grasses. It is caused by members of the luteovirus group, some of which are closely related serologically and share common aphid vectors. BYDV particles occur in very low concentration in phloem tissue and are similar to ribosomes.

A combined ultrastructural and autoradiographic study on leaves of maize, barley and oat, healthy and infected with BYDV, has been carried out in order to detect the possible sites of virus synthesis and to compare the alterations caused by the BYDV in the different hosts. Therefore susceptible varieties of barley (*Hordeum vulgare* var Pirate), maize (*Zea mays* L) and oat (*Avena byzantina* L) were grown in the greenhouse and inoculated with PAV strain of BYDV by the aphid vector *Rhopalosiphum padi* (L). One month after inoculation, small samples of leaf tissues were floated for 20 min and 3 h at 27°C on sterilized water containing 500 µC/ml [H^3]-uridine, and then prepared for EM autoradiography. The ultrathin sections were examined on a Philips CM 10 electron microscope, operated at 80 kV. The EM observations demonstrated that nuclei were the organelles the most sensitive to the BYDV infection. Moreover EM autoradiography showed that [H^3]-uridine was incorporated almost exclusively into the phloem nuclei, which show typical alterations, and after only 3 h of uptake they are also found in the cytoplasm where virus particles were observed. From these experiments it seems that the host cell nuclei are involved in BYDV RNA synthesis.

Geminiviruses: genome organization and protein functions. B Gronenborn [1], M Bendahmane [1], C David [1], C Desbiez [1], F Heyraud [2], I Jupin [1], A Kheyr-Pour [1], J Laufs [1], S Schumacher [1], L Wartig [1] *([1] CNRS, Institut des sciences végétales, F-91198 Gif-sur-Yvette, France; [2] Max-Planck-Institut für Züchtungsforschung, D-50829 Cologne, Germany)*

Geminiviruses are small plant pathogens with a circular single-stranded DNA genome encapsidated within a particle of an architecture unique among all known viruses. The virion consists of 2 incomplete icosahedrons attached to each other forming a 'twin' or 'geminate' particle. The viral genome consists of either 1 (monopartite) or 2 (bipartite) circular molecules of about 2.6 to 3.0 kb.

Geminiviruses are classified into 3 subgroups. Subgroup I comprises monopartite geminiviruses, *eg* maize streak virus (MSV), *Digitaria* streak virus (DSV) or wheat dwarf virus (WDV), all pathogens of Poaceae including important cereal crops as maize, wheat and barley. With the single exception of tobacco yellow dwarf virus (TYDV) the subgroup I geminiviruses infect monocotyledonous plants, and the diseases caused by them occur in both temperate and tropical climates. They are transmitted by cicadellid leafhoppers, for instance, by *Psammotettix alienus* in the case of wheat dwarf virus, the only geminivirus of Poaceae in Europe.

The main member of subgroup II is beet curly top virus (BCTV), a geminivirus that represents an intermediate (recombinant) between leafhopper-transmitted subgroup I and the whitefly-transmitted subgroup III geminiviruses.

The geminiviruses of subgroup III are transmitted by the whitefly *Bemisia tabaci* and occur worldwide in subtropical and tropical habitats. In general, the whitefly-transmitted geminiviruses have bipartite genomes consisting of an A- and a B-DNA component. Exceptions to this rule include the Mediterranean and Near East/African tomato yellow leaf curl viruses (TYLCVs) and tomato leaf curl virus (ToLCV) from Australia, all being monopartite geminiviruses.

Upon infection, the single-strand encapsidated within the twinned virion is converted by host enzymes into a double-stranded, replicative DNA (minichromosome). To initiate the synthesis of the complementary strand, subgroup I geminiviruses encapsidate a small (80 nt) DNA primer molecule. The double-stranded DNA serves as template for divergent transcription of both plus- and minus-strands, originating at promoters within a large intergenic region or in an about 250 bp 'common region' in the case of geminiviruses with a bipartite genome. Central to the large intergenic as well as the common regions is a conserved palindromic sequence able to form a hairpin-loop structure. The loop of this hairpin contains a 9-base sequence motif identical within all known geminiviruses.

This nonanucleotide TAATATT↓[1]AC is part of the replication origin with the penultimate A being the first base of virus-strand DNA synthesis.

Contrary to most plant viruses, geminiviruses do not code for a replicase. Rather, they rely on host polymerases for their genome replication. They do encode, however, a multifunctional protein of M_r 41 kDa essential for their DNA replication, the Rep protein. The Rep protein of the whitefly-transmitted geminiviruses is a single contiguous polypeptide chain (M_r 41 kDa),

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