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

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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 TxD/T 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 TxDD 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.

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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

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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 DxFP 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), 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 DxFP and 18 TtT/P populations. The DxD and TtT/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).

| 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        |
| 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 EcoRI and MseI mixture at 37°C for 3 hr. A 24 µL of ligation mix containing EcoRI and MseI was added to each digest adaptors and used as template for pre-amplification. A 5 µL of pre-amplified DNA template, together with the [γ33P] ATP-labelled EcoRI and unlabelled MseI primers were subjected to selective PCR. The 8 primer combinations of EcoRI and MseI 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 AG2D10 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 |
| 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           | 1.1491     | 0.0509         | 0.0411 |
| 31  | AG6D2           | 1.1491     | 0.0578         | 0.0467 |
| 32  | AG6D3           | 1.1842     | 0.0579         | 0.0476 |
| 33  | AG6D4           | 1.2193     | 0.0786         | 0.0634 |
| 34  | AG6D5           | 1.3509     | 0.0852         | 0.0729 |
| 35  | AG6D6           | 1.2851     | 0.0920         | 0.0751 |
| 36  | AG6D7           | 1.2807     | 0.1061         | 0.0841 |
| 37  | AG6D8           | 1.5395     | 0.1469         | 0.1234 |
| 38  | AG6D9           | 1.1464     | 0.1052         | 0.1292 |
| 39  | AG6D10          | 1.2105     | 0.0631         | 0.0519 |
| 40  | AG3DP1          | 1.2156     | 0.0828         | 0.0668 |
| 41  | AG3DP2          | 1.3026     | 0.0854         | 0.0713 |
| 42  | AG4DP1          | 1.3070     | 0.0828         | 0.0668 |
| 43  | AG4DP2          | 1.3026     | 0.0854         | 0.0713 |
| 44  | AG5DP1          | 1.4825     | 0.1239         | 0.1053 |
| 45  | AG6DP1          | 1.1491     | 0.0467         | 0.0380 |
| 46  | AG6DP2          | 1.2807     | 0.0576         | 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         | 0.1915|
| 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](image1.png)

*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 TdT/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).
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 (RRS) 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.

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