Annals of Botany **XX**: 1–16, 2022
https://doi.org/10.1093/aob/mcac036, available online at www.academic.oup.com/aob

ANNALS OF BOTANY
Founded 1887

RESEARCH IN CONTEXT

Occurrence of triploids in oil palm and their origin

V. Pomiès[1,2,*], N. Turnbull[3], S. Le Squin[3], I. Syahputra[4], E. Suryana[4], T. Durand-Gasselin[3], B. Cochard[3] and F. Bakry[1,2]

[1]CIRAD, UMR AGAP Institut, F-34398 Montpellier, France, [2]UMR AGAP Institut, Univ Montpellier, CIRAD, INRAE, Institut Agro, F-34398 Montpellier, France, [3]PalmElit SAS, 34980 Montferrier sur Lez, France and [4]Pt Socfin Indonesia, Jl.K.L.YosSudarso No. 106, Medan 20115, Sumatera Utara, Indonesia
**For correspondence. E-mail virginie.pomies@cirad.fr*

Received: 10 November 2021 Returned for revision: 28 February 22 Editorial decision: 14 March 2022 Accepted: 16 March 2022

• **Background and Aims** Oil palms showing exceptional vigour and dubbed as 'giant palms' were identified in some progeny during breeding. A panel of phenotypical traits were studied to characterize these trees. The hypothesis that gigantism and other anomalies might be linked to polyploidy was investigated.
• **Methods** Twenty sib pairs of palms from different crosses, each comprising a giant and a normal oil palm, were studied by flow cytometry with rice 'Nipponbare' as standard reference. In parallel, palms were assessed in the field using 11 phenotypic traits. A principal component analysis (PCA) was conducted to define relationships between these phenotypical traits, and a linear discriminant analysis (LDA) to predict ploidy level and giant classification. Finally, a co-dominant molecular marker study was implemented to highlight the sexual process leading to the formation of 2*n* gametes.
• **Key Results** The first group of oil palms presented an oil palm/rice peak ratio of around 4.8 corresponding to diploid oil palms, whereas the second group presented a ratio of around 7, classifying these plants as triploid. The PCA enabled the classification of the plants in three classes: 21 were normal diploid palms; ten were giant diploid palms; while 11 were giant triploid palms. The LDA revealed three predictors for ploidy classification: phyllotaxy, petiole size and circumference of the plant, but surprisingly not height. The molecular study revealed that triploid palms arose from 2*n* gametes resulting from the second division restitution of meiosis in parents.
• **Conclusions** This study confirms and details the process of sexual polyploidization in oil palm. It also identifies three phenotypical traits to assess the ploidy level of the giant oil palms in the field. In practical terms, our results provide a cheap scientific method to identify polyploid palms in the field.

Key words: *Elaeis guineensis*, flow cytometry, sexual polyploidization, 2*n* gamete, phenotypical traits, triploid, simple sequence repeat, phyllotaxy.

INTRODUCTION

The oil palm tree (*Elaeis guineensis* Jacq. $2n = 32$) is a diploid allogamous arborescent monocot that belongs to the Arecaceae family which grows in wild, semi-wild and cultivated forms in the equatorial tropics of Africa, South-east Asia and South America (Hartley, 1988). The oil palm tree is cultivated extensively for the production of palm oil and palm kernel oil. In 2018/2019, world production was 77.3 Mt of crude palm oil (CPO) (Oil World, 2020), accounting for 36 % of all edible oil worldwide. The oil palm is the highest yielding oil crop, with the more productive plantations reaching over 7.5 t of CPO ha^{-1} $year^{-1}$ in Asia (Mathews and Foong, 2010) where the maximal potential is estimated at 18 Mt CPO ha^{-1} $year^{-1}$ (Corley and Tinker, 2016). It is expected that by 2050, the production of palm oil will double in order to meet the world demands based on the need for 25 kg per person per year (Corley, 2009). Therefore, an increase in productivity remains one of the key breeding targets for seed companies.

In the mid-2000s, abnormal palms showing uncommon traits were detected at a very low frequency ($<1 \times 10^{-5}$) in Indonesia in 9- to 20-year-old breeding trials. Later, similar palms were also observed in other trials planted on the same estate and in Latin America. These palms showed an increased height, a wider stem circumference and little to no slope angle when compared with normal palms; they were given the name 'giant palms'. These giant palms produced bunches presenting with sterility problems, which led to poor fruit set. When present, fruits were big with thick shells and could be considered as macrocarya fruits (Beccari, 1914). Over time, these palms have prompted questions on the origin of the abnormality, with some hypotheses suggesting a possible link to polyploidy.

Spontaneous polyploidization is common in higher plants. It is considered as a natural driving force leading to a boost in evolution and adaptation of the species complex (Leitch and Leitch, 2008). Polyploid plants often possess novel morphological and physiological traits that differ from those of diploids (Ramsey and Schemske, 1998). In diploid oil palm, shifts to regular sexual reproduction were first reported by Nelson *et al.* (2009) and Dunwell *et al.* (2010). They selected rare

haploid and doubled-diploid plants in the progeny. This spontaneous production of haploids in oil palm was later confirmed by Ramajayam *et al.* (2014), also at a very low frequency. These haploid plants differ from diploids in the growth of plant organs, leaf colour change and aborted female inflorescences. More recently, in a single nucleotide polymorphism (SNP) marker study, Ngoot-Chin *et al.* (2021) detected spontaneous chromosomal aberrations in progeny but also four putative triploids, with only one complete true triploid while the other three were aberrant. These palms presented extraordinary vigorous growth compared with the diploids in the nursery and during the first year of planting. However, beyond these data, none of these studies provides explanations for the origin of these departures from normal sexual reproduction in oil palm.

In the present study, giant palms previously identified in the field based on the breeder's experience were characterized phenotypically. We established, by flow cytometry, that some of these giant palms were spontaneous triploid palms resulting from crosses between diploid parents. Within these latter giant palms, we were able to identify phenotypic markers that can discriminate triploid palms from diploid palms. In addition, we studied the process of sexual polyploidization at the origin of these triploid palms by simple sequence repeat (SSR) marker analysis. The implications of these results for breeding programme issues are discussed.

MATERIALS AND METHODS

Planting material

The giant palms studied are part of the *Elaeis guineensis* species and were found in crosses planted in trials involving Deli Dura parents and African Tenera/Pisifera parents according to the definition of fruit form by Hartley (1967). Their identification and classification as giant palms were mainly based on their exceptional height, their almost non-existent slope angle, the oversized cross-section of the petioles and, when present, the large size of the fruit. Twenty pairs of palms were selected, 19 pairs each consisting of a giant palm and a normal palm from the same cross and Pair 10 comprising two giant palms and two normal palms (Table 1), for a total of 21 giant palms. This enabled us to compare giant and normal palms while minimizing the genotypic variability between them. All the palms were found in trials planted in PT Socfin Indonesia estate, at Aek Loba (North Sumatra, Indonesia).

Phenotyping measurement

Both giant and normal palms were characterized using 11 different traits, and palms were compared for every pair (Fig. 1).

Height. Measurement of the height was done from the base of the stem to the insertion point of leaf no. 33, identified as the reference point (Jacquemard, 1980).

Stem circumference. The circumference of the stem of the palms was measured using a measuring tape that was unrolled around the palm at the junction of the stem and the root collar (circumference_0). Measurements were directly reported on a scoring sheet with a precision of 1 mm.

Stem diameter. The diameter was measured at 1.5 m from the base of the stem in order to avoid biased observations with bottle-shaped palms (stem diameter_1.5). For each palm, two measures were taken perpendicular to one another using a forester's calliper fitted with extension rods (Berthaud, 1993).

Parastichy vertical slope angle. The pattern of insertion of leaves around the stem caused by the specific phyllotaxy of the oil palm forms a series of spirals know as parastichies (Henry, 1955; Rees, 1964). The oil palm's phyllotaxis is defined by a Fibonacci pattern of 8/13 families of parastichies turning in opposite directions, with the 8-family parastichies being the most conspicuous (Thomas *et al.*, 1969).

The orientation of the turn (right side or left side) of the 8-family parastichies was verified to make sure the correct parastichy angle was measured. The slope angle of the parastichy was measured as the angle between the vertical symmetric axis of the stem and the line drawn by the parastichy pattern. This angle is adjacent to the slope angle described by Vakarelov, 2008 and is easier to measure (Fig. 2A). The angle was measured at 1.5 m height from the base of the stem using a protractor fixed at the tip of a 1.5 m tall pole fitted so that the zero of the tool was aligned to the vertical axis. A 50 cm long ruler was aligned between the centre of the protractor and following the pattern of the 8-family parastichy. The angle was measured with a precision of 1°.

Petiole cross-section. Petiole cross-section was measured at point E, at 50 cm from point C, and at point C (Fig. 3) (Ruer and Varéchon, 1964). Width and height were measured using a ruler; measurements were directly reported on the scoring sheet with a precision of 1 mm (Fig. 2B, C) (Aholoukpè *et al.*, 2013). Measurement of point E was done on a petiole base at 1.5 m height from the bottom of the stem (size_petiole). Ratios (r_Cpoint and r_Cpoint50) were calculated as follows:

$$\text{r\_Cpoint} = \frac{\text{Length of petiole section}}{\text{Width of petiole section}}$$

Petiole length. Measurement of the length of the petiole was done from point C (the junction point of the petiole/pinnae insertion) to the point at which leaf no. 17 had been cut down beforehand.

Canopy projection. The longest leaves on each of three sides were identified. For each leaf, the projection on the ground was determined. The distance between the tip of the leaf and the stem was measured at 1.5 m height. This was repeated for the other two leaves. The canopy projection is calculated as follows:

$$\text{Canopy projection} = \frac{\text{Direction1} + \text{Direction2} + \text{Path}}{3} + \text{Stem radius}$$

Approximation of oil palm leaf area. Measurement of the leaf area index (LAI) was adapted from the method described by Tailliez and Ballo Koffi (1992).

Pinnae size. The ten consecutive longest pinnae were sampled on the left side of leaf no. 17 for measurement of their length

TABLE 1. *List of sample pairs used in the study and information on the crossing and the site of planting.*

Pairs	Origin of tree	Plant identification	Observation	Female		Male	
				Parent	Origin	Parent	Origin
Pair 1	**Indonesia**	OP-12	Normal	P01	**Benin**	P03	**Benin**
		OP-6	Giant				
Pair 2	**Indonesia**	OP-13	Normal	P14	**Benin**	P18	**Benin**
		OP-9	Giant				
Pair 3	**Indonesia**	OP-14	Normal	P17	**Indonesia**	P12	**Indonesia**
		OP-10	Giant				
Pair 4	**Indonesia**	OP-15	Normal	P02	**Indonesia**	P12	**Indonesia**
		OP-3	Giant				
Pair 5	**Indonesia**	OP-42	Normal	P16	**Indonesia**	P10	**Indonesia**
		OP-5	Giant				
Pair 6	**Indonesia**	OP-16	Normal	P08	**Indonesia**	P09	**Indonesia**
		OP-4	Giant				
Pair 7	**Indonesia**	OP-17	Normal	P04	**Benin**	P05	**Benin**
		OP-1	Giant				
Pair 8	**Indonesia**	OP-18	Normal	P06	**Indonesia**	P13	**Indonesia**
		OP-2	Giant				
Pair 9	**Indonesia**	OP-19	Normal	P07	**Indonesia**	P19	**Indonesia**
		OP-11	Giant				
Pair 10	**Indonesia**	OP-20	Normal	P11	**Indonesia**	P15	**Indonesia**
		OP-21	Normal				
		OP-7	Giant				
		OP-8	Giant				
Pair 11	**Indonesia**	OP-22	Normal	P21	**Benin**	P22	**Benin**
		OP-23	Giant				
Pair 12	**Indonesia**	OP-24	Normal	P23	**Benin**	P24	**Benin**
		OP-25	Giant				
Pair 13	**Indonesia**	OP-26	Normal	P23	**Benin**	P25	**Benin**
		OP-27	Giant				
Pair 14	**Indonesia**	OP-28	Normal	P26	**Benin**	P27	**Benin**
		OP-29	Giant				
Pair 15	**Indonesia**	OP-30	Normal	P28	**Indonesia**	P29	**Indonesia**
		OP-31	Giant				
Pair 16	**Indonesia**	OP-32	Normal	P30	**Indonesia**	P13	**Indonesia**
		OP-33	Giant				
Pair 17	**Indonesia**	OP-34	Normal	P31	**Indonesia**	P20	**Indonesia**
		OP-35	Giant				
Pair 18	**Indonesia**	OP-36	Normal	P32	**Indonesia**	P33	**Indonesia**
		OP-37	Giant				
Pair 19	**Indonesia**	OP-38	Normal	P34	**Indonesia**	P35	**Indonesia**
		OP-39	Giant				
Pair 20	**Indonesia**	OP-40	Normal	P36	**Indonesia**	P37	**Indonesia**
		OP-41	Giant				

and width. The mean length and mean width were determined and a ratio (r_pinnae) was calculated as follows:

$$\text{r\_pinnae} = \frac{\text{Mean pinnae length}}{\text{Mean pinnae width}}$$

Leaf sampling

For every pair of palms, leaves were sampled on both the giant and the normal palms. Fresh leaf samples were also collected from the still living parent trees of the palms sampled in the trials planted in Indonesia, despite their advanced age and their great height. These samples came from PT Socfin Indonesia in North Sumatra (Indonesia) and INRAB (Benin). After collection, leaf samples were placed in plastic bags, sealed using a vacuum pump and sent to Cirad (France) for ploidy analysis. In addition, oil palm DNA content was determined from fresh leaf samples taken on an *Elaeis guineensis pisifera* oil palm reference tree grown in a greenhouse at Montpellier (France).

Samples for genotyping analysis were rapidly freeze-dried after collection and then ground. Bottles of ground leaf samples were stored at 4 °C before being sent to Cirad for analysis.

Flow cytometry

Ploidy level of the palms (parents and descendants) collected in the field. The ploidy level of diploid parents and their progeny (Table 2) was estimated with 6′-diamidino-2-phenylindole (DAPI) staining about 10 days after leaf harvest, on non-fresh palm material, leading to less narrow linear scale peaks than fresh palm material. Approximately 0.5 cm² of leaf was chopped in the standard DAPI staining CyStain UV Ploidy solution (Partec GmbH, Münster, Germany, ref: 05-5001) together with leaf tissue of *Oryza sativa* L. ssp *japonica* 'Nipponbare'

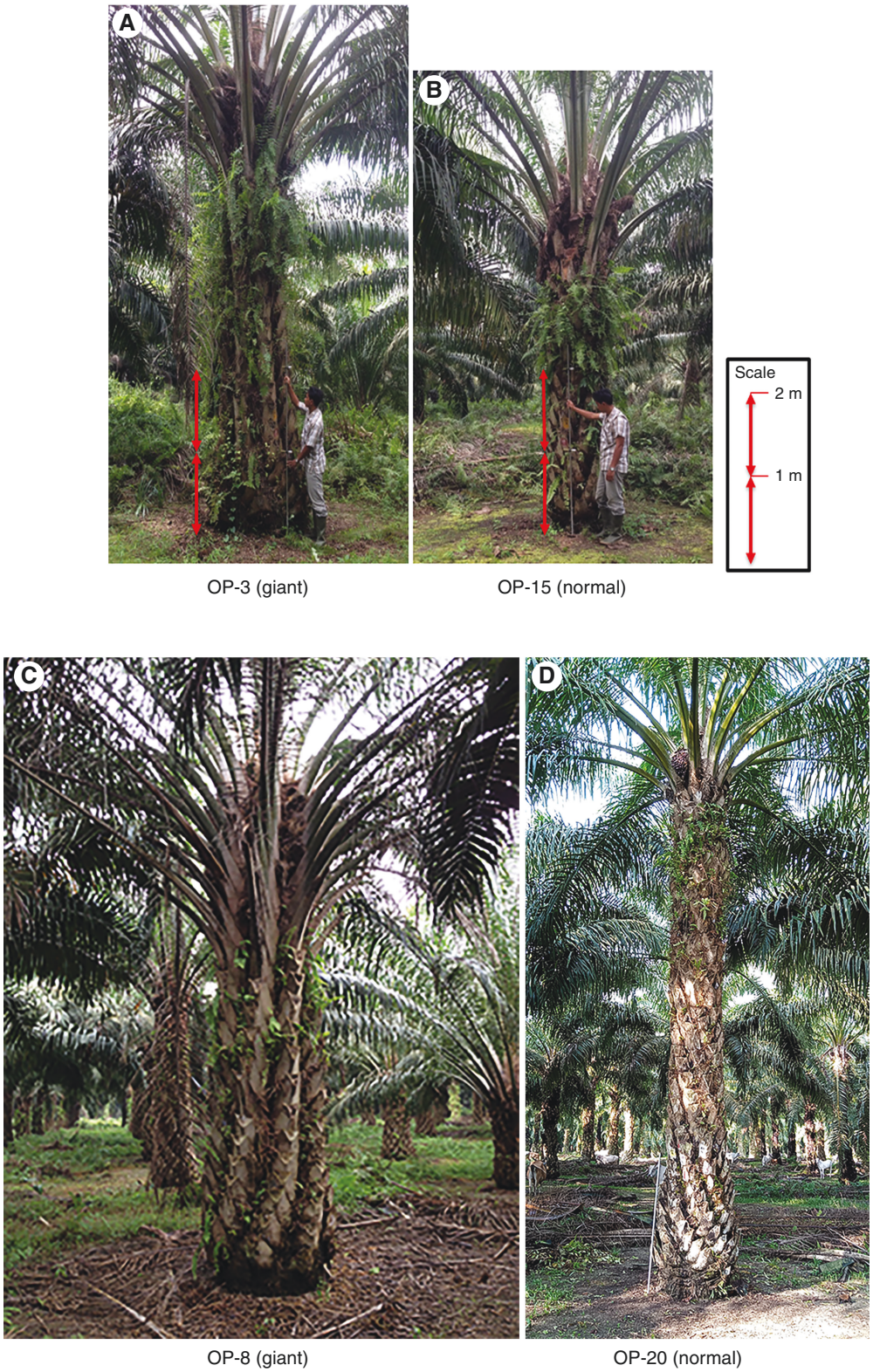


FIG. 1. Pairs of palm trees from family 4 at the same age (A, B) and family 10 (C, D) showing differences in various phenotypic traits. Differences in height, stem circumference and stem diameter can be observed between the giant OP-3 (A) and the normal OP-15 (B) trees. Differences in parastichy slope angle and petiole cross-section can be observed between the giant OP-8 (C) and the normal OP-20 (D) trees.

used as the internal reference standard (haploid genome size 0.91 pg per nucleus). Suspensions were then filtered with a 30 µm nylon mesh and placed in the dark for incubation for 30 min. Subsequently samples were analysed with the Partec PAS II Flow Cytometer (Partec GmbH, Münster, Germany) with a fluorescent excitation UV lamp (350 nm). Each extraction and measurement were repeated once. Results were therefore registered in linear scale and then analysed in logarithmic scale, leading to accurate peak distance determination.

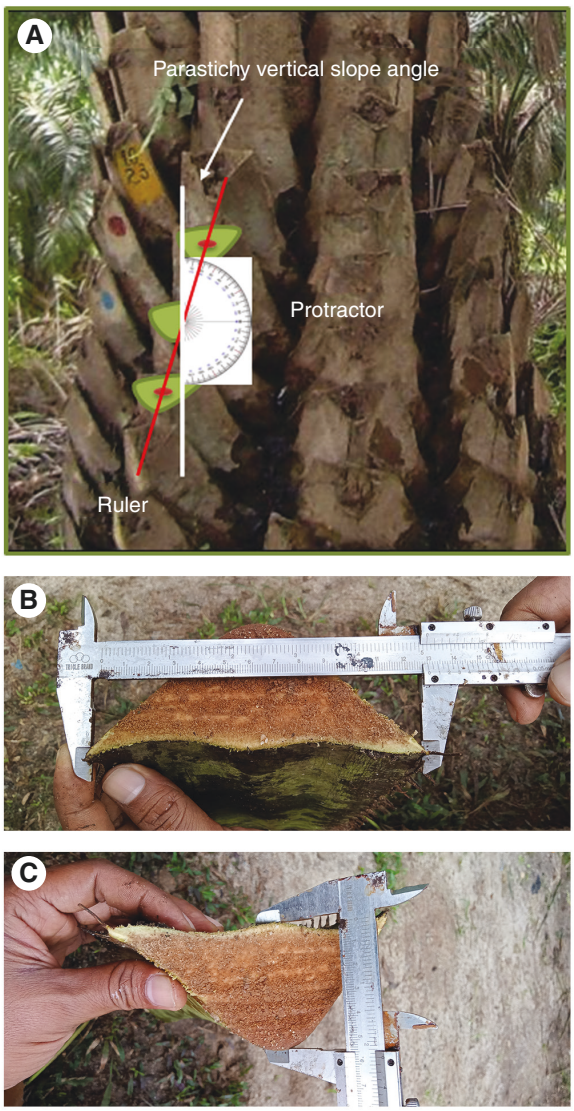


FIG. 2. Parastichy vertical slope angle (A) and petiole cross-section measurements: width (B) and height (C).

DNA content of the reference oil palm. Accurate total DNA content estimation was performed on leaf tissue from a diploid plant located in the Cirad greenhouse which was used as the reference palm. Leaf samples were chopped together with leaf tissue of *O. sativa* L. ssp *japonica* 'Nipponbare' as internal reference. Extraction of nuclei was performed in a modified LB01 buffer (Doležel *et al.*, 1989) in which mercaptoethanol was substituted by 40 mM sodium sulfite (Na_2SO_3). After filtration with a 30 µm nylon mesh to eliminate cell debris, 50 ppm of propidium iodide (PI) + RNase A (50 ppm) was added to stain the nuclei. After 1 h in the dark, analyses were performed with a PAS II flow cytometer (Partec GmbH) using an argon ion laser (488 nm) for PI excitation as described by Doležel and Bartos (2005). Five independent replicates of isolated nuclei were made and measured on the same day. This same protocol was applied to this palm reference with DAPI staining (366 nM) and a UV lamp as the excitation source. The AT/GC content was calculated according to Smarda *et al.* (2008).

Marker selection and genotyping

The SSR markers in this study were selected from a set of 291 markers available in the same oil palm genetic background (Cochard *et al.*, 2015). They were chosen according to the heterozygote patterns of the parents (three or four different alleles for the same locus), their distribution on chromosomes and their distance from the centromeres (Singh *et al.*, 2013). For the study, a good distribution of SSR markers along the chromosome is necessary, with a regular gap of 10 cM. The population of this study comes from relatively consanguineous parents and our SSR requirements greatly reduce the number of usable markers and therefore the number of chromosomes studied. In total, we were able to use the distribution of 62 informative SSR loci on five chromosomes constituted of 61 oil palm SSRs and one transferable coconut (*Cocos nucifera* L.) SSR as detailed in Fig. 4. We genotyped 15 loci on chromosome 2, 12

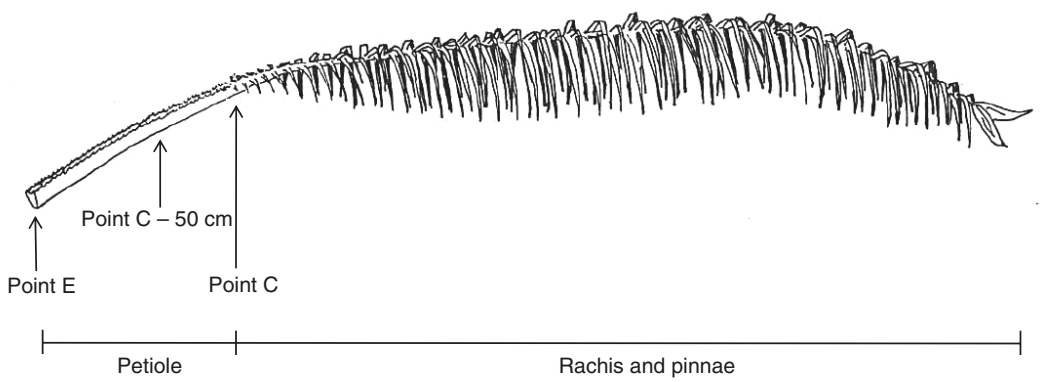


FIG. 3. Localization of the petiole cross-section measurements on an oil palm leaf according to Ruer and Varéchon (1964).

TABLE 2. *List of samples analysed by flow cytometry (FC) and genotyped (G) in the study*

Pairs	Plant identification	Observation	FC	G	Female parent	FC	G	Male parent	FC	G
Pair 1	OP-12	Normal	x		P01	x	x	P03	x	x
	OP-6	Giant	x	x						
Pair 2	OP-13	Normal	x		P14	x	x	P18		x
	OP-9	Giant	x	x						
Pair 3	OP-14	Normal	x		P17		x	P12	x	x
	OP-10	Giant	x	x						
Pair 4	OP-15	Normal	x		P02	x	x	P12	x	x
	OP-3	Giant	x	x						
Pair 5	OP-42	Normal	x		P16		x	P10	x	x
	OP-5	Giant	x	x						
Pair 6	OP-16	Normal	x		P08	x	x	P09	x	x
	OP-4	Giant	x	x						
Pair 7	OP-17	Normal	x		P04	x	x	P05	x	x
	OP-1	Giant	x	x						
Pair 8	OP-18	Normal	x		P06	x	x	P13	x	x
	OP-2	Giant	x	x						
Pair 9	OP-19	Normal	x		P07	x	x	P19		x
	OP-11	Giant	x	x						
Pair 10	OP-20	Normal	x		P11	x	x	P15	x	x
	OP-21	Normal	x							
	OP-7	Giant	x	x						
	OP-8	Giant	x	x						
Pair 11	OP-22	Normal	x		P21			P22		
	OP-23	Giant	x							
Pair 12	OP-24	Normal	x		P23			P24		
	OP-25	Giant	x							
Pair 13	OP-26	Normal	x		P23			P25		
	OP-27	Giant	x							
Pair 14	OP-28	Normal	x		P26			P27		
	OP-29	Giant	x							
Pair 15	OP-30	Normal	x		P28			P29		
	OP-31	Giant	x							
Pair 16	OP-32	Normal	x		P30			P13		
	OP-33	Giant	x							
Pair 17	OP-34	Normal	x		P31			P20		
	OP-35	Giant	x							
Pair 18	OP-36	Normal	x		P32			P33		
	OP-37	Giant	x							
Pair 19	OP-38	Normal	x		P34			P35		
	OP-39	Giant	x							
Pair 20	OP-40	Normal	x		P36			P37		
	OP-41	Giant	x							

Fresh leaves of four parents (P16, P17, P18 and P19) were not available for FC analysis (dead trees). Parents of pairs 11–20 were not studied.

loci on chromosome 3, nine loci on chromosome 7, nine loci on chromosome 9, and 17 loci on chromosome 10.

Genotyping was then applied to selected giant palms and their parents (see Table 2). It was conducted by the commercial laboratory ADNId (Montpellier, France, http://www.adnid.fr). Genotyping results were analysed with GeneMapper® 4.1 software (Applied Biosystems, USA).

Statistical analysis

A principal component analysis (PCA; Pearson, 1901) was performed on the 42 palms and 11 phenotypic variables in order to further define the relationships between phenotypical traits. Giant classification was used as a supplementary qualitative variable.

To predict the ploidy level and giant classification based on morphology information, a linear discriminant analysis (LDA; Fisher, 1936) was applied, with the combination of giant classification and ploidy level as the dependent variable and phenotypic variables as covariates. LDA can be viewed as a dimensionality reduction technique, providing a reduced space that best separates known classes. It can also be used as a predictive model, developing a classification rule, and computing for each individual the probability of belonging to the different classes. The accuracy of prediction was estimated using the misclassification rate estimated by the leave-one-out cross-validation method (Stone, 1974).

All analyses were performed in R 4.0.3 (R Core Team, 2020), using package FactoMineR (Lê *et al.*, 2008) for PCA, MASS (Venables and Ripley, 2002) for LDA and ggplot2 (Wickham, 2016) and ggord (Beck, 2020) for graphical representation.

RESULTS

Ploidy level in progeny

Oil palm/rice peak ratios were on average 4.83 ± 0.07 for diploid parents. For their progeny, two classes were observed: the first one with a peak ratio of 4.81 ± 0.12 and the second one with

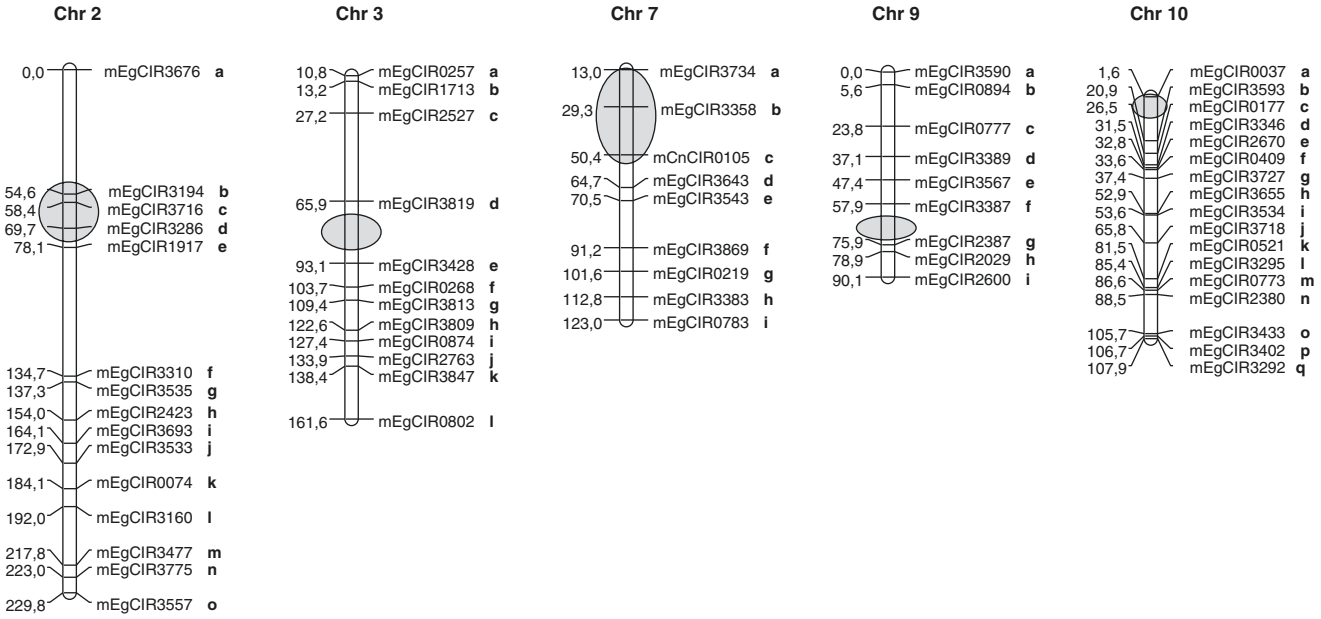


FIG. 4. Localization of 62 SSR markers used on each chromosome with the centromere positioned according to Singh *et al.* (2013).

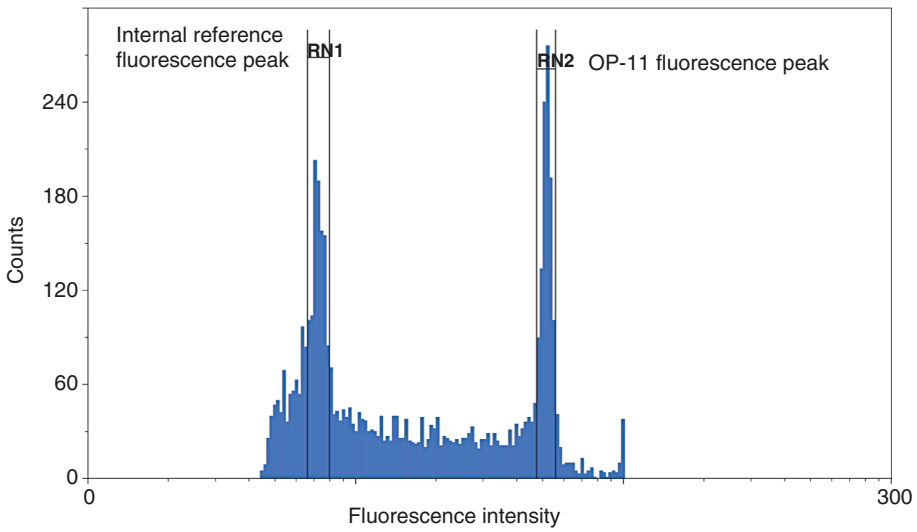


FIG. 5. Fluorescence peaks of the OP-11 triploid sample and the rice internal reference genome size (*Oryza sativa* L. ssp *japonica* 'Nipponbare', 0.91 pg per nucleus). The histogram was obtained by flow cytometric analysis of DAPI-stained nuclei of OP-11 and rice samples, which were isolated, stained and analysed simultaneously. On the *x*-axis is the fluorescence intensity representing the relative nuclear DNA content, and on the *y*-axis is the count of nuclei.

7.07 ± 0.13, showing that both diploids and triploids have been identified in the samples (Fig. 5). The results of the ploidy study of all collected giant palms indicate that out of the 21 giant palms sampled, ten were found to be diploid and 11 were found to be triploid (Table 3). All normal trees sampled in each pair were found to be diploid. It should be mentioned that in addition, we found a tetraploid plant (oil palm/rice peak ratio: 10.27) classified as non-giant in this study.

Oil palm genome size and AT/GC content

In the study, fresh leaf samples of a diploid oil palm tree from the Cirad greenhouse were used. The difference in peak ratio between rice and oil palm with PI and DAPI staining allowed us to calculate the AT/GC content in oil palm (Smarda, 2008). The mean oil palm/rice peak ratio with PI staining was 4.28 and was 4.7 with DAPI staining. The calculated DNA content of our oil palm reference was 3.90 ± 0.01 pg and the AT content was estimated to 58.2 %; this value is higher by about 13 % than that published by genome sequencing (Singh *et al.*, 2013).

Phenotypic characterization

The first and second components on the correlation matrix of the PCA represented 30 and 25 % of the total phenotypic

TABLE 3. *Flow cytometry result obtained with fresh palm material with DAPI staining*

Pairs	Plant identification	Observation	Rice value	Oil palm value	Ratio	Ploidy conclusion
Pair 1	OP-12	Normal	30.81	142.77	4.63	2*x*
	OP-6	Giant	30.50	213.80	7.01	3*x*
Pair 2	OP-13	Normal	30.96	148.21	4.79	2*x*
	OP-9	Giant	29.40	200.80	6.83	3*x*
Pair 3	OP-14	Normal	29.90	146.24	4.89	2*x*
	OP-10	Giant	28.78	206.41	7.17	3*x*
Pair 4	OP-15	Normal	29.74	145.26	4.88	2*x*
	OP-3	Giant	29.72	210.62	7.09	3*x*
Pair 5	OP-42	Normal	26.17	128.56	4.91	2*x*
	OP-5	Giant	27.62	199.95	7.24	3*x*
Pair 6	OP-16	Normal	26.58	131.56	4.95	2*x*
	OP-4	Giant	31.27	222.27	7.11	3*x*
Pair 7	OP-17	Normal	30.39	145.08	4.77	2*x*
	OP-1	Giant	31.10	227.34	7.31	3*x*
Pair 8	OP-18	Normal	30.25	146.36	4.84	2*x*
	OP-2	Giant	30.97	216.27	6.98	3*x*
Pair 9	OP-19	Normal	22.91	112.95	4.93	2*x*
	OP-11	Giant	29.18	203.55	6.98	3*x*
Pair 10	OP-20	Normal	25.23	127.80	5.07	2*x*
	OP-21	Normal	32.97	154.15	4.68	2*x*
	OP-7	Giant	29.19	204.64	7.01	3*x*
	OP-8	Giant	28.83	204.59	7.10	3*x*
Pair 11	OP-22	Normal	30.99	149.15	4.81	2*x*
	OP-23	Giant	30.21	144.25	4.77	2*x*
Pair 12	OP-24	Normal	29.24	142.21	4.86	2*x*
	OP-25	Giant	31.06	141.71	4.56	2*x*
Pair 13	OP-26	Normal	31.06	148.78	4.79	2*x*
	OP-27	Giant	29.64	142.79	4.82	2*x*
Pair 14	OP-28	Normal	29.15	139.91	4.80	2*x*
	OP-29	Giant	30.31	144.60	4.77	2*x*
Pair 15	OP-30	Normal	30.89	147.01	4.76	2*x*
	OP-31	Giant	29.21	138.83	4.75	2*x*
Pair 16	OP-32	Normal	22.68	113.43	5.00	2*x*
	OP-33	Giant	29.80	143.79	4.83	2*x*
Pair 17	OP-34	Normal	30.18	141.64	4.69	2*x*
	OP-35	Giant	29.47	143.35	4.86	2*x*
Pair 18	OP-36	Normal	27.29	140.68	5.16	2*x*
	OP-37	Giant	29.14	139.72	4.79	2*x*
Pair 19	OP-38	Normal	29.88	145.72	4.88	2*x*
	OP-39	Giant	28.63	137.64	4.81	2*x*
Pair 20	OP-40	Normal	29.70	144.99	4.88	2*x*
	OP-41	Giant	30.98	149.64	4.83	2*x*

Rice was the internal reference standard; results were registered on a logarithmic scale.

variation, respectively. The first axis is characterized by high values for height, petiole cross-section, petiole length and stem circumference, and low values for the two cross-sections along the petiole (Fig. 6). The second axis is characterized by high values for stem diameter at 1.5 m height, stem circumference, petiole cross-section and the petiole cross-section near pinnae, and lower values for slope angle and canopy projection. Stem circumference, stem diameter at 1.5 m height and petiole cross-section were highly correlated. Similarly, the variables height, petiole length, oil palm tree leaf area and canopy are correlated. Tall oil palms had long petioles, with a high canopy and oil palm tree leaf area. These tall oil palms did not always have wide petioles, wide circumference or wide stem diameter, or a small slope angle. Finally, oil palms with small angle values had wide petioles, wide circumference or wide stem diameter.

Regarding individual plant grouping, five clusters of palms were identified (Fig. 7). Cluster A and B correspond to giant palms with large stems and large petioles. Plants of cluster A were taller with longer petioles than those of cluster B. The C and D clusters correspond to control palms with narrow stem plants, cluster C grouping smaller palms than cluster D. Cluster E, located in the central position, corresponds to a mix of giant and control plants with average data.

Discrimination of ploidy and giant classification

By combining ploidy level and giant classification, only three classes were highlighted (i) 21 control palms were diploid; (ii) 11 giant palms were triploid; and (iii) ten giant palms were diploid, and each palm was assigned to one of these classes. Phenotypic data were first standardized before running LDA. The percentage separation achieved by the first linear discriminant function (LD1) is about 97 %, and it is 3 % for the second linear discriminant function (LD2). Values of the loadings of the discriminant function are given in Table 4. For LD1, the three most important predictors are slope angle, size of the petiole and circumference. The first discriminant function (*x*-axis) separates the three classes into distinct spaces (Fig. 8).

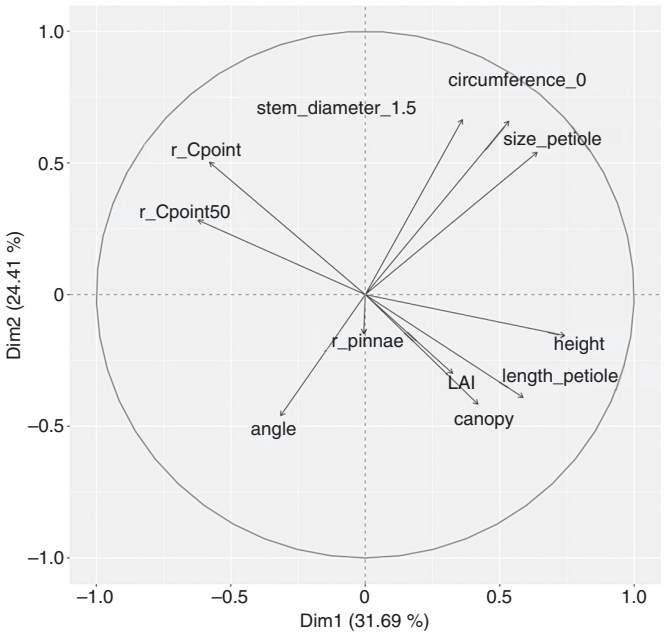


FIG. 6. Correlation circle of phenotypic variables.

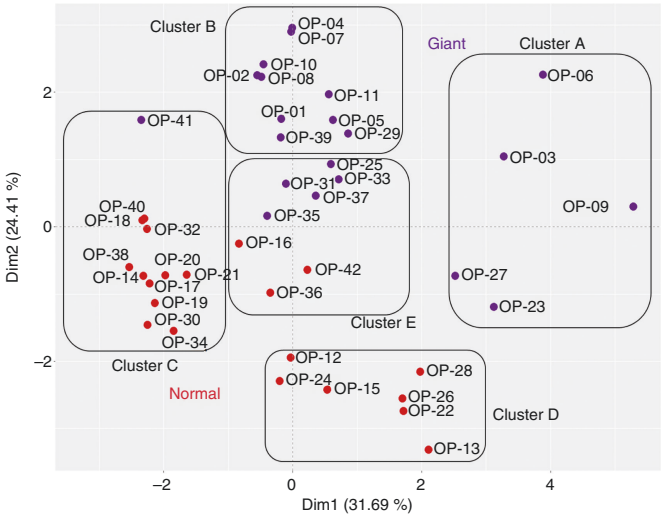


FIG. 7. Individual factor map with giant (purple dots) and normal palms (red dots).

Using prior probabilities as the proportion of observations in each class (21/42 for normal diploids, 11/42 for giant triploids and 10/42 for giant diploids), the misclassification rate of the LDA is about 5 %, with only one giant diploid predicted as giant triploid and one giant triploid predicted as giant diploid.

Considering phenotypic means (see Table 5), giant triploids had a small slope angle (5.91°) with a large petiole (32.91 cm) and large circumference (423.00 cm), following by giant diploids with a normal slope angle (17.30°), a large petiole (30.30 cm) and relatively large circumference (388.30 cm), while control palms had a larger slope angle (19.29°), with smaller petiole (24 cm) and smaller circumference (334.29 cm).

TABLE 4. *Loadings of predictor variables for the first and second linear discriminant (LD) function.*

Phenotypic trait	LD1	LD2
angle	–1.3265	–0.4964
size_petiole	1.0849	–0.5857
circumference_0	0.9496	0.5435
stem_diameter_1.5	0.7732	–0.4757
r_Cpoint	0.6230	–0.3116
LAI	–0.4752	0.2197
length_petiole	–0.2622	–0.3107
canopy	0.2482	–0.5194
r_pinnae	–0.2182	–0.0640
r_Cpoint50	–0.2012	–0.4401
height	0.0036	–0.1994

Origin of polyploid descendants

To understand the origin of triploids in progenies, the formation of diploid gametes was investigated in the parents. SSR analysis was performed on a set of five chromosomes out of the total of 16, considering that the process leading to the formation of a diploid gamete affects one meiocyte only and that the segregating pattern is identical for all chromosomes in the same mitotic cell.

Theoretically, the transmission of co-dominant alleles through the 2*n* gametes suggests that triploid descendants may have two to three alleles depending on the restitution process (Table 6). Loci were considered as informative if it was possible to identify the 2*n* donor between the two parents. For example, for Pair 1, on the mEgCIR3813 locus, the descendant triploid OP-6 had three allele sizes, of which two were from the female parent and one from the male parent (Fig. 9). In contrast, if there were one or two alleles in common in parents, the progeny will have only two alleles; even for triploids, the double dose of alleles in common could not be detected in the genotyping results, thus these loci are not informative.

Therefore, gamete restitution analysis was performed locus by locus along all the five chromosomes belonging to the same triploid palm to identify among all loci those that carry information and contribute to identifying the origin of the 2*n* gamete (Fig. 10).

If we use the same example of triploid palm OP-6 as before, this sample shows numerous loci transmitted in a di-allelic pattern by the maternal parent on the five chromosomes but systematically only one allele from the paternal parent. On chromosome 2, di-allelic PO1 maternal alleles are identified on either side of the centromere. Also identification of maternal SSR markers in di-allelic configuration along the four other chromosomes of this same OP-6 palm confirms this maternal 2*n* gamete restitution hypothesis. In addition, we noticed that loci in the distal position on one of the two arms of chromosome 3 were transmitted via di-allelic inheritance while the SSR markers located closer to the centromere, in the proximal position, were transmitted in a mono-allelic configuration.

A similar analysis was applied to other triploid palms. One sample (OP-11) could not be represented in Fig. 10 because of the absence of informative markers; indeed we observed only one or two alleles for all SSR marker used in the study. In conclusion, six palm trees were identified as originating from maternal

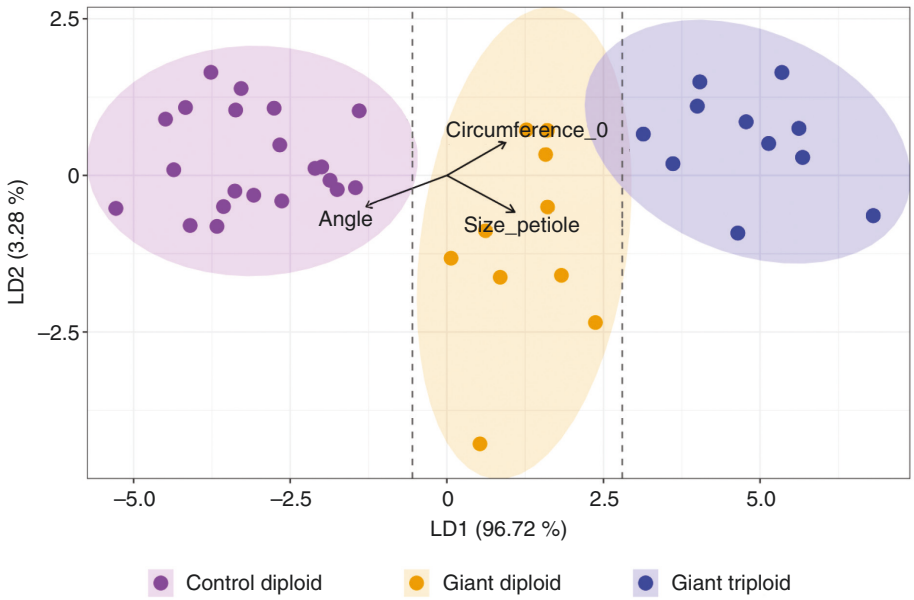


FIG. 8. Biplot of the first two components of the LDA with each point coloured by its classified group level and the three most important predictors for LD1 (circumference, slope angle and petiole size).

TABLE 5. *Phenotypic means for each class*

Phenotypic trait	Control diploid	Giant diploid	Giant triploid
angle	19.29	17.30	5.91
size_petiole	23.97	30.30	32.91
circumference_0	334.29	388.30	423.00
stem_diameter_1.5	61.90	73.25	76.63
r_Cpoint	0.51	0.53	0.52
LAI	10.28	9.58	9.88
length_petiole	269.81	324.60	197.73
canopy	554.08	571.63	525.61
r_pinnae	18.98	18.14	17.60
r_Cpoint50	0.44	0.46	0.43
height	285.62	432.10	364.55

restitution and four from paternal restitution, indicating that 2*n* pollen grains are viable at anthesis in *E. guineensis*.

The preponderance of allele transmission in the heterozygous state for loci positioned on the distal parts of chromosomes suggested that these 2*n* gametes came mainly from second division restitution (SDR) according to Cuenca *et al.* (2011) and De Storme and Geelen (2013), with a crossover probably located between markers transmitted in the mono-allelic and di-allelic mode on the same chromosomal arm. For OP-6 on chromosome 2, we suggested a double crossing-over for this chromosome segment (Cuenca *et al.*, 2011) because of the alternation of maternal mono- and di-allelic markers along these chromosomes. The possibility of restitution by maternal first division restitution (FDR) cannot be excluded for the OP-2 sample. For OP-7 and OP-8, an SDR restitution could be suggested with the little SSR information we had. It is noteworthy that we found no contradictory conclusions on the modes of segregation between the five chromosomes belonging to the same 2*n* gamete, suggesting that the remaining 11 untested chromosomes followed the same segregation pattern during meiosis. We also observed in this study that parental restitution is not limited to a single genetic background but comes from at least 19 different parents either from the female side (six palms) or the male side (four palms).

On another note, the tetraploid plant mentioned above showed a full bi-parental di-allelic pattern for all loci on the five linkage groups. This observation clearly supports a hypothesis of early spontaneous endoduplication of the first cells of the zygote. This post-fertilization event should not be considered as related to any anomalies occurring during meiosis.

DISCUSSION

Flow cytometry applied to Elaeis guineensis

In this study, the DNA content for our greenhouse reference plant of *Elaeis guineensis* was estimated to be 3.90 ± 0.01 pg per nucleus. This DNA content was reported by several authors (Rival *et al.*, 1997; Srisawat *et al.*, 2005; Madon *et al.*, 2008; Camillo *et al.*, 2014), with a range of 3.76–4.32 pg $2C^{-1}$. This variation could be explained by the different references and chromosomal aberrations (Ngoot-Chin *et al.*, 2021).

In addition, the use of field leaf samples that have travelled (a minimum of 10 days) forced us to employ DAPI, which is less sensitive to the freshness of the leaf tissues than the fluorochrome PI. A slight difference in peak ratio value was observed between fresh and non-fresh leaves with DAPI staining. This non-exact matching value for diploids from the field (ratio: 4.8) in comparison with fresh diploid material as reference (ratio: 4.7) may result in a risk of errors due to non-linearity in logarithmic scale as described by Lysák *et al.* (1999) and/or in some small differences in DNA content of different genetic backgrounds as noticed by Madon *et al.* (2008).

In addition, we have provided new information. The use of the UV excitation wavelength coupled with the DAPI fluorochrome on our greenhouse *E. guineensis* reference plant has made it possible to determine the AT/GC ratio in the oil palm genome,

TABLE 6. *Theoretical allelic pattern of triploid descendants*

Allelic pattern of the 2*n* gamete donor parent	Allelic pattern of the *n* gamete donor parent	Theoretical allelic pattern of triploid descendant	
		Allelic heterozygote transmission of the 2*n* gamete	Allelic homozygote transmission of the 2*n* gamete
[AB]	[CD]	[**ABC**]/[**ABD**]	[AC]/[AD]/[BC]/[BD]
[AB]	[CC]	[**ABC**]	[AC]/[BC]
[AB]	[AC]	[AB]/[**ABC**]	[A]/[AC]/[AB]/[BC]

Informative allelic patterns are given in bold.

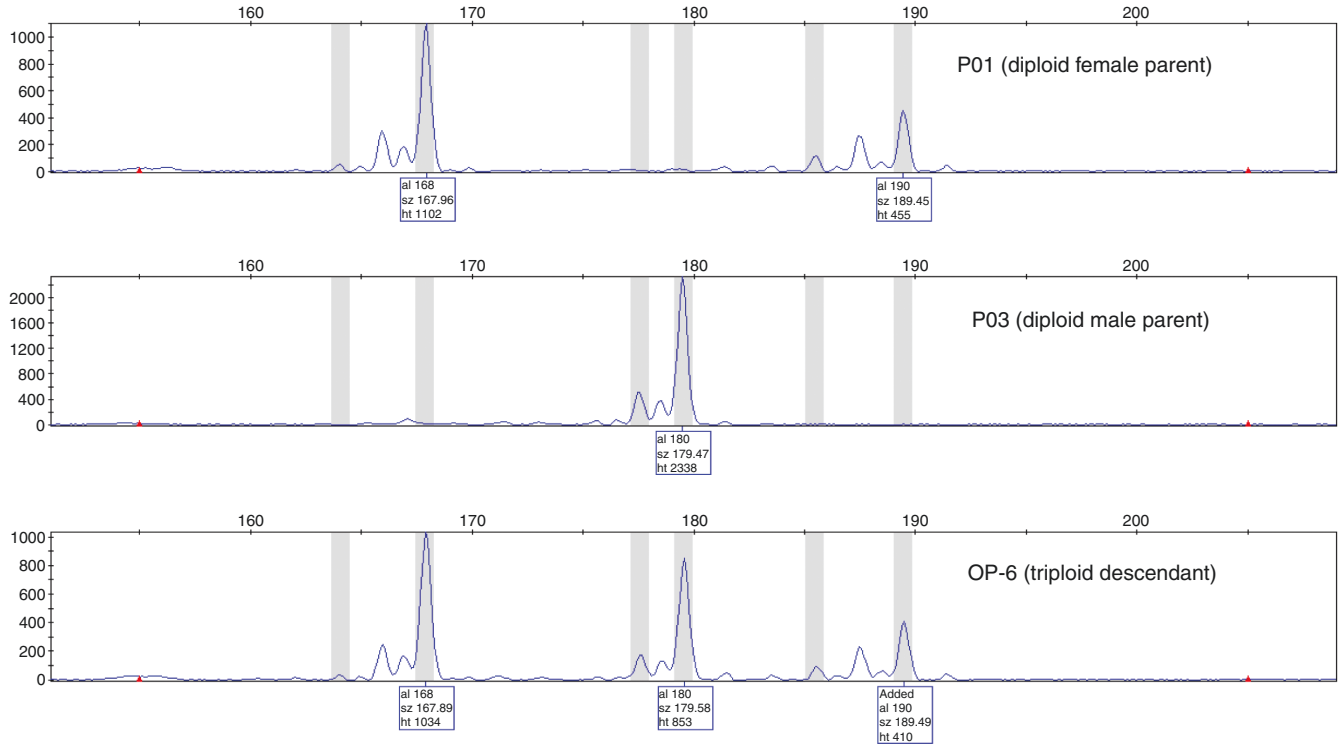


FIG. 9. Electrophoregram of OP-6 and its parent samples performed with the mEgCIR3813 primer. Three allele sizes of 168/180/190 bp are restituted in the progeny (two from the female parent, 168 and 190 bp; and one from the male parent, 180 bp).

the only source of information to date being the sequencing results. By applying the conversion calculation method proposed by Doležel *et al*. (2018), we found that our values obtained by flow cytometry are about 25 % higher. Indeed, by sequencing, Singh *et al.* (2013) estimated the size of the oil palm genome at 1.535 Gbp for 1C while we found a value of 1.907 Gbp by flow cytometry. In addition, it can be seen that the percentage of G + C is 41.8 % in flow cytometry, whereas it was estimated at 37 % by sequencing. These differences in values between sequencing and flow cytometry are quite common in the plant kingdom and could be explained by several hypotheses such as gaps and mistakes due to repetitive sequences in genome assembly (Doležel *et al.*, 2018).

The quality of the peaks on the linear scale was still insufficient with these non-fresh leaf tissues for quality measurement. By converting the linear scale into a logarithmic scale, we were able to obtain peaks narrow enough to measure the DNA content of the parents and their descendants and unambiguously classify the plants from Indonesia into two main categories, diploid and triploid. It should be noted that we also found one tetraploid giant oil palm plant but it is not part of this study on triploids. Sexual polyploidization has already been reported in oil palm. Haploid and doubled-haploid plants have been selected from early greenhouse screenings by several authors (Dunwell *et al.*, 2010; Nasution *et al.*, 2013). Triploids, as well as some tetraploids, have also been reported in the sampling of large seedlings by Nasution *et al.* (2013). However, these underlying mechanisms, reflecting erratic meiosis and/or spontaneous endoduplications, are rare and have never been elucidated before by genetic studies.

Genetic constitution of the triploid plants

As noted by De Storme and Geelen (2013), contrary to the assumption of Stebbins (1950) and Harlan and De Wet (1975), almost all plant species produce 2*n* gametes with some frequency. Today, from a methodological point of view, SSR, as a co-dominant marker, remains an efficient tool to study allelic configuration in triploid descendants as well as their

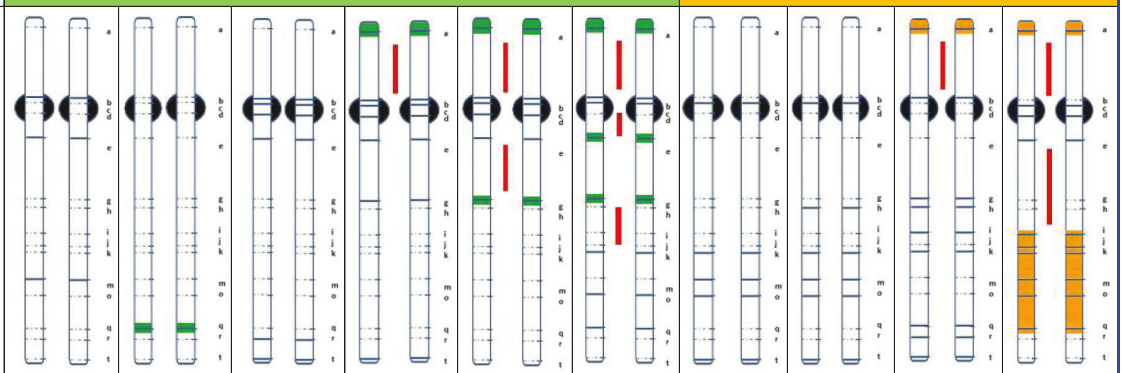
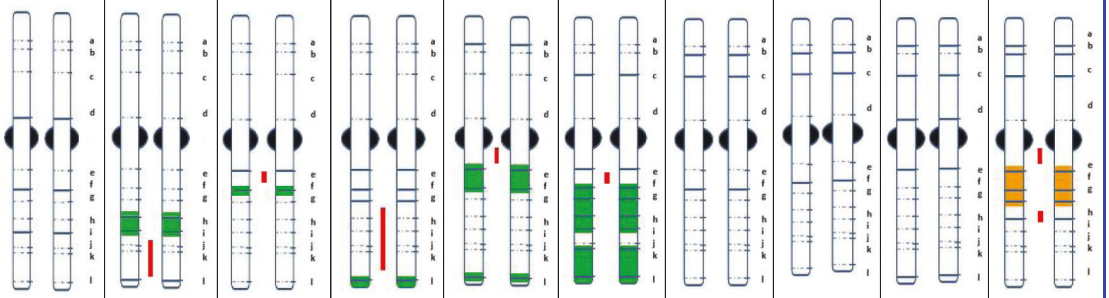
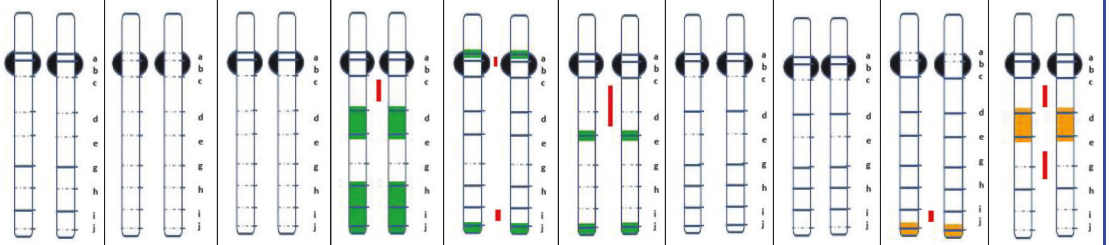
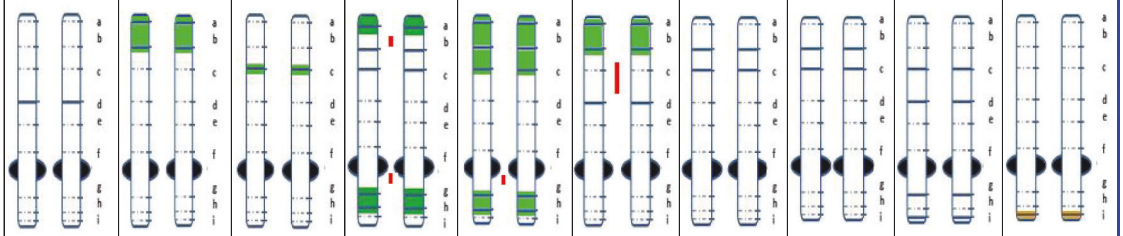
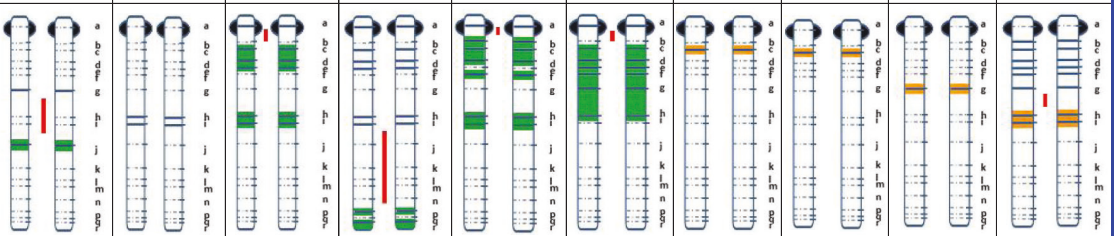
Triploid hybrid	OP-1	OP-2	OP-3	OP-4	OP-5	OP-6	OP-7	OP-8	OP-9	OP-10
Female parent	P04	P06	P02	P08	P16	P01	P11	P11	P14	P17
Male parent	P05	P13	P12	P09	P10	P03	P15	P15	P18	P12
Gamete transmission	Mother restitution						Father restitution			
CHR 2										
CHR 3										
CHR 7										
CHR 9										
CHR 10										
Conclusion	SDR	FDR	SDR	SDR	SDR	SDR	Likely SDR	Likely SDR	SDR	SDR

FIG. 10. Illustration of gamete transmission according to the theoretical allelic pattern on nine triploid samples of the study by using SSR genotyping markers listed in Fig. 4. Non-informative markers are presented with broken lines and informative markers with solid lines (representing homozygotic restitution). Coloured regions indicate the parental allelic restitution (mother/green and father/orange). The red bars represent the hypothetical position of crossing-over, and the black circles indicate the centromere position according to Singh *et al.* (2013).

origin from diploid parents. In this study, we used 62 markers localized on five out the 16 chromosomes present in diploid oil palms to determine the mechanism leading to unreduced gamete formation. This technical approach is based on the postulate that erratic mechanisms leading to unreduced gametes affect all chromosomes belonging to the same cell in the same way at meiosis. To our knowledge, there is no information contradicting this working hypothesis. The analysis of the five linkage groups for the ten triploid descendants clearly indicated that the 2*n* gametes, in our sample, originate both from the maternal parent and from the male parent, producing functional 2*n* pollen. This is rather common in the plant kingdom and has been reported in various contrasting species such as citrus and populus (Liesebach *et al*, 2015; Rouiss *et al.*, 2017*a*, *b*). Moreover, in our study, the restitution of 2*n* gametes is not restricted to a single genotype but to at least 19 different parents.

Global parental heterozygosity transmitted by the 16 linkage groups to triploid progeny throughout the 2*n* gametes was not determined in this study. However, for the five linkage groups investigated, the systematic positioning of SSR markers of the 2*n* donor parent in mono-allelic distribution near the centromeres and, preferentially, in di-allelic configuration in the distal parts of the chromosomes leads to the conclusion that 2*n* gametes derive from SDR. Indeed, in SDR, meiosis I proceeds normally, with correct pairing and recombination followed by restitution of sister chromatids in meiosis II. This anomaly results in 2*n* gametes that are always homozygous from the centromere to the first crossing-over, but retain parental heterozygosity on distal parts of the chromosomes (Ramanna and Jacobsen, 2003). It is in these telomeric parts of the chromosomes that the coding regions are usually located (Park *et al.*, 2007), resulting in a tendency towards functional heterozygosity of SDR-derived 2*n* gametes.

This result is true for most of the triploids studied. However, it should be stressed that for one of the triploid plants (OP-11), we have not been able to differentiate between SDR and FDR 2*n* gamete formation.

Plant vigour and ploidy/heterozygosity

Triploid palms display increased vigour compared with normal diploid palms in old plantations, which is reflected in their greater height, larger trunk and thicker petioles. Also, triploid palms show various degrees of sterility which is a common consequence of polyploidy (Stebbins, 1971); two of these palms were fully abortive, which is not the case for normal diploid palms.

This vigour is different from others already known. Palms that had 100 % of their inflorescences removed were 21 % taller, had a 10 % larger petiole cross-section size and 64 % more dry matter in their trunks compared with the control palms (Corley and Breure, 2008). Likewise, palms which had their fruiting development prevented for 18 months accumulated more than twice as much starch in the trunk (Legros *et al.*, 2009). In the absence of bunch production, these accumulated resources in the trunk may then be reallocated into vegetative growth (Corley and Tinker, 2016). Hence, abortive palms known as pisifera (Chevalier, 1910) with no bunch production present a higher vegetative growth compared with the palms with normal bunch production. Here, our own study suggests that vigour of the triploid plants may also result from their fruit sterility.

Differentiation of giant triploids

As palms were identified and sampled for the study of their possible ploidy, some individuals were found to be difficult to characterize as giant. Three groups were defined: giant, doubtful and normal palms. All the normal palms selected as controls for the giant and doubtful palms were diploids. All the doubtful palms were also found to be diploids. However, three of the phenotypic giant palms were finally found to be diploid and therefore were normal for their ploidy level.

Sampling was based primarily on height and secondarily on the slope angle of the 8-family parastichies and the petiole width. However, the height was not a good criterion because it did not make it possible to discriminate the giant palms; indeed, it was possible to observe large palms but this criterion does not make it possible to distinguish the giant diploid palms from the giant triploid palms. The palms included in this study belong to crosses between heterozygous parents and which present a rather high variability for this trait. The average height increment at 9 years old for progenies with giant palms ranged from 2.3 to 4.8 m, i.e. a span of 208 %.

This study allowed us to identify the most important predictors of the ploidy level among a set of phenotypic traits. The virtual absence of slope angle of the parastichies along with bunch sterility (absence of fruit development) are the main phenotypic traits that discriminate a triploid giant palm from normal palms and giant diploid palms. Petiole cross-section and circumference are useful to distinguish giant diploid palms from normal diploid palms.

Towards a modified phyllotaxy in triploid oil palms

A conspicuous feature of the triploid palms in this study is the change in orientation of the leaf spirals. There are eight regular helixes, starting ‘clockwise/left’ or ‘counter-clockwise/right’ with a slope angle of 16°, the theoretical value modelled by Ochs, 1960 (Fig. 11A) in diploid palms. This value is close to our own measurements of the slope angle on diploid control plants. In these diploids, the eight spirals (or parastichies) continue to rotate in this way, to the right or to the left, throughout the life of the plant. In triploid palms, after a certain period of time, the leaf segments of the same spiral straighten into vertical lines (orthostichies) by superimposition of the leaf scars with a slope angle of 0–5° (see Fig. 1C for example). It should be noted here that all triploid plants in this study have a fixed number of eight orthostichies by straightening the parastichies recognizable in the lower parts of the plants. At the adult stage, these eight orthostichies diverge by an angle of about 45° (360°/8 = 45°) and a divergence angle of 135° between two successive leaves. In this configuration, the leaf of rank $n + 9$ comes to be aligned at the vertical height of the leaf of rank n (Fig. 11B). This reduction of divergence angle between two successive leaves, from 137° in diploids to 135° in triploids, an

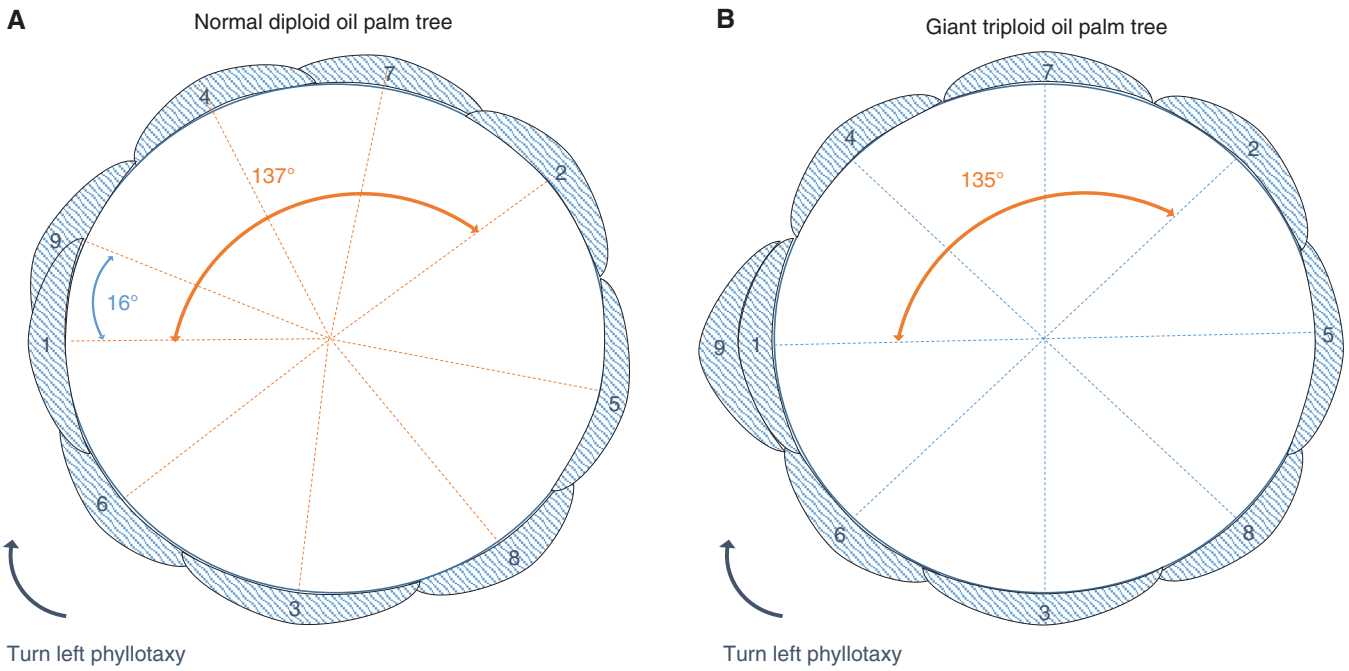


FIG. 11. Schematic comparison of leaf insertion between (A) a normal diploid oil palm tree (from Ochs, 1960) and (B) a giant triploid oil palm tree.

average of 2° per leaf, is sufficient to explain the striking differences in leaf insertion between these two groups of oil palms (see Fig. 1C, D for example).

For the moment, the mechanisms leading to a reduction in the angle of divergence between two successive leaves in triploids remain unclear. One could assume that a change in auxin metabolism in these triploids could influence phyllotatic patterns as polyploidization is known to affect auxin metabolism in plants (Ma *et al.*, 2016). Auxin metabolism in plants is directly involved with the mechanisms linked to phyllotaxy (Kuhlemeier and Reinhardt, 2001; Reinhardt *et al.*, 2003). However, the normal habit of the tetraploid plant with eight parastichies oriented to the left invalidates this hypothesis (Fig. 12).

Conclusion

Spontaneous polyploidization is rare, but seems to be ubiquitous in oil palms, as can be seen in a wide range of progenitors used for seed production. Giant sterile palms are now well identified in Asia, but some observations have also been reported in Latin America and Africa by PalmElit's breeders. It is not a major concern, but these sterile plants contribute to a decline of oil palm productivity in plantations. Our work contributes to a better identification of these abnormal plants so that they can be discarded in the nursery before planting. Indeed, observations in the field have proven that their giant growth patterns are very different from normal diploid palms and they should therefore be easily identified and culled before planting. In a well-managed plantation, by the end of the pre-nursery and main nursery steps, it is usually expected to have 10–15 % and 10–20 % eliminated palms, respectively (Wuidart and Boutin, 1976; Jacquemard, 1998; Rankine and Fairhurst, 1998). Following such stringent standards enables the transfer to the plantation of such abnormal palms to be greatly reduced. Unfortunately, these standards have been lowered in the past years (Serikat Petani Kelapa Sawit, 2016) in an effort to minimize the seed requirements per hectare to lower costs. Moreover, the rapid expansion of oil palm plantations since 2008, especially in Malaysia and Indonesia with an increase of +30 % and +94 %, respectively (Oil World, 2017), has caused a great shortage of well-trained workers. In 2013, this shortage was estimated at 14 % for nursery operators in Malaysia alone (Ismail, 2013). To address these organizational shifts, early detection of off-types in conjunction with routine flow cytometry in the greenhouse could help to eliminate these abnormal plants, leaving only productive plants in the field.

Polyploidy has been widely used in the past to increase yield in the plant kingdom: Simmonds (1980) estimated that about 40 % of all cultivated species were polyploids in 1980. However, every species reacts differently to the phenomenon and, in the case of oil palm, triploidy induces strong fertility dysfunction and thus is not favoured. However, during this study, a tetraploid palm was identified as part of the giant palms. In contrast to triploids, this palm showed stunted growth and canopy size and did not show any sign of sterility. This autopolyploid has been self-fertilized and the resulting progeny have been planted in the field after confirmation of the ploidy level of the plantlets. In this case, polyploidy may be useful for the stunted features it brings; furthermore, polyploidy may positively affect the tolerance to some stresses, such as nutrient deficiency, drought, water deficit, temperature, pests and pathogens (Levin, 2002). In that sense, these tetraploid oil palms may present a favourable possibility for the evolution of the species, especially in the

FIG. 12. Tetraploid oil palm tree in the field.

context of the major climatic changes that our planet will have to face in the future.

ACKNOWLEDGEMENTS

We thank INRAB (Institut National des Recherches Agricoles du Benin) for collecting and sending fresh leaf samples of parent trees. We thank Camille Madec (PalmElit) for the picture of oil palm trees at PT Socfin Indonesia estate.

FUNDING

This work was supported by CIRAD (Centre de coopération Internationale en Recherche Agronomique pour le Développement) and PalmElit SAS.

LITERATURE CITED

Aholoukpè H, Dubos B, Flori A, *et al.* 2013. Estimating aboveground biomass of oil palm: allometric equations for estimating frond biomass. *Forest Ecology and Management* **292**: 122–129.

Beccari O. 1914. *Contributo alla conoscenza della Palma a olio (Elaeis guineensis)*. Firenze: Istituto agricolo coloniale italiano.

Beck MW. 2020. *ggord: ordination plots with ggplot2. R package version 1.1.5*. https://fawda123.github.io/ggord/

Berthaud A. 1993. Les mesures de croissance sur jeune palmier à huile. *Oléagineux* **48**: 429–436.

Camillo J, Leão AP, Alves AA, *et al.* 2014. Reassessment of the genome size in *Elaeis guineensis* and *Elaeis oleifera*, and its interspecific hybrid. *Genomics Insights* **7**: 13–22. doi: 10.4137/GEI.S15522.

Chevalier A. 1910. *Les végétaux utiles de l'Afrique tropicale française. fasc. VII, document sur le palmier à huile*. Paris: Challamel.

Cochard B, Carasco-Lacombe C, Pomiès V, *et al.* 2015. Pedigree-based linkage map in two genetic groups of oil palm. *Tree Genetics and Genomes* **11**: 68. doi: 10.1007/s11295-015-0893-7.

Corley RHV. 2009. How much palm oil do we need? *Environmental Science and Policy* **12**: 134–139.

Corley RHV, Breure CJ. 2008. Fruiting activity, growth and yield of oil palm. I. Effects of fruit removal. *Experimental Agriculture* **28**: 99–109.

Corley RHV, Tinker PB. 2016. *The oil palm*, 5th edn. Chichester, UK: Wiley-Blackwell.

Cuenca J, Froelicher Y, Aleza P, Juarez J, Navarro L, Ollitrault P. 2011. Multilocus half-tetrad analysis and centromere mapping in citrus: evidence of SDR mechanism for 2n megagametophyte production and partial chiasma interference in mandarin cv 'Fortune'. *Heredity* **107**: 462–470. doi: 10.1038/hdy.2011.33.

De Storme N, Geelen D. 2013. Sexual polyploidization in plants – cytological mechanisms and molecular regulation. *New Phytologist* **198**: 670–684. doi: 10.1111/nph.12184.

Doležel J, Bartoš J. 2005. Plant DNA flow cytometry and estimation of nuclear genome size. *Annals of Botany* **95**: 99–110.

Doležel J, Binarová P, Lucretti S. 1989. Analysis of nuclear DNA content in plant cells by flow cytometry. *Biologia Plantarum* **31**: 113–120.

Doležel J, Čížková J, Šimková H, Bartoš J. 2018. One major challenge of sequencing large plant genomes is to know how big they really are. *International Journal of Molecular Sciences* **19**: 3554. doi: 10.3390/ijms19113554.

Dunwell JM, Wilkinson MJ, Nelson S, *et al.* 2010. Production of haploids and doubled haploids in oil palm. *BMC Plant Biology* **10**: 218. doi: 10.1186/1471-2229-10-218.

Fisher RA. 1936. The use of multiple measurements in taxonomic problems. *Annals of Eugenics* **7**: 179–188.

Harlan JR, De Wet JMJ. 1975. On Ö. Winge and a Prayer: the origins of polyploidy. *The Botanical Review* **41**: 361–390.

Hartley CWS. 1967. *The oil palm*. London: Longmans, Green.

Hartley CWS. 1988. *The oil palm (Elaeis guineensis Jacq.)*. Harlow, UK; New York: Longman Scientific & Technical; Wiley.

Henry P. 1955. Note préliminaire sur l'organisation foliaire chez le palmier à huile. *Revue Générale de Botanique* **62**: 127–135.

Ismail A. 2013. The effect of labour shortage in the supply and demand of palm oil in Malaysia. *Oil Palm Industry Economic Journal* **13**: 15–26.

Jacquemard J-C. 1980. Méthode d'observation de la taille des palmiers à huile. *Oléagineux* **35**: 439–442.

Jacquemard J-C. 1998. *Oil palm*. London: Macmillan Education Ltd.

Kuhlemeier C, Reinhardt D. 2001. Auxin and phyllotaxis. *Trends in Plant Science* **6**: 187–189.

Lê S, Josse J, Husson F. 2008. FactoMineR: an R package for multivariate analysis. *Journal of Statistical software* **25**: doi: 10.18637/jss.v025.i01

Legros S, Mialet-Serra I, Clement-Vidal A, *et al.* 2009. Role of transitory carbon reserves during adjustment to climate variability and source–sink imbalances in oil palm (*Elaeis guineensis*). *Tree Physiology* **29**: 1199–1211.

Leitch AR, Leitch IJ. 2008. Genomic plasticity and the diversity of polyploid plants. *Science* **320**: 481–483.
Levin DA. 2002. *The role of chromosomal change in plant evolution*. Oxford: Oxford University Press.
Liesebach H, Ulrich K, Ewald D. 2015. FDR and SDR processes in meiosis and diploid gamete formation in poplars (*Populus* L.) detected by centromere-associated microsatellite markers. *Tree Genetics & Genomes* **11**: 801. doi: 10.1007/s11295-014-0801-6.
Lysák M, Doleželová M, Horry J, Swennen R, Doležel J. 1999. Flow cytometric analysis of nuclear DNA content in Musa. *Theoretical and Applied Genetics* **98**: 1344–1350.
Ma Y, Xue H, Zhang L, *et al.* 2016. Involvement of auxin and brassinosteroid in dwarfism of autotetraploid apple (*Malus* × *domestica*). *Scientific Reports* **6**: 26719. doi: 10.1038/srep26719.
Madon M, Phoon LQ, Clyde MM, Mohd DA. 2008. Application of flow cytometry for estimation of nuclear DNA content in *Elaeis*. *Journal of Oil Palm Research* **20**: 447–452.
Mathews J, Foong L. 2010. Yield and harvesting potentials. *The Planter* **86**: 699–709.
Nasution O, Sitorus AC, Nelson SPC, Forster BP, Caligari PDS. 2013. A high-throughput flow cytometry method for ploidy determination in oil palm. *Journal of Oil Palm Research* **25**: 265–271.
Nelson SPC, Wilkinson MJ, Dunwell JM, *et al.* 2009. Breeding for high productivity lines via haploid technology. In: Proceeding of the 2009 International Palm Oil Congress, Agriculture, Biotechnology and Sustainability. Bangi: Malaysian Palm Oil Board, 203–225.
Ngoot-Chin T, Zulkifli MA, van de Weg E, *et al.* 2021. Detection of ploidy and chromosomal aberrations in commercial oil palm using high-throughput SNP markers. *Planta* **253**: 63. doi: 10.1007/s00425-021-03567-7.
Ochs R. 1960. *Protocole d'une méthode globale d'observations végétatives sur le palmier à huile*. Paris: Institut de recherche pour les huiles et oléagineux.
Oil World. **2017.** Statistics on palm oil. https://www.oilworld.biz/t/statistics/commodities.
Oil World. **2020.** Statistics on palm oil.
Park TH, Kim JB, Hutten RCB, Van Eck HJ, Jacobsen E, Visser RGF. 2007. Genetic positioning of centromeres using half-tetrad analysis in a 4*x*–2*x* cross population in potato. *Genetics* **176**: 85–94. doi: 10.1534/genetics.107.070870.
Pearson K. 1901. On lines and planes of closest fit to systems of points in space. *Philosophical Magazine* **2**: 559–572.
Ramajayam D, Mathur RK, Ravichandran G, Naveen Kumar P, Krishnamurthy H. 2014. Detection of spontaneous haploids in oil palm. *ICAR NEWS* **20**: 1–2.
Ramanna MS, Jacobsen E. 2003. Relevance of sexual polyploidization for crop improvement. *Euphytica* **133**: 3–18.
Ramsey J, Schemske DW. 1998. Pathways, mechanisms and rates of polyploid formation in flowering plants. *Annual Review of Ecology and Systematics* **29**: 467–501. doi:10.1146/annurev.ecolsys.29.1.467.
Rankine IR, Fairhurst TH. 1998. *Field handbook – oil palm series – Vol. 1 Nursery*. Singapore: Potash and Phosphate Institute.
R Core Team. **2020.** *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.
Rees AR. 1964. The apical organization and phyllotaxis of the oil palm. *Annals of Botany* **28**: 57–69. doi:10.1093/oxfordjournals.aob.a083895.
Reinhardt D, Pesce ER, Stieger P, *et al.* 2003. Regulation of phyllotaxis by polar auxin transport. *Nature* **426**: 255–260.
Rival A, Beule T, Barre P, Hamon S, Duval Y, Noirot M. 1997. Comparative flow cytometric estimation of nuclear DNA content in oil palm (*Elaeis guineensis* Jacq) tissue cultures and seed-derived plants. *Plant Cell Reports* **16**: 884–887. doi:10.1007/s002990050339.
Rouiss H, Cuenca J, Navarro L, Ollitrault P, Aleza P. 2017*a*. Unreduced megagametophyte production in lemon occurs via three meiotic mechanisms, predominantly second-division restitution. *Frontiers in Plant Science* **8**: 1211.
Rouiss H, Cuenca J, Navarro L, Ollitrault P, Aleza P. 2017*b*. Tetraploid citrus progenies arising from FDR and SDR unreduced pollen in 4*x* × 2*x* hybridizations. *Tree Genetics and Genomes* **13**: 10–14.
Ruer P, Varéchon C. 1964. Premières observations sur les caractéristiques du petiole de feuille de palmier a huile. *Oléagineux* **8**: 539–542.
Serikat Petani Kelapa Sawit. **2016.** Standar operasional prosedur agronomi untuk petani kelapa sawit. *SOP-AGRO-09/02*. https://spks.or.id/file/publikasi/13__b__SOP_PENGELOLAAN_LINGKUNGAN-Koperasi-BUMP.pdf.
Simmonds NW. 1980. Polyploidy in plant breeding. *SPAN* **23**: 73–75.
Singh R, Ong-Abdullah M, Low ET, *et al.* 2013. Oil palm genome sequence reveals divergence of interfertile species in Old and New worlds. *Nature* **500**: 335–339. doi:10.1038/nature12309.
Smarda P, Bures P, Horova L, Foggi B, Rossi G. 2008. Genome size and GC content evolution of *Festuca*: ancestral expansion and subsequent reduction. *Annals of Botany* **101**: 421–433. doi:10.1093/aob/mcm307.
Srisawat T, Kanchanapoom K, Pattanapanyasat K, Srikul S, Chuthammathat W. 2005. Flow cytometric analysis of oil palm: a preliminary analysis for cultivars and genomic DNA alteration. *Songklanakarin Journal of Science and Technol* **27**: 645–652.
Stebbins CJ. 1950. *Variation and evolution in plants*. New York: Columbia University Press
Stebbins GL. 1971. *Chromosomal evolution in higher plants*. London: Edward Arnold.
Stone M. 1974. Cross-validatory choice and assessment of statistical predictions. *Journal of the Royal Statistical Society, Series B (Methodological)* **36**: 111–133. doi:10.1111/j.2517-6161.1974.tb00994.x.
Tailliez B, Ballo Koffi C. 1992. Une méthode de mesure de la surface foliaire du palmier à huile. *Oléagineux* **47**: 537–545.
Thomas RL, Chan KW, Easau PT. 1969. Phyllotaxis in the oil palm: arrangement of fronds on the trunk of mature palms. *Annals of Botany* **33**: 1001–1008. doi: 10.1093/oxfordjournals.aob.a084328.
Vakarelov II. 2008. Method for practical assessment of cylindrically represented spiral phyllotaxis. *Phytologia Balcanica* **14**: 263–268.
Venables WN, Ripley BD. 2002. *Modern applied statistics with S*, 4th edn. New York: Springer.
Wickham H. 2016. *ggplot2: elegant graphics for data analysis*, 2nd edn. New York: Springer.
Wuidart W, Boutin D. 1976. Palmier à huile. Choix des plants de pépinière. *Oléagineux* **31**: 371–374.