TECHNICAL SHEET No. 4

Virus Detection: *Cucurbit yellow stunting disorder virus* (CYSDV)

Method: RT-PCR

General

Virus detected: *cucurbit yellow stunting disorder virus* (CYSDV) from cucurbit leaves
Method: RT-PCR

Developed by
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Goals

To develop a reliable and fast detection system for CYSDV.

Introduction

Cucurbit yellow stunting disorder virus (CYSDV) is a member of the genus *Crinivirus* within the family *closteroviridae* (Fauquet and Mayo, 1999). CYSDV has a bipartite single-stranded plus-sense RNA encapsidated in long and flexuous particles. It is a whitefly-transmitted viral disease of cucurbits. It was first reported by Hassan and Duffus (1991) in the United Arab Emirates. This virus was also reported in Europe (Celix et al. 1996). Rubio et al. (2001) studied 71 isolates from collected from Jordan, Lebanon, Saudi Arabia, Spain, Turkey and North America. They found that these isolates could be divided into two subpopulations. Symptoms start as interveinal mottle on the middle leaves and develop into severe yellowing as leaves grow older. The fruits look normal but the fruit set is greatly reduced leading to a yield loss of about 50% (Abou-Jawdah et al., 2000). The development of an RT-PCR system would provide an easy, reliable and quick method to detect the disease.

Materials and Methods

Sample collection

Samples of infected cucurbit leaves (cucumber, squash, melon) were collected from greenhouses in the coastal area of Lebanon, at the end of the growing season, when symptoms are most apparent. Generally, the 4^{th} or 5^{th} yellow, papery leaf from the top of the plant was collected as is recommended in the literature.

Total RNA extraction

1. 0.2 g of leaf tissues are ground in liquid nitrogen
2. Add 400 µl of 100 mM Tris-HCl (pH 8) containing 0.2% sodium dodecyl sulfate (SDS) and 10 mM EDTA
3. Extract RNA with 1 volume of (1:1) phenol:chloroform
4. The aqueous phase is then adjusted to 2 M LiCl and incubated overnight at 4°C

5. Centrifuge at 12,000g for 15 min and dissolve the pellet in 100 µl RNAse-free-water

Primer design

Using the NCBI gene bank, the nucleotide sequence of the CYSDV HSP70 gene (AJ223619) was retrieved and used for primer design (Celix et al., 1996). In the sequence below, the bolded areas are the parts used for primer design.

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   1 atggcgaagg ctggtttgga gtttggtact actttctcta ctatcagcag ttatgttaat
  61 ggtgttatga aagttttgaa attgaacgaa actgaattta tccctacctg tttagccata
 121 acgtctaata atgatgtggt tgttggaggt cccgctcaag tattgtctaa tagtgacatg
 181 cctaactgtt acttttatga cttgaaaagg tgggtaggtg ttgacagtat caattataat
 241 gtgataaaaa ccaaaatcaa tccagcgtat gtcaccgagt tacgtggtaa cgacgtgtac
 301 atcactggta tcgatagagg ttatacctgc acttacacag tcaaacaatt gatattgtta
 361 tacattgaaa ctttagtaag attgttttct aaagttgagt ccataaccat aactagtctt
 421 aacgtctctg ttccagctga ttataaatgt aagcagcgta tgtttatgaa atcagtttgt
 481 gacagtctag gtttttcatt acgtagaatt ataaacgaac catctgcagc agctatatac
 541 tttgtttcaa agtatccgca gtataacaac ttcctgatgt atgacttcgg aggaggaact
 601 tttgattctt ctctaatagt c[agagacggt aagtat]gtca cagtggctga tactgaaggt
 661 gattcgtttt tgggaggtag agacattgat aatgctatcg ctgactatat aacaacgacg
 721 tatggtatga aaggtggttt gtccgctgac gtactggcgt ctataaagga ggattgtaat
 781 tctaagggta gagagaattt caatgttata gattcatcag gcaaacttca taatgtgaaa
 841 ttcacaagac aagatctgag tcgttgcatt gaacctttct ctaagaagag catagcactg
 901 cttgataata tggtagtgcg taacataaca aaagattccg ctgtgtttat ggttggaggt
 961 tcatcattgt tgaagaaagt tcaacatgat gtgatgaatt actgtgctag gacgaaacta
1021 gaatgcatca ttgataaaga ccttagatca gcagtgtcat ttggttgctc t[atgtcacat]
1081 [gcccaa]gaag acacaaaaaa tatgatatac atcgattgta attcacatcc cttaatgggc
1141 atatcatatt tttgttctcc aaagattata gttaggaaac ctgtggccat accttatact
1201 ggagtgcgag aagaaacctt aacaagacac tatacaattt tgaacgttta cgagggatca
1261 gatcccttcg ttttgaacaa cgattggttg attagtgcca atatgcagtc caacaagtac
1321 ggtgagatag gtgatacatt acaatatctt tacaaatata atgtagatgg catcttagag
1381 ttggttgtga ggaataaaag aacaggtaag gagacggtac ttccaattcc tttgcttttg
1441 actgagagta taaagaagct ggatgtaaat ctaactcaat tgtcaaatat tgatgaattg
1501 gcaactttgg tagctattat gagttactat aagcctgaat taaagtacct cctgacttat
1561 gtaaagacac caactatttt tgaaaatgaa attaagaaat ttggatctgg tgaagattta
1621 tataaatcct tagctgctct gaataaaaat ttcaagtaa
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Primer 1: 5’ TTG GGC ATG TGA CAT 3’ (410L)
Primer 2: 5’ AGA GAC GGT AAG TAT 3’ (410U)

RT-PCR using the “Access RT-PCR system” (Promega)

PCR mix: 20 µl reaction mixture containing 80 ng of primers and 4 µl of RNA sample to each bead.

PCR cycles:

- 1 cycle at 48°C for 45 min
- 1 cycle at 94°C for 2 min
- 35-40 cycles at 94°C for 30 sec, 42°C for 30 sec, 68°C for 70 sec
- 1 cycle at 68°C for 7 min
- 4°C end of program

The PCR product was visualized by electrophoresis in 1.2% agarose gel in TAE.

Results

Electrophoresis from extracts of virus-infected cucumber, melon and squash plants showed a specific DNA band of about 460 bp that was absent in extracts of the respective healthy controls.

Discussion

This RT-PCR technique provides an easy and consistent procedure for CYSDV detection in plants with yellowing symptoms.

Figure 1. Gel electrophoresis of RT-PCR amplified products in 1.2% agarose gel. Lanes a-c: healthy cucumber, melon and squash, respectively; lane d: 100 bp ladder (Pharmacia); Lanes e & f: infected cucumber, lanes g & h: infected melon and squash, respectively.

References

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