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Natural diversity in the carotene, tocochromanol and fatty acid composition of crude palm oil

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ABSTRACT

Crude palm oil (CPO) is extracted from the mesocarp of oil palm (*Elaeis guineensis)* fruits. CPO is widely consumed in many African countries. Due to its high provitamin A carotenoid content, it is also widely used in programmes designed to prevent vitamin A deficiency. *Elaeis guineensis* occurs naturally across a wide geographical range in Africa. We investigated the carotene, tocochromanol (vitamin E) and fatty acid composition of a large set of genotypes representative of this genetic and geographic diversity. We found considerable intraspecific diversity in most lipid traits. Populations from Côte d'Ivoire were distinguished from other origins by their very low palmitate content and high tocochromanol content. Genotypes from Benin, Côte d'Ivoire and Nigeria were characterized by high carotene contents. Finally, hybrids of crosses between genotypes from Côte d'Ivoire and Nigeria produce CPO with exceptionally high provitamin A and vitamin E contents together with low palmitate content.

1. Introduction

Crude palm oil (CPO), also called red palm oil, is extracted from the mesocarp of *Elaeis guineensis* fruits. CPO has been consumed in Africa for thousands of years (Kay & Kaplan, 2015) and is still widely used in many West African countries (Rafflegeau, Nanda, & Genot, 2018), where it can represent up to 60% of edible oil consumption, as is the case in Cameroon (Rébéna, Rafflegeau, Kanski, Nanda, & Genot, 2019). In countries where CPO is traditionally used for everyday cooking, consumers look for deep colour and high fluidity (Cheyns, Bricas, & Aka, 2004).

The two main pigments responsible for the colour of CPO, α- and β-carotenes, are also provitamin A carotenoids. CPO is one of the richest dietary sources of provitamin A and is thus also used in programmes to prevent long-term vitamin A deficiency (Zagré, Delpeuch, Traissac, & Delisle, 2003; Burri, 2012; Delisle, 2018). Vitamin A deficiency is a public health concern in numerous developing countries. It can affect up to 80% of the population in sub-Saharan countries and 50% in South East Asia (Murphy, 2007; Hamer & Keusch, 2015). Vitamin A deficiency has a plethora of clinical manifestations (night blindness, growth disturbances, and susceptibility to severe infection) and increases the mortality of young children and mothers (Black et al., 2008). Worldwide, more than half a million children under the age of five die every year due to vitamin A deficiency. The identification of oil palm varieties with a high carotene content could thus not only satisfy consumer demand but also help prevent vitamin A deficiency.

A comprehensive literature review revealed that very little is known about intraspecific variability of the carotenoid content of CPO. Most reviews (e.g., Sundram, Sambanthamurthi, & Tan, 2003) provide the same figures for CPO total carotenoid content, i.e. 500–700 ppm, but a closer examination showed that this range of values was based on very few studies. However, a few original studies did show that variations in CPO carotenoid content between oil palm origins can be significantly higher. Among four Nigerian genotypes, carotenoid content was found to vary from 648 to 2 226 ppm (Ames, Raymond, & Ward, 1960). Monde et al. (2009) found a higher carotenoid content (1 532 ppm) in a variety originating from Côte d'Ivoire than in a variety belonging to the Deli group (690 ppm). Finally, a range of 450 to 1 250 ppm was reported

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among 42 progenies collected in Angola (Noh et al., 2002).

Because the specific vitamin activity and bioavailability of the different carotenoids varies considerably (Haskell, 2012), carotenoid composition also warrants attention. In the few original studies available, β-carotene and α-carotene were always the two major carotenoids in CPO. β-carotene, which has twice the vitamin A potential of α-carotene, represented 86% of carotenoids in a variety from Côte d'Ivoire (Monde et al., 2009) but 60% and 72% in two Nigerian varieties (Ikemefuna & Adamson, 1984), suggesting the ratio of β-carotene to α-carotene may also vary among oil palm from different origins in Africa.

CPO is also well known for its high vitamin E content (Schroeder, Becker, & Skibsted, 2006; Teh & Lau, 2021). Tocochromanols are a group of four tocopherols and four tocotrienols that collectively constitute vitamin E, an essential micronutrient in the human diet. A specific feature of CPO is its particularly high concentration of tocotrienols, which may account for 80–95% of total tocochromanols (Monde et al., 2009; Irias-Mata et al., 2017). Tocotrienols are the subject of increasing interest because of their health properties (Trela & Szymanska, 2019; Wallert, Börmel, & Lorkowski, 2021). Because tocochromanols and carotenes protect each other from oxidation during storage or cooking (Schroeder, Becker, & Skibsted, 2006), identifying oil palm origins with high tocochromanol content is not only important as a source of vitamin E but also for provitamin A intake. Again, reviews of CPO tocochromanol contents are based on a limited literature and report different ranges: 500–800 ppm (Sundram, Sambanthamurthi, & Tan, 2003) or 600–1 000 ppm (Hoe, Chan, Ramanan, & Ooi, 2020; Goh, Choo, & Ong, 1985). Original studies are rare but provide a different picture of intraspecific variability of CPO vitamin E content. Luo et al. (2020) recently found values ranging between 172 and 1 288 ppm in a set of 161 oil palm individuals collected in different producer countries. Unfortunately, the African origin of the genotypes they studied was not investigated. Low tocochromanol contents (332 to 525 ppm) were found in three varieties grown in Costa Rica (Irias-Mata et al., 2017), but high vitamin E content (1 124 ppm) was found in the genotype of the Deli group studied by Monde et al. (2009).

Palm oil is known for its high level of palmitic acid (16:0), meaning it has many advantages for the food industry, including high oxidative stability and a high melting point, making it a good alternative to trans fats (hydrogenated oils). The range of 16:0 content usually reported for palm oil is 39–46% (e.g., Sundram, Sambanthamurthi, & Tan, 2003). However, CPO consumers in Africa want their CPO to be fluid at ambient temperature (Cheyns, Bricas, & Aka, 2004), i.e., a CPO with a low 16:0. Little is known about the intraspecific variability of fatty acid composition in *E. guineensis*. However, a few studies reported that oil palms originating from Côte d'Ivoire may have a 16:0 level lower than 39% (Gascon & Wuidart, 1975; Monde et al., 2009; Montoya et al., 2014).

Our knowledge of the variability of CPO carotene, tocochromanol and FA levels linked with the different geographic origins and genetic groups of oil palm thus remains very limited. Oil palm is a native of west and central Africa, from Senegal to Angola along the Atlantic Ocean, and as far as Tanzania in the east. Genetic analyses revealed two major genetic groups within *E. guineensis*: Group I contains collections from Senegal, Guinea, Sierra Leone, and Côte d'Ivoire, while Group II includes all other collections, including those from Benin, Nigeria, Cameroon, Congo, and Angola (Cochard et al., 2009; Bakoumé et al., 2015). A third genetic group, termed Deli, which is of considerable importance for oil palm breeding, contains material introduced from Africa and bred in Indonesia in the middle of the 19th century, but the area of origin of this material in Africa remains unknown (Cochard et al, 2009; Bakoumé et al, 2015). The aim of this study was thus to characterize carotene, tocochromanol and FA composition of CPO in a large set of genotypes representing the three genetic groups of oil palm. We analysed 44 genotypes from Angola, Benin, Cameroon, Congo, Côte d'Ivoire, Nigeria, and the group Deli, and assessed diversity within and between origins in each of the three classes of lipid compounds. Twelve inter-group hybrids were further analysed to test whether new genetic combinations could provide CPO with high provitamin A and vitamin E contents and a low 16:0 level.

2. Material and methods

2.1. Plant material and crude oil extraction in Benin

All the genotypes studied were grown at the Agricultural Research Centre for Perennial Plants (*Centre de Recherches Agricoles Plantes Pérennes,* CRA-PP/INRAB) at Pobè, Benin. Passport data (genotype identifier, country and population of origin, genetic group, planting year) of each genotype are given in Supplementary Table 1. The genetic group of each genotype was determined in a previous study (Cochard et al., 2009). We studied 10 genotypes originating from Côte d'Ivoire belonging to two populations (La Mé, seven genotypes, and Yocoboué, three genotypes), five genotypes from Benin (population Pobè), four genotypes from Nigeria (population Aba-Calabar), five genotypes from Congo (population Yangambi), four genotypes from Angola, belonging to two populations (Cuanza and N'Jala, two genotypes each), and 16 genotypes of the group Deli, divided into two sub-groups (Dabou, 13 genotypes) and (Socfin, three genotypes) (Fig. 1). The 12 inter-group hybrids investigated were chosen because at least one of their two parents did not belong to any of the nine populations (including the two Deli sub-groups) in the CRA-PP field genebank (Fig. 1). For each genotype, the objective was to extract and analyse CPO in triplicate from three independent bunches, i.e., collected on three different dates in the period 2017–2019. Each genotype corresponded to single palm tree in the CRA-PP field genebank. In the end, four bunches were harvested and analysed in six genotypes, three bunches in 47 genotypes and only two bunches in three genotypes (Supplementary Table 1). Forty well-developed undamaged fruits were removed from spikelets collected in

Fig. 1. Origin of the oil palm genotypes studied here. The natural range of *E. guineensis* corresponds to the area hatched in blue. The populations collected and the number of genotypes per population studied are given in the green boxes. The size of the two subgroups of the Deli group, whose exact African origin is unknown, is given in the grey box. Hybrids between populations of different countries are indicated in the blue boxes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the centre of each bunch. The fruits were immediately immersed in boiling water for 20 min or until a final temperature of 75–80 °C was reached in the centre of three fruits per bunch measured with a Stuart SCT1 probe thermometer (Cole-Parmer, Stone, UK). After heating, fruits were left to cool to 60 °C (internal mesocarp temperature) and then pressed using a manual 1.2 L screw-press. The extracted crude oil was stirred while still warm and fluid, and 10 mL were sampled and immediately frozen at −20 °C. At this stage of the process, crude oil still contains water and impurities. Unpurified crude oil samples were stored at −20 °C at Pobè for maximum two months.

2.2. CPO purification

Unpurified crude oil samples were sent to Montpellier, France, by plane and kept cold during transport using eutectic plates. Prior to lipid extraction, after 15 min in a 40 °C water-bath (Firlabo, Meyzieu, France) to melt all the triacylglycerols, the samples were homogenised. Total lipids were extracted from the samples of crude oil using a modified Folch method as described previously (Laffargue, de Kochko, & Dussert, 2007). Briefly, 3 mL of crude oil was homogenised for 30 s in 20 mL of methylene chloride/methanol (2/1) (analytical grade, Merck, Darmstadt, Germany) using a IKA T25 Ultra Turrax homogeniser (Staufen, Germany) prior to filtration (pore size 5) into a glass filter cup. The filtrate was transferred in a 100 mL glass separatory funnel and washed with 5 mL of 0.73% NaCl solution by vigorous hand shaking. After the resulting mixture separated into two phases, the lower phase was recovered and divided into nine aliquots, i.e. three aliquots for each of the three classes of compounds to be analysed (carotenoids, tocochromanols and fatty acids).

2.3. Carotenoid analysis

Carotenoid extraction was adapted from the method described previously (Montúfar et al., 2010), which includes a preliminary triacylglycerol (TAG) removal step. Briefly, CPO samples (15 mg) dried under nitrogen (≥99.999%, Linde, Lyon, France), using an OASys heating system (Organomation, Berlin, MA, USA) were carefully mixed with 10 mL of acetone (analytical grade, Merck) and left for 60 min at −20 °C, leading to TAG crystallisation. After centrifugation (Eppendorf 5810R, Hamburg, Germany) of frozen samples at 900 *g* and −9 °C for 20 min, TAG were separated by rapid sampling of the upper acetone phase, which contained the carotenoids, and filtered through a 0.2 μm Minisart SRP4 PTFE filter (Sartorius, Göttingen, Germany). The 20-μL carotenoid extract was directly injected into the Agilent Infinity II HPLC system (Santa Clara, CA, USA). The column was a polymeric YMC-30 (300 × 4.6 mm, YMC, Kyoto, Japan). Elution was performed at a flow rate of 1 mL/min as follows: (i) 100% A for 1 min, (ii) a linear gradient from 100% A to 10% A and 90% B for 40 min, (iii) a linear gradient to 100% B for 5 min, (iv) a 5-min step with 100% B, and (v) a return to initial conditions for 5 min (A = methanol:water [60:40, v/v], B = methanol: methyl *tert*-butyl ether:water [28.5:67.5:4, v/v/v]). A 15-min rinse step was then carried out using 100% ethyl acetate, followed by re-equilibration with 100% A for 10 min. All solvents were of HPLC grade and were purchased from Merck. The column temperature was maintained at 25 °C. An Agilent G7115A UV–visible photodiode array detector was used to analyse the chromatograms at a detection wavelength of 450 nm. Carotenoids were identified by the combined use of their relative retention times and their wavelength absorption maxima determined using analytical standards (Merck). All analyses were performed in triplicate from three independent carotenoid extractions.

2.4. Tocochromanol analysis

Analysis of tocochromanols was adapted from the method described previously (Montúfar et al., 2010). CPO samples (15 mg) dried under nitrogen were carefully dissolved in 500 μL of *n*-hexane and 30 μL aliquots of the solution were injected into the Agilent Infinity II HPLC system, which was equipped with an Agilent G7121B fluorescence detector set at an excitation wavelength of 290 nm and an emission wavelength of 330 nm. The HPLC column was a Merck Lichrospher Si-60 (250 × 4.6 mm internal diameter, 5 μm particle size). The mobile phase was *n*-hexane/propan-2-ol (99:1, v/v) at a flow rate of 1 mL/min. All the solvents were of HPLC grade and were purchased from Merck. Standard curves were run with commercial standards of α-, β-, γ- and δ-tocopherols (Tocopherol set, Calbiochem, Merck) and α-, β-, γ- and δ-tocotrienols (Cayman Chemical, Ann Harbor, MI, USA). All analyses were performed in triplicate from three independent CPO dissolutions in *n*-hexane.

2.5. Fatty acid analysis

Fatty acid methyl esters (FAME) were prepared according to the ISO-5509 standard from 50 mg CPO samples and analysed using gas chromatography to determine FA composition (Laffargue, de Kochko, & Dussert, 2007). Analyses were performed using an Agilent 7820A Gas Chromatography system with flame ionization detection. A Famewax capillary column (Restek, Lisses, France), (30 m × 0.25 mm × 0.25 m) was used. Analyses were conducted from 185 to 225 °C at 4 °C/min and then kept at 225 °C for 10 min. The carrier gas was helium at 40 cm/s. Both injector and detector were maintained at a temperature of 230 °C. Only peaks identified in the chromatograms with a relative percentage > 0.1% were retained. All fatty acid analyses were performed in triplicate from three independent FAME reactions.

2.6. Statistical analyses

Genotype means were calculated using two to four biological replicates (two to four bunches collected from the same palm tree on different dates) and three technical replicates per biological replicate. Linear regression and one-way ANOVA were performed using Statistica (Statsoft, Tulsa, USA). Linear regression was used to test the relationships between carotene content, tocochromanol content, the β-carotene to α-carotene ratio, and the percentages of tocotrienols, linoleic acid and α- and β-tocochromanol forms. ANOVA was used to test the effect of the country of origin and the population on all chemical traits measured. When the effect of the country of origin or the population was significant, Duncan's post-hoc multiple comparison test was performed using Statistica. The accuracy of the classifications based on carotene, tocochromanol and fatty acid compositions was also tested using discriminant analysis (DA) and canonical analysis (CA). DA and CA were performed using the following active variables: total carotene content, β-carotene to α-carotene ratio, total tocochromanol content, the percentage of tocotrienols, VTE3 and VTE4 activities, and the percentages of 14:0, 16:0, 16:1, 18:0, 18:1*n*-7, 18:2, 18:3, 20:0 and 20:1*n*-9. The population or the country of origin was the grouping variable. DA and CA were performed using Statistica.

3. Results and discussion

3.1. Intraspecific variability of carotene, tocochromanol and fatty acid composition of crude palm oil

Total carotene content varied considerably in the 44 genotypes chosen to represent the geographic and genetic diversity of oil palm: from 77 ppm in the genotype Yang5 to 1 275 ppm in the genotype Pobe3 (Fig. 2a; Supplementary Table 2). The CPO carotene content of 16 out of 44 genotypes was above the limit of the range usually reported in the literature (500–700 ppm), thus supporting the possible identification of high provitamin A genotypes, as previously reported by Monde et al. (2009) and Noh et al. (2002). More unexpectedly, we found that more than half the genotypes studied contained<500 ppm of carotenes. In five genotypes, CPO carotene content was even<200 ppm. We therefore

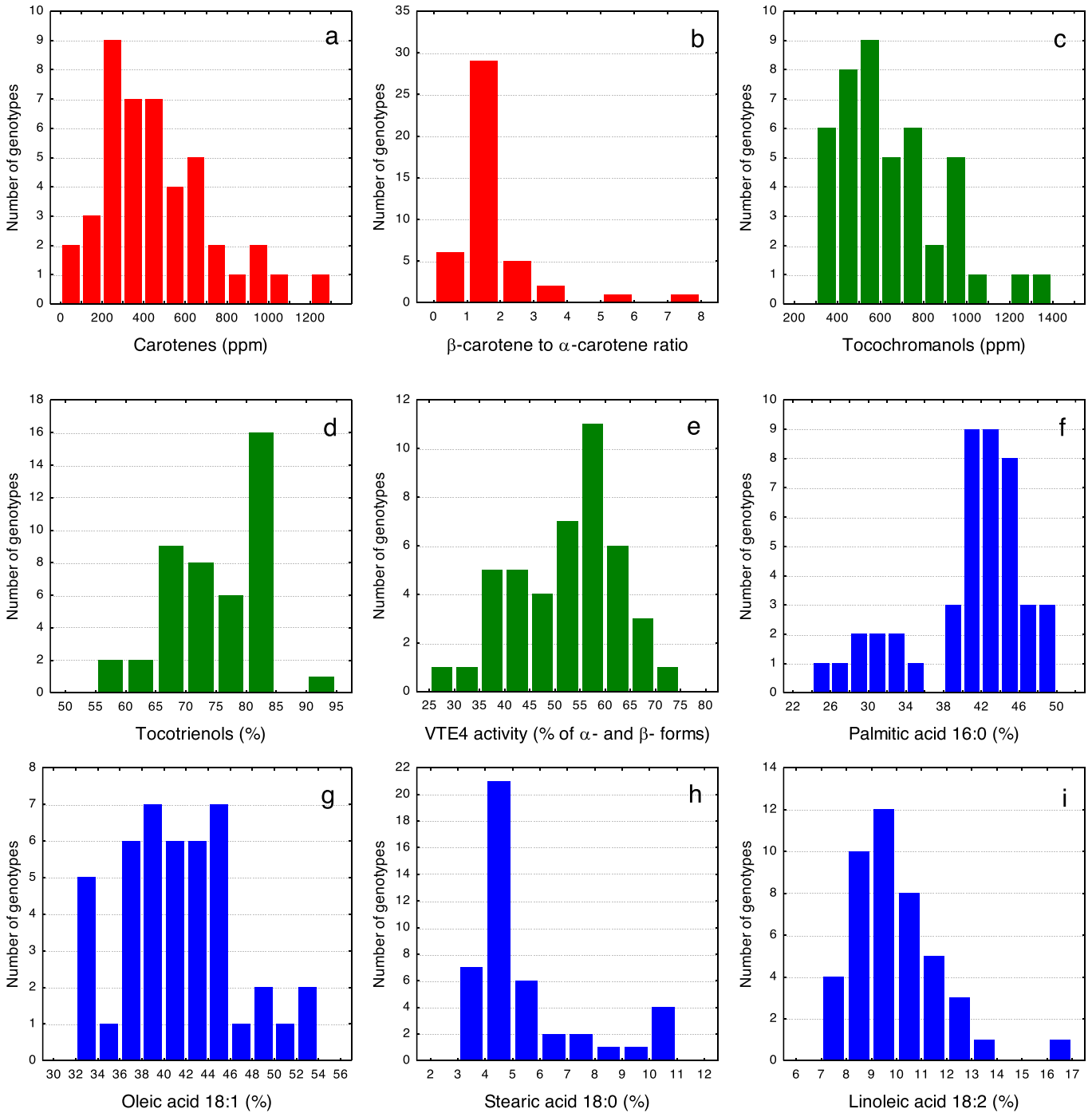

Fig. 2. Distribution of CPO (a) carotene content, (b) β-carotene to α-carotene ratio, (c) tocochromanol content, (d) tocotrienol percentage (e) VTE4 activity, and (f) 16:0, (g) 18:1, (h) 18:0 and (i) 18:2 levels in the 44 genotypes chosen to represent the geographic and genetic diversity of oil palm.

advise caution concerning the origin of the CPO when calculating recommended CPO intakes for use in programmes to prevent vitamin A deficiency (Burri, 2012).

In all the genotypes studied here, α-carotene and β-carotene were the only two major carotenoids. None of the other minor carotenoids (<0.5%) reported in the literature (Sundram, Sambanthamurthi, & Tan, 2003) was identified as a major compound in our large sample set, suggesting that the intraspecific diversity in the different types of carotenoids accumulated in the mesocarp of oil palm fruits is very restricted. In contrast, although the majority of genotypes had the β-carotene to α-carotene ratio usually given for CPO, i.e., between 1.5 and 2, the ratio varied considerably among the 44 genotypes studied (Fig. 2b). In the genotype Pobe5, β-carotene content was 7 times higher than that of α-carotene (518 vs 75 ppm, Supplementary Table 2). Such a high ratio was also observed in one of the two origins studied by Monde et al. (2009). Conversely, in the genotype AbaC1, α-carotene represented 60% of the CPO carotenoids. Considering that the vitamin A equivalence of α-carotene is half that of β-carotene (Haskell, 2012), the choice of the source of CPO also appears to be crucial regarding these characteristics. Finally, it is worth noting that the β-carotene to α-carotene ratio was not correlated with the total carotene content of the CPO (Fig. 3).

Total tocochromanol content also varied considerably within the panel of genotypes investigated (Fig. 2c; Supplementary Table 3), from 310 to 1 362 ppm, a very similar range to that recently found by Luo

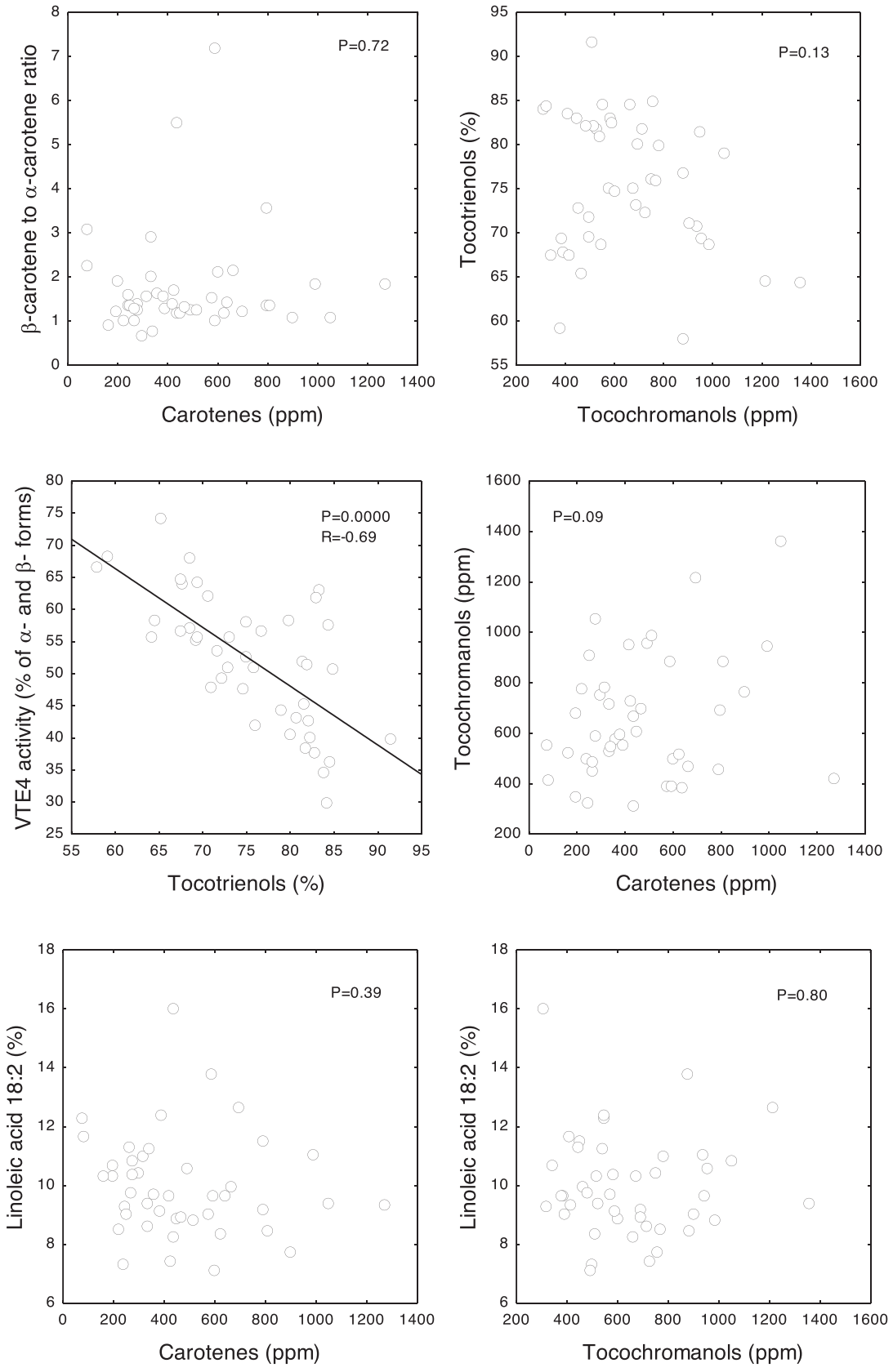

Fig. 3. Relationships between the different components of the carotene, tocochromanol and fatty acid composition of CPO in the 44 genotypes representative of the geographic and genetic diversity of oil palm.

et al. (2020) in a very large set of oil palm individuals characterised to identify the molecular determinants of CPO vitamin E content. Taken together, the results of our study and those of Luo et al. (2020) make it possible to propose that the range for the tocochromanol content of CPO is closer to 200–1 400 ppm than to 500–800 ppm (Sundram, Sambanthamurthi, & Tan, 2003) or 600–1 000 ppm (Hoe, Chan, Ramanan, & Ooi, 2020; Goh, Choo, & Ong, 1985). We also observed very high variability of the proportion of tocotrienols in the tocochromanols accumulated in the oil palm mesocarp (Fig. 2d). The CPO of four genotypes contained a substantial proportion (35–45%) of tocopherols, presenting a different picture of CPO tocochromanol composition from that previously reported, in which tocotrienols largely prevailed (>80%; Monde et al., 2009; Peh, Tan, Liao, & Wong, 2016; Irias-Mata et al., 2017). Given the pharmacological differences between tocopherols and tocotrienols (Peh, Tan, Liao, & Wong, 2016; Trela & Szymanska, 2019; Wallert, Börmel, & Lorkowski, 2021), the present work provides a valuable basis for the identification of genotypes producing tocopherol- or tocotrienol-rich CPO. Moreover, we found no correlation between the tocochromanol content of CPO and the percentage of tocotrienols (Fig. 3), suggesting that these two traits are controlled by distinct genetic determinants.

Using the relative percentages of the different tocochromanols, it is possible to estimate the activities of the enzyme VTE3 (2-methyl-6-phytyl-1,4-hydroquinone methyltransferase), which regulates competition between the biosynthetic branch of γ- and α-tocochromanols and that of δ- and β-tocochromanols, and the enzyme VTE4 (tocopherol O-methyltransferase), which converts γ- and δ-tocochromanols into α- and β- tocochromanols, respectively (Hunter & Cahoon, 2007; Munoz & Munné-Bosch, 2019; Supplementary Fig. 1). VTE3 activity was always very high (>88%) in the panel of genotypes studied here (in other words, α- and γ-tocochromanols were always the major forms of vitamin E; Supplementary Fig. 2). By contrast, VTE4 activity varied considerably among genotypes, ranging from 29% in genotype AbaC4 to 74% in genotype Pobe4 (Supplementary Table 3). As the bioavailability and antioxidant activity of α- and γ-tocochromanols differ significantly (Peh, Tan, Liao, & Wong, 2016), the data presented here may be very important in nutritional considerations of CPO intake. However, a highly significant negative correlation was found between the percentage of tocotrienols and VTE4 activity (Fig. 3), suggesting that in *E. guineensis*, the enzyme VTE4 converts γ-tocopherol into α-tocopherol more efficiently than γ-tocotrienol into α-tocotrienol.

Because the tocochromanol and carotene biosynthesis pathways share the same isoprenoid building blocks, they may be in competition (Asensi-Fabado & Munné-Bosch, 2010). No significant correlation was found between total tocochromanol content and total carotene content in our panel of 44 genotypes (Fig. 3), suggesting that there is no competition for geranylgeranyl-PP, the branch-point between the two pathways (Supplementary Fig. 1). This result also suggests that the activation of the upstream pathway, which produces geranylgeranyl-PP in genotypes that accumulate large amounts of either carotenes or tocochromanols, does not necessarily influence the two downstream pathways. However, this analysis also revealed that several genotypes had high concentrations of both carotenes and tocochromanols, suggesting it is possible to select varieties with high provitamin A and vitamin E CPO.

Palmitic (16:0) and oleic (18:1n-9) acids were the major FA in all genotypes (Fig. 2f and 2 g; Supplementary Table 4), followed by stearic (18:0) and linoleic (18:2) acids (Fig. 2h and 2i). Other minor FA (<1%), including vaccenic (18:1n-7), linolenic (18:3) and arachidic (20:0) acids, were also detected (Supplementary Table 4). Considerable intraspecific variation was found in the levels of 16:0 and 18:1 (Fig. 2f and 2 g), higher than that reported previously (Gascon & Wuidart, 1975; Montoya et al., 2014). Indeed, although a few studies previously pointed to a low 16:0 level in some oil palm varieties, e.g. 31% in Monde et al. (2009), we did not expect to identify genotypes with 25–26% of 16:0. Percentages of 16:0 and 18:1 were highly (R = 0.92) negatively correlated (Supplementary Fig. 3), as previously reported (Montoya et al., 2014). Moreover, the percentage of 18:0 was also negatively correlated with that of 16:0 (Supplementary Fig. 3). This finding may have consequences for the melting temperature of low 16:0 CPO, since 18:0 also increases the melting point of triacylglycerols. In palm oil, 18:0 is mostly incorporated in PSO triacylglycerols (P = 16:0, S = 18:0, O = 18:1; Chen et al., 2007) and PSO has a melting point as high as that of PPO (Berry, 2009). The 18:2 level was not correlated with that of any of the other FA, in agreement with the distinct pathways involved in the biosynthesis of 16:0, 18:0 and 18:1 in the plastid on one hand, and of 18:2 in the endoplasmic reticulum on the other (Dussert et al., 2013).

Because tocochromanols and carotenoids are strong lipid antioxidants, they are believed to be accumulated by plants in seeds and fruits to protect polyunsaturated FA against peroxidation. For instance, a significant relationship was found between the α-tocopherol content and the percentage of 18:2 in 14 oil crops (Kamal-Eldin & Andersson, 1997). Within our representative sample of oil palm diversity, neither total carotene content nor tocochromanol content was significantly correlated with the percentage of 18:2 (Fig. 3).

3.2. Differences in crude palm oil composition among oil palm origins

Significant effects of the country of origin (P = 0.0067) and of the population of origin (P = 0.0092) on the total carotene content of CPO were observed (Fig. 4), although within-country and within-population variability was high in some origins: e.g., 245–992 ppm in Aba-Calabar (Nigeria). Two populations contained only genotypes with high (>500 ppm) carotene content, Yocoboué in Côte d'Ivoire and Pobè in Benin (Fig. 4). However, the populations La Mé (Côte d'Ivoire), Aba-Calabar (Nigeria) and Deli Dabou also contained genotypes that produced a CPO rich in carotenes and were thus categorised at an intermediate level according to the Duncan test. Our results are consistent with those of the few previous studies which compared a limited number of origins. CPO from some Nigerian genotypes had high carotene contents (Ames, Raymond, & Ward, 1960) and the variety from Côte d'Ivoire analysed by Monde et al. (2009) had a higher carotene content than the variety in the Deli group. No significant differences between the different groups were observed in the β-carotene to α-carotene ratio (Supplementary Fig. 4).

Although significant (P = 0.04), the effects of the country and of the population of origin on the CPO tocochromanol content were lower than those found for carotene content (Fig. 4). However, the two populations from Côte d'Ivoire, La Mé and Yocoboué, were again distinguishable because they contained genotypes with very high tocochromanol contents (ca. 1 000 ppm). The Deli Dabou group was classified in an intermediate position, with several genotypes with high tocochromanol contents (>800 ppm), in agreement with the high level found in the Deli genotype investigated by Monde et al. (2009). The dispersion of the percentage of tocotrienols was high in some populations, including in La Mé (Côte d'Ivoire) and Yangambi (Congo) (Fig. 5). However, the effect of the country of origin was highly significant (P = 0.0062) and the chance of identifying varieties with a substantial percentage of tocopherols appears to be greater in Côte d'Ivoire and Benin, while the Deli group showed homogeneously high CPO tocotrienol levels.

The most spectacular difference between the origins studied was in 16:0 and 18:1 contents (P = 0.0000 for both country and population effects; Fig. 5 and Supplementary Fig. 5). The population La Mé from Côte d'Ivoire was clearly separated from all the others with a 16:0 content < 35% in all genotypes (Fig. 5). This finding is in agreement with previous observations by Monde et al. (2009) and Gascon and Wuidart (1975). However, it is worth noting that only one genotype from Yocoboué, the other population from Côte d'Ivoire, had a low palmitate content, highlighting the specificity of the CPO FA composition in the La Mé population.

Discriminant analysis and canonical analysis were also used to assess the accuracy of classification based on carotene, tocochromanol and fatty acid composition. Highly significant classifications were obtained

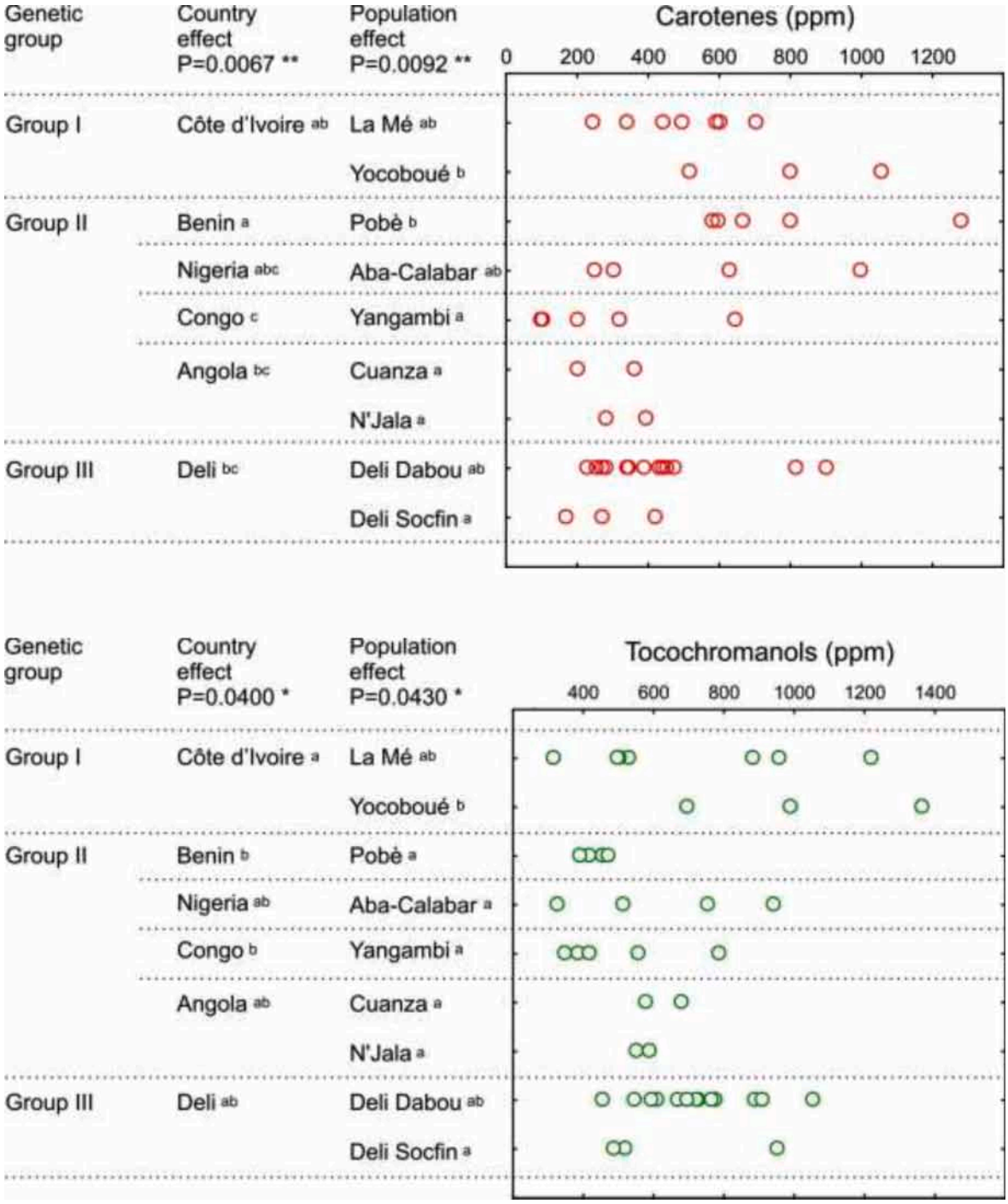

Fig. 4. Effects of the country and the population of origin on CPO carotene and tocochromanol contents. Countries or populations followed by the same letter do not differ significantly according to Duncan's post hoc test.

when either the population or the country of origin was used as the grouping variable, as estimated by P values (<0.0000) associated with Wilks' lambda coefficients (0.00037 and 0.00250, respectively). Percentages of correct classifications were very satisfactory with both grouping variables (Supplementary Fig. 6). Among the 44 palm genotypes analysed, only two were misclassified in both analyses. The CF1-CF2 score plots obtained by canonical analysis of CPO composition clearly separated countries or populations from one another (Supplementary Fig. 6).

On the basis of the carotene, tocochromanol and FA composition of CPO, the material from Côte d'Ivoire was distinguished from the other oil palm origins among the germplasm we studied. Using microsatellite genetic markers, Cochard et al. (2009) first showed that populations from Côte d'Ivoire form a distinct genetic group. Their genetic divergence likely results from a major arid phase in the Dahomey gap to the west of Benin between 20,000 and 14,000 BP. Our data suggest that this genetic separation of West African populations also led to changes in the composition of the oil stored in the fruits of *E. guineensis*. For this reason, it would be interesting to explore the CPO composition of other group I origins, such as populations from Senegal or Guinea. Based on our recent study of fruit lipid composition in 144 palm species, we hypothesise that these changes reflect genetic drift rather than adaptation (Guerin et al., 2020). However, one cannot exclude a role for carotenes as pigments to attract dispersers, assuming that the fauna differed on the two sides of

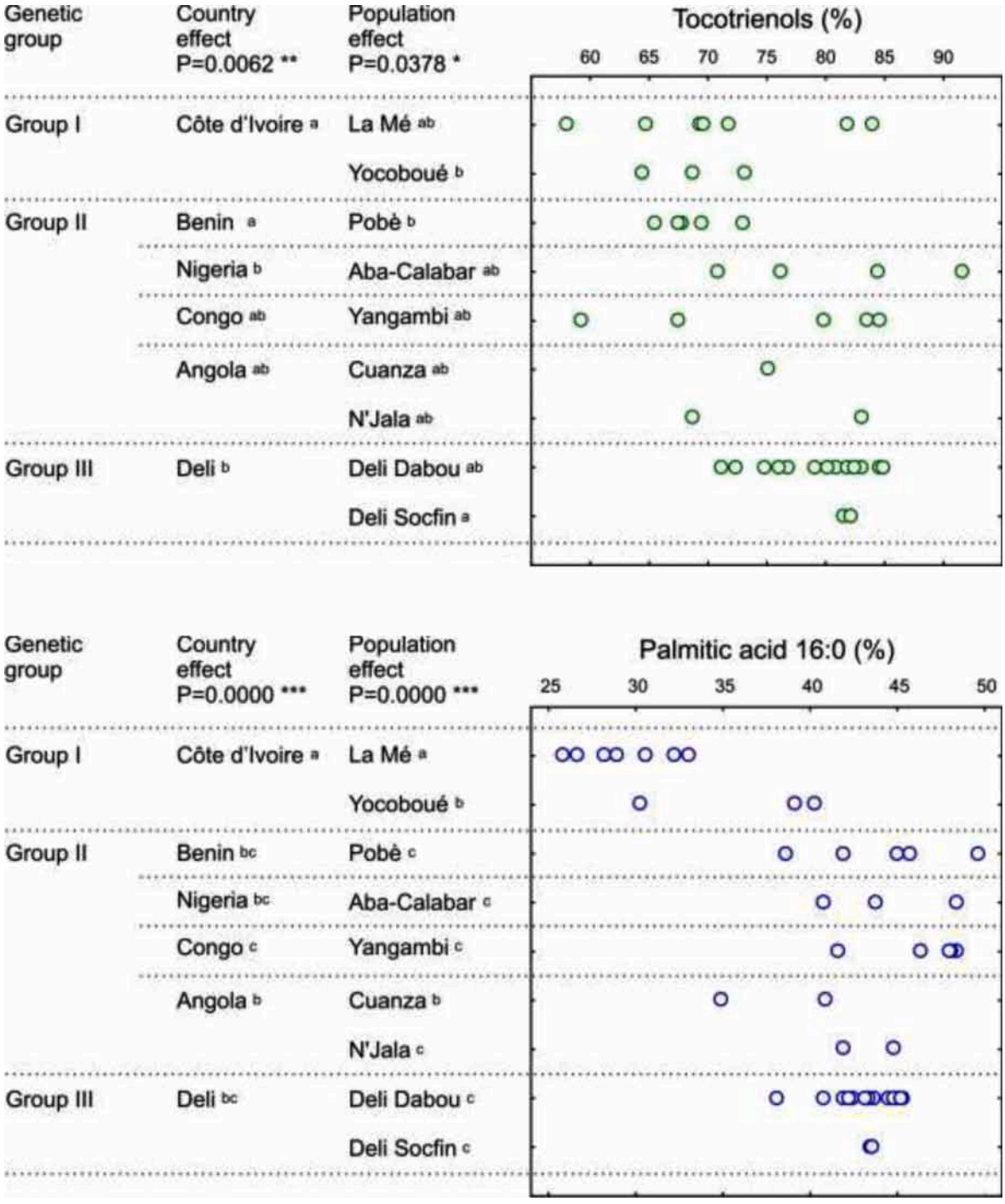

Fig. 5. Effects of the country and the population of origin on CPO tocotrienol and 16:0 percentages. Groups, countries, or populations followed by the same letter do not differ significantly according to Duncan's post hoc test.

the Dahomey gap. Genetic studies also provide evidence that the Deli group (group III) derived from group II, which encompasses Benin, Nigeria, Congo and Angola (Cochard et al., 2009; Bakoumé et al., 2015). The composition of CPO fits this hypothesis, since no major chemical differences were identified that distinguished the Deli genotypes from those from these four countries.

3.3. Identifying genotypes that satisfy CPO consumers and may help in programmes to fight vitamin deficiency

Cheyns, Bricas, and Aka (2004) reported that CPO consumers in Côte d'Ivoire prefer fruits harvested in native palm groves that produce fluid and deep coloured CPO to fruits produced by modern varieties. This traditional usage was confirmed by our data, which showed that genotypes from Côte d'Ivoire had low 16:0 and high carotene contents (Figs. 4 and 5). This prompted us to investigate whether some origins combine high levels of provitamin A and vitamin E and a low level of 16:0. We sorted the 44 genotypes according to decreasing carotene contents and retained those with a 16:0 content of<35% (Table 1). As expected, eight of the nine genotypes that satisfied the 16:0 threshold used were from Côte d'Ivoire. Among these eight genotypes, two genotypes from La Mé and one from Yocoboué had both carotene and

Table 1
CPO composition of the 12 genotypes with a palmitate level below 35%. α-C and β-C: α-carotene and β-carotene. Tocols: tocochromanols. PRO: population of restricted origin.

Type	Genotype	Carotenes (ppm)	β-C to α-C ratio	Tocols (ppm)	Tocotrienols (%)	VTE4 activity (%)	16:0 (%)	18:0 (%)	18:1 (%)	18:2 (%)
Hybrid	LameXNige1	1564	1.5	1330	70.2	58.5	32.3	6.1	49.0	10.7
Hybrid	LameXNige2	1172	1.7	756	66.4	55.9	33.5	6.5	48.4	9.6
PRO	Lame1	698	1.2	1214	64.6	58.2	32.1	7.5	45.6	12.6
PRO	Lame7	601	2.1	495	69.5	55.6	28.8	10.4	51.9	7.1
PRO	Lame4	590	1.0	881	57.9	66.5	30.5	8.4	45.7	13.8
PRO	Yoco3	515	1.2	985	68.6	57.0	30.2	10.2	49.1	8.8
PRO	Lame2	493	1.3	956	69.3	55.2	26.6	7.6	53.5	10.6
PRO	Lame5	439	5.5	310	83.9	34.5	28.1	9.6	44.8	16.0
PRO	Lame3	338	2.9	526	81.7	45.2	25.7	10.9	52.3	9.4
PRO	Lame6	243	1.4	498	71.7	53.6	33.0	10.3	47.8	7.3
Hybrid	LameXSibi1	207	0.5	486	79.3	49.0	32.2	7.4	47.4	11.3
PRO	Cuan1	199	1.9	678	75.1	52.4	34.8	4.2	48.6	10.3

tocochromanol contents higher than 500 ppm. Therefore, oil palm populations from Côte d'Ivoire represent a valuable reservoir to identify varieties that satisfy both traditional CPO demand for cooking (Cheyns, Bricas, & Aka, 2004; Rafflegeau, Nanda, & Genot, 2018; Rébéna, Rafflegeau, Kanski, Nanda, & Genot, 2019) and the requirements of action programmes against vitamin A deficiency (Zagré, Delpeuch, Traissac, & Delisle, 2003; Burri, 2012), combined with increasing vitamin E intake. For instance, the genotype Lame1 showed ca. 700 ppm carotenes, 1 200 ppm tocochromanols and a total saturated FA percentage lower than 40%.

However, none of genotypes from populations of restricted origin combined the extreme values found in each of the three classes of molecules: $>$ 1 000 ppm for both carotenes and tocochromanols and $<$ 35% for 16:0. Consequently, we further investigated 12 hybrids maintained in the CRA-PP field genebank at Pobè, Benin (Supplementary Tables 2–4). The hybrids were chosen because one of their two parents originated from populations that were not represented in the genebank (Fig. 1): Ekona, Mayang and Widikum in Cameroon, Eala and Sibiti in Congo, and a population from Nigeria, whose exact location remains uncertain (and is therefore simply called 'Nigeria'). Two of the 12 hybrids had particularly high carotene contents together with a low level of palmitate (Table 1). They both originated from a cross between individuals from the populations La Mé and Nigeria. Moreover, the hybrid LamexNige1 had the highest carotene content (1 564 ppm) among the 56 genotypes evaluated, exceptionally high vitamin E content (1 330 ppm) and low total saturated FA (38.4%), since it combined low 16:0 (32.3%) and low 18:0 (6.1%) levels. Crosses between La Mé and Nigeria origins thus offer the opportunity to both meet CPO consumer requirements and have very high vitamin contents.

4. Conclusions

Given the major economic importance of oil palm, information on the natural diversity and nutritional potential reported in this study should encourage the oil palm community to continue efforts to characterise CPO composition in the other major oil palm gene banks around the world, including that maintained in Malaysia (Bakoumé et al., 2015). To date, breeding efforts in oil palm have been almost entirely focused on yield and disease resistance (Murphy, 2007). Although successful in terms of these traits, they may have led to a loss in oil fluidity and in concentrations of provitamin A carotenoids in modern varieties. Our investigations showed that crosses between selected origins can combine several key nutritional properties of CPO, not only with respect to vitamins, but also its saturated FA level, whose role in health is frequently questioned and could improve consumer acceptance in Western countries (Murphy, 2007). It is also worth noting that the selection of oil palm varieties combining low palmitate and high provitamin A and vitamin E contents would facilitate both the production of red palm olein and the extraction of CPO phytonutrients (Hoe, Chan, Ramanan, & Ooi, 2020).

CRediT authorship contribution statement

Fabienne Morcillo: Conceptualization, Funding acquisition, Methodology, Supervision, Writing - review & editing. **Virginie Vaissayre:** Methodology, Investigation. **Julien Serret:** Investigation. **Sylvie Avallone:** Methodology, Investigation. **Hubert Domonhédo:** Resources. **Florence Jacob:** Conceptualization. **Stéphane Dussert:** Conceptualization, Funding acquisition, Methodology, Supervision, Writing - original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2021.130638.

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