Molecular Characterization of a New Begomovirus Infecting Tobacco in Kagoshima,Japan : an Evidence for Interspecific Recombination

Were, Hassan Karakacha
Laboratory of Plant Pathology Divisioin of Plant Pathology and Pesticide Science, Department of Applied Genetics and Pest Management, Faculty of Agriculture,Kyushu University

Takeshita, Minoru
Laboratory of Plant Pathology Divisioin of Plant Pathology and Pesticide Science, Department of Applied Genetics and Pest Management, Faculty of Agriculture,Kyushu University

Furuya, Naruto
Laboratory of Plant Pathology Divisioin of Plant Pathology and Pesticide Science, Department of Applied Genetics and Pest Management, Faculty of Agriculture,Kyushu University

Takanami, Yoichi
Laboratory of Plant Pathology Divisioin of Plant Pathology and Pesticide Science, Department of Applied Genetics and Pest Management, Faculty of Agriculture,Kyushu University

https://doi.org/10.5109/4624

出版情報：九州大学大学院農学研究院紀要. 50 (1), pp.83-91, 2005-02-01. Faculty of Agriculture, Kyushu University
バージョン：
権利関係：

J. Fac. Agr., Kyushu Univ., **50** (1), 83–91 (2005)

Molecular Characterization of a New Begomovirus Infecting Tobacco in Kagoshima, Japan: an Evidence for Interspecific Recombination

Hassan Karakacha WERE, Minoru TAKESHITA, Naruto FURUYA and Yoichi TAKANAMI*

Laboratory of Plant Pathology, Division of Plant Pathology and Pesticide Science, Department of Applied Genetics and Pest Management, Faculty of Agriculture, Kyushu University, Fukuoka 812–8581, Japan
(Received November 5, 2004 and accepted November 11, 2004)

A virus infected tobacco sample (KG5Tob) showing leaf curl symptoms was collected in Kagoshima, Japan. The complete DNA–A like molecule of KG5Tob was determined and found to comprise 2762 nucleotides with a typical begomovirus genome organization. A comparison of the DNA–A sequences revealed that KG5Tob shared 87% with its closest relatives, *Honeysuckle yellow vein mosaic virus* (HYVMV) and *Tobacco leaf curl Yamaguchi virus* (TbLCYV), however, on comparison of the intergenic region (IR) nt sequences, the isolate shared only 66 and 64% of the IR sequences with the former and the latter, respectively. Phylogenetic analysis of full length DNA–A component clustered KG5Tob in a lone cluster but close to HYVMV and TbLCYV. When the CPs were aligned, similar results were observed and KG5Tob was placed in a lone cluster. A comparison of the Other ORFs showed that different parts of the genome had different affinities, suggesting that recombination could have played a major role in the evolution of this isolate. Molecular data show that KG5Tob isolate is an isolate of HYVMV, although the isolate was obtained from tobacco.

INTRODUCTION

Tobacco leaf curl disease is a viral disease of tobacco first reported in South Africa in 1902 (Lucas, 1958), the Netherlands in 1917 (Lucas, 1975), East Africa (Storey, 1931), Brazil (Costa and Forster, 1939), Venezuela (Wolf *et al.*, 1949) and Japan (Osaki and Inouye, 1978). The disease has since been reported in many other countries (Brown *et al.*, 1991; Nicolaeva *et al.*, 1995; Paximadis *et al.*, 1999). Symptoms of the disease vary greatly (Osaki and Inouye, 1978; Valand and Muniyappa, 1992; Paximadis and Rey, 1997) most probably due to differences in location and origin of the causal agent.

In Japan, only one begomovirus, *Honeysuckle yellow vein mosaic virus* (HYVMV) has been reported infecting honeysuckle while three begomoviruses, *Tobacco leaf curl Kochi virus* (TbLCKV–KK), *Tobacco leaf curl Japan virus* (TbLCJV), TbLCJV–[JP3], TbLCJV–[JP2] and *Tobacco leaf curl Yamaguchi virus* (TBLCYV) infect tomato (Ikegami *et al.*, 1987; Onuki, 2003) but their antigenic and genomic affinities have not been studied in detail. Moreover it remains to be seen whether begomoviruses infecting tobacco are the same ones infecting honeysuckle.

To gain a better understanding of the genomic variation and evolution of the Japanese begomoviruses, we collected samples of tobacco leaves showing symptoms of viral

* Corresponding author (E-mail: takanami@agr.kyushu-u.ac.jp)

infection, in Kagoshima, Japan and characterized their molecular biological and genetic properties.

MATERIALS AND METHODS

Isolates

A virus-infected symptom expressing tobacco (*Nicotiana tabacum* L.) plant herein referred to as KG5Tob (Fig. 1) was collected at a field in Kagoshima Prefecture, Japan in 2002.

Fig. 1. Leaf curling and vein enation symptoms on begomovirus-infected tobacco plant in the field.

PCR-mediated amplification and cloning of DNA-A

Total DNA extraction from infected plants, primers for PCR, and amplification of DNA-A, were fully described in Were *et al.* (2005).

Sequence analysis

Tobacco begomovirus DNA-A sequences were phylogenetically compared to DNA-A of other tobacco, honeysuckle, *Eupatorium* and tomato begomoviruses as described in Were *et al.* (2005). Percent DNA-A nucleotide (nt) and ORF amino acid (aa) sequence identities between virus isolates were calculated using the distance between all pairs of sequences in the multiple alignments.

The begomovirus DNA-A sequences used for comparison and their database accession numbers were as follows: *Honeysuckle yellow vein mosaic virus* (HYVMV, AB020781; HYVMV-UK1, AJ542540), *Tobacco leaf curl Yamaguchi virus* (TbLCYV, AB079765), *Tobacco leaf curl Kochi virus* (TbLCKV-KK, AB055009), *Tobacco leaf curl Japan virus* (TbLCJV, AB028604; TbLCJV-JP2, AB055008; TbLCJV-JP3, AB079689), *Eupatorium yellow vein virus* (EpYVV, AB007990; EpYVV-Yamaguchi, AB079766; EpYVV-MNS2, AJ438936), *Tomato leaf curl Taiwan virus* (ToLCTV, U88692),

Ageratum yellow vein China virus–[Hn2] (AYVCNV–Hn2, AJ495813), *Ageratum yellow vein Sri Lanka virus* (AYVSLV, AF314144), *Tomato yellow leaf curl China virus*–Tb[Y5] (TYLCCNV–Tb[Y5] AJ319674), *Tobacco leaf curl Yunnan virus*–[Y3] (TbLCYNV–Y3, AF240674), *Tobacco leaf curl Zimbabwe virus* (TbLCZV, AF350330), *Malvastrum yellow vein virus*–[Y47] (MYVV–[47], AJ457824), *Tomato yellow leaf curl Thailand virus* (TYLCTHV, AY514630) and East African cassava mosaic Zanzibar virus (EACMZV, AF422174). Recombination analysis was done using a recombination detection software, RDP version 2.0.

RESULTS

Sequence analysis

The complete DNA–A nucleotide sequence of KG5Tob was determined to be 2762 nucleotides (AB178949). The genome organization of KG5Tob was similar to that of other monopartite begomoviruses with 2 open reading frames ORFs [AV1 (CP) and AV2] in the virion sense strand and 4 in the complementary sense strand (AC1, AC2, AC3 and AC4). The intergenic region (IR) separated the virion and complementary sense genes and the isolate contained a putative stem loop structure with the conserved nonanucleotide sequence 5'–TAATATTAC–3' that is characteristic of the *Geminiviridae* in the loop. In addition, it contained the TATA Box, identified in the IR sequence at nucleotides, 258–261.

Alignment of the retrieved complete DNA–A nt sequence with those from the DNA data bases revealed a high degree of sequence variability ranging from 41% to 87%

Table 1. Percentage nucleotide or amino acid sequence identities between KG5Tob and 19 other begomoviruses from Asia, Africa and Europe.

Virus	DNAn	IRn	V1a	V2a	C1a	C2a	C3a	C4a
EpYVV	72	48	81	82	84	70	72	85
EpYVV–[MNS2]	72	51	81	81	87	73	74	83
EpYVV–[Yamaguchi]	73	52	82	80	86	74	75	83
HYVMV	87	66	93	87	91	86	90	80
TbLCYV	87	64	93	88	92	83	83	87
HYVMV–[UK1]	71	64	85	83	87	81	82	–
TbLCKoV–[KK]	72	89	86	89	93	83	81	87
TbLCJV–[JP2]	71	85	83	85	90	83	84	79
TbLCJV–[JP3]	71	86	84	84	90	83	86	81
TbLCJV	71	68	83	85	90	83	85	–
AYVCNV–[Hn2]	58	47	44	59	58	61	65	68
ToLCTV	59	46	42	76	58	60	63	71
TLCYNV–[Y3]	56	50	44	60	51	59	63	52
TYLCTHV	58	50	44	64	58	58	62	68
TYLCCNV–tb[Y5]	57	51	40	61	54	60	62	69
AYVSLV	41	47	41	64	57	55	62	68
MYVV–[Y47]	42	48	43	54	55	58	60	68
EACMZV	54	43	40	53	50	55	61	–
TbLCZV	52	54	41	63	53	50	62	–

[n] Nucleotide sequence identity. [a] Amino acid sequence identity.

depending on geographical location and or different hosts/diseases (Table 1). KG5Tob showed a high degree of sequence similarity with other begomoviruses found in Japan and rest of Asia than those found in Africa. For instance the isolate shared 87% with each of HYVMV and TbLCYV, 71–73% with Japanese tobacco begomoviruses (JTBVs) as well as Japanese *Eupatorium* viruses and 41–58% with Asian and African begomoviruses.

Phylogenetic analysis of the complete DNA–A components divided the Japanese begomoviruses into three groups, the Japanese *Eupatorium* infecting begomoviruses (JEpBVs) group, Japanese honeysuckle infecting begomovirses (JHBVs) group and the Japanese tomato infecting tobacco begomoviruses (JTBVs) group with KG5Tob being placed in a lone cluster within (JHBVs) (Fig. 2). The begomoviruses from China, Taiwan and Thailand were clustered together and so were those from Sri Lanka and Africa. Hosts also played a big role in the separation of the viruses, *Eupatorium* geminiviruses were clearly separated from those of honeysuckle and tobacco (Fig. 2).

A detailed analysis of the aligned CP nt as well as aa sequences revealed that the 5' terminal and the core region was the most conserved area of the CP in all begomoviruses

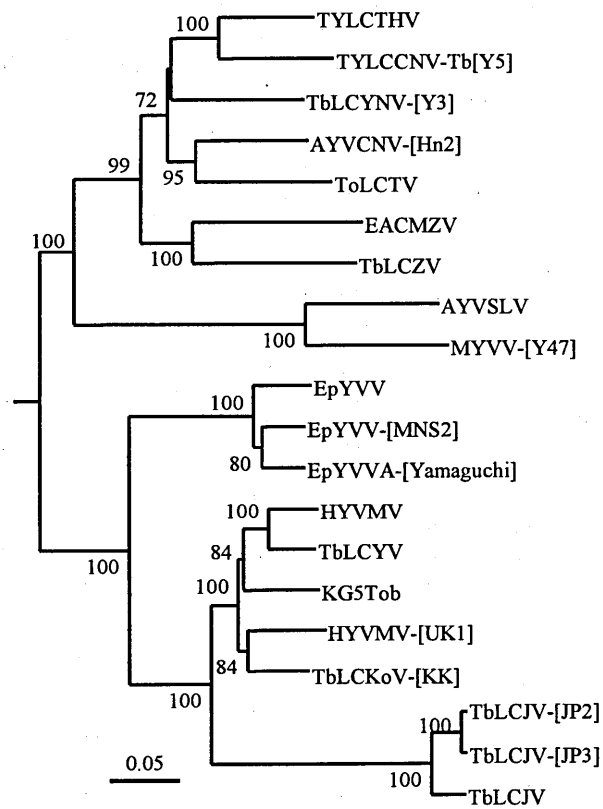

Fig. 2. Phylogenetic tree generated from alignment of full-length DNA–A nucleotide sequences of honeysuckle, tobacco, *Eupatorium* and other Asian, African and European begomoviruses.

compared. Furthermore, KG5Tob shared a high degree of sequence identity (81–93%) with the Japanese begomoviruses and 40–44% with those found in rest of Asia and Africa. Phylogenetic analysis showed that the CP was the most conserved part of the genome. Begomoviruses from the same geographical location were clustered together resulting in a tree with two major clusters, one, in which KG5Tob was clustered contained the Japanese begomoviuses while the other, begomoviruses found in the rest of Asia and Africa (Fig. 3).

When the IR nt sequences were compared, KG5Tob shared only 64% with TbLCYV but a high percetage (66–89%) with JTBVs. Other begomovirsues from rest of Asia and Africa compared poorly with the isolate. The IR had the grossest variation of the whole DNA–A component.

A comparison of the C1 sequences revealed that KG5Tob shared a high degree (92–95%) of sequence identity with JHBVs and JTBVs than with HYVMV–[UK1] 87%. The C1 of Japanese begomoviruses was more related than that from rest of Asia and/or Africa. Phylogenetic analysis of the C1 revealed a tree structure in which KG5Tob was

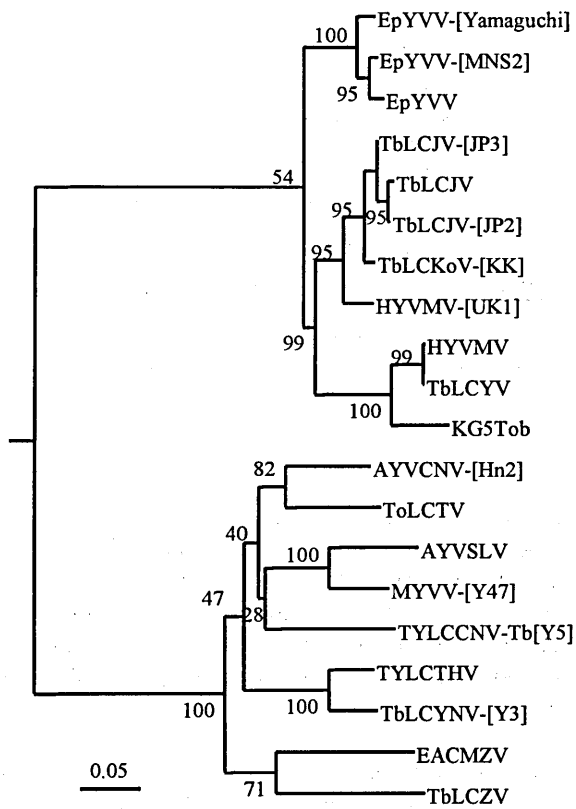

Fig. 3. Phylogenetic tree generated from CP amino acid sequence alignments of honey suckle, tobacco, *Eupatorium* and other Asian, African and European begomoviruses.

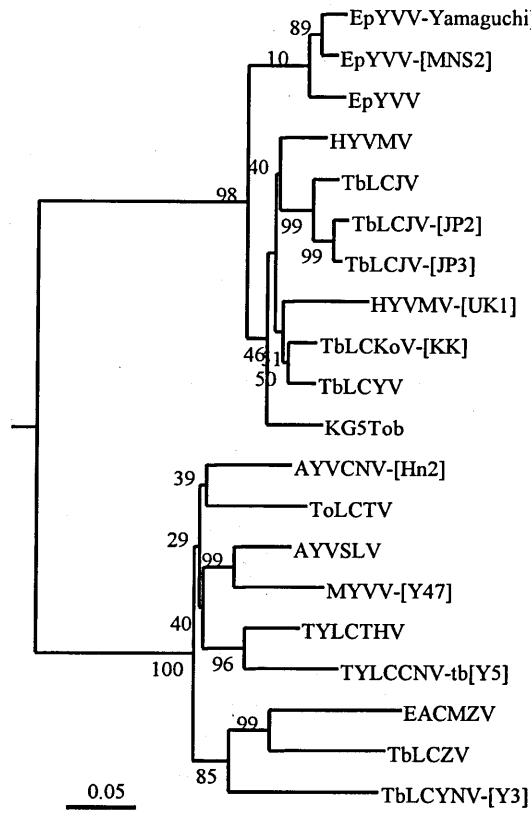

Fig. 4. Phylogenetic tree generated from C1 amino acid sequence alignments of honeysuckle, tobacco, *Eupatorium* and other Asian, African and European begomoviruses.

clustered alone but more related to JTBVs (Fig. 4).

When individual encoded proteins were compared, the highest amino acid sequence similarity was between KG5Tob and HYVMV with 93% CP, 87% V2, 91% C1, 84% C2, 90% C3 and 80% C4. Generally, the C1 was the most conserved sequence of begomovirus genome among the Japanese geminiviruses followed by CP and V2 in that order. The CP of KG5Tob was more related to that of JHBVs than to that of JTBVs (Table 1). The IR, which was found to be most variable part of the begomovirus genome was more related to JTBVs than to JHBVs.

Several significant as well as insignificant recombination events were detected in KG5Tob. For instance there was an event (2178–2533) spanning part of C1 and the entire C4, whereby KG5Tob sequence formed part of a group comprising HYVMV–OT2, HYVMV, EpYVV, and TbLCYV. The other event (2680–40 nt), covered almost the entire IR and involved TbLCJV–[JP2] and TbLCKoV–[KK] as major and minor parents, respec-

tively.

DISCUSSION

Observations made in the field and in the glasshouse revealed that KG5Tob induced symptoms in tobacco plants typical of begomovirus infection. Comparison of the nucleotide sequence of KG5Tob with those from the databases, explored their relationship in great detail. It was shown that KG5Tob shares low DNA–A sequence homology (>87%) with all the begomoviruses from the databases compared. Additionally, the IR also compared poorly with the database ones but was close to that of HYVMV and TLCYV. However, within the Japanese begomoviruses the CP and C1 had higher (83–93%) nt sequence homology than the rest of the ORFs compared. All these differences point to the fact that different parts of the same sequence have different affinities. The finding that only Japanese begomovirus CPs shared a high degree of sequence homology confirms earlier findings (Harrison and Robinson, 1988; Harrison *et al.*, 1997; Harrison *et al.*, 2002) that begomoviruses from different hosts in the same geographical area tend to be more closely antigenically related to one another than to those causing indistinguishable diseases in other regions. It also indicates that the Japanese begomoviruses have evolved independently from other begomoviruses resulting in the KG5Tob CP varying significantly from that of begomoviruses found outside Japan.

Padidam *et al.* (1995), observed that strains of anyone virus should share >90% of their DNA–A sequence, later, Mayo and Pringle (1998) stated that, they should share > 90% of their CP sequences in addition. However, the multiplicity of variants due to recombination and other factors associated thereto, increasingly complicate and consequently harden the decision on whether two virus isolates should be considered strains or distinct viruses (Harrison and Robinson, 1999). When a complete DNA–A of KG5Tob was carefully examined different blocks of the sequence had different affinities indicating that genetic recombination had occurred. For instance KG5Tob shared 93% of the CP aa sequence identity with HYVMV and TbLCYV but the percentage of their IR sequences shared was very low. It has been shown that IR sequences of begomoviruses give the most sensitive indication of the extent of viral similarity and difference (Padidam *et al.*, 1999) because it contains the presumed Rep binding site (Laufs *et al.*, 1995; Orozco and Hanley–bowdoin, 1996). Therefore, it is highly unlikely that KG5Tob with so huge variation in that IR will replicate the other viruses' genome even though they share 93% of their CPs. Additionally, KG5Tob shared 87% overall sequence identity with HYVMV and TbLCYV, a figure that is too low to warrant placement of the two viruses in the same species. Based on that sequence comparisons, it can be deduced that the high sequence homology within the CP and C1 could be due to recombination in the areas affected resulting in KG5Tob being distinguished from HYVMV and JTBVs as belonging to a different virus species. This was confirmed by the results of Recombination Detection Program kindly provided by Darren Martin. Using the program, it was discovered that part of the CP and much of the Rep belonged to different honeysuckle begomoviruses while the N–terminal of the Rep and the IR belonged to different tobacco begomoviruses. Interspecific recombination has been reported in begomoviruses infecting cassava (Berrie *et al.*, 2001; Zhou *et al.*, 1997) but none had been reported in tobacco begomoviruses. The possession of different parts having different affinities by KG5Tob could be due to

the presence of several begomovirus groups in the same geographical location such as that reported in Pakistan and Brazil (Harrison *et al.*, 1997; Krause *et al.*, 1998). Since these groups are capable of either infecting honeysuckle or tobacco plants, the possibility of having many frequent multiple begomovirus infections is high. The multiple infections provide fertile ground for the creation of recombinants and hence new viruses. Varma and Malathi (2003) observed that the ease with which geminivirus recombinants arise suggests that their occurrence in nature must be much great than yet realized. They attributed the high frequency of recombination to the rampant occurrence of mixed infections since the begomoviruses are transmitted by the same whitefly species.

Sequence analyses have demonstrated that KG5Tob is a new previously unrecognized virus. However, since the reservoir host of KG5Tob in tobacco in winter must be geminivirus–infected honeysuckle, we propose the name, HYVMV–KG5Tob, for KG5Tob.

ACKNOWLEDGEMENTS

We thank Mr. Hong–Soo Choi of the National Institute of Science and Technology, Suwon, South Korea for help with sequencing. This work was funded by the Japan Society for the Promotion of Science, Postdoctoral Fellowship for Foreign Researchers.

REFERENCES

Berrie, L. C., E. P. Rybicki and M. E. C. Rey 2001 Complete nucleotide sequence and host range of *South African cassava mosaic virus*: further evedence of recombination amongst begomoviruses. *J. Gen. Virol.*, **82**: 53–58

Brown, J. K., R. Lastra and J. Bird 1991 First documentation of whitefly transmitted geminiviruses causing widespread disease in cotton, tobacco and tomato in Dominican Republic and in tomato in Puerto Rico. *Fitopathologia*, **26**: 47

Costa, A. S. and R. Forster 1939 Una suspeita de virus de fomo (*Nicotiana tabacum* L.) sekelhante 'leaf curl' fpresente no Estrado de Sao Palo. *J. Agron. Piracicaba*, **2**: 296–302

Harrison, B. D. and D. J. Robinson 1988 Molecular variation in vector–born plant viruses: Epidemiological significance. *Phil. Tran. R. Soc. London* Ser. B, **321**: 447–462

Harrison, B. D. and D. J. Robinson 1999 Natural genomic and antigenic variation in whitefly–transmitted geminviruses (begomoviruses). *Ann. Rev. Phytopathol.*, **37**: 369–389

Harrison, B. D., Y. L. Liu, S. Khalid, S. Hameed and G. W. Otim–Nape 1997 Detection and relationship of Cotton leaf curl virus and allied whitefly transmitted geminiviruses occurring in Pakistan. *Ann. Appl. Biol.*, **130**: 61–75

Harrison, B. D., M. M. Swanson and D. Fargette 2002 Begomovirus coat protein: serology and functions. *Physiol. Mol. Plant Pathol.*, **60**: 257–271

Ikegami, M., T. Osaki and T. Inouye 1987 Single stranded DNA in tobacco leaf curl virus. *Ann. Phytopath. Soc. Jpn.*, **53**: 269–273

Krause, R., J. J. Fermandes, A. C. de Avila, I. C. Bezerra and I. C. Rebeiro 1998 Widespread occurrence of tomato geminiviruses in Brazil associated with the new biotype of the whitefly vector. *Abst. Int. Workshop on Bemisia and Geminiviruses*, Puerto Rico, poster **46**

Laufs, J., I. Jupin, C. David, S. Schumacher, F. Heyraud–Nitschke and B. Gronenborn 1995 Geminivirus replication: Genetic and biochemical characterization of Rep protein function, a review. *Biochemie*, **77**: 765–773

Lucas, G. B. 1958 *Diseases of tobacco* Scarecrow Press, New York

Lucas, G. B. 1975 *Diseases of tobacco*, 2nd ed. Harold E Parker and Sons, North Carolina

Mayo, M. A. and C. R. Pringle 1998 Virus taxonomy–1997. *J. Gen. Virol.*, **79**: 649– 657

Nicolaeva, V. B., J. K. Brown, C. C. Gariby and R. E. Rivera–Bustamante 1995 Incidence and distribution of geminiviruses in tobacco in Mexico. *Agronomy and Phytopathology joint meetings* (CORESTA),

Oxford

Onuki, M. 2003 Studies on Molecular identification of Begomoviruses isolated in Japan Ph.D Thesis, Kyushu University, Japan, 171 pp.

Osaki, T. and T. Inouye 1978 Resemblance in morphology and internuclear appearance of viruses isolated from yellow dwarf diseased tomato and leaf diseased tobacco. *Ann. Phytopath. Soc. Jpn.*, **44**: 167–178

Orozco, B. M. and L. Hanley–bowdoin 1996 A DNA structure is required for geminivirus origin function. *J. Virol.*, **270**: 148–158

Padidam, M., R. N. Beachy and C. M. Fauquet 1995 Classification and identification of geminviruses using sequence comparisons. *J. Gen. Virol.*, **76**: 249–263

Padidam, M., S. Sawyer and C. M. Fauquet 1999 Possible emergence of new geminiviruses by frequent recombination. *Virology*, **265**: 218 – 225

Paximadis, M. and M. E. C. Rey 1997 Aetiology of tobacco leaf curl in Southern Africa. *Ann. Appl. Biol.*, **131**: 449–457

Paximadis, M., A. M. Idris, I. Torres–Jerez, A. Villarreal, M. E. C. Rey and J. K. Brown 1999 Characterization of tobacco geminiviruses in the Old and New World. *Arch. Virol.*, **144**: 703–717

Storey, H. H. 1931 A new virus disease of the tobacco plant. *Nature*, **128**: 187–188

Valand, G. B. and V. Muniyappa 1992 Epidemiology of tobacco leaf curl virus in India. *Ann. Appl. Biol.*, **120**: 257–267

Varma, A. and V. G. Malathi 2003 Emerging geminivirus problems: A serious threat to crop production. *Ann. Appl. Biol.*, **142**: 145–164

Were, H. K., M. Takeshita, N. Furuya and Y. Takanami 2005 Molecular characterization of a new begomovirus infecting honeysuckle in Kobe, Japan. *J. Fac. Agr., Kyushu Univ.*, **50**: 61–71

Wolf, F. A., W. H. Whitcomb and W. C. Mooney 1949 Leaf curl of tobacco in Venezuela. *J. Mitchell. Sci. Soc.*, **65**: 38–47

Zhou, X., Y. Liu, L. Calvert, D. Munoz, G. W. Otim–Nape, D. J. Robinson and B. D. Harrison 1997 Evidence that DNA–A of a geminivirus associated with severe cassava mosaic disease in Uganda has arisen by interspecific recombination. *J. Gen. Virol.*, **78**: 2101–2111