Comparison of the complete sequences of three different isolates of *Pepino mosaic virus*: Size variability of the TGBp3 protein between tomato and *L. peruvianum* isolates*

Brief Report

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Received November 24, 2003; accepted March 26, 2004
Published online December 10, 2004 © Springer-Verlag 2004

Summary. The complete nucleotide sequence of the genomes of two Spanish isolates (LE-2000 and LE-2002) from tomato and one Peruvian isolate (LP-2001) from *Lycopersicon peruvianum* of the *Pepino mosaic virus* (PepMV) were determined. The tomato isolates share identities higher than 99%, while the genome of LP-2001 had mean nucleotide identities of 95.6% to 96.0% with tomato isolates. The predicted amino acid sequences showed similarities ranging between 95.2% and 100% with TGBp3 and TGBp2 and CP proteins, respectively. In LP-2001 two main differences were found with respect to the tomato isolates; (i) the 5′ untranslated region (UTR) was 2 nt shorter by deletion at position 12–13 and it had some polymorphims at the putative promoter sequence reported for PepMV tomato isolates and other potexviruses, which could be functionally significant for RNA replication, and (ii) the TGBp3 protein had two extra amino acids in the C-terminal region.

*Pepino mosaic virus* (PepMV), a member of the *Potexvirus* genus [7], is the causal agent of a serious disease associated with tomato crops [23]. PepMV was first described on pepino (*Solanum muricatum*) crops in Peru in 1980 [7], and it is now known to occur in most tomato plants growing in European and North-American

*Nucleotide sequence data reported here are available in the EMBL database under the accession numbers AJ606359, AJ606360 and AJ606361.*
countries [5, 8, 18, 23, 26]. No specific vectors have been identified so far for this
virus, and the only known way of transmission is by infected plant material or
direct contact [7]. The virus was detected by serology in tomato seeds, but it was
not seed transmissible [19]. PepMV isolates differ in the biological characteristics
and symptoms they cause in various host species and cultivars [24]. Symptoms
in infected tomato plants can include yellow or dark green mosaic, chlorosis, leaf
distortions, green striations in stems and fruit loss, which appear to be dependent
on the environmental conditions [8, 13] and on the PepMV isolate [5, 23]. The
genome regions involved in the expression of any of these symptoms are presently
unknown.

The PepMV genome is a single-stranded, positive-sense RNA molecule, with
a length of 6,410 nucleotides (nt), organised in 5 open reading frames (ORFs), two
untranslated regions (UTRs) of 86 and 64 nt at the 5′ and 3′ termini, respectively,
and a 3′ terminal poly(A) tail. ORF1 encodes a 164 kDa replicase protein contain-
ing the putative methyltransferase, NTPase/helicase and RNA-dependent RNA
polymerase domains [1, 2]. Adjacent to the viral replicase is a genetic module of
three partially overlapping ORFs (2 to 4) typical of potexvirus, termed the “triple
gene block (TGB)” encoding proteins of 26, 14 and 9 kDa respectively, which
are required for virus cell-to-cell movement. ORF5 encodes the coat protein (CP)
of 25 kDa required for encapsidation [1, 2, 17]. Presently, complete genomic
sequences are available for two tomato isolates, Sp-13 from Spain [1] and the
French isolate from France [2], and the sequence of the 3′ terminal region has been
determined for another isolate from UK [17], with nucleotide identities between
them being higher than 99%. To increase knowledge of the molecular variability
among PepMV isolates and in an attempt to correlate this variability with their
biological diversity; in this study, the complete genomic sequence was determined
from two Spanish tomato PepMV isolates that differ in symptom expression and
a Peruvian asymptomatic isolate from Lycopersicon peruvianum.

Isolates LE-2000 and LE-2002 were obtained from tomato infected plants
in the regions of Murcia and Almería (both in the East of Spain) and kept in a
screenhouse as part of the “Centro de Conservación y Mejora de la Agrodiversidad
Valenciana” (COMAV) collection. These two isolates induced severe mosaics and leaf distortions in L. esculentum and L. peruvianum plants. Isolate LE-2000
additionally induced vein banding in L. esculentum. The Peruvian isolate LP-
2001 was obtained from an asymptomatic L. peruvianum plant in the Arequipa
region (Peru) [22]. This isolate induced very mild mosaic and leaf distortions in L. peruvianum, after mechanical inoculation in a greenhouse, although it was
symptomless in L. esculentum. Quantitative ELISA measured at 405 nm of optical
density were performed 10 and 30 days after inoculation shown that accumulation
of LP-2001 isolate in tomato plants was ten fold smaller than those of tomato
isolates.

Preparations enriched in double-stranded RNA (dsRNA) were extracted from
tomato leaf samples with buffer-saturated phenol and fractionated by column
chromatography on non-ionic cellulose (CF-11, Whatman) as previously de-
scribed [16]. To prepare cDNA, full-genome length PepMV dsRNAs were eluted