

Early selection and genetic analysis of susceptibility to tapping panel dryness by applying an intense harvesting system to a segregating population in *Hevea brasiliensis*

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ABSTRACT

Tapping Panel Dryness (TPD) is a physiological syndrome affecting natural rubber production in *Hevea brasiliensis* that is thought to be exacerbated by climate change stress. TPD is associated with high latex viscosity and agglutination of rubber particles. Although many studies have been carried out on the physiological and molecular mechanisms associated with TPD, little has been done in the way of genetic improvement. Intensive harvesting systems with high tapping frequency and ethephon stimulation of rubber trees are known to induce early TPD occurrence. A harvesting system with daily tapping and monthly ethephon stimulation was applied for one year to a segregating population of 189 individuals obtained from a cross between the TPD-susceptible clone PB 260 and the TPD-tolerant clone SP 217. This treatment induced a dramatic increase in dry cut length after six months for 26 % of the genotypes. Heritability also peaked at 88 % four months after treatment. Of the seven quantitative trait loci identified for the dry cut length, three were uniquely detected after application of the intensive harvesting system, and two quantitative trait loci were only observed after opening of trees under a standard harvesting system. Several genes underlying quantitative trait loci revealed functions previously identified through transcriptomic analyses. These results suggest a complex genetic basis of TPD.

1. Introduction

Natural rubber is an essential raw material for the production of tyres and other products, with consumption reaching 13.86 million tonnes in 2023 (IRSG, 2024). This bio-sourced polymer is synthesised in a specialised organelle, rubber particles, located in latex of *Hevea brasiliensis*. These cells differentiate in the phloem tissue and form an articulated network that enables a large quantity of latex to be harvested after each tapping of the soft bark. Latex regeneration takes about 48 hours after tapping, which determines the frequency of tapping. Ethephon, an ethylene releaser, is the most widely used product to stimulate latex regeneration and flow of some rubber clones with low or less than optimal latex metabolism. A physiological syndrome known as Tapping Panel Dryness (TPD) affects natural rubber production and can be induced by a combination of stresses, including harvesting systems and the environment (Herlinawati et al., 2022). Production losses have been

estimated at 15–20 % of the annual rubber production with 20–50 % of productive trees affected by TPD (Senevirathna et al., 2007). In a context of climate change, this stress-induced TPD is likely to be more significant and to dramatically affect natural rubber production worldwide (Cahyo et al., 2024).

The International Rubber Research and Development Board Workshop on Tapping Panel Dryness definitively concluded in 2005 that TPD is directly linked to over-exploitation (excessive tapping and intense ethylene stimulation), and that there is a progressive evolution from tapping cut dryness (reversible TPD) to brown bast (irreversible TPD) (Jacob, 2005). Under standard tapping conditions, a coagulation process at the cut surface occurs several hours after tapping to stop the latex flow. The coagulation of rubber particles has been widely documented (Zhang et al., 2017). Lutoids are lysosomal micro-vacuoles that play a major role in the stoppage of flow after tapping (d'Auzac, 1989). Lutoids contain Hevein, a lectin-like protein involved in the agglutination of

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rubber particles (Gidrol et al., 1994). Some authors proposed a model of latex aggregation by Hevein (Berthelot et al., 2016). This process of agglutination of rubber particles is exacerbated in trees affected by TPD that have a high latex viscosity, which hampers latex flow after tapping (Zhang et al., 2017). Reversible TPD is associated with a ROS production and consequent oxidative stress in the laticifers, leading to luteoid instability (Chrestin, 1989; Junaidi et al., 2022; Zhang et al., 2017). Irreversible TPD - or brown bast - was linked to an alteration in cyanide metabolism due to an oxidative stress in the bark tissues that spreads throughout the tree (Chrestin et al., 2004). Putranto and collaborators have proposed calling these forms ROS-TPD and BB-TPD (Putranto et al., 2015).

Several bark treatments exist to cure trees by scraping bark affected by brown bast and applying a product to control pathogens and regenerate the bark (Feng, 2012; Siswanto, 1997; Suwandi et al., 2018). However, these treatments are costly and time-consuming. Moreover, a latex diagnosis (LD) has been established to monitor the physiological status of rubber trees and adjust harvesting systems to optimise latex yield and prevent TPD occurrence (Jacob et al., 1989). LD comprises four main parameters: sucrose (Suc), inorganic phosphorus (Pi), thiols (RSH) and total soluble content (TSC). The need for a biochemical laboratory close to the plantations has meant that LD has only been developed by estate plantation companies and research centres, whereas 85 % of production comes from smallholders who cannot benefit of such a technology (Junaidi et al., 2023). TPD incidence was higher in high-metabolism than low-metabolism clones (Putranto et al., 2015). However, some new rubber clones such as IRR 112 and IRR 118 in Indonesia with a high metabolism reflected by a high Pi content, have a low TPD susceptibility associated with a high sucrose content (Herlinawati et al., 2022). These clones showed low dry cut length (DCL) under an intensive harvesting system (IHS), S/2 d1 ET 2.5 % 12/y, where DCL is a way to quantify TPD. Interestingly, the application of IHS induced a high DCL and homogenised the clonal response in the TPD-susceptible clone PB 260. Molecular studies reported a low gene expression in the latex and bark of TPD-affected trees, particularly for genes involved in the rubber biosynthesis pathway (Liu et al., 2015; Montoro et al., 2018; Putranto et al., 2015). Some authors also reported the involvement of hormonal signalling pathways linked to stress and regulation of the redox system (Zhang et al., 2019). Depending on the severity of TPD, programmed cell death pathways may be triggered (Deng et al., 2016; Liu et al., 2019; Yue et al., 2024).

Progress in our knowledge of physiological and molecular mechanisms involved in TPD has not yet facilitated the development of TPD-tolerant rubber clones. Phenotyping F1 populations for susceptibility to TPD is hampered by the low number of individuals per genotype in small-scale clone trials and by the slow onset of TPD under standard growing conditions. A slow progression of this physiological syndrome is estimated at one to several per cent of trees per year according to the clones, agroclimatic conditions and harvesting systems. A significant incidence of TPD may require many years of tapping. Although TPD may be governed by heritable genes (Chaendaekattu and Mydin, 2014), environmental influence makes it difficult to study the heritability of this trait (Omokhafa and Aniamaka, 2000). TPD markers have been reported from expressed sequence tag libraries without any further application (Li et al., 2013, 2012).

In this context, the application of IHS to trigger the early onset of TPD in *Hevea* populations would appear to be an interesting way of identifying TPD-susceptible genotypes. Rubber clone PB 260 is known to be susceptible to TPD, whereas clone SP 217 is tolerant (Putranto et al., 2015). An F1 population was created from the cross of these two rubber clones (Ismawanto et al., 2024). Juvenile material was first phenotyped for drought tolerance (Cahyo et al., 2022). The population was also planted in a small-scale clone trial for studying the genetic factors linked to latex production (Ismawanto et al., 2024) and tolerance to circular leaf fall disease (Darojat et al., 2024).

In this study, IHS was applied on the small-scale clone trial in the

second year of tapping after one year of tapping every three days. DCL and yield were monitored monthly and at each tapping, respectively. LD was performed once a year during the peak of latex production. For the first, DCL was monitored only every three months and these data used as a baseline. Twenty-six percent of genotypes had an elevated DCL within six months of IHS application. Physiological markers derived from latex diagnosis have a low correlation, making it difficult to predict TPD susceptibility using these markers. IHS also increased the heritability for DCL, and this treatment led to identify seven major QTLs. These results suggest that the IHS treatment is effective in accelerating TPD occurrence. Genotypes with high DCL may be excluded from further large-scale clone trials and recommendations. Finally, analysis of genes underlying the QTLs confirmed the previous findings obtained from transcriptomic analyses.

2. Materials and methods

2.1. Plant material and harvesting systems

An F1 population was created in 2012 at the Sembawa Research Centre of the Indonesian Rubber Research Institute (Ismawanto et al., 2024). A small-scale clone trial was planted in November 2016 with 189 genotypes of the F1 population and eight control clones (AVROS 2037, GT1, IRR 112, IRR 39, PB 260, PR 261, RRIC 100, SP 217). The GPS location coordinates of this trial are: 2°57'42" S 104°3'3" E. The trial consisted of five replicates (plots), and each replicate contained all the genotypes and control clones planted in duplicate (two trees per genotype per plot). The trial was initiated in January 2021. The harvesting system applied from January to December 2021 was half spiral tapping every 3 days (S/2 d3). From January 2022 up to March 2023, the intensive harvesting system with daily tapping and ethephon stimulation every two weeks (S/2 d1 ET 2.5 % 12/y) was carried out according to the protocol established by Herlinawati et al. (2022).

2.2. Determination of dry cut length, latex yield, girth and LD parameters

The level of TPD occurrence was assessed monthly by visually evaluating the percentage of dry cut length (DCL) that did not instantly produce latex after tapping before latex flow from the upper part of the groove.

The yield was measured for each tapping from January 2021 to March 2023. Latex after tapping was left uncollected until full coagulation in the collecting cup to form a cup lump. Before the next tapping, the cup lump was collected and weighed. The rubber yield was calculated based on the dry rubber content (DRC) of the cup lump that was measured periodically (Ismawanto et al., 2024).

The trunk girth was measured at 150 cm from the graft. In order to be able to compare tree production without the effect of the girth, the latex yield was converted in gram of rubber per cm of tapping cut per month.

Latex diagnosis was carried out once a year at the peak rubber production period in South Sumatra from February to April. It consists of four parameters, i.e., sucrose (Suc), inorganic phosphorus (Pi), thiol (RSH) and total solid content (TSC). The determination methods used are those of the 1995 IRRDB Manual described by Ismawanto et al. (2024). Suc, Pi and RSH were determined using 0.5 mL of latex supplemented with 4.5 mL of trichloroacetic acid (TCA) solution at 2.5 %. The serum was filtered with filter paper to obtain clear latex serum. Suc was measured using the anthrone method, while Pi and RSH were determined using the ammonium molybdate and the acid dithiobis nitrobenzoic (DTNB) methods, respectively. The TSC was measured by drying 2 g of fresh latex sample at 90°C for 48 hours. The dry rubber content was obtained from the ratio of the dry weight and fresh weight of latex.

2.3. Data analysis

Data from each tree was stored in a database developed with Microsoft Access (Washington, D.C., USA) and analysed with Microsoft Excel (Washington, D.C., USA) and XLStat (Denver, CO, USA). For each genotype, the value of each replicate was obtained by the average of the two duplicates. LS-means were calculated from the five replicates using ANOVA. K-means clustering, classification using the quantile method, and principal component analysis were performed with XLStat.

2.4. Heritability calculation and QTL detection

Genotype-level heritability (h_f) and best linear unbiased prediction (BLUP) values per genotype were calculated using the lme4 R package for the mixed model equation (Bates et al., 2015). The broad sense heritability at the genotype level is defined as:

$$\frac{V_g}{(V_g + \frac{V_e}{n})}$$

where h_f is the heritability at genotype level, V_g the genetic variance, V_e the environmental variance and n the number of replicates.

QTL detection was performed using MapQTL6 (Kyazma B.V., 1996–2011, Wageningen, Netherlands) with three input files: BLUP values (file.qua) from this study and genotypic files (file.map and file.loc) from a previous study (Ismawanto et al., 2024). The genetic map was built with 3,106 SNPs and 273 SSR markers for 183 individuals. The map covers a total length of 2,046.294 cM with an average distance between markers of 0.60 cM. A non-parametric Kruskal-Wallis (KW) test was carried out because DCL values did not follow a normal distribution. QTLs were selected for a k value higher than 10 with a p -value < 0.0001 for several consecutive months. The figure of QTL mapping was performed with Spidermap v1.7.1 application (Dr Rami, CIRAD, Montpellier, France).

2.5. Identification of genes underlying QTLs

The methods used to identify putative genes within the identified QTLs were identical to those previously described in Ismawanto et al. (2024). Briefly, the HiFi PacBio draft assembly of clone PB 260, obtained as part of the RUBIS project (<https://zenodo.org/doi/10.5281/zenodo.10281548>) was annotated using the Reyan 7–33–97 clone 58,062 predicted full-length cDNA sequences (Tang et al., 2016), using mini-map2 (Li, 2018), and UCSC Kent tools v385 and Bedtools v2.30.0 (Quinlan and Hall, 2010). Functional annotation of PB 260 predicted proteins was obtained using InterProScan-5.67–99.0 (Jones et al., 2014). Gene Ontology (GO) term enrichment was performed by testing terms frequency within individual QTLs versus what is found in the full proteome with the hypergeometric test of the GOfuncR v1.22.2 package (Grote, 2024). Only GO terms with a corrected p -value, i.e. a family-wise error rate (FWER) lower than 0.05 were considered significant and the associated genes were reported.

3. Results

3.1. Dynamic analysis of latex yield and DCL

The distribution of latex yield and DCL was analysed per month from January 2021 to March 2023 for the two harvesting systems used (Fig. 1). Under the d3 harvesting system, yield and DCL ranged from 0.1 to 6.6 g rubber/cm/month and 0–70 %, respectively. The average yield peaked at 1.6 g/cm/month under d3, reaching 7.2 g/cm/month on average under IHS treatment. By contrast, DCL did not change in the same way. However, the box plot showed that the max DCL increased. DCL ranged from 0 % to 70 % under d3 and 0.5–98 % under IHS with an average of 15.1–24 % under d3 and 14.2–36.3 % under IHS, depending on the month.

The k-means clustering for DCL shows three clusters for the F1 population and control clones from January 2021 to March 2023 (Supplemental Table 1). This dynamic analysis of DCL per month revealed that clusters 1, 2 and 3 consist of 98, 61 and 30 individuals,

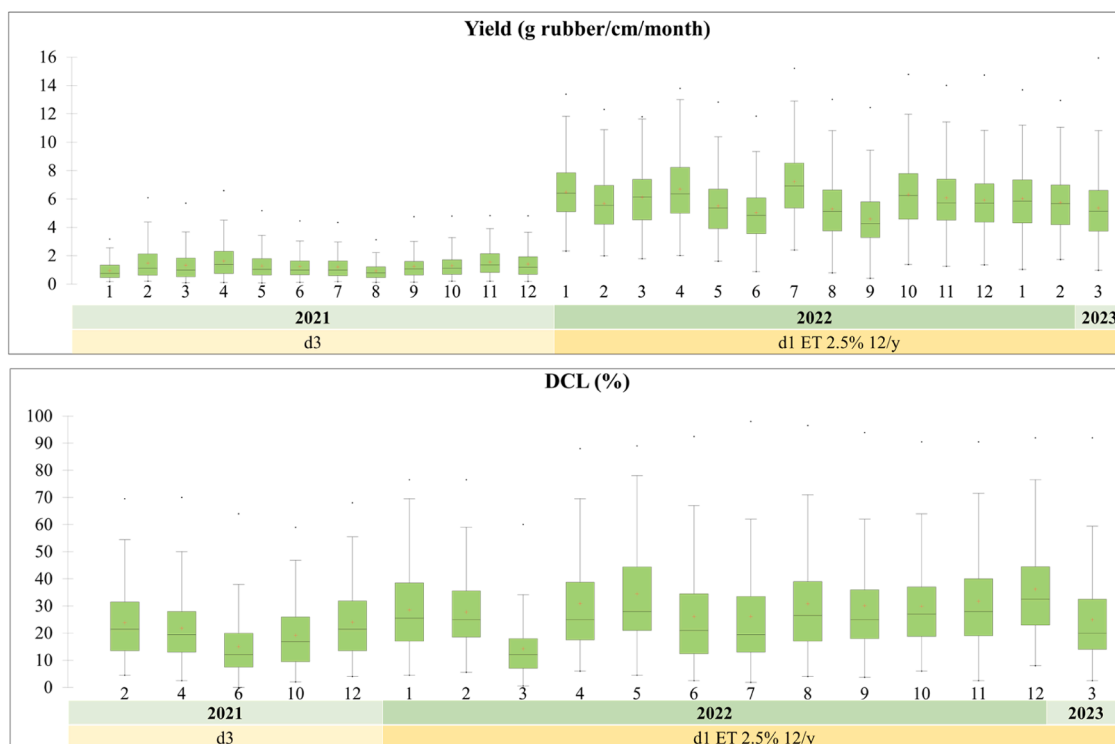


Fig. 1. Box plots illustrating the distribution of yield and DCL per month within the F1 population from January 2021 to March 2023.

respectively (Fig. 2). Individuals of cluster 1 and cluster 2 have the lowest (about 8–26 %) and the intermediate (12–42 %) levels of DCL and did not show any obvious variation between d3 and IHS. By contrast, individuals of cluster 3 has the highest level of DCL under d3 (24–45 %) and under IHS. For the latter, the level of DCL dramatically increased after 3 months of IHS, to vary between 53 % and 70 %.

Based on this result, the average of DCL during the second half year was selected as the best period to identify the TPD-susceptible genotypes. A classification of control clones and F1 population individuals was carried out using the quantile method (Table 1). Seventeen genotypes are in class 1, corresponding to the lowest DCL level, followed by class 2 with 31 genotypes and control clone SP217. Intermediate classes 3 and 4 consist of 45 and 47 genotypes and most of the control clones (class 3: GT1, IRR 112, IRR 39, RRIC 100; class 4: AVROS 2037, PR 261). Finally, classes 5 and 6, for the highest level of DCL, consist of 29 and 20 genotypes. Control clone PB 260 is positioned in class 5.

3.2. Correlation between DCL and other agronomic and physiological traits

Latex diagnosis parameters – sucrose, Pi, RSH and TSC – were determined once a year at the peak season of production from February to April (Supplemental Table 2). The distribution of these parameters was compared for the F1 population to the average of DCL per year (DCL21 and DCL22), the girth measured before harvesting treatment (G20/12 for December 2020) and one year after each treatment (G21/12 and G22/12 for December 2021 and December 2022), as well as the total latex yield per year (Y21 and Y22) (Fig. 3, Supplemental Table 2). The mean of the F1 population for annual average of DCL slightly increased during the application of IHS in 2022 from 20.8 % to 29.0 %. Conversely, the girth increment decreased from 4.4 cm in 2021 to 2.6 cm in 2022. The yield dramatically increased from 15.5 to 70.9 g of rubber per cm per year during IHS. TSC and sucrose content dramatically decreased after 3 months under IHS and slightly increased after 15 months of treatment. Interestingly, the variability inside the population decreased, in particular for sucrose compared to its content in 2021: 3.5–23.6 mM in 2021 to 1.7–5.9 mM in 2022 and 2.7–11.7 mM in 2023. By contrast, the mean of Pi rapidly increased after 3 months from 6.7 on d3 to 19.5 mM under IHS and remained quite stable (20.8 mM) in 2023. For RSH, it decreased from 0.69 to 0.57 mM after one year under IHS application.

A principal component analysis (PCA) was performed using LS-means of measured and calculated agronomic and physiological variables for each genotype (Fig. 4). These parameters are listed in Supplemental Table 2 as: average DCL in 2021 (DCL21), 2022 (DCL22), and second half year 2022 (DCL22S2), total yield in 2021 (Y21) and 2022

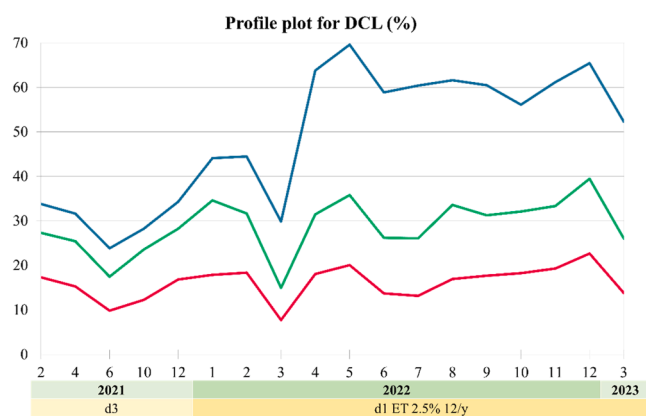


Fig. 2. Dynamic analysis of DCL per month of three k-means clusters for the F1 population and control clones from January 2021 to March 2023. Cluster 1 (red), cluster 2 (green) and cluster 3 (blue).

Table 1

Quantile classification of control clones and genotypes of the F1 population.

Class	Control clone (Name)	Genotype (N°)
1	None	17
2	SP 217	31
3	GT1, IRR 112, IRR 39, RRIC 100	45
4	AVROS 2037, PR 261	47
5	PB 260	29
6	None	20

(Y22), girth in December 2020 (G20), 2021 (G21) and 2022 (G22), and latex diagnosis parameters measured at the peak season in 2021, 2022 and 2023 (Suc21, Suc22, Suc23, Pi21, Pi22, Pi23, RSH22, RSH23, TSC21, TSC22, TSC23). The eigen values and correlations between variables and factors show five and three variables explaining only 42 % of the total variability on the two first axes F1 and F2 (Supplemental Table 3). The F1 axis explains 25 % of the total variability for Y22, G20, G21, G22 and TSC22. The influence of DCL can be observed on the F2 axis, which explain 17 % of the variability. Although the level of explanation is low for this axis, there is a significant negative correlation between the DCL (DCL22 (-0.574) and DCL22S2 (-0.560)), and Y21 (0.601) and Pi21 (0.613).

3.3. Genetic analysis of DCL

Heritability was calculated for DCL at different observation dates in the SSCT1 trial (Supplemental Table 4 and Fig. 5). Hf varies from 0.74 to 0.83 in 2021 under the d3 harvesting system, and from 0.78 to 0.88 under IHS, with a peak from April to September 2022 under IHS.

Given that DCL data do not follow a normal distribution, a non-parametric Kruskal-Wallis test was implemented to detect QTLs. Altogether, seven QTLs were identified (Table 2, Supplemental Table 5) and mapped on the high-density map (Fig. 6). Two QTLs (QTL3 and QTL4) located on linkage groups 5 and 14 had high k values (peaks at 24 and 20) from June 2021 to January 2022, revealing a specific effect of the d3 harvesting system. Among the five QTLs with high k values under d1 ET 2.5 % 12/y, QTL5 on LG17 and QTL6 on LG17 started to have significant k values (9 and 13) in October 2021 under d3, increasing under IHS and peaking in November 2022 for QTL5 (k = 16) and January 2022 for QTL6 (k = 17). Two QTLs located on LG3 and LG18 are more specific of IHS with significant and high k values from July to December 2022 for QTL1 (k_{max}=17) and from May to September 2022 for QTL7 (k_{max}=14).

3.4. Identification of genes underlying QTLs

Genes underlying QTLs were identified in chromosomal regions of the genome sequence of clone PB 260 (Table 3). The size of these QTL regions ranged from 1,022,658 to 58,349,730 bp. The QTL regions contain from 28 to 264 genes. The frequency of GO terms associated with the genes within the QTLs was calculated to identify over- and underrepresented annotations in QTL (Supplemental Table 6). Genes with significant GO terms were found for four QTLs: 24 genes for QTL2, two for QTL3, four for QTL5 and 28 for QTL7. Annotation of these regions revealed several proteins reported linked to with TPD in transcriptomic studies such as transcription factors associated with developmental processes or response to stimuli, including the ethylene signalling pathway, ROS-scavenging systems and water transport channels (Supplemental Table 7).

4. Discussion

4.1. TPD susceptibility cannot be easily predicted by physiological markers

A comparison of the intensity of DCL under IHS in 2022 with latex

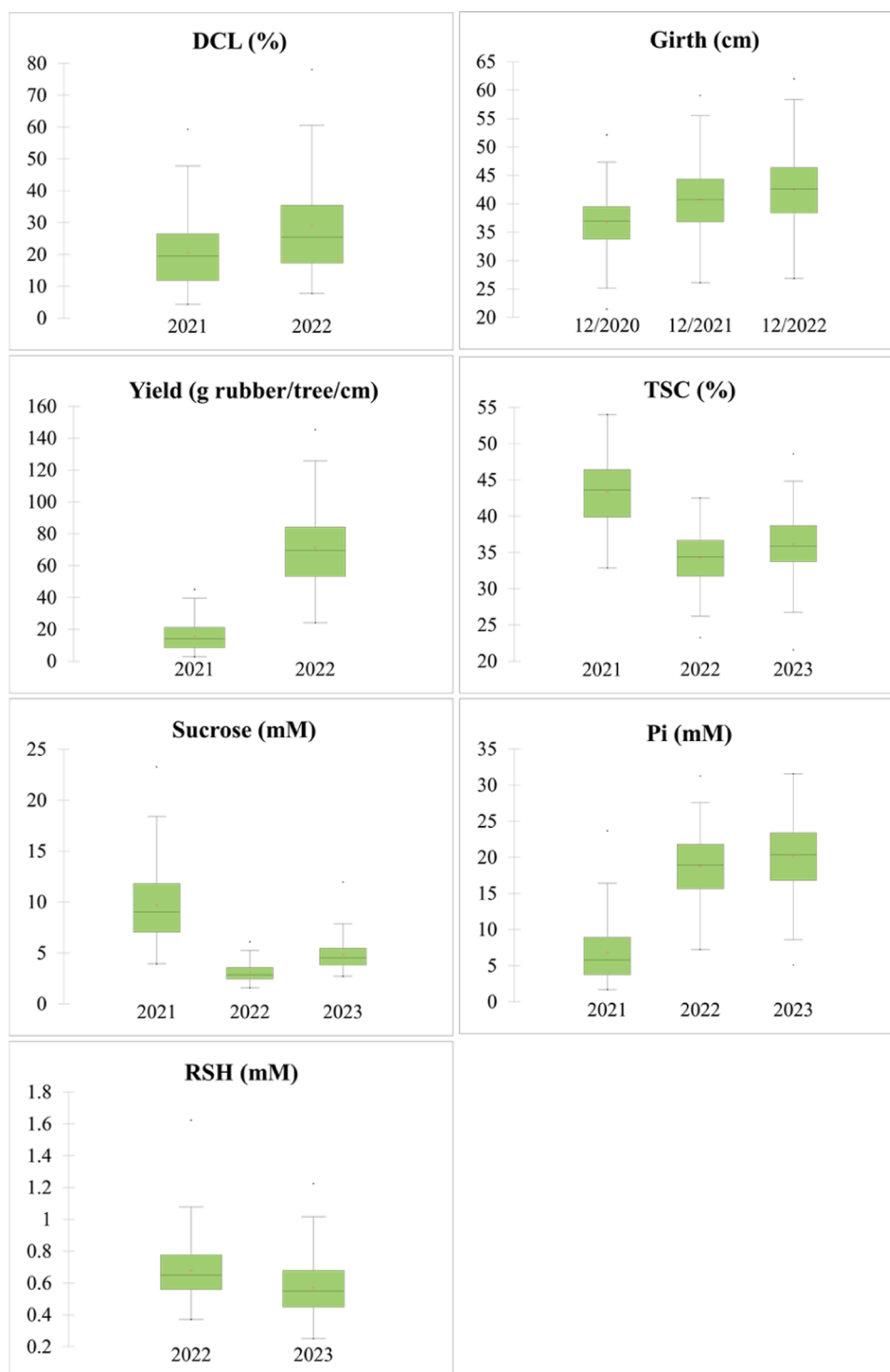


Fig. 3. Box plots illustrating the ranges of variation within the F1 population for the year average of DCL in 2021 and 2022, for the girth in December 2020, 2021 and 2022, for the total yield calculated per cm of tapping per tree in 2021 and 2022, and for latex diagnosis parameters (total soluble content (TSC), sucrose, inorganic phosphorus (Pi), and reduced thiols (RSH)) determined in March 2021, 2022 and 2023.

yield, girth and LD parameters for all harvesting systems revealed a weak correlation. LD parameters, especially from the d3 harvesting system in 2021, cannot be exclusively used to predict the TPD-susceptibility of rubber genotypes observed under IHS. Yield and LD parameters followed a predictable evolution under IHS based on latex physiology. High tapping frequency and ethephon stimulation induced a rapid increase in latex yield and Pi due to the activation of latex metabolism. Pi dramatically increased after three months of IHS application

and then slightly even after one year of IHS treatment. This result suggests that the response of genotypes to IHS is lower after one year under IHS. Conversely, TSC and Suc were reduced as of the first three months of IHS application. TSC decreased due to water uptake in the laticifers induced by ethephon stimulation (Zou et al., 2015). Suc dramatically decreased in 2022, revealing a high sucrose consumption for rubber production under IHS. DCL22 and DCL22S2 showed a weak negative Pearson correlation with Y21 (-0.23 and -0.20) and Pi21 (-0.05 and

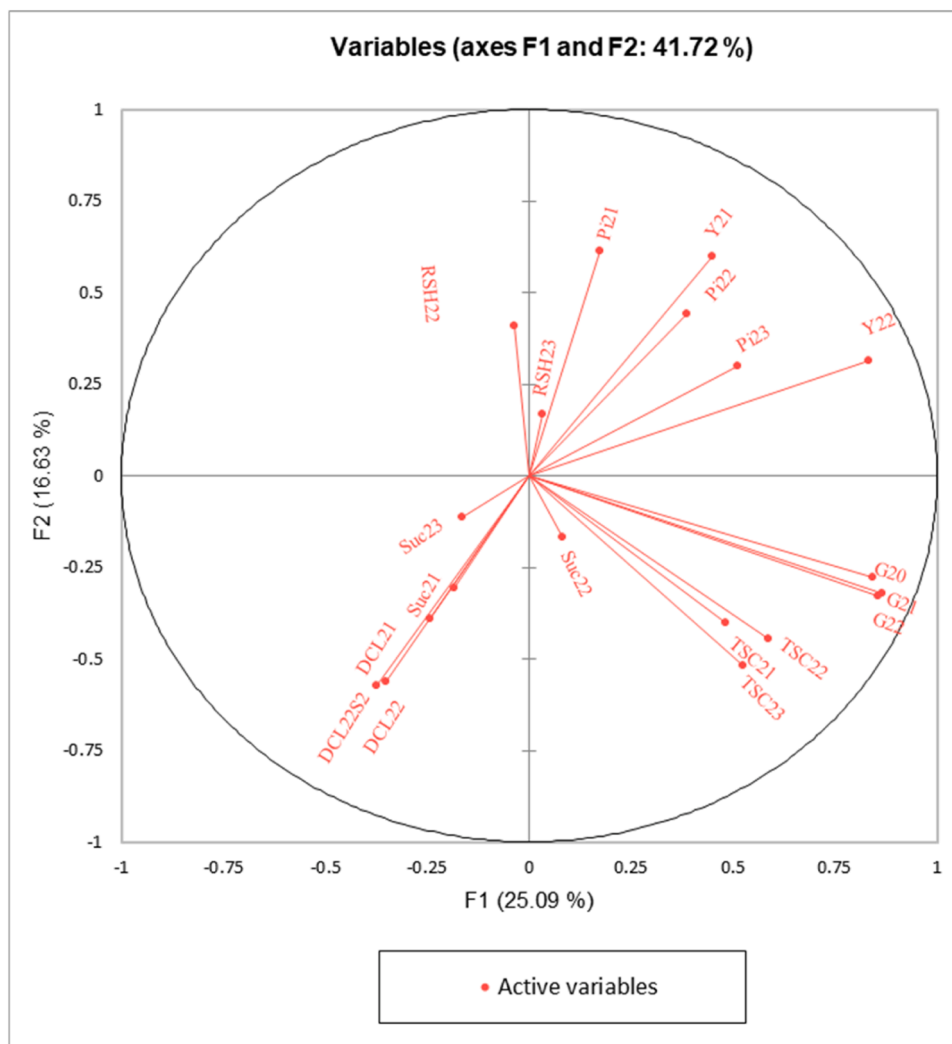


Fig. 4. Principle component analysis of agronomic (yield (Y) and girth (G)) and latex diagnosis (sucrose (Suc), inorganic phosphorus (Pi), thiols (RSH) and total soluble content (TSC)) variables for years 2021, 2022 and 2023.

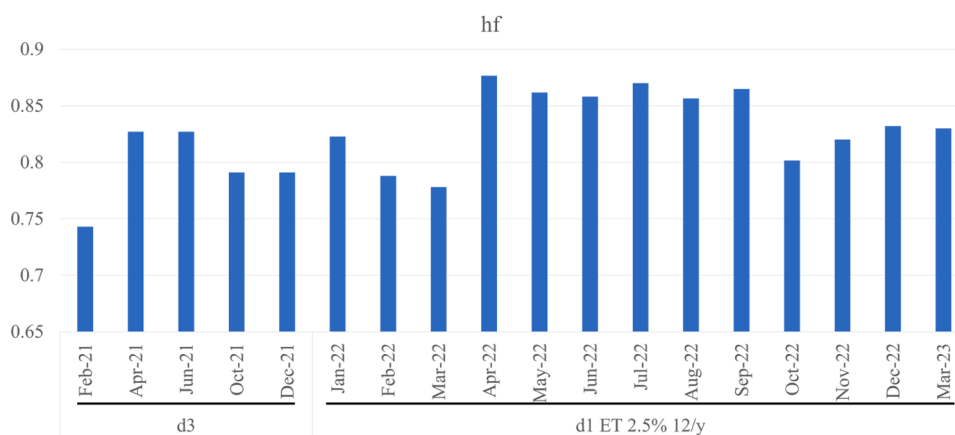


Fig. 5. Family means heritability (hf) of dry cut length at different observation dates.

-0.03). The negative correlation between DCL and sucrose reported by Herlinawati et al. (2022) was not confirmed in this study, suggesting that Suc21 cannot be exclusively used as a physiological marker.

RSH is a stress marker for laticifers used to adjust harvesting systems and prevent TPD occurrence. However, no correlation with DCL was

found in this study. According to a meta-analysis of LD parameters, RSH is not well interpreted in the literature and RSH ranges from 0.11 to 1.03 mM (Junaidi et al., 2023). In this study, RSH could not be determined in 2021. Interestingly, RSH ranged from 0.38 to 1.58 mM three months after application of IHS in 2022, revealing the wide range of RSH

Table 2

QTLs identified for dry cut length under d3 in 2021 and IHS in 2022 and 2022 using the Kruskal-Wallis test. k: Kruskal-Wallis test value; k values in bold are significant with a p-value < 0.0001. Green colour scale is used to highlight high k values.

QTL (N°)	Linkage group (N°)	Position (cM)	Locus	k value																
				2021				2022												2023
				Apr	Jun	Oct	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Mar
				d3				d1 ET 2.5 % 12/y												
QTL1	3	43.731	SNP0935	1	3	1	1	0	8	8	4	9	7	12	11	17	17	15	10	9
QTL2	3	58.616	SNP0979	3	4	4	3	2	11	8	2	8	9	9	11	20	15	19	9	14
QTL3	5	38.517	SNP1716	7	11	17	14	24	7	10	5	2	5	7	2	5	2	3	1	2
QTL4	14	62.876	SNP5515	8	19	19	20	14	10	8	1	2	3	2	3	7	7	5	7	7
QTL5	15	24.761	SNP5780	3	3	9	6	8	13	8	11	8	10	5	7	14	11	16	13	13
QTL6	17	23.783	g17SSH166	3	7	13	7	8	17	16	15	9	11	12	5	7	6	5	9	10
QTL7	18	112.343	g18T1067	1	3	5	2	1	6	6	6	12	13	14	11	12	8	10	6	13

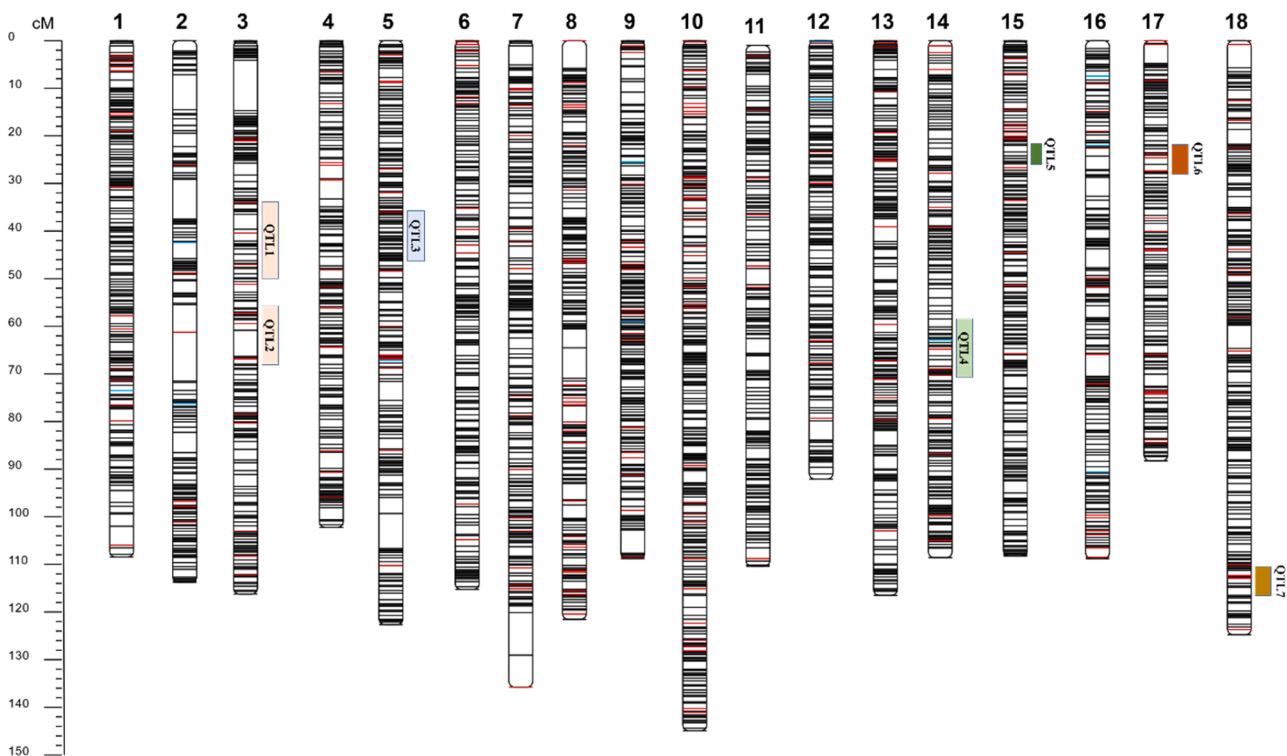


Fig. 6. Linkage map and position of QTLs for DCL. SSR and SNP markers are in red and black bars, respectively.

Table 3

Length of QTL sequences, number of genes underlying the different QTLs, number of genes associated with gene ontology (GO) terms, and number of GO terms per QTL for dry cut length observed in the SSCT1 field trial.

QTL	Linkage group	Contig	Length of QTL sequence	Gene	Gene with significant GO term	Under-represented		Over-represented	
(N°)	(N°)	(N°)	(bp)	(N°)	(N°)	GO (N°)	Gene (N°)	GO (N°)	Gene (N°)
QTL1	3	2	2 796 292	139	0	-	-	-	-
QTL2	3	2	58 349 730	264	24	1	14	3	10
QTL3	5	6	2 923 745	173	2	-	-	1	2
QTL4	14	7	2 812 362	160	0	-	-	-	-
QTL5	15	1	1 022 658	28	4	-	-	1	4
QTL6	17	16	2 432 546	141	0	-	-	-	-
QTL7	18	3	4 086 989	73	28	1	3	11	25

in the F1 population studied and the high level of RSH never recorded (up to 1.58 mM) for some genotypes. In 2023, more than one year after application of IHS, RSH tends to decrease. This is in line with a previous comprehensive analysis of reduced and oxidised antioxidants (Junaidi et al., 2022). Under a short stress (even an intensive stress), laticifers can cope with ROS by supplying antioxidants, whereas after a long stress or TPD occurrence, RSH tends to decrease. It is worth mentioning that sucrose is both a source of carbon for rubber biosynthesis and of energy for latex metabolism. Junaidi et al. (2022) proposed that sucrose is an essential factor to support the synthesis of antioxidants. For that reason, the Suc drop and its low variability in the population in 2022 and 2023, when the population was subjected to IHS stress, compared to 2021, suggests that several factors are responsible for susceptibility to TPD. The low correlation between DCL and both yield and physiological parameters calls into question the hypothesis that quick-starter clones are more susceptible to TPD. For instance, clones IRR 112 and IRR 118 can have a high yield and high Pi, as well as a high sucrose content and low TPD occurrence (Herlinawati et al., 2022; Mydin et al., 2015). The lack of a strong correlation between the percentage of DCL and physiological parameters makes it necessary to apply the intensive harvesting system to identify TPD-susceptible genotypes.

4.2. TPD-susceptible genotypes can be rapidly identified using intensive harvesting

The identification of TPD-susceptible genotypes takes many years under standard harvesting systems. Conventional *Hevea* breeding programmes consist of four main trials: seedling evaluation trial, small-scale and large-scale clone trials, followed by adaptation trials in multiple locations. The whole process takes about 35 years, with the three final steps lasting 10 years, including five years of immature period and five years of tapping. Low incidence of TPD was assessed after six years of small-scale clone trials in India (Mydin and Gireesh, 2016). TPD occurrence can be significantly determined only in large-scale and adaptation trials after 11 years in Brazil (Goncalves et al., 2007) and 16 years in India (Mydin, 2019). Mydin and collaborators then classified clones from the lowest to the highest TPD occurrence, with clone PB 280 (11 % of TPD trees) and PB 314 (27 %), respectively.

Application of IHS induced the early onset of TPD within five months in clone PB 260 (Putranto et al., 2015), and within six months in the segregating population of 189 individuals (this study). Selection under stress conditions such as drought, salinity, heat or nutrient deficiency is common in plants. Stress should be uniformly applied across the breeding population to ensure that observed differences in performance are primarily due to genetic factors. Selection of drought-tolerant rubber genotypes has been attempted under control conditions in the greenhouse (Cahyo et al., 2022). This study is the first to use IHS to select rubber genotypes tolerant to intensive harvesting conditions.

Heritability was dramatically increased under IHS treatment. Heritability is often used by breeders to measure the accuracy of a trial to compute the response to selection (Piepho and Möhring, 2007; Schmidt et al., 2019). Heritability depends on the genetic variance in the population, the environment, and the accuracy of observations (Covarrubias-Pazarán, 2019). In this study, heritability at the family level ranged from 0.74 under d3, to peak at 0.88 under IHS, indicating that 74–88 % of all the phenotypic variation in the severity of TPD is due to variation in genotypes for that trait. This level of heritability is high compared to previous studies: 8 % (Omokhafa and Aniamaka, 2000), 50 % (Chaendaekattu and Mydin, 2014) and 68 % (Mydin et al., 1999). It was already shown that broad sense heritability can increase or decrease under high plant density or drought (Bogale, 2012; Soro et al., 2023).

A high variability of the F1 population for DCL has been observed in this study. This phenotypic variability is often associated with the high heterozygosity of *Hevea brasiliensis* (Liu et al., 2020). Some genotypes have lower and higher DCL levels than parent clones, SP 217 and PB

260, respectively. Early induction of TPD using IHS is a means of exacerbating the susceptibility of rubber genotypes and performing preliminary screening of genotypes at the small-scale clone trial stage. Genotype ranking offers two options for selecting genotypes, either by retaining genotypes with low DCL or by excluding genotypes with high DCL. Given the current state of knowledge, these results suggest excluding the 49 genotypes with high DCL from classes 5 and 6. The DCL genetic value of the 140 remaining genotypes can be compared to other traits studied (yield, LD, leaf disease resistance, etc.) in order to perform a selection combining multiple agronomic and physiological traits.

4.3. Genes underlying QTLs are in line with the literature on molecular mechanisms

This study revealed three groups of QTLs that could be associated with two mechanisms. The first mechanism is linked to the intrinsic viscosity of clones at tree opening. QTL3 and QTL4 were detected under d3 only. Indeed, some genotypes show high DCL at tree opening until complete activation of the laticifer metabolism, either after successive tappings or stimulation by ethephon. Some clones have early high latex viscosity, such as clone PB 260 (Putranto et al., 2015). These genotypes require several ethephon stimulations to activate the metabolism and improve the latex flow during the first tappings after tree opening. The second mechanism is associated with the presence of dry spots in the phloem tissues that make it possible to observe DCL (Herlinawati et al., 2022). QTL1, QTL2 and QTL7 were specifically identified after the induction of TPD under IHS, whereas QTL5 and QTL6 started to be significant 10 months after opening under the d3 harvesting system. QTL6 disappeared after 6 months of IHS.

The three groups of QTLs are located in linkage groups 5 and 14 (high and early viscosity of tree at opening), 15 and 17 (early TPD at the end of d3), and 3 and 7 (high DCL after IHS induction). These QTLs for DCL on LG 3, 5, 14, 15, 17, and 18 are all different than those identified by Ismawanto and colleagues for yield (LG 2 and 16), sucrose (LG1 and 9), Suc loading (LG1), Pi (LG 5, 9, 16) and TSC (LG12 and 16). Pi shares one QTL at LG5 with DCL, but in different positions: 38.5 and 67.2 cM, respectively, without any overlapping (Ismawanto et al., 2024).

Several functions of genes underlying QTLs were previously reported in transcriptomic analyses comparing healthy and TPD-affected trees. Some of these candidate genes were characterized in molecular studies on the involvement of programmed cell death during the onset of TPD (Deng et al., 2018; Liu et al., 2019, 2016; Peng et al., 2011) and the production of ROS (Yang et al., 2022; Yuan et al., 2021; Zhang et al., 2019), ethylene and jasmonate (Putranto et al., 2015), as well as a general repression of gene expression (Montoro et al., 2018). Several Ethylene Response Factors (ERF) were reported to be associated with TPD and linked functions such as *HbERF-Iib2*, an orthologue of ORA47 controlling jasmonate biosynthesis, *HbERF-VIIIa14*, a repressor of programmed cell death regulation, and *HbAP2-10*, a developmental regulator (Putranto et al., 2015). Transcriptomic data may provide additional evidence of the involvement of these genes and their regulation under TPD occurrence within QTLs.

Functional analysis of these candidate genes associated with TPD occurrence is necessary to better understand the mechanisms involved. An efficient genetic modification procedure is available in rubber (Blanc et al., 2006; Leclercq et al., 2010). This method made it possible to carry out several functional analyses of candidate genes (Leclercq et al., 2012; Lestari et al., 2017; Martin et al., 2018; Montoro et al., 2008). However, this approach is restricted to traits observed in plants growing in greenhouses. Although marker-assisted selection is not easy to implement, genome-wide association studies of clonal populations in various agro-climatic conditions and studies of the response of new TPD-tolerant clones to harvesting and environmental stresses appear to be complementary way of demonstrating these mechanisms.

5. Conclusions

This study is the first to use an intensive harvesting system to rapidly determine TPD-susceptible genotypes in an F1 rubber population at the small-scale clone trial stage of breeding programmes. It is also the first genetic analysis of TPD to reveal different genetic bases for intrinsic latex viscosity at tree opening and TPD susceptibility. A high-density genetic map was used to identify genes underlying the seven QTLs. These candidate genes support the hypotheses established by transcriptomic studies and call for further functional analysis studies. It is hoped that this will contribute to a better understanding of TPD and to the development of genetic markers for early selection through marker-assisted selection.

The intensive harvesting system can be used as an effective phenotyping method to identify TPD-susceptible genotypes. Many rubber smallholders tap their trees daily (Cahyo et al., 2024). Development of rubber clones adapted to smallholders' practices is now possible by excluding clones sensitive to high tapping frequency and susceptible to TPD. Tolerance to TPD will make it possible to extend the production cycle in plantations and to retain a large number of productive trees in mature plantations. These new rubber clones should also be tested under extreme agro-climatic conditions to assess their response to climate change.

CRedit authorship contribution statement

Sigit Ismawanto: Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **David Lopez:** Writing – review & editing, Writing – original draft, Validation, Resources, Methodology, Investigation, Formal analysis, Data curation. **Martini Aji:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Pascal Montoro:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Fetrina Oktavia:** Writing – review & editing, Supervision, Resources, Funding acquisition.

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. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.indcrop.2024.120443](https://doi.org/10.1016/j.indcrop.2024.120443).

Data availability

The data are available in the supplementary data file linked to this manuscript

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