Journal of Oil Palm Research
DOI: https://doi.org/10.21894/jopr.2022.0073

AN ASSESSMENT OF THE MALAYSIAN OIL PALM BREEDING POPULATIONS USING AFLP MARKERS

MAIZURA ITHNIN[1]*, RAJANAIDU NOOKIAH[2], RAJINDER SINGH[1] and AHMAD KUSHAIRI[3]

ABSTRACT

The narrow genetic diversity coupled with intensive selection has reduced the variability of yield components and vegetative traits of oil palm breeding populations. Thus, the objective of the current study is to evaluate the genetic variability of oil palm breeding populations using AFLP markers. The eight AFLP primer pairs utilised in this study generated 228 bands across 67 populations. As expected, populations created from intercrossing revealed relatively higher levels of genetic diversity compared with those derived from selfings. The dendrogram and Principal Component Analysis (PCA) of the DxD palms indicated a high genetic similarity. Among palms from the TxT/P crosses, the groupings were aligned according to the agency, signifying the accumulation of distinctive sets of alleles, likely due to the different selection pressure imposed by the respective agency. In PCA, the TxT palms, however, revealed a close genetic relationship indicating the need for incorporating new genetic resources to widen the genetic base. Based on the results, it is recommended the introduction of new genetic resources into the dura *and* tenera/pisifera *populations. Additionally, crossing palms from populations of high genetic distances and adopting intercrossing scheme should result in off-springs with considerable diversity for selection gain in future breeding programmes.*

Keywords: AFLPs, breeding populations, genetic assessment, oil palm.

Received: 3 June 2022; **Accepted:** 11 October 2022; **Published online:** 11 November 2022.

INTRODUCTION

Selection in plant breeding is aimed at achieving uniformity and improvement of desirable traits of a cultivated variety. However, selection may also lead to the narrowing of genetic diversity and elimination of allelic variants, which can affect crops' ability to withstand any future environmental challenges (Bhandari *et al.*, 2017; Govindaraj *et al.*, 2015). Although uniformity in high yield, quality, growth and maturity are necessary for advanced farming technology, genetically uniformed crops may hold a hidden danger. A classic example demonstrating the vulnerability of a uniformed crop is the Corn Belt hybrid that was wiped out due to the devastating disease, southern corn leaf blight (SCLB) caused by the fungus, *Helminthosporium maydis* T. (Ullstrup, 1972). History has further revealed that genetic diversity among cultivated varieties and breeding populations is necessary for sustainability and selection gains.

In oil palm, the cultivated variety *dura* x *pisifera* (DxP) or the resultant *tenera* is produced by hybridizing selected *dura* (female) and *pisifera* (male). The *dura* and *pisifera* parental stocks are maintained and improved separately in recurrent selections. DxP progeny testing is carried out to evaluate agronomic performances of various parental combinations (Soh, 1999). The *dura* lines used in commercial DxP seed production in Malaysia are mainly descendants of four palms planted at the Bogor Botanical Garden, Indonesia in 1848 (Rosenquist, 1986). Kushairi (1992) has reviewed the oil palm breeding programmes in

[1] Malaysian Palm Oil Board,
6 Persiaran Institusi, Bandar Baru Bangi,
43000 Kajang, Selangor, Malaysia.

[2] Taman Danau Desa,
Kuala Lumpur, Malaysia

[3] Tiara Heights,
Sepang Selangor, Malaysia

* *Corresponding author e-mail: maizura@mpob.gov.my*

Malaysia. From Bogor, oil palm seeds reached the "Public Garden" in Kuala Lumpur in 1905, and planted in "Experimental Plantation KL", which subsequently became the source of *dura* seeds for the Serdang Avenue (SA) palms. Through an alternative route, seeds from Ulu Remis were brought into Malaysia and laid down at Genetic Blocks I to V and VII in Chemara. These SA and the Ulu Remis materials were later intercrossed and planted at Genetic Block (GB) IIIA, Chemara, Layang-layang. Some of the introgressed populations were established at Kelanang Bharu, Banting and used in breeding programmes.

Unlike the *duras*, the genetic source of *pisifera* breeding populations is slightly more diverse. In Malaysia, oil palm breeders utilised *pisifera* populations from Congo, Nigeria and Ivory Coast, among others (Yong, 1992). Two well-known *pisifera* populations from Congo are Yangambi and AVROS. Yangambi lines were developed by National Institute for Agronomic Study of the Belgian Congo (INEAC) in Eala and nine other *teneras* from Yawenda, N'gazi and Isangi (Hardon *et al*., 1976). A descendant of the Yangambi palm, SP540 was developed in Sungei Panchur, Indonesia. The SP540 palm was crossed to an African *tenera* by *Algemeene Vereniging van Rubberplanters ter Oostkust van Sumatra* (AVROS) (Rosenquist, 1986), where *pisifera* descendants of the crosses were simply called AVROS *pisifera* and also used in breeding and evolved into Ulu Remis *tenera* (URT) (Rosenquist, 1986).

In oil palm, breeding and selection had reduced the coefficient of variation (CV) for fruit bunch yield (Kushairi, 1992; Kushairi *et al.,* 1994; Lee *et al.,* 1990) and decreased the variability in terms of fresh fruit bunch (FFB) components and vegetative traits among some DxP progenies (Noh *et al*., 2010). Diversity of an intended crop can be investigated by both, phenotypic traits and DNA-based methods such as Restriction Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), microsatellites and Single Nucleotide Polymorphism (SNP) (Bhandari *et al.,* 2017). AFLP has been applied for genetic diversity studies in a wide range of plant species including teak (Vaishnaw *et al.,* 2015), orchardgrass (Zhang *et al.,* 2018), potato (Bryan *et al.,* 2017), legume shrub (Fan *et al.,* 2017), winged bean (Mohanty *et al*., 2019), blackberry (Garrido *et al.,* 2020), Jatropha (Avendaño *et al.,* 2015) and *Brassica oleracea* (El-Esawi *et al.,* 2016). The main advantage of AFLP markers is that several loci can be assayed simultaneously, where the technique is amenable to automation and more importantly shows high stability and reproducibility (Thaipong *et al.,* 2017; Todd *et al.,* 2011)

In this study, the genetic variability of 67 oil palm breeding populations in Malaysia was estimated by employing eight AFLP primer combinations. The information generated could help breeders to plan and strategize crossing schemes to prevent inbreeding and maintain sufficient variability among breeding populations for selection gain in future breeding programmes.

MATERIALS AND METHODS

Planting Materials

A total of 67 oil palm breeding populations were sampled from six Malaysian oil palm research organisations (*Table 1*). Of the 67 populations, 39 were DxD, 10 DxP and 18 TxT/P populations. The DxD and TxT/P populations were derived from 10 and 6 different genetic backgrounds respectively. Between 8 and 10 palms were sampled for each population. DNA was extracted from leaf materials sampled from each palm using a modified CTAB method (Rahimah *et al.,* 2006).

TABLE 1. LIST OF DXD, DXP, TXT/P POPULATIONS INCLUDED IN THE STUDY

No.	Population name	Crossing scheme	Agency	Cross type
1	AG1D1	self	Agency 1	DxD
2	AG1D2	intercross	Agency 1	DxD
3	AG1D3	intercross	Agency 1	DxD
4	AG1D4	intercross	Agency 1	DxD
5	AG2D1	self	Agency 2	DxD
6	AG2D2	intercross	Agency 2	DxD
7	AG2D3	intercross	Agency 2	DxD
8	AG2D4	intercross	Agency 2	DxD
9	AG2D5	self	Agency 2	DxD
10	AG2D6	self	Agency 2	DxD

TABLE 1. LIST OF DXD, DXP, TXT/P POPULATIONS INCLUDED IN THE STUDY (continued)

No.	Population name	Crossing scheme	Agency	Cross type
11	AG2D7	intercross	Agency 2	DxD
12	AG2D8	intercross	Agency 2	DxD
13	AG2D9	intercross	Agency 2	DxD
14	AG2D10	intercross	Agency 2	DxD
15	AG2D11	intercross	Agency 2	DxD
16	AG3D1	self	Agency 3	DxD
17	AG3D2	self	Agency 3	DxD
18	AG3D3	self	Agency 3	DxD
19	AG3D4	self	Agency 3	DxD
20	AG3D5	self	Agency 3	DxD
21	AG3D6	intercross	Agency 3	DxD
22	AG3D7	self	Agency 3	DxD
23	AG3D8	intercross	Agency 3	DxD
24	AG3D9	self	Agency 3	DxD
25	AG3D10	self	Agency 3	DxD
26	AG4D1	self	Agency 4	DxD
27	AG4D2	intercross	Agency 4	DxD
28	AG5D1	intercross	Agency 5	DxD
29	AG5D2	intercross	Agency 5	DxD
30	AG6D1	intercross	Agency 6	DxD
31	AG6D2	self	Agency 6	DxD
32	AG6D3	intercross	Agency 6	DxD
33	AG6D4	self	Agency 6	DxD
34	AG6D5	intercross	Agency 6	DxD
35	AG6D6	self	Agency 6	DxD
36	AG6D7	self	Agency 6	DxD
37	AG6D8	intercross	Agency 6	DxD
38	AG6D9	self	Agency 6	DxD
39	AG6D10	self	Agency 6	DxD
40	AG3DP1	intercross	Agency 3	DxP
41	AG3DP2	intercross	Agency 3	DxP
42	AG4DP1	intercross	Agency 4	DxP
43	AG4DP2	intercross	Agency 4	DxP
44	AG5DP1	intercross	Agency 5	DxP
45	AG6DP1	intercross	Agency 6	DxP
46	AG6DP2	intercross	Agency 6	DxP
47	AG6DP3	intercross	Agency 6	DxP
48	AG6DP4	intercross	Agency 6	DxP
49	AG7DP1	intercross	Agency 6	DxP
50	AG6T1	intercross	Agency 6	TxP
51	AG6T2	intercross	Agency 6	TxP
52	AG6T3	intercross	Agency 6	TxP
53	AG6T4	intercross	Agency 6	TxP

TABLE 1. LIST OF DXD, DXP, TXT/P POPULATIONS INCLUDED IN THE STUDY (continued)

No.	Population name	Crossing scheme	Agency	Cross type
54	AG6T5	intercross	Agency 6	TxP
55	AG6T6	intercross	Agency 6	TxP
56	AG1T1	intercross	Agency 1	TxT
57	AG1T2	intercross	Agency 1	TxT
58	AG3T1	intercross	Agency 3	TxT
59	AG3T2	self	Agency 3	TxT
60	AG3T3	intercross	Agency 3	TxT
61	AG3T4	self	Agency 3	TxT
62	AG3T5	intercross	Agency 3	TxT
63	AG3T6	self	Agency 3	TxT
64	AG3T7	intercross	Agency 3	TxT
65	AG3T8	self	Agency 3	TxT
66	AG4T1	self	Agency 4	TxT
67	AG4T2	self	Agency 4	TxT

AFLP and Data Analysis

The AFLP procedure was performed with AFLP Analysis System 1 from GIBCO BRL, USA following the manufacturer's recommended protocol. Some 300 ng of each DNA sample was digested with *Eco*RI and *Mse*I mixture at 37°C for 3 hr. A 24 µL of ligation mix containing *Eco*RI and *Mse*I was added to each digest adaptors and used as template for pre-amplification. A 5 µL of pre-amplified DNA template, together with the [γ^{33}] ATP-labelled *Eco*RI and unlabelled *Mse*I primers were subjected to selective PCR. The 8 primer combinations of *Eco*RI and *Mse*I are listed in *Table 2*. The PCR products were electrophoresed on 6% polyacrylamide gels at 1600 V for 3 hr. Gels were exposed against X-ray films for 4-5 days in -80°C freezer.

Autoradiograms were scored manually, band presence being indicated by '1' and absence by '2'. Genetic diversity analysis was performed using PowerMarker software (Liu and Muse, 2005). Among the parameters estimated were major allele frequency, allele number, gene diversity and Polymorphic Information Content (PIC). These parameters were computed for each marker as well as population. Gene diversity refers to the probability of two randomly chosen alleles from the population are different whereas PIC measures the polymorphism degree of the markers and populations (Botstein *et al.*, 1980; Serrote *et al.*, 2020). Genetic distance among individuals was computed using Darwin software (Perrier *et al.*, 2003). Dendrograms were constructed for each DxD and TxT/P palms, following the unweighted pair group method with arithmetic mean (UPGMA) method to visualise the genetic relationship between the palms. Principal Coordinate Analysis (PCA) was carried out to illustrate the coordinates of palms for each DxD and TxT/P crosses.

RESULTS

Genetic Diversity Revealed by AFLPs

The number of scorable bands revealed by the 8 primer combinations varied between 14 and 48 (*Table 2*), contributing to a total of 225 bands scored for data analysis. On average, the AFLP markers recorded gene diversity and PIC values of 0.2274 and 0.2008, respectively (*Table 3*). The PIC of the markers ranged between 0.0628 to 0.4226 (data not shown). The genetic variability measures for each population are presented in *Table 4*. Among the DxD populations, the values for allele number ranged from 1.0658 to 1.6404, while that for gene diversity and PIC ranged from 0.0210 to 0.1502 and 0.0172 to 0.1292 respectively. Population AG2D1 of Agency 2 recorded the lowest score for these parameters whereas the highest was observed for population AG6D10 from Agency 6. Among the DxP populations, progeny AG6DP1 had the lowest number of alleles (1.1491), the lowest score for gene diversity (0.0467) and PIC (0.0380), while progeny AG4DP2 exhibited the highest scores for these parameters (1.5833, 0.1989 and 0.1613) respectively. As for the TxT/P crosses, gene diversity (0.0575) and PIC (0.0472) were lowest in AG1T1 but highest in AG6T6 (0.2689 and 0.2144). Progeny AG6T1 exhibited the lowest number of alleles (1.1491), while AG6T6 recorded the highest (1.1886).

TABLE 2. PRIMER COMBINATIONS USED AND THE NUMBER OF BANDS OBTAINED IN THE AFLP ANALYSIS

Primer combinations	Number of bands
E-AAC/M-CAG	32
E-ACA/M-CTT	33
E-ACC/M-CTC	15
E-ACG/M-CAA	17
E-ACT/M-CTG	29
E-ACG/M-CAT	40
E-AGG/M-CAC	14
E-AAG/M-CTA	48

TABLE 3. GENETIC DIVERSITY PARAMETERS NAMELY, MAJOR ALLELE FREQUENCY, GENE DIVERSITY AND POLYMORPHIC INFORMATION CONTENT (PIC) ESTIMATED FOR EACH PRIMER COMBINATION APPLIED IN THE STUDY

Primer combination	Major allele frequency	Gene diversity	PIC
AAC/CAG	0.8436	0.2372	0.2101
ACA/CTT	0.8229	0.2773	0.2452
ACA/CTT	0.8944	0.1758	0.1590
ACC/CTC	0.8899	0.1912	0.1752
ACG/CAA	0.7976	0.3069	0.2685
ACT/CTG	0.8690	0.2012	0.1733
AGC/CAT	0.8776	0.2026	0.1825
AAG/CAC	0.8741	0.1968	0.1737
Mean	0.8545	0.2274	0.2008

TABLE 4. GENETIC DIVERSITY PARAMETERS NAMELY, ALLELE NUMBER, GENE DIVERSITY AND POLYMORPHIC INFORMATION CONTENT (PIC) ESTIMATED FOR POPULATIONS ANALYSED IN THE STUDY

No.	Population name	Allele no.	Gene diversity	PIC
1	AG1D1	1.1579	0.0536	0.0435
2	AG1D2	1.2939	0.0903	0.0745
3	AG1D3	1.4035	0.1131	0.0944
4	AG1D4	1.3947	0.1274	0.1044
5	AG2D1	1.0658	0.0210	0.0172
6	AG2D2	1.1623	0.0419	0.0354
7	AG2D3	1.1316	0.0423	0.0344
8	AG2D4	1.2061	0.0494	0.0425
9	AG2D5	1.1842	0.0498	0.0420
10	AG2D6	1.1667	0.0512	0.0421
11	AG2D7	1.1667	0.0532	0.0436
12	AG2D8	1.2544	0.0545	0.0480
13	AG2D9	1.1886	0.0641	0.0519
14	AG2D10	1.2675	0.0740	0.0617
15	AG2D11	1.3947	0.1255	0.1037
16	AG3D1	1.1272	0.0363	0.0303
17	AG3D2	1.1316	0.0428	0.0349

TABLE 4. GENETIC DIVERSITY PARAMETERS NAMELY, ALLELE NUMBER, GENE DIVERSITY AND POLYMORPHIC INFORMATION CONTENT (PIC) ESTIMATED FOR POPULATIONS ANALYSED IN THE STUDY (continued)

No.	Population name	Allele no.	Gene diversity	PIC
18	AG3D3	1.1623	0.0494	0.0406
19	AG3D4	1.1491	0.0549	0.0438
20	AG3D5	1.1579	0.0566	0.0452
21	AG3D6	1.1886	0.0578	0.0476
22	AG3D7	1.1842	0.0587	0.0481
23	AG3D8	1.2500	0.0703	0.0590
24	AG3D9	1.3904	0.1051	0.0882
25	AG3D10	1.4167	0.1052	0.0895
26	AG4D1	1.2368	0.0738	0.0608
27	AG4D2	1.3070	0.0933	0.0772
28	AG5D1	1.2807	0.0760	0.0640
29	AG5D2	1.5395	0.1324	0.1139
30	AG6D1	1.1404	0.0436	0.0357
31	AG6D2	1.1491	0.0509	0.0411
32	AG6D3	1.1667	0.0578	0.0467
33	AG6D4	1.1842	0.0579	0.0476
34	AG6D5	1.2193	0.0786	0.0634
35	AG6D6	1.3509	0.0852	0.0729
36	AG6D7	1.2851	0.0920	0.0751
37	AG6D8	1.2807	0.1061	0.0841
38	AG6D9	1.5395	0.1469	0.1234
39	AG6D10	1.6404	0.1502	0.1292
40	AG3DP1	1.2105	0.0631	0.0519
41	AG3DP2	1.2456	0.0828	0.0668
42	AG4DP1	1.3026	0.0854	0.0713
43	AG4DP2	1.5833	0.1989	0.1613
44	AG5DP1	1.4825	0.1239	0.1053
45	AG6DP1	1.1491	0.0467	0.0380
46	AG6DP2	1.2807	0.0676	0.0581
47	AG6DP3	1.3465	0.0924	0.0776
48	AG6DP4	1.3728	0.1256	0.1032
49	AG7DP1	1.5921	0.1755	0.1456
50	AG6T1	1.1491	0.0598	0.0476
51	AG6T2	1.2719	0.1047	0.0844
52	AG6T3	1.5833	0.1617	0.1366
53	AG6T4	1.5746	0.2111	0.1688
54	AG6T5	1.4956	0.2203	0.1713
55	AG6T6	1.7149	0.2689	0.2144
56	AG1T1	1.1886	0.0575	0.0472
57	AG1T2	1.3596	0.1175	0.0961

TABLE 4. GENETIC DIVERSITY PARAMETERS NAMELY, ALLELE NUMBER, GENE DIVERSITY AND POLYMORPHIC INFORMATION CONTENT (PIC) ESTIMATED FOR POPULATIONS ANALYSED IN THE STUDY (continued)

No.	Population name	Allele no.	Gene diversity	PIC
58	AG3T1	1.2807	0.0749	0.0629
59	AG3T2	1.3202	0.0982	0.0808
60	AG3T3	1.4298	0.1045	0.0891
61	AG3T4	1.3991	0.1204	0.1002
62	AG3T5	1.3904	0.1235	0.1021
63	AG3T6	1.6623	0.1463	0.1275
64	AG3T7	1.6798	0.1796	0.1515
65	AG3T8	1.5965	0.2042	0.1648
66	AG4T1	1.3509	0.1017	0.0846
67	AG4T2	1.5570	0.1551	0.1303

The gene diversity and PIC values for DxD and TxT crosses were averaged according to their crossing schemes (intercrossing and selfing) (*Figure 1*). Populations derived from intercrossings showed on average higher PIC than those produced from selfings. In general, *dura* populations had lower PIC scores than those observed in TxT and TxP populations.

Population Structure Analysis

The dendrograms based on genetic distances estimated among all individual palms are presented in *Figure 2* (DxD populations) and *Figure 3* (TxT/P populations). Apart from the main cluster (denoted as '1'), the majority of the DxD palms formed small sub-clusters, not according to genetic background or agency. In the main cluster, two sub-populations were observed; the first was confined to DxD palms from Agency 2 only while the second sub-cluster consisted of palms from six agencies. The palms of the TxT/P populations also formed several sub-clusters but each sub-cluster consisted of palms from the same agency (*Figure 3a*). The TxT/P palms attained from Agency 3 formed sub-clusters with mixed origins. The Principal Component Analysis (PCA) of the DxD (*Figure 4a*) and TxT (*Figure 4b*) revealed that the majority of palms from different agencies, derived from various genetic backgrounds were positioned at the centre of the PCA, signifying a close genetic relationship.

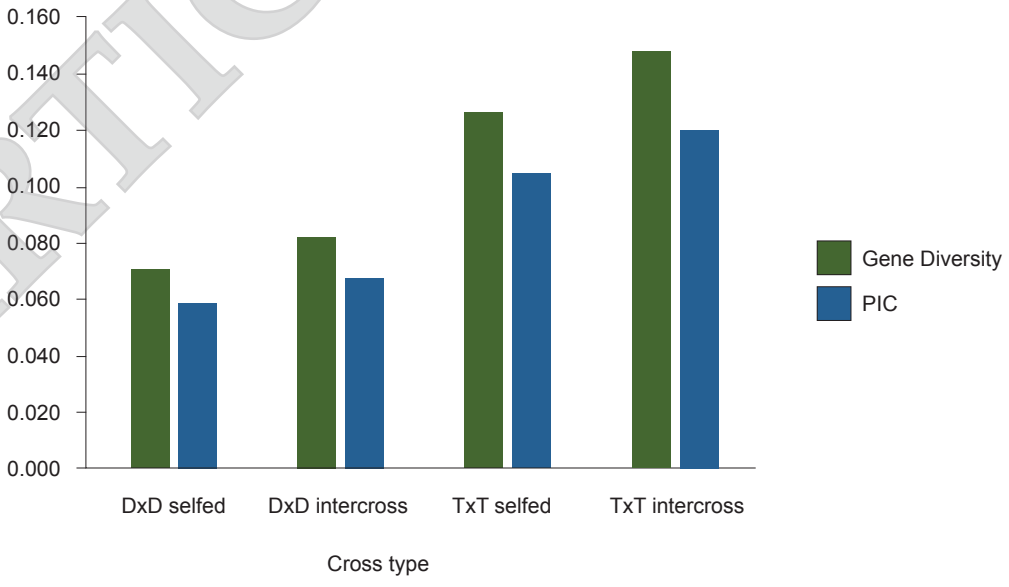

Figure 1. Barplots showing the differences in Gene diversity and PIC values between selfed and intercrossed populations for both DxD and TxT/P crosses. In general, selfed population exhibited lower diversity values compared to intercrossed.

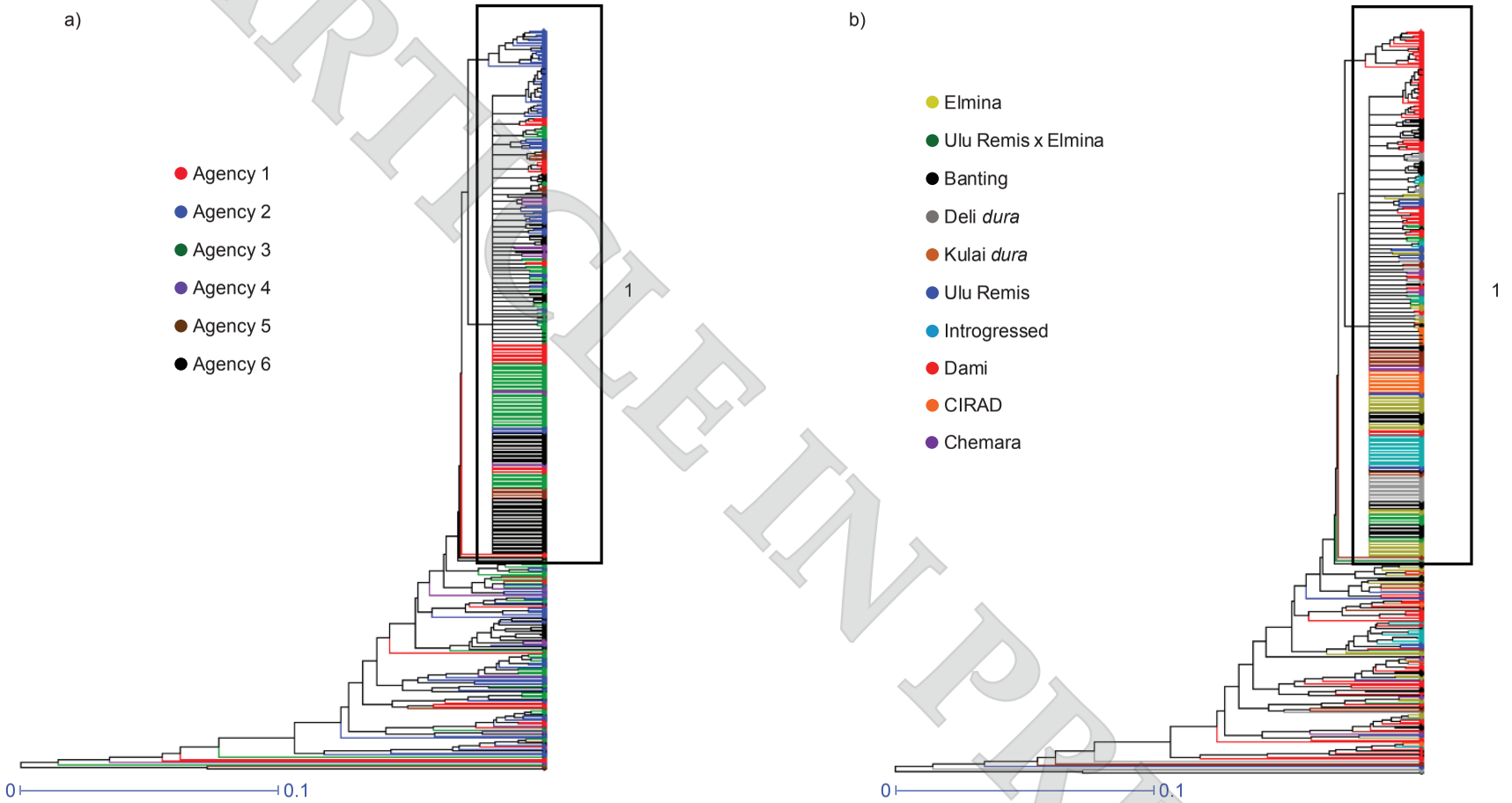

Figure 2. Dendrograms presenting the genetic relationship between DxD individual palms analysed in the study. Each branch represents individual palm, coloured respectively according to a) 6 oil palm agencies, b) genetic backgrounds. The main cluster (marked as 1) is composed of palms from various agencies. The sub-populations observed at the bottom contained palms from mixed agencies and genetic backgrounds.

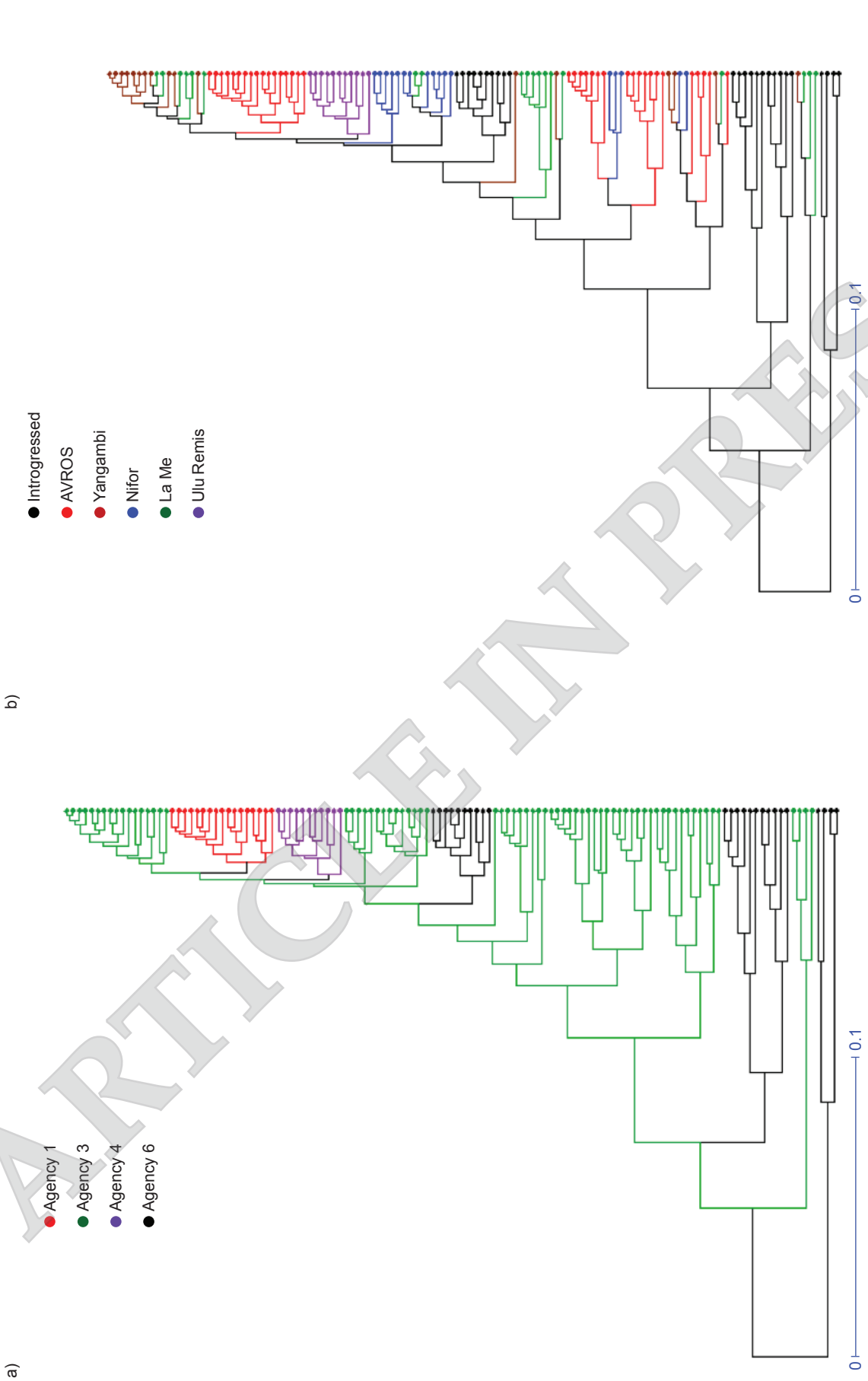

Figure 3. Dendrogram presents the genetic relationship between TxT/P individual palms analysed in the study. Each branch represents individual palm, coloured respectively according to a) 6 oil palm agencies, b) genetic backgrounds. Palms from agency 3 (green dots in (a)) originated from various genetic backgrounds are mixed in the clusters (b).

Figure 4. Principal Component Analysis showing coordinates of a) DxD and b) TxT individual palms. Dots are coloured corresponding to the 6 oil palm agencies. Most palms are located close to the centre of PCA. No clear groupings are observed.

DISCUSSION

The AFLP technique, which has proven reliable in plant genetic studies over the last 20 years, was utilised to obtain an overview of the diversity of the advanced oil palm breeding populations in Malaysia. In this study, although on average, the AFLP markers exhibited moderate levels of informativeness (PIC = 0.2008), there are markers that recorded PIC above 0.3000, signifying high discriminatory power for determining the diversity of the breeding populations. However, as AFLP is a dominant marker, it is not able to detect recessive alleles or select for palms that are homozygous or heterozygous at a particular locus for breeding purposes. Despite these limitations, the AFLP markers provided useful genetic information for oil palm genetic and breeding studies. AFLP markers can be linked to specific loci under selection (Jump and Penuelas, 2007) and Kirschner *et al*. (2021) were of the view that since the AFLP technique can analyse a large number of loci for polymorphism with a single primer pair, it is comparable to Restriction-Site Associated DNA (RAD) sequencing in its effectiveness at genetic analysis.

Oil palm breeding and improvement programmes in Malaysia utilised genetic materials from restricted sources. The impact of selection and breeding on breeding populations with narrow genetic bases can be more prominent as recombination involving recessive alleles or undesirable genes can affect population fitness (Rosenquist, 1986), indicating the importance of continuously evaluating the genetic diversity of the oil palm advanced breeding populations. In this study, oil palm breeding populations originating from various genetic backgrounds provided by six oil palm research agencies were analysed. The phylogenetic tree of the DxD individual palms displayed one main cluster and several sub-clusters with no clear preference to genetic background or agency. The PCA showed that majority of the palms were positioned at the center, demonstrating genetic similarity, except a few that were outside the cluster suggesting that the DxD palms analysed had close genetic relationship. This finding was similar to those reported in previous analyses on oil palm breeding populations (Budiman *et al*., 2019; Chun *et al*., 2018; Purba *et al*., 2000).

It would appear that the *dura* breeding populations at the different agencies had very similar genetic background. Although the populations analysed in this study were from different selection programmes (e.g., Banting, Kulai *dura*, Dami, Elmina Chemara, Ulu Remis), they nevertheless originated from the four Bogor palms established at the Bogor Botanical Garden, Indonesia in 1848. The narrow gene pool among the *dura* populations had resulted in the accumulation of a mostly common set of alleles despite different selection pressure imposed by the agencies in their breeding programmes. This also contributed to the lower average gene diversity and PIC in the DxD compared with those of the TxT/P populations. Low diversity among breeding lines has also been observed in breeding programmes of other plant species such as cauliflower (Lee *et al.*, 2020), cucumber (Zhu *et al*., 2018), cacao (Aikpokpodion *et al.*, 2009), conifer (*Platycladus orientalis*) (Jin *et al.*, 2016), sunflower (Filippi *et al*., 2020) and cotton (Billings *et al*; 2021).

The dendrogram for the TxT/P palms generally displayed grouping according to the agency. The *tenera* populations in Malaysia originated from several African breeding programmes. Thus, when subjected to breeding and selection, distinctive sets of alleles likely accumulated in materials sourced from the different agencies. It was observed that the populations from Agency 3 are mixed across several clusters. Agency 3 had likely sourced materials of different genetic backgrounds for incorporation into their breeding programme as part of the initiative to develop planting materials with slower vertical growth, bigger fruit bunches and larger fruits. Similar to the DxD palms, the TxT/P palms were also positioned at the centre of the PCA plot signifying the need for introducing new genetic resources. Although the global oil palm industry had benefited from hybrid vigour, sustaining variability among the parental lines is essential for further genetic improvement.

The conventional breeding programme involves improving the maternal *dura* and paternal *pisifera* lines separately where the parental lines are subsequently hybridised via controlled pollination to realise the hybrid vigour in the commercial DxP seedlings. The two breeding schemes employed are reciprocal recurrent selection (RSS) and modified reciprocal recurrent selection (MRRS), depending on the overall objective of the improvement programme. The RRS scheme often involves selfing of palms prior to seed production, as a means to improve the homozygosity of parental lines and reduce variation of subsequent commercial progenies. The MRRS scheme although accommodates selfing, intercrossing of selected lines is widely practised in parental improvement programmes. As expected, the study showed that populations derived from intercrossing recorded relatively higher diversity than selfing. It seems that selfing is inducing homozygosity as intended, which is in maize inbred lines and can contribute towards hybrid vigour when outcrossed to selected *pisifera*. Crossing between palms from DxD and TxT/P populations that recorded high homozygosity, indicated by low gene diversity and PIC values estimated in this study, can likely result in higher uniformity in the resulting hybrids as desired by commercial estates. However, intercrossing as largely practised in the MRRS scheme in Malaysia, can help maintain diversity within the parental improvement programme, especially if the breeders select and hybridise palms from specific populations that record high genetic distance values. These resulting parental populations will thus possess a higher number of different alleles, thus hybridization between them would result in hybrids with relatively higher genetic diversity. Sritharan *et al*. (2017) had shown that hybrids generated from more diverse parental backgrounds can result in higher oil yield than those from a limited and highly inbred genetic background. In fact, the breeders have also started incorporating genetic source from the Nigerian germplasm as a pollen source to enhance diversity and generate new planting materials known as PS1.1 that showed high yield with slow height increment (Arolu, 2017; Kushairi *et al*., 2001; Kushairi and Amiruddin, 2020). The outcome from such an approach can reduce the risk of inbreeding depression as well as safeguard genetic variability for selection gain in oil palm in the future. In summary, marker-assisted efforts can help select palms for crossing to realise the desired hybrid vigour while also assisting in increasing variability among breeding populations, in the effort to develop more resilient varieties against diseases and climate change. The overview of the advanced breeding populations in Malaysia obtained via AFLP in this study can be further complemented by other co-dominant markers such as SSR and SNPs in the future.

CONCLUSION

AFLP marker analysis revealed high genetic similarity among selected oil palm (*Elaeis guineensis* Jacq.) breeding populations utilised in Malaysia, due to the narrow gene pool. The introduction of new genetic resources into the *dura* and *tenera*/*pisifera* populations is highly recommended. Such efforts can help increase genetic variability among the breeding populations to ensure selection gain and maintain survival against diseases and extreme climate change in the future.

ACKNOWLEDGEMENT

We wish to gratefully acknowledge MPOB for providing resources to conduct the study.

REFERENCES

Aikpokpodion, P O; Motamayor, J C; Adetimirin, V O; Adu-Ampomah, Y; Ingelbrecht, I; Eskes, A B; and Kolesnikova-Allen, M (2009). Genetic diversity assessment of sub-samples of cacao, *Theobroma cacao* L. collections in West Africa using simple sequence repeats marker. *Tree Genet. Genomes, 5(4)*: 699-711.

Arolu, I W; Rafii, M Y; Marjuni, M; Hanafi, M M; Sulaiman, Z; Rahim, H A; Zainol Abidin, M I; Amirudin, M D; Ahmad Kushairi, D; and Nookiah, R (2017). Breeding of high-yielding and dwarf oil palm planting materials using Deli *dura* × Nigerian *pisifera* population. *Euphytica, 213*: 154. DOI: https://doi.org/10.1007/s10681-017-1943-z.

Avendaño, R; Díaz, E G; Valdez-Melara, M; Solano, N C; Villalobos A M; Cascante F A; Benavides B W and Solis-Ramos L Y (2015). Genetic diversity analysis of *Jatropha* species from Costa Rica using AFLP markers. *Am. J. Plant Sci., 6(14)*: 2426.

Bhandari, H R; Bhanu, A N; Srivastava, K; Singh, M N; and Shreya, H A (2017). Assessment of genetic diversity in crop plants-an overview. *Adv. Plants Agric. Res, 7(3)*: 279-286.

Billings, G T; Jones, M A; Rustgi, S; Hulse-Kemp, A M and Campbell, B T (2021). Population structure and genetic diversity of the Pee Dee cotton breeding program. *G3 Genes Genomes Genet., 11(7)*: jkab145. DOI: https://doi.org/10.1093/g3journal/jkab145.

Botstein, D; White, R L; Skolnick, M and Davis, R W (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Amer. Jour. of Hum. Genet., 32(3)*: 314-331.

Bryan, G J; McLean, K; Waugh, R and Spooner, D M (2017). Levels of intra-specific AFLP diversity in tuber-bearing potato species with different breeding systems and ploidy levels. *Front. Genet., 8*: 119.

Budiman, L F; Apriyanto, A; Pancoro, A D I and Sudarsono, S (2019). Genetic diversity analysis of *tenera*×*tenera* and *tenera*×*pisifera* crosses and D self of oil palm (*Elaeis guineensis*) parental populations originating from Cameroon. *Biodivers. J. Biol. Divers., 20(4)*: 937-949.

Chun, T C; Ling, CK; Hee, L C; Choo, C S; Barin, J; Fong, A; Abdullah, M P and Guan, T S (2018). Genetic diversity and inbreeding level in deli *dura* and AVROS advanced breeding materials in oil palm (*Elaeis guineensis* Jacq.) using microsatellite markers. *J. Oil Palm Res., 30*: 366-379.

El-Esawi, M A; Germaine, K; Bourke, P and Malone, R (2016). AFLP analysis of genetic diversity and phylogenetic relationships of *Brassica oleracea* in Ireland. *Comptes. Rendus. Biol., 339(5-6)*: 163-170.

Fan, Y; Zhang, C; Wu, W; He, W; Zhang, L and Ma, X (2017). Analysis of genetic diversity and structure pattern of *Indigofera pseudotinctoria* in Karst Habitats of the Wushan Mountains using AFLP markers. *Molecules, 22(10)*: 1734.

Filippi, C V; Merino, G A; Montecchia, J F; Aguirre, N C; Rivarola, M; Naamati, G; Fass, M I; Álvarez, D; Di Rienzo, J; Heinz, R A and Contreras Moreira, B; (2020). Genetic diversity, population structure and linkage disequilibrium assessment among international sunflower breeding collections. *Genes, 11(3)*: 283.

Garrido, P; Morillo, E and Vásquez-Castillo, W (2020). Genetic diversity of the Andean blackberry (*Rubus glaucus* Benth.) in Ecuador assessed by AFLP markers. *Plant Genet. Resour., 18(4)*: 243-250.

Govindaraj, M; Vetriventhan, M; and Srinivasan, M (2015). Importance of genetic diversity assessment in crop plants and its recent advances: An overview of its analytical perspectives. *Genet. Res. Int., 431487.* http://dx.doi.org/10.1155/2015/431487.

Hardon, J J; Gascon, J P; Noiret, J M; Meunier, J; Tan, G Y and Tan, T K (1976). Major oil palm breeding programmes. *Oil Palm Research* (Corley R H V; Hardon J J and Wood, B J eds.). Elsevier, Amsterdam. p. 107-125.

Jin, Y; Ma, Y; Wang, S; Hu, X G; Huang, L S; Li, Y; Wang, X R and Mao, J F (2016). Genetic evaluation of the breeding population of a valuable reforestation conifer *Platycladus orientalis* (Cupressaceae). *Sci. Rep., 6(1)*: 1-9.

Jump, A S and Penuelas, J (2007). Extensive spatial genetic structure revealed by AFLP but not SSR molecular markers in the wind-pollinated tree, *Fagus sylvatica*. *Mol. Ecol., 16(5)*: 925-936.

Kirschner, P; Arthofer, W; Pfeifenberger, S; Záveská, E; Schönswetter, P; Steiner, F M and Schlick-Steiner, B C (2021). Performance comparison of two reduced-representation based genome-wide marker-discovery strategies in a multi-taxon phylogeographic framework. *Sci. Rep., 11(1)*: 1-12.

Kushairi A (1992) *Prestasi baka sawit dura x pisifera di Malaysia*. Masters. Thesis. Universiti Kebangsaan Malaysia.

Kushairi, A; Rajanaidu, N; Jalani, B S and Zakri, A H (1994). Variation in Malaysian *dura* × *pisifera* planting materials. I. Bunch yield. *J. Oil Palm Res., 6(1)*: 14-24.

Kushairi, A and Amiruddin, M D (2020). Development of new oil palm cultivars in Malaysia. *J. Oil Palm Res., 32(3):* 420-26.

Kushairi, A; Rajanaidu, N; Rafii, M Y; Mohd Din, A and Mohd Isa, Z A (2001). Population 12 - pollen for the development of dwarf PS1 - type oil palm planting materials. *MPOB Information Series No.100.*

Lee, C H; Yong, Y Y; Donough, C and Chiu S B (1990). Selection progress in Deli *dura* populations. *Proc. of Intl. Workshop on Oil Palm Breeding Populations*. PORIM, Malaysia, p. 81-89.

Lee, H Y; Kim, J G; Kang, B C and Song, K (2020). Assessment of the genetic diversity of the breeding lines and a genome wide association study of three horticultural traits using worldwide cucumber (*Cucumis* spp.) germplasm collection. *Agronomy, 10(11)*: 1736.

Liu, K and Muse, S V (2005). PowerMarker: An integrated analysis environment for genetic marker analysis. *Bioinformatics, 21(9)*: 2128-2129.

Mohanty, C S; Singh, V; Gupta, P; Srivsatava, A; Pattanayak, R; Jaiswal, V and Dikshit, N (2019). Estimation of genetic diversity, structure and trait association of winged bean (*Psophocarpus tetragonolobus* (L.) DC.), genotypes through AFLP and ITS markers. *Indian J. Biotech. Vol., 18*: 235-245.

Noh A; Rafii M Y; Saleh G and Kushairi A (2010). Genetic performance of 40 Deli *dura* x AVROS *pisifera* full-sib families. *J. Oil Palm Res. 22:* 781-795.

Perrier, X; Flori, A and Bonnot, F (2003). Data analysis methods. *Genetic Diversity of Cultivated Tropical Plants*. (Hamon, P; Seguin, M; Perrier, X and Glaszmann, J C eds.). Science Publishers, Montpellier. p. 43-76.

Purba, A R; Noyer, J L; Baudouin, L; Perrier, X; Hamon, S and Lagoda, P J L (2000). A new aspect of genetic diversity of Indonesian oil palm (*Elaeis guineensis* Jacq.) revealed by isoenzyme and AFLP markers and its consequences for breeding. *Theor. Appl. Genet., 101(5)*: 956-961.

Rahimah, A R; Cheah, S C and Rajinder, S (2006) Freeze drying of oil palm (*Elaeis guineensis*) leaf and its effect on the quality of extractable DNA. *J Oil Palm Res., 18*: 296-304.

Rosenquist, E A (1986). The genetic base of oil palm breeding populations. *Proc. of the Intl. Workshop on Oil Palm Germplasm and Utilization*. MPOB, Bangi. p. 27-56.

Serrote, C M L; Reiniger, L R S; Silva, K B; dos Santos Rabaiolli, S M and Stefanel, C M (2020). Determining the polymorphism information content of a molecular marker. *Gene, 726:144175.*

Soh A (1999). Breeding plans and selection methods in oil palm. *Proc. of the Symposium on the Science of Oil Palm Breeding*. PORIM, Bangi. p. 65-95.

Sritharan, K; Subramaniam, M; Arulandoo, X and Yusop, M R (2017). Yield and bunch quality component comparison between two-way crosses and multi-way crosses of DxP oil palm progenies. *Sains Malaysiana, 46(9):* 1587-1595.

Thaipong, K; Promchot, S; Auvuchanon, A and Boonprakob, U (2017). Genetic analysis of guava germplasm using AFLP markers. *Int. J. Agric. Technol., 13*: 741-752.

Todd, J; Wu, Y Q; Wang, Z and Samuels, T (2011). Genetic diversity in tetraploid switchgrass revealed by AFLP marker polymorphisms *Genet. Mol. Res., 10*: 2976-2986.

Ullstrup, A J (1972). The impacts of the southern corn leaf blight epidemics of 1970-1971. *Ann. Rev. of Phytopathology, 10(1):* 37-50.

Vaishnaw, V; Mohammad, N; Wali, S A; Kumar, R; Tripathi, S B; Negi, M S and Ansari, S A (2015). AFLP markers for analysis of genetic diversity and structure of teak (*Tectona grandis*) in India. *Cana. J. For. Res., 45(3)*: 297-306.

Yong, Y Y (1992). The oil palm breeding programme in Malaysia. *Symposium on the Science of Oil Palm Breeding* (Rajanaidu D and Jalani B S eds.). PORIM, Malaysia. p. 141-64.

Zhang, C; Sun, M; Zhang, X; Chen, S; Nie, G; Peng, Y; Huang, L and Ma, X (2018). AFLP-based genetic diversity of wild orchardgrass germplasm collections from Central Asia and Western China, and the relation to environmental factors. *PloS One, 13(4):* p.e0195273.

Zhu, S; Zhang, X; Liu, Q; Luo, T; Tang, Z and Zhou, Y (2018). The genetic diversity and relationships of cauliflower (*Brassica oleracea* var. *botrytis*) inbred lines assessed by using SSR markers. *PloS One, 13(12)*: e0208551.