Sugarcane streak mosaic virus: distribution, prevalence and severity in the integrated farming units of Zuénoula and Borotou-Koro, Côte d'Ivoire

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ABSTRACT

Sugarcane streak mosaic is an emerging viral disease caused by sugarcane streak mosaic virus (SCSMV) recently reported in Ivorian sugar production areas. A 5-year study was performed to determine the spatial distribution, prevalence and severity of SCSMV on commercial varieties in two of the three sugarcane production basins of Côte d'Ivoire. In Zuénoula, 104 plots were evaluated between 2018 and 2021. In Borotou-Koro, 72 plots were evaluated between 2019 and 2022. Disease prevalence and severity were assessed using a 0 to 4 rating scale based on the percentage of symptomatic leaf area.

In Zuénoula, overall disease prevalence was 98% in 2018, 100% in 2020 and 98% in 2021. Varieties M1400/86, M2593/92, R570 and SP711406 were monitored during the three years. M1400/86 and M2593/92 showed a moderately susceptible profile, whereas R570 was susceptible to moderately susceptible and SP711406 was susceptible. In Borotou-Koro, overall disease prevalence was 21% in 2019, 48% in 2020 and 61% in 2022. Varieties Co449, M1400/86, R570 and SP711406 were monitored during the three years. Co449 remained moderately susceptible throughout the monitoring, while M1400/86, R570 and SP711406 varied from partially resistant to moderately susceptible.

This study showed that SCSMV is highly prevalent in the Zuénoula sugar complex and is rapidly spreading in the Borotou-Koro complex. In this epidemic context, varieties showed either a stable or increasing susceptibility profile. *In vitro* regeneration permitted significant reduction in prevalence and severity but efforts must be maintained to achieve higher levels of resistance.

Keywords: Côte d'Ivoire, *Poacevirus*, *Poacevirus sacchari*, *Potyviridae*, sugarcane, varietal susceptibility

INTRODUCTION

Sugarcane cultivation has played an important role in the economy of Côte d'Ivoire since its expansion in the 1970s. It is produced on an area of 35,337 ha in four agro-industrial complexes located in Borotou-Koro (North-West), Ferkessédougou (Ferké 1 and Ferké 2, North) and Zuénoula (Centre) (Ilboudo, 2023 ; SUCRIVOIRE, 2022). These complexes are specialized in the industrial production and processing of sugarcane. In addition, there are also small sugarcane farms around the large plantations. National sugar production is estimated at over 214,000 t/year, representing 3.3% of agricultural Gross Domestic Product (GDP), or 1% of national GDP and providing over 10,000 employments (FAO, 2019).

Numerous biological constraints, including pests and diseases, contribute to limiting sugarcane production. *Eldana saccharina* is the main sugarcane stem-boring insect in Côte d'Ivoire (Kouamé et al., 2010). There are many fungal diseases of sugarcane, but in Côte d'Ivoire the most damaging are sugarcane smut (*Sporisorium scitamineum*) and orange rut (*Puccinia kuehnii*) (Yao et al., 2021, Saumtally et al., 2011). Leaf scald (*Xanthomonas albilineans*) is the main bacterial disease (N'guessan et al., 2019). Viral diseases include yellow leaf disease, characterized by varying degrees of vein yellowing caused by sugarcane yellow leaf virus (SCYLV, species newly renamed *Polerovirus SCYLV* by the International Committee on Virus Taxonomy of Viruses (ICTV, 2024)) and mosaic disease. Several viruses of the family *Potyviridae* cause sugarcane mosaic disease (Lu et al., 2021 ; Daugrois et al., 2024): sugarcane mosaic virus (SCMV, newly renamed *Potyvirus sacchari* by ICTV, 2024) , sorghum mosaic virus (SrMV, newly renamed *Potyvirus sorghitessellati* by ICTV, 2024) and sugarcane streak mosaic virus (SCSMV, newly renamed *Poacevirus sacchari* by ICTV, 2024) . SCMV was considered by scientists to be the only virus causing sugarcane mosaic before the 1990s (Lu et al., 2021). Molecular analyses revealed that SrMV and SCSMV also cause sugarcane mosaic. SCSMV and SrMV have been independently classified as new mosaic virus species by the ICTV. Symptoms of mosaic disease caused by SCMV, SrMV and SCSMV are similar (Hall et al., 1998; Viswanathan et al., 2007). Among the viruses causing sugarcane mosaic diseases, SCSMV is one of the most damaging (Lu et al., 2021). The first report of streak mosaic virus was made by Hall et al. (1998) from quarantined germplasm imported from Pakistan to the USA and showing mosaic symptoms. Subsequently, the virus has also been reported in most Asian countries such as Bangladesh, India, Indonesia, Iran, Sri Lanka, Thailand, Vietnam and China (for a review, see Daugrois et al., 2024). SCSMV belongs to the genus *Poacevirus* in the family *Potyviridae* (Li et al., 2011). The natural hosts of SCSMV are plants belonging to the *Poaceae* family, including sugarcane, maize and sorghum (Srinivas et al., 2010; Fu et al., 2015 ; Daugrois et al., 2024). Putra et al. (2014) have shown that this virus can cause yield losses in sugarcane tonnage and sugar of 16-17% and 19-22%, respectively in Indonesia. SCSMV can be mechanically transmitted through cutting tools during harvest, but no biological vector(s) of the virus has been described so far (He *et al.*, 2014 ; Daugrois et al., 2024).

SCSMV has been described mainly in Asian countries, but was recently discovered in sugar complexes in Côte d'Ivoire, West Africa (Sorho et al., 2020; Daugrois et al., 2020; Lu et al., 2021). Sorho et al. (2020) showed that serological tests carried out on infected sap using a DAS-ELISA kit (DSMZ, RT-0166) were negative for SCMV, and no amplification products were obtained by RT-PCR using primers specific to the SCMV coat protein (CP) gene, analyzing 82 symptomatic samples collected from all sugarcane production sites in Côte d'Ivoire. Daugrois et al (2024) also showed that SCMV and SrMV were absent in symptomatic samples of sugarcane mosaic in Côte d'Ivoire. However, these samples were positive for SCSMV. Finally, no study has yet revealed the presence of SCMV and SrMV on sugarcane in Côte d'Ivoire. On the basis of this previous information, we considered that the mosaic symptoms observed in sugarcane production sites in Côte d'Ivoire were caused solely by SCSMV.

In order to assess the threat that this emerging virus could represent for the Ivorian sugar industry, an in-depth study was launched in two sugar complexes in Côte d'Ivoire. The objectives were to assess the spatial distribution, prevalence and severity of SCSMV, as well as the level of susceptibility of the sugarcane varieties grown in these complexes.

MATERIALS AND METHODS

Study areas

The study was carried out on SUCRIVOIRE's Integrated Agricultural Units (IAU) located in Zuénoula and Borotou-Koro. In Zuénoula, the study was carried out from October 2018 to October 2021 and in Borotou-Koro, the study was carried out from November 2019 to April 2022. The Zuénoula IAU covers an area of 11,000 hectares, including 6,223 hectares of industrial plantations, either irrigated (pivots) or rainfed between latitudes 7°30 and 7°40 North and longitudes 6°5 and 6°15 West. It is located in a transition zone between forest and savannah, with an equatorial and subtropical climate. The Borotou-Koro IAU covers 9,702 hectares of industrial plantations (irrigated or rainfed) between latitudes 8°20 and 8°40 North, and longitudes 7°5 and 7°15 West. It belongs to the sub-Sudanese zone, with a humid tropical Sudano-Guinean climate.

Plant material

The evaluation of the spatial distribution, prevalence and severity of SCSMV was carried out at three periods on sugarcane plots that were between 3 and 8 months old. For virgin plots, i.e. plots planted with cuttings originating from nurseries, the age of the plots was determined from the planting of cuttings from the nursery, while the age of the regrowth plots (ranging from 1 to 5 regrowths) was determined from the previous harvest date. For Zuénoula, the first period took place between February and October 2018 on 9 commercial varieties (55 irrigated plots and 12 rainfed plots), the second in October 2020 on 6 commercial varieties (21 irrigated plots and 1 rainfed plot) and the third in October 2021 on 12 commercial varieties (18 irrigated plots and 7 rainfed plots). For Borotou-Koro, the first period took place between October and November 2019 on 8 commercial varieties (21 irrigated plots and 6 rainfed plots), the second in October 2020 on 8 commercial varieties (16 irrigated plots and 6 rainfed plots) and the third from March to April 2022 on 10 commercial varieties (20 irrigated plots and 3 rainfed plots). These varieties were of diverse origins (Table 1). Selected plots represented the crop age range generally observed in

ivorian agro-industrial complexes (virgin crop established from cuttings from nurseries to 5-cycle regrown crops). They were different for each evaluation campaign.

Assessment of symptoms

The size of the rainfed plots at both sites ranged from 1 to 30 hectares, and that of the quarter pivot plots from 12 to 36 hectares. A M-type device with 5 microplots (quadrats) per plot was adopted for symptom assessment (Fig. 1). Each quadrat was 100 m^2 (10 m x 10 m) with 7 rows. Observations were made on 30 sugarcane plants randomly selected over 10 m for each of the 7 rows, for a total of 210 plants per quadrat and 1,050 plants per plot. A severity score (Si, $i \in [0;4]$) was assigned to each plant based on the percentage of symptomatic area on the third extended leaf below the apex using a rating scale adapted from Putra et *al*. 2014 (Fig. 2; Table 2). Mean prevalence (P) and mean severity (S) were calculated per quadrat and per plot with the following formulas:

$$P(\%) = \frac{\sum_{i=1}^{4} Ni}{N} \times 100$$

$$S = \frac{\sum_{i=1}^{4} Si \times Ni}{N}$$

with Si the severity score, Ni the number of plants with severity score Si in the area unit considered (quadrat or plot), N the number of plants observed per area unit considered (quadrat or plot).
Maps of spatial distribution of SCSMV were established based on the prevalence of SCSMV with the following categories: Healthy (P =0 %), Low ($0\% < P \leq 10\%$), Moderate ($10\% < P \leq 70\%$) and High ($70\% < P \leq 100\%$).

Sampling method and sample conditioning

The calculations of mean prevalence presented previously are only reliable if the score 0 (absence of symptoms) assigned based on visual observation corresponds to absence of virus. For 8 of the 22 plots assessed in 2020 in Borotou-Koro, visual scoring on the rated leaf was coupled with leaf testing for SCSMV infection by reverse transcriptase polymerase chain reaction (RT-PCR). Three to four samples were taken from each of the 5 quadrats of the M-type plot assessment device, for a total of 147 samples tested in the laboratory.

To assess the impact of sample dehydration and conditioning methods on the detection of SCSMV by RT-PCR, all samples were dehydrated and conditioned in duplicate using two different methods.

1) calcium chloride dehydration (CCD) according to the method of Bos (1969): a 6.5g leaf subsample was finely chopped with a single-use razor blade and packaged in an absorbent paper pad inserted in a jar containing 3.5g calcium chloride ($CaCl_2$), hermetically sealed and stored at 4°C;

2) hot-air dehydration (HAD): sampled leaves were roughly cut into strips, packed in kraft paper and oven-dried at 30°C for 48 hours in the laboratory.

Molecular detection of SCSMV

Total RNA extraction

Samples were prepared in individual extraction bags (Bioreba): 0.3 g dehydrated leaves were rehydrated in 4 mL phosphate solution (Na_2HPO_4 0.03M + diethyldithiocarbamate (DIECA) 0.2% w/v) for 2 h at 4°C before grinding. Two more ml of phosphate solution were added to the extraction bag after grinding. A 250 µL aliquot of crude extract was collected and stored at -20°C until use. Total RNAs were extracted with Tri-reagent (Molecular Research Center, Cincinnati, OH) with some modifications of the manufacturer's protocol. A 500 µL volume of Tri-reagent was added to the 250 µL sample, homogenized by vortexing for 2-3 s and left for 5 min at room temperature. Next, 100 µL of chloroform was added to each tube, homogenized by vortexing and left for 10 min at room temperature. The tubes were centrifuged at 14,000 rpm (18400g) and 4°C, for 10 min. At the end of the first centrifugation, 450 µL of the upper aqueous phase was removed from each tube and added to 150 µL of isopropanol and 150 µL of sodium acetate (3M pH 5,2). After precipitation at -20°C for at least 30 min, a second centrifugation was performed at 14,000 rpm (18400 g) and 4°C for 15 min. The pellet was washed with 500 µL of 70% ethanol. After centrifugation at 14,000 rpm (18400 g) and 4°C for 5 min, the supernatant was discarded and the pellet was resuspended in 20 µL of RNAse-free distilled water and stored at -20°C.

Amplification of viral cDNA by RT-PCR

The fifteen complete nucleotide sequences of SCSMV available in GenBank and belonging to the two major molecular groups known for this virus (Kasemsin et al., 2016) were downloaded and aligned with ClustalW included in MEGA6 (Tamura et al., 2013) : KJ187047- KJ187048 KJ187049, JF488066, JF488064, JF488065, JN163911, JN941985, GQ246187, GQ388116, KX430771, KX430772, KX823354, KX823355, KX823356 (Fig S1). Primers were chosen on either side of the N-terminal region of the capsid, in regions as conserved as possible between all the sequences: SCSMV-9280-R 5'-CCACTTGTACGCCAATTCGCC-3' and 5'-SCSMV-8450-F CCAAARCTATCACGAGAACG-3', the names of the primers indicating the position of their 5' extremity in the genome, yielding an expected 830-nt fragment (Fig S2). RNAs were denatured by adding 8 µl of sterile water to 2 µl of total RNA and heating at 80°C for 3 min. Fifteen microliters of RT-PCR mix were added to each tube of 10 µl denatured RNA to obtain a total reaction volume of 25 µl, with a final composition of 1X RT-PCR buffer (Sigma), 1.5 mM MgCl2, 0.25 mM dNTPs, 0.5 µM of each primer, 2.5 U of MMLV reverse transcriptase (Promega), 2.5 U of Taq hot start DNA polymerase (Promega). One-step RT-PCR was performed with a reverse transcription (RT) at 42°C for 1 h, denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, primer hybridization at 60°C for 30 s, elongation at 72°C for 50 s and final elongation at 72°C for 10 min. Next, 2.5 µl of the products obtained from each RT-PCR tube were analyzed on a 1.5% electrophoresis agarose gel.

Statistical analysis

Statistical analyses were performed using STATISTICA 7.1 and XLSTAT 2016 in EXCEL. A Shapiro-Wilks normality test was performed for the variables and residuals of the variables of prevalence and severity of different sugarcane varieties. The test showed that both variables follow the normal distribution. A test of homogeneity of variances was carried out using the Bartlett test.

The test showed that there was no equality between the variances. The effect of plant variety on SCSMV mean prevalence and severity was investigated through nonparametric Kruskal-Wallis tests. When the null hypothesis of equality was rejected, Dunn's pairwise multiple comparisons were performed using the Bonferroni correction.
Distribution maps of sugarcane streak mosaic prevalence were produced using ArcMap 10.8 software, at a scale of 1:80,000 for maps of the Zuénoula IAU and 1:100,000 for maps of the Borotou-Koro IAU.

RESULTS

Spatial distribution and overall SCSMV prevalence in Zuénoula

Symptoms were widespread on all cane plots (nurseries, industrial plots) in the three sectors of the Zuénoula IAU. All varieties evaluated showed typical sugarcane streak mosaic symptoms. Streaks were distributed over the entire leaf surface and tended to be more diffuse on older leaves. For varieties Co449, M1400/86, M2593/92, R570, SP711406Rég, M1954/91, R98/4001, R98/4158, R96/2569, R97/0391 and R93/0136 symptoms were very visible on the five youngest leaves and less visible on the older leaves. For Co997, M1176/77, M2580/95, R96/2116 and R579, symptoms were visible on almost all leaves, generally with the same intensity. For Co997, R579 and SP711406, some plants were severely stunted with leaf yellowing and shortened internodes (Fig. 3). In 2018, the overall prevalence of sugarcane streak mosaic symptoms was 98%. Mean prevalences were 100% for 60/67 plots (high) and below 70 % (moderate) for 7/67 plots (Fig. 4a). In 2020, prevalence was 100% (high) in all observed plots (Fig. 4b). In 2021, overall prevalence was 98%. Mean prevalences were 100% (high) for 18/22 plots and below 70% (moderate) for 4/22 plots (Fig. 4c).

Mean prevalence and severity of SCSMV on sugarcane varieties in Zuénoula

In 2018, mean prevalences were very high (98-100%) for 8 of the 9 varieties assessed (Fig. 5a). Only variety M2593/92 had a significantly lower prevalence than the others (85% ; $P<0.005$). Mean severity was high for M2580/95, Co997, R579, M1176/77, SP711406 and Co449 (susceptible). The mean severity of varieties R570, M1400/86 and M2593/92 was significantly lower than the previous ones, they were partially susceptible (Fig. 5b).

In 2020, only 6 sugarcane varieties were evaluated. Some varieties were excluded from the industrial plots due to their high susceptibility to streak mosaic. Mean prevalence was 100% for all 6 varieties tested (Fig. 5c). Mean severity was high for Co997, SP711406 and R570. The mean severities of varieties M1400/86, M2593/92 and M1954/91 were lower although the difference with SP711406 and R570 was not significant for M1400/86 and M2593/92 (Fig. 5d).

In 2021, 12 sugarcane varieties were evaluated. New varieties were introduced in industrial plots and one variety (SP711406Rég) was regenerated by *in vitro* cultivation. Mean prevalences were very high for all varieties assessed: 100% for 5 varieties and 77% to 99% for 7 varieties (Fig. 5e). Mean severity was high for SP71106 and R96/2116. The mean severities of varieties R570, M1400/86, R97/0391, SP711406Rég (regenerated), M1954/91, M2593/92, R96/2569, R98/4158 and R98/4001 were lower than the previous ones but the difference was not always significant

(Fig. 5f). The *in vitro* regenerated variety SP711406Reg showed a significant reduction in prevalence (-22%) and severity (-45%) compared to its parent variety.

SP711406, M1400/86, M2593/92 and R570 were monitored during the three years. SP711406 showed a susceptible profile during the three years: prevalence was 100% and severity was consistently higher than 2. R570 showed a susceptible to moderately susceptible profile: prevalence was 100% and severity was close to 2. M1400/86 and M2593/92 showed a moderately susceptible profile: prevalence was higher than 80% and severity was between 1 and 2.

Spatial distribution and overall prevalence of SCSMV in Borotou-Koro

All varieties evaluated showed typical sugarcane streak mosaic symptoms. Streaks were distributed over the entire leaf surface and tended to be more diffuse on older leaves. For varieties Co449, M1400/86, R570, SP711406Rég, R98/4158, R97/0391, R93/0136, RB725147 and FR8783, symptoms were very visible on the five youngest leaves and less visible on the older leaves. For Co997, M1176/77, M2580/95 and R579, symptoms were visible on almost all leaves and generally at the same intensity. In 2019, the overall prevalence of the disease was 21%. The spatial distribution of the disease presented a disparity between sectors: high prevalences (around 100%) for 4 plots exclusively located in sector E, composed solely of rainfed plots. Prevalences in the other sectors (B, C, D) were moderate for 9 plots (10% to 50%) and low (< 10%) for 14 plots (Fig. 6a). In 2020, the overall prevalence of sugarcane streak mosaic was 48%, with high prevalences for 3 plots in sector E and moderate to low prevalences in sectors B, C and D (Fig. 6b). In 2022, overall prevalence was 61%. Sugarcane streak mosaic was distributed across all sectors, with plots showing high prevalence in all sectors, plots showing moderate prevalence only in sectors B and C and plots showing low prevalence restricted to sector D (Fig. 6c).

Mean prevalence and severity of SCSMV in sugarcane varieties in Borotou-Koro

In 2019, the highest mean prevalence was recorded for Co449 (54 %), R570 (36 %) and Co997 (30 %). Varieties SP711406, R579, RB725147, M1400/86 and M1176/77 showed significantly lower prevalences with 13%, 11%, 10%, 8% and 3% respectively (Fig. 7a). The mean severity of Co449 was 1.29, while that of varieties Co997, R570, SP711406, R579, M1400/86, RB725147 and M1176/77 was significantly lower, at 0.75, 0.57, 0.22, 0.21, 0.15, 0.13 and 0.06 respectively (Fig. 7b).

In 2020, the prevalences of varieties Co449, SP711406, Co997, R570, M2580/95, R579, and M1176/77 varied from 45% to 60%. Disease prevalence was significantly lower in M11400/86, at 25% (Fig. 7c). The mean SCSMV severities of varieties Co449, SP711406, M2580/95, Co997, R579, R570 were 2.01, 1.85, 1.85, 1.75, 1.58, 1.48 respectively. Mean severity was significantly lower on varieties M1176/77 (0.98) and M1400/86 (0.46) (Fig. 7d).

In 2022, 10 sugarcane varieties were evaluated. Mean prevalences exceeded 80% on varieties R93/0136, Co449, R97/0391, M1400/86, R570. M2580/95, SP711406, R98/4158, FR8783 and SP711406Rég showed significantly lower prevalences with respectively 65%, 46%, 23%, 17%, 12%. (Fig. 7e). Mean severities of varieties R93/0136, M1400/86, M2580/95, R570, R97/0391, Co449, SP711406 was 1.45, 1.44, 1.41, 1.40, 1.38, 1.20, 1.13 respectively. Mean

severity was significantly lower on FR8783, R98/418, SP711406Rég than on the previous varieties, with 0.32, 0.24, 0.23 respectively (Fig. 7f). The *in vitro* regenerated variety SP711406Reg showed a significant reduction in prevalence (-75%) and severity (-80%) compared to its parent variety.

A follow-up was carried out on the following 4 varieties: Co449, R570, SP711406 and M1400/86, that were planted during the 3 years of surveys. For these 4 varieties, prevalence increased considerably over time from 54% to 94% for Co449, from 36% to 83% for R570 and from 8% to 83% for M1400/86, with the exception of SP711406 whose prevalence decreased slightly in the third year of evaluation compared with the second year (-13%). The same was true for mean severity, which varied from 1.2 to 2 for Co449, from 0.6 to 1.4 for R570, from 0.1 to 1.4 for M1400/86 and from 0.2 to 1.8 for SP711406.

Virus detection as a function of sample storage type and severity score

Both CCD and HAD conditioning methods enabled amplification of SCSMV (Fig. S3,). For asymptomatic samples (severity score=0), no amplification was observed whatever the type of sample storage (Table 3). For symptomatic samples (severity score>0), the molecular detection method developed enabled amplification in 88% of cases. For score 1, the percentage of detection was 80% and 60% for CCD and HAD dehydration respectively, but the number of samples tested was low. The percentage of detection was over 90% from score 2 to 4, whatever the type of sample storage.

DISCUSSION

This 5-year study provides a detailed assessment of the evolution of the prevalence and severity of SCSMV in two of the three sugarcane production basins of Côte d'Ivoire. The assessment was based on a visual assessment of the presence and intensity of foliar symptoms. A molecular detection test targeting SCSMV was developed and implemented on samples of varying severity. The asymptomatic samples (severity score=0) were never amplified, confirming that the visual assessment of leaf symptoms allows a reliable assessment of the sanitary status of the plant: a plant with a severity score of 0 could be considered healthy with confidence. This information is particularly important for epidemiological surveillance and prophylaxis management in nurseries in case of introduction and evaluation of new sugarcane varieties or on-site multiplication for planting new plots. Indeed Putra et al. (2015) showed that to avoid increased spread of SCSMV, the use of virus-free plant material is recommended. For symptomatic plants (severity score>0), molecular detection was function of the intensity of foliar symptoms with a minimum probability of 91 % of detecting SCSMV when the severity score was greater than 1. Low detection efficiency obtained for samples with a severity score of 1 could be explained by a lower viral titer in the plants associated to a greater degradation of the viral RNA during storage and/or non-optimal specificity of the primers. The presence of other mosaic-inducing viruses, not detected with SCSMV primers, can't be completely excluded, although there has been no evidence for the presence of such viruses in Côte d'Ivoire so far (Daugrois et al., 2020 ; Daugrois et al., 2024 ; Ouattara et al., 2024). For instance, two badnaviruses have been described on sugarcane in Côte d'Ivoire (Ouattara et al., 2024) but there is no evidence that they are related to any symptoms.

Regarding the conditioning method, hot-air dehydration and calcium chloride dehydration were found to be equally effective. This result allows us to prioritize the hot-air dehydration method for future studies because it is easier and faster to implement.

The epidemiological situation of sugarcane streak mosaic was very different in the two sites monitored. In Zuénoula, the overall prevalence was extremely high (>98%) throughout monitoring. The virus was widespread in the three sectors of this site with moderate to high mean prevalences in the plots, which confirms the build-up of strong viral pressure since its first observation in 2015 by Kouamé (pers. comm.). Conversely, in Borotou-Koro, the epidemiological situation evolved gradually throughout monitoring: the overall prevalence was 21% in 2019, 48% in 2020 and 61% in 2022. Sector E, made up entirely of rainfed plots, was the most attacked sector. This could be explained by the multiplication of varieties such as Co997, also known to be susceptible to to mosaic (SCSMV and SCMV) in India (Krishna et al. 2023). These results corroborate those of Putra et al. (2014) who showed in Indonesia that SCSMV had spreads rapidly due to the extension and planting of the highly susceptible variety PS 864, and that the prevalence increased from 0.44% to 86.75% between 2007 and 2011. Putra et al. (2014) also showed that the rapid spread of SCSMV in fields can be facilitated by mechanical transmission with knives during plantation preparation or harvest. Nineteen varieties of sugarcane from various origins were evaluated during the study. Their sensitivity to SCSMV was assessed using a severity scale and the distinct epidemiological situations between the two sites made it possible to test their behaviour in contrasting contexts. In Zuénoula, the varieties M2580/95, Co997, R579, M1176/77, SP711406 and Co449 were the most susceptible, with mean severities greater than 2. The variety SP711406 was susceptible throughout the three years of evaluation. Varieties M1400/86 and M2593/92 remained in the moderately susceptible range ($1 \leq S < 2$) throughout the three years of evaluation, while variety R570 was classified as either susceptible or moderately susceptible depending on the year of assessment. In Borotou-Koro, four varieties were monitored throughout the three years of evaluation: Co449, M1400/86, R570 and SP711406. Co449 remained in the moderately susceptible range ($1 \leq S < 2$) throughout the three years of evaluation, while M1400/86, R570 and SP711406 moved from partially resistant ($0.1 \leq S < 1$) to moderately susceptible ($1 \leq S < 2$) during the study. On both sites, the *in vitro* regenerated variety SP711406Reg showed a significant reduction in prevalence and severity compared to its parent variety but was not completely virus-free, demonstrating that cultivation of vitroplants is a means of reducing risk but does not eliminate it. Either the *in vitro* regeneration did not completely eliminate the virus (Lu et al., 2021 ; Cheong et al., 2012 ; Subba et al., 2011), or the recontamination was fast during the growing season. On both sites, SP711406Rég was tested only during the last year of the survey, so there is no information about the evolution of virus prevalence and severity after several years of field multiplication. *In vitro* culture has been described as an efficient way to reduce virus prevalence (Lu et al., 2021, Subba et al., 2011). It can be associated with the use of partially or highly resistant varieties (Krishna et al., 2023). Indeed, the rational selection and distribution of disease-resistant varieties are the most economical and effective prevention and control measures against viruses (Lu et al., 2021).

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DECLARATIONS

Competing interests The authors have no competing interests that are relevant to the content of this article to declare.

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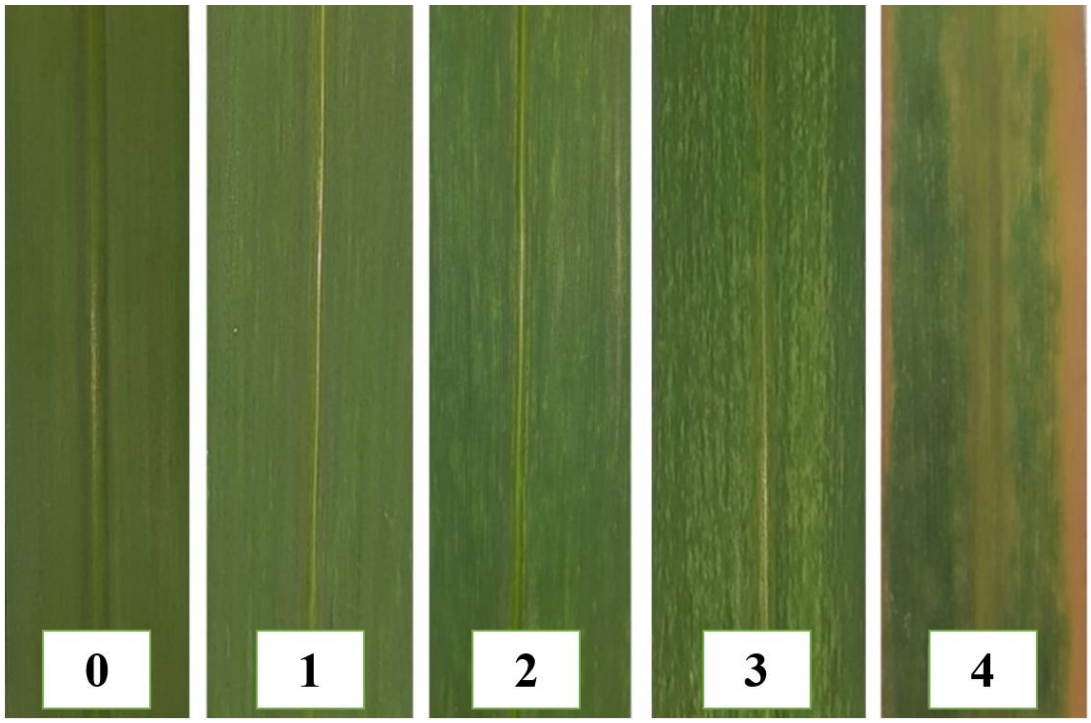

Fig 2. Visual assessment scale of SCSMV used in Zuénoula and Borotou-Koro (see Table 2 for further description)

Fig 3. Yellowing and stunting of SCSMV-infected sugarcane plants (variety R93/0136) in Zuénoula

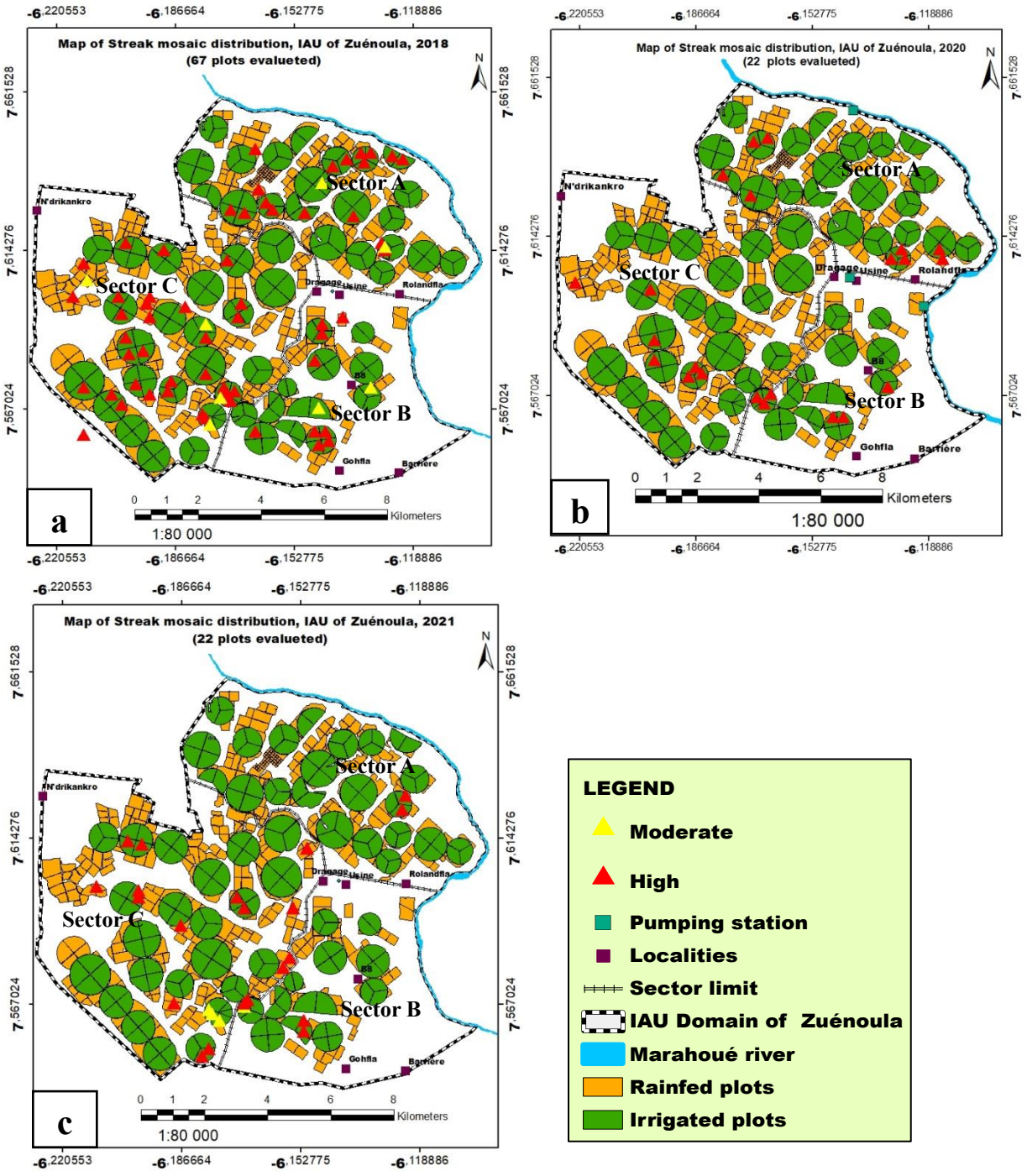

Fig 4. Spatial distribution of SCSMV in Zuénoula in October 2018 (a), October 2020 (b) and October 2021 (c)

« Low », « Moderate » and « High » : are defined according to prevalence

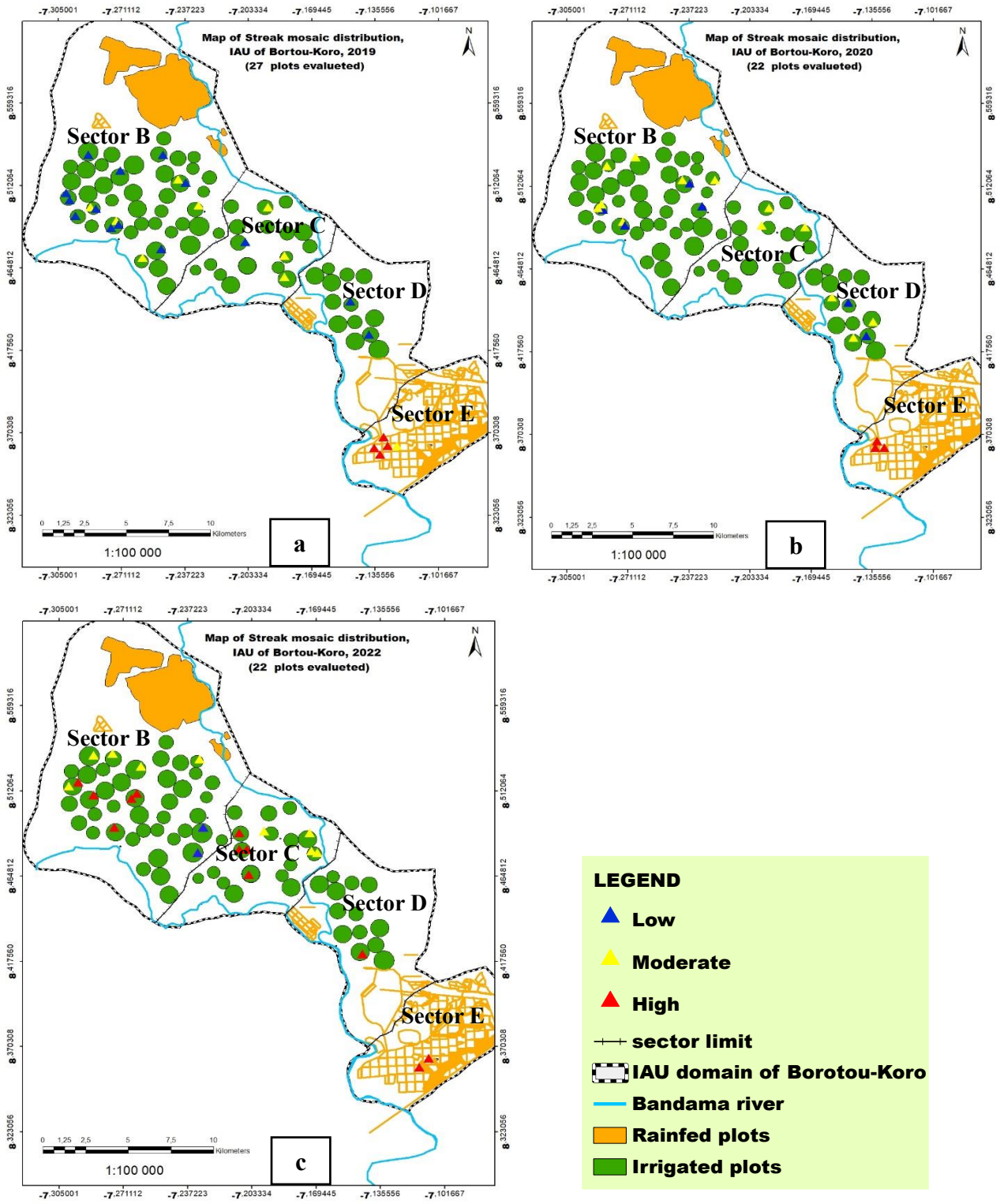

Fig 6. Spatial distribution of SCSMV in Borotou-Koro in November 2019 (a), October 2020 (b) and April 2022 (c)

« Low », « Moderate » and « High » : are defined according to prevalence

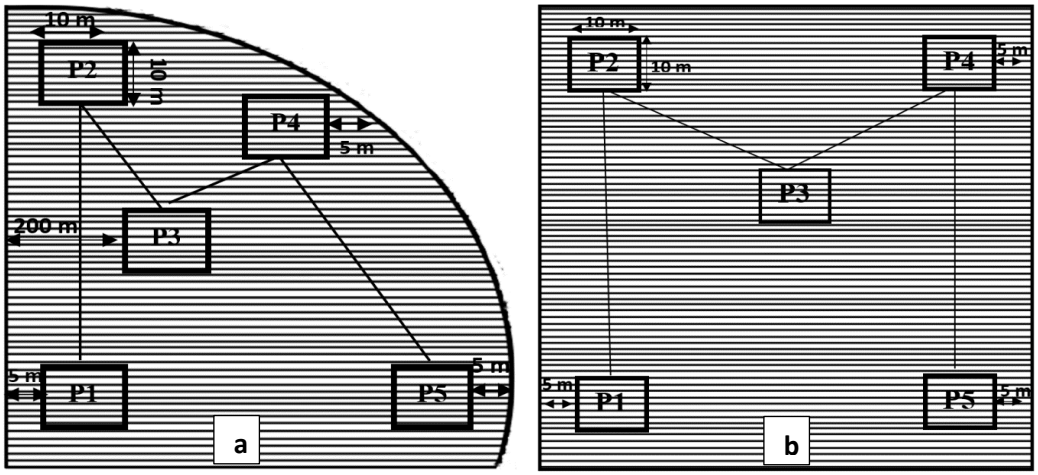

Fig 1. M-type device used to assess the prevalence and severity of SCSMV in pivot-irrigated (a) and rainfed (b) sugarcane plots in Zuénoula and Borotou-Koro

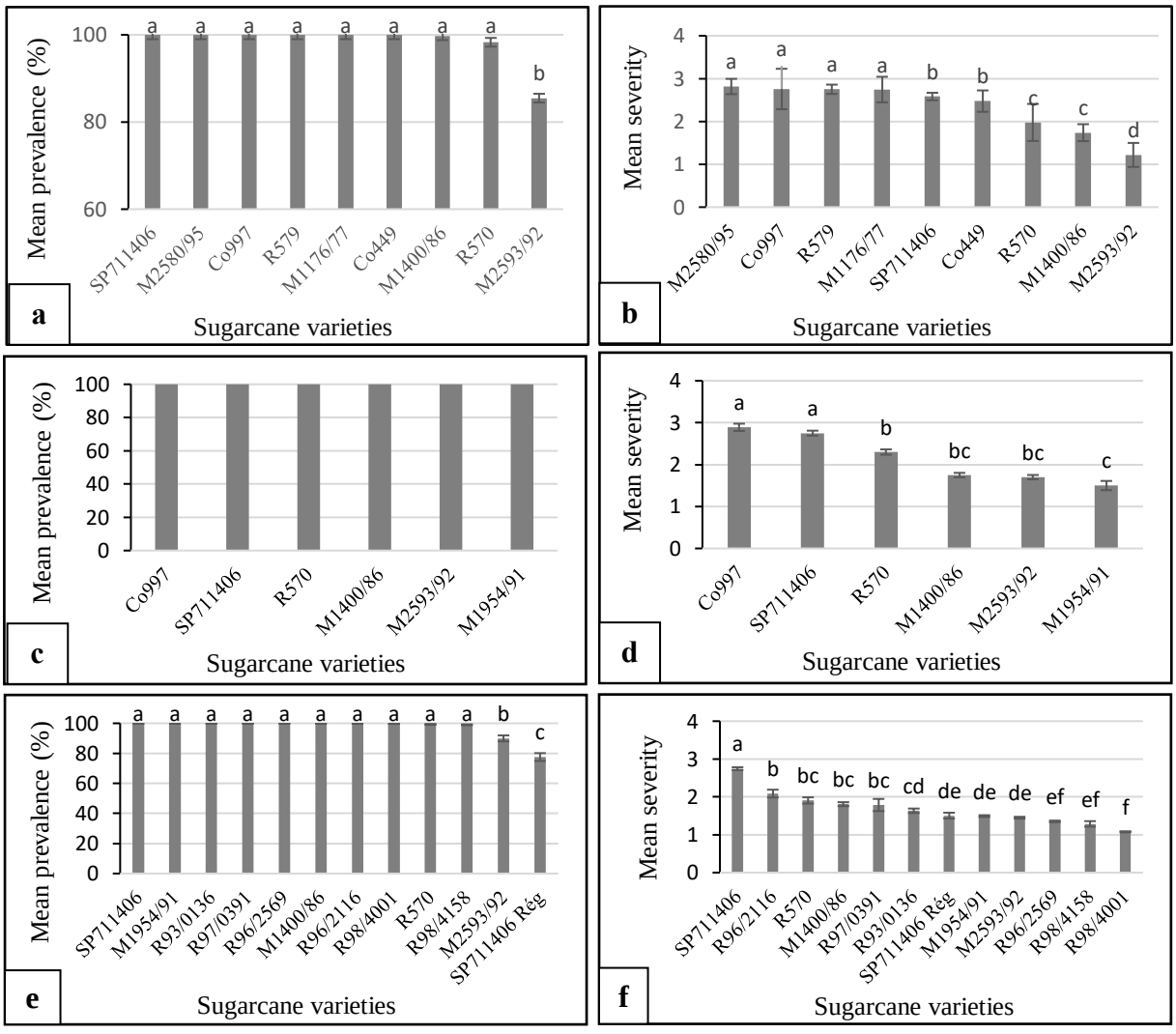

Fig 5. Mean prevalence and severity of sugarcane streak mosaic on sugarcane varieties grown in Zuénoula.

a- Mean prevalence (October 2018), b- severity (October 2018),

c- Mean prevalence (October 2020), d- severity (October 2020)

e- Mean prevalence (October 2021), f- severity (October 2021)

Histogram bars topped by the same letters are not significantly different at the 5% threshold according to Dunn's pairwise multiple comparisons test using Bonferroni correction.

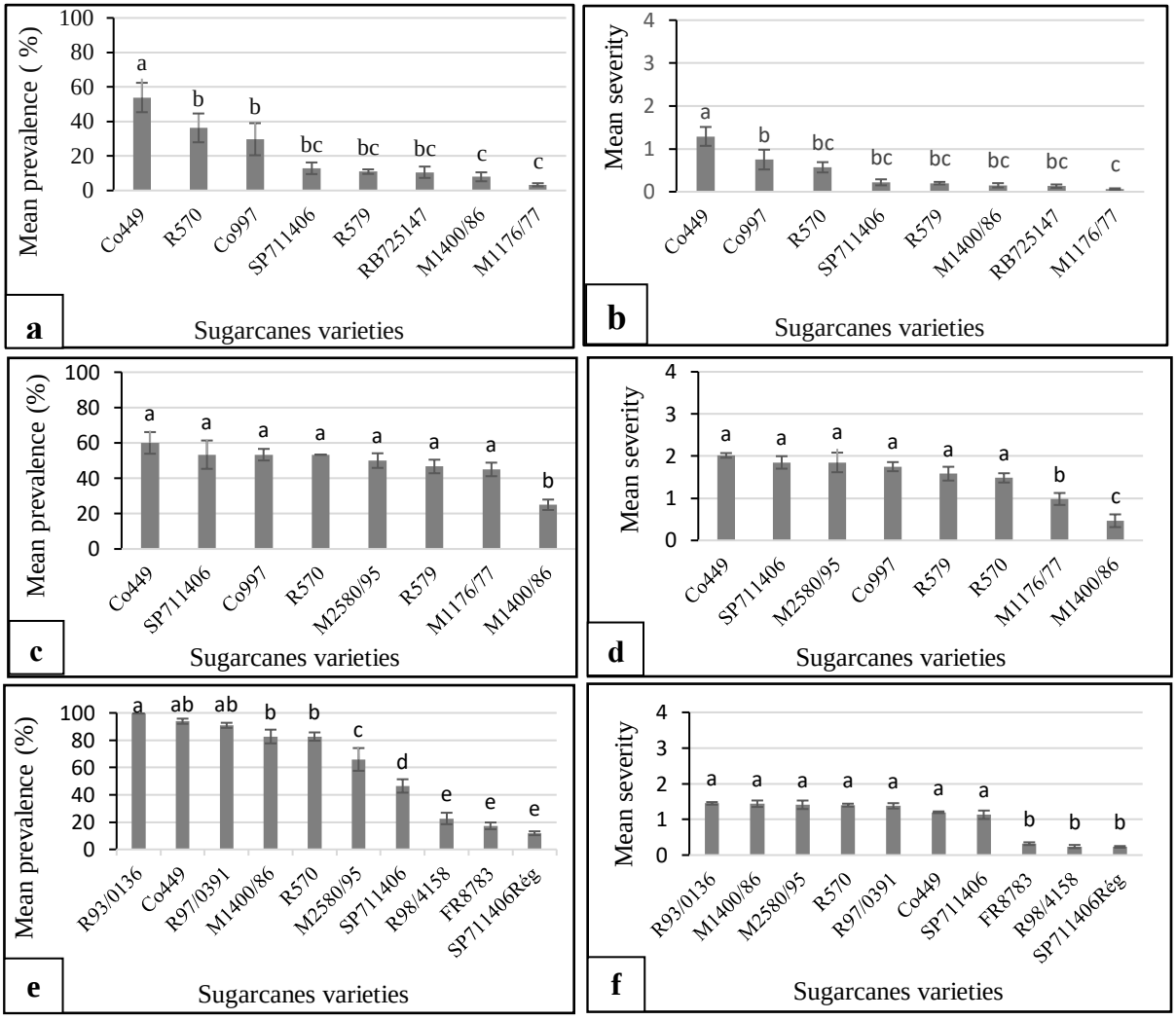

Fig 7. Mean prevalence and severity of SCSMV on 8 sugarcane varieties grown in Borotou-Koro

a- Mean prevalence (November 2019), b- severity (November 2019),

c- Mean prevalence (October 2020), d- severity (October 2020)

e- Mean prevalence (April 2022), f- severity (April 2022)

Histogram bars topped by the same letters are not significantly different at the 5% threshold according to Dunn's pairwise multiple comparisons test using Bonferroni correction.

Table 1. Sugarcane varieties evaluated for SCSMV susceptibility between 2018 and 2022 in Zuénoula and Borotou-Koro

			Zuénoula Number of plots			Borotou-Koro Number of plots		
Cultivar	**Origin**	**Year of probable introduction in Côte d'Ivoire**	**2018**	**2020**	**2021**	**2019**	**2020**	**2022**
Co449	Coimbatore (India)	1963	2			4	3	1
Co997	Coimbatore (India)	1981	10	4		4	3	
FR8783	Guadeloupe, France	2019						2
M1176/77	Mauritius	2015	4			4	3	
M1954/91	Mauritius	2015		1	3			
M1400/86	Mauritius	2015	4	4	4	2	3	3
M2580/95	Mauritius	2015	2				1	1
M2593/92	Mauritius	2015	6	4	1			
R570	Reunion Island, France	1988	20	4	3	4	3	3
R579	Reunion Island, France	1994	7			3	3	
R93/0136	Reunion Island, France	2019			2			4
R96/2116	Reunion Island, France	2019			2			
R96/2569	Reunion Island, France	2019			1			
R97/0391	Reunion Island, France	2019			2			2
R98/4001	Reunion Island, France	2019			1			
R98/4158	Reunion Island, France	2019			3			1
RB725147	Brazil	1987				2		
SP711406	São Paulo (Brazil)	1987	12	4	2	4	3	3
SP711406Rég*	São Paulo (Brazil)	2021			1			3
Diverse**				1				
Total			**67**	**22**	**25**	**27**	**22**	**23**

** variety regenerated by in vitro culture*

*** nursery with several sugarcane varieties*

Table 2. Rating scale used to assess the severity of SCSMV in sugarcane plots in Zuénoula and Borotou-Koro

Severity score (Si)	% of leaf area with symptoms	Characteristics
0	0	Apparently healthy plant (a variety is considered highly resistant if mean severity S=0)
1	1-10 %	Slight presence of mottling or streaking on the plant (a variety is considered partially resistant if S∈]0 ; 1])
2	11-30 %	Moderate presence of mottling or streaking on the plant (a variety is considered moderately susceptible if S∈]1 ; 2])
3	31-50 %	Strong presence of mottling or streaking, stunting of leaves, plant continues to grow (a variety is considered susceptible if S ∈]2; 3])
4	51-100 %	Heavy mottling or streaking, stunting of leaves and/or plant, plant no longer growing or dead (a variety is considered highly susceptible if S∈]3 ; 4])

Table 3. SCSMV detection from samples varying in severity score and dehydration technique: calcium chloride dehydration (CCD) and hot-air dehydration (HAD)

Severity score	Number of plants sampled (duplicates)	Number of amplifications after CCD	Number of amplifications after HAD	% detection after CCD	% detection after HAD
0	9	0	0	0	0
1	5	4	3	80	60
2	32	30	32	94	100
3	77	70	74	91	96
4	24	24	23	100	96
Total	**147**	**127**	**131**		

Supplementary material

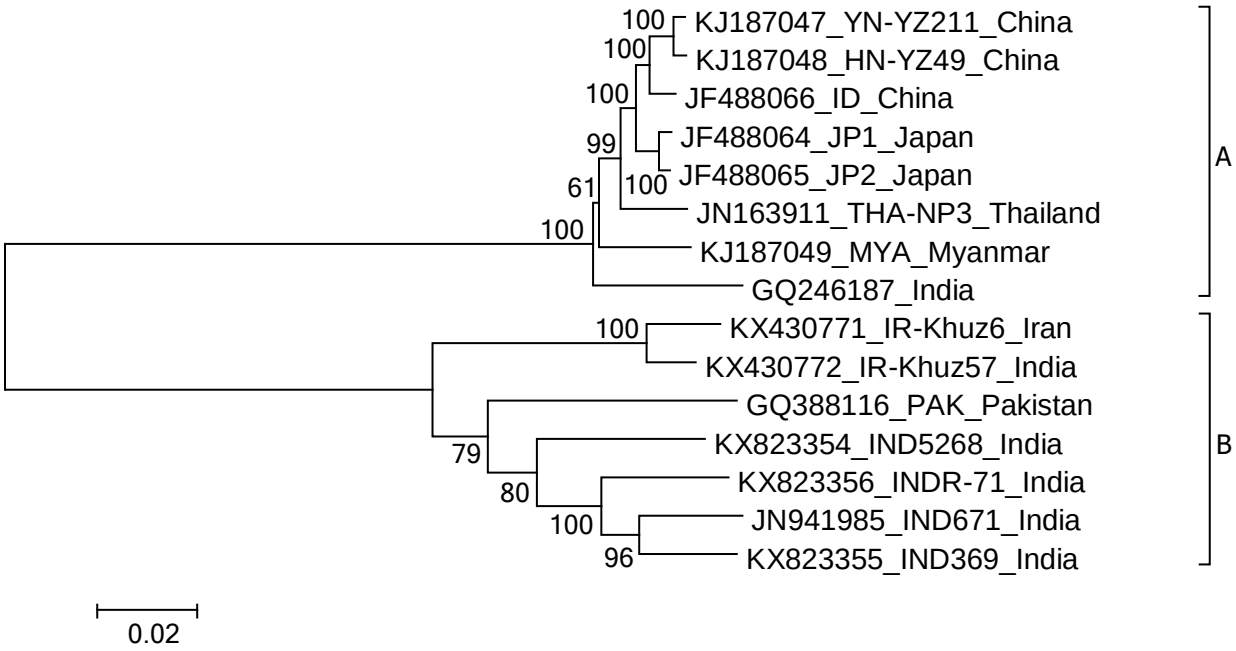

Fig S1. Neighbour-joining tree of complete SCSMV nucleotide sequences. Bootstrap values (n=500) are shown for each node

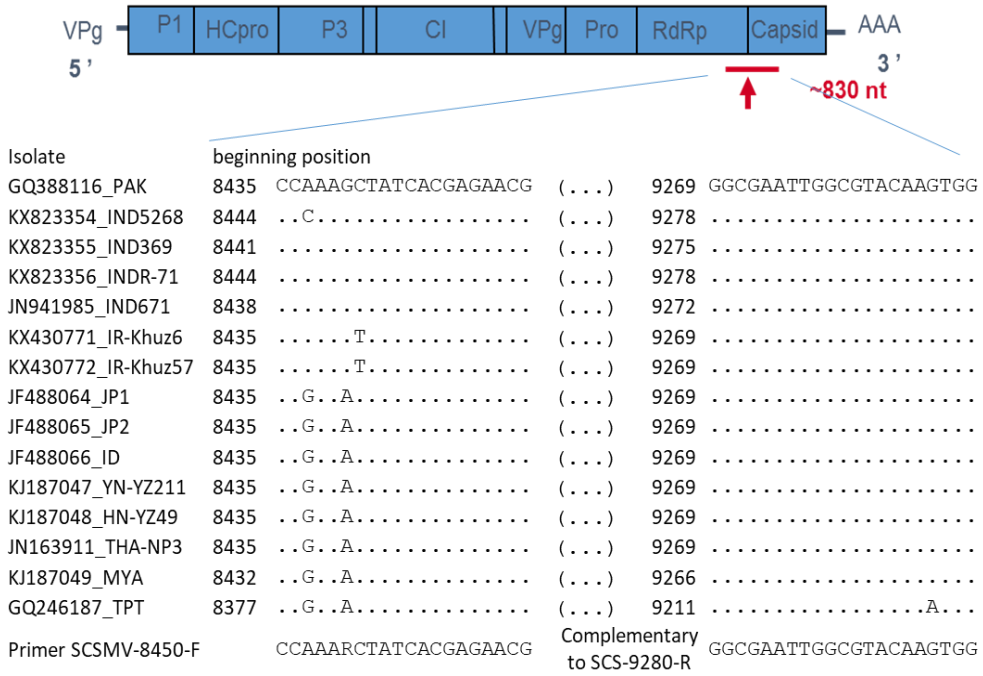

Fig S2. Polypeptide structure of SCSMV showing the site of choice of the specific primer pair at the N-terminal part of the protein capsid

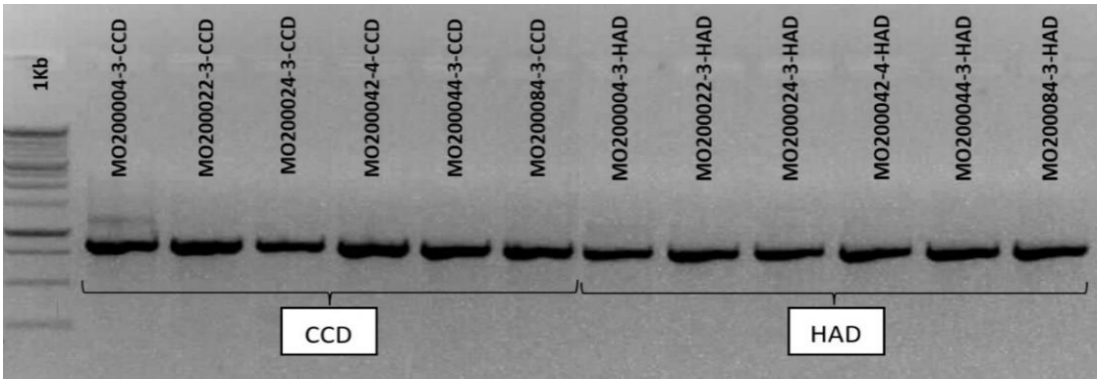

Fig S3. Amplification of SCSMV from samples stored after calcium chloride dehydration (CCD) and hot-air dehydration (HAD)