Optimization of an on-farm multiplication and sanitation technique for plantain banana

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Optimization of an on-farm multiplication and sanitation technique for plantain banana

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Summary

Introduction – Plantain banana is a major crop for food supply in tropical areas, while facing pests and diseases affecting fruit yield and quality. Within the agroecological transition context, the development of prophylactic methods aimed at avoiding the use of pesticides is an avenue worth exploring. Among these methods, an *in vivo* technique of mass propagation of shoots called PIF (from the French '*Plants Issus de Fragment de tige*', meaning 'shoots resulting from corm fragments'), was developed in Cameroon to multiply and sanitize plantain shoots at the farm level. Despite showing promising results, studies on factors that could improve its efficiency are lacking. *Materials and methods* – The effects of three main factors (temperature, hormone and light) were investigated in separate assays within semi-controlled environmental conditions, in Guadeloupe, French West Indies, to measure how these factors affect the efficiency of the PIF technique during the reproductive stage. Five response variables were used to assess the number and the robustness of daughter shoots produced. *Results and discussion* – PIF technique performance increased with temperatures above 30 °C (>15 shoots per corm, >25 roots per shoot, >80 cm root length), LED light application for 15 minutes per day, and synthetic hormone supplementation. A moderate but significant virus sanitation potential of this technique was found, with up to 36.7% daughter plants sanitized from banana mild mosaic virus (BanMMV) infected mother plants. These results open perspectives for larger scale assays to refine an appropriate methodology allowing farmers to become more autonomous in healthy planting material satisfying the principles of agroecological transition.

Keywords

agroecology, *Musa* spp., AAB, PIF technique, prophylaxis

Significance of this study

What is already known on this subject?
- Relative humidity over 80% combined with average air temperatures of 30 °C increases the efficiency of the PIF technique in the Cameroon context.

What are the new findings?
- The performances also increased with light application and synthetic hormone supplementation. The technique partially sanitized shoots contaminated with BanMMV virus.

What is the expected impact on horticulture?
- These results could allow farmers or nurserymen to become more autonomous in healthy planting material satisfying the principles of agroecological transition.

Introduction

Banana is one of the most important food crops in the world (Kwa and Temple, 2019). The Cavendish variety (*Musa acuminata*, AAA triploid), part of highly structured market channel, is the main banana variety exported in the world and is consumed as a fruit. In 2021, almost 125 Mt have been produced worldwide (FAO, 2023). The other main type of banana is plantain (*Musa* spp., AAB triploid), which is consumed as a cooked vegetable, whose production amounted to more than 45 Mt worldwide (FAO, 2023). Crop practices and plant physiology are significantly less documented for plantain than for Cavendish, despite the staple importance of the former for many households around the world, especially in the tropical regions of sub-Saharan Africa (Dépigny and Damour, 2022; Kwa and Temple, 2019).

Both plantain and Cavendish face a number of telluric pests, such as *Cosmopolites sordidus* weevils and *Radopholus similis* nematodes (Kwa and Temple, 2019) and aerial fungi (European Food Safety Authority (EFSA), 2008; Pegg *et al.*, 2019). Banana is also prone to viral diseases, with varying impacts on yield (Mukwa Fama Tongo, 2016). Since the incidence of telluric pests in a crop depends on the sanitary status of both the planting material and the soil (Gold *et al.*, 2001; Haegeman *et al.*, 2010), prophylactic solutions are an avenue to explore. Most plantain farmers cannot grow their plantations longer than two cycles on the same land, and must use crop rotation to prevent decreasing yields associated with the weevil threat (Mboula, 2014). Uprooting of the crops after two years of cultivation represents a significant financial burden for plantain producers. Besides crop rotation, the use of healthy planting material combined with soil sanitation through improved fallows is another practice that is gaining momentum (RITA Guadeloupe, 2019). The production of healthy shoots can be implemented in two ways. Firstly, farmers could clean the banana shoots mechanically with a machete, and/or chemically with baths based on household products (chlorine-based), without guaranteeing the healthy sanitary status. Secondly, farmers can use virus-

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TABLE 1. Average seasonal temperature, global radiation and relative humidity measured at the experimental site in 2021 and 2022. The dry season takes place from January to June and the humid season from July to December. Numbers between parentheses are standard errors.

2021	Temperature (°C)	Global radiation (DaJ cm⁻²)	Relative humidity (%)
Dry season	24.02 (1.25)	1,940.56 (485.75)	86.55 (3.68)
Humid season	25.40 (1.09)	1,863.70 (451.99)	88.39 (88.39)
2022			
Dry season	24.02 (1.42)	1,984.78 (455.39)	88.05 (3.36)
Humid season	26.18 (0.58)	2,024.01 (522.40)	89.78 (3.19)

free vitroplants, ensuring the lowest sanitary risk (Sadom *et al.*, 2010), but at a prohibitive initial cost imposed by buying vitroplants from private biotechnology companies (Olumba and Onunka, 2020). Alternatively, farmers could use a technique, referred to as PIF (from the French *Plants Issus de Fragment de tige* – shoots resulting from corm fragments) with which new shoots are obtained from the fragmentation of banana corms.

The PIF technique was developed by the CARBAP (African Center for Research on Banana and Plantain) in Cameroon to tackle the lack of healthy shoots. This method involves activating latent buds by cutting corms to produce plantain shoots in large amounts and within a short period of time (Kwa, 2003). Two experiments, led by the CARBAP between 1993 and 1998 to focus on the influence of global environmental conditions on the production of PIF shoots showed that a relative humidity over 80% in the macropropagation chamber, combined with an average air temperature of 30 °C (7 °C above outdoors temperature) and an average substrate temperature of 24 °C (2 to 4 °C above outdoors) were appropriate to obtain results, with 4 to 15 more shoots than controls (Kwa, 2003; Tomekpe *et al.*, 2011).

Visible light, especially in the red wavelengths (700–740 nm), was found to have a significant impact on the banana shoot length (Kwa and Temple, 2019).

In the literature, the comparison of the impact of two types of light source (white fluorescent bulb *vs.* LED) was carried out for Cavendish *in vitro* multiplication and better results were obtained with LED light (Bhaya and Al-RazzaqSalim, 2019). LEDs are commonly used to study shoot physiology (Jackson *et al.*, 1985). Research has shown the role of light flashes (0.01–10 seconds) on dormancy termination and on germination process (Batlla and Benech-Arnold, 2014; Yan and Chen, 2020). They demonstrated that a single flash could induce a reaction, but that repeated flashes (two or more) were often required for the full germination process to occur. However, these works have been carried out on seeds and on model species such as *Arabidopsis thaliana* and cannot be extended to banana shoot activation and growth. Coconut water, which contains a diversity of hormones such as auxin, cytokinins and gibberellins (Yong *et al.*, 2009), has been shown to activate latent bud growth in three banana *Musa* AAA varieties (Bora Lukando, 2013) as well as in *Dioscorea* spp. yams (Dibi *et al.*, 2016).

Although the PIF technique has been shown to produce healthy shoots (Tomekpe *et al.*, 2011), as long as safe practices are used, another study has highlighted the potential risk of activation of endogenous viral sequences (Mukwa Fama Tongo, 2016), even if the symptoms caused by these viruses are limited (European Food Safety Authority, 2008). However, a recent long-term study carried out in Guadeloupe, has shown that the viral activation risk is negligible on plantain 'French clair' variety (Umber *et al.*, 2022).

In this study we hypothesize that (i) the PIF technique performance increases with optimal temperature, light and hormone supplementation, and that (ii) it allows sanitizing plantain shoots contaminated with BanMMV virus.

For this purpose, the effects of these three factors (temperature, light and hormone supplementation) on five response variables were investigated in three separate assays under semi-controlled environmental conditions. In each assay, we investigated whether the studied factor played a significant role on top of the global environmental conditions. Last, the presence of the BanMMV virus was assessed before and after PIF implementation in order to assess the potential of this technique for sanitization.

Materials and methods

Study area and experimental setup

Field experiments were carried out between 2021 and 2022 in Guadeloupe (French Caribbean), at the INRAE outstation (Domaine de Duclos, geographical coordinates 16°12′12.0″N, 61°39′41.9″W). Climatic conditions at the experimental site are those of a tropical climate (Table 1).

Shoots of cultivar 'French clair' (named '*Blanche*' in Guadeloupe), which is the most present variety in the territory (Scherschel, 2017), were used as planting material. These shoots had been produced locally at the INRAE outstation during a previous system experiment (Bezard *et al.*, 2023b).

The effects of three main factors (temperature, light and hormone supplementation) were investigated in three separate assays under semi-controlled environmental conditions. Here, we define semi-controlled conditions as the intervention to vary one environmental factor among the global environmental conditions. During each assay, twenty-seven corms were planted individually in 27 pots containing 11 L of substrate, composed of pine wood chips. The pots were distributed equally (according to their weight) to three growth chambers, each containing 9 pots, corresponding to the maximum capacity of growth chambers. At the beginning of the experiment, the weight of each corm was measured. Watering was done with a water pump according to Pourrat (2022). The growth chambers were lit with red-reinforced (55.6% red, 630–660 nm; 22.2% blue, 430–460 nm; 5.5% infrared, 730 nm; 5.5% ultraviolet and 11% white) LED lights (477 cd, 15 W) for 12 h per day from 6:00 am to 6:00 pm (which is the average photoperiod in Guadeloupe).

Sensors were used to monitor the environmental variables inside the growth chambers: thermo-hygrometers for air temperature and relative humidity, PAR (Photosynthetically Active Radiation) sensors for the light radiation, TDR (Time Domain Reflectometry) sensors for substrate water content, and thermocouples for substrate temperature. Measures were made every 5 min with a CR1000 data logger (Campbell Scientific, U.S.A.).

At the end of each experiment, the following response variables were measured: (i) the number of shoots produced per corm, (ii) the average size of shoots produced per corm (in cm) for shoots longer than 2 cm, (iii) the average number of leaves per shoot produced, (iv) the number of roots per corm, and (v) the length of the largest corm root (in cm). In this study, we consider that the higher these variables, the higher the performance of the technique.

Production of planting material through PIF technique

Tools were disinfected with 4% household bleach (Kapadia and Patel, 2021). Plantain shoots with no central leaf were selected for the following procedures (Kwa and Temple, 2019). On a shaded and clean work surface, corms were cleaned mechanically, from the base of the leaves, with a knife (Figure 1A). Damaged or symptomatic parts, as well as prominent buds, are removed. Corms were then cleaned by soaking in 80 L containers filled with water and 4% bleach for 5 min (Figure 1B), then for 5 min in a solution of 2% Limocide (Vivagro, Martillac, France), a commercial mix of essential oils authorized in organic agriculture, and 2% black soap. Twenty-four hours later (Figure 1C), about five leaves were incised 2 mm above the meristematic line in order to preserve lateral buds (Figures 1D, E) (Bezard *et al.*, 2023c). The remaining of the pseudostem was cut off and a cross-shaped incision was made in the center of the apical meristem to inactivate it without damaging the lateral buds (Figure 1F). The incised corms, referred to as explants, were placed in the growth chamber, filled with a substrate made of moistened pine wood chips (Figure 1G), and then totally covered with a second layer of substrate a few centimeters thick. The pots were placed in the growth chambers (Figure 1H).

Experiment A: Temperature factor variation

To test the effect of temperature variation, growth chambers were set up with three temperature modalities: 25 °C, 30 °C and 35 °C. The 30 °C modality was used as control, since it corresponds ambient temperature around. To achieve 25 °C and 35 °C, the growth chambers were placed in a room regulated to 25 °C with an air conditioner or heated with a regulated electric heater, respectively.

Experiment B: Hormones factor variation

Two types of hormone preparations were used to test the effect of hormone supplementation: fresh coconut water and synthetic hormone. Coconut water was extracted from fresh coconuts of most common variety found at the research outstation an was prepared by heating to 80–100 °C for 10 min with continuous stirring in order to precipitate proteins, fats, and other compounds (George *et al.*, 2008; Nasib *et al.*, 2008). Precipitates were removed by filtration through coffee filters (20 microns approximately) and filtrated coconut water was let to cool down to ambient temperature. Synthetic hormone solution containing 100 mg L^{-1} of indole-3-acetic acid was prepared by dissolving commercially available pills (Rhizopon®).

Hormone supplementation was achieved by immersing 18 corms in each of the preparations for about 19 h (coconut water treatment, 9 corms) or 5 sec (synthetic hormone treatment, 9 corms). After immersion, the corms were left to dry for five hours, and planted in individual pots. Non-treated shoots prepared according to the PIF protocol were used as control (9 corms).

Experiment C: Light factor variation and combination of optimum conditions

For the third assay, we added red-reinforced light and we combined the optimal conditions (temperature and hormone treatment) devised from the first two experiments. For this, a LED device providing light between 380 and 700 nm wavelength was turned on in the growth chamber every morning at 5 am for 15 min.

Experiment D: Viral status assessment of banana shoots

In this work, the purpose of this sanitary risk stage is to follow the presence of the BanMMV in the shoot material and

FIGURE 1. The different steps of the PIF (in French, *Plants Issus de Fragment de tige* – Shoots resulting from corm fragment) technique protocol based on Bezard *et al.* (2023c). A. Mechanical cleaning; B. Chemical cleaning; C. Drying of shoots; D. Buds' exposure; E. Leaf removing; F. Apical meristem inactivation; G. Setting up in the micropropagation chamber; H. Transparent tarpaulin setting up; I. Small shoots emergence.

evaluate its transmission from one generation to another after PIF preparation.

The complete viral status of a panel of 75 plantain trees, variety 'French clair', collected in the largest production area of Guadeloupe (corresponding to an entire plot at INRAE outstation), was evaluated for the six banana-infecting viruses. Sampled leaves were processed as described by the two extraction methods, depending on the detected virus. Firstly, total nucleic acids (TNAs) were extracted using the procedure 2 described by Foissac *et al.* (2005) for RNA viruses detection (BanMMV, BBrMV, BVX and CMV); cDNA was synthetized according to Umber *et al.* (2022). Detection primers were designed by Foissac *et al.* (2005) for BanMMV, Iskra-Caruana *et al.* (2008) for BBrMV, Mansoor *et al.* (2005) for BBTV, Teycheney *et al.* (2007) for BVX, and Blas *et al.* (1994) for CMV. Then, for BSV detection, in order to avoid the detection of endogenous viral sequences, virus indexing was performed by immunocapture-PCR (IC-PCR) according to Le Provost *et al.* (2006) modified by Umber *et al.* (2016), using a polyclonal antibody purchased from Neogen (Ayr, Scotland).

As only BanMMV was detected in 'French clair' plantains in Guadeloupe, leaves of 32 mother suckers, *i.e.*, suckers used for PIF production, were collected just before performing the PIF technique, processed as described before and indexed only for BanMMV. Then, 30 shoots originated from BanMMV-infected corms were collected at the three-leaf stage, *i.e.*, when the shoots are well developed but still attached on the mother corm and samples were indexed for BanMMV. The integrity of synthetized cDNA was verified by using housekeeping primers targeting *Musa actin* gene (Gayral *et al.*, 2008). All sequences of primers and PCR conditions used in this study are described in Supplemental Table S1.

Dataset and statistical analysis

All statistical analysis were processed with R software (R Core Team, 2022).

Data of each assay were used to investigate:
– The relationship between the variation of one environmental factor (temperature, hormone or light supplementation) and response variables;
– If the varied environmental factor plays a significant role among the overall the global experimental conditions;
– The sanitizing potential of the PIF technique for the BanMMV virus.

The relationships between the temperature, hormone or light supplementation and the response variables was investigated using descriptive statistics and to go further, a Kruskal-Wallis test and a Wilcoxon test were done for pairwise mean comparisons, in order to compare the response variables between chambers.

To study whether the varied environmental factors play a specific role among global conditions and thus, discuss experimental conditions (air and substrate temperature, air and substrate humidity and light radiation), the hourly average was calculated for each factor, mean comparison tests and Bonferroni-adjusted post-hoc tests (to make a pairwise comparison) were used to highlight the significant differences.
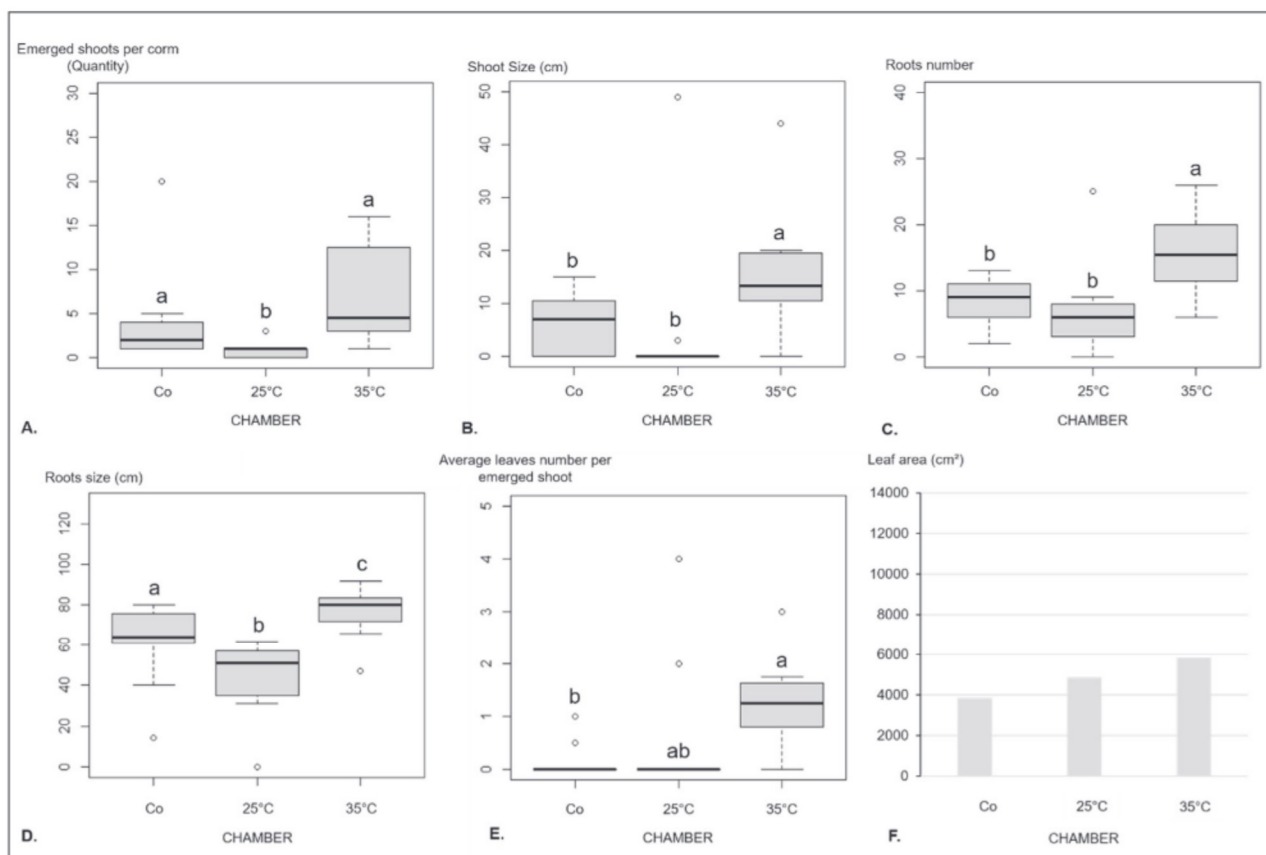
Figure 2. Response variable for the temperature assay (Experiment A). A. Quantity of emerged shoots per corm; B. The 'Shoot size' variable corresponds to the average size of emerged shoots (for shoots over 2 cm); C. The 'Roots number' to the average number of roots per corm; D. The 'Root size' to the largest corm roots; E. The 'Leaves' to the average leaves number per emerged shoots; F. Leaf area. The Co modality corresponds to the control chamber (ambient temperature, with an average of 30 °C), the 25 °C modality to the chamber in air conditioning conditions and the 35 °C modality to the heated chamber.

Results and discussion

The detailed measures of the response variables, in the different conditions, are presented in Supplemental Table S2.

Significantly higher performances with increasing temperature

Significant differences were identified for each response variable for the Experiment A (temperature assay). Looking at the chamber separately with the post-hoc test (pairwise Wilcox test), the response variables all increased significantly with increasing temperature (Figure 2), except for the number of emerged shoots per corm for which there was not significant difference between the control chamber (30 °C) and heated chamber (35 °C). The other response variables are all significantly higher in the heated chamber (35 °C). The leaf area is also the highest in the heated chamber (35 °C) (Figures 2 and 5).

This result is coherent with the results obtained by Kwa (2003) and Tomekpe *et al.* (2011) in Cameroon and by Turner *et al.* (2007) on the positive correlation between a higher Growing-Degree-Days (GDD) and the bunch initiation development.

Several studies have been carried out to investigate the physiological and molecular mechanisms involved, particularly in situations of stress for banana plants (water deficit, nitrogen deficit, *etc.*) (Tong *et al.*, 2023; Zhao *et al.*, 2024). They identified that the GRAS gene family plays a key role in banana growth and development, since it is associated with the metabolic pathways of phytohormones such as gibberel-

lin. Tong *et al.* (2023) showed that the expression of these genes was higher in leaves than in roots under stress. Rising temperatures increase transpiration rates and can generate water stress which can have a direct impact on root and plant development (Panigrahi *et al.*, 2021). Lobo and Rojas (2020) show that over 38 °C the stomata close leading to growth arrest. It would therefore be interesting to go further and carry out molecular or even genetic analyses to identify the mechanisms at work when temperatures rise during the initiation phase of the PIF technique.

Higher performances with synthetic hormone and light addition but not statistically significant

In Experiment B, the addition of coconut water negatively affected performance, since the size of the largest root was significantly smaller, on average, than in the control and hormone synthesis chambers (Figure 3). Looking at the individual results, the greatest number of emerged shoots per corm was of 32 emerged shoots for one corm in the synthetic hormone modality (Supplemental Table S2). In the coconut water modality, we immersed shelled corms into coconut water during 19 hours. Of the 9 initial corms, only 4 produced emerged shoots, the other were affected by rot (Supplemental Table S2). We can assume that it was the length of the bath and the fact that the corms were naked that caused the rot. Therefore, two alternatives could be explored. On the one hand, a bath shorter than 19 h (Opata *et al.*, 2020). On the other hand, a bath, before the stage of corms preparation, thus with corms that are not naked, as the addition of
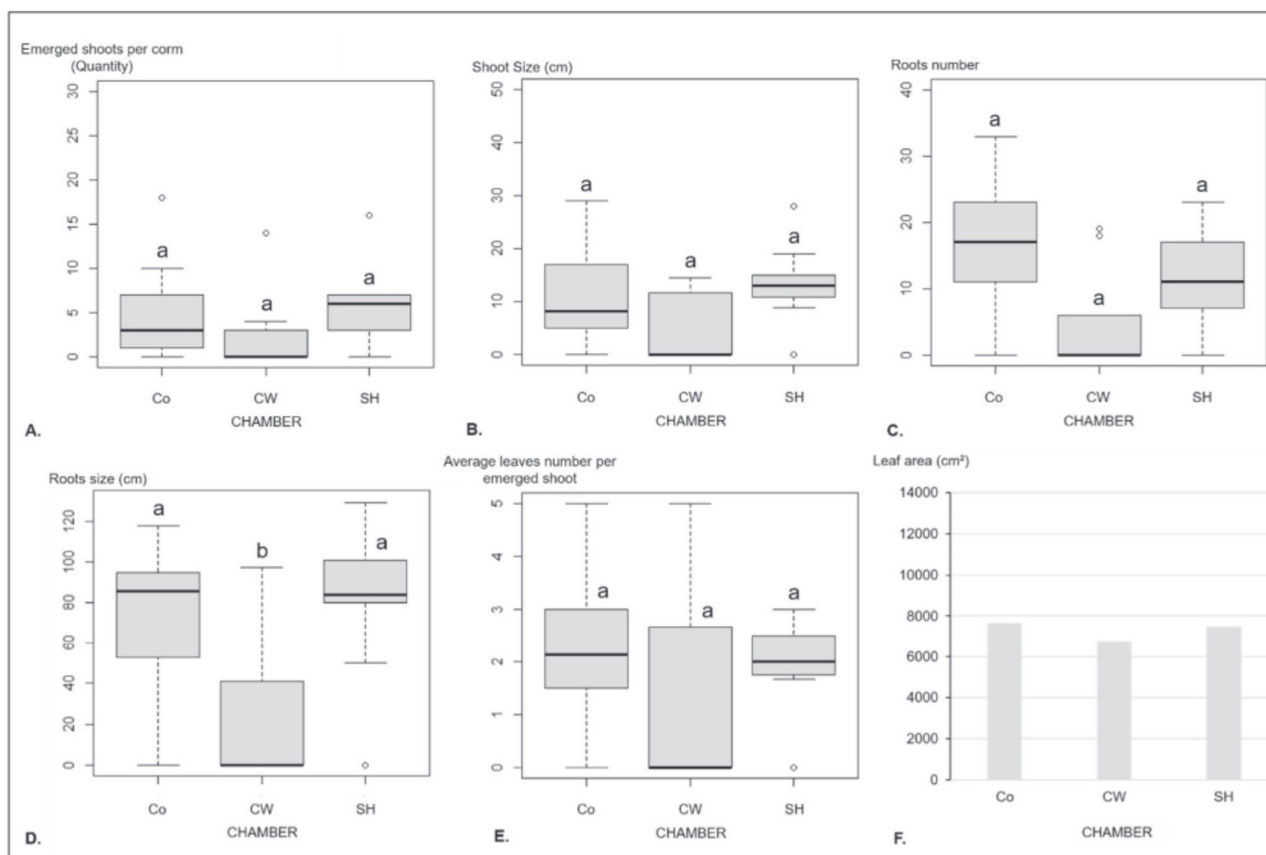
FIGURE 3. Response variable for the hormone assay (Experiment B). A. Quantity of emerged shoots per corm; B. The 'Shoot size' variable corresponds to the average size of emerged shoots (for shoots over 2 cm); C. The 'Roots number' to the average number of roots per corm; D. The 'Root size' to the largest corm roots; E. The 'Leaves' to the average leaves number per emerged shoots; F. Leaf area. The Co modality corresponds to the control modality, the CW to the Coconut modality and the SH modality to the Synthetical hormone modality.

coconut water has been shown to induce the regeneration of new shoots, particularly in the case of Meyer lemon (*Citrus × meyeri*) for *in vitro* propagation (Qiao Er Wong *et al.*, 2024). The characterization of the composition of the coconut water would also be interesting to discuss since it could not be characterized, and in particular its hormone concentration. Indeed the physico-chemical composition of coconut water depends on the variety (Ma *et al.*, 2008) and on the phenological state (Jackson *et al.*, 2004). Coconut water from immature fruits was reported to produce better results than water from mature fruits (Yong *et al.*, 2009). To go further in increasing performance with the addition of synthetic hormone, it would be interesting to test varying concentrations in order to identify the optimal concentration in terms of the number of plantlets obtained, as was done by Qiao Er Wong *et al.* (2024) for the shoot regeneration of Meyer lemon.

We could not demonstrate that adding light or combining optimal conditions increase significantly the performance of the PIF technique during Experiment C (Figure 4). Moreover, the latter experiment allows identifying trends: several variables (number and size of roots, number of leaves per shoot) are higher in the chamber with the 'light' modality. The highest value for the 'emerged shoots per corm' variable, for this assay, is reach in the optimum chamber modality with up to 20 emerged shoots (Supplemental Table S2).

We hypothesize that the non-significance of the results is related to the small sample size and that it would be appropriate to conduct tests on a larger scale to confirm or not the identified trends. This could be an opportunity to go further in characterizing the impact of different wavelength, since it

has been shown that different spectra affect the physiology of a wide range of plants (Rehman *et al.*, 2024).

Partial viral sanitation with PIF technique (Experiment D)

In order to assess the sanitary risks of the PIF technique, the potential of vertical transmission (from infected corms to generated shoots) of virus was evaluated using molecular detection tools. Firstly, the viral status of 'French clair' variety in Guadeloupe was assessed using 75 suckers randomly selected throughout production areas. After indexing for the six banana-infecting viruses (BanMMV, BBrMV, BBTV, BSV, BVX and CMV), only BanMMV was detected with a very high prevalence of 74.7% (56/75; Supplemental Table S3). BanMMV indexing was therefore undertaken for 32 mother suckers (suckers used for PIF technique) from leaves collected just before preparing the corm for PIF technique, and BanMMV was detected in 13 mother suckers (Supplemental Table S3). Following the PIF technique implementation, shoots from BanMMV-infected mother suckers were also indexed only for BanMMV. Among the 30 analyzed samples, 11 were negative for BanMMV, although the quality of their cDNA was good, as it produced adequate PCR amplification with housekeeping primers (Supplemental Table S3).

Unlike the use of vitroplants as planting material, the use of shoot from PIF technique does not guarantee the sanitary status of planting material. Indeed, even if foliar fungi contamination does not occur with the use of shoots from PIF technique as leaves were removed during the preparation, nematode and weevil larvae may remain if the corm
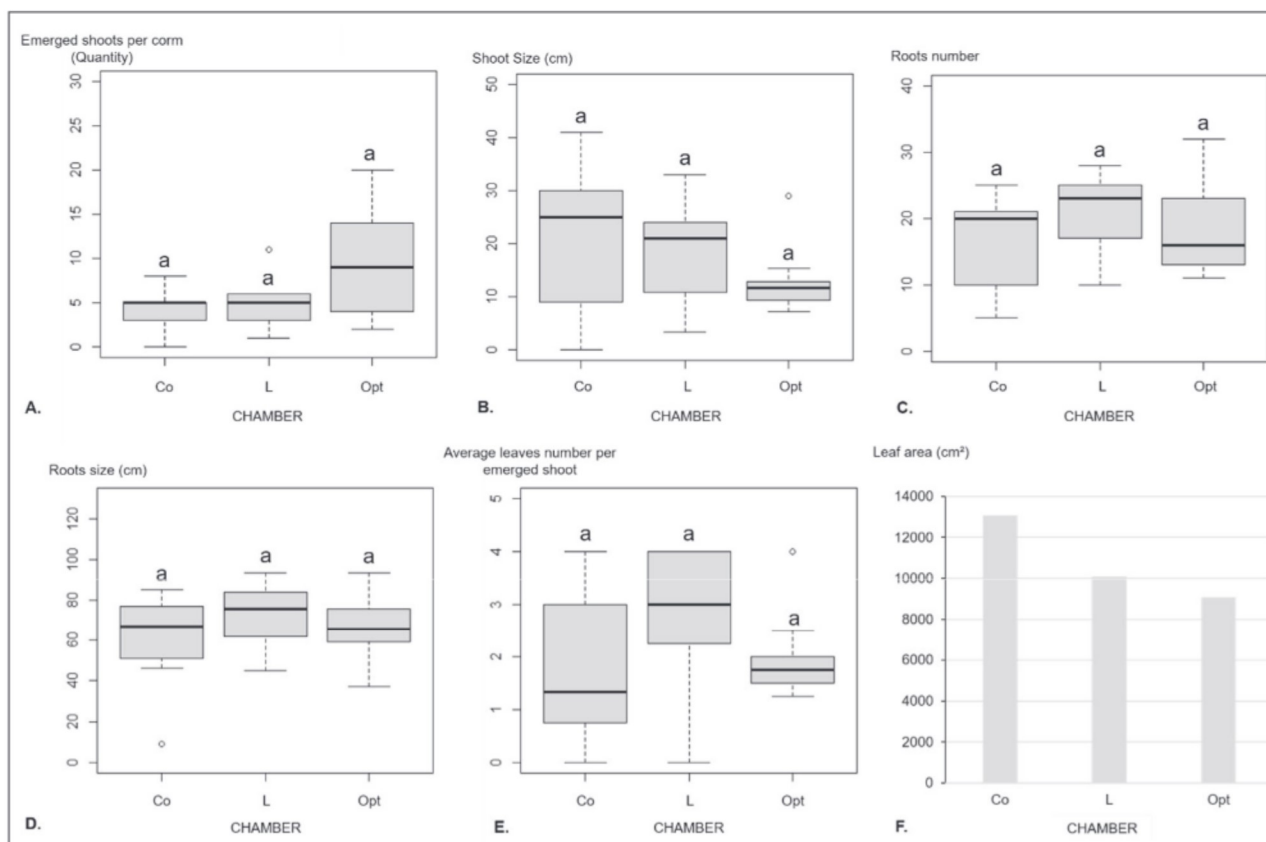
FIGURE 4. Response variable for the light and optimum assay (Experiments C and D). A. Quantity of emerged shoots per corm; B. The 'Shoot size' variable corresponds to the average size of emerged shoots (for shoots over 2 cm); C. The 'Roots number' to the average number of roots per corm; D. The 'Root size' to the largest corm roots; E. The 'Leaves' to the average leaves number per emerged shoots; F. Leaf area. The Co modality corresponds to the control modality, the L modality corresponds to the Light addition modality and the Opt modality to the optimal modality.

was not cut properly. Regarding viral contamination, vegetative multiplication, like PIF, can lead to the spread of viruses from contaminated shoots. However, we demonstrated that the PIF technique might be a way to eliminate some viruses, like BanMMV, with a moderate sanitation rate of 36.7% (11 sanitized shoots/30 shoots produced from BanMMV-infected mother suckers). The sanitation of BanMMV can be explained by the fact that shoots generated during the PIF process directly originated from meristematic cells, which are in most cases virus-free. Thus, considering that BanMMV has a weak impact on banana production (European Food Safety Authority (EFSA), 2008) and that the PIF technique may eliminate this virus, the PIF technique does not cause high sanitary risks in Guadeloupe, if done properly. However, further studies are required to improve the sanitation rate of BanMMV by the PIF technique.

Influence of specific environmental factors yet to be identified, but showing trends

We also identified that between the different chambers, several environmental factors (air and substrate temperature, substrate temperature, light radiation, substrate water content and relative humidity) varied as only one was modulated. These results mean that, during each assay, the varied factor was not the only one which has influenced the differences between response variables.

For the Experiment A (temperature), post-hoc Bonferroni-adjusted tests confirmed that the differences were significant between each chamber for all the environmental factors. The results were the same for the Experiment B (hormone), post-hoc Bonferroni-adjusted tests confirmed that the differences were significant between each chamber for all the environmental factors. For the Experiments C and D (light and optimum), post-hoc Bonferroni-adjusted tests confirmed
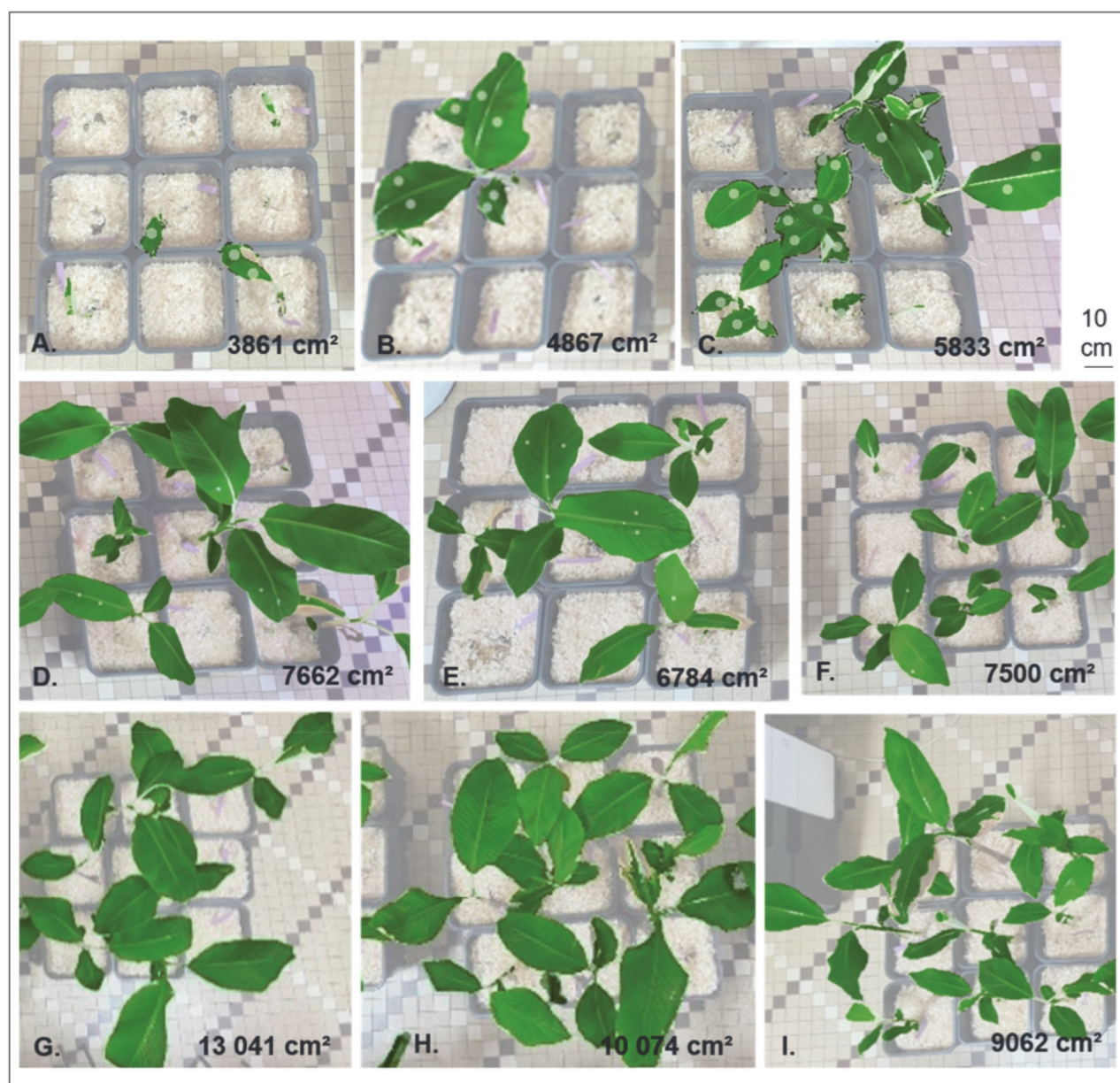
FIGURE 5. Estimation of 'Leaf area' with Mesurim software (Madre, 2013). A. Control modality (ambient temperature, with an average of 30 °C) (Experiment A); B. 25 °C modality (air conditioning conditions) (Experiment A); C. 35 °C modality (heated chamber) (Experiment A); D. Control modality (Experiment B); E. Coconut water modality (Experiment B); F. Synthetic hormone modality (Experiment B); G. Control modality (Experiments C and D); H. Light modality (Experiment C); and I. Optimum modality (light and heater) (Experiment D).

that the differences were significant between each chamber for all the environmental factors but were more important for T_{AIR} between chamber 1 and 3 and 2 and 3 ($P = 2e\text{-}16$) than between chamber 1 and 2 ($P = 1.2e\text{-}8$). The differences were significant between each chamber for all the other factors ($P = 2e\text{-}16$).

Indeed, some environmental factors are correlated, the substrate temperature (TS) and the air temperature (TAIR) on the one hand, and the TDR (Time-Domain Reflectometry) and relative humidity (HR) on the other hand. In addition, the relative humidity depends on the temperature (Bergeron and Naud, 1995), thus it explains why it is more a chamber effect rather than the effect of an isolated environmental factor. The interconnection between two other factors (Photosynthetically Active Radiation (PAR) and temperature was clearly shown that there is a link between light and hormone production by weed seeds, for germination process, such as gibberellin (Batlla and Benech-Arnold, 2014).

Other factors were not tested in this experiment but could be tested in later assays. In particular the variety effect (Dépigny and Damour, 2022) which was tested by Kwa (2003) in combination with other factors (temperature, humidity, *etc.*) or the substrate effect, since Monono *et al.* (2018) showed that there were significant differences according to the type of substrate and in particular with the use of palm male inflorescence.

Thus, the performance of PIF technique, measured through environmental factors, could be better explained by the effect of overall conditions, but we can assume that these initial results represent trends that may be confirmed in subsequent trials.

Experimental setup

This experimentation made it possible to test a setup for semi-controlled conditions. It allowed a comfort of experimentation as well as the statistical confirmation of the trends identified by Kwa (2003). It also allowed identifying new trends (with the light and the synthetical hormone addition). However, some points need to be improved, especially the use of heating or air conditioning as it impacts the relative humidity, as suggested by Fouda and Melikyan (2011). It would also be interesting to test the various trends identified on a larger scale since the sample size was limited, in this experiment, by the availability of measuring equipment and the quantity of growth chamber.

Specific context

The experiment presented in this work was carried out in Guadeloupe, where the dissociation between Cavendish intended for export in structured channel market and plantain intended for the local market is also present. In this context, the PIF technique has already been adopted by some farmers, but they highlighted a number of difficulties, including the time required to set up this technique and the small number of produced shoots (Bezard *et al.*, 2023a–c). However, by optimizing this technique, farmers will be able to reduce their production costs and increase their autonomy.

Conclusion

In this experiment, we highlighted the important factors for optimizing the PIF technique. These factors can easily be varied, which can be of major interest to farmers who lack plantain shoots. Furthermore, this technique, set up by respecting the good practices, does not present any sanitary risk. These results open perspectives for research. Larger scale tests could confirm or refute the trends identified. The experimental setup could be improved to statistically discriminate the environmental impacting factors with the addition of a fogger to control humidity as the temperature increases.

For the farmers, these results are interesting since the factors identified as significantly impacting are factors that can be varied by creating 'greenhouse' devices with a warm and humid environment. The other factors identified in the trends could present interesting perspectives in nursery type production conditions.

Declaration of competing interest

The authors declare that they have no conflicts of interest.

Authors' contributions

Marie Bezard, David Hammouya, Thierry Bajazet, Marie Umber, Jean-Louis Diman and Harry Ozier Lafontaine contributed to the study conception and design. All authors contributed to the development of the methodology. The data collection was performed by Marie Bezard, Marion Villard and Simon Pourrat. Marie Bezard and Marie Umber performed the molecular viral indexation. The data visualization was prepared by Marie Bezard, the first draft of the manuscript was written by Marie Bezard and all authors commented on earlier versions of the manuscript. All authors read and approved the final manuscript.

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SUPPLEMENTAL INFORMATION

SUPPLEMENTAL INFORMATION – TABLE S1. Sequence of primers and PCR parameters used in this study.

Name	Targeted virus or plant	Sequence	Reference	Primers (nM)	MgCl$_2$ (mM)	Annealing temperature	Size of amplification product
PDO-F1i	BanMMV	5' TIT TYA TKA ARW SIC ARY WIT GIA C 3'	Foissac et al., 2005	800	3	49°C	362 pb
PDO-F2i		5' GCY AAR GCI GGI CAR ACI YTK GCI TG 3'					
PDO-R1i		5' TCH CCW GTR AAI CKS ATI AII GC 3'					
PDO-R3i		5' GCR CAC ATR TCR TCI CCI GCR AAI IA 3'					
PDO-R4i		5' ARI YIC CAT CCR CAR AAM ITI GG 3'					
Bract N1	BBrMV	5' GGR ACA TCA CCA AAT TTR AAT GG 3'	Iskra-Caruana et al., 2008	400	1.5	57°C	280 pb
Bract NR		5' GTG TGC YTC TCT AGC CCT GTT 3'					
BBTV-FN	BBTV	5' GGC GCG ATA TGT GGT ATG CTG G 3'	Mansoor et al., 2005	400	2	58°C	290 pb
BBTV-RN		5' CCA AAC TCG AAG GGA CCT TCG 3'					
Gf-F	BSGFV	5' TCG GTG GAA TAG TCC TGA GTC TTC 3'	Le Provost et al., 2006 Umber et al., 2016	60	1.5	58°C	476
Gf-R		5' ACG AAC TAT CAC GAC TTG TTC AAG C 3'					
Im-F	BSIMV	5' CAC CCA GAC TTT TCT TTC TAG C 3'		200			384
Im-R		5' TGC CAA CGAATA CTA CAT CAAC 3'					
Mys-F	BSMYV	5' TAA AAG CAC AGC TCA GAA CAAACC 3'		400			589
Mys-R		5' CTC CGT GAT TTC TTC GTG GTC 3'					
OL-F	BSOLV	5' GCT CAC TCC GCA TCT TAT CAG TC 3'		120			522
OL-R		5' ATC TGAAGG TGT GTT GAT CAA TGC 3'					
MonF2	*Musa* DNA	5' GTC GAC ACA TGG GAG GAC TT 3'		300			300
MonR2		5' CTT GTT GGG TCT TCA GAG GAA 3'					
BVX1	BVX	5' GCC AAA CTC TCG CTT GTT TC 3'	Teycheney et al., 2007	800	3	53°C	410 pb
BVX3		5' CCA TTC AAT TTG TAC CTC AAAA 3'					
CMV1-F	CMV	5' GTA GAC ATC TGT GAC GCGA 3'	De Blas et al., 1998	200	1.5	53°C	540 bp
CMV1-R		5' GCG CGA AAC AAG CTT CTT ATC 3'					
Actine1F	*Musa actin* gene	5' TCC TTT CGC TCT ATG CCA GT 3'	Gayral et al., 2008	200	1.5	58°C	420 pb
Actine1R		5' GCC CAT CGG GAA GTT CAT AG 3'					

Supplemental Information – Table S2a. Response variables.

Internal code	Weight (g)	Quantity	Plant size (cm)	Roots number	Root size (cm)	Leaves number
			Temperature step			
			Control (average 30 °C) modality			
PT10	200	1	7	6	40	0
PT22	240	1	0	3	62	0
PT4	280	5	10.5	6	80	0.5
PT12	360	2	15	9	76	0
PT23	380	4	0	9	80	0
PT19	500	1	10	2	14	0
PT25	560	1	14	11	64	1
PT17	680	20	0	11	61	0
PT18	780	4	0	13	67	0
			25 °C (air-conditioning) modality			
PT2	120	0	0	0	0	0
PT5	220	1	0	9	35	0
PT24	300	0	0	8	42	0
PT3	340	1	3	7	57	2
PT7	400	1	0	3	51	0
PT26	480	3	0	3	56	0
PT15	520	1	0	6	62	0
PT16	760	0	0	2	31	0
PT27	940	1	49	25	62	4
			35 °C (heater) modality			
PT1	120	14	10.25	11	47	1.75
PT13	300	4	19	6	80	1
PT11	340	16	11.25	18	78	1.5
PT21	380	11	15.5	26	82	1.5
PT8	440	5	10.8	12	80	0.6
PT14	580	3	44	22	92	3
PT9	740	1	20	14	66	1
PT6	760	3	0	17	85	0

SUPPLEMENTAL INFORMATION – TABLE S2b. Response variables.

Hormone step						
Internal code	Weight (g)	Quantity	Plant size (cm)	Roots number	Root size (cm)	Leaves number
Control modality						
PH12	260	4	22.00	20	92	3.00
PH18	240	0	0.00	0	0	0.00
PH23	360	3	17.00	33	86	3.00
PH10	100	18	6.17	17	95	2.50
PH24	440	1	29.00	13	118	5.00
PH11	200	0	0.00	0	0	0.00
PH30	540	7	12.00	31	80	1.50
PH6	240	10	8.19	23	104	2.13
PH25	320	1	5.00	11	53	2.00
Coconut water modality						
PH9	300	0	0	0	0	0.00
PH7	240	0	0	0	0	0.00
PH13	420	14	11.67	18	42	3.33
PH8	180	1	14.50	5	34	5.00
PH22	460	4	12.33	19	97.5	2.33
PH5	220	0	0	0	0	0.00
PH27	660	0	0	0	0	0.00
PH17	260	0	0	0	0	0.00
PH26	340	3	10.67	6	41	2.67
Synthetic hormone modality						
PH21	280	3	14.50	17	50	2.50
PH15	240	32	10.83	11	101	2.33
PH29	380	6	11.25	11	90	1.75
PH4	140	0	0.00	0	0	0.00
PH31	460	7	13.00	14	120	2.00
PH20	220	16	28.00	23	129	3.00
PH28	560	6	8.83	7	82	1.67
PH1	240	3	15.00	7	84	2.00
PH14	320	5	19.00	17	80	3.00

Supplemental Information – Table S2c. Response variables.

Internal code	Weight (g)	Quantity	Plant size (cm)	Roots number	Root size (cm)	Leaves number
**Light and optimum step**
*Control modality*
PL9	502	0	0.00	5	9	0.00
PL17	375	5	2.90	7	46.5	0.00
PL35	244	3	26.00	10	77	4.00
PL25	278	5	41.00	21	80	1.33
PL1	572	6	9.00	16	70.5	0.75
PL6	482	5	30.00	20	67	3.00
PL38	175	1	25.00	20	85.5	4.00
PL29	333	3	10.50	25	51	1.00
PL31	778	8	38.00	24	59	3.00
*Light modality*
PL30	512	3	21	13	84	2.50
PL37	282	11	9.75	17	76	2.25
PL5	240	1	24.00	24	45	4.00
PL12	414	5	25.00	23	62	4.00
PL16	523	5	21.00	27	93.5	3.50
PL20	192	1	23	25	81.5	4.00
PL11	473	6	11	19	90	1.00
PL24	290	6	33	28	72	3.00
PL2	813	6	3.25	10	45	0.00
*Optimum (light + heater) modality*
PL8	492	4	9.25	32	37	1.50
PL3	345	20	9.33	20	76	1.50
PL19	252	19	8.88	11	66	1.25
PL18	264	5	11.63	16	50	2.50
PL21	645	4	29.00	11	72	4.00
PL14	484	2	15.25	23	62	2.00
PL34	151	13	7.06	13	59.5	1.50
PL27	341	9	11.88	14	93.5	1.75
PL23	646	14	12.75	32	85	2.00

SUPPLEMENTAL INFORMATION – TABLE S3. Indexing results for all samples analyzed in this study.

Varieties	Internal code	Species	Date of collection	Country of origin	Municipality	Ban-MMV	BBrMV	BBTV	BSGFV	BSIMV	BSMYV	BSOLV	BVX	CMV
	Samples			Location		Virus indexing results								
French Clair	B1	*Musa* spp.	24-7-2017	Guadeloupe	Capesterre Belle-Eau	+	-	-	ND	ND	ND	ND	-	-
French Clair	B2	*Musa* spp.	24-7-2017	Guadeloupe	Capesterre Belle-Eau	+	-	-	ND	ND	ND	ND	-	-
French Clair	B3	*Musa* spp.	24-7-2017	Guadeloupe	Capesterre Belle-Eau	+	-	-	ND	ND	ND	ND	-	-
French Clair	B4	*Musa* spp.	24-7-2017	Guadeloupe	Capesterre Belle-Eau	+	-	-	ND	ND	ND	ND	-	-
French Clair	B5	*Musa* spp.	24-7-2017	Guadeloupe	Capesterre Belle-Eau	+	-	-	ND	ND	ND	ND	-	-
French Clair	B6	*Musa* spp.	24-7-2017	Guadeloupe	Capesterre Belle-Eau	+	-	-	ND	ND	ND	ND	-	-
French Clair	B7	*Musa* spp.	24-7-2017	Guadeloupe	Capesterre Belle-Eau	+	-	-	ND	ND	ND	ND	-	-
French Clair	B8	*Musa* spp.	24-7-2017	Guadeloupe	Capesterre Belle-Eau	+	-	-	ND	ND	ND	ND	-	-
French Clair	B9	*Musa* spp.	24-7-2017	Guadeloupe	Capesterre Belle-Eau	+	-	-	ND	ND	ND	ND	-	-
French Clair	B10	*Musa* spp.	24-7-2017	Guadeloupe	Capesterre Belle-Eau	+	-	-	ND	ND	ND	ND	-	-
French Clair	B11	*Musa* spp.	24-7-2017	Guadeloupe	Capesterre Belle-Eau	+	-	-	ND	ND	ND	ND	-	-
French Clair	B12	*Musa* spp.	24-7-2017	Guadeloupe	Capesterre Belle-Eau	+	-	-	ND	ND	ND	ND	-	-
French Clair	B14	*Musa* spp.	24-7-2017	Guadeloupe	Capesterre Belle-Eau	+	-	-	ND	ND	ND	ND	-	-
French Clair	B15	*Musa* spp.	24-7-2017	Guadeloupe	Capesterre Belle-Eau	+	-	-	ND	ND	ND	ND	-	-
French Clair	B16	*Musa* spp.	24-7-2017	Guadeloupe	Capesterre Belle-Eau	+	-	-	ND	ND	ND	ND	-	-
French Clair	B17	*Musa* spp.	24-7-2017	Guadeloupe	Capesterre Belle-Eau	+	-	-	ND	ND	ND	ND	-	-
French Clair	B18	*Musa* spp.	24-7-2017	Guadeloupe	Capesterre Belle-Eau	+	-	-	ND	ND	ND	ND	-	-
French Clair	B19	*Musa* spp.	24-7-2017	Guadeloupe	Capesterre Belle-Eau	+	-	-	ND	ND	ND	ND	-	-
French Clair	B20	*Musa* spp.	24-7-2017	Guadeloupe	Capesterre Belle-Eau	+	-	-	ND	ND	ND	ND	-	-
French Clair	B21	*Musa* spp.	24-7-2017	Guadeloupe	Capesterre Belle-Eau	+	-	-	ND	ND	ND	ND	-	-
French Clair	B22	*Musa* spp.	24-7-2017	Guadeloupe	Capesterre Belle-Eau	+	-	-	ND	ND	ND	ND	-	-
French Clair	B23	*Musa* spp.	24-7-2017	Guadeloupe	Capesterre Belle-Eau	-	-	-	ND	ND	ND	ND	-	-
French Clair	B24	*Musa* spp.	24-7-2017	Guadeloupe	Capesterre Belle-Eau	-	-	-	ND	ND	ND	ND	-	-
French Clair	B25	*Musa* spp.	24-7-2017	Guadeloupe	Capesterre Belle-Eau	-	-	-	ND	ND	ND	ND	-	-
French Clair	B26	*Musa* spp.	24-7-2017	Guadeloupe	Capesterre Belle-Eau	+	-	-	ND	ND	ND	ND	-	-
French Clair	B27	*Musa* spp.	24-7-2017	Guadeloupe	Capesterre Belle-Eau	+	-	-	ND	ND	ND	ND	-	-
French Clair	B28	*Musa* spp.	24-7-2017	Guadeloupe	Capesterre Belle-Eau	+	-	-	ND	ND	ND	ND	-	-
French Clair	B29	*Musa* spp.	24-7-2017	Guadeloupe	Capesterre Belle-Eau	+	-	-	ND	ND	ND	ND	-	-
French Clair	B30	*Musa* spp.	24-7-2017	Guadeloupe	Capesterre Belle-Eau	+	-	-	ND	ND	ND	ND	-	-
French Clair	B31	*Musa* spp.	24-7-2017	Guadeloupe	Capesterre Belle-Eau	+	-	-	ND	ND	ND	ND	-	-
French Clair	B32	*Musa* spp.	24-7-2017	Guadeloupe	Capesterre Belle-Eau	+	-	-	ND	ND	ND	ND	-	-
French Clair	B33	*Musa* spp.	24-7-2017	Guadeloupe	Capesterre Belle-Eau	+	-	-	ND	ND	ND	ND	-	-
French Clair	B34	*Musa* spp.	24-7-2017	Guadeloupe	Capesterre Belle-Eau	+	-	-	ND	ND	ND	ND	-	-

SUPPLEMENTAL INFORMATION – TABLE S3. Continued.

Varieties	Samples Internal code	Species	Date of collection	Location Country of origin	Municipality	Ban-MMV	BBrMV	BBTV	BSGFV	BSIMV	BSMYV	BSOLV	BVX	CMV
French Clair	B35	*Musa* spp.	24-7-2017	Guadeloupe	Capesterre Belle-Eau	+	-	-	ND	ND	ND	ND	-	-
French Clair	B36	*Musa* spp.	24-7-2017	Guadeloupe	Capesterre Belle-Eau	+	-	-	ND	ND	ND	ND	-	-
French Clair	B37	*Musa* spp.	24-7-2017	Guadeloupe	Capesterre Belle-Eau	+	-	-	ND	ND	ND	ND	-	-
French Clair	B38	*Musa* spp.	24-7-2017	Guadeloupe	Capesterre Belle-Eau	+	-	-	ND	ND	ND	ND	-	-
French Clair	B39	*Musa* spp.	24-7-2017	Guadeloupe	Capesterre Belle-Eau	+	-	-	ND	ND	ND	ND	-	-
French Clair	B40	*Musa* spp.	24-7-2017	Guadeloupe	Capesterre Belle-Eau	+	-	-	ND	ND	ND	-	-	-
French Clair	B100	*Musa* spp.	29-8-2017	Guadeloupe	Capesterre Belle-Eau	+	-	-	-	-	-	-	-	-
French Clair	B101	*Musa* spp.	29-8-2017	Guadeloupe	Capesterre Belle-Eau	+	-	-	-	-	-	-	-	-
French Clair	B103	*Musa* spp.	29-8-2017	Guadeloupe	Capesterre Belle-Eau	-	-	-	-	-	-	-	-	-
French Clair	B104	*Musa* spp.	29-8-2017	Guadeloupe	Capesterre Belle-Eau	-	-	-	-	-	-	-	-	-
French Clair	B105	*Musa* spp.	29-8-2017	Guadeloupe	Capesterre Belle-Eau	-	-	-	-	-	-	-	-	-
French Clair	B106	*Musa* spp.	29-8-2017	Guadeloupe	Capesterre Belle-Eau	-	-	-	-	-	-	-	-	-
French Clair	B111	*Musa* spp.	29-8-2017	Guadeloupe	Capesterre Belle-Eau	+	-	-	-	-	-	-	-	-
French Clair	B112	*Musa* spp.	29-8-2017	Guadeloupe	Capesterre Belle-Eau	-	-	-	-	-	-	-	-	-
French Clair	B113	*Musa* spp.	29-8-2017	Guadeloupe	Capesterre Belle-Eau	+	-	-	-	-	-	-	-	-
French Clair	B114	*Musa* spp.	29-8-2017	Guadeloupe	Capesterre Belle-Eau	+	-	-	-	-	-	-	-	-
French Clair	B115	*Musa* spp.	29-8-2017	Guadeloupe	Capesterre Belle-Eau	+	-	-	-	-	-	-	-	-
French Clair	B117	*Musa* spp.	29-8-2017	Guadeloupe	Capesterre Belle-Eau	+	-	-	-	-	-	-	-	-
French Clair	B119	*Musa* spp.	29-8-2017	Guadeloupe	Capesterre Belle-Eau	-	-	-	-	-	-	-	-	-
French Clair	B122	*Musa* spp.	29-8-2017	Guadeloupe	Capesterre Belle-Eau	-	-	-	-	-	-	-	-	-
French Clair	B124	*Musa* spp.	29-8-2017	Guadeloupe	Capesterre Belle-Eau	+	-	-	-	-	-	-	-	-
French Clair	B129	*Musa* spp.	29-8-2017	Guadeloupe	Capesterre Belle-Eau	-	-	-	-	-	-	-	-	-
French Clair	B130	*Musa* spp.	29-8-2017	Guadeloupe	Capesterre Belle-Eau	+	-	-	-	-	-	-	-	-
French Clair	B131	*Musa* spp.	29-8-2017	Guadeloupe	Capesterre Belle-Eau	+	-	-	-	-	-	-	-	-
French Clair	B132	*Musa* spp.	29-8-2017	Guadeloupe	Capesterre Belle-Eau	+	-	-	-	-	-	-	-	-
French Clair	B133	*Musa* spp.	29-8-2017	Guadeloupe	Capesterre Belle-Eau	-	-	-	-	-	-	-	-	-
French Clair	B134	*Musa* spp.	29-8-2017	Guadeloupe	Capesterre Belle-Eau	+	-	-	-	-	-	-	-	-
French Clair	B135	*Musa* spp.	29-8-2017	Guadeloupe	Capesterre Belle-Eau	-	-	-	-	-	-	-	-	-
French Clair	B136	*Musa* spp.	29-8-2017	Guadeloupe	Capesterre Belle-Eau	-	-	-	-	-	-	-	-	-
French Clair	B137	*Musa* spp.	29-8-2017	Guadeloupe	Capesterre Belle-Eau	-	-	-	-	-	-	-	-	-
French Clair	B138	*Musa* spp.	29-8-2017	Guadeloupe	Capesterre Belle-Eau	-	-	-	-	-	-	-	-	-
French Clair	B139	*Musa* spp.	29-8-2017	Guadeloupe	Capesterre Belle-Eau	+	-	-	-	-	-	-	-	-
French Clair	B140	*Musa* spp.	29-8-2017	Guadeloupe	Capesterre Belle-Eau	+	-	-	-	-	-	-	-	-

SUPPLEMENTAL INFORMATION – TABLE S3. Continued.

Varieties	Samples			Location		Virus indexing results								
	Internal code	Species	Date of collection	Country of origin	Municipality	Ban-MMV	BBrMV	BBTV	BSGFV	BSIMV	BSMYV	BSOLV	BVX	CMV
French Clair	B141	*Musa* spp.	29-8-2017	Guadeloupe	Capesterre Belle-Eau	+	-	-	-	-	-	-	-	-
French Clair	B142	*Musa* spp.	29-8-2017	Guadeloupe	Capesterre Belle-Eau	-	-	-	-	-	-	-	-	-
French Clair	B143	*Musa* spp.	29-8-2017	Guadeloupe	Capesterre Belle-Eau	+	-	-	-	-	-	-	-	-
French Clair	B144	*Musa* spp.	29-8-2017	Guadeloupe	Capesterre Belle-Eau	-	-	-	-	-	-	-	-	-
French Clair	B145	*Musa* spp.	29-8-2017	Guadeloupe	Capesterre Belle-Eau	+	-	-	-	-	-	-	-	-
French Clair	B146	*Musa* spp.	29-8-2017	Guadeloupe	Capesterre Belle-Eau	+	-	-	-	-	-	-	-	-
French Clair	B147	*Musa* spp.	29-8-2017	Guadeloupe	Capesterre Belle-Eau	+	-	-	-	-	-	-	-	-
French Clair	B148	*Musa* spp.	29-8-2017	Guadeloupe	Capesterre Belle-Eau	+	-	-	-	-	-	-	-	-
French Clair	B149	*Musa* spp.	29-8-2017	Guadeloupe	Capesterre Belle-Eau	+	-	-	-	-	-	-	-	-
French Clair	L32 mp	*Musa* spp.	8-3-2022	Guadeloupe	Petit-Bourg	+	ND	ND	ND	ND	ND	ND	ND	ND
French Clair	L32-1	*Musa* spp.	26-7-2022	Guadeloupe	Petit-Bourg	+	ND	ND	ND	ND	ND	ND	ND	ND
French Clair	L32-2	*Musa* spp.	26-2-2022	Guadeloupe	Petit-Bourg	+	ND	ND	ND	ND	ND	ND	ND	ND
French Clair	L32-3	*Musa* spp.	26-2-2022	Guadeloupe	Petit-Bourg	+	ND	ND	ND	ND	ND	ND	ND	ND
French Clair	PH13 mp	*Musa* spp.	26-7-2022	Guadeloupe	Petit-Bourg	+	ND	ND	ND	ND	ND	ND	ND	ND
French Clair	PH13-1	*Musa* spp.	7-11-2022	Guadeloupe	Petit-Bourg	-	ND	ND	ND	ND	ND	ND	ND	ND
French Clair	PH30 mp	*Musa* spp.	26-7-2022	Guadeloupe	Petit-Bourg	+	ND	ND	ND	ND	ND	ND	ND	ND
French Clair	PH30-1	*Musa* spp.	7-11-2022	Guadeloupe	Petit-Bourg	+	ND	ND	ND	ND	ND	ND	ND	ND
French Clair	PH30-2	*Musa* spp.	7-11-2022	Guadeloupe	Petit-Bourg	+	ND	ND	ND	ND	ND	ND	ND	ND
French Clair	PL1 mp	*Musa* spp.	7-11-2022	Guadeloupe	Petit-Bourg	+	ND	ND	ND	ND	ND	ND	ND	ND
French Clair	PL1-1	*Musa* spp.	18-1-2023	Guadeloupe	Petit-Bourg	-	ND	ND	ND	ND	ND	ND	ND	ND
French Clair	PL1-2	*Musa* spp.	18-1-2023	Guadeloupe	Petit-Bourg	-	ND	ND	ND	ND	ND	ND	ND	ND
French Clair	PL2 mp	*Musa* spp.	7-11-2022	Guadeloupe	Petit-Bourg	+	ND	ND	ND	ND	ND	ND	ND	ND
French Clair	PL2-1	*Musa* spp.	18-1-2023	Guadeloupe	Petit-Bourg	-	ND	ND	ND	ND	ND	ND	ND	ND
French Clair	PL2-2	*Musa* spp.	18-1-2023	Guadeloupe	Petit-Bourg	-	ND	ND	ND	ND	ND	ND	ND	ND
French Clair	PL3 mp	*Musa* spp.	7-11-2022	Guadeloupe	Petit-Bourg	+	ND	ND	ND	ND	ND	ND	ND	ND
French Clair	PL3-1	*Musa* spp.	18-1-2023	Guadeloupe	Petit-Bourg	+	ND	ND	ND	ND	ND	ND	ND	ND
French Clair	PL3-2	*Musa* spp.	18-1-2023	Guadeloupe	Petit-Bourg	+	ND	ND	ND	ND	ND	ND	ND	ND
French Clair	PL3-3	*Musa* spp.	18-1-2023	Guadeloupe	Petit-Bourg	-	ND	ND	ND	ND	ND	ND	ND	ND
French Clair	PL5 mp	*Musa* spp.	7-11-2022	Guadeloupe	Petit-Bourg	-	ND	ND	ND	ND	ND	ND	ND	ND
French Clair	PL6 mp	*Musa* spp.	7-11-2022	Guadeloupe	Petit-Bourg	+	ND	ND	ND	ND	ND	ND	ND	ND
French Clair	PL6-1	*Musa* spp.	18-1-2023	Guadeloupe	Petit-Bourg	+	ND	ND	ND	ND	ND	ND	ND	ND
French Clair	PL6-2	*Musa* spp.	18-1-2023	Guadeloupe	Petit-Bourg	+	ND	ND	ND	ND	ND	ND	ND	ND
French Clair	PL8 mp	*Musa* spp.	7-11-2022	Guadeloupe	Petit-Bourg	-	ND	ND	ND	ND	ND	ND	ND	ND

SUPPLEMENTAL INFORMATION – TABLE S3. Continued.

Varieties	Samples Internal code	Species	Date of collection	Location Country of origin	Municipality	Ban-MMV	BBrMV	BBTV	BSGFV	BSIMV	BSMYV	BSOLV	BVX	CMV
French Clair	PL9 mp	Musa spp.	7-11-2022	Guadeloupe	Petit-Bourg	-	ND	ND	ND	ND	ND	ND	ND	ND
French Clair	PL11 mp	Musa spp.	7-11-2022	Guadeloupe	Petit-Bourg	-	ND	ND	ND	ND	ND	ND	ND	ND
French Clair	PL12 mp	Musa spp.	7-11-2022	Guadeloupe	Petit-Bourg	-	ND	ND	ND	ND	ND	ND	ND	ND
French Clair	PL14 mp	Musa spp.	7-11-2022	Guadeloupe	Petit-Bourg	+	ND	ND	ND	ND	ND	ND	ND	ND
French Clair	PL14-1	Musa spp.	18-1-2023	Guadeloupe	Petit-Bourg	+	ND	ND	ND	ND	ND	ND	ND	ND
French Clair	PL14-2	Musa spp.	18-1-2023	Guadeloupe	Petit-Bourg	+	ND	ND	ND	ND	ND	ND	ND	ND
French Clair	PL16 mp	Musa spp.	7-11-2022	Guadeloupe	Petit-Bourg	+	ND	ND	ND	ND	ND	ND	ND	ND
French Clair	PL16-1	Musa spp.	18-1-2023	Guadeloupe	Petit-Bourg	+	ND	ND	ND	ND	ND	ND	ND	ND
French Clair	PL16-2	Musa spp.	18-1-2023	Guadeloupe	Petit-Bourg	-	ND	ND	ND	ND	ND	ND	ND	ND
French Clair	PL17 mp	Musa spp.	7-11-2022	Guadeloupe	Petit-Bourg	+	ND	ND	ND	ND	ND	ND	ND	ND
French Clair	PL17-1	Musa spp.	18-1-2023	Guadeloupe	Petit-Bourg	+	ND	ND	ND	ND	ND	ND	ND	ND
French Clair	PL17-2	Musa spp.	18-1-2023	Guadeloupe	Petit-Bourg	+	ND	ND	ND	ND	ND	ND	ND	ND
French Clair	PL18 mp	Musa spp.	7-11-2022	Guadeloupe	Petit-Bourg	-	ND	ND	ND	ND	ND	ND	ND	ND
French Clair	PL19 mp	Musa spp.	7-11-2022	Guadeloupe	Petit-Bourg	-	ND	ND	ND	ND	ND	ND	ND	ND
French Clair	PL20 mp	Musa spp.	7-11-2022	Guadeloupe	Petit-Bourg	+	ND	ND	ND	ND	ND	ND	ND	ND
French Clair	PL20-1	Musa spp.	18-1-2023	Guadeloupe	Petit-Bourg	+	ND	ND	ND	ND	ND	ND	ND	ND
French Clair	PL20-2	Musa spp.	18-1-2023	Guadeloupe	Petit-Bourg	+	ND	ND	ND	ND	ND	ND	ND	ND
French Clair	PL21 mp	Musa spp.	7-11-2022	Guadeloupe	Petit-Bourg	-	ND	ND	ND	ND	ND	ND	ND	ND
French Clair	PL23 mp	Musa spp.	7-11-2022	Guadeloupe	Petit-Bourg	-	ND	ND	ND	ND	ND	ND	ND	ND
French Clair	PL24 mp	Musa spp.	7-11-2022	Guadeloupe	Petit-Bourg	-	ND	ND	ND	ND	ND	ND	ND	ND
French Clair	PL25 mp	Musa spp.	7-11-2022	Guadeloupe	Petit-Bourg	+	ND	ND	ND	ND	ND	ND	ND	ND
French Clair	PL25-1	Musa spp.	18-1-2023	Guadeloupe	Petit-Bourg	-	ND	ND	ND	ND	ND	ND	ND	ND
French Clair	PL25-2	Musa spp.	18-1-2023	Guadeloupe	Petit-Bourg	-	ND	ND	ND	ND	ND	ND	ND	ND
French Clair	PL25-3	Musa spp.	18-1-2023	Guadeloupe	Petit-Bourg	-	ND	ND	ND	ND	ND	ND	ND	ND
French Clair	PL25-4	Musa spp.	18-1-2023	Guadeloupe	Petit-Bourg	-	ND	ND	ND	ND	ND	ND	ND	ND
French Clair	PL27 mp	Musa spp.	7-11-2022	Guadeloupe	Petit-Bourg	-	ND	ND	ND	ND	ND	ND	ND	ND
French Clair	PL29 mp	Musa spp.	7-11-2022	Guadeloupe	Petit-Bourg	-	ND	ND	ND	ND	ND	ND	ND	ND
French Clair	PL30 mp	Musa spp.	7-11-2022	Guadeloupe	Petit-Bourg	-	ND	ND	ND	ND	ND	ND	ND	ND
French Clair	PL31 mp	Musa spp.	7-11-2022	Guadeloupe	Petit-Bourg	-	ND	ND	ND	ND	ND	ND	ND	ND
French Clair	PL34 mp	Musa spp.	7-11-2022	Guadeloupe	Petit-Bourg	-	ND	ND	ND	ND	ND	ND	ND	ND
French Clair	PL35 mp	Musa spp.	7-11-2022	Guadeloupe	Petit-Bourg	-	ND	ND	ND	ND	ND	ND	ND	ND
French Clair	PL37 mp	Musa spp.	7-11-2022	Guadeloupe	Petit-Bourg	+	ND	ND	ND	ND	ND	ND	ND	ND
French Clair	PL37-1	Musa spp.	18-1-2023	Guadeloupe	Petit-Bourg	+	ND	ND	ND	ND	ND	ND	ND	ND

SUPPLEMENTAL INFORMATION – TABLE S3. Continued.

| | Samples | | | Location | | Virus indexing results | | | | | | | | |
Varieties	Internal code	Species	Date of collection	Country of origin	Municipality	Ban-MMV	BBrMV	BBTV	BSGFV	BSIMV	BSMYV	BSOLV	BVX	CMV
French Clair	PL37-2	*Musa* spp.	18-1-2023	Guadeloupe	Petit-Bourg	+	ND	ND	ND	ND	ND	ND	ND	ND
French Clair	PL37-3	*Musa* spp.	18-1-2023	Guadeloupe	Petit-Bourg	+	ND	ND	ND	ND	ND	ND	ND	ND
French Clair	PL38 mp	*Musa* spp.	7-11-2022	Guadeloupe	Petit-Bourg	-	ND	ND	ND	ND	ND	ND	ND	ND
French Clair	PL39 mp	*Musa* spp.	7-11-2022	Guadeloupe	Petit-Bourg	-	ND	ND	ND	ND	ND	ND	ND	ND
French Clair	PL40 mp	*Musa* spp.	7-11-2022	Guadeloupe	Petit-Bourg	-	ND	ND	ND	ND	ND	ND	ND	ND

Banana plant tested for the evaluation of viral status.

Mother plant used for PIF production and tested for BanMMV detection.

Shoots produced from PIF method and tested for BanMMV detection.

ND   Not determined.