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Running head: Pepper viruses in Côte d'Ivoire

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Abstract

The prevalence of viruses in pepper crops grown in open fields in the different agro-ecological zones (AEZs) of Côte d'Ivoire was surveyed. *Pepper veinal mottle virus* (PVMV; genus *Potyvirus*) and *Cucumber mosaic virus* (CMV; genus *Cucumovirus*) were the most frequent viruses among those surveyed, while tobamoviruses (genus *Tobamovirus*) were detected at low frequency. PVMV showed a high heterogeneity across AEZs, which may be related to climatic, ecological or agronomical conditions, whereas CMV was more homogeneously distributed. The molecular diversity of CMV and PVMV were analyzed from partial genome sequences. In spite of a low number of CMV isolates characterized, two molecular groups were revealed, one corresponding to IA subgroup and the other to reassortants between IA and IB subgroups. RNAs 1 and 3 of the reassortants clustered with the IB subgroup of CMV isolates whereas their RNA 2 clustered with the IA subgroup. Importantly, RNA 1 of CMV isolates of the IB subgroup has been shown to be responsible for adaptation to pepper resistance. The diversity of PVMV in the VPg- and coat protein-coding regions revealed multiple clades. The central part of the VPg showed a high level of amino acid diversity and evidence of positive selection, which may be a signature of adaptation to plant recessive resistance. As a consequence, for efficient deployment of resistant pepper cultivars, it would be desirable to examine the occurrence of virulent isolates in the CMV or PVMV populations in Côte d'Ivoire and to follow their evolution as the resistance will become more largely deployed.

Introduction

Pepper belongs to the family Solanaceae and the genus *Capsicum*. It is grown in all regions of Côte d'Ivoire throughout the year. Pepper crops are generally rainfed but irrigation is necessary during the dry season spanning from November to May. The best yields are obtained with plantations in March-April for rainy season production and in September-October for dry season production (Fondio *et al*., 2009). The latest official reports of FAO showed that the pepper production in Côte d'Ivoire could reach 32,900 tons/year and is therefore an important cash crop for growers (FAOSTAT, 2012).

Diseases, including viral ones, are one of the major limitations to pepper production throughout the world by reducing fruit quality and quantity. In Côte d'Ivoire, four pepper viruses have been described: *Cucumber mosaic virus* (CMV; genus *Cucumovirus*, family *Bromoviridae*), *Pepper veinal mottle virus* (PVMV; genus *Potyvirus*, family *Potyviridae*), *Tobacco mosaic virus* (TMV) and *Pepper mild mottle virus* (PMMoV; genus *Tobamovirus*, family *Virgaviridae*) (Fauquet & Thouvenel, 1987). Recently, *Pepper vein yellows virus* (PepVYV; genus *Polerovirus*, family *Luteoviridae*) was found in association with PVMV and CMV on pepper in the East of Côte d'Ivoire (Bolou Bi *et al*., 2015).

PVMV, which is the most devastating pepper virus in West Africa, was found for the first time in Ghana (Brunt & Kenten, 1971), then in Nigeria, the African country with the highest pepper production (Lana *et al*., 1975), and in Côte d'Ivoire on *Nicotiana tabacum* (De Wijs, 1973), the weed *Physalis angulata* and *Capsicum* spp. (Fauquet & Thouvenel, 1987). In Côte d'Ivoire, 100% losses of marketable fruits due to PVMV infection have been reported, causing field abandonment before harvest and making cultivation of pepper not profitable in some areas (Fauquet & Thouvenel, 1987). Pepper plants infected by PVMV show various symptoms depending on the isolate agressiveness, age of plants at the time of infection and pepper cultivar. The symptoms exhibited on leaves include mild mottle, mosaic, vein banding, ringspots,

various types of necrosis, discoloration and deformation. Severe stunting of the whole plant is also frequently observed (Green & Kim, 1991). In addition, symptoms may appear on leaves, stems, flowers and fruits. In Côte d'Ivoire, PVMV was shown to be transmitted in non-persistent and non-circulative manner by aphids of the species *Aphis gossypii, Aphis spiraecola* and *Toxoptera citricidus* (De Wijs, 1973). CMV has a very broad host range, including over 1000 species in more than 85 botanical families. As PVMV, it is transmitted from plant to plant in natural epidemic conditions in a non-permanent and non-circulative manner by aphids. TMV, in addition to pepper, infects tomato, tobacco, eggplant and some wild species in Côte d'Ivoire (Fauquet & Thouvenel, 1987). PMMoV, in contrast, has a more restricted natural host range that includes mostly members of the genus *Capsicum* (Brunt *et al*., 1996). Like other tobamoviruses, TMV and PMMoV are highly contagious and the primary sources of infection are contaminated seeds and soil (Toyoda *et al*., 2004). Secondary infections occur through contact between plants and plant handling. Because of the non-persistent mode of transmission of PVMV and CMV, insecticide spraying is often inefficient to control them in the field (Afouda *et al*., 2013). Therefore, control methods include mostly prophylaxis and plant genetic resistance (Caranta *et al*., 1996; Fajinmi & Fajinmi, 2010).

The goal of the present study was to estimate the prevalence and diversity of the main viruses infecting pepper crops in Côte d'Ivoire in order to evaluate the possibility to control them with adequate resistance genes.

Materials and Methods

Sample collection

Leaf samples were collected from plants showing symptoms of possible viral etiology from 2014 to 2015 in the different agro-ecological zones (AEZs) of Côte d'Ivoire (Fig. 1; Supplementary Table 1). A total of 525 pepper plants were collected in open fields, including

73 *Capsicum annuum* and 452 *C. chinense* plants. Two to five plants with symptoms were sampled per field, depending on the field acreage. Immediately after collection, samples were placed in a plastic bag and stored in a mobile refrigerator. They were subsequently kept desiccated on calcium chloride and stored at 4°C.

Virus detection

Samples were tested by double antibody sandwich-enzyme-linked immunosorbent assay (DAS-ELISA) for infection by PVMV, CMV, PVY, TMV and PMMoV with polyclonal antisera produced by INRA Pathologie Végétale unit (Moury *et al*, 2005; Janzac *et al*., 2008). Samples were considered positive when absorbance values at 405 nm (A_{405}) were at least three times greater than the mean absorbance value of five healthy control samples.

Total RNAs were extracted from 5 mg of dried leaf tissue from samples that reacted positively to PVMV or CMV in DAS-ELISA using the Tri-Reagent kit (Molecular Research Center, Cincinnati, USA) according to the manufacturer's instructions. Two-step reverse transcription-polymerase chain reactions (RT-PCR) were then performed using PVMV- or CMV-specific primers (Supplementary Table 2) as described by Moury *et al*. (2005). For PVMV, two different genome regions were targeted, encoding either the viral genome-linked protein (VPg) or coat protein (CP). For CMV, three different regions corresponding to the three genomic RNAs were targeted. RT-PCR amplicons were sequenced directly with primers PVMV-VPg-rv, PVMV-CP-rv, 1Cons-AS1, G2-CrFLP-AS or G3-CrFLP-AS (Supplementary Table 2) by Genoscreen (Lille, France).

Molecular variability of PVMV and CMV isolates

Sequence quality was checked using ChromasPro version 1.7.6 (Technelysium Pty Ltd, Australia). Sequence alignments were performed using the ClustalW program implemented in

the MEGA7 software (Kumar *et al*., 2016) and gaps, only present at the beginning or end of the sequence alignments, were removed. Phylogenetic analyses were performed using the neighbour-joining (NJ), maximum-likelihood (ML) and maximum-parsimony (MP) methods implemented in MEGA7 and the robustness of the tree topology was evaluated with 500 bootstrap resamplings. These methods were run with the optimal nucleotide-substitution model selected by MEGA7. Sequences obtained from isolates from Côte d'Ivoire were also compared to sequences from reference isolates or to additional sequences retrieved from sequence databases using the BLASTn program (Altschul *et al.*, 1990).

To identify codon sites with positive selection, *i.e.* sites showing a dN/dS ratio between the nonsynonymous (dN) and the synonymous (dS) substitution rates in an alignment of amino acid–coding sequences significantly higher than 1.0, ML approaches implemented in the HyPhy (Kosakovsky Pond & Frost, 2005a) package were performed using the NJ phylogenetic tree obtained previously. In HyPhy, the SLAC (single-likelihood ancestor counting), FEL (fixed-effects likelihood), IFEL (internal fixed-effects likelihood), REL (random-effects likelihood) and FUBAR (fast unbiased Bayesian approximation) methods (Kosakovsky Pond & Frost, 2005a, 2005b; Kosakovsky Pond *et al*., 2006; Murrell *et al*., 2013) were used to detect individual codon positions subjected to positive selection. In addition, the Branch-site REL method (Kosakovsky Pond *et al*., 2011) was used to detect episodic positive selection along particular branches of the phylogenetic tree and the MEME (mixed-effects model of evolution) method (Murrell *et al*., 2012) was used to detect episodic positive selection affecting individual codon positions.

Results

Virus prevalence

From all samples showing virus-like symptoms, 27.4% (142/525) were positive for at least one virus (Table 1). There was no clear association between symptoms observed in the field and the detected viruses, maybe because of coinfection of plants with several viruses or because of the confounding effect of other biotic or abiotic stresses. This may also be due to the presence of other, untested viruses like poleroviruses (Bolou Bi *et al*., 2015), which detection is inefficient on desiccated leaf material, or begomoviruses. Begomoviruses have not been described in pepper crops in Côte d'Ivoire but are responsible for yellow vein disease in pepper in neighboring countries like Burkina Faso and Mali (Tiendrébéogo *et al*., 2011). The most prevalent virus was PVMV with 21.2 % (92/525) positive samples, followed by CMV detected in 11% (52) of samples. PMMoV and TMV were detected at low frequencies, with respectively 2.5% (13) and 1.3% (7) positive samples. Since serological cross-reactivity occurs between TMV and other tobamoviruses like ToMV (*Tomato mosaic virus*), the precise species to which belongs the virus detected by DAS-ELISA remains to be determined. No sample was found infected by PVY. Overall, the prevalence of PVMV was significantly higher than that of CMV, and both PVMV and CMV showed highly significantly higher prevalence than TMV, PMMoV or PVY (Table 1).

A statistical analysis of virus prevalence among AEZs was performed for the four AEZs (I, II, IV and VI) where more than 12 plants were sampled. The highest virus prevalence (combining the four detected viruses) was recorded in AEZ II (52.2%). It was significantly higher than virus prevalence in AEZ IV (33.3%), and both were significantly higher than virus prevalence in AEZs I (18.1%) and VI (11.8%). The prevalence of PVMV was highly heterogeneous among AEZs. PVMV was not detected in AEZ VI, whereas it reached a maximum of 36.7% in AEZ II. In contrast, the prevalence of CMV was more homogeneous among AEZs, varying from 5.7 (AEZ I) to 14.0% (AEZ IV), if we consider only the AEZs with substantial sampling effort (n>11). Consequently, climate and/or ecological differences in the different AEZs seem to play

a major direct or indirect role in determining the distribution of PVMV infection, and to a lower extent of CMV infection on pepper in open fields in Côte d'Ivoire.Molecular variability of CMV isolates

Partial sequences, 307 to 521 nucleotide long, were obtained for the three RNAs of Ivorian CMV isolates. Some sequences showed a large number of ambiguous nucleotides (double peaks) in the sequence chromatograms and were consequently discarded in further analyses. These ambiguities result most probably from mixed infections of plants by several isolates. However, sequences were kept when there was a marked difference in the peak heights and at these positions, the predominant nucleotide in the virus population was considered in further analyses. Sequence accession numbers are MG334359 to MG334384. Similar tree topologies were obtained with the three different phylogenetic methods NJ, ML and MP. Concerning RNAs 1 and 3, two groups of Ivorian isolates could be distinguished, clustering either with group IA or IB reference isolates (Fig. 2A and 2C). Since the IB subgroup of CMV is not monophyletic for RNAs 1 and 3 (Tepfer *et al*., 2016), we looked for the IB CMV isolates that shared the highest nucleotide identity with the Ivorian isolates using BLASTn. For RNA 1, the most similar isolate was from Egypt (accession number KT921314, 97% nucleotide identity) followed by isolates from India, Malaysia and Italy (Vir isolate) (89-94% identity). For RNA 3, the most similar isolates were from China and Thailand (98% identity), followed by a large number of isolates from South-east Asia and Tunisia (96-98% identity).

In contrast, all RNA 2 sequences clustered with the subgroup IA reference isolate (Fig. 2B). These groups were supported by high bootstrap values (≥76%). No Ivorian isolate clustered with the group II reference isolate. Sequences of the three viral RNAs could be analyzed for four Ivorian isolates only, named 350, 406, 563 and 566. Isolates 350 and 406 had a typical genome structure of IA group, whereas isolates 563 and 566 had a reassortant type of genome structure, clustering with group IB for RNAs 1 and 3 and with group IA for RNA 2. All five

isolates clustering with group IB for one or the other genome segment were collected in the region of Bongouanou (AEZ IV, central-Western part of Côte d'Ivoire; Fig. 1). In contrast, isolates of group IA are distributed in most agro-ecological zones.

Molecular variability of PVMV isolates

As with CMV, a number of VPg- and CP-coding sequences of PVMV isolates could not be exploited because of a large number of nucleotide ambiguities, most likely the result of nucleotide variation within the virus population. A 479-nucleotide-long alignment of VPg-coding sequences was obtained with 42 Ivorian isolates collected for this study (accession numbers MG334290 to MG334331), one older Ivorian reference isolate (accession number DQ009807; date and location of collection unknown) and one Ghanaian isolate (accession number DQ645484). Using the best-fit nucleotide substitution model (Tamura 3 + Γ) in the MEGA7 software, the mean pairwise distance between sequences was 0.15 (maximum pairwise distance 0.41). Similar tree topologies were obtained with the different phylogenetic methods. Based on bootstrap supports ≥69% in the maximum-likelihood tree, five PVMV groups could be distinguished, one (group 3) including the older Ivorian isolate (Fig. 3A). The Ghanaian isolate and two Ivorian isolates did not cluster with additional isolates based on this bootstrap threshold or a lower stringency threshold of 50% bootstrap support. The largest group 1 contained isolates from different regions of Côte d'Ivoire. Group 2 contained two isolates from central Côte d'Ivoire (Yamassoukro and Gonaté). Group 3 contained three isolates from the South-East (Gonzagueville) and one isolate from central Côte d'Ivoire. Group 4 contained three isolates from central (Yamassoukro) or south-western (Gagnoa) part of Côte d'Ivoire and group 5 contained four isolates from central Côte d'Ivoire (Yamassoukro and Gonaté). Consequently, there was no clear association between geography and genetic clustering of PVMV isolates.

For the CP-coding region, a 588-nucleotide-long sequence alignment was obtained with 26 Ivorian isolates collected for this study (accession numbers MG334332 to MG334357), the older Ivorian reference isolate (accession number MG334358) and 13 sequences from African or Asian isolates available in GenBank. Using the best-fit nucleotide substitution model (Tamura 3 + Γ) in the MEGA7 software, the mean pairwise distance between sequences was 0.08 (maximum pairwise distance 0.29). Again, similar tree topologies were obtained with the different phylogenetic methods. Based on bootstrap supports ≥62% in the maximum-likelihood tree, three PVMV groups could be distinguished (Fig. 3B). The majority of Ivorian isolates (15/26) clustered in group 3. This group contains isolates from different parts of Côte d'Ivoire. Four isolates collected in Gonaté, in the central-Western region of Côte d'Ivoire, clustered in group 1, which is related to a group of isolates collected in Ghana and Asia. Four isolates collected in the regions of Bongouanou or Gonzagueville (Eastern or South-eastern Côte d'Ivoire) clustered in group 2. One isolate (number 447) collected in the region of Bongouanou clustered with an isolate from Senegal. Finally, two Ivorian isolates also collected in the region of Bongouanou (isolates 563 and 573) did not cluster with additional isolates based on a threshold of 50% bootstrap support.

Several isolates belonged to different phylogenetic groups for the VPg- and CP-coding regions (Fig. 3). For example, isolate 465 clustered in group 5 with isolates 516, 517 and 518 for the VPg-coding region, whereas these isolates were split into two separate groups for the CP-coding region (group 3 for isolate 465 and group 1 for isolates 516, 517 and 518). Similarly, isolates 228, 438 and 439 clustered together in group 2 for the CP-coding region, whereas they were split into group 3 (isolate 228), group 1 (isolate 439) or were not related to any group (isolate 438) for the VPg-coding region. This suggests that recombination events may have affected PVMV evolution. An alternative hypothesis is that some plants were mixed-infected by several PVMV variants and that the primers used amplified preferentially one or another variant

depending on the genome region. The tree of the CP-coding region, which includes a set of non-Ivorian PVMV isolates, is also indicative of long-range dissemination of PVMV. This is suggested by the clustering of Ivorian isolate 447 with an isolate from Senegal and by the proximity between group 1 of Ivorian isolates and a group containing all Asian isolates characterized to date. This group includes also a Ghanaian isolate.

The sequences of the VPg-coding region of PVMV were analyzed for amino acid polymorphism and evidence of positive selection. The region spanning from amino acid position 103 to 125 of PVMV VPg is of particular relevance since it corresponds to the region of PVY VPg (amino acid positions 101 to 123) which determines adaptation to pepper recessive resistance mediated by the eukaryotic translation initiation factor 4E (eIF4E) encoded by alleles of the *pvr2* gene (Ruffel *et al*., 2002; Ayme *et al*., 2006; Charron *et al*., 2008) (Fig. 4A). Moreover, alleles of the *pvr2* gene and of the *pvr6* gene, encoding the eIFiso4E isoform, were shown to confer resistance to PVMV (Caranta *et al*., 1996; Ruffel *et al*., 2006). Ten different PVMV haplotypes were observed based on the amino acid diversity in this VPg region for the Ivorian isolates collected in this study (Fig. 4B). The older Ivorian isolate and the Ghanaian isolate (accession DQ645484) represent two more haplotypes. One haplotype was predominant (24/42 isolates), one had an intermediate prevalence (7/42) and the others were represented once or twice only. A large amino acid diversity was observed at positions 117 and 122, with four and five different amino acids, respectively. Since these positions correspond to positions in PVY VPg that are virulence determinants and/or are subjected to positive selection (Fig. 4A), we performed an analysis of positive selection for PVMV as well, using the Hyphy software suite (Table 2). Among the six algorithms used, two (SLAC and FUBAR) failed to detect positive selection in the VPg-coding sequence. Position 82 of the VPg was detected by the FEL and IFEL methods. Positions 117 and 119 were detected by one method only (REL and MEME, respectively). The strongest evidence of positive selection was obtained for position 122 that

was detected by three different methods and, for two of them, with strong statistical support (*p*-value $\leq 10^{-4}$). Interestingly, positions 117 and 122 of PVMV VPg aligned with positions 115 and 120 of PVY VPg that were shown to be responsible for adaptation to eIF4E-mediated recessive resistance in pepper (Ayme *et al*., 2006). In addition, the Branch-site REL algorithm identified four internal branches in the phylogenetic tree that corresponded to episodic positive selection (Fig. 3A). These branches linked groups 2, 3 and 4 of PVMV isolates, and a subgroup of group 1 excluding isolate 419, to the rest of the tree.

Discussion

In this survey of viruses infecting open-field pepper crops in Côte d'Ivoire, PVMV and CMV were found most prevalent. Prevalence of PVMV was highly heterogeneous. It was absent from AEZ VI corresponding to Sudanian savanna and reached 36.7% of samples in AEZ II (Table 1). Environmental conditions, including climate and soil, may directly or indirectly affect virus prevalence. AEZ VI was the driest sampled zone, even though it corresponds to a humid tropical savanna area. Indirect environmental effects on virus prevalence include vegetation type (different crops and weeds that may host the virus) and structure (more or less isolated patches of host plants), aphid populations that are virus vectors and agricultural practices. Contrasting with PVMV, prevalence of CMV was more homogeneous between AEZs, spanning from 5.7% to 14.0% for AEZs significantly sampled (>11 samples). CMV has a much larger host range than PVMV, including many alternative hosts to pepper. By ensuring the possibility for CMV to persist in alternative hosts during the period of absence of pepper crops, this difference may explain the observed relative homogeneity of CMV prevalence.

Control of CMV or PVMV epidemics relies mainly on prophylaxis and plant genetic resistance. Polygenic resistance to CMV, combining several QTLs (quantitative trait loci) acting on

different steps of plant infection, has been used to create highly-resistant pepper cultivars, including F_1 hybrids (Caranta *et al*., 1997a, 2002; Palloix & Ordon, 2011). However, a recent epidemiological survey in Tunisia showed that several CMV isolates were adapted to the resistant pepper cultivar 'Milord' (Ben Tamarzizt *et al*., 2013). One common property shared by all CMV isolates virulent towards pepper resistance was the fact that they had a IB-type RNA 1. Noticeably, RNA 1 of IB-type CMV isolates determines also virulence towards on plants carrying the *Cmr1* resistance gene that restricts the systemic movement of CMV group IA in pepper (Kang et al. 2012). As a consequence, since several Ivorian CMV isolates are reassortants with a IB-type RNA 1 (Fig. 2), their virulence towards pepper resistance should be carefully examined before resistance deployment.

Resistance in pepper towards PVMV has also been studied extensively. A bigenic recessive resistance to PVMV has been identified (Caranta *et al*., 1996), which results from the combination of resistance alleles at the *pvr2* locus encoding the eIF4E and a natural knock-out allele of the *pvr6* gene encoding the eIFiso4E isoform (Ruffel *et al*., 2006). The phenotype is a total resistance, with no virus detection in the inoculated organs or at the systemic level. Among resistance alleles at the *pvr2* locus, only alleles $pvr2^1$, $pvr2^2$ and $pvr2^7$ were shown to complement *pvr6* for PVMV resistance (Rubio *et al*., 2009). PVMV-resistant pepper cultivars (with the $pvr2^1$ + *pvr6* or $pvr2^2$ + *pvr6* combinations) are being released in Africa but are yet poorly deployed in Côte d'Ivoire (Bolou Bi A, personal observations). A polygenicresistance against potyvirus E, that belongs to a particular strain of PVMV (Moury *et al*., 2005), has also been studied in pepper. It has a quantitative phenotype, with symptoms at the systemic level being milder and expressing later, and it includes two QTLs encompassing the *pvr2* and *pvr6* loci (Caranta *et al*., 1997b). This resistance has not been included in breeding programs. The central part of the VPg of PVY shows a large amino acid diversity and evidence of positive selection at some amino acid positions (Moury *et al*., 2014; Ben Khalifa *et al*., 2012). The high

rate of amino acid substitutions in this small region was explained by the selection of virulence properties against eIF4E-mediated recessive resistance in several crops belonging to the family Solanaceae including pepper (Ayme *et al*., 2006; Moury *et al*., 2014; Ben Khalifa *et al*., 2012). The observation of a similarly high level of amino acid diversity and evidence of positive selection in the corresponding region of PVMV VPg (Table 2; Fig. 4) is surprising because of the rarity of grown PVMV-resistant pepper cultivars. Several hypotheses can be raised to explain this evolution pattern of PVMV VPg. First, virulence towards the *pvr2 + pvr6* combination of resistance genes in pepper involving amino acid substitutions in PVMV VPg could have been selected in spite of the low frequency of grown resistant pepper cultivars. Second and more plausibly, amino acid substitutions in PVMV VPg may have been responsible for PVMV adaptation to other, unstudied plant resistances in pepper, other crops that are hosts of PVMV like tomato or African eggplant (*Solanum aethiopicum*) or wild plants. Third, the high amino acid substitution rate in the central part of VPg may not exclusively be related to plant host adaptation. This protein region was shown to show a high level of intrinsic disorder (Hébrard *et al*., 2009), which may allow the virus to tolerate a high amino acid substitution rate. Whatever the reason, this high diversity raises some threats about the future deployment of PVMV-resistant pepper cultivars, since some VPg mutations may be responsible for the occurrence of virulent variants in PVMV populations or may facilitate the future acquisition of virulence properties through additional mutations, which would impair resistance durability. Surveys of PVMV diversity and evolution as the resistance will be deployed would therefore be highly desirable to predict potential resistance breakdowns.

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Table 1 Distribution and prevalence of pepper viruses in Côte d'Ivoire.

AEZ[a]	Characteristics	PVMV	CMV	PMMoV	TMV	PVY	Total of infected samples – infection rate	Total of samples
I	Southern humid forest	18 - 9.3% **b**[b]	11 – 5.7% **b**	4 – 2.1%	4 – 2.1%	0	35 - 18.1% **c**	193
II	Western humid forest	22 - 36.7% **a**	8 – 13.3% **ab**	3 – 4.9%	1 – 1.6%	0	32 - 52.2% **a**	61
IV	Half-humid forest	44 - 25.7% **a**	24 - 14.0% **a**	3 – 1.8%	1 – 0.6%	0	57 - 33.3% **b**	171
VI	Sudanian savanna	0 **c**	6 – 8.9% **ab**	3 – 4.4 %	1 – 1.5%	0	8 - 11.8% **c**	68
III	Mountain humid forest	2 - 16.7%	0	0	0	0	2 - 14.3%	12
V	Transition humid forest	2 - 15%	3 – 25%	0	0	0	4 - 33.3%	12
VII	Guinea savanna	4 – 50%	0	0	0	0	4 - 50.0%	8
	Total of infected samples – infection rate	92 - 21.2%^{A}	52 - 11%^{B}	13 - 2.5%^{C}	7 - 1.3%^{C}	0%^{C}	142 - 27.0%	525

[a] AEZ: agro-ecological zone.

[b] Number of positive samples – Percentage of positive samples among all samples collected for a given AEZ. Statistical comparisons of virus prevalence (Fisher exact tests) were performed for the entire dataset (among viruses) or for datasets containing more than 12 collected samples (among AEZs). Lower-case letters indicate significant (p<0.05) differences in the infection rates across AEZs for PVMV, CMV or all tested viruses. Upper-case letters indicate significant (p<0.05) differences in infection rates across viruses.

Table 2 Amino acid positions of the VPg of PVMV isolates significantly affected by positive selection[a]

Amino acid position in VPg	Algorithm							
	FEL		IFEL		REL		MEME	
	dN/dS	*P*	dN/dS	*P*	dN/dS	*P*	dN/dS	*P*
82	∞[b]	0.084	∞[b]	0.017				
117					2.5	$<10^{-5}$		
119							NA[c]	0.077
122			5.7	0.030	2.6	10^{-4}	NA	5×10^{-6}

[a]The analysis was performed with the HyPhy software suite (Kosakovsky Pond and Frost 2005a) and conducted on 38 non-redundant sequences containing the eight 3'-proximal codons of the 6K2-coding region and the 151 5'-proximal codons (on a total of 191) of the VPg-coding region. When available, the estimated dN/dS value is indicated for detected codon positions. A p-value threshold of 0.10 was considered.

[b]Estimate of dN/dS was infinite because of the lack of synonymous substitutions (dS=0).

[c]NA, not available.

Figure 1 Sampling sites in the different agro-ecological zones ("Zones agro-écologiques"-ZAE) of Côte d'Ivoire. Black stars indicate the different sampling sites.

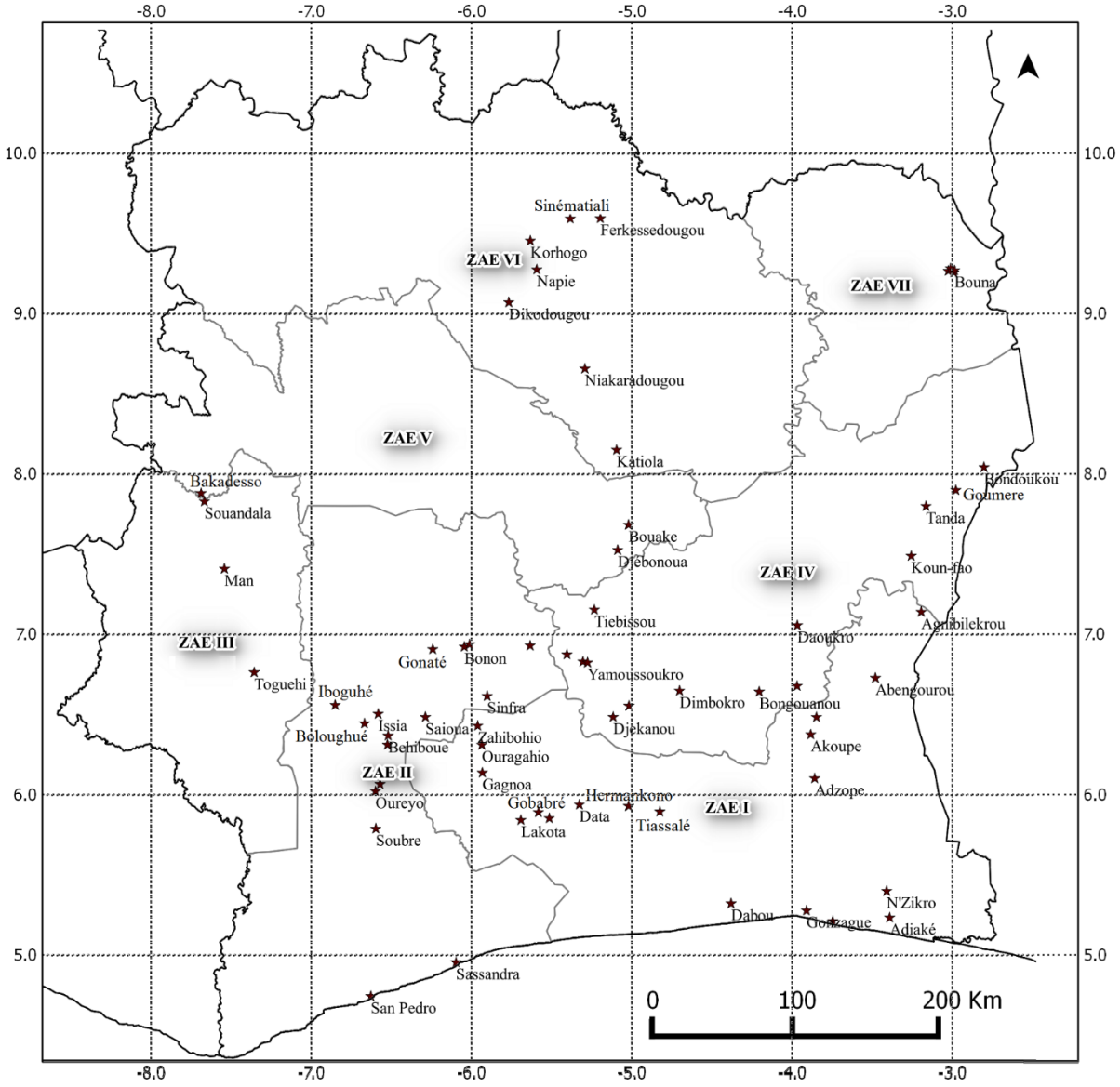


Figure 2 Unrooted maximum-likelihood phylogenetic trees of partial sequences corresponding to the three genomic RNAs of Ivorian *Cucumber mosaic virus* (CMV) isolates and reference isolates corresponding to three main groups of CMV, IA, IB and II. Reference isolates are identified by their accession numbers and underlined (isolate I17F for group IA, Vir for group IB and R for group II). Bootstrap percentages (among 500 resamplings) above 50% are shown. Scale bars indicate branch lengths in substitutions per nucleotide site. Ivorian isolates for which the three genome segments have been sequenced are boxed, reassortant ones with a white box and non-reassortants with a gray box. **A.** Tree from a 521-nucleotide-long alignment of RNA1 with Tamura 3-parameter model + Γ. **B.** Tree from a 479-nucleotide-long alignment of RNA2 with Kimura 2-parameter model + Γ. **C.** Tree from a 521-nucleotide-long alignment of RNA3 with Kimura 2-parameter model + Γ.

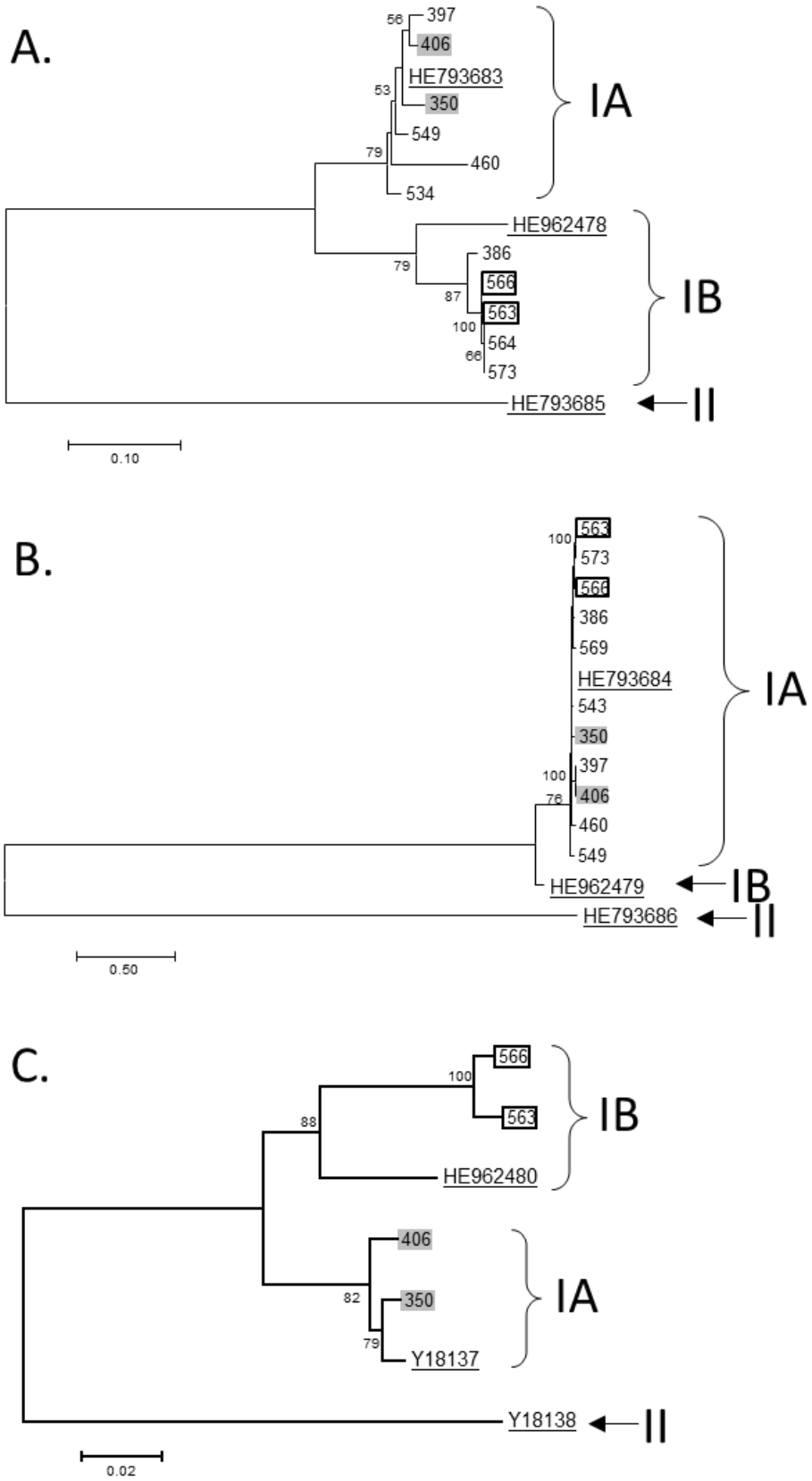

A.
397
406
HE793683
350
549
460
534
IA
HE962478
386
566
563
564
573
IB
HE793685
II
0.10
B.
563
573
566
386
569
HE793684
543
350
397
406
460
549
IA
HE962479
IB
HE793686
II
0.50
C.
566
563
IB
HE962480
406
350
Y18137
IA
Y18138
II
0.02

Figure 3 Unrooted maximum-likelihood phylogenetic trees of partial sequences of Ivorian *Pepper veinal mottle virus* (PVMV) isolates and reference isolates (identified by their accession numbers). Bootstrap percentages (among 500 resamplings) above 50% are shown. Scale bars indicate branch lengths in substitutions per nucleotide site. Asterisks indicate isolates that did not cluster in a particular clade with other isolates with a bootstrap support >50%. **A.** Tree from a 477-nucleotide-long alignment of the VPg-coding region with Tamura 3-parameter model + Γ. Branches highlighted in deep gray were shown to correspond to episodic positive selection using the MEME algorithm in the Hyphy software (Kosakovsky Pond & Frost 2005a). **B.** Tree from a 585-nucleotide-long alignment of the coat protein-coding region with Tamura 3-parameter model + Γ.

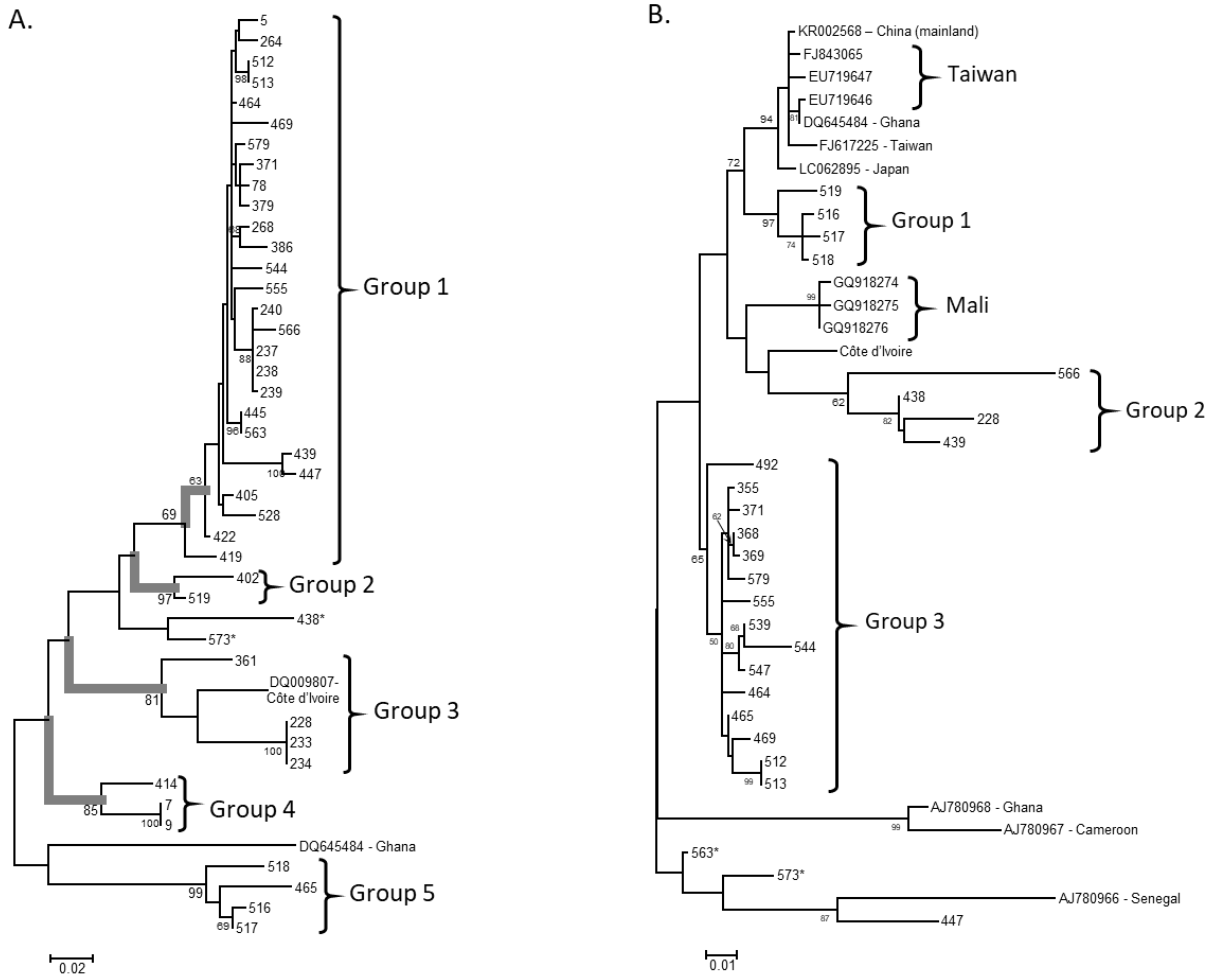
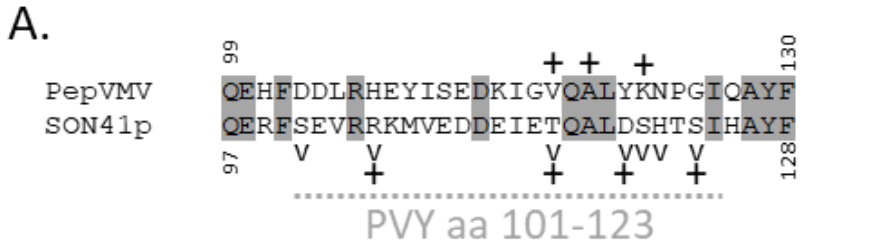


Figure 4 Amino acid diversity of central part of the VPg of PVMV isolates. **A.** Alignment of the central part of the VPg of PVMV (isolate Ghana; accession number DQ645484) and PVY (isolate SON41p; accession number AJ439544). PVY virulence determinant corresponds to amino acid positions 101 to 123. Positions subjected to positive selection in PVMV (this study) or in PVY (Moury et al. 2004) are indicated by the '+' symbol above or below the amino acid sequences, respectively. Positions involved in PVY virulence towards *pvr2*-mediated resistance in pepper (Ayme *et al*., 2006, 2007) are indicated by the 'v' symbol. **B.** Haplotypes identified based on amino acid sequences of positions 103 to 125 of PVMV isolates, aligning with the virulence determinant against recessive eIF4E-mediated resistance in pepper (*pvr2* locus) identified in *Potato virus Y* (PVY). The number of isolates representing each haplotype is indicated between parentheses when >1.



B.

	103 125	
Haplotype 1	DDLRHEYISEDKIEWQAIYSNPG	(24)
Haplotype 2	.............V..L.K...	(7)
Haplotype 3	.............R.....	(2)
Haplotype 4	..................A...	(2)
Haplotype 5	................K..A...	(2)
Haplotype 6	..................T...	
Haplotype 7	.............QR.....	
Haplotype 8	................T..G...	
Haplotype 9	........A..........	
Haplotype 10	..................A...	
Isolate Côte d'Ivoire	.......M......M..L.KT..	
DQ645484 - Ghana	.............GV..L.K...	

Supplementary Table 1: Characteristics of the seven agro-ecological zones (AEZs) surveyed in Côte d'Ivoire (Halle & Bruzon, 2006).

AEZ	Localities sampled	Samples collected	Characteristics	Altitude (m)	Pluviometry (mm)	Average annual temperature in °C (Standard deviation)
I	Abidjan, Dabou, N'zikro, Adiaké, Akoupé, Adzopé, Abengourou, Tiassalé, Data, Hermankono, Lakota, Gobabré, Gagnoa, Ouragahio, Zahibohio, Agnibilekrou	193	Southern dense rainforest	0-200	1400 - 2500 (bimodal)	29 (5.6)
II	Bonon, Gonaté, Sinfra, Behiboué, Iboguhé, Bolouguhé, Issia, Oureyo, Soubré, San-Pédro, Sassandra,	61	Western dense rainforest	~1000 (Daloa)	1300- 1750	23.5 (13.4)
III	Duekoué (Toguehi), Man, Souandala, Bakadenso	12	Western semi-montainous forest	> 1000 (Man)	1300- 2300	24.5 (7.7)
IV	Toumodi, Yamoussoukro, Djekanou, Tiébissou, Bongouanou, Daoukro, Koun-fao, Tanda, Goumeré, Bondoukou	171	Semi deciduous dense rainforest	0-200	1300- 1750	23.5 (13.4)
V	Bouaké, Djebonoua	12	Transitional forest	300-600	1300- 1750 (unimodal)	23.5 (13.4)
VI	Katiola, Niakaradougou, Dikodougou, Napié, Korhogo, Ferkessedougou, Sinématiali	68	Humid tropical savanna	300- 500	1150- 1350 (unimodal)	26.7 (1.1)
VII	Bouna	8	Dry tropical savanna area	300-500	1150- 1350	26.7 (1.1)

Reference: Halle A, Bruzon E, 2006. Étude du profil environnemental de la Côte d'Ivoire, Rapport d'étude pour la Commission Européenne, Abidjan, Côte d'Ivoire, 128 pp.

Supplementary Table 2 Primers used for reverse transcription or PCR amplifications and for sequencing of *Cucumber mosaic virus* (CMV) and *Pepper veinal mottle virus* (PVMV).

Primer name	Polarity	Primer sequence (5' to 3')[a]	Binding site	RNA and/or gene target
CMV				
1consAS1	-	ACAGTCGGACATTCATTAAG	2406-2425[b]	RNA1, ORF 1a
1consS1	+	GCTCAGACACGTTCCCCCAT	1821-1840[b]	RNA1, ORF 1a
G2cRFLPAS	-	GGATGGACAACCCGTTCACC	2844-2863[b]	RNA2, ORF 2b
G2cRFLPS	+	GGCTGCTTTAATGTTAGGCG	2257-2276[b]	RNA2, ORF 2a
G3cRFLPAS	-	CTGGATGGACAACCCGTTC	2011-2029[b]	RNA3, ORF 3b, CP gene
G3cRFLPS	+	CGTCCAACTATTAACCACCC	1405-1424[b]	RNA3, ORF 3b, CP gene
PVMV				
PVMV-VPg-fw	+	CAGTTTGTYATGGAYTCATTT	5709-5729[c]	VPg cistron
PVMV-VPg-rv	-	TGATAATCACYGAWCCAAATCC	6429-6450[c]	VPg cistron
PVMV-CP-fw	+	GGTGARACHGTNGATGCHGG	8610-8629[c]	CP cistron
PVMV-CP-rv	-	ARRAGRTTGTGCATATTCC	9382-9400[c]	CP cistron

[a]Y: C or T; W: A or T; R: A or G; H: A, C or T; N: A, C, G or T.

[b]Referring to CMV isolate I17F (accession numbers HE793683, HE793684 and Y18137 corresponding to RNAs 1, 2 and 3, respectively).

[c]Referring to PVMV isolate ns1 (accession number FJ617225).