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INDUCTION OF TYLCV RESISTANT PLANTS THROUGH TOOLS OF MOLECULAR BIOLOGY

BY

ADEL ABD EL-SABOUR A. REZK

B.Sc. Agric. Sciences (Plant Pathology), Cairo University, 1993 M.Sc. Agric. Sciences (Plant Pathology), Ain Shams University, 2001

THESIS

Submitted in partial fulfillment of the requirements for the degree of

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In

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INDUCTION OF TYLCV RESISTANT PLANTS THROUGH TOOLS OF MOLECULAR BIOLOGY

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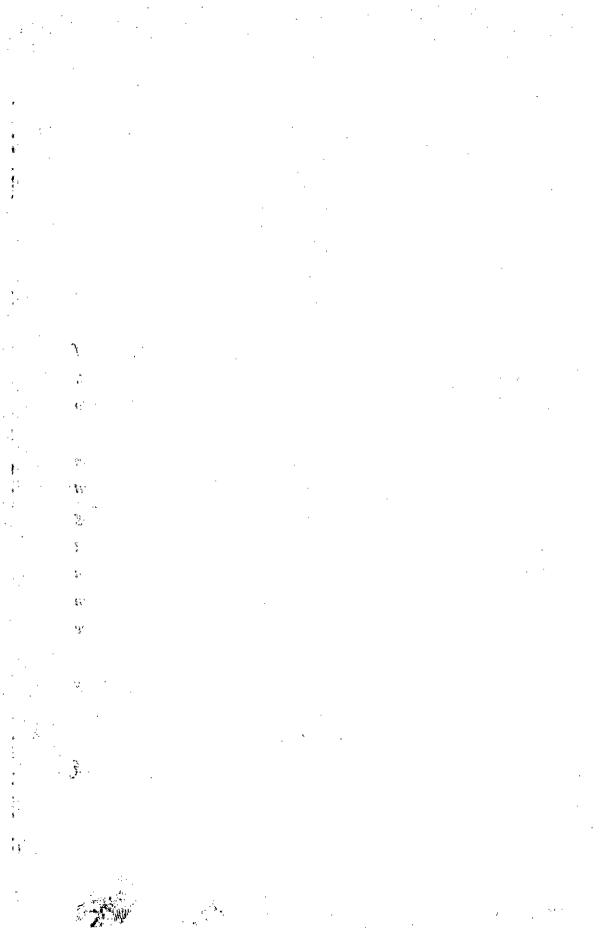
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ABSTRACT

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Control of diseases caused by plant viruses requires an understanding of the virus, its replication, the vectors that spread the virus, and the deployment of useful genes of resistance in high-yielding varieties. As many crops, tomatoes are infected by many diseases. However, the disease caused by Tomato yellow leaf curl virus (TYLCV) is the most limiting factor causing remarkable yield losses. Recent advances in molecular biology and the development of new techniques to introduce foreign genes into plant cells gave us a chance to development of new strategies to control diseases caused by plant viruses. Pathogen-Derived Resistance (PDR) is among the most promising strategy. The concept of PDR based on the introduction of gene(s) derived from the pathogen (virus) into the cell of the host plant to trigger resistance against that pathogen. Post-transcriptional gene silencing (PTGS) is a nucleotide sequence specific RNA turnover mechanism produced by plant cell as a natural mechanism against virus infection. Double strands of short (21-25 nt) interference RNA (siRNA) mediate this natural defense mechanism. The PTGS mechanism is becoming powerful tools for reducing expression of a certain viral gene and controlling infection. In the purpose of controlling the TYLCV, three regions from the viral genome located in the overlap regions between C1/C2, C2/C3 and V1/V2 were used for studying their efficiency in working as siRNA to target viral DNA accumulation in plant cells. Constructs were prepared by cloning each of the three regions both in the sense or sense/antisense directions in the binary vector pFGC5941 and introduced into tomato and tobacco plant tissues via Agro-infiltration technique. Additional construct containing the three regions together in the sense/antisense directions was also used as a control to prove that either one of the selected regions may activate the siRNA mechanism. All plants were challenged with virus using the TYLCV-Eg infectious clone 15 days post infiltration. The obtained results revealed that only sense/antisense (as) constructs inhibit or reduce accumulation of viral genome due to the induction of siRNA mechanism. However, infiltered plants with the construct containing the asC1/C2 region proved to be the best region for initiating PTGS as it inhibited the accumulation of the viral genome completely as well as it prevented the appearance of viral symptoms in all tested plants. This may be due to its role in silencing the expression of two very important viral genes which express the replication associated protein (Rep) and transactivator protein for the viral sense promoter. However, the other two regions showed a high, but not a complete, level of interference for genome accumulation and viral infection symptoms. The obtained results may also be explained based on the length of the cloned fragments, as it was only 116 bp for C1/C2 region compared to the other regions (280 bp for C2/C3 and 260 bp for V1/V2) and / or the type of the genes they interfere. It can be concluded that this mechanism can result in higher viral resistance in tomato against TYLCV.