to be included in the hybridization programme on the basis of genetic diversity, high cluster means for yield and its components should be given due importance (Kumar & Singh, 2006). Moreover, the germplasm from these clusters may be exploited for direct use as high yielding varieties or could be used in hybridization programs to

develop materials with desirable characters (Ajmal *et al.*, 2013). Hybridization between accessions of different clusters with high cluster means for quality traits will result into palms which will perform better than their parents (Kumar *et al.*, 2010). Also, the maximum inter cluster diversity exhibited by the accessions grouped into the different cluster will produce a tremendous prospect for oil palm improvement through hybridization programs by crossing accessions from different clusters. Findings follow similar trend as that of Ahmad *et al.* (2014) in multivariate analysis of breadwheat.

Genetic relations in crop species is a significant component of crop species, as it serves to provide information about genetic diversity and also a platform for stratified sampling of breeding populations (Mohammadi & Prasanna, 2003). In the current study, oil palm germplasm with highest genetic distance between them were in different clusters while those with the least genetic distances belong to the same clusters and according to Rahman and Al-Mansur (2009), higher inter and intra cluster distances indicate higher genetic variability between and within clusters respectively and the minimum inter and intra cluster distances indicate closeness among the accessions of two clusters and within the clusters. In view of this, it can be anticipated that crossing between morphologically distant populations will result to maximum degree of heterosis (Odewale *et al.*, 2012; Singh *et al.*, 2008; Mohammadi & Prasanna, 2003). Hence, the use of these populations for breeding programs should be given utmost importance for maximum heterosis as new recombinants with desired characters and high hybrid vigor will be produced. On the other hand, crossing between populations with the least genetic distance between them should be avoided (Odewale *et al.*, 2012). However, it was stated by Rahim *et al.* (2010) that genotypes with minimum distance between them could be used for backcross breeding program.

Genetic diversity is usually associated with geographical diversity but genetic diversity is not necessarily directly related with geographical distribution (Rahman & Al-Mansur, 2009). In this study, grouping of the oil germplasm by the two clustering methods did not follow a particular pattern. Some accessions from same location of collection were grouped together while others from different locations were clustered together. This implies that there is no correspondence between population and their geographic origin. Though Kjaer *et al.* (2004) found correlation between genetic distance and geographic locations in sago palm (a member of the oil palm family; Arecaceae). Zulkifli *et al.* (2012) also found a strong association between genetic distance and geographic location in evaluation of MPOB oil palm germplasm. However, the absence of association between genetic distance and geographic location in this study suggests that the populations of different locations have genetic similarity and could have been derived from the same breeding materials (Tahir *et al.*, 2013) or the random distribution of populations into various clusters from different geographic location implicates that drives other than geographic influence such as exchange of breeding material, genetic drift, natural and artificial selections are responsible for diversity as reported by Murthy and Arunachalm (1966). These findings agree with studies on crops like groundnut (Kumar *et al.* 2010; Makinde & Ariyo, 2010),

sugarcane (Tahir *et al.*, 2013), lime (Rahman & Al-Mansur, 2009), sesame (Seymus & Uzun, 2010), safflower (Khan *et al.*, 2009), coconut (Odewale *et al.*, 2012), soybean (Iqbal *et al.*, 2008) etc.

Acknowledgements

This work was supported by the Research Management Centre of the Universiti Sains Islam Malaysia (USIM) under the grant USIM/FRGS/FST/32/50213. The authors also express their profound gratitude and thanks to the Malaysian Palm Oil Board (MPOB) for providing the data for this study.

CONCLUSION

Cluster analysis as a multivariate analysis has proven as a useful tools in a number of ways. Firstly, it has helped to group the oil palm accessions into groups based on similarity. Secondly, it has helped to identify populations that can best be combined for specific traits and lastly accessions that are morphologically diverse have been identified. The results from the study also showed that there was no consistency of genetic distance with geographic location as accessions from different region were similar and those from same region differed in traits. This diversity could serve as a source of elite materials for oil palm improvement.

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How to cite this article:

Che Wan Zanariah C W Ngah *et al.*, Genetic Diversity In Oil Palm Germplasm As Shown By Hierarchical Clustering Methods. *International Journal of Recent Scientific Research Vol. 6, Issue, 6, pp.4866-4872, June, 2015*
