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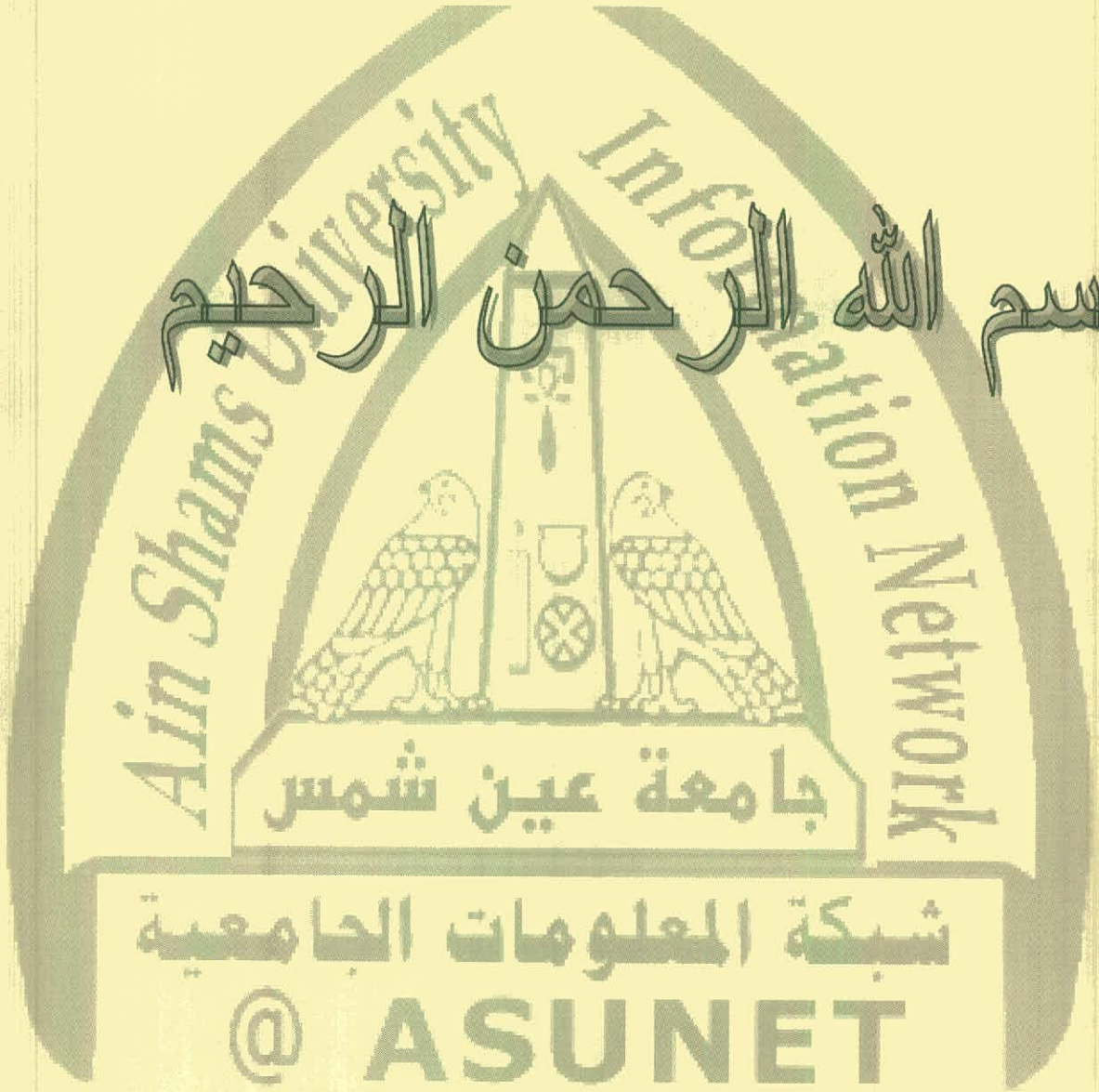

بسم الله الرحمن الرحيم

شبكة المعلومات الجامعية

التوثيق الالكتروني والميكروفيلم

شبكة المعلومات الجامعية

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التوثيق الالكتروني والميكروفيلم

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BIOLOGICAL AND MOLECULAR CHARACTERISTICS OF MAIZE YELLOW STRIPE VIRUS AND ITS RELATIONSHIP WITH THE LEAFHOPPER VECTOR

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THESIS
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ABSTRACT

Maize yellow stripe virus (MYSV) causes a disease of maize in Egypt and is transmitted by the leafhopper *Cicadulina chinai*. Based on the morphology of the virus particles and on some other features, it was described as a tenuiviurs-like virus. Further studies were carried out to confirm this preliminary classification using molecular and biological approaches. Maize stripe virus (MStV), a definite member of the genus *tenuivirus* was used in most of the tests as a control.

The viral nucleic acid was extracted from purified virus or directly from infected plants. Four dsRNA fragments of 3.5, 2.6, 2.5 and 1.5 kb were obtained from MStV-R-infected maize plants and three from MYSV-infected maize plants of 3.3, 2.4 and 1.1 kb. Conserved sequences at the ends of tenuivirus RNAs (Tenuivirus-specific primer), were used to amplify the extracted RNAs by RT-PCR. Five PCR fragments of 2.5, 1.8, 1.6, 1.3 and 0.9 kb for MYSV and four major fragments of 2.5, 1.5, 0.64 and 0.42 kb for MStV-R were amplified when ds RNA was used as template. Several cDNA clones were obtained by cloning of the PCR fragments. In Northern blot hybridization, ^{32}P labeled cDNA probes hybridized with the homologous viral RNA extracted from virus infected plants and viruliferous insects. No hybridization was obtained with RNAs from healthy plants and no cross-reactions were detected between MYSV and MStV-R. In Northern blot hybridization, the 5 MYSV cDNA probes detected 6 single stranded formaldhyde-denatured RNA fragments of >9.5 kb (RNA1), 2.3 and 1.3 kb (RNA2), 2.1 kb (RNA3), 1.6 kb (RNA4) and 1.6 kb (RNA5). MStV-R clones were hybridized with 2 viral RNA fragments RNA1 and RNA3.

At least four distinct RNA fragments were detected with the 5 cDNA probes confirming the segmented nature of MYSV genome. No

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significant sequence similarities were detected between the 5 MYSV cDNAs and MStV-R, tenuiviruses or any other viruses for which GenBank sequences were available either through nucleotide comparisons or through predicted amino acid sequence comparisons. However, as expected, similarities were found between cDNA components of MStV-R and RNA1 and RNA3 of tenuiviruses. Sequence analysis showed that the 5' and 3' termini (16-20 nucleotides) of the individual MYSV genomic RNA segments (RNA 2, 3, 4 and 5) are complementary. Similar complementary sequences were previously detected for viruses of the genus *Tenuivirus*.

Based on western blot, the suspected serological relation between MYSV and MStV was confirmed using capsid and non capsid protiens.

MYSV multiplication in its leafhopper vector *C. chinai* was studied using dot-blot hybridization test. MYSV was not detected in leafhoppers collected 1 day after the end of a 2-day acquisition access period (AAP) of experiment 1, and in those collected 0, 1 and 2 days after the end of the 1-day AAP of experiment 2. However the virus was detected in the leafhoppers collected later, on days 5, 10, 15 and 20 of the 1st experiment and in the leafhoppers collected on day 4, 6, 10 and 20 of the 2nd experiment, indicating that MYSV multiplies in its vector. This is a basic characteristic of other tenuiviruses.

Taken togrther, the above findings suggest that although MYSV is distantly related to tenuiviruses, it should probably be placed in a taxonomically separate genus related to the genus *tenuivirus*. We suggest that a new family called *Tenuiviridae* would contain MYSV and previously confirmed tenuiviruses as two distantly related genera i.e. genus *Delphacitenuivirus* containing the planthopper transmitted tenuiviruses and a second novel genus designated as *Cicaditenuivirus* containing the leafhopper transmitted tenuiviruses (MYSV).

Key words:- maize yellow stripe virus, maize stripe virus, tenuivirus, serology, genomic characteristics, dot blot hybridization, vector relations, *Cicadulina chinai*, *Peregrinus maidis*, leafhopper, planthopper, maize, Egypt, Reunion island.

E.D. Amal

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