ECOLOGICAL AND MOLECULAR STUDIES ON MAIZE YELLOW STRIPE VIRUS WITH IMPLICATION FOR ITS INSECT VECTOR (*Cicadulina chinai* Ghauri)

By

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B.Sc.Agric. Sci. (Agric. Prod.), Fac. Agric., Cairo Univ., Egypt, 1999

THESIS

Submitted in Partial Fulfillment of the Requirements for the Degree of

MASTER OF SCIENCE

In

Agricultural Sciences
(Economic Entomology)

Department of Economic Entomology and Pesticides
Faculty of Agriculture
Cairo University
EGYPT

2009

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ABSTRACT

Maize Yellow Stripe Virus (MYSV) is a leafhopper-borne Tenuivirus that has induced some epidemic on maize plants earlier in Egypt. In this study, field samples from maize plantations were taken during the summer and Nily growing seasons of 2004 and 2005 to estimate the occurrence of MYSV on maize in ten governorates of Middle and Lower Egypt. Disease severity and percentage of occurrence for both MYSV and viruliferous *Cicadulina chinai* were recorded. The relationship between meteorological data and incidence of both MYSV and viruliferous insect were studied for early prediction of virus infection. Also, deleterious effects of MYSV on its leafhopper *C. chinai* vector was estimated. Visual examination of MYSV symptoms, serodiagnosis using DAC-ELISA and sometimes molecular tools (IC/RT-PCR and hybridization) were used to detect the disease occurrence in maize fields. SSCP and sequencing of MYSV-RNA3 were used to determine viral strain recognition. It is also aimed at determination of whether 3 different MYSV-infection types are different strains or symptom development using genomic studies. Generally, MYSV occurrence was higher in Middle than in Lower Egypt. Also, it was higher in Nily than in summer plantations. However, MYSV incidence in the summer season of 2004 was higher than in Nily season of the same year. It was found that 15% of MYSV-viruliferous *C. chinai* leads to 25% MYSV infection. It was determined that different symptoms (Fine stripe, Coarse stripe and Chlorotic stunt) caused by MYSV, are symptom development. MYSV can decrease females lifespan by 49.7 % and males 19%.It decreases fecundity by 86.2%. It was also determined that MYSV population structure in this study has very high homology by (97-98%)of genomic structure for Egyptian isolate and less homology with other tenuiviruses studied using sequence analysis of MYSV RNA3 core region. MYSV ecology and *C. chinai* transmission persistency lead to cyclical infection of the virus by 9-year intervals. This study predicts one more MYSV, as emerging disease, severe outbreak during 2009 maize growing season.

Key words: Maize Yellow Stripe Virus , *C. chinai*, ELISA , molecular tools, ecology.

ACKNOWLEDGEMENT

I thank my god for his help which, without his peacemaking I can't do any thing.

I am appreciating working with my supervisor ***Dr. El-Desouky Aboul-Yazeed Ammar****, Prof. of Economic Entomology, Faculty of Agriculture, Cairo University. I would like to thank, him for his valuable advice, patience , helps and directions.*

I would like to thank my supervisor ***Dr.Mahmoud Mostafa Elbolok*** *, Prof. of Economic Entomology, Faculty of Agriculture, Cairo University for his valuable advice, patience, directions, helps, continues support, kindness and correction of the manuscript.Also, special thanks to* ***Dr. Aboul Ata El-Nady Aboul Ata****, Head Researcher of Virus and Phytoplasma, Plant Pathology Research Institute, Agricultural Research Center for his actual work with me,valuable advice, patience, directions, helps, continues support, kindness and corrections all time of the manuscript.*

I would like to thank, ***Dr. Hala Abdulla Amin****, Researcher of Virus and Phytoplasma, Plant Pathology Research Institute, Agricultural Research Center for her helps in the Molecular experiments as well as her advices.*

دراسات بيئية وجزيئية على فيروس التخطط الأصفر في الذرة وناقله الحشرى *Cicadulina chinai* Ghauri

رسالة مقدمة من

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للحصول على

درجة الماجستير

فى

العلوم الزراعية
(حشرات إقتصادية)

قسم الحشرات الاقتصادية والمبيدات
كلية الزراعة
جامعة القاهرة
مصر

٢٠٠٩

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التاريخ : ٢٩ / ٤ /٢٠٠٩

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عنـوان الرسـالة: دراسـات بيئيـة وجزيئيـة علـي فيـروس الـتخطط الأصـفر فـي الـذرة وناقلـه الحشـرى *Cicadulina chinai* Ghauri

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المستخلص العربى

يعتبر فيروس التخطط الأصفر فى الذرة احد الفيروسات الخيطية (Tenuiviruses) المحمولة بواسطة نطاطات الاوراق والذى احدث اصابات وبائية لنباتات الذرة الشامية فى مصر أكثر من مرة. فى هذه الدراسة تم تقدير نسبة انتشار مرض التخطط الأصفر أثناء موسمى زراعة الذرة الشامية الصيفى والنيلى لعامى ٢٠٠٤و٢٠٠٥ فى عشر محافظات ، فى مناطق الوجه البحرى و مصر الوسطى عن طريقة جمع عينات ورقية من حقول زراعات الذرة الشامية. تم تسجيل الشدة المرضية للاصابة بالاضافة الى نسبة الإصابة بالمرض و كذلك نسبة الحشرات الحاملة له. ولقد درست العلاقة بين العوامل المناخية و كلا من نسبة انتشار المرض والحشرات الحاملة له للتنبؤ مبكرا بحدوث الإصابة الفيروسية. كما تم تقدير التأثير الضار لفيروس التخطط الأصفر على ناقله. ولقد استخدم كل من الفحص الظاهرى للأعراض المميزة للمرض واحد طرق التشخيص السيرولوجية (DAC-ELISA) و بعض الطرق الجزيئية (IC/RT-PCR) واختبار تهجين الاحماض النووية (Hybridization) للكشف عن المرض. هذا بالاضافة الى انه تم عمل اختبار SSCP وكذلك عمل تحليل تتابع قواعد (Sequencing) الحمض النووى RNA3 الخاص بفيروس التخطط الأصفر لتحديد سلالة الفيروس وبهدف تحديد ما اذا كانت الأعراض الثلاثة المختلفة التى يحدثها المرض (التخطط الدقيق، التخطط الواسع، إ صفرار مع تقزم) سلالات مختلفة أو أنها تطور للأعراض وذلك باستعمال الدراسات الجينية .

بصفه عامة وجد ان انتشار مرض التخطط الأصفر فى الذرة الشامية كان بنسبة أعلى فى مصر الوسطى عنه فى الوجه البحرى. كما أن نسبة انتشاره فى زراعات الذرة الشامية للعروة النيلى كانت أعلى منه فى العروة الصيفية ومع ذلك أثناء عام ٢٠٠٤ كانت نسبة انتشاره فى العروة الصيفية اعلى منه فى العروة النيلية لنفس العام. كما وجد أن تواجد الحشرات الحاملة بنسبة ١٥% تؤدى الى تواجد المرض بنسبة ٢٥ %. كماحددت الدراسة أن الأعراض الثلاثة المختلفة سابقة الذكر ما هى إلا تطورًالأعراض المرض. كما أدى هذا المرض الى خفض طول عمر الحشرات الحاملة الإناث بنسبة ٤٩.٧ % والذكور بنسبة ١٩% وخفض درجة الخصوبة بنسبة 86.2%.

أضافت هذه الدراسة أن جينوم الفيروس خاصة الجزء القاعدى للحامض النووى (RNA3) متشابه بدرجة كبيرة مع جينوم العزلة المصرية المدروسة سابقا وغير متشابها مع جينوم الفيروسات الخيطية الاخرى (Tenuiviruses).

الدراسات البيئية لمرض التخطط الأصفر فى الذرة والنقل الباقى لهذا الفيروس بواسطة ناقله *C. chinai* تؤدى الى العدوى الدورية للمرض بحلول كل ٩ سنوات. وتتوقع هذه الدراسة تفشى حاد للمرض أثناء موسم زراعة الذرة الشامية لعام ٢٠٠٩ .

الكلمات الدالة: فيروس التخطط الأصفر فى الذرة ،*Cicadulina chinai* ، اليزا ، الطرق الجزيئية ، الدراسات البيئية.

CONTENTS

INTRODUCTION

Maize (*Zea mays* L.) is one of the major cereal crops, it ranks third in production following wheat and rice in the world. Also, it is one of the important strategic cereal crops in Egypt. Most of the maize cultivated area is planted in the spring (summer plantation), while the remaining area is planted in late summer (Nily plantation). In 2007 season, the total national production of maize reached about 6.142 million tons that resulted from an area of 1.782 million feddans, mostly of white grains with an average of 24.62 ard. /fed. (Anonymous, 2007).

Chiykowski (1981) indicated about 52 virus or virus-like diseases of maize that are transmitted by leafhoppers or aphids have been recorded world wide. Thottappilly *et al*. (1993) pointed out the endemic nature of some viral diseases of maize as one of the major factors responsible for low production in many countries**.** Maize yellow stripe virus is one of the most important virus diseases of maize in Egypt. This disease was first recorded in Egypt by Aboul-Ata (1983) and Ammar *et al*. (1984). Ammar *et al*. (1987 and 1990a) reported that during 1984 and 1985 incidence of MYSV-infection was very high, particularly in late-sown maize plants in Giza and some other regions in Egypt (reaching 50-70%). Aboul-Ata *et al*. (1996) proved that MYSV-like-diseased samples of sorghum from Sudan gave high positive reaction with the MYSV antiserum. In addition to maize, MYSV infects sorghum, wheat, barley, oat and other Graminaceous wild hosts (Sewify, 1994 and Thouvenel *et al*., 1996**)**. Three types of symptoms are associated with this disease: fine stripe, coarse stripe and

chlorotic stunt. Filamentous particles of 4-8 nm in diameter were observed in purified extracts of MYSV (Ammar *et al*., 1989 and Hussein, 1994).MYSV is not transmitted by mechanical inoculation or seed transmission. It is transmitted in a persistent- manner by the leafhopper *Cicadulina chinai* Ghauri but not by *C. bipunctella zeae* China. Recently, Ammar *et al*. (2007) proved that MYSV multiplies in its vector *C. chinai* but not transovarially transmitted. MYSV has several similarities with viruses of genus Tenuivirus however, viruses of this genus are known to be transmitted in a persistent-propagative manner by planthoppers (Delphacid, Homoptera) and not by cicadellid leafhoppers as the case of MYSV. So it is suggested that MYSV could be a tentative member of the Tenuivirus genus (Ammar *et al*., 1990a and Hussein, 1994).

Tenuivirus contains four or five single-stranded RNA. All of these virus family, the 5`and 3`ends (approx. 18 nt) of the RNA segments are complementary, potentially forming a panhandle structure and this probably explains the circularity that can be observed in the individual ribonucleoproteins (RNPs) in the electron microscope (Ramirez and Haenni, 1994). MYSV causes heavy losses in maize plantations whereas, severe stunting and yellowing were observed in maize fields in middle Egypt (Giza, Beni-suef, Fayoum and Minia). Severe outbreak, caused by MYSV, during 1991 was recorded and some maize fields were removed in Fayoum and Beni-suef (Anonymous, 1992 and Aboul-Ata *et al*., 1996). Also, one more severe outbreak, caused by MYSV, has occurred during summer season 2000 in maize fields in Sohag, Quena, Beni-suef and Giza (Anonymous, 2001).

The present work aimed at surveying maize fields for MYSV-infection incidence in ten governorates. Ecology of MYSV was studied to estimate behavior of MYSV and explain the outbreaks-causing factors. Tools of advanced techniques [*i.e.*, symptomatology, serological (DAC-ELISA) and molecular (IC/RT-PCR and hybridization)] were used for virus detection. SSCP analysis was used to explain whether difference in symptom type and intensity are different strain or symptom development. Sequence and alignment analyses were performed to confirm the SSCP outputs.

REVIEW OF LITERATURE

1. Maize viruses in Egypt

Maize is the third important strategic cereal crop in Egypt. Wheat (*Triticum aestivum* L.) and rice (*Oryza sativa* L.) are the first and second important cereals. It is widely used in bread making in rural areas of the country. Recently, maize flour is mixed with wheat flour by 20% in bread making in order to reduce amount of the imported wheat. It is used as feed for livestock and poultry, either as green fodder and silage or as a main component (grain) of dry feed. In addition, it is used as a row material for several industries such as starch, fructose and maize oil (Anonymous, 2001).

Viruses are considered very important diseases which cause high losses in maize plantation. Chiykowski (1981) indicated about 52 virus or virus-like diseases affecting maize, those are transmitted by leafhoppers or aphids have been recorded world wide. Thottappilly *et al*. (1993) pointed out the endemic nature of some viral diseases of maize as one of the major factors responsible for low production in many countries.

In Egypt, Abou-Zeid (1975) isolated maize dwarf mosaic virus (MDMV) from naturally infected maize plants showing dwarf and mosaic symptoms at Giza Agricultural Experiment Station using mechanical and insect transmission. The virus was mechanically transmissible to maize and other susceptible graminaceous hosts. Three aphid species namely, *Rhopalosiphum maidis* Fitch, *Myzus persicae* Sulzer and *Aphis gossypii* Glover were vectors. *R. maidis* and *M.*

persicae were almost equally efficient in transmitting the virus (36%) whereas; *Aphis gossypii* was much less effective in this respect causing only 12% infection. He explained that MDMV is a strain of the sugarcane mosaic virus.

Ammar *et al*. (1982) found symptoms like maize streak virus (MSV) on maize plants adjacent to sugarcane fields in Upper Egypt during 1975, whereas percentages of streaked maize plants were 0-4% in July, 11-14% in August, 77-78% in September and 25-58% in October. Ammar (1983) identified maize streak virus by serological studies, electron microscopy and transmission of the virus using the leafhopper *Cicadulina bipuncetella zeae.* Also, Aboul-Ata and Ammar (1989) studied the incidence of maize streak virus in Giza, Qalyobia, Munofia and Gharbia during summer and Nily plantation. The virus was not transmitted by sap, Gemini mono-particles 20 x 30 nm diameters occurring in pairs were detected by electron microscope. Egyptian isolate reacted with antiserum to an isolate of MSV from Kenya. The disease symptoms are broken narrow translucent chlorotic streaks on maize or sugarcane leaves.

Maize yellow stripe virus (MYSV) is one of the most important virus diseases of maize in Egypt. This disease was first recorded in Egypt by Aboul-Ata (1983) and Ammar *et al*. (1984)**,** it was found at four different locations in Egypt during the field inspection in 1981 and 1982. Ammar *et al*. (1987) reported that during 1984 and 1985 incidence of MYSV was very high, particularly in late-sown maize plants in Giza and some other regions in Egypt (reaching 50-70%) and it was the most widespread and most important virus disease of maize