

Metagenomic discovery, worldwide distribution and genetic diversity of novel macluraviruses infecting yams (*Dioscorea spp.*)

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Blast annotations of 44,164 CAP3-assembled sequences of public ESTs (NCBI) revealed the presence of a putative new macluravirus species infecting South-East Asian tropical yam species *D. alata* in Nigeria. Based on this new information, the diversity of macluraviruses was investigated in tropical yam germplasm using four complementary approaches: 1) Sanger sequencing of cloned yam-macluravirus-specific RT-PCR amplicons, 2) virion-associated nucleic acids (VANA), 3) dsRNA and 4) siRNA deep sequencing.

Primers were designed in the *cp* gene of yam macluraviruses, based on the alignment of ESTs sequences and sequences of known viral species (*Chinese yam necrotic mosaic virus* and *Yam chlorotic necrotic mosaic virus*), and used to screen the worldwide yam germplasm collection maintained by Guadeloupe's Biological Resource Centre for Tropical Plants (CRB-PT), by RT-PCR performed on total nucleic acids. Additional samples from India and South Pacific Islands were also screened. Sanger sequencing of the RT-PCR amplicons revealed the existence of two novel macluravirus species in tropical yams: one species tentatively named *Dioscorea alata* macluravirus was identified in *D. alata* and *D. nummularia*, whereas a distinct species, tentatively named *Dioscorea esculenta* macluravirus, was identified in *D. esculenta*. These two species appear to be present in Nigeria, Guadeloupe, India and in some South Pacific Islands (Palau, Papua New Guinea, Tonga, Vanuatu), suggesting a worldwide distribution.

siRNA sequences from a *D. alata* plant collected in India were assembled in contigs, leading, after Blast annotation, to the complete genomic sequence of a strain of *Dioscorea alata* macluravirus, which was confirmed by Sanger sequencing of RT-PCR and 3'-RACE amplicons. Using 454 pyrosequencing of VANA or cDNA synthesized from purified viral dsRNAs, RT-PCR and RACE analyses, the complete genomic sequence of another strain of *Dioscorea alata* macluravirus was obtained.

Mots-clés : Macluravirus, *Dioscorea*, yam, diversity, deep sequencing, complete genome.