Meta-Analysis of a Large Industrial Latex Diagnosis Database Provides Insight on *Hevea brasiliensis* Clonal Adaptation and Site-Specific Yield Potential in Western Africa

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Summary

A meta-analysis of the Latex Diagnosis (LD) database gathering all LD data stored from 2006 to 2018 in SIFCA/SIPH plantations of Côte d'Ivoire (SAPH), Ghana (GREL) and Nigeria (RENL) has been performed. Average clonal LD data comprising Sucrose (Suc), Inorganic Phosphorus (Pi), Reduced Thiols (RSH) and latex Total Solid Content (TSC) were analyzed and compared for different plantation sites of SAPH (Toupah, Ousrou, Bongo, Rapides Grah, Digahio, Divo and Bettie), GREL (Abura) and RENL (Osse River, New Land, Utagba Uno, Araromi, Waterside and Iguobazuwa). The database was filtered in order to keep only the LD data obtained either from conventional and standard tapping systems (S/2 downward and S/4 upward), excluding the intensified period before slaughtering. Data were processed and analyzed on clones GT1, RRIM600, PR107, AV2037, RRIC100, AF261, PB5/51, PB217, PB235, PB260, PB312, PB314, IRCA18, IRCA41, IRCA109, IRCA111, IRCA130, IRCA209 and IRCA230. All clones were tapped either in d4 or d5 6d/7 tapping frequencies. Stimulation was applied on panel (Pa), under industrial stimulation rates adapted to each clone metabolism and clonal sugar loading characteristics. Based on the relation between Suc and Pi, the analysis of these LD data confirms the latex physiological positioning in clonal typology of all clones. It confirms in particular the high latex sugar loading capacity of clones PB217 (as this clone obtains the highest latex Suc level whatever the plantation site), IRCA109, IRCA41 and IRCA230. It also reveals a systematic positive correlation for all sites between RSH and Suc latex contents: Clones with high latex Suc maintain higher RSH latex concentrations than clones with lower Suc, confirming at industrial scale earlier research results. The physiological hypothesis is that a high clonal latex sugar loading, allowing higher stimulation rates and improved stimulation response, would as well maintain higher latex RSH levels resulting in an improved resistance to oxidative stress resulting from latex metabolic activation. This improved scavenging protection would therefore have positive effects on membranes integrity, on latex stability, on latex flow and on tapping panel dryness (TPD) onset. Moreover, as tapping intensity (tapping frequency and stimulation) was almost similar in all sites, we also make the hypothesis that the latex RSH level and the Pi x RSH value might be accurate indicators to describe, at plantation site level, the local clonal suitability as well as the global stress conditions of trees on each plantation site. This study will be completed later on in order to set up a standard interpretation method of such LD databases.

Keywords: *Hevea brasiliensis*, meta-analysis, latex harvesting, latex diagnosis, latex physiological parameters, database, clonal typology, latex sugar loading, metabolism, oxidative stress.

1. Introduction

Latex Diagnosis (LD) is currently used worldwide by most of rubber agroindustries as a routine physiological tool to optimise, at block level, the rubber yield production of the rubber plantations. During the peak production period (August to November in Northern Hemisphere, February to May in Southern Hemisphere), the simultaneous analysis and comparison of:

- latex Sucrose content (Suc, indicator representing the possibilities of exploitation intensification);
- latex Inorganic Phosphorus content (Pi, indicator of the energetic level of latex cells metabolism);
- latex Reduced Thiols content (RSH, indicators of cell and lutoidic membranes protection against Reactive Oxygen Species (ROS) released during latex metabolic activation);
- latex DRC/TSC (indicator of the balance between water importation to the latex cells and cytoplasmic biosynthesis resulting from latex regeneration,

provides a functional picture of the physiological condition of latex cells at the moment of sampling/analysis and permits to derive a diagnosis of normal, under or over exploitation (Eschbach *et al.*, 1984, Jacob *et al.*, 1985, 1988a, 1988b, 1995a, 1997, 1998, d'Auzac *et al*., 1997, Gohet *et al*., 2008).

LD interpretation depends on former set up LD parameters reference values. These ones are established for the 4 parameters used in LD: latex sucrose content (Suc, $mM.l^{-1}$), latex inorganic phosphorus content (Pi, $mM.l^{-1}$), latex reduced thiols content (RSH, $mM.l^{-1}$) and DRC/TSC (%). These LD reference values are established for 5 limit levels (very low, low, normal, high and very high), for each LD parameter (Suc, Pi, RSH and DRC/TSC), either at regional scale or, in case of large estates and companies, at plantation scale when local LD parameters database is large enough. LD reference values are usually set up for each clone, not taking into account panel cut positions or implemented tapping systems. LD interpretation lays in the combination of the levels of each LD parameter (Suc, Pi, RSH and DRC/TSC) in comparison with the corresponding parameter reference values. Therefore, it is exactly the same principle as that used for interpretation of blood analyses in human or veterinarian medicine.

To set up correctly these LD reference values, it is required to know what can be the general evolution of the 4 LD parameters (Suc, Pi, RSH and DRC/TSC) depending on exploitation intensity. In the agro-industry, tapping frequency is usually fixed, as it is mostly determined by manpower availability and cost in regard of the rubber price, as well as housing and social facilities. For these reasons, stimulation intensity is the most often used parameter used in rubber agro industries using reduced tapping frequencies to modulate their exploitation intensity (Gohet *et al*., 1991, Gohet *et al*., 2008).

To perform an accurate LD interpretation, it is therefore essential to know and to understand what can be the effect of stimulation on each LD parameter.

INFLUENCE OF ETHEPHON STIMULATION ON LD PARAMETERS (SUC, Pi, RSH and TSC/DRC).

- **Effect on Latex Sucrose content (Suc, $mM.\ l^{-1}$): Figure 1**

Whatever the tapping frequency, increase in Ethephon stimulation intensity generally results in a hyperbolic decrease of Latex Sucrose content. This decrease (from very high Suc values to very low Suc values) is due to increased sucrose consumption from the latex regeneration metabolism. It is however remarkable that overexploitation, due to excessive stimulation intensity, results in a simultaneous drop of production and Latex Sucrose content (Lacrotte 1991, Gohet 1996, Gohet *et al*.,1997a, Gohet *et al*., 2008).

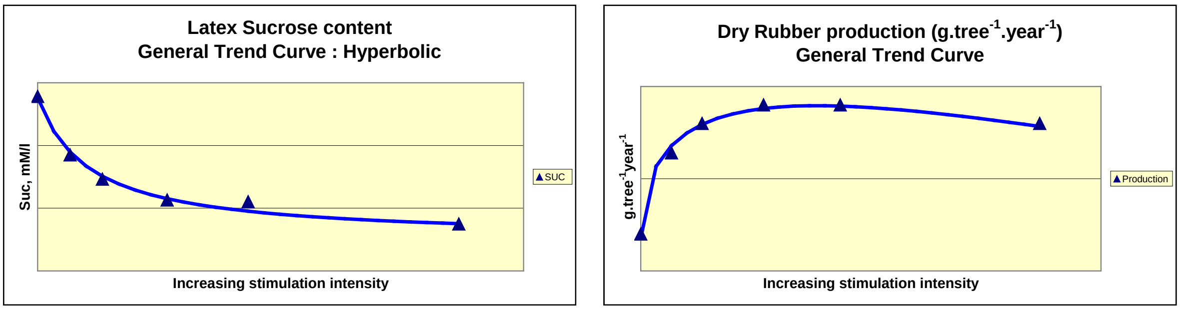


Figure 1: Simultaneous effect of Ethephon stimulation intensity on Latex Sucrose Content (Suc, mM.l^{-1}) and on dry rubber production (g.tree^{-1}.year^{-1}). Clone GT1 in Côte d'Ivoire. Average values after 7 years of tapping.

- **Effect on Latex Pi content (Pi, mM.l^{-1}): Figure 2**

Whatever the tapping frequency, increase in Ethephon stimulation intensity generally results in a parabolic evolution of Latex Pi content. For lower stimulation intensities, increase of stimulation results in metabolic activation (increase of Pi, until reaching a maximum level). It is afterwards followed by a drop of Pi for higher intensities. This drop of Pi is a typical and significant sign of overexploitation and is always associated with a drop of production. (Gohet *et al*., 1995, 1996, Gohet 1996, Gohet *et al*., 2008).

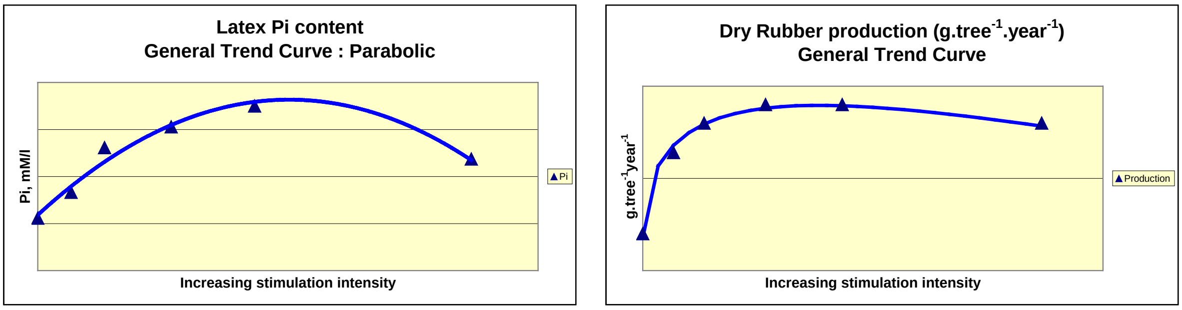
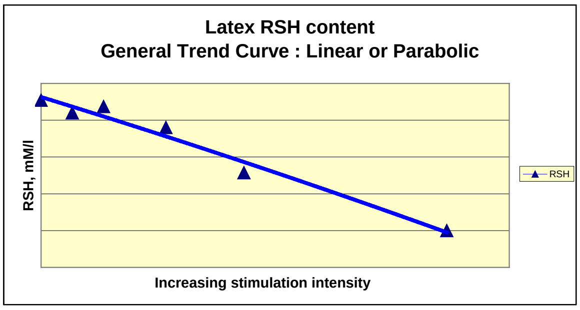


Figure 2: Simultaneous effect of Ethephon stimulation intensity on Latex Pi Content (Pi, mM.l^{-1}) and on dry rubber production (g.tree^{-1}.year^{-1}). Clone GT1 in Côte d'Ivoire. Average values after 7 years of tapping.

- **Effect on Latex RSH content (RSH, mM.l^{-1}): Figure 3**

Whatever the tapping frequency, increase in Ethephon stimulation intensity generally results in a linear decrease of Latex RSH content. This decrease (from very high RSH values to very low RSH values) is due to an enhanced synthesis of reactive oxygen species (ROS) produced during latex cells metabolic activation. These released ROS oxidise RSH groups, leading to the decrease of their content in the latex. Sometimes, for low intensities of stimulation and/or for low metabolism clones, RSH curve may show a slight increase before starting decreasing again. Such a transient increase of RSH shows in such case the effect of metabolic activation on RSH synthesis, as this synthesis requires energy (Gohet 1996, Gohet *et al.* 1997, Gohet *et al*. 2008).



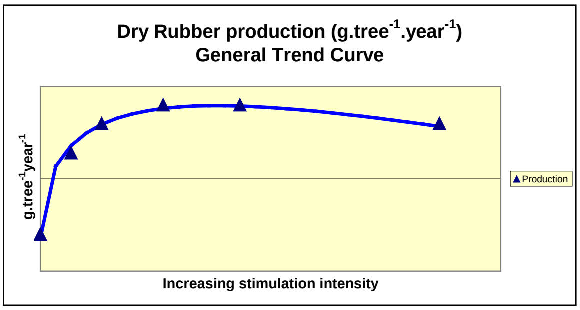
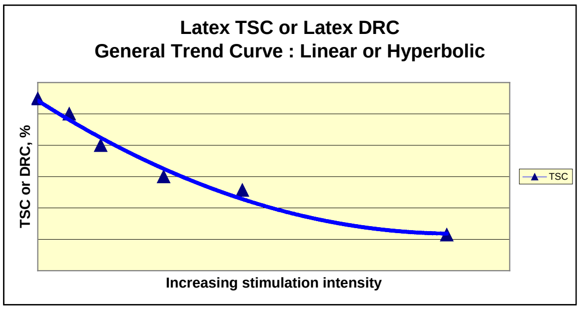


Figure 3: Simultaneous effect of Ethephon stimulation intensity on Latex RSH Content (RSH, mM.l^{-1}) and on dry rubber production (g.tree^{-1}.year^{-1}). Clone GT1 in Côte d'Ivoire. Average values after 7 years of tapping.

1. Effect on TSC/DRC (%): Figure 4

Whatever the tapping frequency, increase in Ethephon stimulation intensity generally results in a linear or hyperbolic decrease of Latex DRC/TSC. This decrease (from very high DRC/TSC values to very low DRC/TSC values) is first due to enhanced water importation to the latex (activation of water transport). Sometimes, for low intensities of stimulation and/or for low metabolism clones, DRC/TSC curve may show a slight increase before starting decreasing again. Such a transient increase of DRC/TSC shows in such case the effect of metabolic activation on total cytoplasm syntheses, permitted by the metabolic activation of latex cells and concurrent release of metabolic energy (Gohet 1996). In fact, DRC and TSC give a global view of the balance between two antagonist effects of stimulation: Enhancement of water importation into the latex (Decrease of DRC/TSC) and enhancement of syntheses following metabolic activation (Increase of DRC/TSC). DRC/TSC is therefore an integrated parameter whose significance is less precise than those of the three other parameters (Suc, Pi and RSH). DRC/TSC is therefore mainly used as a confirmation of LD interpretation derived from combination of Suc, Pi and RSH levels.



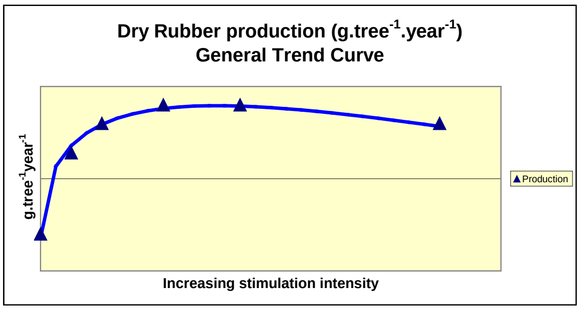


Figure 4: Simultaneous effect of Ethephon stimulation intensity on TSC or DRC (%) and on dry rubber production (g.tree^{-1}.year^{-1}). Clone GT1 in Côte d'Ivoire. Average values after 7 years of tapping.

INDUSTRIAL LATEX DIAGNOSIS: PRINCIPLES AND APPLICATIONS.

In plantations where LD is performed and where it has been used as a physiological tool to pilot this exploitation intensity at local (block per block) level, the strategy is as follows:

1. First step.

During the first months of the physiological year (For instance in Northern hemisphere: April to End–August / Mid - September, that is 5 months after completed refoliation), a unique tapping frequency and an associated stimulation intensity are applied to every homogenous cultural unit (Same clone, same planting year, same planting material, same date of opening, same tapping panel position and same plantation division). This implemented stimulation intensity must be as precise as possible. It must take into account the clonal latex physiological characteristics and therefore a latex clonal typology (Jacob *et al*. 1995b, Gohet *et al.* 1997, 2005, Thanh and Thuy 2005), but also factors like year of tapping, tapping panel position, tapping direction (downward tapping, upward tapping, combined upward/downward…), tapping cut length (½S, ⅓S or ¼S) and tapping frequency (d/3, d/4, d/5 or d/6).

The latex clonal typology established by Cirad lays in a classification of *Hevea brasiliensis* clones in a 2 dimensions-matrix (Table 1) containing five different metabolic types (low, low-medium, medium, medium-high and high) and three different latex sugar loading types (low, medium and high). This typology permits in particular to describe the response of any rubber tree clone to ethephon stimulation: stimulation intensity is to be increased when clonal latex metabolic activity decreases and/or when clonal latex sugar loading capacity increases. Conversely, stimulation intensity is to be decreased when clonal latex metabolic activity increases and/or when clonal latex sugar loading capacity decreases. Latex Clonal Typology therefore greatly simplifies stimulation recommendations, as only five different levels of stimulation intensity (very high, high, medium, low and very low) provide accurate stimulation recommendations to all physiological types of clones, depending on their respective position in the matrix (Jacob *et al*. 1995a, Gohet *et al*. 2005, Gohet *et al*. 2008, Lacote *et al*. 2010, Gohet *et al*. 2016, 2017).

This physiological modelling thus allows predicting, in case of use of reduced tapping frequencies (d/3, d/4, d/5 and d/6), the recommended ethephon stimulation intensity that will be required for the clonal yield potential expression. In fact, these stimulation recommendations can be associated to 5 matrix diagonals, limiting to 5 the total number of possible stimulation recommendations for all clones

- **Very high stimulation intensity (diagonal 1): classes c2 and c5**
  - Low metabolism x medium sugar loading (c2: AF 261…)
  - Low-medium metabolism x high sugar loading (c5: PB 217…)

- **High stimulation intensity (diagonal 2): classes c1, c4 and c8**
  - Low metabolism x low sugar loading (c1: AV2037…)
  - Low-medium metabolism x medium sugar loading (c4: PR 107…)
  - Medium metabolism x high sugar loading (c8: RRIC 121…)

- **Medium stimulation intensity (diagonal 3): classes c3, c7 and c11**
  - Low-medium metabolism x low sugar loading (c3)
  - Medium metabolism x medium sugar loading (c7: GT1, RRIC 100…)
  - High medium metabolism x high sugar loading (c11: IRCA 41, IRCA 19, RRIM 921…)

- **Low stimulation intensity (diagonal 4): classes c6, c10 and c13**
  - Medium metabolism x low sugar loading (c6)
  - Medium-high metabolism x medium sugar loading (c10: RRIM 600, BPM 24, IRCA 18, PR 255…)
  - High metabolism x high sugar loading (c13: IRCA 230, RRIM 712…)

- **Very Low stimulation intensity (diagonal 5): classes c9 and c12**
  - Medium-high metabolism x low sugar loading (c9)
  - High metabolism x medium sugar loading capacity (c12: PB 235, PB 260, PR 261…)

This modelling is of great importance as it also permits significant reduction of time usually required to introduce newly selected clones into rubber estates. As a matter of fact, a few yield and physiological data obtained from these new clones, compared to those of control clones under same tapping conditions, are sufficient to precise their position in the typology matrix and therefore to perform early and accurate stimulation recommendations for these new clones. Long-lasting tapping system experiments usually set up to optimise stimulation recommendations are therefore less and less necessary.

Table 1: **Cirad Clonal Latex Typology, 2019 update**

Clonal Metabolic Typology. CIRAD

Physiological basis for tapping systems recommendations (tapping frequency, stimulation)

	Low Metabolism Met-	**Low-Medium Metabolism Met -=**	**Medium Metabolism Met =**	**Medium-High Metabolism Met =+**	**High Metabolism Met +**
Low Sugar Loading (Suc -)	***Typology c1*** ***Met - Suc -*** AVROS 2037	***Typology c3*** ***Met -= Suc -*** FDR5597	***Typology c6*** ***Met = Suc -*** CDC56 **Low Stim** **c6, c10, c13**	***Typology c9*** ***Met =+ Suc -*** **Very low Stim** **c9, c12**	***Low probability***
Medium Sugar Loading (Suc =)	***Typology c2*** ***Met - Suc =*** AF 261 FDR5283 MDX624 MDX607 FDR5802 **Very high Stim** **c2, c5**	***Typology c4*** ***Met -= Suc =*** PB 86 PR 107 FDR5665 **High Stim** **c1, c4, c8**	***Typology c7*** ***Met = Suc =*** GT1, IRCA840 **RRIC 100**, IRCA323 FDR5240, IAN710 CDC312 **Medium Stim** **c3, c7, c11**	***Typology c10*** ***Met =+ Suc =*** RRIM 600, PB 5/51 BPM 1, IRCA18 BPM 24, RRIM703 RRIC 110, **RRIT251** PR 255, **PB280** RRIM 937, IRCA 209 RRIM706, RRIM934	***Typology c12*** ***Met + Suc =*** PB 235, **RRIM 911** **PB 260**, PR 261 PB 340, IRCA 111 RRIM 901, IRCA 130 PB 312, **PM10** PB 314, IRCA804 **IRCA317**, **PB350**
High Sugar Loading (Suc +)	***Low probability***	***Typology c5*** ***Met -= Suc +*** **PB 217**	***Typology c8*** ***Met = Suc +*** RRIC 121	***Typology c11*** ***Met =+ Suc +*** **IRCA 19**, **IRCA109** **IRCA 41**, **IRCA331** **RRIM 921**, **PB330** **PB254**	***Typology c13*** ***Met + Suc +*** **IRCA 230**, FDR4575 **RRIM 712**, FDR5788 **PB255**, PMB1

In Bold, Cirad recommended clones for industrial plantings

In Blue, CMS Clones

Diagonals of the [5,Met x 3,Suc] matrix : Homogenous stimulation recommendations

Updated 03/2019

2. Second step.

At the end of the first period (End August until Mid October in Northern Hemisphere, End February until Mid April in Southern Hemisphere), LD (latex field sampling, analysis of physiological parameters and interpretation) is performed on each homogenous cultural unit of each plantation. For each one, LD is performed to assess the latex physiological status on the cultural unit after at least 5 months of tapping and stimulation under the initial stimulation intensity. After LD interpretation, 5 types of conclusions and decisions may be taken. These conclusions and associated decisions are summed up in the following table (**Table 2**):

A new stimulation rhythm is generally applied from October to January (Northern Hemisphere) or from April to July (Southern Hemisphere), the peak yielding months. This permits the fine local optimisation (block per block) of tapping and stimulation intensities during the peak production period and therefore permits to get a maximum and sustainable rubber yield, balanced with the actual physiological yield potential of each plantation block, creating a safe and significant added production and value.

The process has to be renewed every year in order to take into account the physiological modifications of the latex induced by age, tapping position and direction on panel, etc. After a while, a large database is created, which permits to get more and more accurate and precise clonal Latex Diagnosis reference values, which are the median values observed.

Table 2: Recommended modifications of stimulation intensity following LD interpretation (Gohet *et al* 2008).

Latex Diagnosis Interpretation	Decision regarding stimulation intensity	Stimulation planning modification (rounds per year)	Example
Overexploitation status (LD --)	Stimulation to be decreased , depending on clonal typology and risk level strategy	-2/Y or -1/Y	Et2.5% 8/Y ↓ Et2.5% 6/Y or Et2.5% 7/Y
Light overexploitation status (LD -)	Stimulation to be or slightly decreased or maintained, depending on clonal typology and risk level strategy	-1/Y or 0/Y	Et2.5% 8/Y ↓ Et2.5% 7/Y or Et2.5% 8/Y
Optimal physiological status (LD =)	Stimulation to be maintained or slightly increased, depending on clonal typology and risk level strategy	0/Y or +1/Y	ET2.5% 8/Y ↓ Et2.5% 8/Y or Et2.5% 9/Y
Light underexploitation status (LD +)	Stimulation to be increased, depending on clonal typology and risk level strategy	+1/Y or +2/Y	ET2.5% 8/Y ↓ Et2.5% 9/Y or Et2.5% 10/Y
Underexploitation status (LD ++)	Stimulation to be increased, depending on clonal typology and risk level strategy	+2/Y or +3/Y	ET2.5% 8/Y ↓ Et2.5% 10/Y or Et2.5% 11/Y

2. Material and Methods

From 2006, after installation of 3 latex diagnosis laboratories in SAPH, GREL and RENL and training by Cirad, this strategy was implemented in all SIFCA/SIPH rubber plantations: For each plantation site, all stimulations were implemented at block level according to Cirad clonal typology, in compliance with industrial SIFCA/SIPH standard stimulation rates. Then they were fine-tuned after latex diagnosis interpretation to adjust the stimulation intensity to the local physiological status. All clones were tapped either in d4 or d5 6d/7 tapping frequencies.

The objective of this presentation is to analyze the LD database stored for all plantation sites. This is to provide insight on the clonal local behavior and adaptation and to help to set up accurate recommendations for further replantings. Another objective is to get insight on each plantation site characteristics as related to their yield potential and general trees physiological conditions.

A meta-analysis of the Latex Diagnosis (LD) database gathering all LD data stored from 2006 to 2018 in SIFCA/SIPH plantations of Côte d'Ivoire (SAPH), Ghana (GREL) and Nigeria (RENL) has therefore been performed. Average clonal LD data comprising latex Sucrose content (Suc), latex Inorganic Phosphorus content (Pi), latex Reduced Thiols (RSH) and latex Total Solid Content (TSC) were analyzed and compared for different plantation sites of:

- SAPH in Côte d'Ivoire (7 plantations: Toupah (TPH), Ousrou (OU), Bongo (BG), Rapides Grah (RG), Digahio (DGH), Divo (DV) and Bettie (BT))
- GREL in Ghana (1 plantation: Abura (GREL)
- RENL in Nigeria (6 plantations: Osse River (OR), New Land (NL), Utagba Uno (UU), Araromi (ARA), Waterside (WS) and Iguobazuwa (IBZ).

The database was filtered in order to keep only the LD data obtained either from conventional and standard tapping systems (S/2 downward and S/4 upward), excluding the intensified period before slaughtering. Data were processed and analyzed on clones GT1, RRIM600, PR107, AV2037, RRIC100, AF261, PB5/51, PB217, PB235, PB260, PB312, PB314, IRCA18, IRCA41, IRCA109, IRCA111, IRCA130, IRCA209 and IRCA230. Newly introduced and promising clones as IRCA317 and IRCA331 were discarded from the meta-analysis as fewer LD data were available from those clones.

Analysis comprised 7387 LD data for each of the 4 parameters (2831 for SAPH Côte d'Ivoire, 1893 for GREL Ghana and 2663 for RENL Nigeria), each LD data being itself the average of 4 elementary analyses (LD is performed on 4 tapping tasks).

3. Results

COMPARISON BETWEEN SITES

- **Per country.**

The table 3 shows the global averages (all clones, systems and ages) of Suc, Pi, RSH and TSC values calculated per each of the 3 countries (Côte d'Ivoire, Ghana and Nigeria) from 2006 to 2018.

Table 3. Average LD values observed from 2006 to 2018 in the 3 countries (Côte d'Ivoire / CI, Ghana /GH and Nigeria /NGA). All clones, all systems.

All Clones	Suc	Pi	RSH	TSC
Côte d'Ivoire (CI)	9.01	20.83	0.43	45.63
Ghana (GH)	9.41	16.19	0.54	50.15
Nigeria (NGA)	8.17	21.10	0.34	45.62
CV%	7%	14%	22%	6%

An overall interpretation at this global scale indicates a trend of lower metabolic activation (higher Suc, RSH and TSC, lower Pi) in Ghana than in Côte d'Ivoire and in Nigeria. In the contrary, the low RSH associated with lower Suc and higher Pi observed in Nigeria indicate a general higher metabolic activation. The most balanced profile is the one observed in Côte d'Ivoire.

- **Per plantation site.**

The table 4 shows the global averages (all clones, systems and ages) of Suc, Pi, RSH and TSC values calculated per each of the 14 plantations from 2006 to 2018. Ranking is from the highest RSH value, obtained in the Nigerian plantation of Iguobazuwa, to the lower RSH value, obtained in the Nigerian plantation of Utagba Uno.

Table 4. Average LD values observed from 2006 to 2018 in the 14 SIFCA/SIPH plantations. All clones, all systems. RSH ranking from highest to lowest value.

All Clones	Plantation	Suc	Pi	RSH	TSC
Nigeria (NGA)	IBZ	7.32	17.87	0.54	46.11
Ghana (GH)	GREL	9.41	16.19	0.54	50.15
Côte d'Ivoire (CI)	TPH	7.73	21.93	0.53	45.03
Côte d'Ivoire (CI)	OU	8.04	21.71	0.45	45.88
Côte d'Ivoire (CI)	BG	8.83	26.93	0.44	46.07
Côte d'Ivoire (CI)	BT	11.88	14.59	0.42	46.41
Côte d'Ivoire (CI)	DV	6.87	20.36	0.40	46.52
Nigeria (NGA)	OR	9.91	21.14	0.39	44.90
Côte d'Ivoire (CI)	DGH	10.90	17.62	0.36	45.94
Nigeria (NGA)	WS	8.12	21.63	0.35	45.92
Nigeria (NGA)	ARA	7.53	21.66	0.35	45.68
Nigeria (NGA)	NL	8.20	21.47	0.34	45.19
Côte d'Ivoire (CI)	RGH	9.52	14.49	0.33	44.19
Nigeria (NGA)	UU	8.32	19.22	0.30	45.98
CV%		16%	17%	20%	3%

With a variation coefficient of only 3%, TSC cannot be considered as an efficient parameter to discriminate the different sites. Except for the plantation site of Iguobazuwa, RSH measured in Nigerian sites are mostly lower (<0.40 mM/l) than those observed in Côte d'Ivoire or in Ghana. In Côte d'Ivoire, only the plantations of Divo (DV), Digahio (DGH) and Rapides Grah (RGH) get similar RSH values to Nigerian plantation sites. Those lower values of RSH can't be correlated in any way with those of Suc and Pi, quite similar for Côte d'Ivoire and Nigeria plantations. For instance, the metabolic parameters (Suc and Pi) are quite similar in Ousrou / Côte d'Ivoire (respectively 8.04 mM Suc and 21.71 mM Pi) and for Waterside / Nigeria (respectively 8.12 mM Suc and 21.63 mM Pi), but RSH are however very different between the 2 sites (0.45 mM RSH in Ousrou, 0.35 mM RSH in Waterside).

COMPARISON BETWEEN SYSTEMS

- **Per country.**

The table 5 shows the global averages (all clone and ages) of Suc, Pi, RSH and TSC values calculated per each of the 3 countries from 2006 to 2018, depending on the tapping systems (downward tapping S/2 or upward tapping S/4U).

Table 5. Average LD values observed from 2006 to 2018 in the 3 countries (Côte d'Ivoire / CI, Ghana /GH and Nigeria /NGA). All clones. Comparison between S/2 (downward) and S/4U (upward) systems.

All Clones	System	Suc	Pi	RSH	TSC
Côte d'Ivoire (CI)	S/2	7.22	21.84	0.44	44.27
Côte d'Ivoire (CI)	S/4U	11.44	19.47	0.41	47.47
Ghana (GH)	S/2	8.46	17.35	0.53	50.05
Ghana (GH)	S/4U	11.10	14.16	0.56	50.33
Nigeria (NGA)	S/2	7.22	21.75	0.36	46.19
Nigeria (NGA)	S/4U	9.70	20.07	0.32	44.69
CV%		20%	15%	21%	6%

An overall interpretation at this global scale confirms the trend of lower metabolic activation (higher Suc, RSH and TSC, lower Pi) in Ghana than in Côte d'Ivoire and in Nigeria. This trend to under-exploitation seems more significant in S/4U (lower Pi, higher RSH) than in S/2. The low RSH observed in Nigeria are encountered on both S/2 and S/4U. The most balanced profiles is those observed in Côte d'Ivoire, both for S/2 and S/4U. In all 3 countries, the 2 systems differ especially by their Suc content (higher Suc in S/4 U), which is logical (higher Suc supply due to higher carbohydrate reserves and direct supply by phloem flow).

- **Per plantation site.**

The table 6 shows the global averages (all clones and ages) of Suc, Pi, RSH and TSC values calculated per each of the 14 plantations from 2006 to 2018, for S/2 and S/4U systems. Ranking is from the highest RSH value, obtained in the Ghanean plantation of GREL (S/4U) to the lowest RSH value, obtained in the Nigerian plantation of Utagba Uno (S/4U).

Table 6. Average LD values observed from 2006 to 2018 in the 14 SIFCA/SIPH plantations. All clones. S/2 and S/4U systems. RSH ranking from highest to lowest value.

All Clones	Plantation	System	Suc	Pi	RSH	TSC
Ghana (GH)	GREL	S/4U	11.10	14.16	0.56	50.33
Nigeria (NGA)	IBZ	S/2	7.32	17.87	0.54	46.11
Côte d'Ivoire (CI)	TPH	S/2	7.17	22.42	0.53	44.64
Ghana (GH)	GREL	S/2	8.46	17.35	0.53	50.05
Côte d'Ivoire (CI)	TPH	S/4U	9.80	20.13	0.53	46.49
Côte d'Ivoire (CI)	OU	S/4U	10.68	20.87	0.47	47.16
Côte d'Ivoire (CI)	BG	S/2	7.43	26.60	0.44	44.76
Côte d'Ivoire (CI)	BT	S/2	7.86	17.62	0.44	43.39
Côte d'Ivoire (CI)	OU	S/2	6.67	22.15	0.43	45.22
Côte d'Ivoire (CI)	BG	S/4U	10.39	27.29	0.43	47.54
Côte d'Ivoire (CI)	DV	S/2	6.52	20.58	0.41	46.03
Côte d'Ivoire (CI)	BT	S/4U	13.83	13.12	0.40	47.89
Nigeria (NGA)	OR	S/2	8.09	22.69	0.40	45.06
Nigeria (NGA)	OR	S/4U	11.68	19.63	0.38	44.75
Nigeria (NGA)	NL	S/2	7.25	22.86	0.37	46.81
Côte d'Ivoire (CI)	DGH	S/4U	13.29	15.86	0.36	49.17
Côte d'Ivoire (CI)	DGH	S/2	7.24	20.33	0.36	40.97
Nigeria (NGA)	ARA	S/2	6.93	22.23	0.36	45.74
Nigeria (NGA)	WS	S/2	7.17	21.77	0.36	46.50
Côte d'Ivoire (CI)	RGH	S/2	7.55	15.05	0.34	42.00
Côte d'Ivoire (CI)	RGH	S/4U	11.45	13.94	0.33	46.34
Nigeria (NGA)	WS	S/4U	10.75	21.23	0.32	44.30
Nigeria (NGA)	ARA	S/4U	8.51	20.71	0.32	45.57
Côte d'Ivoire (CI)	DV	S/4U	8.47	19.37	0.32	48.76
Nigeria (NGA)	UU	S/2	7.37	19.89	0.31	46.48
Nigeria (NGA)	NL	S/4U	9.18	20.05	0.30	43.52
Nigeria (NGA)	UU	S/4U	9.75	18.22	0.29	45.24
CV%			23%	18%	20%	5%

With a variation coefficient of only 5%, TSC cannot be considered as an efficient parameter to discriminate the different sites and tapping systems combinations.

Except for the plantation site of Iguobazuwa, RSH measured in Nigerian sites are confirmed mostly lower (<0.40 mM/l) than those observed in Côte d'Ivoire or in Ghana, whatever the tapping system. In Côte d'Ivoire, only the plantations of Divo (DV, S/4U), Digahio (DGH, S/2 and S/4U) and Rapides Grah (RGH, S/2 and S/4U) get similar RSH values to Nigerian plantation sites. Those lower values of RSH cannott be correlated in any way with those of Suc and Pi, for any of the 2 systems S/2 or S/4U: With similar Suc and Pi values as Côte d'Ivoire and Ghana, the plantations of Nigeria get lower latex RSH.

Except for the plantation of Iguobazuwa, younger than others and where there upward tapping has not yet been implemented, all other 13 plantation sites show a logical higher Suc content with S/4U compared to S/2, resulting from an improved latex sugar supply compared to downward tapping (direct supply to the cut + stored reserves moboilization).

CLONAL LATEX DIAGNOSIS VALUES

- **All systems and locations**

The table 7 shows the global averages of Suc, Pi, RSH and TSC values calculated for each clone present in the database from 2006 to 2018, for all systems. Ranking is from the highest RSH value, obtained for the clone IRCA41 to the lowest RSH value, obtained for the clone PB5/51.

Table 7. Average LD values observed from 2006 to 2018 for the 19 clones analyzed. All plantation sites and systems. RSH ranking from highest to lowest value.

CLONE	Suc	Pi	RSH	TSC	PixRSH
IRCA41	**7.98**	**19.76**	**0.54**	**47.34**	**10.69**
IRCA145	**7.98**	**21.65**	**0.54**	**46.55**	**11.68**
PR107	**12.01**	**22.66**	**0.53**	**43.94**	**11.97**
PB217	**13.93**	**20.53**	**0.49**	**44.82**	**10.03**
GT1	**11.27**	**15.65**	**0.46**	**47.76**	**7.22**
RRIC100	**7.41**	**22.37**	**0.44**	**45.48**	**9.89**
IRCA230	**6.20**	**23.19**	**0.44**	**44.05**	**10.10**
PB260	4.97	20.73	0.42	47.99	8.64
PB312	6.28	26.18	0.36	44.68	9.37
IRCA111	4.77	23.27	0.35	44.29	8.08
IRCA109	8.40	20.18	0.34	47.29	6.94
AVROS2037	7.05	10.24	0.34	46.44	3.46
IRCA130	6.15	24.36	0.34	44.53	8.23
IRCA18	4.98	23.11	0.33	46.70	7.70
IRCA209	6.49	17.65	0.33	46.60	5.81
PB314	6.64	21.76	0.31	43.80	6.69
AF261	11.73	15.14	0.29	42.34	4.44
PB235	4.63	20.03	0.27	50.38	5.48
PB5/51	4.71	20.32	0.24	47.81	4.93

The clone showing the highest Suc content is PB217. A detailed analysis of the database shows that this clone obtains the highest latex sugar, both for upward tapping and downward tapping in all plantation sites. It therefore confirms the exceptional sugar loading capacity of this clone under the eco-climatic conditions of Western Africa. High Suc values are also observed on clones PR107, GT1, AF261, IRCA109, IRCA41 and IRCA145.The highest RSH contents are observed on IRCA41, IRCA145, PR107, PB217, GT1, RRIC100 and IRCA230. It is noticeable that the 5 highest RSH contents (IRCA41, IRCA145, PR107, PB217 and GT1) also present the highest Suc contents. It is also noticeable that typical "quick starter" clones (PB5/51, PB235, PB314, PB312, IRCA111, IRCA130, IRCA18, IRCA209, PB260) showing lower Suc also show lower RSH, the only exception being IRCA230. Among these quick starter clones, IRCA230 is the only one classified with a high sugar loading capability and this may explain why the clone maintains higher thiols than others (Gohet *et al.*, 1997b).

Except very low starter clones like AV2037 or AF261, it is remarkable that all clones almost show similar Pi values. This indicates that the stimulation implemented by SIFCA/SIPH, tailored to clones according to clonal typology, can reduce the initial metabolic differences between clones, bringing all of them to a near maximum metabolic activity. Under this similar metabolic activity of all clones stimulated at their optimum, the yield potential will then depend mainly on latex Suc (indicator for the latex regeneration capability) and latex RSH (indicator for membranes protection against oxidative stress, latex stability and flow).

The figure 5 shows the general positive correlation existing between the clonal latex RSH content (Y axis) and the corresponding clonal latex Suc content, all systems included, on average on the 14 SIFCA/SIPH plantations present in the database.

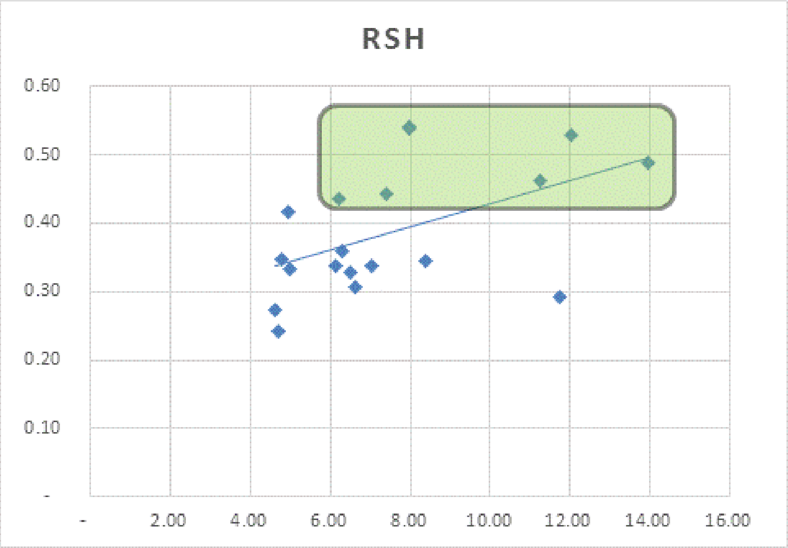


Figure 5. Positive interclonal relation between latex RSH content (Y axis) and Suc content (X axis). Average values observed on the 14 SIFCA/SIPH plantations (all systems and ages). Each point is one of the 19 clones analyzed from the database. 7379 LD analyses.

Hypothesis for this positive clonal relation between Suc and RSH is that a higher latex Suc content will ease the scavengers molecules (as RSH) synthesis and regeneration. Clones with high latex Suc maintain higher RSH latex concentrations than clones with lower Suc, confirming for the first time at industrial scale earlier research results (Gohet 1996, Gohet *et al.* 1997). The physiological hypothesis is that a high clonal latex sugar loading, allowing higher stimulation rates and improved stimulation response, would as well maintain higher latex RSH levels, resulting in an improved resistance to oxidative stress resulting from latex metabolic activation. This improved scavenging protection would therefore have positive effects on membranes integrity, on latex stability, on latex flow and on tapping panel dryness (TPD) onset (Gohet 1996, Gohet *et al.* 1997b).

It is therefore easy to identify the clones presenting the highest RSH content as a function of their latex Suc content, moreover considering that the metabolism is here maximized and homogenized by the use of stimulation tailored to each metabolic class. If the best physiologies (clonal adaptation to the local environment) are then determined by the highest RSH contents, the physiologically interesting clones to be retained for further recommendations are those included in the green shadowed area of figure 5 (clones with highest RSH).

In average for the 14 SIFCA/SIPH plantation sites, 7 clones can be identified having such a better physiological profile than others, among the 19 clones studied from the database. Those include the most planted historic clones in Western Africa (GT1, PR107 and RRIC100) and others introduced more recently like PB217, IRCA41, IRCA145 and IRCA230

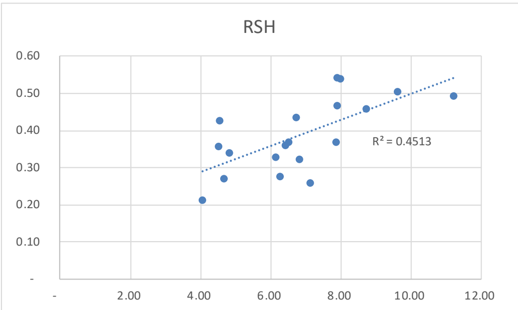
The metabolic classes of those 7 clones are:

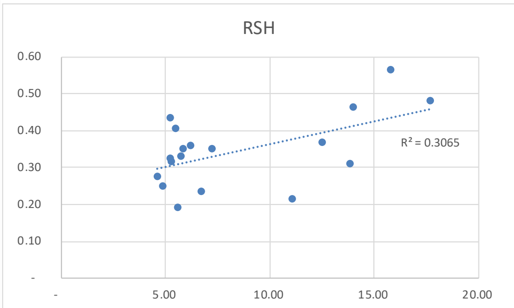
- Low-medium metabolism x high sugar loading (c5: PB 217)
- Low-medium metabolism x medium sugar loading (c4: PR 107)
- Medium metabolism x medium sugar loading (c7: GT1, RRIC 100)
- High medium metabolism x high sugar loading (c11: IRCA 41, IRC145)
- High metabolism x high sugar loading (c13: IRCA 230)

From these 7 clones, 4 have been selected for their high sugar loading and improved potential compared to the “classic clones as GT1, PR107 or RRIC100. They are PB217, IRCA41, IRCA145 and IRCA230. They cover the whole metabolic pattern from low metabolism (“slow starters”: PR107, PB217) to high metabolism (“quick starters”: IRCA 230), having in common to obtain the highest RSH and Suc contents of their class. PB217, IRCA41 and IRCA230 are currently the 3 mostly recommended clones by Cirad in Western Africa, based on their yield potential and these positive physiological characteristics based on improved sugar loading. Other new clones, like IRCA331 are already recommended, although absent from this study, on the same bases.

- **Per system (S/2 or S/4U, all locations)**

The positive clonal relation between RSH and Suc is observed whatever the system S/2 (figure 6) or S/4U (figure 7). The correlation is however higher for S/2 downward tapping system than for S/4 upward tapping system. However, the positive trend remains clearly marked.



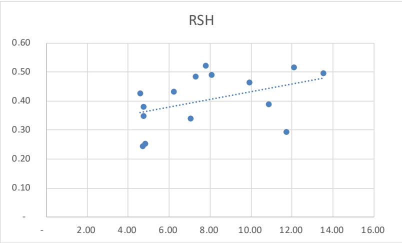


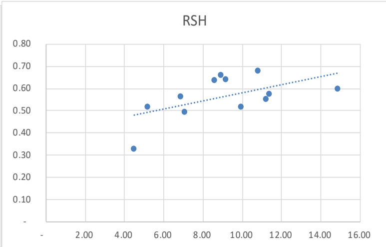
Figures 6 and 7. Positive interclonal relation between latex RSH content (Y axis) and Suc content (X axis). Average values observed on the 14 SIFCA/SIPH plantations

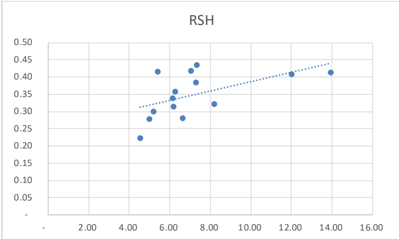
- figure 6, left: S/2 downward, 4472 LD analyses
- figure 7, right: S/4U upward 2907 LD analyses
- All ages. Each point is one of the 19 clones analyzed and present in the database.

- **Per country (all systems)**

The positive clonal relation between RSH and Suc is observed whatever the country: Côte d’Ivoire (figure 8), Ghana (figure 9) or Nigeria (figure 10). The highest Suc level is observed in all 3 countries on clone PB217.







Figures 8 (left, Côte d'Ivoire, 2831 LD), 9 (middle, Ghana, 1893 LD) and 10 (right, Nigeria, 2663 LD)). Positive interclonal relation between latex RSH content (Y axis) and Suc content (X axis). Average values observed on the 14 SIFCA/SIPH plantations. All ages. Each point is one of the 19 clones analyzed and present in the database.

The 5 clones presenting the highest RSH contents and therefore the most balanced physiological profiles are, per decreasing order:

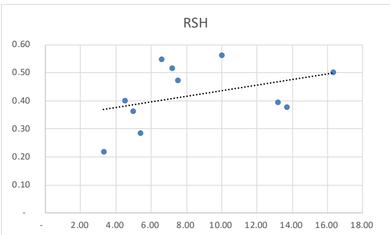
- In Côte d'Ivoire: IRCA41; PR107; PB217; IRCA230, RRIC100.
- In Ghana: PR107, IRCA145, RRIC100, IRCA41, PB217.
- In Nigeria: IRCA41, IRCA145, IRCA230, PB217, GT1.

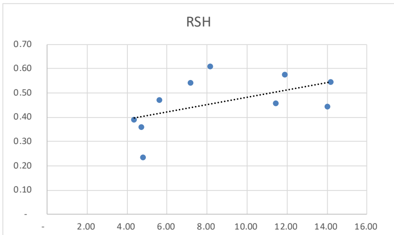
- **Per plantation site (all systems)**

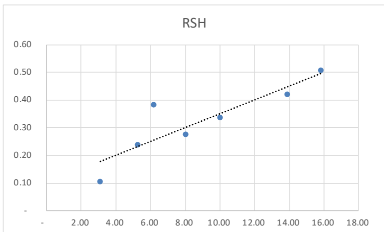
The positive clonal relation between RSH and Suc is observed whatever the plantation in all sites of:

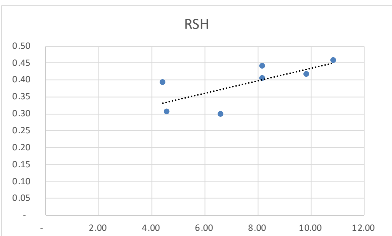
- **Côte d'Ivoire:**

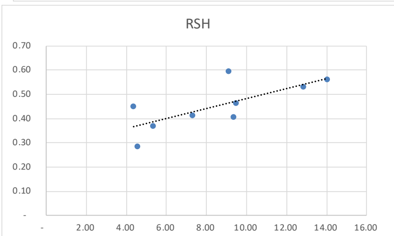
The positive clonal relation between RSH and Suc is observed whatever the plantation, although less significant on the plantation of Rapides Grah. The highest Suc level is observed in all 7 plantations on clone PB217 (figures 11 to 17).

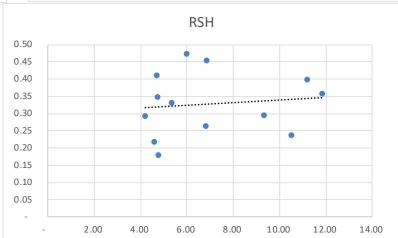


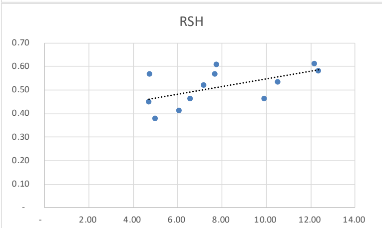












Figures 11 (up, left, Bettié), 12 (up, middle, Bongo), 13 (up, right, Digahio), 14 (middle, left, Divo), 15 (middle, middle, Ousrou), 16 (middle, right, Rapides Grah) and 17 (down, left, Toupah). Positive clonal relation between latex RSH content (Y axis) and Suc content (X axis). Average values observed on the

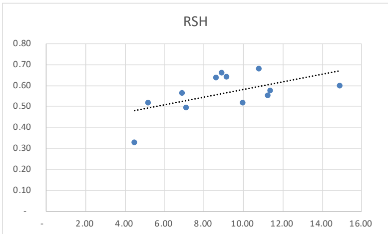
SIFCA/SIPH plantations of Côte d’Ivoire. All ages. Each point is one of the 19 clones analyzed and present in the database.

The 5 clones presenting the highest RSH contents (and therefore the most balanced physiological profiles) are, per decreasing order:

- In Bettié (BT) plantation: IRCA230, IRCA41, RRIC100, PB217 and PB5.51.
- In Bongo (BG) plantation: IRCA41, PR107, PB217, RRIC100 and PB314.
- In Digahio (DGH) plantation: PR107, PB217, PB260, GT1 and IRCA230
- In Divo (DV) plantation: PB217, IRCA41, GT1, RRIC100 and PB260
- In Ousrou (OU) plantation: IRCA230, PB217, PR107, IRCA41 and PB260
- In Rapides Grah (RGH) plantation : IRCA41, IRCA230, PB260, PR107 and PB217
- In Toupah (TPH) plantation : PB217, IRCA230, PR107, IRCA41 and PB260

- **Ghana :**

The positive clonal relation between RSH and Suc is observed as well in Grel plantation, The highest Suc level is observed as well on clone PB217 (figure 18).



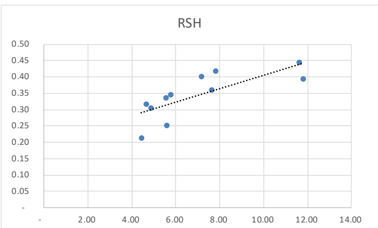
Figures 18. Positive clonal relation between latex RSH content (Y axis) and Suc content (X axis). Average values observed on the SIFCA/SIPH plantation of Ghana (GREL). All ages. Each point is one of the 19 clones analyzed and present in the database.

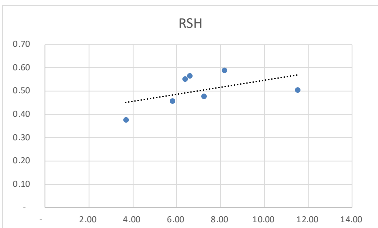
The 5 clones presenting the highest RSH contents (and therefore the most balanced physiological profiles) are, per decreasing order:

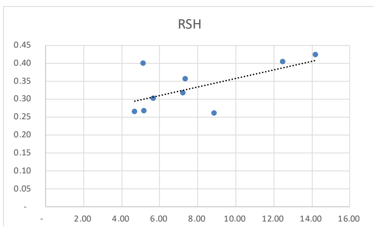
- In GREL plantation: PR107, IRCA145, RRIC100, IRCA41 and PB217.

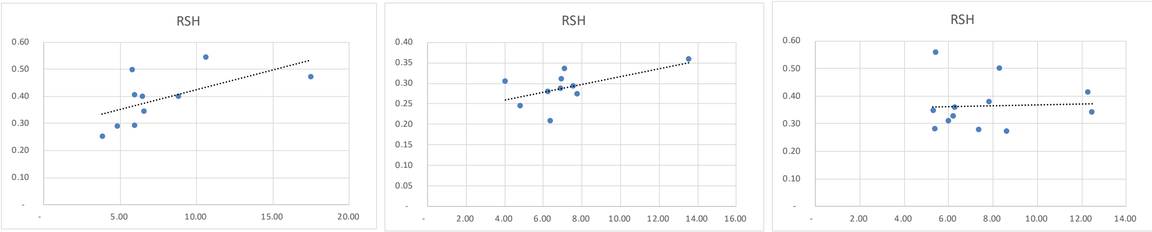
- **Nigeria:**

The positive clonal relation between RSH and Suc is observed whatever the plantation, although less significant on the plantation of Waterside. The highest Suc level is observed in all 6 plantations on clone PB217 (figures 19 to 25).









Figures 19 (up, left, Araromi), 20 (up, middle, Iguobazuwa), 21 (up, right, New Land), 22 (down, left, Osse River), 23 (down, middle, Utagba Uno), 24 (down, right, Waterside). Positive clonal relation between latex RSH content (Y axis) and Suc content (X axis). Average values observed on the SIFCA/SIPH plantations of Nigeria. All ages. Each point is one of the 19 clones analyzed and present in the database.

The 5 clones presenting the highest RSH contents (and therefore the most balanced physiological profiles) are, per decreasing order:

- In Araromi (ARA) plantation: PB217, RRIC100, IRCA41, GT1 and IRCA109
- In Iguobazuwa (IBZ) plantation: GT1, IRCA41, RRIC100, PB217 and IRCA109.
- In New Land (NL) plantation: PB217, GT1, IRCA230, RRIC100, IRCA130
- In Osse River (OR) plantation: IRCA145, IRCA130, PB217, IRCA230 and RRIC100
- In Utagba Uno (UU) plantation: PB217, RRIC100, IRCA109, IRCA145 and IRCA41
- In Waterside (WS) plantation : IRCA230, IRCA41; GT1, RRIC100, PB312

4. Discussion and conclusion

The meta-analysis of the Latex Diagnosis (LD) database gathering all LD data stored from 2006 to 2018 in SIFCA/SIPH plantations of Côte d'Ivoire (SAPH), Ghana (GREL) and Nigeria (RENL) has been performed. Average clonal LD data comprising Sucrose (Suc), Inorganic Phosphorus (Pi), Reduced Thiols (RSH) and latex Total Solid Content (TSC) were analyzed and compared for different plantation sites of SAPH (Toupah, Ousrou, Bongo, Rapides Grah, Digahio, Divo and Bettie), GREL (Abura) and RENL (Osse River, New Land, Utagba Uno, Araromi, Waterside and Iguobazuwa). The database was filtered in order to keep only the LD data obtained either from conventional and standard tapping systems (S/2 downward and S/4 upward), excluding the intensified period before slaughtering. Data were processed and analyzed on clones GT1, RRIM600, PR107, AV2037, RRIC100, AF261, PB28/59, PB5/51, PB217, PB235, PB260, PB312, PB314, IRCA18, IRCA41, IRCA109, IRCA111, IRCA130, IRCA209 and IRCA230. All 19 clones were tapped either in d4 or d5 6d/7 tapping frequencies. Stimulation was applied on panel (Pa), under industrial stimulation rates adapted to each clone metabolism and clonal sugar loading characteristics, according to the clonal typology described by Cirad (Gohet 1996, Gohet *et al.*, 1996, 1997a, 2003, 2008, 2016, Lacote *et al.*, 2010).

Based on the relation between Suc and Pi, the analysis of these LD data confirms the latex physiological positioning in clonal typology of all clones. It confirms in particular the high latex sugar loading capacity of clones PB217 (as this clone obtains the highest latex Suc level whatever the plantation site), IRCA109, IRCA41 and IRCA230. It also reveals a systematic positive correlation for all plantation sites sites between RSH and Suc latex contents: Clones with high latex Suc maintain higher RSH latex concentrations than clones with lower Suc, confirming at industrial scale earlier research results (Gohet *et al.*, 1997b). The physiological hypothesis is that a high clonal latex sugar loading, allowing higher stimulation rates and improved stimulation response, would as well maintain higher latex RSH levels resulting in an improved resistance to oxidative stress resulting from latex metabolic activation. This improved scavenging protection would therefore have positive effects on membranes integrity, on latex stability, on latex flow and on tapping panel dryness (TPD) onset. Moreover, as tapping intensity (tapping frequency and stimulation) was almost similar in all sites, we also make the hypothesis that the latex RSH level and the Pi x RSH value might be accurate indicators to describe, at plantation site level, the local clonal suitability as well as the global stress conditions of trees on each plantation site. For instance, RSH are systematically lower in Nigeria than in Ghana or Côte d'Ivoire, with same clones, same tapping and stimulation intensities, and same levels of the metabolic physiological parameters (Suc and Pi). We formulate the hypothesis that RSH latex content does not represent only the effect of the oxidative stress consecutive to tapping, but the integration of all stress incurred by the tree in his environment (including soil and climate). As the ecoclimatic conditions of Nigeria are quite different of those of Côte d'Ivoire and Ghana (longer dry season, higher rainfall during rainy months…), this difference in ecoclimatic conditions could be one of the possible causes of lower RSH levels observed in Nigeria.

The analysis showed as well that, whatever the conditions (country, location, tapping system, downward or upward tapping), the clones showing the best balanced physiological profiles (highest RSH and Suc under maximum metabolic activation) were mainly, for the SIFCA/SIPH plantations of Western Africa, PB217, IRCA41, IRCA230. RRIC100, PR107, IRCA145 and GT1. IRCA109 also showing good profiles under specific conditions of Nigeria. As a conventional quick starter (active metabolism with low latex sugar content), the clone PB260 was the only one to appear interesting in some plantations of Côte d'Ivoire. In the contrary, all clones showing the highest RSH levels always coincided with those with higher latex sugar, either because of a low or medium metabolism (PR107, GT1, RRIC100), either because of a high sugar loading capability (IRCA230, IRCA41, IRCA145, IRCA109). The clone PB217 combined these two characteristics (low medium metabolism associated with high sugar loading), explaining its exceptionally high latex sugar content and its excellent physiological profile on all LD physiological parameters (high Pi, high Suc and high RSH) in 13 of the 14 plantation sites.

This study will be completed later on in order to set up a standard interpretation method of such LD databases, in order to get the maximum information on local adaptation of clones.

Acknowledgement

Our thanks are addressed to SIPH and SIFCA, to Michelin, as well as to the SIFCA/SIPH plantations of Nigeria (RENL), Ghana (GREL) and Côte d'Ivoire (SAPH), for making those data Latex Diagnosis data available for publication.

5. References

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