Virus Genes 22:3, 329–333, 2001

Phylogenetic Placement of a Novel Tenuivirus from the Grass *Urochloa plantaginea*

JOACHIM R. DE MIRANDA[1,2,*], MIGUEL MUÑOZ[1,3], RAY WU[3] AND ANA M. ESPINOZA[1]

[1] *CIBCM, University of Costa Rica, San José, Costa Rica (E-mail: mmunoz@cariari.ucr.ac.cr)*
[2] *Department of Entomology, 548 ASI, Penn State University, PA 16802, USA (E-mail: jxd35@psu.edu)*
[3] *Department of Biochemistry, Cornell University, Ithaca, NY 14850, USA (E-mail: ves3@cornell.edu)*

Received October 27, 2000; Accepted January 9, 2001

Abstract. Evidence is presented that a tenuivirus recovered from the grass *Urochloa plantaginea* is probably a novel tenuivirus species, to be called *Urochloa hoja blanca* virus (UHBV). It is related to both *Echinochloa hoja blanca virus* (EHBV) and *Rice hoja blanca virus* (RHBV), and these three form a group distinct from *Maize stripe virus* (MStV) and *Rice stripe virus* (RStV). Phylogenetic analysis of the sequence data for RNA-3 and RNA-4 of these viruses supports the hypothesis that EHBV and UHBV may have evolved from an ancestral form of RHBV, precipitated by the introduction of *Echinochloa colona* and *Urochloa plantaginea* to America.

Key words: tenuivirus, *Urochloa*, *Caenodelphax*, planthopper, evolution

The intensification of farming practices has resulted in a corresponding evolution of pests and pathogens particular to human development. Globalization of trade in produce and materials has spread many of these around the world, often with disastrous consequences. One such consequence is the proliferation and adaptation of the pathogens of cultivation into native flora and fauna. This may have been the case in the fine ecological and molecular distinction between two closely related tenuiviruses, *Rice hoja blanca virus* (RHBV) and *Echinochloa hoja blanca virus* (EHBV), whose ecological independence despite physical proximity (*Echinochloa colona* is a common weed of rice cultivation) was due largely to the host specificity of their respective planthopper vectors; *Tagosodes orizicolus* and *Tagosodes cubanus* [1]. Here we attempt a similar characterization of a novel tenuivirus, discovered in March 1996 in Alexander grass (*Urochloa plantaginea* (Link) Webster; Poaceae: Panacoideae) in San Pedro, Costa Rica. The symptoms, a light green striated mosaic with beige tips, were similar to those described in 1958 for this plant [2] and are consistent with tenuivirus infection [3]. The plants were colonized by the planthopper *Caenodelphax teapae* (Fowler); (Hemiptera: Delphacidae). Both plant and planthopper are very common and have a wide distribution throughout Latin America, principally in drier pastures and savanas, up to 1500 m above sea level [4]. Our 1992–1994 RHBV surveys in Costa Rica had shown that both urochloa field samples and seedlings inoculated by *C. teapae* reacted positively in ELISA to RHBV antiserum. Groups of five planthoppers associated with the original source plants were placed on two week old urochloa seedlings which reproduced the tenuivirus symptoms, confirmed subsequently by Northern blots with UHBV cDNA probes, identifying *C. teapae* as a vector of the virus.

The tenuivirus genome consists of two negative stranded RNA segments (RNA-1 and RNA-5) each encoding a single gene, and three ambisense RNA segments (RNAs-2, -3 and -4), each containing two genes separated by a large intergenic spacer [5]. Genes with known or suspected function are the 330 kD replicase (vcRNA-1), a putative 94 kD membrane glycoprotein (vcRNA-2), the 35 kD nucleoprotein (vcRNA-3) and the 20 kD major noncapsid protein (vRNA-4) [5–7].

*Author for all correspondence.

Tenuivirus RNA was purified from the inoculated urochloa seedlings, cloned and sequenced using established protocols [8,9], producing complete sequences of RNA-3 and RNA-4 and partial sequence of RNA-1 (covering nt 1-1737). A partial sequence of RHBV RNA-1 (covering nt1-1805) was recovered from a previously constructed RHBV cDNA library [10]. These sequences were aligned to homologous sequences from other tenuiviruses using the GCG DNA analysis software [11]. Pairwise analyses show 80–82% nucleotide similarity among RHBV, EHBV and the new virus for the coding regions and only 47–57% nucleotide similarity for the intergenic regions, which also differ greatly in size (Table 1). Since geographic and temporal strains of a tenuivirus species are more than 95% and 90% identical for their coding and intergenic regions respectively [12,13], these viruses are molecularly distinct. This separation, together with the narrow plant host ranges and unique planthopper host-vectors for each virus, suggests that these three viruses are independently evolving lineages [1], which would make *Urochloa hoja blanca virus* (UHBV) a separate tenuivirus species [14].

There are three types of mechanisms that can precipitate such genetic isolation and subsequent divergence between the viruses [15]. The first involves strict co-evolution of the virus with its plant or planthopper hosts and since tenuiviruses are primarily transovarially transmitted [3,16] co-evolution with the planthopper is a distinct possibility [17]. The second type of mechanism involves an ecological change of plant host and planthopper host-vector [15], through (temporary) overlaps in the host ranges of the planthoppers [17,18]. In both scenarios the genetic isolation of the viruses would be reinforced by geographic isolation, adaptation, transovarial transmission and the host specificity of the planthoppers [3,18] which would limit the opportunity for interaction and competition between the viruses and their progenitors. The third mechanism is through recombination with other viruses, instantly creating new virus species with unique molecular and biological characteristics [15,19,20]. Although common in several virus taxons [15,20], it can be disregarded here since there is no indication of any major recombination or segment re-assortment between the tenuivirus genomes, as indicated by the similar relationships between the viruses for the different RNA segments (Table 1; [21]).

RHBV, EHBV and UHBV and their respective vectors are almost exclusively Neotropical [3,22,23] suggesting that the divergence between these viruses took place in America. By contrast, the principal plant hosts are all non-native. Rice (*Oryza sativa*) was domesticated around 5000 BC in Asia and was cultivated around the Mediterranean sea by 700 AD [24]. It was one of many cereals taken to the New World by Columbus with the first successful harvest in Puerto Rico, in 1535 [25], followed by other Atlantic coast areas [24]. It reached Mexico and Peru during the 16th century and was widely cultivated in colonial times [24,25]. *Echinochloa colona* is an ancient millet that was harvested in pre-dynastic Egypt and is widely grown in India [26]. The *Urochloa* genus is native to old world tropics, mainly Africa [27] and contains many forage species although *U. plantaginea* is currently regarded a weed [28]. Both these grasses were introduced to America during the mid-late nineteenth century as forage crops, probably from Africa [4]. Like many introduced grasses they are widespread and aggressive colonizers and are common weeds of rice and other crops [2,3]. Their natural and cultivated abundance would have made them attractive targets for colonization by endemic planthoppers and their viruses. Since tenuiviruses are not seed transmitted [3] the divergence between RHBV, EHBV and UHBV cannot have been through co-evolution with their plant hosts. The hypothesis proposed is therefore that the ancestral RHBV, established in America, escaped from rice into these grasses through overlap in the host-ranges of their respective planthoppers to evolve into EHBV and UHBV, placing the earliest date for this divergence at around 1850 AD. The alternative hypothesis is that the viruses and planthoppers had co-evolved before this date, on various native plant hosts, and subsequently acquired their new principal plant hosts when these were introduced to America.

These hypotheses were partly tested by phylogenetic analyses of the multiple sequence alignments of the tenuivirus RNA segments, found at http://www.personal.psu.edu/jxd35/tenui-alignments.-htm, using Maximum Likelihood criteria as implemented by PAUP-4.0b4a and the branch-and-bound tree searching algorithm [29]. The non-coding regions were excluded from these analyses since their large size differences made the assignment of positional homology impossible. A general-time-reversible model allowing for variable nucleotide substitution rates, rate heterogeneity between sites and rate heterogeneity between lineages was found to be optimal, as determined by likelihood ratio tests [30]. Bootstrapping

Table 1. Percentage nucleotide similarity between the tenuiviruses for the coding regions (upper triangles) and the intergenic regions (lower triangles) of the four major RNA segments.

	% nucleotide similarity							GenBank accession
RNA-1	RStV	MStV	RHBV	EHBV	UHBV	RGSV		
RStV		-	56.4	-	55.2	43.7		D31879
MStV			-	-	-	-		-
RHBV				-	**79.6**	47.8		AF009569
EHBV					-	-		-
UHBV						47.3		U82448
RGSV								AB009656
RNA-2	RStV	MStV	RHBV	EHBV	UHBV	RGSV	**IR-2**	
RStV		61.4	53.2	-	-	45.4	300 nt	D13176
MStV	49.1		53.6	-	-	43.9	121 nt	U53224
RHBV	45.8	45.9		-	-	42.7	364 nt	L54073
EHBV	-	-	-		-	-	-	-
UHBV	-	-	-	-		-	-	-
RGSV	44.9	41.5	40.9	-	-		568 nt	AB010376
RNA-3	RStV	MStV	RHBV	EHBV	UHBV	RGSV	**IR-3**	
RStV		65.9	55.4	54.4	55.1	46.2	742 nt	X53563
MStV	40.3		55.0	55.2	54.7	44.1	651 nt	M57426
RHBV	46.1	43.7		**81.2**	**81.6**	44.4	517 nt	L07940
EHBV	42.7	42.1	**57.1**		**82.4**	44.1	565 nt	L75930
UHBV	41.4	43.0	**47.6**	**48.9**		43.5	489 nt	U82447
RGSV	43.8	39.0	45.0	41.7	44.7		878 nt	AB000403
RNA-4	RStV	MStV	RHBV	EHBV	UHBV	RGSV	**IR-4**	
RStV		70.9	60.5	61.4	60.7	49.2	653 nt	D10979
MStV	47.2		61.1	61.5	61.2	49.4	734 nt	L13438
RHBV	33.1	34.7		**81.5**	**80.2**	50.7	522 nt	AF004657
EHBV	35.7	40.1	**48.3**		**81.6**	51.3	407 nt	L48441
UHBV	34.2	36.4	**50.8**	**52.4**		50.7	394 nt	U82446
RGSV	44.2	44.2	44.5	44.8	43.0		914 nt	AB000404

The data for RHBV, EHBV and UHBV are indicated in bold. Also shown are the lengths of the intergenic regions of RNA-2 (IR-2), RNA-3 (IR-3) and RNA-4 (IR-4) and the GenBank accession numbers of the sequences.

procedures followed the same algorithms. *Rice grassy stunt virus* (RGSV) RNAs 5 and 6 are functionally and evolutionarily homologous to tenuivirus RNAs 3 and 4 [31] and were used as outgroup sequences. The most likely trees for the coding regions of RNA-3 and RNA-4 are very similar both in branching pattern and branch lengths (Fig. 1). The main difference is that for RNA-3 there has been considerably more evolution on the *hoja blanca virus* (HBV) branch than for RNA-4. There has also been considerably more evolution on the EHBV and UHBV branches than on the RHBV branch, suggesting that the ancestor of these three viruses was much closer to present-day RHBV than to EHBV or UHBV. This supports the hypothesis that the common ancestor of these three viruses most likely was an ancestral form of RHBV. The evolution between RHBV, EHBV and UHBV appears to have been a two-step process; first from ancestral RHBV into a common EHBV/UHBV ancestor and subsequently into EHBV and UHBV. The short branch length indicates that these events occurred within a very short time span, which supports the hypothesis that this evolution may have been precipitated by the simultaneous introduction of *Echinochloa colona* and *Urochloa plantaginea* to America. The lower bootstrap support for the internal HBV branches may reflect a degree of ecological and molecular interaction between the viruses during this early divergence. Finally, the phylogenetic relationships between the planthoppers do not match those of their viruses, since *Tagosodes orizicolus* and *Tagosodes cubanus* (vector-hosts of RHBV and EHBV) are much more closely related to each other than to *Caenodelphax teapae*, the vector-host of UHBV [22,23]. All these observations favour host switching over strict co-evolution as the primary cause of evolutionary divergence between these viruses. There are

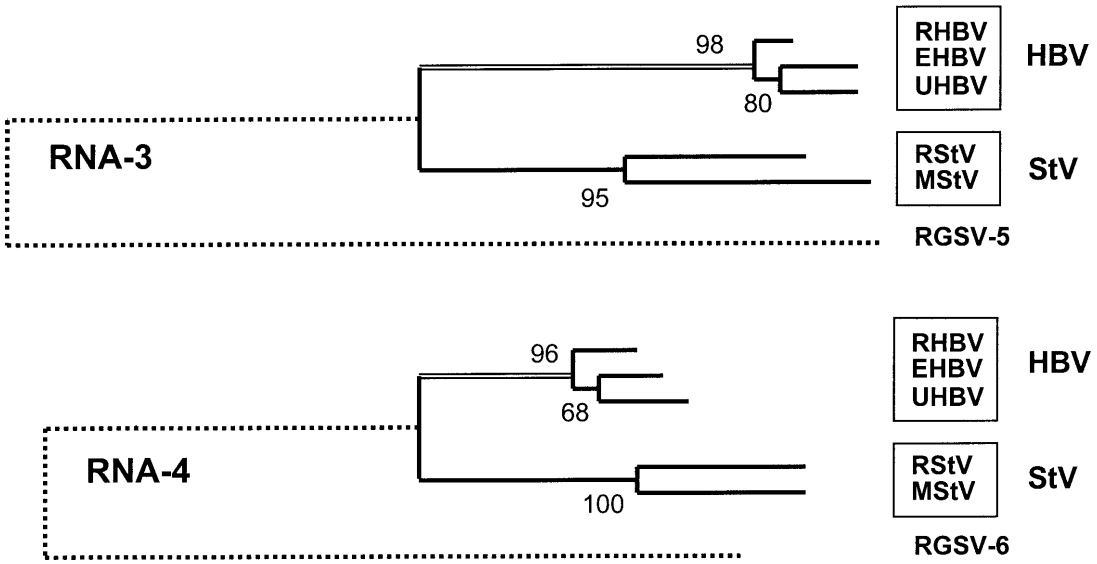

Fig. 1. Maximum Likelihood trees for the coding regions of tenuivirus RNA-3 and RNA-4. Coding regions are defined by positions 98-751 and 1730-2772 (RNA-3) and positions 69-630 and 1671-2676 (RNA-4) of the multiple alignments found at http://www.personal.psu.edu/jxd35/tenui-alignments.htm. Shown is the percentage bootstrap support for each node. RHBV, EHBV, UHBV and RGSV are as defined in the text. MSTV and RSTV refer to *Maize stripe virus* and *Rice stripe virus* respectively.

many other examples of host-switching in virus evolution, often driven by recombination or reassortment [15,19,32] although definitive proof is sometimes difficult to obtain [15]. To solidify the hypothesis that plant host introductions can serve as conduits for major demarcations in tenuivirus evolution, further support will have to be sought from viruses of other native and introduced grass species and, ideally, from historical virus samples.

Acknowledgements

Many thanks to Drs. Mayra Montiel (University of Costa Rica) and M.R. Wilson (International Institute of Entomology, London) for help with the classification of *Urochloa plantaginea* and *Caenodelphax teapae* respectively, and to the DNA-services unit of Cornell University for the sequencing. This work was supported by the Rockefeller Foundation and the European Community DG-XII.

References

1. Madriz J.A., de Miranda J.R., Cabezas E., Oliva M., Hernandez M., and Espinoza A.M., J Phytopath *146*, 305–308, 1998.
2. Green V.E. and Orsenigo J.R., Pl Dis Reporter *42*, 342–345, 1958.
3. Gingery R.E., in The Plant Viruses. Plenum Press, New York, Vol. 4, 1988, pp. 297–329.
4. Pohl W.R., Flora Costaricensis, Family #15 Gramineae. William Burger, New York, 1980, p. 608.
5. Ramirez B.-C., and Haenni A.-L., J Gen Virol *75*, 467–475, 1994.
6. de Miranda J.R., Muñoz M., Wu R., and Espinoza A.M., Virus Genes *12*, 231–238, 1996.
7. Toriyama S., Takahashi M., Sano Y., Shimizu T., and lshihama A., J Gen Virol *75*, 3569–3579, 1994.
8. de Miranda J.R., Espinoza A.M., and Hull R., J Virol Meth *56*, 109–114, 1996.
9. de Miranda J.R., Muñoz M., Wu R., and Espinoza A.M., Virus Genes *12*, 131–134, 1996.
10. de Miranda J.R., Hernandez M., Hull R., and Espinoza A.M., J Gen Virol 75, 2127–2132, 1994.
11. Genetics Computer Group, Wisconsin GCG Package Version 9.0, Madison, Wisconcin, 1996.
12. de Miranda J.R., Ramirez B.-C., Muñoz M., Lozano I., Wu R., Haenni A.-L., Espinoza A.M., and Calvert L.A., Virus Genes *15*, 191–193, 1997.
13. Qu Z., Liang D., Harper G., and Hull R., Virus Genes *15*, 99–103, 1997.
14. van Regenmortel M.H.V., Fauquet C.M., Bishop D.H.L., Carstens E.B., Estes M.K., Lemon S.M., Maniloff J., Mayo M.A., McGeoch D.J., Pringle C.R., and Wickner R.B., Virus Taxonomy. The Classification and Nomenclature of Viruses. VIIth Report of the ICTV. Academic Press, San Diego, 2000, p. 1167.
15. Gibbs A.J., Keese P.L., Gibbs M.J., and Garcia-Arenal F., in Domingo E., Webster R.G., and Holland J.J. (eds), *Origin*

and Evolution of Viruses. Academic Press, New York, 1999, pp. 263–285.
16. Galvez G.E., Phytopathology *58*, 818–821, 1968.
17. Nault L.R., in Denno F.R. and Perfect J.T. (eds), *Planthoppers: Their Ecology and Managemen*ts. Chapman and Hall, New York, 1994, pp. 429–448.
18. Wilson S.W., Mitter C., Denno F.R., and Wilson M.R., in Denno F.R. and Perfect J.T. (eds), *Planthoppers: Their Ecology and Management*. Chapman and Hall, New York, 1994, pp. 7–114.
19. White P.S., Morales F.J., and Roossinck M.J., Virology *207*, 334–337, 1995.
20. Roossinck M.J., Annu Rev Phytopathology *35*, 191–209, 1997.
21. de Miranda J.R., Muñoz M., Wu R., and Espinoza A.M., Virus Genes *12*, 231–238, 1996.
22. Asche M. and Wilson M.R., Syst Ent *15*, 1–42, 1990.
23. Fennah R.G., Trans R Ent Soc Lond *117*, 95–125, 1965.
24. Sauer J.D., Historical Geography of Crop Plants: A Select Roster. CRC Press, London, 1993, p. 309.
25. del Rio-Morero J.L., Los Inicios de la agriculture Europea en el nuevo mundo (1492–1542). Edicion conjunta bajo rural de Huelva y bajo rural de Sevilla, Sevilla, Spain, 1991, p. 214.
26. de Wet J.M.J., in Smartt J. and Simmonds N.W. (eds), *Evolution of Crop Plants* (2nd edn). Longman Scientific and Technical, Harlow, England, 1995, pp. 202–208.
27. Hacker J.B., in Smartt J. and Simmonds N.W. (eds). Evolution of Crop Plants (2nd edn). Longman Scientific and Technical, Harlow, England, 1995, pp. 229–235.
28. Wiersema J.H. and Leon B., World Economic Plants: A Standard Reference. CRC Press, New York, 1999, p. 749.
29. Swofford D.L., PAUP*: Phylogenetic Analysis using Parsimony (*and other methods) version 4.0,β4a. Sinauer associates, Sunderland, Massachusetts, 1998.
30. Huelsenbeck J.P., and Crandall K.A., Annu Rev Ecol Syst *28*, 437–466, 1997.
31. Toriyama S., Kimishima T., Takahashi M., Shimizu T., Minaka N., and Akutsu K., J Gen Virol *79*, 2051–2058, 1998.
32. Rodriguez L.L., Fitch W.M., and Nichol S.T., Proc Nat Acad Sci USA *93*, 13030–13035, 1996.