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## Research Article

# Shedding light on the existence of Furan fatty acids in latex lipids across a wide diversity of *Hevea brasiliensis* genotypes



Chaisiri Kitpaosong<sup>a,b,c,d</sup>, Siriluck Liengprayoon<sup>b</sup>, Erwann Durand<sup>e,f</sup>, Tucksin Lerksamran<sup>b</sup>, Aurélie Daval<sup>g,h</sup>, Eric Gohet<sup>i,j,k</sup>, Lim Khan Tiva<sup>k</sup>, Phean Chetha<sup>k</sup>, Kong Mengchheang<sup>k</sup>, Phen Phearun<sup>k</sup>, Ung Rattana<sup>k</sup>, Kim Chandyy<sup>k</sup>, Pol Sokea<sup>k</sup>, Un Kim Eng<sup>k</sup>, Kittipong Rattanaporn<sup>a</sup>, Bruno Barea<sup>e,f</sup>, Laurent Vaysse<sup>c,d,\*</sup>

<sup>a</sup> Department of Biotechnology, Faculty of Agro-Industry, Kasetsart University, Bangkok 10900, Thailand

<sup>b</sup> Kasetsart Agricultural and Agro-industrial Product Improvement Institute (KAPI), Kasetsart University, Bangkok 10900, Thailand

<sup>c</sup> CIRAD, UPR BioWooEB, Montpellier F-34398, France

<sup>d</sup> BioWooEB, CIRAD, Université de Montpellier, Montpellier F-34398, France

<sup>e</sup> CIRAD, UMR Qualisud, Montpellier F-34398, France

<sup>f</sup> Qualisud, Avignon Université, CIRAD, Institut Agro, IRD, Université de Montpellier, Université La Réunion, Montpellier F-34398, France

<sup>g</sup> CIRAD, UMR AGAP Institut, Montpellier F-34398, France

<sup>h</sup> AGAP Institut, CIRAD, INRAE, Institut Agro, Université de Montpellier, Montpellier F-34398, France

<sup>i</sup> CIRAD, UMR ABSys, Phnom Penh 1337, Cambodia

<sup>j</sup> ABSys, CIHEAM, CIRAD, INRAE, Institut Agro, Université de Montpellier, Montpellier F-34398, France

<sup>k</sup> Cambodian Rubber Research Institute (CRRRI), Phnom Penh 1337, Cambodia

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## ABSTRACT

Furan fatty acids produced by plants and bacteria, and present in some edible resources, have attracted significant scientific attention for their health benefits. They include 10,13-epoxy-11-methyl-octadecan-10,12-dienoic acid, which has been identified in the lipid fraction of latex from two *Hevea brasiliensis* genotypes commonly known as the source of natural rubber. Those two genotypes, namely RRIM501 and PB235, are from Rubber Research Institute of Malaysia (RRIM) and Prang Besar, Malaysia (PB), respectively. This research aimed to undertake the first ever investigation into the existence of this potential high value-added co-product in the lipid fraction of 48 *Hevea brasiliensis* genotypes, seeking to study the widest possible clonal variability. The results showed furan fatty acid exists in all lipid fractions of their latices. Its content varied significantly, ranging from 0.01% to 0.71% (w/w in latex), the highest concentrations were found in genotypes from the Institut de Recherche sur le Caoutchouc (IRCA) in Côte d'Ivoire, Prang Besar (PB) in Malaysia, and Rubber Research Institute of Vietnam (RRIV) in Vietnam breeding programs. A positive correlation with total fatty acid content was observed when its content exceeded 0.10%, suggesting an additive rather than a substitutive role with the other fatty acids present. Interestingly, linoleic and palmitoleic acids strongly correlated with the furan fatty acid concentration, indicating a possible biosynthetic pathway linkage. In terms of yield per tapping PB235, RRIV4, RRIV2, IRCA41, IRCA18, PB324, IRCA814, IRCA323, and IRCA109 genotypes showed the highest production potential, with yields range of 1 367–2 446 mg furan fatty acid per tree per tapping.

\* Corresponding author.

E-mail address: [laurent.vaysse@cirad.fr](mailto:laurent.vaysse@cirad.fr) (L. Vaysse).

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Notably, the biochemical markers of natural rubber productivity (sucrose, inorganic phosphorus, thiols, and total solid content) showed no direct involvement in furan fatty acid biosynthesis during latex regeneration between tappings. Based on knowledge of the parentage of the studied clones, a trait heritability study was conducted and genotype PB5/51 was identified as a very worthwhile genitor for improving furan fatty acid contents in a breeding population.

## 1. Introduction

Natural rubber (NR), primarily comprising the *cis*-1,4-polyisoprene polymer, is obtained from the latex of the rubber tree (*Hevea brasiliensis*) through bark tapping (Bottier, 2020). This bio-elastomer is a key material for various manufactured products, such as tyres and anti-vibration parts. Global NR production reached 14 512 million tons in 2023, with 72% coming from Southeast Asia, notably led by Thailand (International Rubber Study Group, 2024).

Fresh *Hevea* latex contains approximately 60% water, 35% polyisoprene, and 5% non-isoprene molecules, which include proteins, lipids, minerals, and carbohydrates (Vaysse et al., 2012). Investigating these non-isoprene components is crucial, as they can significantly affect NR quality (Liu et al., 2023). A set of biochemical analyses was therefore developed to assess the physiological status of rubber trees in relation to their latex yield, i.e., latex diagnosis, consists of determining sucrose (carbon sources), inorganic phosphorus (metabolic energy), and thiol compound (oxidative stress resistance) concentrations in latex. In addition to these water-soluble components, lipids are the primary non-isoprene components found in dry natural rubber (Vaysse et al., 2012).

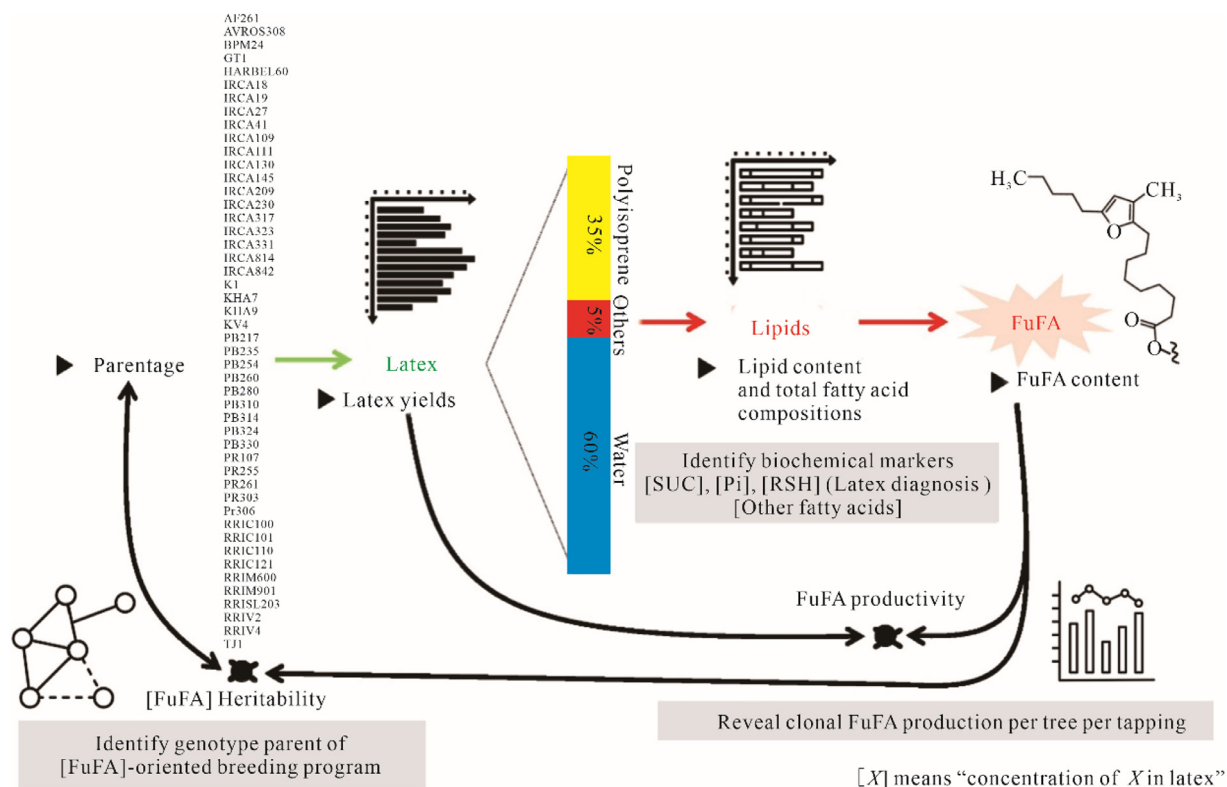
Several breeding programs were developed in Malaysia and Indonesia during 20th century: one can cite major contributor such as Rubber Research Institute of Malaysia (RRIM), Prang Besar (PB) in Malaysia or Balai Penelitian Medan (BPM) in Indonesia. The fatty acid (FA) composition of lipid extracts varies depending on the genotype, as previously documented for RRIM501 (Hasma and Subramaniam, 1986), PB260 (Siler et al., 1997), and for genotypes BPM24, PB235, and RRIM600 (Liengprayoon et al., 2011). These lipids consist of both saturated and unsaturated fatty acids with aliphatic chains ranging from 14 to 20 carbons, linoleic acid (C18:2) being the predominant fatty acid. Interestingly, genotypes RRIM501 and PB235 exhibit low levels of C18:2, but they are rich in a furan ring-containing fatty acid. Indeed, Hasma and Subramaniam (1978) identified a unique fatty acid with a furan ring, referred to as a furan fatty acid or 10,13-epoxy-11-methyloctadecan-10,12-dienoic acid (FuFA-F2) in the latex of RRIM501. Later, Liengprayoon et al. (2013) found that FuFA-F2 makes up >70% of the total fatty acids in the latex of genotype PB235, meaning that this molecule is present at approximately 0.4% w/V in latex (or 1.1% w/w in NR), which is significant given its potential. Indeed, there has recently been significant scientific interest in the family of furan fatty acid (FuFA), particularly due to their health benefits. Although the information on their biosynthesis is relatively limited, these molecules belong to the oxylipin class and may result from the enzymatic oxidation of fatty acids derived from linoleic acid (C18:2), *cis*-vaccenic acid (C18:1) or hexadecadienoic acid (C16:2), occurring in both bacteria and plants (Batna et al., 1993; Shirasaka et al., 1997; Lemke et al., 2020). The FuFA can be part of the human diet, as they are found in fruits, vegetables, vegetable oils, butter, fish, and fungi (Wakimoto et al., 2011; Xu et al., 2017; Müller et al., 2022). Although *in vivo* studies are limited, likely due to the absence of sufficient available supply, the potential health benefits of FuFA are noteworthy, hence the interest in FuFA-F2 found in latex. The FuFA may protect lipids from peroxidation, reduce inflammation, and lower the risk of cardiovascular diseases (Spiteller, 2005; Wakimoto et al., 2011; Xu et al., 2017; Tovar et al., 2017; Li et al., 2018). Additionally, a FuFA catabolite, the 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid (CMPF), is believed to prevent fatty liver development in animal models of obesity and in humans (Dai et al., 2018; Prentice et al., 2018). Recent studies suggest that FuFA-F2 extracted from PB235 latex could increase muscle mass and oxidative metabolism (Pelletier et al., 2023), or reduce metabolic disorders while mimicking positive effects of physical activity in obesogenic diet-fed mice models (Dore et al., 2023).

With growing consumer demand for natural products that are environmentally friendly and health-conscious, manufacturers are shifting towards such alternatives. The promising therapeutic and pharmacological potential of FuFA, combined with FuFA-F2 abundance in the natural rubber industry, is a unique opportunity for socio-economic development. A bio-sourced molecule like this, which could potentially serve as a co-product from rubber plantations, can tackle public health challenges in line with the sustainable development goals outlined by the United Nations. This work therefore was set out to assess and document the clonal variability of FuFA in latex lipids for the first time. This was done by collecting latex from 48 clones from a small-scale clone trial at the Cambodian Rubber Research Institute (CRRRI) plantation in Tbong Khmum province (Cambodia) to cover the widest possible clonal variability. The lipid content and fatty acid concentrations (including FuFA) for each genotype were then assessed. Combined with an evaluation of the latex diagnosis parameters, this dataset enabled us to determine the production capacity of this bioactive molecule for each clone and to conduct a trait heritability study (Fig. 1). To our knowledge, this is unprecedented research that has never been published before.

## 2. Materials and methods

### 2.1. Materials

The ammonia solution with analytical grade was bought from Merck, Germany. Trichloroacetic acid (TCA), ethylenediaminetetraacetic acid (EDTA) with the American Chemical Society (ACS) reagent grade, and aqueous sodium chloride solution with the purity



**Fig. 1.** Diagrammatic illustration of the study design evaluating the genetic variability of furan fatty acid (FuFA) content, along with an assessment of the FuFA production capacity of each clone and the study of heritability to identify possible genitor of future breeding programs.

Notes: IRCA, Institut de Recherche sur le Caoutchouc; PB, Prang Besar; RRIV, Rubber Research Institute of Vietnam; AF, Africa; AVROS, Algemene Vereniging Rubberplanters Oostkust Sumatra; BPM, Balai Penelitian Medan; GT, Gondang Tapen; HARBEL, Harbel, Liberia; K, Khmer; KHA, Khmer Agriculture; KV, Kampuchea Vietnam; PR, Proefstation voor Rubber; RRIC, Rubber Research Institute of Ceylon; RRIM, Rubber Research Institute of Malaysia; RRISL, Rubber Research Institute of Sri Lanka; TJ, Tjirandji; SUC, Sucrose; Pi, inorganic phosphorus; RSH, thiols.

of 99.99% were provided by Merck, Germany. Chloroform and methanol with ACS reagent grades were bought from RCI Labscan, Ireland. The *n*-hexane with laboratory reagent grade and ACS reagent grade were bought from Honeywell, South Korea and Carlo Erba, France, respectively. Diethyl ether with gas chromatography (GC) grade and methanol with high performance liquid chromatography (HPLC) grade were bought from Honeywell, Germany. Methyl heptadecanoate (C17:0 fatty acid methyl ester (FAME)) with purity  $\geq 99\%$  was bought from Sigma-Aldrich, USA. Sodium methoxide (25% w/v in methanol) and acetyl chloride with purity  $\geq 99.0\%$  were bought from Sigma-Aldrich, Germany. Methyl laurate (C12:0 FAME) with GC standard grade, methyl palmitate (C16:0 FAME) with purity  $\geq 98.5\%$ , methyl palmitoleate (C16:1 FAME) with GC standard grade, methyl heptadecanoate (C17:0 FAME) with purity  $\geq 99\%$ , methyl linoleate (C18:2 FAME) with GC standard grade, and methyl linolenate (C18:3 FAME) with GC standard grade were bought from Sigma-Aldrich, USA. Methyl myristate (C14:0 FAME) with the purity of 99.5% was bought from Fluka AG, Switzerland. Methyl stearate (C18:0 FAME) with the purity of 99% and methyl arachidate (C20:0 FAME) with the purity of  $\sim 99\%$  were bought from Sigma, France. Methyl oleate (C18:1 FAME) with the purity of 99% was bought from Aldrich, France.

The natural rubber latex samples were collected from the plantation of the Cambodian Rubber Research Institute (CRRRI) in Tbong Khmum province, Cambodia. The *Hevea* trees used were planted on red basaltic latosols. It consists of the 48 genotypes indicated in Table 1. The rubber trees were planted in 2008 and opened in 2016. They were tapped every 3 d with a half spiral cut (Vijayakumar et al., 2009) with 3 block replications planted in a randomized complete block design (RCBD) (small-scale clone trial, 13 trees per unit plot). The 48 selected clones were representative of the currently planted genotypes in the world. The latex samples were collected once for each genotype on tapping panel BO-2 in October 2022. The cumulative rainfall in October 2022 was 257 mm while the monthly average rainfall for 2022 was 185 mm. The maximum and minimum temperatures in the plantation in October 2022 were 32.7 and 23.1 °C, respectively.

## 2.2. Fresh latex collection

Fresh latex was collected from the cup of each latex-producing tree in the plot 30 min after tapping in the early morning (around 6 a.m.). It was filtered through a sieve into a plastic bottle. The total collection volume was at least 200 mL per plot. The latex bottle was immediately stored in an ice bucket and transferred to the laboratory for latex diagnosis and lipid extraction.

**Table 1**  
List of the genotypes studied.

Genotype (alphabetical order)					
AF261	IRCA41	IRCA323	PB217	PB330	RRIC110
AVROS308	IRCA109	IRCA331	PB235	PR107	RRIC121
BPM24	IRCA111	IRCA814	PB254	PR255	RRIM600
GT1	IRCA130	IRCA842	PB260	PR261	RRIM901
HARBEL60	IRCA145	K1	PB280	PR303	RRISL203
IRCA18	IRCA209	KHA7	PB310	PR306	RRIV2
IRCA19	IRCA230	KHA9	PB314	RRIC100	RRIV4
IRCA27	IRCA317	KV4	PB324	RRIC101	TJ1

Notes: AF, Africa; AVROS, Algemene Vereniging Rubberplanters Oostkust Sumatra; BPM, Balai Penelitian Medan; GT, Gondang Tapen; HARBEL, Harbel, Liberia; IRCA, Institut de Recherche sur le Caoutchouc; K, Khmer; KHA, Khmer Agriculture; KV, Kampuchea Vietnam; PB, Prang Besar; PR, Proefstation voor Rubber; RRIC, Rubber Research Institute of Ceylon; RRIM, Rubber Research Institute of Malaysia; RRISL, Rubber Research Institute of Sri Lanka; RRIV, Rubber Research Institute of Vietnam; TJ, Tjirandji.

### 2.3. Latex analysis parameter

Rubber production was determined by the latex volume and dry rubber content (DRC) of the latex. The DRC measurement protocol was adapted from ISO 126:1995 (Latex, Rubber, Natural Concentrate: Determination of Dry Rubber Content, Switzerland) for fresh latex, as indicated in the following equation

$$\text{Dry rubber content} = \text{Dry rubber weight} / \text{Fresh latex weight} \times 100\% \quad (1)$$

Latex dry matter or total solid content (TSC) was measured following standard ISO 124:1997 (Latex, Rubber: Determination of Total Solid Content, Switzerland) adapted for fresh latex as indicated in Eq. (2)

$$\text{Total solid content} = \text{Dry matter weight} / \text{Fresh latex weight} \times 100\% \quad (2)$$

For practical reasons, the latex used to measure DRC and TSC may have been ammoniated by 28%–30% ammonia solution at a concentration of 0.6% (w/w in latex). The corresponding corrected fresh latex weight (without ammonia) was used for TSC and DRC calculation.

The latex serum to determine tree physiology indicators was extracted from collected fresh latex by mixing with trichloroacetic acid and ethylenediaminetetraacetic acid according to the latex diagnosis method reported by Zaw et al. (2022) to determine the concentrations of sucrose (SUC), thiol (RSH), and inorganic phosphorus (Pi). The concentration was expressed in millimoles per liter of fresh latex.

Rubber yield data were routinely collected by CRRRI expressed in grams of dry rubber per tree and grams of dry rubber per tree per tapping.

### 2.4. Lipid extraction from fresh latex

Lipids were extracted from fresh latex following the procedure described by Liengprayoon et al. (2008). Briefly, diluted latex (fresh latex:distilled water = 10mL:10 mL) was extracted with 100 mL of chloroform-methanol mixture (2:1, V/V), followed by washing with 20 mL of 0.9% aqueous sodium chloride solution (adapted from Folch et al. (1956)). After clear phase separation, the lower phase was collected and the solvent was evaporated by rotary evaporator followed by nitrogen gas flow. The dry lipid extract was weighed, dissolved in chloroform and stored at 4 °C. The coagulum obtained after lipid extraction was dried and weighed. The latex lipid content (lipid extract) was calculated by the Eq. (3):

$$\text{Lipid extract} = \text{Dry lipid weight} / (\text{Dry coagulum weight} + \text{Dry lipid weight}) \times \text{Dry rubber content of latex} \times 100\% \quad (3)$$

### 2.5. Purification of triacylglycerol-FuFA and production of FuFA methyl ester standard

The triacylglycerol- FuFA (TAG-FuFA) was purified from PB235 lipid extract by liquid chromatography (Reveleris® X2, Büchi, Switzerland). The lipid (400 mg) was deposited on a silica 6 nm column (RediSep®Rf, Teledyne ISCO, USA) with the packing amount of 4 g and silica particle size of 40–63 μm. The 1st mobile phase, *n*-hexane, was eluted for 30 min at a flow rate of 1 mL/min. The 2nd mobile phase, the mixture comprising *n*-hexane and diethyl ether (90:10, V/V), was eluted for 1 h at a flow rate of 5 mL/min. The lipid-containing fraction detected through a high signal on an evaporative light scattering detector and an ultraviolet detector (230 nm) was characterized by the thin layer chromatography technique proposed by Liengprayoon et al. (2013) with PB235 lipid

extract as a control deposit. The fraction with the highest TAG-FuFA purity was selected to be methylated as the in-house standard FuFA methyl ester (FuFAME).

## 2.6. Preparation of fatty acid methyl ester

The (15 ± 5) mg of dry lipid extract and 1 mg of methyl heptadecanoate (as internal standard) were trans-esterified in a screw-cap test tube with 1 mL of 0.5 mol/L sodium methoxide-methanol reagent (10:90, V/V) at (65 ± 5) °C for 15 min. The test tube was placed on ice for 10 min and the sample was then esterified by adding 1 mL of 7.4% (V/V) acetyl chloride in methanol and heating at (65 ± 5) °C for 15 min. The test tube was placed on ice for 10 min, and then the reaction mixture containing fatty acid methyl ester (FAME) was extracted with 1 mL of *n*-hexane (ACS reagent grade) and 1 mL of distilled water. The supernatant phase was then collected for further analysis (Section 2.7).

## 2.7. Analysis of fatty acid methyl ester by gas chromatography with flame-ionization detection

The FAME was analyzed by gas chromatography (GC) with a flame-ionization detector (FID), using a FOCUS GC (Thermo Electron Corporation, USA). The GC-column phase was CP-Sil 88 for FAME (Agilent Technologies, Inc., USA). The column length was 50 m with a 0.25 mm inner diameter and a 0.25 μm film thickness. The sample injection volume was 1 μL with a split ratio of 15. The flow rate was adjusted to provide a constant pressure of 241 kPa. The oven temperature was started from a holding temperature at 160 °C for 0.5 min then increased at a rate of 10 °C/min for 6 min and held at 220 °C for 5 min. The injector and detector temperatures were set to 250 and 270 °C, respectively. The total elution time was 11.5 min.

## 2.8. Calculation of total fatty acid content in lipid extract and latex

The concentration of fatty acids detected in lipid extracts was determined using 8-level calibration curves obtained by GC-FID for each of the following standard FAME: C12:0 FAME, C14:0 FAME, C16:0 FAME, C16:1 FAME, C17:0 FAME, C18:0 FAME, C18:1 FAME, C18:2 FAME, C18:3 FAME, C20:0 FAME, and FuFAME. The FuFAME was obtained by (trans)esterification (cf. 2.6) of the purified TAG-FuFA (cf. 2.5). The total fatty acid content in lipid was estimated by the difference between the initial lipid weight before the (trans)esterification step and the total weight of fatty acids detected by GC-FID (the total fatty acid weight was calculated based on the measured weight of FAME) as shown in the following equation:

$$\text{Total fatty acid content of lipid extract} = \text{Total fatty acid weight} / \text{Initial lipid weight for (trans)esterification} \times 100\% \quad (4)$$

The total fatty acid content in latex was calculated by multiplying the total fatty acid content of lipid extract in Eq. (4) by the lipid extract content in latex from Eq. (3) as in the following equation:

$$\text{Total fatty acid content in latex} = \text{Total fatty acid content of lipid extract} \times \text{Lipid extract} \times 100\% \quad (5)$$

## 2.9. Data processing for trait heritability estimation

The FuFA concentration in latex was assessed on the diversity population of 48 clones planted in the small-scale clone trial (SSCT) as previously mentioned. The degree of relatedness (called pedigree) of each clone was taken into account, and Pedimap software was used to map the pedigree (Voorrips et al., 2012). The heritability of FuFA concentration in latex was estimated using the restricted maximum likelihood and best linear unbiased predictor (REML/BLUP) linear mixed model (de Villemereuil, 2018). The statistical model was implemented using the ASReml-R package (Butler et al., 2009) as in Eq. (6):

$$y_{ijk} = \mu + R_i + C_j + \varepsilon_{ijk} \quad (6)$$

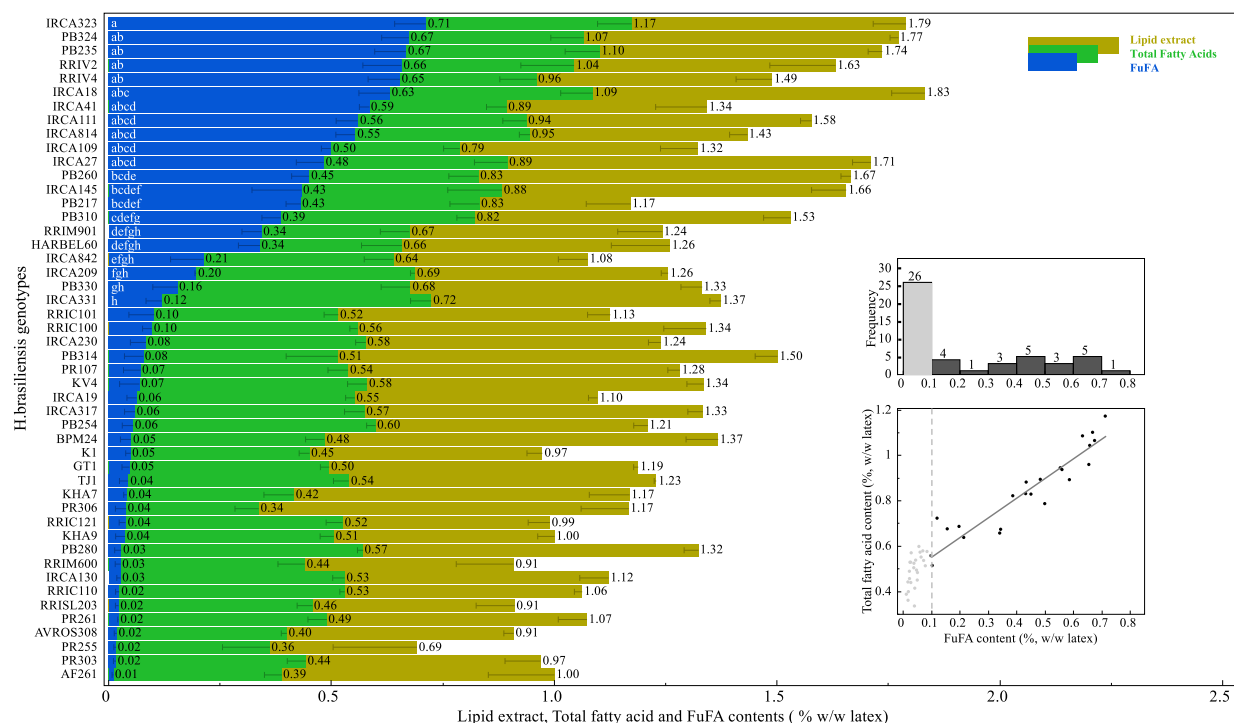
where  $y_{ijk}$  is the  $k$ th observation of FuFA concentration in the latex of the  $i$ th replication (R) for the  $j$ th clone;  $\mu$  is the overall mean;  $R_i$  is the fixed replication effect ( $i = 1, 2, 3$ ),  $C_j$  is the random  $j$ th clone effect that links to the effect of the relationship information by the pedigree-based kinship matrix  $\mathbf{K} \sim N(0, J\sigma_j^2)$ ;  $\varepsilon_{ijk}$  is the random residual effect.

The best linear unbiased predictor (BLUP) of each clone in the kinship matrix (tested clones and parental clones) was found on the  $\mathbf{J}$  vector, corresponding to the vector of random genetic value prediction. For each descendant studied, the random genetic value corresponds to its own genetic value, i.e., the specific combining ability (SCA), and for the related parent to the mean descendant values, i.e., the breeding value (BV), or general combining ability (GCA) (Isik et al., 2017). Broad-sense heritability ( $H_{BS}^2$ ) was estimated from the genetic and total phenotypic variances as in Equation (7)

$$H_{BS}^2 = \frac{V_G}{V_P} = \frac{\sigma_j^2}{\sigma_p^2} \quad (7)$$

where  $\sigma_j^2$  is the variance of the clone effect and  $\sigma_p^2$  is the total phenotypic variance.





**Fig. 2.** Lipid extract, total fatty acids, and FuFA contents of latices from 48 genotypes and the distribution of FuFA content in latices and the relation between FuFA and total fatty acid content (Notes: Half error bars represent the standard error and genotypes with  $<0.1\%$  FuFA in latex are in grey.) *H. brasiliensis*, *Hevea brasiliensis*. a–h, the average comparison of the first eight statistical groups with by Tukey-Kramer test ( $P < 0.05$ ).

## 2.10. Statistical analysis

The data were analyzed using the all pairs Tukey-Kramer honestly significant difference (HSD) test,  $P < 0.05$ , and a Pearson correlation coefficient ( $r$ ) analysis with JMP Pro software (version 14, SAS Institute Inc., Caru, USA).

## 3. Results and discussion

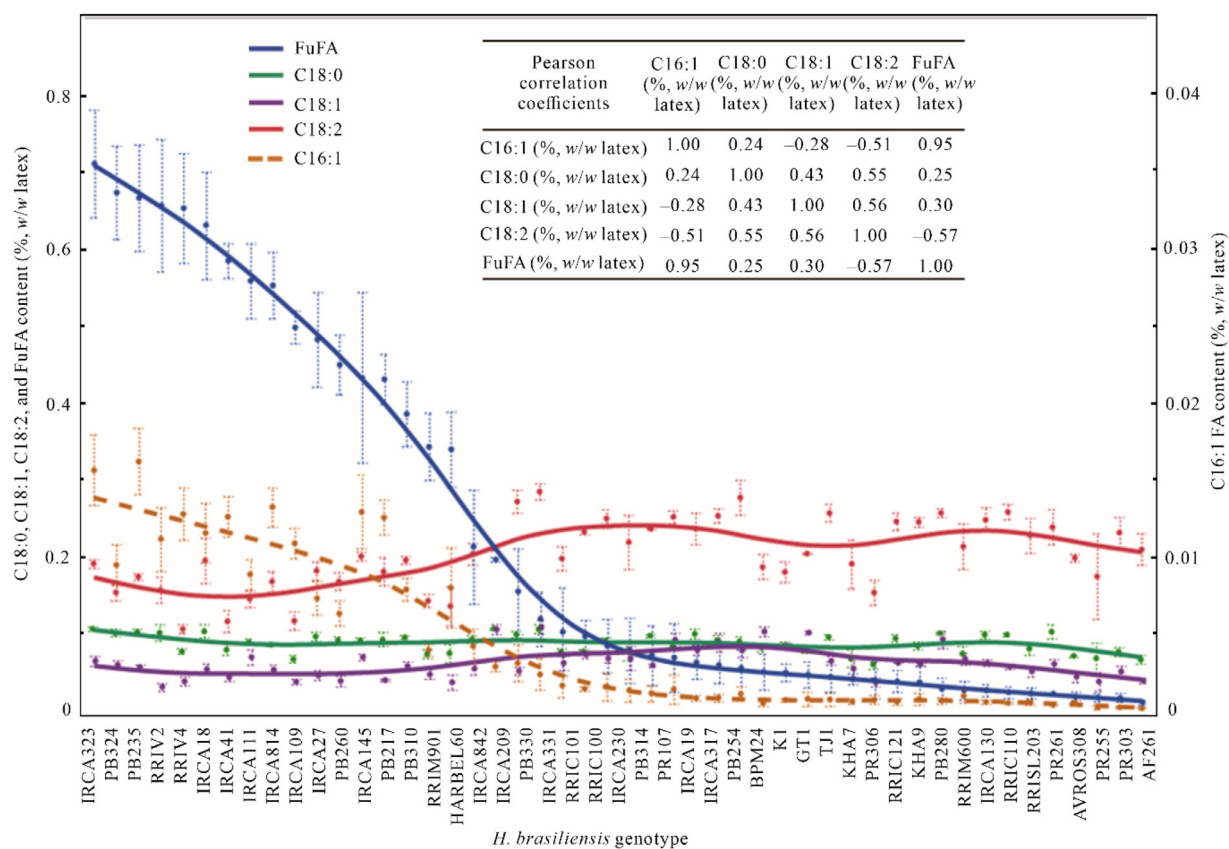
### 3.1. Lipids, total fatty acids, and furan fatty acid contents in latices

The lipid content was extracted from the latices of 48 *Hevea brasiliensis* genotypes and the total quantity of fatty acid in the resulting lipid extracts was measured. The composition of these total fatty acids, including FuFA concentration, was also analyzed for each latex. Fig. 2 shows the lipid extract, total fatty acids, and FuFA content for each genotype. The genotypes were ranked on the x-axis in descending order of FuFA content in their latex.

The lipid contents of the latices ranged from 0.7% to 1.8% (w/w in latex). Statistical analysis showed a significant genotype effect on the lipid extract, and several statistical groups were found as shown in Fig. S1 and Table S1. The statistical group of genotypes with the highest lipid extracts (1.4%–1.8%) comprised the genotypes IRCA18, IRCA323, PB324, PB235, IRCA27, PB260, IRCA145, Rubber Research Institute of Vietnam (RRIV) 2, IRCA111, PB310, PB314, RRIV4, and IRCA814. The genotypes with the lowest lipid content (0.7%–1.1%) were Proefstation voor Rubber (PR) 255, RRIM600, Algemene Vereniging Rubberplanters Oostkust Sumatra (AVROS) 308, Rubber Research Institute of Sri Lanka (RRISL) 203, PR303, Khmer (K) 1, Rubber Research Institute of Ceylon (RRIC) 121, Africa (AF) 261, Khmer Agriculture (KHA) 9, RRIC110, PR261, IRCA842, IRCA19, IRCA130, and RRIC101. The lipid contents of the latex from genotypes PB235 and RRIM600 were consistent with the results of Liengprayoon et al. (2017).

The FuFA contents were detected in all lipid fractions and the distribution ranged widely from very low (0.01%, w/w in latex) to high (0.71%, w/w in latex) (Fig. S2 and Table S2). However, most of the genotypes (26/48) had  $<0.1\%$  FuFA in the lipid fraction of their latex as inserted in Fig. 2.

The latices with the highest FuFA content (0.5%–0.7%, w/w in latex) came from the following genotypes in descending order: IRCA323, PB324, PB235, RRIV2, RRIV4, IRCA18, IRCA41, IRCA111, IRCA814, IRCA109, and IRCA27 (group a in Fig. 2). It is worth noting that they originated from only three breeding programs: IRCA (Institut de Recherche sur le Caoutchouc, Côte d'Ivoire), PB (Prang Besar, Malaysia), and RRIV (Rubber Research Institute of Vietnam, Vietnam). Genotype PB235, which was previously considered to have the highest FuFA content in its latex, ranked third in this study, at 0.67% (Liengprayoon et al., 2011; 2013). As shown in the insert of Fig. 2, when FuFA content exceeded 0.1%, the presence of FuFA in the latex was positively linked to an increase in



**Fig. 3.** The C16:1, C18:0, C18:1, C18:2 and furan fatty acid contents of the latices from the 48 studied genotypes and the corresponding Pearson correlation coefficients as inserted (Note: Error bars represent the standard error.).

total fatty acid content (linear regression  $R^2 = 0.82$ ). It can be suggested that the presence of FuFA in latex is more additive than substitutive compared to other fatty acids.

Fig. 3 shows the main individual fatty acid contents for all the latices (detailed data provided in Table S3). Firstly, the individual fatty acid content of all the latex samples from the studied genotypes was consistent with previously published data (Siler et al., 1997; Liengprayoon et al., 2013). Secondly, FuFA emerged as the most concentrated fatty acid in 17 of the 48 genotypes. Thirdly, excluding genotypes with the highest FuFA concentration ( $>0.3\%$  w/w in latex), linoleic acid (C18:2) was the most prevalent fatty acid (0.12%–0.28% w/w in latex), followed by stearic acid (C18:0), oleic acid (C18:1), and palmitic acid (C16:0). Linolenic acid (C18:3), arachidic acid (C20:0), and palmitoleic acid (C16:1) were found in fatty acid concentrations of  $<0.03\%$  (w/w in latex).

To identify a possible metabolic relationship between fatty acids, Pearson correlation coefficients ( $r$ ) were calculated to assess correlations between their concentrations (as inserted in Fig. 3). Interestingly, linoleic acid (C18:2 FA), which is described as one of the potential biochemical precursors of FuFA (Spiteller 2005; Alvarado et al., 2021), was negatively correlated ( $r = 0.57$ ) with FuFA content in latex. This result may provide support for the hypothesis that furan-ring formation originates from the oxidation and cyclization of C18:2 FA. Although present in quantities under  $0.02\%$  (w/w, in latex), the palmitoleic acid (C16:1 FA) content showed a strong correlation with FuFA ( $r = 0.95$ ). This correlation suggested that C16:1 FA might be a short-life biochemical compound linked to the anabolism or catabolism of FuFA. To date, C16:1 FA has never been considered as a precursor of FuFA. Indeed, the recently reported precursors of pentyl FuFA were *cis*-vaccenic acid (C18:1 *cis*-11 FA) in bacteria (Shirasaka et al., 1997; Lemke et al., 2020), or linoleic acid (C18:2 FA), and C16:2 (9,12-hexadecadienoic acid) in algae (Batna et al., 1993; Spiteller 2005). No other noticeable correlation of FuFA content was observed with the other fatty acids. It could be noted that C18:2 FA content was positively correlated with the content of all the other fatty acids, except C16:1 FA and FuFA.

### 3.2. Potential yield of FuFA production

At this stage, we have identified the genotype with the highest FuFA content in its latex. However, it is well known that the quantity of latex produced per tree per tapping depends on the genotype, indicating that the ability of a tree to provide FuFA would also depend on its latex production capacity. The latex yield per tree per tapping was measured and averaged over an



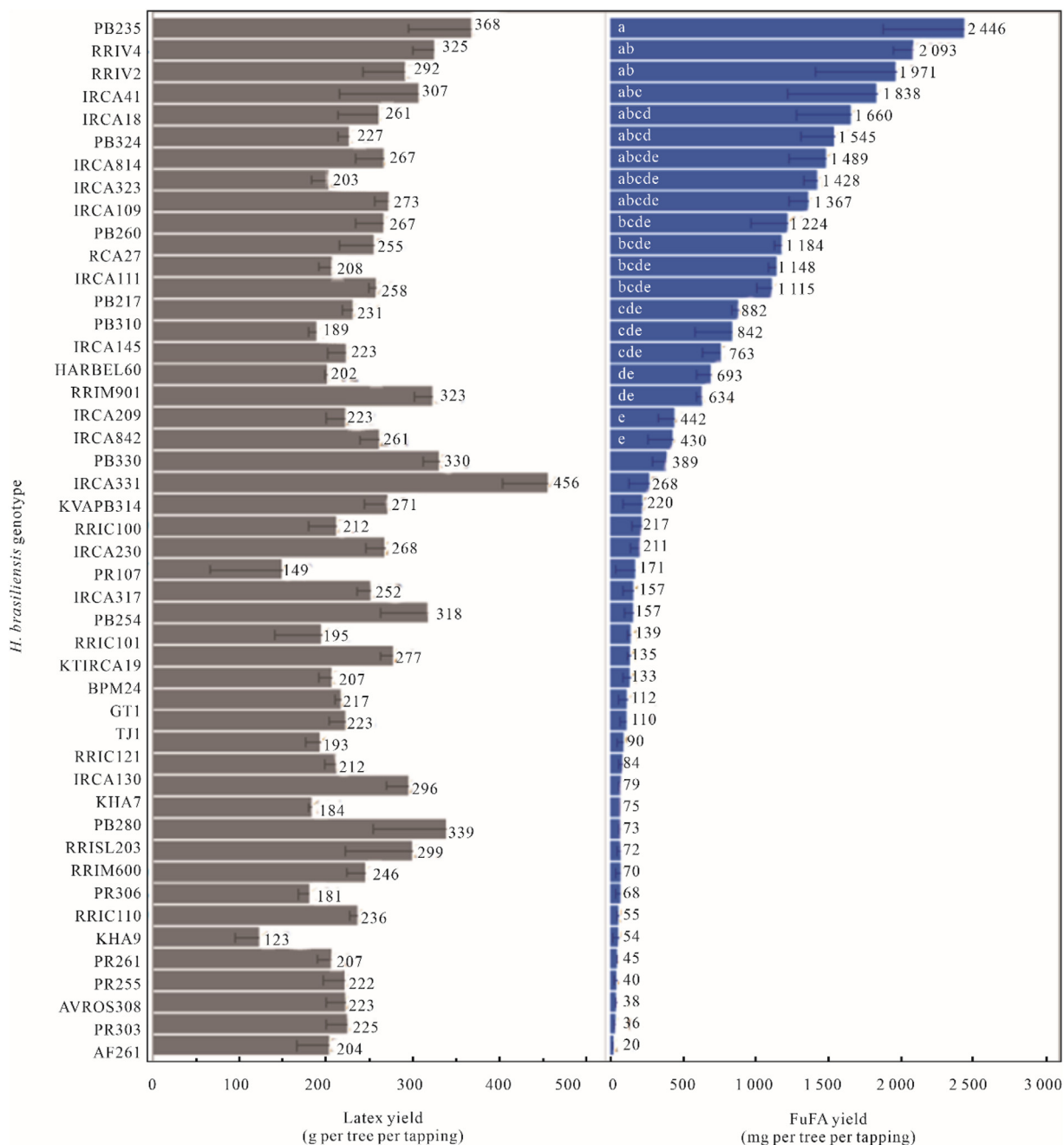
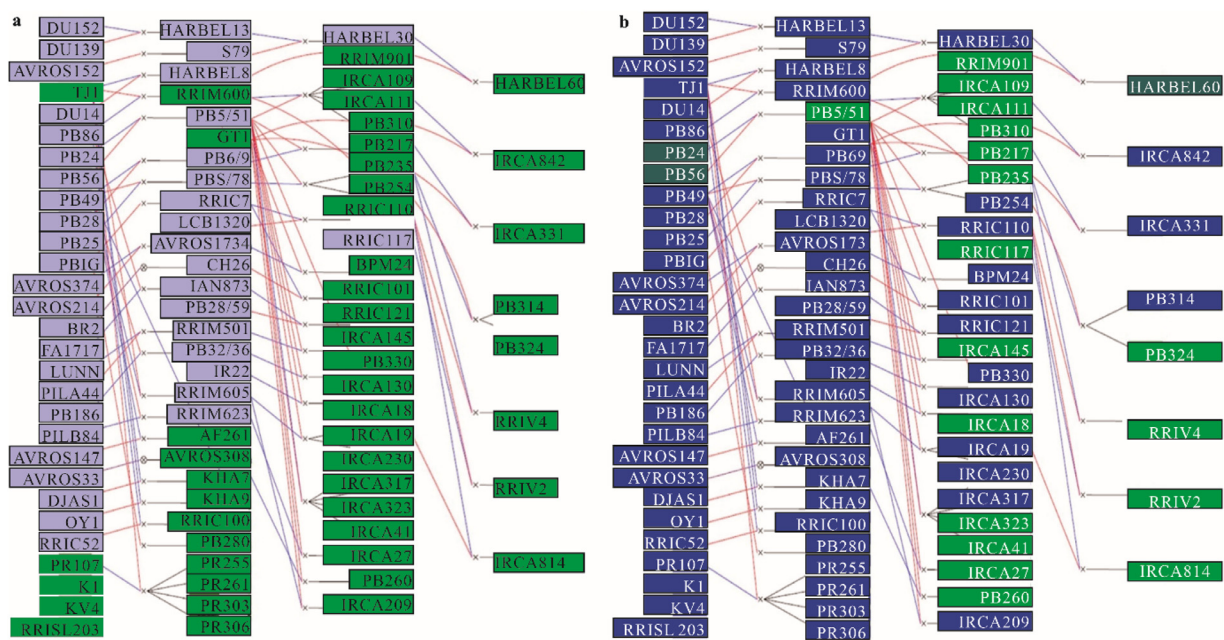


Fig. 4. Average latex yield (2022–2023) and corresponding FuFA yield (Notes: Half error bars represent the standard error; The first five statistical groups (Average comparison by Tukey-Kramer test,  $P < 0.05$ ) are indicated by the letters a to e.).

agronomical year (April 2022 – February 2023) for each genotype and each replication block. Fig. 4 shows latex yields and the corresponding FuFA yields expressed in milligrams of FuFA per tree per tapping, with genotypes ranked in descending order of FuFA yields. The first five statistical groups were indicated by the letters a to e (average comparison by the Tukey-Kramer test,  $P < 0.05$ ). In terms of harvested yield per tapping, the genotypes showing the greatest potential for FuFA production were PB235, RRIV4, RRIV2, IRCA 41, IRCA 18, PB324, IRCA 814, IRCA 323, and IRCA109, with values ranging from 2 446 mg FuFA per tree per tapping down to 1 367 mg FuFA per tree per tapping. Genotype IRCA 323, which produced the latex with the highest FuFA concentration (Fig. 2), ranked only 8th when considering its FuFA yield per tapping, showing a 40% lower yield than that of PB235. In our study, genotype PB235 offered the best combination of high FuFA concentration in latex and high latex yield.

**Table 2**  
Pearson correlation coefficients between FuFA yield per tapping and the biochemical and physical indicators of latex diagnosis.

Pearson correlation coefficients (r)	Concentration of thiols (mmol/L)	Concentration of inorganic phosphorus (mmol/L)	Concentration of sucrose (mmol/L)	Total solid content (%)
FuFA yield (mg of FuFA per tree per tapping)	$r = 0.11$	$r = 0.15$	$r = -0.15$	$r = 0.12$



**Fig. 5.** Pedigree of the 48 genotypes. (a) Information of observed genotypes (green) and unobserved relatives (light purple); (b) The breeding value prediction of individual values for each parental clone (The genetic value is used as a color fill, with the corresponding genetic value increasing from dark blue to green.)

Each box corresponds to 1 individual. Red and blue lines correspond to the female and male parent, respectively.

### 3.3. Correlation of FuFA production with biochemical parameters of latex cells

Latex diagnosis has been developed to measure key indicators that illustrate the physiological status of latex cells with respect to latex production yields (Jacob et al., 1985; 1989). These indicators include the concentration of sucrose ([SUC]), concentration of inorganic phosphorus ([PI]), concentration of thiols ([RSH]), and total solid content (TSC). These were measured in the same latex samples as those used for FuFA quantification in Table S4 to investigate whether they could also be linked to a biosynthetic metabolic pathway for FuFA in latex. It is important to note that no hormonal stimulation was applied to these trees, despite the tapping frequency typically involving such stimulation in production plots (Gohet et al., 1996). However, in the context of small-scale clone trials, it is common to forego stimulation to evaluate the inherent yield potential of clones. Table 2 gives the Pearson correlation coefficient of those indicators with FuFA production (mg) per tapping per tree. Under the conditions of the experiment, none of the biochemical markers measured in latex diagnosis (sucrose, inorganic phosphorus, thiols, and TSC) strongly correlated with the FuFA yield. Further studies are therefore needed to identify biomolecules that might be linked to FuFA biosynthesis in latex during its regeneration between two tappings.

### 3.4. Heritability estimation and prediction of the parental genotype effect

The relatedness information for the 48 genotypes studied is indicated in a pedigree (Fig. 5a) of 91 genotypes. For three observed genotypes (K1, Kampuchea Vietnam (KV) 4, and RRISL203), no relatedness information was available. For eight observed genotypes (Tjirandji (TJ) 1, PR107, RRM600, Gondang Tapen (GT) 1, IRCA111, PB217, PB235, and RRIC110), they were also parents of observed genotypes. For the other genotypes, relatedness information was available and they were descendants of unobserved parents.

The estimated broad-sense heritability was around 93%, the genetic effect (here the clonal effect) was very strong and explained almost all the variability of the phenotypic data. The distribution of the data (Fig. 2) and the high heritability suggested high genetic control with a low environmental effect (Würschum, 2012; Gouvêa et al., 2024). The raw distribution was a typical distribution of

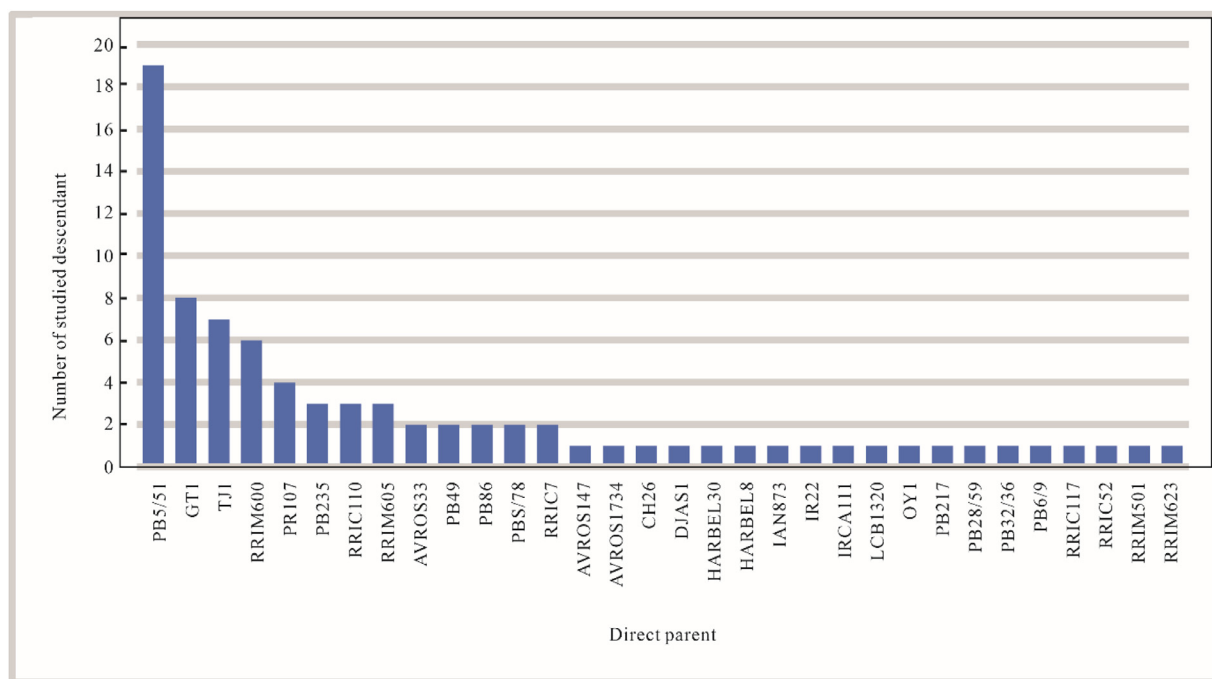


Fig. 6. Histogram of the number of descendants for each direct parent.

traits under monogenic or oligogenic control (Le Roy, 1992), like traits close to metabolic pathways, or certain disease resistance traits.

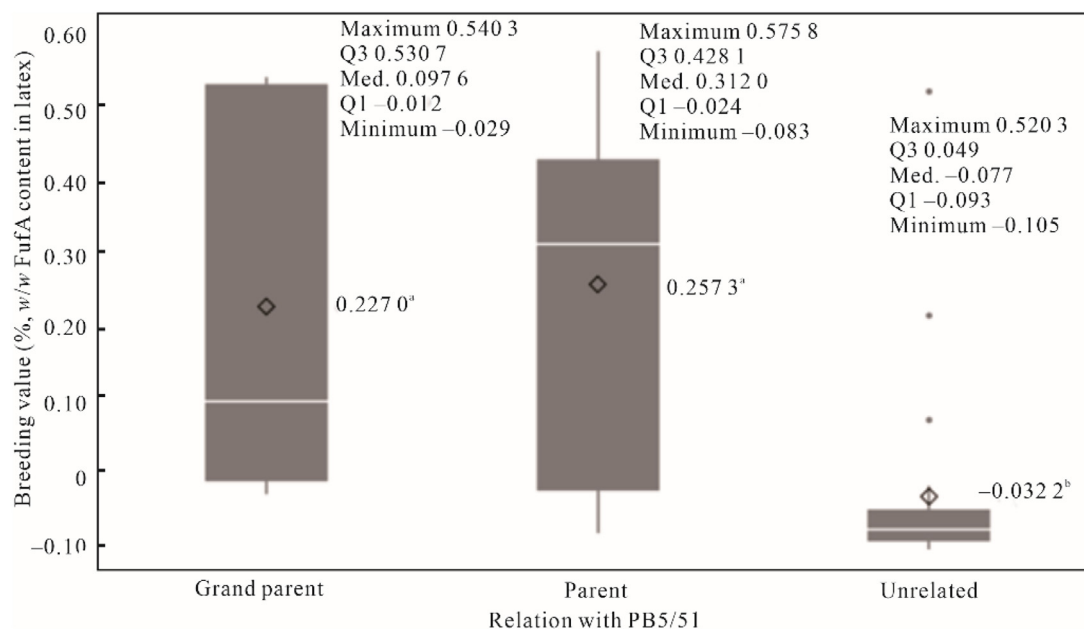
The breeding value prediction (Fig. 5b) of individual values for each parental clone was based on the performance of the studied (descendant) clones and of the studied clones themselves (Isik, 2017). The ranking of the breeding value for the studied clone was clustered in a way consistent with the analysis in Section 3.1., with all the clones mentioned in group a at the top of the breeding value list (Table S5).

However, the specificity of data from breeding programs, such as unbalanced phenotypic data sets due to the selection process, could reduce the confidence in breeding value prediction for a parental clone represented by a small number of progenies (Wang et al., 2012). Indeed, the BV was only relevant for 7 parents (Fig. 6). The Prang Besar (PB) genotype PB5/51 was involved in 19 crosses as the male or female parent, GT1, TJI, and RRIM600 were engaged in >5 crosses and studied genotypes, which increased the level of confidence in the predicted breeding value, and PR107, PB235 and RRIC110 were involved in >3 crosses and also studied in the trial.

It is interesting to observe the effect of the parental genotype PB5/51, which is the most represented parent. This genotype was predicted to have a high breeding value and almost all the top ten of the studied genotypes had PB5/51 as a parent or grandparent (except for one clone, RRIV2) (Table S5). The genotypes having PB5/51 as a grandparent or parent were found to have respectively >0.23% and 0.26% FuFA content in their latex than the mean of the trial (Fig. 7). The genotypes unrelated to PB5/51 had 0.03% less FuFA in their latex than the mean of the trial. According to the Tukey test, the first two groups were significantly different from the third. Genotype PB5/51 seemed to be a very worthwhile genitor for improving FuFA content within the breeding population.

#### 4. Conclusions

In this study, FuFA was detected in all the lipid fractions of the 48 *Hevea brasiliensis* genotypes involved. The FuFA distribution ranged widely from 0.01% to 0.71% (w/w in latex). The genotypes with the highest content came from three breeding programs: IRCA, PB, and RRIV. Moreover, when FuFA content exceeded 0.1%, it correlated with an increase in total fatty acid content, suggesting that its presence in latex complements rather than substitutes for the other fatty acids present. Notably, this work confirmed that C18:2, known as a potential precursor of FuFA in algae, showed a negative correlation with FuFA content from *Hevea* latex. Although present in small quantities (<0.02% w/w in latex), C16:1 strongly correlated with FuFA levels, indicating a possible linkage in biochemical pathways. As regards yield per tapping, PB235, RRIV4, RRIV2, IRCA 41, IRCA 18, PB324, IRCA 814, IRCA 323, and IRCA109 showed the highest potential for FuFA production, with yields ranging from 2 446 mg FuFA per tree per tapping down to 1 367 mg FuFA per tree per tapping. Interestingly, under our experimental conditions, which did not involve hormonal stimulation, no clear relationship was found between latex cell metabolic status assessed by latex diagnosis and FuFA production. This suggested that biochemical markers, such as sucrose, inorganic phosphorus and thiols contained in latex were not direct limiting factors for FuFA biosynthesis during latex regeneration between tapplings. Finally, a trait heritability study based on the pedigree of the studied genotypes revealed



**Fig. 7.** Boxplots of the breeding value of the studied clones having Prang Besar 5/51 (PB5/51) as a grandparent, parent, or being unrelated (average comparison by Tukey-Kramer test,  $P < 0.05$ )

Q1, first quartile; Q3, third quartile; Méd., median (indicated by the white line); mean is indicated by a losange.

genotype PB 5/51 to be a very worthwhile genitor for improving FuFA content in the breeding population. To conclude, the selection of *Hevea brasiliensis* genotypes plays a crucial role in FuFA production. In addition to the promising health benefits, producing FuFA-F2 from *Hevea brasiliensis* could catalyze the on-going transformation towards sustainability in the natural rubber value chain. As a high-value co-product, it would indeed provide additional economic value, it would lead to greater financial benefit from the land planted to *Hevea* and it could contribute to social improvement by increasing the modest incomes of natural rubber producers, who are mainly family farmers.

#### 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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#### Availability of data

Data is available on request from the authors.

#### Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.jobab.2024.11.005](https://doi.org/10.1016/j.jobab.2024.11.005).

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