Application of microsatellite markers for hybrid verification and genetic analysis of oil palm (*Elaeis guineensis* Jacq.)

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**Abstract**

The legitimacy of parents and progenies used in crop improvement programmes is vital for any meaningful progress in selection. While acknowledging the shortcomings of controlled pollination in oil palm breeding and commercial seed production, the legitimacy of 20 oil palm progenies from the Nigerian Institute for Oil Palm Research (NIFOR) breeding programme was determined using 16 fluorescently-labeled microsatellite markers. The genotyping of parents and progenies was conducted by capillary electrophoresis using the ABI 3730 DNA Genetic Analyzer (Applied Biosystems, USA). Results revealed a complementary expression of the parents’ alleles in 18 out of the 20 individual progenies screened, confirming their hybridity and genetic identity. The two illegitimate progenies detected could be attributed to pollination and planting errors, respectively. A subset of three sufficiently informative loci (sMg00016, sMg00179 and sMo00102) was identified for routine quality control genotyping. The detection of illegitimate progenies provided ample evidence to substantiate the importance of assessing hybrid fidelity in breeding programmes. Furthermore, the usefulness of microsatellite markers as a reliable technique for routine assessment and unambiguous identification of oil palm crosses was established. The implications of microsatellite-based hybrid identification in oil palm varietal improvement programmes have been adequately discussed.

**Key words:** Contamination, Genetic identity, Illegitimacy, NIFOR, Parent-Progeny relationships, seed production

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**Introduction**

Over the past eight decades at the Nigerian Institute for Oil Palm Research (NIFOR), there has been a steady progress in oil palm breeding with the highest average oil yield of about 4-5 t/ha/yr (Ataga et al., 2018). In accordance with the economic diversification efforts of the federal government to boost national palm oil production, a policy to invest $500 million in oil palm plantation development was enunciated. This policy aims at increasing the annual
local production of palm oil by 700% over the next eight years (2019 to 2027) to attain self-sufficiency in the commodity (USDA 2019). To meet this projected target, future breeding progress will depend on the legitimacy of individual parents as well as their progeny for breeding crosses and commercial hybrids. While acknowledging the susceptibility of oil palm controlled pollination to various sources of errors (during pollination, seed collection, germination, and field planting), the need for accurate hybrid verification and identification (genetic fingerprints) of oil palm crosses cannot be overlooked (Corley and Tinker, 2016). Therefore, the success of hybrid oil palm production beside other factors depends on the production and timely supply of genetically pure planting materials to the oil palm growers. This ensures that the gains of heterosis can be harnessed through enhanced yield by growing pure F₁ hybrid crops.

A major concern to the sustainability of Nigerian oil palm industry is the fidelity of planting materials. The out-crossing behaviour of the crop coupled with the challenges associated with controlled pollination, inadvertently deviate the expected or theoretical Mendelian segregation ratios, leading to contamination or illegitimacy in fruit forms. Hence the need to objectively confirm the genetic identity of hybrids in a crossing programme for breeding and seed production. This situation is further exacerbated by adulteration of oil palm planting materials by illegal seed or seedling hawkers/producers, who handpick seeds from plantations and raise them as seedlings for sale to farmers (Okwuagwu, 2001). These traffickers market their illegitimate materials to unsuspecting farmers on the pretense of being NIFOR agents to support their fraudulent actions. More worrisome is the inability of the farmers to differentiate between NIFOR tenera planting material and the material procured from the seed/seedling vendors. This problem is equally shared by oil palm breeders who find it difficult in identifying the hybrids of crossed progenies before planting and production of fruit bunches. As a step to safeguard the very vulnerable farmers and the oil palm industry from illegal seed traffickers, several measures including microsatellite marker, genetic fingerprinting scheme were initiated to characterize breeding crosses and commercial planting materials for early identification at or before planting stage (Okooye 2016).

The shell thickness gene (Sh) in oil palm fruit forms (dura, pisifera and tenera) plays a major role in identification of fruit type and also influences palm oil yield (Singh et al., 2013). Illegitimacy and contamination (some illegitimate palms in a family) in oil palm is conventionally assayed by using the traditional method of shell thickness assessment and segregation pattern of the fruit forms which are often ambiguous and vulnerable to long term field evaluation. Essentially, oil palm must be grown ±3-4 years before production of fruit bunches for fruit form determination and subsequent verification of hybrid legitimacy. A reliable method for hybrid identification of oil palm at early seedling stage is therefore crucial both for the integrity of a durable breeding programme and for assuring good quality planting material to the farmers.

Unlike the morphological means (fruit-form determination) of identifying contamination in oil palm, molecular markers are good alternatives because they are not subject to environmental influences and can be readily detected in all plant tissues, notwithstanding the growth or developmental stage (Mondini et al., 2009). The use of microsatellites or simple sequence repeats (SSR) marker technique is well established and accepted for genotype identity and hybrid verification because of their abundance in the genome, co-dominant inheritance, high polymorphism and reproducibility (Amos et al., 1996; Smith et al., 1997). Several studies have successfully employed microsatellite markers as a routine quality control approach to address the issue of illegitimacy and contamination in oil palm breeding programmes and commercial seed production. Especially promising is the current application of microsatellite markers for identity checking in selection and seed production processes by Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD) and PalmElit (Pomiès et al., 2019), and Sime Darby Plantation R&D Centre, Malaysia (Teh et al., 2019).

The legitimacy of oil palm materials currently used in the NIFOR oil palm main breeding programme has not been assessed and documented using any molecular marker technique. As part of a quality control approach in the breeding and seed production programme, we present a preliminary fingerprinting report of
some oil palm breeding crosses with molecular markers. Specifically, this study 1) sought out to employ fluorescently-labeled microsatellite markers for confirmation of parentage and hybrid purity of 20 oil palm progenies, and 2) to identify a subset of highly informative SSR markers for routine and low-cost quality control genotyping.

**Materials and Methods**

Twenty five (25) oil palm genotypes comprising two parental genotypes with contrasting yield components and twenty of their resulting progenies, and three advanced experimental selections from Malaysian Palm Oil Board (MPOB) were evaluated in this study (Table 1). The parental genotypes consist of the thick-shelled *dura* (female) and thin-shelled *tenera* (male) genotypes. The *dura* parent genotype was selected for high bunch weight and low bunch number while the *tenera* genotype was selected for high bunch number and low bunch weight. These two sequentially developed traits are highly heritable and determine the yield of fresh fruit bunch in the oil palm (Okoye et al., 2001). The three advanced experimental selections were used as outgroup in the multivariate analysis to assess the efficiency of the employed molecular markers in genetic differentiation and discrimination among different oil palm genotypes.

Young leaf samples were collected from unopened spears of 22 individual palms planted at the NIFOR Main Station, Benin City, Nigeria. The samples were stored at -80°C at the Bioscience Centre, International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria prior to DNA isolation. In addition, DNA samples of the three advanced experimental selections were obtained from MPOB Malaysia.

DNA was extracted from the individual palms following the Cetyl-Trimethyl Ammonium Bromide (CTAB) procedure (Doyle and Doyle 1990) with an additional chloroform extraction step. The extracted DNA quality of each sample was assessed using 1% (w/v) agarose gel and the quantity of DNA was confirmed using NANODROP® (ND-1000) Spectrophotometer (Thermo Fisher Scientific Inc., Denver). DNA concentrations were normalized at 25 ng/µl in sterile water and stored at 4°C until polymerase chain reaction (PCR) amplification at the Advanced Biotechnology and Breeding Centre (ABBC), MPOB Selangor, Malaysia.

**Table 1**: List of parental genotypes along with their putative hybrids and three elite oil palm populations used as an outgroup in this study

| S/N | Genotype | Origin                      | Code |
|-----|----------|-----------------------------|------|
| 1   | P1♀      | Serdang Avenue Deli         | DD1  |
| 2   | P2♂      | Umuabi OP                   | T6   |
| 3   | DT_47/593| Putative hybrid P1 x P2     | DT1  |
| 4   | DT_47/649| Putative hybrid P1 x P2     | DT2  |
| 5   | DT_47/793| Putative hybrid P1 x P2     | DT3  |
| 6   | DT_47/820| Putative hybrid P1 x P2     | DT4  |
| 7   | DT_47/916| Putative hybrid P1 x P2     | DT5  |
| 8   | DT_47/945| Putative hybrid P1 x P2     | DT6  |
| 9   | DT_47/1040| Putative hybrid P1 x P2    | DT7  |
| 10  | DT_47/1041| Putative hybrid P1 x P2    | DT8  |
| 11  | DT_47/1096| Putative hybrid P1 x P2    | DT9  |
| 12  | DT_47/1150| Putative hybrid P1 x P2    | DT10 |
| 13  | DT_47/1151| Putative hybrid P1 x P2    | DT11 |
| 14  | DT_47/1207| Putative hybrid P1 x P2    | DT12 |
| 15  | DT_47/1468| Putative hybrid P1 x P2    | DT13 |
| 16  | DT_47/1679| Putative hybrid P1 x P2    | DT14 |
| No. | Code          | Description                          | Location  |
|-----|---------------|--------------------------------------|-----------|
| 17  | DT_47/1803    | Putative hybrid P1 x P2              | DT15      |
| 18  | DT_47/1846    | Putative hybrid P1 x P2              | DT16      |
| 19  | DT_47/1847    | Putative hybrid P1 x P2              | DT17      |
| 20  | DT_47/1874    | Putative hybrid P1 x P2              | DT18      |
| 21  | DT_47/2042    | Putative hybrid P1 x P2              | DT19      |
| 22  | DT_47/2043    | Putative hybrid P1 x P2              | DT20      |
| 23  | 013_0338/0391*| MPOB Kluang  *Dura*                 | Kl-D1     |
| 24  | 014_0174/0655*| MPOB Kluang  *Pisifera*             | Kl-P1     |
| 25  | 016_911101/1203*| MPOB KULIM  *Pisifera*           | Ku-P2     |

♀- female parent, ♂- male parent, OP- open pollinated, *elite breeding populations forming the outgroup

A total of sixteen fluorescently-labeled microsatellite markers were used to fingerprint progenies and their parents. Nine of these markers (sMg00156, sEg00154, sMo00102, sMg00228, sMg00016, sMg00120, sEg00151, sMg002179 and sMg00087) were developed at MPOB by Singh et al., (2008). A further set of seven markers (mEgCIR3813, mEgCIR0793, mEgCIR0425, mEgCIR0328, mEgCIR3828, mEgCIR3519, mEgCIR0790 and mEgCIR3745) were obtained from CIRAD (Billotte et al., 2005; http://tropgenedb.cirad.fr/). Preliminary screening of these markers on the parental genotypes for polymorphism allowed the selection of eight informative loci used to confirm the identity of the 20 oil palm hybrids (Figure 1).

**Figure 1:** Screening of parents and a hybrid with four SSR markers prior to capillary electrophoresis analyses.

Polymerase chain reaction was conducted in a Perkin Elmer 9700 thermocycler (Life Technologies, Thermo-Fisher Scientific, USA) using the composition and conditions described in Ting et al., (2010). The total reaction volume was 10 µl containing (prepared in order listed): 2 µl of 25 ng genomic DNA, 6.625 µl MilliQ water, 1× PCR standard buffer (NEB, USA), 0.2 µl of 10mM deoxynucleotide triphosphates (dNTPs) (NEB, USA), 0.025 µl of each primer (M13 tailed forward primers and untailed reverse primers), 0.025 µl dye, and 0.1µl of Taq DNA polymerase (5 U/µl) (NEB, USA). The amplification cycle consisted of an initial 3 min denaturation at 95 °C, followed by 35 cycles of denaturation at 95 °C for 30 sec, primer annealing for 30 sec at 50 - 58 °C depending on the primer annealing temperature and an extension temperature of 72 °C for 30 sec, followed by an additional extension at 72 °C for 2 min. Amplified fragments were estimated by capillary electrophoresis on a DNA Genetic Analyzer-ABI PRISM 3730 (Applied Biosystems, USA). The alleles were sized with reference to GS 500 LIZ, a formamide containing red DNA size standard. Fragment size in base pairs was determined using GeneMapper® software version 4.1 (Applied Biosystems, USA). Sample plots were generated and genotype data for all the markers scored in an Excel matrix. The scoring of the genotype data was performed manually with reference to allele and peak size, except for stutters. Null alleles were assigned to sample individual genotypes that were confirmed to have no amplification products under standard conditions.
The peaks present in the parents and their respective progenies were scored for each of the SSR markers used. These were termed alleles and according to the GeneMapper® software were referred to by their size in base pairs rounded to the appropriate unit. Comparison of the parents’ alleles to those of their progenies allowed for the determination of legitimate and illegitimate hybrids. A progeny was considered legitimate if one of its two alleles was maternal and the other one paternal showing that they were inherited from its two parents. On the contrary, a progeny was considered illegitimate or contaminant if at least one of its alleles was not inherited from its parents at a minimum of two microsatellite markers. If only maternal alleles were present, the possibility of the progeny being a product of self-pollination and not that of a cross between putative parents was not discounted. Correspondingly, the presence of an unexpected allele with a maternal allele infers a non-hybrid progeny from contamination by foreign pollen. The number of alleles per locus (Na), unbiased gene diversity (He; Nei 1978) and the observed heterozygosity (Ho) along with their standard error of means were calculated with Genetic Analysis in Excel computer package 6.5 (Peakall and Smouse, 2012). The software Cervus 3.0.7 was used to assess the probability of identity (PID); the probability that two individuals drawn at random from a population will have the same genotype at multiple loci (Marshall et al. 1998). PID was calculated for the entire data set and on a per locus basis as described in Paetkau et al., (1995) to measure the power of each marker set for individual identification. The polymorphic information content (PIC), according to Anderson et al., (1993), was calculated using PowerMarker v3.25 software (Liu and Muse 2005). The percentage of hybrid genetic purity was calculated using scored data according to the purity index of Bohra et al., (2011): Hybrid purity (%) was determined by dividing the number of true hybrids (comprising alleles from both parents) by the total number of hybrids screened, then multiplied by 100. Pairwise comparisons of the proportion of shared alleles between individual genotypes (plants) were determined by simple matching dissimilarity index. The resulting genetic dissimilarity coefficient was then transformed into a distance matrix averaging over 1000 bootstraps. Cluster analysis was generated from the distance matrix by the unweighted pair group method using the arithmetic averages (UPGMA) algorithm for a better visualization of the genetic relationships among the parent-offspring and MPOB oil palm genotypes used as outgroup. These calculations were performed by the computer program DARwin v5 (Perier and Jacquemoud-Collet 2006).

Results and Discussion

Screening the parents and progenies using SSR markers

The choice of 16 microsatellite markers used for screening the oil palm parent genotypes and their putative hybrids was based on their ability to generate polymorphic bands under optimized PCR conditions. Based on our genotyping results, eight microsatellite markers which were polymorphic among the parents were employed for further analysis of their hybrids (Table 2).

| Locus       | Linkage Group | Repeat Motif | Na  | uHe  | Ho   | PID  | PIC  |
|-------------|---------------|--------------|-----|------|------|------|------|
| mEgCIR0793  | 2             | (GA)15       | 3.00| 0.623| 0.955| 0.230| 0.5318|
| sEg00154    | 6             | (CAG)5       | 5.00| 0.672| 0.955| 0.177| 0.5979|
| sMo00102    | 7             | (AG)11       | 4.00| 0.722| 1.000| 0.141| 0.6494|
| sMg00016    | 9             | (GA)13       | 6.00| 0.717| 1.000| 0.141| 0.6496|
| mEgCIR3519  | 10            | (GA)15(GT)8 | 5.00| 0.672| 0.955| 0.177| 0.5979|
| mEgCIR0790  | 12            | (GA)19       | 4.00| 0.644| 0.955| 0.204| 0.5628|
| sEg00151    | 13            | (CAG)8       | 2.00| 0.512| 0.909| 0.375| 0.3750|
| sMg00179    | 14            | (AAAG)6      | 5.00| 0.772| 1.000| 0.104| 0.7103|

Table 2: Genetic diversity measures for eight polymorphic microsatellite loci tested in 22 oil palm genotypes
The eight polymorphic SSR loci exhibited a total of 34 alleles among the parents and putative hybrids with a mean value of 4.25 alleles per locus (Table 2). The highest number of alleles (6 alleles per locus) was detected for sMg00016, whereas loci sEg00154, mEgCIR3519 and sMg00179 presented 5 alleles per locus. Similar studies involving the analysis and characterization of microsatellites in oil palm have also detected loci with highly variable number of alleles, as well as the polymorphic information content (2 to 6 alleles per locus, Bakoumé et al., 2011; 3 to 8 alleles per locus, Hama-Ali et al., 2015; 5 to 11 alleles per locus, Budiman et al., 2019). The highest polymorphism information content (PIC) value was detected for the locus sMg00179 (0.7103), while the lowest PIC value of 0.3750 was recorded for the locus sEg00151 with a mean value of 0.5843. This was comparable to PIC values found in previous studies (0.488 to 0.777, Hama-Ali et al., 2014; 0.41 to 0.84, Budiman et al., 2019). The high level of polymorphism observed for the described microsatellite markers support their application in genetic studies of oil palm. Number of alleles detected and PIC value based on the frequencies of different alleles by a particular marker indicates the quality (discriminatory power) of the marker (Powell et al., 1996). The range of gene diversity varied between 0.512 (sEg00151) and 0.772 (sMg00179) with a mean value of 0.667. The observed heterozygosity differed among the locus from 0.909 (sEg00151) to 1.000 (sMg00102, sMg00016 and sMg00179). Lower but comparable results were obtained in a study that involved the analysis of 16 microsatellites, the cumulative probability to obtain identical genotypes among the two parents and the 20 putative--hybrids were 1.14 x 10⁻⁷. This result reflects a relatively high genetic polymorphism despite the two generations of selective breeding on the investigated genotypes.

### Table 3: Allele combinations (in base pairs) at eight microsatellite loci in the oil palm parents and hybrids

| Loci        | mEgCIR0793 | sEg00154 | sMo00102 | sMg00016 | mEgCIR3519 | mEgCIR00790 | sEg00151 | sMg00179 |
|-------------|------------|----------|----------|----------|------------|-------------|----------|----------|
| P1♀         | 170/170    | 260/266  | 254/258  | 283/289  | 241/241    | 231/231     | 239/239  | 237/242  |
| P2♂         | 163/171    | 261/261  | 252/260  | 284/297  | 260/267    | 233/243     | 236/236  | 232/238  |
| DT1         | 163/170    | 260/261  | 258/260  | 284/289  | 241/260    | 231/243     | 236/239  | 232/242  |
| DT2         | 163/170    | 260/261  | 254/260  | 284/289  | 241/260    | 231/233     | 236/239  | 232/242  |
| DT3         | 163/170    | 261/266  | 254/260  | 284/287  | 241/267    | 231/243     | 236/239  | 232/242  |
tested.

Alleles and illegitimate progenies inherited both parents' alleles and were heterozygous at all the SSR loci.

One allele from each of the two intercrossing parents was amplified at each SSR locus of the hybrid. A hybrid was also considered illegitimate if one or both of its two alleles were not inherited from its two parents. The hybrid purity index for the entire tested locus was 90%. Out of 20 individual palms screened, two illegitimate hybrids were observed (Table 3). A closer examination of the illegitimate/contaminated hybrids revealed that one of the progenies (DT4) presented single allele genotype mismatch at three loci, with all matching alleles derived from the female parent. This suggests that pollen from different male parents may have been used to pollinate the maternal tree rather than the intended pollen, or the pollen used was contaminated with pollen from a different source. Overall however, a pollination error or a planting error could possibly explain this event. The other illegitimate sample (DT12) did not have any allele in common with either parent (Table 3). An error during the seed and seedling handling stages (incorrect labeling or mixing of families in nurseries) is the most likely explanation here. All the legitimate progenies inherited both parents' alleles and were heterozygous at all the SSR loci tested.

Notwithstanding the long history of crossing programmes in oil palm breeding, controlled pollination is difficult and susceptible to various sources of error resulting in illegitimacy or contamination in the controlled crosses. The reasons for illegitimacy or contamination in oil palm breeding programmes are several and have been elaborately discussed by Corley (2005) and Corley and Tinker (2016). More recent utilization of SSR for characterizing genetic diversity of oil palm breeding populations by Budiman et al., (2019), revealed four illegitimate individuals among two dura self progenies of PT Astra Agro Lestari (AAL) breeding materials in Indonesia. In a study that used 30 microsatellite markers for illegitimacy and sibship assignments in oil palm, Hama-Ali et al., (2015) found three illegitimate palms among 200 progenies of four half sib families of FELDA's breeding materials in Malaysia. Thongthawee et al., (2012) used eight SSRs for parentage analysis in six full sib families of the breeding plantation of Univanich Palm Oil Public Company Ltd., Krabi, Thailand and detected four non-hybrids. They speculated that illegitimate palms probably resulted from pollen contamination during the control cross and errors during the nursery or planting stage. Taken
together, these observations underscore obvious risk of errors from pollen collection to field planting of controlled crosses, and the need for careful supervision of the process coupled with strict adherence to quality control procedures. The two non-essential genotypes found in this study can be considered excellent when compared to previous reports (Budiman et al., 2019; Hama-Ali et al., 2015; Thongthawee et al., 2012) as well as other studies on different plant species (Subashini et al., 2014).

**Selection of informative markers for quality control**

The selection of informative microsatellite markers for hybrid confirmation is vital in the reciprocal recurrent selection (RRS) method of oil palm breeding. This is cogent, in view of the possibility of contamination at the different stages of oil palm breeding activities. Another concern, mostly from a practical standpoint, is the initial high cost, research equipment, and tedious procedures involved in microsatellite marker development (Bakoumé et al., 2011). Corley (2005) in his review, suggested that the number of markers necessary for hybrid confirmation might be fewer than five due to the high polymorphism of microsatellites at the locus level. Out of the eight detected polymorphic SSR markers, a suite of three markers (sMg00016, sMg00179 and sMo00102) presented sufficient information content among the 22 genotypes representing two parental palms and their 20 hybrids. This marker suite had the highest expected heterozygosity (uHe), PIC values and the lowest probabilities of identity (PID; Table 2). They are located in three different oil palm linkage groups (or chromosomes) which explains their significant discriminatory power to distinguish closely related oil palm genotypes. The efficiency of the cited markers in genetic differentiation and discrimination among different oil palm genotypes was further validated using multivariate analysis. The results of the UPGMA dendrogram constructed based on shared allele frequency showed grouping of the 25 genotypes (22 NIFOR parent-progeny palms and 3 MPOB advanced lines used as outgroup) into two major clusters (Figure 2). Cluster I consisted of the two parents and their hybrids while the MPOB experimental selections were grouped in cluster II. This outcome indicated the usefulness of the markers to differentiate among closely related genotypes as well as the 25 oil palm genotypes under investigation. In a related study, Singh et al., (2007) obtained satisfactory separation of six oil palm ortet-racet sets using five SSR loci.

![Figure 2. UPGMA dendrogram showing the ability of the three sets of SSR loci to discriminate among the parental genotypes, their hybrids and the three elite populations used as outgroup. The dendrogram was](image)
Implications to oil palm varietal improvement

Reports show that molecular markers such as microsatellites or simple sequence repeats (SSR) are more faithful in the acceleration of conventional oil palm breeding with respect to verification and identification of hybridity (Singh et al., 2007; Thawaro and Te-chato, 2010; Thongthawee et al., 2012; Bakoumé et al., 2011; Hama-Ali et al., 2015; Budiman et al., 2019). Results from this study indicate that it is possible to accurately and rapidly determine true hybrids in oil palm using PCR-based techniques compared to the cumbersome morphological observation that has been adopted in the NIFOR oil palm breeding programme. In contrast to the morphological method of hybrid identification which usually takes 3-4 years, SSR analysis takes only 1-3 months depending on the number of populations or sample size. Nevertheless, given the occurrence of inter-type contamination in oil palm, it is not easy for SSR markers to distinguish dura fruit form from tenera or pisifera fruit forms. Therefore, the traditional method of hybrid identification will continue to complement molecular marker technology. Early detection of true hybrids indicates precise selection of plants in the field. In an effort to develop marker-assisted selection (MAS) tools for economically important agronomic traits as well as disease resistance in the oil palm breeding programme, these legitimate hybrids could be used as mapping population. The use of MAS will increase selection efficiency, reduce the breeding cycle and enhance variety development. Introduction of an additional phase involving SSR markers in the modified RRS programme of NIFOR will be desirable for the identification of contaminants in addition to selection of recombinant genotypes that will maximize heterosis among populations. According to Babu et al., (2017), significant cost savings could be made by eliminating illegitimate crosses before field planting.

Conclusion

This is the first study confirming the ascendance of some progenies in NIFOR oil palm main breeding programme using microsatellite markers. Eight polymorphic microsatellite markers were employed for the verification of some oil palm progenies from the breeding programme. In principle, results from the progeny screening highlight the efficiency of the microsatellite markers for analysis of genetic variation and parentage verification. Two individual palms were off-types probably due to genotype mix-ups from pollen contamination during controlled crosses and planting errors, respectively. This also draws attention to the need for constant care, control and organization at all the stages of seed production; from raising of seedlings in the nursery, to planting in the field. It is therefore recommended as a standard practice to test the legitimacy of all crosses in the breeding programme. The three most informative SSR markers (sMg00016, sMg00179 and sMo00102) could be confidently utilized for routine fingerprinting, hybrid purity tests and certification of controlled crosses in oil palm breeding programmes. Although more numbers of plant samples will be required for large scale application in commercial seed production, minor modifications in terms of measurement precision and probabilities will be worked out to circumvent technical drawbacks. The legitimate progenies identified will be useful for reliable inheritance studies and comparison of genetic diversity determined by microsatellite markers to that revealed by agronomic markers, which have rarely been reported in literature.

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