REVIEW ARTICLE

A Review of Cucurbit yellow stunting disorder virus (CYSDV) - a "New" Virus Affecting Melons in the Lower Rio Grande Valley

Jonathan W. Sinclair and Kevin M. Crosby

Texas A&M University, Texas Agricultural Experiment Station, Weslaco, 78596.

ABSTRACT

Cucurbit yellow stunting disorder virus (CYSDV) is a relatively new virus that affects cantaloupe (*Cucumis melo*) production in the Rio Grande Valley of south Texas and northern Mexico. Cucurbits along with lettuce are the only known hosts to date. CYSDV is a member of the newly assigned bipartite, ssRNA, *Crinivirus* Genus, in the *Closteroviridae* family. The virus is vectored by *Bemisia tabaci* biotype B in North America. Initial symptoms include interveinal chlorosis and green spots on the oldest leaves. Severe symptoms include complete leaf yellowing (except veins) and overall brittleness. Fruit quality is severely affected causing economic losses. Positive virus detection in plants is possible through recently developed RT-PCR and ELISA based methods. Virus detection in *B. tabaci* is also now possible through the use of DIG-labeled probes. Low genetic diversity (< 1%) in the major CP gene exists between geographically distant isolates of CYSDV, which may be related to the rapid spread along with *B. tabaci*. Since chemical control has proven ineffective at containment, genetic resistance is likely the best method for controlling the virus. A melon selection from Zimbabwe ("TGR1551") contains resistance to CYSDV, which is controlled by a dominant allele at one locus. Breeding work is in progress to incorporate resistance, but no commercial cultivars resistant to CYSDV are currently available.

RESUMEN

El virus de la enfermedad del enanismo y amarillamiento de las cucurbitáceas (CYSDV) es un virus relativamente nuevo que afecta la producción de melón (Cucumis melo) en el Valle del Río Grande en el sur de Texas y en el norte de México. Tanto las cucurbitáceas como la lechuga son los únicos hospederos conocidos hasta la fecha. CYSDV es un miembro del recientemente asignado genero de virus bipartido *Crinivirus* consistente de RNA de cadena sencilla perteneciente a la familia Closteroviridae. Este virus se transmite en Norteamérica por el vector *Bemisia tabaci* biotipo B. Entre los síntomas iniciales, se incluyen clorosis entre las nervaduras y manchas verdes en las hojas mas viejas. Los síntomas severos incluyen el amarillamiento completo de la hoja (exceptuando las venas) y una debilidad generalizada. La calidad del fruto es afectada severamente lo que ocasiona pérdidas económicas. La detección del virus en las plantas es posible mediante la recientemente desarrollada técnica de PCR de transcripción inversa y ELISA. La detección del virus en *B. tabaci* también es ahora posible mediante el uso de sondas marcadas con digoxigenina. Existe poca diversidad genética (< 1 %) en el gen principal de la cubierta proteínica entre los aislamientos de CYSDV distantes geográficamente, lo cual puede estar relacionado con la rápida dispersión asociada con *B. tabaci*. El uso de resistencia genética es probablemente el método mas eficaz para controlar el virus ya que los métodos de control químico han sido ineficaces. Una selección de melón de Zimbabwe ("TGR1551") presenta resistencia al CYSDV, la cual es controlada por un alelo dominante presente en un locus. Se están realizando intentos de mejoramiento genético para incorporar resistencia pero en la actualidad no existen cultivares comerciales resistentes al CYSDV.

Additional index words: Cucumis melo, Whitefly, Bemisia tabaci, transmission, resistance, breeding

Since 1980 many 'new' viruses infecting cucurbits around the world have been described; several have become widespread causing economic damage following multiplication and spread of the vectors that transmit them (Lecoq et al., 1998). Cucurbit yellow stunting disorder virus (CYSDV) is one such virus now affecting melon production in

North America (Kao et al., 2000).

Origin and Spread of the Virus. First detection of CYSDV was in the United Arab Emirates in 1982 (Hassan and Duffus, 1991) where it remains in epidemic proportions (Duffus, 1995). CYSDV has since spread throughout the Mediterranean region (Celix et al., 1996) including Egypt, Israel, Jordan, Spain, Turkey (Sese et al., 1994; Wisler, et al., 1998; Cohen and Ben-Joseph, 2000), Lebanon (Abou-Jawdah et al., 2000), Portugal (Louro et al., 2000), and Morroco (Desbiez et al., 2000) where it is has caused major economic damage to cucurbit crops (Celix et al., 1996; Livieratos et al., 1999; Rubio et al., 1999; Abou-Jawdah et al., 2000; Louro et al., 2000). CYSDV has also been introduced to North America, specifically the Rio Grande Valley of southern Texas and northern Mexico (Kao et al., 2000).

Plant Description. Melon (*Cucumis melo* L.) contains a horticulturally valuable and economically important group of crops grown throughout the world. *C. melo* is in the *Cucurbitaceae* family and appears to have originated in Africa (Kerje and Grum, 2000). It comprises seven different horticulturally important groups of melon (McCreight et al., 1993), each being annuals and vine-like in growth habit (Wang et al., 1997). Melons are a diploid species having a base chromosome number of x = 12, 2n = 24 (McCreight et al., 1993). The most popular melons in North America are orange-fleshed and heavily netted, commonly referred to as cantaloupes or muskmelons (McCreight et al., 1993). Texas is a major producer of cantaloupes averaging 11,033 acres harvested and $56,291,000 worth of production over the past three years (1999-2001) ranked third in the US over the same time period (NASS, 2002).

Virus Symptoms. CYSDV produces initial symptoms of severe interveinal chlorosis and green spots on oldest leaves which appear between 14 and 22 days post inoculation; definite symptoms are visible after 30 days (Sese et al., 1994; Celix et al., 1996). Leaves may also develop prominent yellow sectors. Severe symptoms include complete yellowing of the leaf lamina (except for the veins) and rolling and brittleness of the leaves (Celix et al., 1996). Fruit quality is severely affected; yield, fruit size, and sugar content are reduced, making fruit unacceptable for sale on the commercial market resulting in economic losses for melon growers.

Since criniviruses produce symptoms mainly in older leaves, CYSDV symptoms may easily be confused with physiological disorders, nutritional deficiencies, inadequate water, insect damage, natural senescence, or pesticide damage (Wisler et al., 1998). Growers, diagnosticians, and researchers may have a hard time visually recognizing such virus infections (Lecoq et al., 1998; Wisler et al., 1998). Further complicating correct identification is the fact that CYSDV symptoms are indistinguishable from those caused by *Beet pseudo-yellows virus* (BPYV) (Wisler et al., 1998), and are also quite similar to those caused by *Lettuce infectious yellows* virus (LIYV) (Sese et al., 1994).

Virus Description. CYSDV is a relatively "new" virus that affects cantaloupe and honeydew production in the Rio Grande Valley of south Texas and northern Mexico. The three other major cucurbit species under cultivation worldwide:

Cucumis sativus (cucumber), *Citrullus lanatus* (watermelon), and *Cucurbita pepo* (squash) are also affected by CYSDV (Hassan et al., 1991; Celix et al., 1996; Berdiales et al., 1999; Louro et al., 2000). Cucurbits and lettuce are the only known hosts to date (Duffus, 1995).

CYSDV is a member of the newly assigned *Crinivirus Genus*, in the *Closteroviridae* family along with several other viruses that have mostly been discovered within the past 12 yr (Lecoq et al., 1998) such as: *Abutilon yellows virus* (AYV), *Lettuce chlorosis virus* (LCV), *Lettuce infectious yellows virus* (LIYV), *Sweet potato chlorotic stunt virus* (SPCSV), *Tomato chlorosis virus* (ToCV), and *Tomato infectious chlorosis virus* (TICV) (Martelli et al., 2000). The CYSDV coat protein (CP) shares the highest level of similarity with those of SPCSV (36%) and LIYV (26%) (Livieratos et al., 1999). Criniviruses are long, flexible particles transmitted naturally by whiteflies (Duffus, 1995; Lecoq et al., 1998; Livieratos et al., 1998; Wisler et al., 1998; Livieratos et al., 1999; Liu et al., 2000). Closteroviruses use polyprotein processing, translational frameshifting, and subgenomic RNA's to express their genomes (Lecoq et al., 1998).

CYSDV is a phloem-limited virus making diagnosis, isolation, and purification difficult (Wisler et al., 1998). However, it has been purified with differential centrifugation and determined to have particle lengths ranging from 825 to 900 nm (Celix et al., 1996). The virus has a bipartite genome consisting of two single strand plus sense RNA segments estimated at ~9 kb (RNA1) and ~8 kb (RNA2) encapsulated separately (Celix et al., 1996). More recently, leaf dip preparations have suggested somewhat shorter particle lengths from 750 to 800 nm (Liu et al., 2000). CYSDV contains a heat shock protein (HSP70) coding region that is unique to closteroviruses (Celix et al., 1996; Tian et al., 1996). Sequence information has been estimated for four complete CYSDV genes (first three oriented 5' to 3') and one incomplete gene. The first open reading frame (ORF) corresponds to HSP70 (1659 nt long, encoding for a protein estimated at 62 kDa), the second ORF corresponds to p58 (1524 nt long, encoding for a protein estimated at 58 kDa), the third ORF corresponds to p9 (240 nt long, encoding for a protein estimated at 9 kDa), and the fourth ORF represents a putative CP gene (756 nt long encoding for a 28.5 kDa protein) (Livieratos et al., 1999).

Virus Transmission. CYSDV is transmitted in a semi-persistent, non-circulative manner by whiteflies (Duffus, 1995). Virus particles are transmitted efficiently world wide by *Bemisia tabaci* biotype B (also known as *B. argentifolii*), commonly known as the silverleaf whitefly (Soria et al., 1995; Celix et al., 1996), and biotype Q in Spain (Berdiales et al., 1999). It is also transmitted by Bemisia tabaci biotype A, but inefficiently. However, CYSDV is not transmitted by the greenhouse whitefly (*Trialeurodes vaporariorum*) which transmits BPYV and has recently been displaced over most of its former range by *B. tabaci* (Celix et al., 1996; Berdiales et al., 1999). CYSDV can persist in the vector for 9 days and has a half life of 72.2 hr which is the longest documented retention time of any known whitefly transmitted closteroviruses (Wisler et al., 1998). It cannot be transmitted mechanically (Sese et al., 1994; Celix et al., 1996). Whitefly population required for virus transmission

has been studied and although one individual is able to transmit the virus, 60 individuals per plant are required for a 100 percent transmission rate. As little as 2 hr of feeding time on infected plants is sufficient for whitefly acquisition of CYSDV resulting in a 50 percent transmission rate, and in as little as 24 hr of feeding time individual whiteflies have the ability to transmit the virus at close to a 100 percent infection rate (Sese et al., 1994).

Virus Detection and Differentiation. Although it produces symptoms similar to other members of the closteroviridae family, such as BPYV and LIYV, CYSDV can be distinguished by host range, insect transmission characteristics, and serology (Duffus, 1995). Specific probes and primers are available for accurate identification (Rubio et al., 1999). Random cDNA cloning of viral dsRNA has been performed, and a virus-specific cDNA clone (p410) of 557 nucleotides that hybridized with the smaller of the two viral dsRNA species has been identified. Heat shock protein 70 (HSP70) homologous gene amplified with primers 410U (5'-AGAGACGGTAAGTAT-3') and 410L (5'-TTGGGCATGTG ACAT-3') has allowed reverse transcription polymerase chain reaction (RT-PCR) detection of CYSDV in plants (Celix et al., 1996). Closterovirus degenerate primers have also been used along with RT-PCR to generate, clone, and characterize cDNA's from CYSDV for use in detecting plant infections (Tian et al., 1996). Oligonucloetide primers have been designed based on the CYSDV clone p410, allowing the use of RT-PCR and hybridization assays for detection of CYSDV and differentiation from BPYV in melon plants (Livieratos et al., 1998). The complete CYSDV CP gene has been cloned and purified and used to develop antiserum. As a result, reliable immunoblot and indirect enzyme-linked immunosorbent assay (ELISA) like tests have been developed for detecting CYSDV in infected plant extracts which can be used in extensive epidemiological studies (Livieratos et al., 1999). A new method of using digoxigen-labelled probes for estimating the amount of CYSDV in *B. tabaci* has recently been developed, which may allow better monitoring of the virus as well as the ability to develop action thresholds for managing the spread of CYSDV in the future (Ruiz et al., 2002).

Genetic Diversity of the Virus. Work on characterizing the genetic variability in CYSDV isolates from different countries of the world has been done through single-strand conformation polymorphism (SSCP) and nucleotide sequence analysis of the CP gene (Rubio et al., 1999; Rubio et al., 2001). Based on these results, isolates were divided into two genetic groups: a 'Western' group containing samples from Spain, Jordan, Turkey, Lebanon, and North America (Rio Grande Valley of Texas and Mexico), and an 'Eastern' group containing samples from Saudi Arabia (Rubio et al., 1999; Rubio et al., 2001). The surprisingly low genetic diversity found in the geographically broad 'Western' group (nucleotide identity > 99%) may be due to the rapid expansion of CYSDV along with its vector (*B. tabaci*), negative selection related to constraints of virus-encoded proteins, or constraints due to secondary structure (Rubio et al., 2001). Also, CYSDV is only transmitted by one vector and host plants are annuals, so infections are usually less than 60 days old (Rubio et al., 2001) not allowing as much time for mutations.

Virus control strategies. Virus control strategies in cucurbits have been based on the use of cultural practices intent on preventing or delaying virus spread through vectors (Lecoq et al., 1998). Time of planting and other epidemiological factors may be important in determining virus severity (Berdiales et al., 1999). Since chemical control has proven ineffective at containing the spread of CYSDV, genetic resistance is the most likely method for controlling the virus.

Virus resistance. A *C. melo* genotype from Zimbabwe ("TGR1551") was found to be resistant to CYSDV (Gomez-Guillamon et al., 1995). Research indicates that the resistance in "TGR1551" is controlled by a dominant allele at one locus (Sese et al., 1999; Lopez-Sese and Gomez-Guillamon, 2000). The locus which has been named designated with the symbol Cys for cucurbit yellow stunting; Cys is the first resistance gene related to a whitefly transmitted virus infecting melon to be described (Lopez-Sese and Gomez-Guillamon, 2000). Resistance may be related to the existence of mechanisms that inhibit vascular transport of the pathogen, changes in cellular membranes that impede the diffusion or transport of virus particles from cell to cell, or an inhibition of virus particle replication in tissue of resistant hosts (Lopez-Sese and Gomez-Guillamon, 2000).

Breeding for Virus Resistance. Researchers in Spain have made reciprocal crosses utilizing TGR1551 and two commercial Spanish cultivars ('Piel de Sapo' and Bola de Oro') (Sese et al., 1999; Lopez-Sese and Gomez-Guillamon, 2000), but currently there are no commercial varieties of *C. melo* available exhibiting resistance to CYSDV. Cucurbit viruses are one of the most complex pathosystems in the world (Lecoq et al., 1998) making breeding for virus resistance a challenge. Breeding virus resistant varieties is generally slow and inefficient due to several factors. First, environmental conditions may have a large effect on the expression of virus symptoms. Second, many viruses have multiple strains, some able to overcome resistance genes. Locating molecular markers linked to virus resistance is expected to make breeding for virus resistance more efficient and will lead to faster development of resistant cultivars (Danin-Poleg et al., 2000). Since resistance to CYSDV in "TGR1551" is conditioned by a single dominant allele at one locus (Sese et al., 1999), effective breeding and marker utilization should be much easier than otherwise.

TGR1551 has several undesirable characteristics including elongated fruit shape, orange color, poor fruit weight and size, white flesh color, and soluble solid content as low as 4% (Gomez-Guillamon et al., 1995). These characteristics make it a poor hybrid parent for commercial seed production, but because of its resistance to CYSDV it may be very useful in developing resistant commercial varieties over a longer period of time (Gomez-Guillamon et al., 1995). Breeding work is currently in progress to incorporate resistance from "TGR1551" mainly through backcrossing with commercially available cultivars.

LITERATURE CITED

Abou-Jawdah, Y., H. Sobh, A. Fayad, H. Lecoq, B. Delecolle, and J. Trad-Ferre. 2000. Cucurbit yellow stunting disorder

virus - a new threat to cucurbits in Lebanon. J. Plant Pathol. 2000. 82:55-60.

Berdiales, B., J.J. Bernal, E. Saez, B. Woudt, F. Beitia, and E. Rodriguez-Cerezo. 1999. Occurrence of Cucurbit yellow stunting disorder virus (CYSDV) and beet pseudo-yellows virus in cucurbit crops in Spain and transmission of CYSDV by two biotypes of *Bemisia tabaci*. Eur. J. Plant Pathol. 105:211-215.

Celix, A., A. Lopez-Sese, N. Almarza, M.L. Gomez-Guillamon, and E. Rodriguez-Cerezo. 1996. Characterization of Cucurbit Yellowing Stunting Disorder Virus, a Bemisia tabaci-transmitted closterovirus. Phytopathology 86:1370-1376.

Cohen, S. and R. Ben-Joseph. 2000. The dynamics of viruses affecting cucurbits in Israel: 40 years since 1960. Acta Hort. 510:321-325.

Desbiez, C., H. Lecoq, S. Aboulama, and M. Peterschmitt. 2000. First report of Cucurbit yellow stunting disorder virus in Morocco. Plant Dis. 84:596 (abstract).

Danin-Poleg, Y., G. Tzuri, N. Reis, Z. Karchi, and N. Katzir. 2000. Search for molecular markers associated with resistance to viruses in melon. Acta Hort. 510:399-403.

Duffus, J.E. 1995. Whitefly transmitted yellowing viruses of cucurbitaceae. p. 12-16 In: G.E. Lester and J.R. Dunlop (eds.) Cucurbitaceae '94: Evaluation and Enhancement of Cucurbit Germplasm, 1-4 Nov. 1994. Gateway Printing, Edinburg, TX.

Gomez-Guillamon, M.L., J.A. Tores, C. Soria, and A.I. Sese. 1995. Screening for resistances to *Sphaerotheca fuliginea* and to two yellowing disesaes in *Cucumis melo* and related *Cucumis* species. p. 205-208 In: G.E. Lester and J.R. Dunlop (eds.) Cucurbitaceae '94: Evaluation and Enhancement of Cucurbit Germplasm, 1-4 Nov. 1994. Gateway Printing, Edinburg, TX.

Hassan, A.A. and J.E. Duffus. 1991. A review of a yellowing and stunting disorder of cucurbits in the United Arab Emirates. Emir. J. Agr. Sci. 2:1-16.

Hassan, A.A., N.E. Quronfilah, U.A. Obaji, M.A. Al-Rays, and M.S. Wafi. 1991. Screening of domestic and wild *Citrullus* germplasm for resistance to the yellow stunting disorder in the United Arab Emirates. Cucurbit Genetics Coop. Rept. 14:98-101.

Kao, J., L. Jia., T. Tian, L. Rubio, and B.W. Falk. 2000. First report of cucurbit yellow stunting disorder virus (genus *Crinivirus*) in North America. Plant Dis. 84:101 (abstract).

Kerje, T. and M. Grum, 2000. The origin of melon, *Cucumis melo*: a review of the literature. Acta Hort. 510:37-44.

Lecoq, H., G. Wisler, and M. Pitrat. 1998. Cucurbit viruses: The classics and the emerging. p 126-142. In: J.D. McCreight (ed.). Cucurbitaceae' 98: Evaluation and enhancement of cucurbit germplasm. ASHS Press, Alexandria, Va.

Liu, H.Y., G.C. Wisler, and J.E. Duffus. 2000. Particle lengths of whitefly-transmitted criniviruses. Plant Dis. 84:803-805.

Livieratos, I.C., A.D. Avgelis, and R.A. Coutts. 1999. Molecular characterization of the cucurbit yellow stunting disorder virus coat protein gene. Phytopathology. 89:1050-1055.

Livieratos, I.C., N. Katis, and R.H. Coutts. 1998.

Differentiation between cucurbit yellow stunting disorder virus and beet pseudo-yellows virus by a reverse transcription-polymerase chain reaction assay. Plant Pathol. 47:362-369.

Lopez-Sese, A.I. and M.L. Gomez-Guillamon. 2000. Resistance to Cucurbit Yellowing Stunting Disorder Virus (CYSDV) in *Cucumis melo* L. HortScience 35:110-113.

Louro, D., M. Vicente, A.M. Vaira, G.P Accotto, and G. Nolasco. 2000. Cucurbit yellow stunting disorder virus (genus *Crinivirus*) associated with the yellowing disease of cucurbit crops in Portugal. Plant Dis. 84:1156.

Martelli, G.P., A.A Agranovsky, M. Bar-Joseph, D. Boscia, T. Candresse, R.H. Coutts, V.V. Dolja, J.E. Duffus, B.W. Falk, D. Gonsalves, W. Jelkmann, A.V. Karasev, A. Minafra, A Murant, S. Namba, C.L. Niblett, H.J. Vetten, and N. Yoshikawa. 2000. Family *Closteroviridae*. pp. 943-952. M.H.V. Van Regenmortel, C.M. Fauquet, D.H. Bishop, E.B. Carstens, M.K. Estes, S.M. Lemon, J. Maniloff. M.A. Mayo, D.J. McGeoch, C.R. Pringle, and R.B. Wickner (eds.) In: Seventh Report of the International Committee on Taxonomy of Viruses. Academic Press, New York, NY.

McCreight, J.D., H. Nerson, and R. Grumet. 1993. Melon Cucumis melo L. pp. 267-294. In: Kalloo, G., Berch B.O. (eds.) Genetic improvement of vegetable crops. Pergamon Press, Oxford, England.

National Agricultural Statistics Service. 2002. Vegetables— Annual Summary PVG-BB). http://usda.mannlib.cornell. edu/reports/nassr/fruit/pvg-bban/. January 2002.

Rubio, L., Y. Abou-Jawdah, H.X. Lin, and B.W. Faulk. 2001. Geographically distant isolates of the crinivirus cucurbit yellow stunting disorder virus show very low genetic diversity in the coat protein gene. J. Gen. Virol. 82:929-933.

Rubio, L., J. Soong, J. Kao, and B.W. Falk. 1999. Geographic distribution and molecular variation of isolates of three whitefly-bourne closteroviruses of cucurbits: Lettuce Infectious Yellows Virus, Cucurbit Yellow Stunting Disorder Virus, and Beet Pseudo-Yellows Virus. Phytopathology 89:707-710.

Ruiz, L., D. Janssen, L. Velasco, E. Segundo, and I.M. Cuadrado. 2002. Quantitation of cucurbit yellow stunting disorder virus in *Bemisia tabaci* (Genn.) using digoxigen-labelled hybridisation probes. J. Virol. Meth. 101:95-103.

Sese, A.L., F. Sanchez, and M.L. Gomez-Guillamon. 1999. Evaluation of melon F1 hybrids resistant to cucurbit yellowing stunting disorder virus (CYSDV).
Acta Hort. 492:341-347.

Sese, A.L, M.L. Gomez-Guillamon, and J.R. Diaz-Ruiz. 1994. Appearance of a possible new melon yellowing disease in Spain. Cucurbit Genetics Coop. Rept. 17:72-73.

Soria, C., A.I. Sese, M.L. Gomez-Guillamon. 1995. Specificity of transmission of melon yellowing viruses by *Trialeurodes vaporarium* and *Bemisia tabaci*. Cucurbit Genetics Coop. Rept. 18:44.

Tian, T., V.A. Klaassen, J. Soong, G. Wisler, J.E. Duffus, and B.W. Falk. 1996. Generation of cDNA specific to Lettuce infectious yellows closterovirus and other whitefly-transmitted viruses by RT-PCR and degenerate

oligonucleotide primers corresponding to the closterovirus gene encoding the heat shock protein 70 homolog. Phytopathology. 86:1167-1173.

Wang, Y.H., C.E. Thomas, and R.A. Dean. 1997. A genetic map of melon (*Cucumis melo* L.) based on amplified fragment length polymorphism (AFLP) markers. Theor. Appl. Genet. 95:791-798.

Wisler, G.C., J.E. Duffus, H.Y. Liu, and R.H. Li. 1998. Ecology and epidemiology of whitefly-transmitted closteroviruses. Plant Dis. 82:270-280.