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Assessment of an oil palm population from Nigerian Institute for Oil Palm Research (NIFOR) for simple sequence repeat (SSR) marker application

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Oil palm (*Elaeis guineensis* Jacq.), a monocotyledonous plant belonging to the Arecaceae family, is one of the most important oil crops in the world. In Nigeria, oil palm has benefited immensely from conventional breeding efforts resulting in high yields that have been achieved with this breeding material. However, oil palm breeding is slow and time-consuming due to a breeding cycle of about 10 years. In addition, the process of outcrossing leads to high variation in yield components and vegetative traits. Although DNA marker technologies offer great possibilities for plant breeding through marker-assisted selection, there are so far no reports of its application to oil palm breeding in Nigeria. In this study, 32 SSR markers were used for the assessment of marker application in an oil palm breeding population coming from the extensive breeding program at the Nigerian Institute for Oil Palm Research (NIFOR). Seven SSR markers out of the 32 tested (22%) segregated in the progeny 12 (*tenera* x Deli *dura*). SSR markers mEgCIR0059, mEgCIR1917, mEgCIR3260, mEgCIR3275, mEgCIR3533 and mEgCIR3557 proved to be fully informative markers following a segregation ratio of 1:1:1:1, while marker mEgCIR0074 segregated in a 1:1 ratio.

Key words: Oil palm, microsatellite marker, marker-assisted selection, NIFOR.

INTRODUCTION

Oil palm (*Elaeis guineensis* Jacq.; Arecaceae), a mono-cotyledonous plant, is one of the most important oil crops in the world (Low et al., 2008). There are currently two species assigned to the genus *Elaeis*: the African oil

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Abbreviations: AVROS, Algemene Verenigung van Rubberplanters ter Oostkust van Sumatra; **bp,** base pairs; **CNRA**, Centre National de Recherche Agronomique; **cM,** centiMorgan; **DF,** degree of freedom; **IRD,** infrared dye**; LG**, linkage group; **NCBI**, National Center for Biotechnology Information; **NIFOR**, Nigerian Institute for Oil Palm Research; **PCR,** polymerase chain reaction; **QTL,** quantitative trait loci; **RPO**, red palm oil; **SSR**, simple sequence repeat.

palm (*E. guineensis*) and the South American oil palm (*Elaeis oleifera*). The chromosome numbers for *E. guineensis* and *E. oleifera* were determined with 2n=2x=32 (Madon et al., 1998). The African oil palm (*E. guineensis*) has three fruit forms differing in their shell thickness: *dura* (thick-shell), *pisifera* (shell-less) and *tenera* (thin-shell), which results from a cross between *dura* and *pisifera* (Hartley, 1988). Shell thickness is controlled by one major co-dominant gene called *Sh* (Beirnaert and Vanderweyen, 1941) and plays a major role in oil yield. In addition, different fruit types (*virescens, albescens, nigrescens* and *poissoni*) are distributed among the three fruit forms (Corley and Tinker, 2003). Oil palms allow the production of two distinct kinds of oils from two different tissues within the fruits: (red) palm oil from the fruit pulp (mesocarp) around the nut and palm kernel oil from the kernel (Akapanabiatu et al., 2001; Asemota et al., 2004). Red palm oil as a saturated fat contains 44% palmitic acid, 39% oleic acid and smaller percentage of stearic, linolenic, lauric, myristic and linoleic acid (Burri, 2012; Oguntibeju et al., 2009). In addition, RPO is rich in provitamin A carotenoids (alpha- and beta-carotene) as well as vitamin E (both tocopherols and tocotrienols) (Burri, 2012). Palm kern oil consists mostly of lauric acid, followed by myristic acid, oleic acid and palmitic acid. Comparing palm kernel oil from Nigeria (Akapanabiatu et al., 2001) with palm kernel oil from Malaysia (Kok et al., 2011), considerable differences in oleic acid and myristic acid content can be observed. Nigerian palm kern oil contained 15-19% oleic acid and only 45-48% lauric acid, whereas the palm kern oil from Malaysia had only 9-12% oleic acid and 51-53% lauric acid. About 90% of the palm oil produced is used for food products (Sambanthamurthi et al., 2000). Although the unrefined red palm oil has a high nutritional value (provitamin A and vitamin E), the refined commercial palm oil (color- and odorless) is used due to its low costs and its high oxidative stability (Burri, 2012; Ong and Goh, 2002; Matthäus, 2007).

In Nigeria, oil palm yield has benefited immensely from conventional breeding efforts. For example, average yields from the second cycle planting materials coming from the Nigerian Institute for Oil Palm Research range between 20 to 25 tones fresh fruit bunches per hectare per year in a well-managed plantation (NIFOR, 2008). However, oil palm breeding is slow and time consuming due to a breeding cycle of about 10 years. As an outcrossing crop, high variations in yield components or vegetative traits are observed in the offspring (NIFOR, 2008). Regarding the long generation time in oil palm breeding, the possibility of selection at the nursery stage has often been considered desirable. Exploitation of DNA marker technologies combining the knowledge from research in molecular genetics and genomics offers great possibilities in plant breeding (Collard and Mackill, 2008). However, there have been no reported cases of its application in breeding of oil palms in Nigeria, so far (NIFOR, 2008).

DNA markers can be used to detect the presence of allelic variation in the genes underlying traits of interest and have been applied to a range of crop species such as *Flammulina velutipes* (Physalacriaceae) (Zhang et al., 2010), *Brassica rapa* (Brassicaceae) (Kapoor et al., 2009), cereals (Gethi et al., 2002; Li et al., 2008; McCough et al., 2002; Shehata, et al., 2009; Zheng et al., 2008), *Cucumis sativus* (Cucurbitaceae) (Hu et al., 2010), *Spartina* spp. (Poaceae) (Baisakh et al., 2009), *Glycine max* (Fabaceae) (Xia et al., 2007), *Nelumbo nucifera* (Nelumbonaceae) (Kubo et al., 2009; Pan et al., 2007). In oil palm, a set of SSR markers have been developed and together with AFLP markers have been integrated into a general genetic map (Billotte et al., 2005). This genetic map consisted of 944 markers on 16 linkage groups, corresponding to the expected haploid chromosome number of oil palm, and covered 1,743 cM. Linkage group 4 carried the *Sh* gene responsible for the shell thickness. The progeny was obtained from a cross between two heterozygous *Elaeis guineensis* Jacq. parents: LM2T (*tenera* palm belonging to the CNRA La Mé oil palm breeding program, Ivory coast) and DA10D (*dura* palm selected from a Deli population introduced to Indonesia in the 19th century). QTL for phenotypic traits regarding fruit variety, yield components and vegetative growth were localized together with the *Sh* gene for shell thickness on this map (Billotte et al., 2010). Additional genetic maps were constructed by Seng et al. (2011), Singh et al. (2009) and Ting et al. (2013). In between, the *Sh* gene was isolated from *E. guineensis* (*dura* [Sh+/Sh+] and *pisifera* [Sh-/Sh-] form) and two independent mutations were identified in the *pisifera* forms, Congo AVROS and Nigerian T128, that lead to non-functional MADS box transcription factors that cannot support the formation of the shell (Singh et al., 2013a).

Simple sequence repeat markers (SSR), also known as microsatellites, are particularly interesting for plant breeding due to the properties of genetic co-dominance, high reproducibility, multi-allelic variation, easy amplify-cation by PCR, and production of results that are easy to interpret in self-pollinating species (Collard and Mackill, 2008). In addition, the technology is relatively easy to transfer from one laboratory to the other (Zheng et al., 2008). In view of the above mentioned advantages, SSR markers have been used for marker-assisted selection e.g. in rice (Shen et al., 2001), in sunflower (Tang et al., 2003) and peach (Sajer et al., 2012). However, in outbreeding crops like e.g. oil palm, eucalyptus, loblolly pine, cassava or potato, the identification of the segregation patterns of the SSRs in the progenies is challenging. Whereas, in inbred lines, the maximum number of alleles that can segregate in crosses is two (van Ooijen, 2011), the number of segregating alleles per locus in the offspring derived from a cross between two individuals of an outcrossing species (full-sib) family can range between one and four. This represents a major distinction between a progeny of inbred lines and a full-sib

family. An additional challenge has to be faced if the parents of the experimental breeding populations are not available. For mapping markers under these circumstances, the reconstruction of the parental genetic constitution from the observed segregation patterns in the full-sib family is required. Markers that allow this are called informative markers (van Ooijen, 2011). Markers with segregation type *ab x cd* provide information regarding the genotypes of the parents of the population. In this case, the four genotypes (*ac*, *ad*, *bc* and *bd*) can be considered the complete genotypes in an allogamous full-sib family. In addition, markers with a segregation type *ef x eg* also provide complete information regarding the genotypes of the parents of the population (van Ooijen, 2011). These fully informative markers follow segregation ratios of 1:1:1:1 as deduced from their banding patterns and the reports of Lespinasse et al. (2000) and Billotte et al. (2005).

The objective of this study was to carry out an assessment of an oil palm breeding population from NIFOR for its suitability for marker-assisted selection (MAS) using a new DNA isolation protocol and SSR markers. The parental material was not available, so that the genetic constitution had to be derived from the observed segregation patterns in the progeny. This is the first of series of experiments undertaken by NIFOR towards the incorporation of molecular markers into its breeding programs. SSR markers were chosen for this study because of the reliable ability to detect polymerphisms among closely related individuals (Shehata et al., 2009).

MATERIALS AND METHODS

Breeding population

The oil palm population (progeny 12) used for this study was derived from the Nigerian Institute for Oil Palm Research experimental oil palm breeding program (second cycle population) planted in 1987. Progeny 12 is a cross between 13.386 T (*tenera*) and 26.1074 D (Deli *dura)*. Ninety-two (92) individuals of the population were used for the molecular investigations.

DNA extraction

Genomic DNA was extracted from 92 individuals of progeny 12. Between 0.15 to 0.2 g of fresh leaf tissue was ground quickly in 800 µl of DNA extraction buffer (100 mM Tris-HCl, pH 8.0; 50 mM EDTA, pH 8.0; 500 mM NaCl; 5% SDS). The homogenate (buffer and ground tissue) was transferred into 1.5 ml Eppendorf tube. Extra 200 µl of DNA extraction buffer was added, mixed well and centrifuged for 2 min at 10,000 x g (4°C). The supernatant was collected into another Eppendorf tube and labeled. Then 200 µl of 5 M potassium acetate were added and mixed well. An equal volume of phenol-chloroform-isoamylalcohol (25:24:1) was added and mixed very well. The mixture was centrifuged for 5 min at 10,000 x *g* (4°C). The supernatant was carefully transferred into another Eppendorf tube and 800 µl of absolute ethanol was added. To precipitate the DNA, the mixture was kept on ice for 20 min and later centrifuged for 5 min at 10,000 x *g* (4°C). Finally, the DNA pellet was washed twice with 70% ethanol, air dried at ambient temperature and re-suspended in 200 µl of TE (10 mM Tris-HCl, pH 8.0; 1 mM EDTA, pH 8.0) and stored at -20°C.

DNA quantification and standardization

The DNA concentrations (ng/µl) and the optical density (OD) ratios 260/280 were calculated by measuring the OD at UV wavelength of 260 and 280 nm using a spectrophotometer (UltrospecTM 2 3100 pro, GE Healthcare Europe GmbH, Freiburg, Germany). The DNA was then diluted to 50 ng/µl and used for SSR analyses.

SSR analyses

The SSR loci that were used for this study were sequenced and mapped by Billotte et al. (2005). Sequences for a total of 32 SSR loci (Table 1) were downloaded from National Center for Biotechnology Information (NCBI) using the accession numbers given in Billotte et al. (2005). Due to the special breeding interest in linkage group 4 because of the presence of the *Sh* gene as well as QTLs for yield, bunch components and vegetative traits located on this linkage group (Billotte et al., 2010), most SSR markers came from this linkage group. Forward and reverse SSR primers were designed from the sequences (Table 1) using the online Primer3 version 4 (http://frodo.wi.mit.edu/).

Primer tailing

An M13 tailing procedure (Oetting et al., 1995) was applied for fluorescence labeling of the PCR amplification products. An M13 tail (5'-TTTCCCAGTCACGACGTT-3') was added to the 5'-end of all the designed forward primers. All unlabeled primers were ordered from Invitrogen and the M13-IRD800 (=DY-781) labeled primer was ordered from biomers.net (Ulm, Germany).

PCR amplification

The PCR amplification was performed in a GeneAmp® PCR System 2700 thermal cycler (Applied Biosystems, Darmstadt, Germany). For the PCR, 4 µl of DNA (50 ng/µl) was mixed with 11 µl master mix. The master mix contained 0.3 µl dNTPs (10 mM), 8.7 µl H_2O, 1.5 µl 10x PCR buffer (for FIREPol*Taq* polymerase), 0.15 µl M13-IRD800 primer (5 pmol/µl) for labeling, 0.05 µl FIREPol*Taq* polymerase (5 U/µl), 0.15 µl forward primer (5 pmol/µl) and 0.15 µl reverse primer (5 pmol/µl). The PCR conditions included an initial denaturation at 95°C for 5 min, followed by 36 cycles of 95°C for 20 s, 50°C for 20 s and 72°C for 30 s and a final elongation step at 72°C for 5 min.

Polyacrylamide gel analysis

The PCR products were mixed with equal volume (15 µl) of loading buffer (98 ml formamide, 2 ml of 0.5M EDTA pH 8.0 and 37.5 mg bromophenol blue). This mixture of PCR products and loading buffer was denatured for 2 min at 94°C and transferred onto ice before loading 0.5 µl into the well for separation. The PCR products were separated on 6% denaturing polyacrylamide gels using the DNA Analyzer Model 4300 (LI-COR Biosciences, NE, USA).

Segregation analyses

The number of alleles that segregated in our study was determined

Table 1. SSR primer combinations tested in progeny 12 from the NIFOR breeding program. SSR markers were named according the SSR loci sequenced by Billotte et al. (2005).

Marker name	NCBI accession number	LG	Primer sequences[1] (5'-3')	Product size (bp)[2]
mEgCIR3275	AJ578630	4	F-GGTGGAAGCTTTTTGTCTGC R-ATTGAAGAGGGCAGGGTTTT	192
mEgCIR3716	AJ578711	4	F-CAGACATGGCAGCAAAAAGA R-ATCTTGTCTGGGGGATGTTC	229
mEgCIR3194	AJ578625	4	F-TGGTGGTTGGGAGTGATT R-TGTTAGTTTGCTAACTTGAAACAGG	262
mEgCIR3413	AJ578665	4	F-GGAAGGAAAGAAGCGAGAGG R-ACATGTCCATGCTGTTGGAA	255
mEgCIR3286	AJ578633	4	F-ATTTTGGGGTCAGGGTTTGT R-CAGGTCCATGGGAAAAGAGA	184
mEgCIR3526	AJ578673	4	F-TGACAGAGAGAAAAGGGAGAGG R-TGGTGTTCATTCTGCTCGTC	216
mEgCIR1917	AJ578575	4	F-GCAATGGAAAGAGCTGGAAG R-GGTGGATCAGTCGAGCATTT	208
mEgCIR0786	AJ578541	4	F-AGTTCCGTGCACCCACTTAC R-CAGCAGACAGGGAGCTAACC	266
mEgCIR3439	AJ578670	4	F-TGACAAGCCAACTTGAAAGC R-GTTGACAACCTGACCAAGCA	248
mEgCIR3232	AJ578627	4	F-CAAGCCCCTTAGCTGCATAC R-TGGAGGAGCAGCTTTAGCAT	213
mEgCIR3310	AJ578644	4	F-TGGCCGATCTGTATTAACCA R-AAATTCTGAGCCCATGCCTA	169
mEgCIR3535	AJ578676	4	F-AAAAACAAAAGGTGGGGAAA R-CCAGCCATTGCCGTATCTAT	199
mEgCIR2423	AJ578598	4	F-ACCCCATGAGGAATTTGGAT R-TGTGCCCATATGTGTGTGTG	184
mEgCIR1716	AJ578569	4	F-TTTGTGGTAACATGTGGTTGC R-CCCCTTCCGCATGTAAATTAT	181
mEgCIR3693	AJ578706	4	F-TTGGCCACTTTGAAGAATCC R-TTTTCTGGTCAGGGTTAGCTG	170
mEgCIR3533	AJ578674	4	F-ACGGTCTATGGCTCTGTCGT R-ACATGAGGAAAGCGCTAGGA	199
mEgCIR1753	AJ578573	4	F-CGATTCATGAACATTACAAGCA R-TCCAAGGTGATGGTCTGTGA	189
mEgCIR3769	AJ578723	4	F-TACTTCCTACTGGCCCATGC R-CGGATAGCTGGTGACATCCT	225
mEgCIR0074	AJ578500	4	F-CGATGATGAGGCTTGTGCTA R-TGAATGGCTATGACCGTGAA	225
mEgCIR2595	AJ578611	4	F-CAATATCAAAGAGCCGCACA R-AATGCATCTCTGGTCCTTGC	265

Table 1. Contd.

mEgCIR3705	AJ578709	4	F-AACCATCCACCATGCAGAA R-TTCCACAATTCCATTCATTCAA	234
mEgCIR3040	AJ578622	4	F-GATCTCTTGTGGGTGCGTTA R-AGGTCCTCATCCGACTTGTG	228
mEgCIR3477	AJ578671	4	F-TAGCATGCAGACCACACACA R-ATGCTGGGAAAATCATGCTG	222
mEgCIR0059	AJ578499	4	F-TGCAGGGGATGCTTTTATTT R-GGCCCTTAATTCCTGCCTTA	189
mEgCIR3775	AJ578724	4	F-ATGTGGGAACTCCTGAAACG R-TCCTTAGCGGCTTCACTTGT	168
mEgCIR3557	AJ578682	4	F-CATTGCCATTCCCTTCAAGT R-TCCCCTCTGTTCACTCAAGC	226
mEgCIR0369	AJ578516	3	F-AACCAAGGGGTAGCAAACCT R-TTTTAATCCCTGCCTGATGC	212
mEgCIR3260	AJ578628	3	F-GGGCAAGTCATGTTTCCTACA R-TAAGGGCGAGGTATTTCTGC	236
mEgCIR0408	AJ578519	2	F-AGCGCAGTTGCTCGGTATAA R-CCCTGCAGTGTCCCTCTTTA	163
mEgCIR3683	AJ578703	2	F-CATCAGTAGCTTGAACCTGAAAAA R-CTGAGGTCTACAGGGCATGTT	190
mEgCIR0874	AJ578558	1	F-TGCTCCAGTTGTCGAGTTGT R-TTGCAGTTTATTTGGCTACCAG	185
mEgCIR3788	AJ578728	1	F-TGACCAAAGACAGCATGAGC R-CATGAGCGCAACATCAGACT	194

[1]Each forward primer contained in addition the M13 tail (TTTCCCAGTCACGACGTT) at the 5'-end. [2]The visible size on the gel will be 18 bp larger due to the M13 tail.

following the genotype configuration demonstrated by Ritter et al. (1990), Lespinasse et al. (2000) and Billotte et al. (2005). Alleles were labeled A_i with i ranging from 0 (null allele = no amplification product) to 4 in case of four segregation alleles. Patterns for the individual SSR markers were identified in the full-sib family (Tab. 2). Chi square tests $\chi^2 = \Sigma (o-e)^2/e$, in which o represents the observed values and e the expected values and P-value calculation were performed using the statistics functions in Excel to verify if the observed segregation pattern corresponds to the expected segregation pattern at $p > 0.05$. Degrees of freedom were calculated as the number of categories (patterns) in this population minus 1.

RESULTS

DNA isolation using the novel DNA protocol

High quality genomic DNA could be extracted from 92 individuals of progeny 12 (*tenera* x *Deli dura*) each showing one major band larger than 23 kilobase (kb) on an ethidium bromide agarose gel. No visible signs of degradation of the DNA were detectable (Figure 1). Estimation of the DNA concentrations showed OD 260/280 ratios ranging between 1.7 and 2.0, supporting the visual picture. This proved that the developed simplified DNA extraction protocol allows efficient isolation from oil palm leaves.

SSR analyses in progeny 12 (NIFOR)

From 32 tested primer combinations for SSR markers coming from four linkage groups (1, 2, 3 and 4), nine gave reproducible patterns in progeny 12 (13.386 T × 26.1074 D). Two of these SSR markers (mEgCIR3439 and mEgCIR3535) resulted in monomorphic banding patterns. However, seven SSR markers out of the 32 tested (22%) segregated in the progeny (Table 2). Their banding patterns (except for mEgCIR0074) are shown in Figures 2 and 3. One of the segregating SSR markers (mECIR3260) belongs to linkage group 3, all other SSR

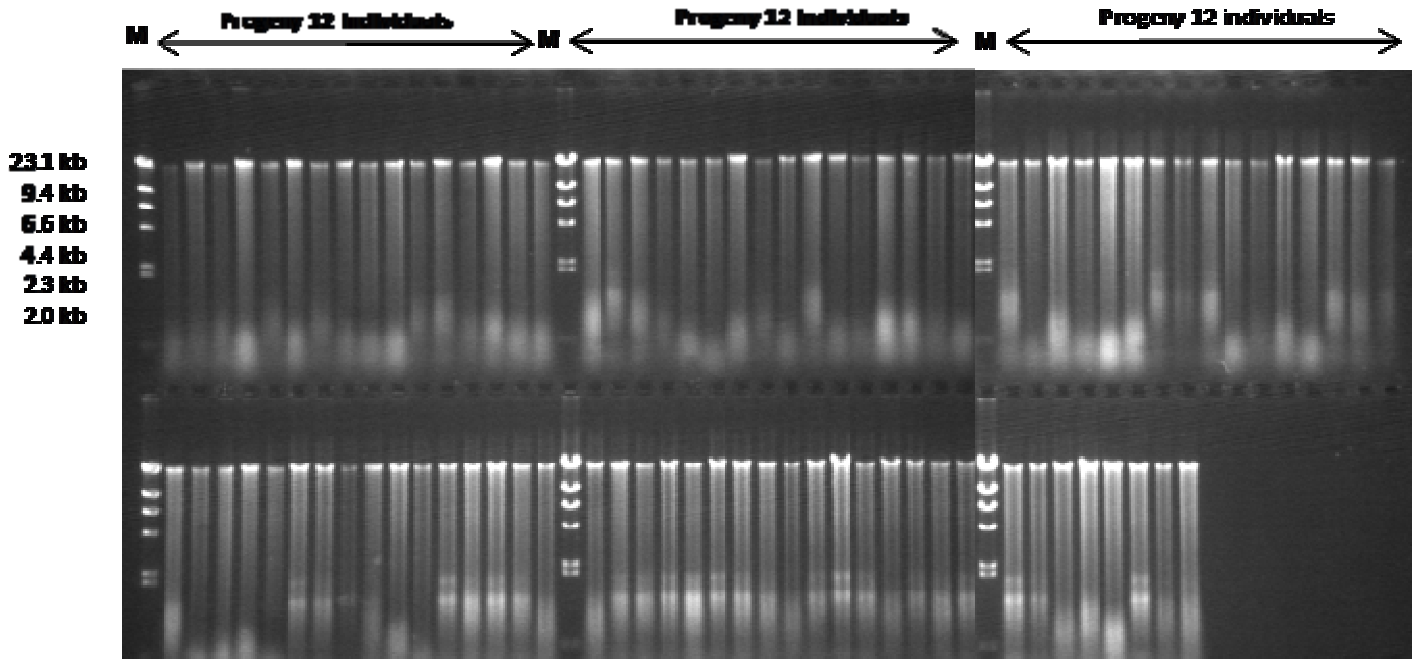


Figure 1. Separation of extracted total DNA from oil palm leaves of progeny 12 (92 individuals) for quality control. M: Lambda DNA HindIII digested (New England Biolabs) as marker. Five microliters of each sample (50 ng/µl) extracted with the new DNA extraction protocol were loaded on a 1% agarose gel and run for 30 min at 80V. The gel was stained with ethidium bromide.

markers to linkage group 4 covering the whole group. SSR marker mECIR3275 is the closest marker to the *Sh* gene. SSR marker mEgCIR1917 showed an allele pattern corresponding to a genotype configuration defined by Ritter et al. (1990) for one with four alleles segregating in the cross between heterozygous parents, whereas for the markers mEgCIR0059, mEGCIR3275, mEgCIR3260, mEgCIR3275, mEgCIR3533, and mEgCIR3557 the SSR allele patterns can be explained from genotype configurations resulting from three alleles segregating in the full-sib cross (Table 2).

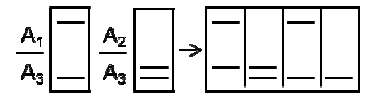
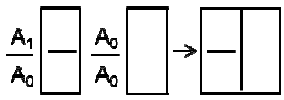
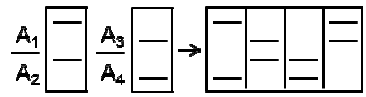
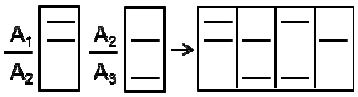
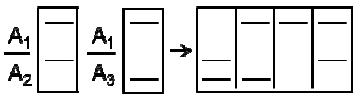
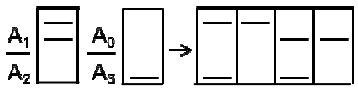
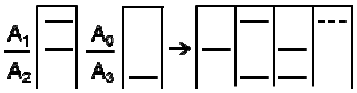
Marker mEgCIR0074 showed a segregation ratio of 1:1 corresponding to the segregation of one allele and a null allele in the second parent. Four SSR markers mEgCIR0059, mEgCIR3260, mEgCIR3533 and mEgCIR3557 showed a distorted segregation ($p < 0.05$). For mEgCIR3557, one allele pattern is missing indicating that the combination A_1A_0 may be selected against or is deleterious.

Screening progeny 12 with these SSR markers also proved that one individual showed a different SSR pattern as marked by 'X' for SSR marker mEgCIR1917 (Figure 3C). This was supported by data for mEgCIR3275 and mEgCIR0059 (data not shown). This palm tree is not part of this progeny and will be excluded from future segregation analyses. The results show that the SSR markers developed for Billotte et al. (2005) can be successfully transferred and used for marker-assisted selection in progeny 12 of the NIFOR breeding program.

DISCUSSION

Due to their long reproductive cycles and the time until traits of the mature tree can be evaluated marker-assisted selection is particularly attractive for tree breeding (Kumar et al., 2012; Thavamanikumar et al., 2013). In outcrossing crops like oil palm the use of markers is challenging as the segregation patterns observed in he progenies are more complex than in inbreeding species (Sewell and Neale, 2000; van Ooijen, 2011). An additional challenge has to be handled if in a breeding program the parents of the progeny are not available anymore for example, got lost due to diseases during the long time of cultivation. In this case, the constitution of the parents has to be reconstructed from the observed segregation pattern in the progeny (Billotte et al., 2005; Lespinasse et al., 2000) to allow mapping of the markers using mapping programs like JoinMap 4.0 (van Ooijen and Voorrips, 2001) and MapQTL 5 (van Ooijen, 2004). Markers that allow this are called informative markers (van Ooijen, 2011). In this study, we showed that for the progeny 12 (13.386 T x 26.1074 D) of the NIFOR oil palm breeding program that this situation could be successfully handled by identifying the segregation patterns for individual SSR markers in the progeny and deducting from this the genetic constitution of the parents at the locus (Table 2). The SSR analysis presented for the progeny 12 showed that the number of segregating alleles per locus in this full-sib family ranged between one

Table 2. Number of alleles segregating in progeny 12 and the corresponding segregation ratios.

Primer no.	Progeny 12 (*Tenera* x *Deli Dura*)							
	Lg	No Of Alleles Segregating	Parents P1 P2	Allelic pattern	Segregation ratio	DF	χ^2 value	P value
mEgCIR0059	4	3	$\frac{A_1}{A_3}$ $\frac{A_2}{A_3}$		1:1:1:1	3	22.091*	0.0
mEgCIR0074	4	1	$\frac{A_1}{A_0}$ $\frac{A_0}{A_0}$		1:1	1	1.103	0.294
mEgCIR1917	4	4	$\frac{A_1}{A_2}$ $\frac{A_3}{A_4}$		1:1:1:1	3	1.515	0.679
mEgCIR3260	3	3	$\frac{A_1}{A_2}$ $\frac{A_2}{A_3}$		1:1:1:1	3	8.378*	0.039
mEgCIR3275	4	3	$\frac{A_1}{A_2}$ $\frac{A_1}{A_3}$		1:1:1:1	3	6.205	0.102
mEgCIR3439	4	Monomorph						
mEgCIR3533	4	3	$\frac{A_1}{A_2}$ $\frac{A_0}{A_3}$		1:1:1:1	3	58.910*	0.0
mEgCIR3535	4	Monomorph						
mEgCIR3557	4	3	$\frac{A_1}{A_2}$ $\frac{A_0}{A_3}$		1:1:1:1	3	61.587*	0.0

*Significant at $p < 0.05$

and four, varying from locus to locus. Six SSR markers mEgCIR1917, mEgCIR0059, mEgCIR3260, mEgCIR3275, mEgCIR3533 and mEgCIR3557 proved to be fully informative markers following a segregation ratio of 1:1:1:1. However, SSR marker mEgCIR0074 belongs to a genotype configuration where in one parent only one allele was amplified, the other being a null allele with no amplification occurring in this parent (Table 2). Such markers segregate in either 1:1 or 3:1 ratios (Billotte et al., 2005; Lespinasse et al., 2000). Also, such markers provide complete information about one of the parents but no information on the other parent (van Ooijen 2011).

The efficiency of molecular markers lies in the fact that they can be used as a tool to detect sequence variation that exist between and within species (Doveri et al., 2008) and also to identify inbred lines (Shehata et al., 2009). SSR markers are particularly interesting in this regard and have been extensively used in oil crops (Cloutier et al., 2011; Rotondi et al., 2011; Xie et al., 2012). From the findings observed in the present study, the SSR markers segregated in the breeding population with up to four alleles. No immediate reason could be given why not all the SSR markers, which were used for the study, produced amplification products other than the fact that the primers (forward and/ or reverse) did not anneal due to sequence differences between the populations. Progeny 12 is quite different from the population used to develop the markers by Billotte et al. (2005). However, 22% of the SSR markers were polymorph in the NIFOR population. This is about in the

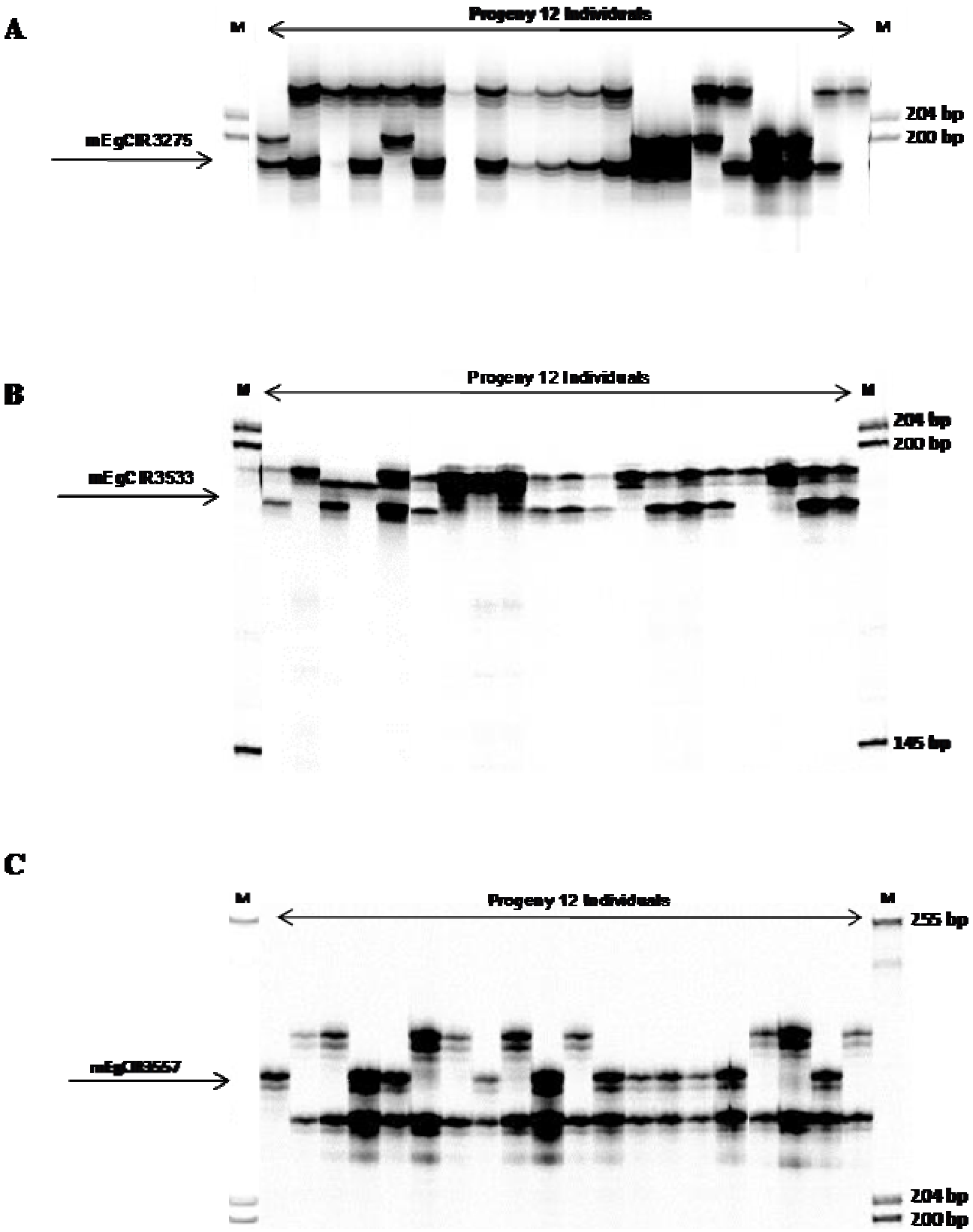


Figure 2. SSR marker analyses in progeny 12 using: **(A)** marker mEgCIR3275, **(B)** marker mEgCIR3533 and **(C)** marker mEgCIR3557. M: IRDye® 800 sizing standard 50-700 bp (LI-COR Biosciences) as marker. Twenty individuals of the segregating population are shown, which represent the observed segregation patterns in the progeny (Table 2), respectively. Samples were separated on a 6% polyacrylamide gel using a DNA analyzer 4300.

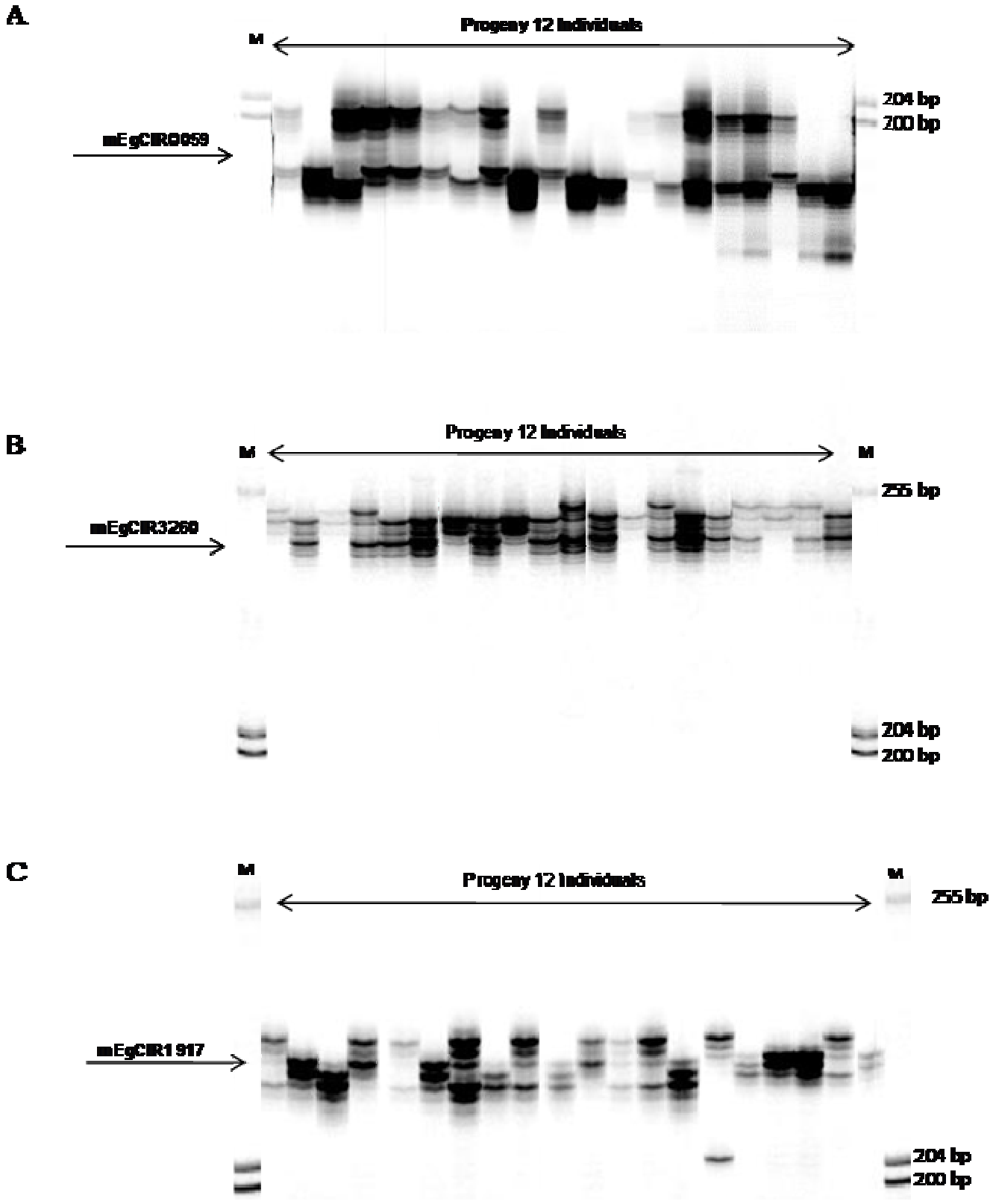


Figure 3. SSR marker analyses in progeny 12 individuals using (A) marker mEgCIR0059, (B) marker mEgCIR3260 and (C) marker mEgCIR1917. 'X' represents banding pattern for an individual, which does not belong to the population. M: IRDye® 800 sizing standard 50-700 bp (LI-COR Biosciences) as marker. Twenty individuals of the segregating population are shown, which represent the observed segregation patterns in the progeny (Table 2), respectively. Samples were separated on a 6% polyacrylamide gel using a DNA analyzer 4300.

range for transferability of SSR markers observed in other families for example, Rosaceae (Sajer et al., 2012), where 25% of the tested SSR markers proved on average to be polymorph in another mapping population.

Segregation distortion describes a phenomenon that observed genotypic frequencies deviate from the expected Mendelian frequencies (Sandler and Novitski, 1957). In progeny 12, distortions were observed for some of the SSR markers at $p > 0.05$. This was also reported by Billotte et al. (2005), however, for different markers, indicating in our case the detection of population-specific or population dependent distorted markers. In rice, Xu et al. (1997) investigated segregation distortion of markers on a large scale with six mapping populations and 1558 markers. A total of 17 chromosomal regions with distorted markers distributed over all 12 chromosomes were detected. Marker-assisted selection in distorted regions can be extremely useful to increase the frequency of the favorable allele by selecting for recombinants in the distorted region (Xu et al., 1997). Distorted markers are usually discarded prior to QTL mapping because unexpected consequences are feared (Zhan and Xu, 2011; Montoya et al., 2013). However, the former authors claim that segregation distortion can be actually helpful in the detection of QTLs (Xu, 2008; Xu and Hu, 2010) and developed a generalized linear mixed model for segregation distortion analyses (Zhan and Xu, 2011).

The application of molecular markers to the existing breeding programs at NIFOR has been considered. It is expected that with molecular markers higher accuracy, precision and earlier release of improved planting materials can be achieved within the shortest possible time when compared with the existing approach that is based on phenotypic observation. For the successful application of molecular markers for marker-assisted selection, high levels of variation should exist in the chosen breeding population. From the result of this study, it is clear that the progeny 12 can be used for the application of markers in crop improvement programs. The result of this study indicates that SSR markers can be of great benefit for breeding purposes at NIFOR if fully exploited. Even with the genome sequence (Singh et al., 2013b) and several transcriptome resources for fruit mesocarp maturation and ripening and other traits available (Bourgis et al., 2011; Tranbarger et al., 2011; Shearman et al., 2013), traits of interest still need to be localized on the genome using markers like for example, SSRs. In addition, breeding programs require markers for marker-assisted selection. Cost efficiencies of markers in breeding programs have been estimated for some crops and proved to be very efficient when integrated at the right generation into the breeding program (Dreher et al., 2003; Kuchel et al., 2005; Miah et al., 2013; Morris et al., 2003; Slater et al., 2013). Progeny 12 (13.386 T x 26.1074 D) was used in this first assessment of SSR markers for marker-assisted selection in the NIFOR breeding program. In addition, 14 additional mapping populations of the same size are available from the NIFOR breeding program (Okwuagwu 1989, NIFOR 2008). Shared parents in some of these populations will even allow multi-parent QTL analysis as performed by Billotte et al. (2010).

This is the first report of successfully applying SSR markers developed for the cross La Mé x Deli Dura (Bilotte et al., 2005) to the NIFOR oil palm breeding program opening the field to future breeding strategies applying marker-assisted selection.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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