Detection, Identification and Assessment of Variation of Whitefly-Transmitted Geminiviruses

B.D. Harrison

Department of Biological Sciences, University of Dundee,
Dundee DD2 5DA, United Kingdom

Abstract

Harrison, B.D. 1994. Detection, Identification and Assessment of Variation of Whitefly-Transmitted Geminiviruses. Arab J. Pl. Prot. 12 (2): 120-116

Whitefly-transmitted geminiviruses (WTGs) characteristically have serologically related particles. Thus although several of the viruses have been detected in plant sap by ELISA or immunosorbent electron microscopy with polyclonal antibodies, these tests do not identify them. Monoclonal antibodies (MAbs) raised against particles of three of the viruses, and used in triple-antibody-sandwich ELISA, have provided a sensitive detection test for >30 WTGs from >20 crop and weed species in >50 countries. Moreover, most of the viruses can be identified by their relative reactivities with sub-sets of 30 of the MAbs. Some crops such as cassava and tomato were shown by these tests, supported by nucleotide sequence data, to be infected with different WTGs in different geographical regions.

WTG genomes consist of two DNA molecules, or in some viruses only one. The molecule always present has a well conserved sequence except for the large intergenic region; the other molecule, where it occurs, is not well conserved in different WTGs. WTGs can therefore be detected by hybridization with DNA probes for conserved sequences or by a polymerase chain reaction (PCR) with primers based on these sequences. Many of the viruses can be differentiated by analysis of the fragments produced by restriction endonuclease digestion of the PCR products, or by using probes or primers specific for sequences of one virus. WTGs can be detected and identified by PCR in extracts of single whiteflies (*Bemisia tabaci*).

Key words: *Bemisia tabaci*, serology, ELISA, cDNA probes, PCR.

Introduction

Geminiviruses are characterised by having bisegmented (geminate) particles, genomes consisting of circular single-stranded DNA, and insect vectors, which may be leafhoppers, treehoppers or whiteflies (6). Whitefly-transmitted geminiviruses (WTGs) all have the same vector, *Bemisia tabaci*, and are widely distributed in tropical and sub-tropical regions, where they cause some of the world's most economically important virus diseases. Their genomes mostly are made up of two DNA molecules, but in some instances only one, each molecule containing 2500 to 2800 nucleotides. The genome segment which is always present (DNA-A) encodes the viral coat protein (mol. wt. *c.* 30,000) (18) and proteins involved in virus replication (11). The other segment (DNA-B) encodes proteins that control the transport of viral DNA from nucleus to cytoplasm and from cell to cell (13). In this paper, I outline methods of detecting and distinguishing WTGs, based on current knowledge of their serological and molecular properties, and describe the variation found among virus isolates associated with the same disease.

Serological methods

Tests with polyclonal antisera to WTGs have shown that the particles of almost all the viruses are serologically related. A consequence of these relationships is that a large majority of the viruses can be detected, in double antibody sandwich ELISA (DAS-ELISA) (23) or immunosorbent electron microscopy (ISEM) (15), with the aid of polyclonal antibodies from a good quality antiserum raised to any one of them. Such tests can be valuable for detecting a wide range of WTGs but they are of little use for distinguishing the different viruses from one another. In general, DAS-ELISA is more widely applicable than ISEM because some WTGs have particles which seem to be unstable and so are not easily recognised in ISEM, although their coat protein is nevertheless detectable by ELISA.

To provide reagents with a greater ability to distinguish among different WTGs, panels of murine monoclonal antibodies (MAbs) have been raised against the particles of some of the viruses, notably African cassava mosaic (23), Indian cassava mosaic (1) and okra leaf curl (19) viruses. These antibodies have proved to be of great value both for detecting and for differentiating a wide range of WTGs when used in triple antibody sandwich ELISA (TAS-ELISA; Table 1). In this test, viral antigen is trapped on microtitre plates by polyclonal antibody, the trapped antigen is exposed to one of the MAbs, and the bound MAb is then detected by incubation first with antibody to mouse γ-globulin that is conjugated to alkaline phosphatase and then with a substrate of the enzyme. Non-specific background reactions typically are lower than in DAS-ELISA with polyclonal antibodies, and can be further decreased by using 5% defatted milk powder to block the surface of the plate wells after they are coated with polyclonal antibody (12). In practice, TAS-ELISA can usually detect the homologous virus in extracts of naturally infected leaf tissue at a dilution of 1:100, and frequently at 1:1000. The best results are obtained with young symptom-bearing leaves from plants which have recently developed systemic symptoms. The viruses are less readily detected, or may be undetectable, in symptom-free (5) or older symptom-bearing leaves.

Individual MAbs differ in the extent of their reactivity with heterologous WTGs. Some MAbs react only with the homologous virus, and perhaps not with all isolates (19), whereas others also react with some or many heterologous WTGs. Tests of the reactivity of a virus against a panel of MAbs can thus be used to establish its epitope profile. We have found that most WTGs have a characteristic epitope profile, which distinguishes each virus from most others (8, 20, 21, 22). TAS-ELISA therefore provides a reliable method of detecting and differentiating WTGs. It can also be used quantitatively to compare concentrations of virus particle antigen in different tissues or different plant genotypes, a potentially valuable aid to selection in resistance-breeding programmes.

Table 1. Whitefly-transmitted geminiviruses detected by TAS-ELISA*

Virus	Source countries†
Abutilon mosaic	Britain (+1)
African cassava mosaic	Africa (17)
Ageratum yellow vein	Singapore (+1)
Asystasia golden mosaic	Nigeria
Bean calico mosaic	Mexico
Bean dwarf mosaic	Colombia
Bean golden mosaic	Puerto Rico (+3)
Bhendi yellow vein mosaic	India
Chino del tomate	Mexico
Cotton leaf crumple	USA
Cotton leaf curl	India (+1)
Croton yellow vein mosaic	India
Dolichos yellow mosaic	India
East African cassava mosaic	Kenya (+4)
Euphorbia mosaic	USA
Honeysuckle yellow vein mosaic	Britain
Horsegram yellow mosaic	Indian
Indian cassava mosaic	India (+1)
Jatropha mosaic	Puerto Rico
Limabean yellow mosaic	India
Malvastrum yellow vein mosaic	India (+2)
Mungbean yellow mosaic	India (+1)
Okra leaf curl	Ivory Coast (+7)
Pepper mild tigré	Mexico
Potato yellow mosaic	Venezuela
Rhynchosia mosaic	Colombia
Serrano golden mosaic	Mexico
Squash leaf curl	USA (+1)
Texas pepper	USA
Tobacco leaf curl	India (+2)
Tomato golden mosaic	Brazil
Tomato leaf crumple	USA
Tomato leaf curl and tomato yellow leaf curl	Pantropical (>25)
Watermelon chlorotic stunt	Saudi Arabia (+3)

* Monoclonal antibodies to African cassava mosaic and Indian cassava mosaic viruses were used for detection in tests at Dundee.

† Origin of virus isolates detected.

The application of epitope profiling to virus isolates from different countries has led to some unexpected findings. For example, WTGs from mosaic-affected cassava in >20 countries in Africa and the Indian subcontinent were found to be of three types which have different, barely overlapping, geographical distributions (7, 20), and are now recognised on the basis of their antigenic and biological differences, and their nucleotide sequences, to constitute three separate viruses (10). Similarly, viruses associated with tomato leaf curl or tomato yellow leaf curl diseases in different countries or regions are distinguishable both antigenically (9) and by nucleotide sequence analysis (14).

Conversely, viruses associated with diseases of different plant species in the same country have unexpectedly similar epitope profiles, as found for WTGs from India (8, 12) or the Americas (21). These serological data are supported by comparisons of the deduced amino acid sequences of WTG coat proteins, which show that relationships among the proteins can be represented as three branches which correspond to Asia, the Americas and the Mediterranean region/Africa, respectively and do not relate to the host ranges of the viruses (Y.G. Hong and B.D. Harrison, unpublished data). The explanation of this state of affairs may involve two other factors: the probable importance of coat protein specificity in determining whitefly transmissibility (15, 2), and the occurrence of different biotypes of *B. tabaci* in different geographical areas (3) and (P.F. McGrath and B.D. Harrison, unpublished data). Indeed, there is preliminary evidence for differential adaptation of WTGs for transmission by the *B. tabaci* biotype occurring in the same area (McGrath and Harrison, unpublished). Such adaptations would tend to result in conservation of coat protein structure among WTGs from the same region.

Use of cDNA probes

WTG DNA is readily detected in southern blots by radioactive probes derived from cloned DNA of the homologous virus. Comparison of the nucleotide sequences of different WTGs shows that there is a strong tendency for sequence conservation within DNA-A. A probe for DNA-A therefore typically detects the DNA of heterologous WTGs, although with varying efficiencies (15), depending on the amount of sequence conservation in the region from which the probe was derived. This type of test can therefore be used to detect WTGs in general but is not the method of choice for discriminating among them. In contrast, DNA-B sequences are much less conserved among different WTGs, so that probes derived from DNA-B typically fail to detect heterologous WTGs (15). A strong reaction with a DNA-B probe therefore gives a firm indication of the identity of the virus in the sample, especially if stringent conditions for hybridization are used.

Tests of these kinds have roughly the same sensitivity as TAS-ELISA but they have less accuracy quantitatively. Also, weak reactions are more difficult to interpret because extracts of WTG-free plants give background reactions on occasion. Moreover, the tests employ radioactive materials, which are not readily available in many of the countries where WTGs occur. However, the growing availability and effectiveness of tests with non-radioactive probes promises to remove this last difficulty. For example, Swanson *et al.* (21) used sulphonated probes derived from DNA-A and/or DNA-B of 8 WTGs in tests to detect the homologous and 17 heterologous viruses. In general, the DNA-A probes detected almost all WTGs from the same continent as the homologous virus but few others. The DNA-B probes detected few if any viruses other than the homologous one. Tests with biotinylated probes can also be expected to give good results.

Detection by the polymerase chain reaction (PCR)

PCR is rapidly replacing the use of cloned cDNA probes for detecting viral DNA. The test is extremely sensitive and can be used to detect a range of WTGs in plants (17) and in whiteflies (4). The viruses can be detected in single viruliferous *B. tabaci*, even in specimens dried some weeks previously.

These tests detect but do not distinguish WTGs. To test for a single WTG, two approaches can be made. In the first, the primers represent sequences that are found only in the virus which is to be detected. This approach has the disadvantage that the nucleotide sequences of the virus in question must be known. In an alternative approach, primers representing a conserved sequence are used to amplify a piece of viral DNA, which is then treated with one or more restriction endonucleases to give fragments that can be separated by gel electrophoresis to give patterns typical for each virus. This procedure adds to the work needed to analyse each sample but has been used successfully both with WTG-infected plants and with viruliferous *B. tabaci* (4). It has the advantages that the nucleotide sequence of the virus sought does not need to be known, and that other WTGs are also detected and distinguished.

Conclusions

Several modern methods are available for detecting and identifying WTGs. TAS-ELISA is probably the most useful method for testing plants for WTGs but several MAbs must be used if the viruses are to be distinguished.

When this is done, putative alternative natural hosts of a WTG can be tested (12) and only those containing virus isolates with the correct epitope profile need to be tried as sources of inoculum for transmission to the usual host. TAS-ELISA can handle large numbers of samples and is also suitable for comparing virus concentrations. However, further development work is needed to devise a protocol in which the polyclonal antibody (which is in limited supply) used for coating microtitre plates is replaced by one or more MAbs.

Of the tests for viral DNA, PCR is much the most sensitive and does not require radioactive materials. It is the only reliable method of detecting WTGs in single whiteflies. Relatively crude tissue extracts can be tested, and defective isolates that do not produce detectable viral coat protein (16) will presumably be detectable when appropriate primers are used. Nucleic acid hybridization tests with cloned DNA probes are no longer favoured for routine testing but will continue to be useful for research purposes.

الملخص

هاريسون، برايان. 1994. طرائق لكشف الفيروسات التوأمية وتعريفها في النباتات وفي حشرة الذبابة البيضاء الناقلة لها. مجلة وقاية النبات العربية. 12 (2): 116-120

أوضحت الإختبارات التي أجريت على أكثر من 30 فيروساً توأمياً تنقلها حشرات الذباب الأبيض وتصيب أكثر من 20 محصول زراعي في أكثر من 50 بلد في العالم أن هناك علاقة مصلية فيما بينها. وتستطيع الأمصال المتعددة الكلونات الكشف عن العديد من الفيروسات في عصارة النباتات دون تعريفها. واستعملت الأجسام المضادة الوحيدة الكلون، التي تم انتاجها بعد حقن ثلاثة من هذه الفيروسات، في الكشف عنها بطريقة السندويش الثلاثي للأجسام المضادة (اليزا)، وكانت أكثر حساسية في الكشف عن هذه الفيروسات. ولقد أمكن تعريف فيروسات مفردة من هذه الفيروسات نتيجة لتفاعلاتها النسبية مع تحت-مجموعات من 30 جسم مضاد وحيد الكلون. وأثبتت النتائج بأن الأمراض التي لايمكن التمييز فيما بينها والتي تحدثها فيروسات توأمية تنقلها الذبابة البيضاء كتلك التي تصيب البندورة أو الكاسافا مثلاً، تختلف فيما بينها بصفاتها البيولوجية الأخرى وتوزعها الجغرافي. ولقد تم تأكيد التفريق فيما بينها بمقارنة تتالي نيكلوتيدات الحمض النووي لعزلات ممثلة لها. ان المادة الوراثية للفيروسات التوأمية التي تنقلها الذبابة البيضاء تتكون من جزيئيين من الحمض النووي (DNA) وفي بعض الحالات من جزئ واحد. ويمتلك جزئ الحمض النووي الموجود دائماً تتابعاً ثابتاً للنوكليوتيدات باستثناء منطقة ما بين الجينات؛ أما الجزيء الآخر، عند وجوده، فيكون متغيراً في الفيروسات التوأمية التي ينقلها الذباب الأبيض، وعليه يمكن كشف هذه الفيروسات من خلال تهجينها مع مجسات الحمض النووي بالنسبة للحالة الأولى، أو بوساطة تفاعل البوليمراز المتسلسل باستخدام بادئات (primers) مبنية على أساس تتالي النيوكليوتيدات في الأجزاء الثابتة للحمض النووي. ويمكن الكشف عن هذه الفيروسات في مجموعات من الحشرة الناقلة *Bemisia tabaci* بواسطة الاختبارات السيرولوجية، الاّ أنه يمكن الكشف عنها في حشرة مفردة بواسطة تفاعل البوليميراز المتسلسل فقط.

كلمات مفتاحية: *Bemisia tabaci*، اختبارات مصلية، اليزا، مجسات أحماض نووية، تفاعل البوليمراز التسلسلي.

References

1. Aiton, M.M. and B.D. Harrison. 1989. Monoclonal antibodies to Indian cassava mosaic geminivirus (ICMV). Report of the Scottish Crop Research Institute for 1988, p. 175.

2. Briddon, R.W., M.S. Pinner, J. Stanley and P.G. Markham. 1990. Geminivirus coat protein gene replacement alters insect specificity. Virology 177: 85-94.

3. Costa, H.S. and J.K. Brown. 1991. Variation in biological characteristics and esterase patterns among populations of Bemisia tabaci, and the association of one population with silverleaf symptom induction. Entomologia Experimentalis et Applicata 61: 211-219.

4. Deng, D., P.F. McGrath, D.J. Robinson and B.D. Harrison. 1994. Detection and differentiation of whitefly-transmitted geminiviruses in plants and vector insects by the polymerase chain reaction with degenerate primers. Annals of Applied Biology 125: 327-336.

5. Fargette, D., J-C. Thouvenel and C. Fauquet. Virus content of leaves of cassava infected by African cassava mosaic virus. 1987. Annals of Applied Biology 110: 65-73.

6. Harrison, B.D. 1985. Advances in geminivirus research. Annual Review of Phytopathology 23: 55-82.

7. Harrison, B.D. and D.J. Robinson. 1988. Molecular variation in vector-borne plant viruses: epidemiological significance. Philosophical Transactions of the Royal Society of London B 321: 447-462.

8. Harrison, B.D., V. Muniyappa, M.M. Swanson, I.M. Roberts and D.J. Robinson. 1991a. Recognition and differentiation of seven whitefly-transmitted geminiviruses from India, and their relationships to African cassava mosaic and Thailand mung bean yellow mosaic virus. Annals of Applied Biology 118: 299-308.

9. Harrison, B.D., M.M. Swanson, P.F. McGrath and D. Fargette. 1991b. Patterns of antigenic variation in whitefly-transmitted geminiviruses. Report of the Scottish Crop Research Institute for 1990, pp. 88-90.

10. Hong, Y.G., D.J. Robinson and B.D. Harrison. 1993. Nucleotide sequence evidence for the occurrence of three distinct whitefly-transmitted geminiviruses. Journal of General Virology 74: 2437-2443.

11. Lazarowitz, S.G. 1992. Geminiviruses: genome-structure and gene function. Critical Reviews in Plant Science 11: 327-349.

12. Muniyappa, V., M.M. Swanson, G.H. Duncan and B.D. Harrison. 1991. Particle purification properties and epitope variability of Indian tomato leaf curl geminivirus. Annals of Applied Biology 118: 595-604.

13. Noueiry, A.O., W.J. Lucas and R.L. Gilbertson. 1994. Two proteins of a plant virus coordinate nuclear and plasmodesmal transport. Cell 7: 925-932.

14. Padidam, M., R.N. Beachy and C.M. Fauquet. 1995. Tomato leaf curl geminivirus from India has a bipartite genome and coat protein is not necessary for infectivity. Journal of General Virology 76: 25-35.

15. Roberts, I.M., D.J. Robinson and B.D. Harrison. 1984. Serological relationships and genome homologies among geminiviruses. Journal of General Virology 65: 1723-1730.

16. Robinson, D.J., B.D. Harrison, J.C. Sequeira and G.H. Duncan. 1984. Detection of strains of African cassava mosaic virus by nucleic acid hybridisation and some effects of temperature on their multiplication. Annals of Applied Biology 105: 483-493.

17. Rojas, M.R., R.L. Gilbertson, D.R. Russell and D.P. Maxwell. 1993. Use of degenerate primers in the polymerase chain reaction to detect whitefly-transmitted geminviruses. Plant Disease 77: 340-347.

18. Stanley, J. and M.R. Gay. 1983. Nucleotide sequence of cassava latent virus DNA. Nature 301: 260-262.

19. Swanson, M.M. and B.D. Harrison. 1993. Serological relationships and epitope profiles of isolates of okra leaf curl geminivirus from Africa and the Middle East. Biochimie 75: 707-711.

20. Swanson, M.M. and B.D. Harrison. 1994. Properties, relationships and distribution of cassava mosaic geminiviruses. Tropical Science 34: 15-25.

21. Swanson, M.M., J.K. Brown, B.T. Poulos and B.D. Harrison. 1992a. Genome affinities and eptiope profiles of whitefly-transmitted geminiviruses from the Americas. Annals of Applied Biology 121: 285-296.

22. Swanson, M.M., A. Varma, V. Muniyappa and B.D. Harrison. 1992b. Comparative epitope profiles of the particle proteins of whitefly-transmitted geminiviruses from nine crop legumes. Annals of Applied Biology 120: 425-433.

23. Thomas, J.E., P.R. Massalski and B.D. Harrison. 1986. Production of monoclonal antibodies to African cassava mosaic virus and differences in their reactivities with other whitefly-transmitted geminivirus. Journal of General Virology 67: 2739-2748.