whereas subgroup I geminiviruses, for instance, WDV, express both an M_r 30 kDa and, *via* differential splicing, an M_r 41 kDa Rep protein, whose amino-terminal domain comprises most of the M_r 30 kDa polypeptide sequence. Rep proteins cleave the nonanucleotide after base 7 and become covalently linked to the 5'-phosphate of the cleaved strand until one round of replication is completed. Rep then catalyzes the cleavage of the newly synthesized strand and concomitantly transfers and ligates the DNA linked to it with the cleaved nonanucleotide sequence generating one circular single-stranded genomic DNA molecule.

Details of geminivirus replication, movement and transmission will be discussed.

Sequence du génome d'un clone infectieux de *maize streak virus* (MSV) de la Réunion, génétiquement distinct des isolats africains. M Peterschmitt [1], M Granier [1], R Frutos [1], M Isnard [1], B Reynaud [2] *([1] CIRAD-CA, IGEPAM, BP 5035, F-34032 Montpellier cedex 1; [2] CIRAD-CA, 7, chemin de l'IRAT, F-97410 Saint-Pierre, France)*

Le génome d'un isolat de *maize streak virus* provenant de l'ile de la Réunion (MSV-R) a été cloné et séquencé. Son pouvoir infectieux a été démontré sur des plantes de maïs à l'aide d'*Agrobacterium tumefaciens* par la technique d'agro-inoculation. À partir de ces plantes, le virus est transmissible par cicadelle, *Cicadulina mbila*. Il provoque des symptômes graves, comparables à ceux causés par l'isolat qui a servi pour le clonage.

D'après la séquence de ce génome de 2 685 nucléotides et des séquences peptidiques qui en sont déduites, le MSV-R diffère des 3 isolats africains de MSV dont les séquences génomiques sont connues : l'isolat du Nigéria (MSV-N), du Kenya (MSV-K), et l'isolat d'Afrique du Sud (MSV-S). Le génome de MSV-R est plus long que celui de MSV-K de 4 nucléotides mais plus court que celui de MSV-N et celui de MSV-S de 2 et 5 nucléotides respectivement. Les cadres ouverts de lecture (ORFs) sont portés par les 2 brins de l'ADN génomique. Les ORFs du brin viral sont identiques en position et en taille avec ceux des 3 souches africaines. Par homologie de séquence avec MSV-N, l'ORF qui code potentiellement pour une protéine de 27,0 kDa, correspond au gène de la protéine de capside. Les ORFs du brin complémentaire sont identiques en taille et en position avec ceux de l'isolat MSV-N mais diffèrent de ceux des 2 autres isolats. MSV-R contient 2 régions intergéniques comparables à celles des isolats africains.

Les homologies de séquences nucléotidiques calculées entre les génomes des différents isolats révèlent que l'isolat réunionnais (MSV-R) diffère des isolats africains qui forment entre eux un groupe distinct. Cette distinction de MSV-R est confirmée en comparant uniquement la séquence intergénique contenant les régions promotrices, et en comparant les séquences d'acides aminés des protéines potentielles de 10,9 kDa et de 31,4 kDa. En revanche, on observe une certaine homogénéité de la protéine de capside.

D'autres isolats réunionnais sont en cours d'analyse. Un isolat a été sélectionné pour sa forte pathogénie par passage récurrent sur une lignée partiellement résistante. À partir de cet isolat, 40 clones ont pu être obtenus et classés en 4 groupes RFLP. Les séquences partielles de ces clones confirment la distinction moléculaire des isolats réunionnais par rapport à ceux d'Afrique. Des plantes de maïs ont été inoculées par agro-infection avec un clone de chaque groupe RFLP. Les 4 isolats ainsi obtenus sont transmis par *C mbila* à des lignées de maïs présentant différents niveaux de résistance. D'après la gravité des symptômes observés, la pathogénie des isolats n'est pas homogène, mais toujours inférieure à celle de l'isolat d'origine.

Geminivirus replication: analysis of Rep protein functions. F Heyraud-Nitschke [1], J Laufs [2], S Schumacher [2], S Schaefer [1], J Schell [1], B Gronenborn [2] *([1] Max-Planck-Institut fuer Zuechtungsforschung, Carl-von-Linné-Weg 10, D-50829 Cologne, Germany; [2] Institut des sciences végétales, CNRS, avenue de la Terrasse, F-91198 Gif-sur-Yvette cedex, France)*

Geminiviruses are the agents of plant diseases which are of agronomic importance in many parts of the world. They are transmitted by either leafhoppers or whiteflies and are characterized by twinned icosahedral particles that contain circular single-stranded DNA. Geminiviruses multiply in the nucleus of infected cells *via* a double-stranded DNA intermediate that is subsequently used as template for rolling-circle replication of the viral-strand DNA. Apart from a single viral encoded protein (thereafter referred to as the Rep protein), geminivirus DNA multiplication entirely relies on the DNA replication apparatus from the host cell. In order to study the molecular

interactions between the M_r 41 kDa viral Rep protein and the host factors, Rep was first over-expressed in *Escherichia coli* and its biochemical properties have been investigated. Using wheat dwarf virus (WDV) and tomato yellow leaf curl virus (TYLCV) as examples, we have demonstrated that Rep acts as a sequence specific endonuclease at the viral origin of replication. It becomes covalently linked to the 5'-end of the cleaved strand and subsequently joins single-stranded origin sequences *in vitro*. In addition, deletion analysis has shown that this nicking–joining activity maps to the amino-terminal domain of the Rep protein. Hence, the carboxy-terminal domain that contains a conserved nucleoside triphosphate (NTP) binding site is not necessary for the *in vitro* cleavage–joining activity; however, a functional NTP-binding domain is required for the full activity of the Rep protein in the replication cycle of the virus. *In vitro* phosphorylation assays have shown that cell extracts contain kinase activities that specifically phosphate Rep. Elucidation of the *in vivo* significance of the biochemical properties of the geminivirus Rep protein will help to unravel the molecular mechanisms involved in virus–host interactions.

Identification and characterization of wheat dwarf geminivirus from France. M Bendahmane [1], F Jouanneau [1], F de Kouchkovsky [1], H Lapierre [2], I Lebrun [2], B Gronenborn [1] *([1] CNRS, Institut des sciences végétales, F-91198 Gif-sur-Yvette; [2] INRA, station de pathologie végétale, F-78026 Versailles, France)*

Wheat dwarf, a prevalent disease in Northern and Eastern Europe, is transmitted by the leafhopper *Psammotettix alienus* (Dahlb). The disease 'pieds chétifs' of wheat, which has been reported in several departments of central France, is also transmitted by *P alienus*. The typical symptoms of leaf streaking, severe stunting and reduced seed set resemble very much wheat dwarf. By molecular analyses we have identified the causative agent of 'pied chétifs' as wheat dwarf geminivirus (WDV). A novel simple and rapid method for purification of circular DNA from small amounts of plant tissue was developed and used to clone the genome of WDV-F (isolate France). We have sequenced the complete genome of WDV-F and have proven its infectivity by agroinoculation. Transmission by *P alienus* of WDV-F from agroinoculated wheat (*Triticum aestivum* L) to healthy tester plants resulted in the development of disease symptoms characteristic of wheat dwarf. The DNA sequence of the WDV-F genome consists of 2 750 bases and differs by only 1.3 and 1.4% from that of WDV isolates from Sweden and Czechoslovakia, respectively. A comparison of the sequence variation among the European WDV isolates with that of various isolates of maize streak virus (MSV) and other geminiviruses of Poaceae is presented. The implications for geminivirus evolution and distribution is discussed.

Transmission of geminiviruses by vectors. PG Markham *(John Innes Centre, Colney Lane, Norwich, Norfolk, NR4 7UH, UK)*

Geminiviruses are single-stranded DNA viruses with genomes of 2.5–3 kb, encapsidated in quasi-isometric particles which place them in the family *Geminiviridae*. Geminiviruses are transmitted by either whiteflies or leafhoppers (one exception is a treehopper). Discussed here are the leafhopper-transmitted geminiviruses, which infect monocotyledenous hosts, particularly maize streak virus (MSV). The actual losses caused by these viruses are difficult to establish but MSV has certainly limited production in many areas of Africa for at least the last century, and still causes epidemics. MSV is one of a complex of streak viruses that effect monocotyledons in Africa. There are a number of similar geminiviruses found in monocotyledons in Australia, Vanuatu, Japan, Asia and Europe. Indeed all these viruses may have a common origin in African grasses. All are leafhopper-transmitted and appear to have specific vector species. MSV is transmitted by several species of *Cicadulina* but the efficiency of transmission varies between species. *Cicadulina mbila* is the most efficient vector and has adapted readily to maize. In Australia the striate mosaic viruses are transmitted by different biotypes of *Nesoclutha pallida*, adapted to different hosts. The success of a virus–vector relationship depends on a number of interactions: symptoms, localisation of the virus in the plant, the feeding behaviour of the insect, the variety of plant, ecological factors, environment and other influences.

Monocot-infecting geminiviruses have a genome with 4 open reading frames, which code for replicative genes in the complementary sense (C) and a movement protein and the coat protein (CP) in the virion sense (V). In MSV point mutations in either C1 or V1 affect host range or have an effect on symptoms. The coat protein is