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PHYLOGENETIC COMPARISON OF THREE ISOLATES OF TOMATO YELLOW LEAF CURL VIRUS FROM HORMOZGAN PROVINCE (IRAN) AND WORLD ISOLATES OF VIRUS

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ABSTRACT

Tomato yellow leaf curl virus (TYLCV) is a member of the genus *Begomovirus,* family Geminiviridae. Because of existence of symptoms of the virus in the Hormozgan province, in winter and spring of 2013-2014 some samples of tomato plants with disease symptoms were collected from farms of Minab and Bandar Lengeh. Then, after extraction of DNA from infected samples a segment of DNA with about 550 bp length was amplified using a pair of degenerate primers of Begomoviruses in polymerase chain reaction method (PCR).Finally, amplified segments were separated via electrophoresis and PCR products were sent to sequencing. For comparison and phylogenetic analysis, the sequences Blasted in Gen bankand using CLUSTALW and Mega4 software the phylogenetic tree was drawn via maximum parsimony method. Results showed variation among Hormozgan province.

Keywords: Begomovirus, CLUSTAL W, DNA, MEGA4, PCR, Phylogenetic, TYLCV

INTRODUCTION

Almost all the plants cultivated by human become infected by at least one virus. [1]. One of the most destructive viruses that infect tomatoes is the tomato yellow leaf curl virus in many tropical and subtropical areas of the world [2]. This virus is transmitted by whiteflies, *Bemisiatabaci* (Aleyrodidae, Hemiptera). Tomato yellow leaf curl virus is classified in the family Geminiviridae, genous Begomovirus[3]. One of the most severe diseases caused by Geminiviruses in tomatoesis the leaf curl disease which causes bythe members of genus Begomovirus called tomato yellow leaf curl virus (TYLCV) and tomato leaf curl virus (TLCV), with a single part genome. This complex of virus, produced two types of diseases, the first type causes

yellowing of the leaf margins while the second type is without of yellowing symptoms. Symptoms of Tomato yellow leaf curl virus (TYLCV) include leaf upward rolling, yellowing of leaf margins, a significant reduction in the leaf size, mild mosaic, falling of flowers, severe stunting and yield reduction. The serological and molecular methods can be used for identification of these diseases. Since the first report, TYLCV and similar viruses have spread rapidly in the Middle East. The first report of this disease was from Israel [4]. Full sequences of some isolates of TYLCV are reported from Iran. For example: TYLCV-Ayazpour [5] TYLCV-ir[6] and TYLCV-kahnoj [7] and TYLCV-abadeh [8]. Today Gemini viruses have been reported as one of the most important plant pathogens in tropical and subtropical regions of the world. One way to manage this disease is to detect the spreeding centers, the activities of vectors and methods of its survival in the fields from one year to another. The study aims were to determine the diversity of TYLCV in the Hormozgan Province (city of Minab and Bandar Lengeh) and compare the nucleotide sequence of Hormozgan isolates with the other isolates from around the world.

MATERIALS AND METHODS

Samplings of this study were conducted during winter of 2012, spring and summer of 2013from the major areas of tomato fields of Hormozgan including Minab and Bandar Lengeh. After detection of infected farms, suspected samples with the symptoms of severe leaf curling with small size and yellowing of margins of the leaf, loss of flowers and plant stunting were collected and transferred to the laboratory. To identify the virus in infected plants by polymerase chain reaction, firstly the total nucleic acid of plant was extracted by the method of Dellaporta *et al*., [9] with minor changes.

Polymerase chain reaction with the volume of 25 microliter containing 10-20 nanogram of template DNA, 1.5 mM $MgCl_2$, 200 mM of each of the four nucleotides (dTTP, dGTP, dCTP, dATP), one micromolar of each primers and two units of Taq polymerase enzyme (company Cinnagen). Temperature program of the PCR was 95 °C for 5 min, 35 cycles of one minute at 95 °C, 1.5 minutes at 55 °C, two minutes at 72 °C and final extension at 72°C for 20 minutes. Then amplified DNA was studied by 1% agarose gel electrophoresis. After amplifying of DNA, PCR products were sent to FazaPajuh Co. for sequencing. Sequences submitted to NCBI and their accession numbers are KP635445.1 (MM13 isolate), KP635444.1 (MM3B isolate) and KP635443.1 (MM3 isolate).

RESULTS

After sampling in winter of 2012 and spring and summer of 2013, from the tomato farms of Minab and Bandar Lengeh, Polymerase chain reaction was designed to detect the TYLCV using coat protein specific primers. PCR results have proven the existence of tomato yellow leaf curl virus in these samples and amplified a DNA fragment with about 550bp length (Fig. 1) that had been expected. Asample from Minab and twosamples from Bandar Lengeh (samples No. 5 and 6 of Lengeh and sample No.7of Minab) which were positive were selected for sequencing.

To study phylogeny were compared some TYLCV sequences obtained from NCBI and DNA sequences of TYLCV isolates of Hormozgan province. A multiple sequence alignment was performed by using Clustal Wand a phylogenetic tree was constructed by MEGA software Version 4 [10], using maximum parsimony method with 1000bootstrap replications (Figure 2).

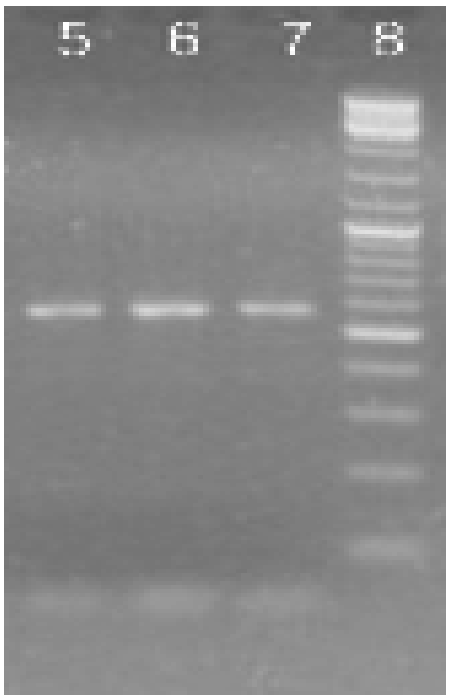

Figure 1.Results of Polymerase chain reaction onagarose gel (1%), No.8: Marker, No.7: sample of Minab and No.6 and 7: samples of Bandar Lengeh

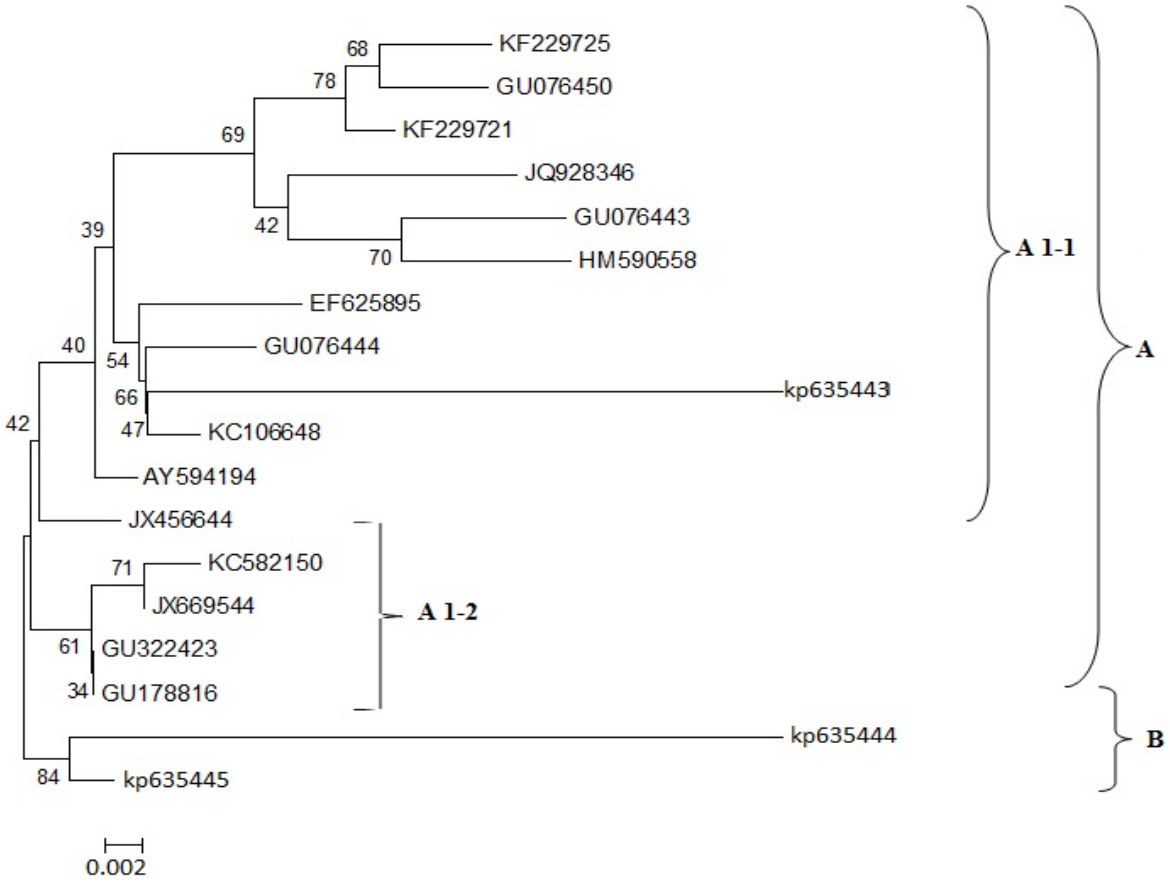

Figure 2: Maximum parsimony tree with bootstrap values of 18 isolates, 3 samples in this work and 11 isolates published inthe GenBank

Based on the phylogeny tree, isolates studied were classified in two separated groups named with letters A and B. Group A was divided into two sub-groups of A1 and A2, A1 is divided to two subgroups A1-1 and A1-2. In this classification the isolateKP635443 (Minab) is closed to the isolates of other countries, including KF229725 (Oman), KF229721 (Oman), HM590558 (Saudi Arabia), EF625895 (Venezuela), AY 594149 (China) and isolates investigated in the country, including GV076450 (Jiroft), JQ928346 (Mashhad), GU076443 (Minab), GU076444 (Shiraz), BTY1 (Minab), KC106648 (Tehran). A1-2 subgroup is also included of isolates of X456644 (China) and the A2 subgroup included isolates: KC582150 (Tehran), JX669544 (China), GU322423 (Hawaii), GU178816 (Australia). Group B only includes isolates of KP635444 (BandarLengeh) and KP635445 (BandarLengeh).

DISCUSSIONS

Using polymerase chain reaction (PCR) is a powerful and highly sensitive technique in basic biological studies, especially amplification of genomic sequences, sequencing and identification of viruses. In 1992 Navotet al.[11] used polymerase chain reaction techniques and some of primer pairsfor amplifying full-length viral

genomic of TYLCV isolated from both infected tomato plants and the vector, showed that the PCR technique is a powerful tool in such studies.In 2010, phylogenetic analysis of TYLCV was performed by Li et al.[12] in South Korea. They noted that prior to 2008 there was no report of TYLCV in South Korea, but then it was very rapidly spread in some parts of the country.Their phylogenetic studies showed that TYLCV divided into two subgroups.According to the results reported byShirazi et al [13] Iran isolates are put in at least two groups, the first group of Hormozgan, Kerman and Khuzestan isolates, which are closely related to the isolates reported from Oman (TYLCV-Albitaneh) and the second group included isolates of Bushehr, Fars, and central areas of Iran that closed to isolates of Egypt (TYLCV-egypt) and Palestine (TYLCV-mid). In present study, KP635443.1 (MM3 isolate) was classified in groups A. Oman isolate and isolates of Kerman and Khuzestan also placed in this group so confirmed previous studies of Shirazi et al. [13]. However, two other isolates of Bandar Lengeh including isolates with accession numbersKP635444 and KP635445placed in group B, separately. Another study was also conducted by comparison of some CP sequences and rep-PCR method could divide some isolates in separated groups [7].Results of the study of Jafarpoor *et al*. [14] showed that high recombination of DNA viruses (including TYLCV) leads to new generations of the viruses. Also, Navas-Castillo et al. [15] also confirmed the existence of recombination in the viral strains and demonstrated in their study. Comparison of all sequences of all isolates of TYLCV-Is showed that the regions between the gene and the 5 prime rep gene of isolates of Iran and Israel were not similar to other isolates. The phylogenetic analysis showed that isolates of Iran and Palestine may be the chimeric caused by recombination between ancestors of TYLCV-Is and agent viruses caused diseases like tomato leaf curlvirus (ToLCV) [16]. Separation of Bandar Lengeh isolates also confirmed above results and states that diversity of the virus in Iran is more than other countries.

CONCLUSION

According to the results of previous studies and this research, it can be concluded that the recombinant is a major factor of virus diversity and also use of the coating protein sequence and complete sequence of TYLCV virus is an important way to identify and classify virus isolates.

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