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Molecular Characterization of a New Begomovirus Infecting Honeysuckle in Sapporo, Japan

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A virus isolated from honeysuckle collected from Sapporo (SP1), Japan, showing symptoms of complete leaf yellowing and stunting was characterized. The complete DNA–A like molecules of SP1 was determined and found to comprise 2771 nucleotides (nts) with a typical begomovirus genome organization. Additionally, SP1 had a DNA β deletion mutant molecule of 426 nts. A comparison of the complete DNA–A nt sequences revealed that SP1 shared 87–88% nt sequence identity with the other three isolates of Japanese *Honeysuckle yellow vein mosaic virus* (HYVMV) but 91% with HYVMV–[UK1], however, the isolate shared 95–98% of CP aa sequence identity with the Japanese isolates of HYVMV and only 86% with HYVMV–[UK1]. Phylogenetic analysis of full length DNA–A component clustered SP1 with Japanese tobacco begomoviruses but analysis of the aligned CP aa sequences clustered the virus with the Japanese honeysuckle begomoviruses. Molecular data show that P1 is an isolate of HYVMV.

INTRODUCTION

A geminivirus isolated from honeysuckle (*Lonicera japonica* Thunb.) was collected in Sapporo (SP1), Japan, showing symptoms of complete leaf yellowing and stunting. Geminiviruses are plant viruses that have globally emerged as important agricultural pathogens (Were *et al.*, 2005). We report here characterization of a new distinct begomovirus infecting honeysuckle in Sapporo, Japan.

MATERIALS AND METHODS

Sample collection and sap inoculation

Honeysuckle samples (stem cuttings) showing severe symptoms of a viral disease (Fig. 1) herein referred to as SP1 were obtained in Sapporo, Japan in 2000. The plants were commercially sold in a garden shop and its origin was unknown. The cuttings were potted and kept in an insect–free greenhouse maintained at 24–27 °C.

PCR–mediated amplification and cloning of SP1 DNA–A and DNA β

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

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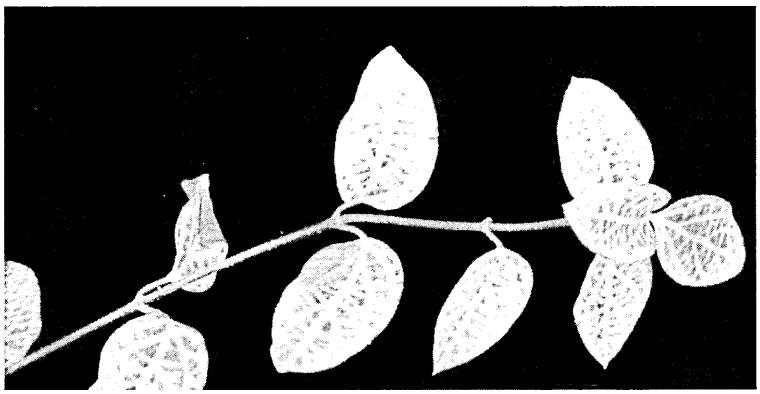

Fig. 1. Honeysuckle plant infected with a begomovirus (SP1) collected in Sapporo, Japan showing

Sequence determination and analysis

SP1 DNA–A and DNA β sequences were phylogenetically compared to those of other honeysuckle, tobacco, *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 sequences used for comparison and their database accession numbers were for DNA–A: *Honeysuckle yellow vein mosaic virus* (HYVMV, AB020781; HYVMV–FK1, AB17845; HYVMV–HY12, AB178946; HYVMV–OT1, AB178947; HYVMV–OT2, AB178948; HYVMV–KG5Tob, AB178949; 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), *Tomato yellow leaf curl Thailand virus* (TYLCTHV, AY514630) and *East African cassava mosaic Zanzibar virus* (EACMZV, AF422174), for DNA β: tobacco leaf curl disease associated DNA (TbLCDA, AJ316033 and AJ316034) tomato begomovirus satellite DNA (Tombegsat, AJ566749), tobacco leaf curl Yunnan disease satellite DNA (TbLCYsat, AJ536628), tomato yellow leaf curl Thailand satellite DNA (TYLCTHsat, AJ566748), tobacco curly shoot satellite DNA (TbCSsat, AJ 457822), Eupatorium yellow vein associated DNA (EpYVass, AJ438939) and honeysuckle yellow vein mosaic disease associated DNA (HYVMDA, AJ316140). Recombination analysis was done using a recombination detection software, RDP version 2.0.

RESULTS

Symptoms

Symptoms exhibited by SP1 in honeysuckle plants collected in Sapporo are shown in (Fig. 1). They consist of complete yellowing of leaves and stunting of the plants. The stems are thinner and some leaves have small green vein enations.

DNA–A Sequence determination and analyses

The SP1 complete DNA–A nucleotide sequence was determined. It comprised 2771 nucleotides (Accesion. No. AB182261) and a genome organization similar to that of other monopartite begomoviruses with two open reading frames ORFs [AV1 (CP) and AV2] in

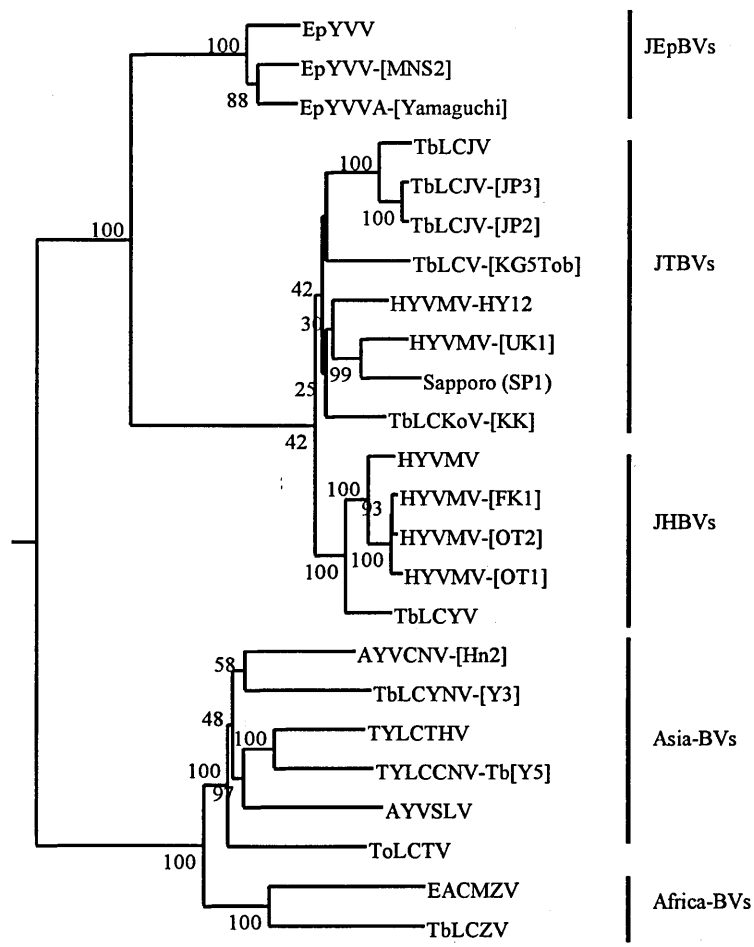

Fig. 2. Phylogenetic tree generated from alignment of full–length DNA–A nucleotide sequences of SP1 and 23 other begomviruses from Asia, Africa and Europe.

Table 1. Percentage nucleotide or amino acid sequence identities between SP1 and 23 other begomoviruses from Asia, Africa and Europe.

Virus	DNA[n]	IR[n]	V1[a]	V2[a]	C1[a]	C2[a]	C3[a]	C4[a]
EpYVV	63	47	81	85	85	66	62	80
EpYVV-[MNS2]	64	53	82	85	85	70	61	81
EpYVV-[Yamaguchi]	64	52	83	81	84	70	61	79
HYVMV-[OT1]	87	67	97	90	88	80	68	79
HYVMV-[FK1]	88	67	95	90	89	82	69	77
HYVMV-[OT2]	88	68	98	90	90	81	67	77
HYVMV	88	75	97	89	89	82	67	74
TbLCYV	88	72	98	91	91	79	66	84
HYVMV-[UK1]	91	98	86	85	96	79	66	-
HYVMV-[KG5Tob]	85	67	92	79	87	84	67	83
HYVMV-[HY12]	86	69	88	91	88	80	66	77
TbLCKoV-[KK]	86	66	87	83	90	81	69	85
TbLCJV-[JP2]	85	66	85	80	89	90	86	75
TbLCJV-[JP3]	85	67	86	79	89	90	87	75
TbLCJV	85	76	85	86	90	90	87	-
AYVCNV-[Hn2]	44	50	43	56	54	62	54	63
ToLCTV	43	49	43	72	54	59	54	66
TLCYNV-[Y3]	43	46	44	58	48	59	52	52
TYLCTHV	43	49	44	60	54	60	52	63
TYLCCNV-Tb[Y5]	43	49	42	57	51	60	55	66
AYVSLV	42	46	42	59	56	58	53	65
EACMZV	39	31	41	49	47	56	52	-
TbLCZV	39	44	42	63	49	50	52	-

[n] Nucleotide sequence identity. - : C4 absent. [a]: Amino acid sequence identity.

the virion sense strand and four in the complementary sense strand (AC1, AC2, AC3 and AC4). The intergenic region (IR) separated the virion and complementary sense genes and contained a putative stem loop structure with the conserved nonanucleotide sequence 5'-TAATATTAC-3' that is characteristic of the *Geminiviridae* in the loop. The TATA Boxes were identified in the IR sequences at nucleotides, 262-266.

Alignment of the retrieved complete DNA-A nt sequences with those from the DNA data bases revealed a high sequence variability ranging from 39% to 91% depending on geographical location and or different hosts/diseases (Table 1). SP1 showed a higher sequence similarity with other begomoviruses found in Japan and rest of Asia than those found in Africa. For instance the isolate shared 88% with each of HYVMV, HYVMV-[OT1] and HYVMV-[FK1], 85% with Tobacco leaf curl begomoviruses from Japan, but only 46% with AYVCNV-[Hn2] and 39% with TbLCZV and EACMZV, respectively. Interestingly, SP1 shared unexpectedly high nt sequence homology (91%) with HYVMV-[UK].

Phylogenetic analysis of the complete DNA-A components divided the Japanese begomoviruses into two major groups, the Japanese honeysuckle, tomato and tobacco infecting begomovirses (JTBVs) group and the Japanese eupatorium infecting begomoviruses JEpBVs group. The former was further subdivided into two subgroups, the first comprising tomato infecting tobacco begomoviruses (JTBVs) while the 2[nd], honeysuckle infecting begomoviruses (JHBVs). Honeysuckle group comprised HYVMV,

HYVMV–[OT1], HYVMV–[FK1], HYVMV–[OT2] and TbLCYV while the later comprised SP1, HYVMV–[HY12], HYVMV–[UK], TbLCKoV–[KK] and other Japanese geminiviruses (Fig. 2). The begomoviruses from China, Taiwan, Thailand and the rest of Asia were clustered together and so were those from Africa, indicating that begomoviruses in a given geographical location tend to evolve independently. Hosts also played a significant role in the separation of the viruses, *Eupatorium* geminiviruses were clearly separated from those of honeysuckle and tobacco (Fig. 2).

When the CP nt as well as the deduced amino acid (aa) sequences were aligned, there was a high nt sequence identity (92–98%) between SP1 and JHBVs but less than

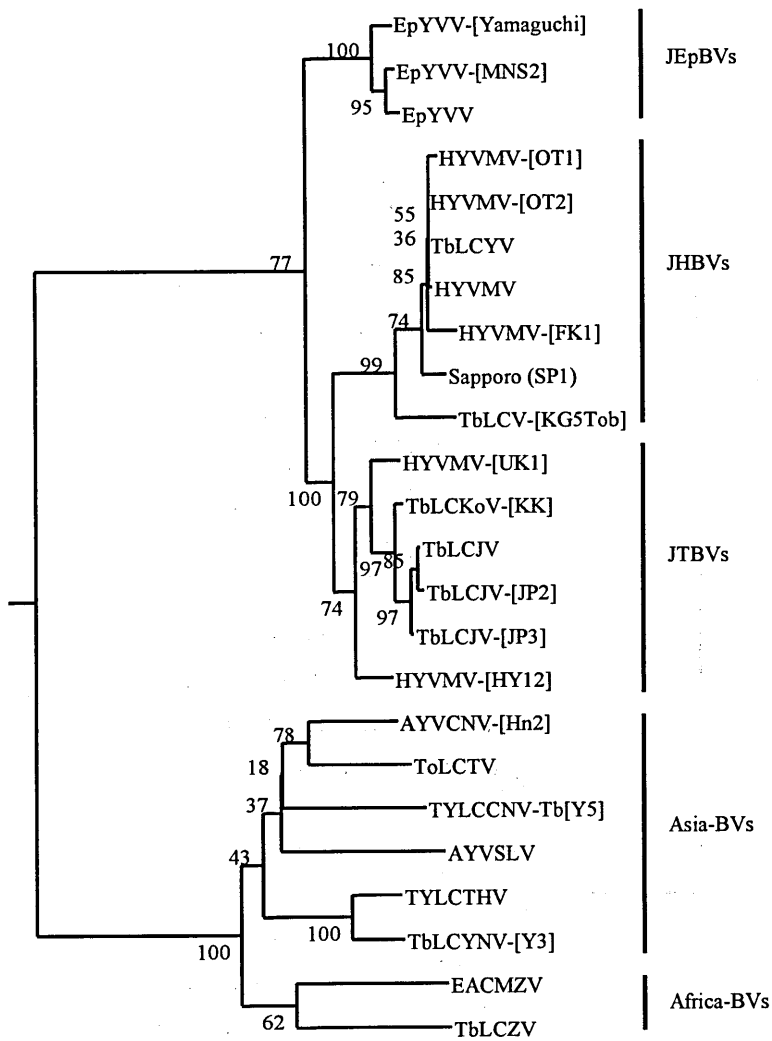

Fig. 3. Phylogenetic tree generated from CP amino acid sequence alignments of SP1 and 23 other begomoviruses found in Asia, Africa and Europe.

87% nt sequence identity with the tobacco viruses and HYVMV–[UK1] (Table 1). Generally there was a higher percent aa sequence identity between SP1 and Japanese begomoviruses than between the isolate and those found in the rest of Asia and Africa. Phylogenetic analysis of the CP revealed a tree structure in which SP1 was clustered with JHBVs (Fig. 3).

When the C1 was aligned, SP1 was more closely related to HYVMV–[UK1] than to other honeysuckle begomoviruses (Fig. 4). A comparison of the IR nt sequences revealed that SP1 shared surprisingly a high percent nt sequence homology (98%) with

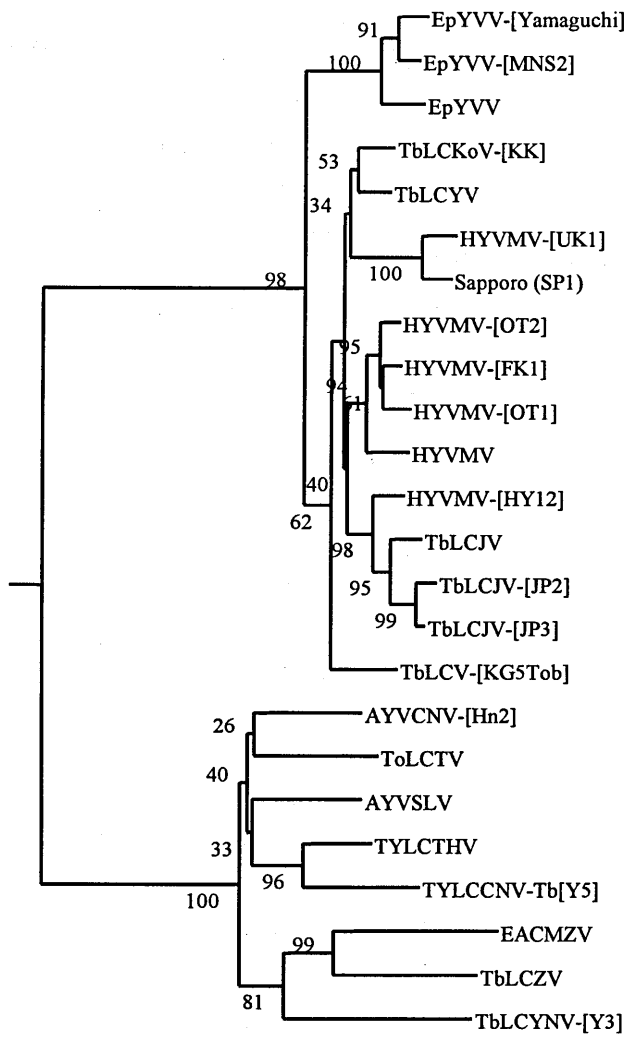

Fig. 4. Phylogenetic tree generated from C1 amino acid sequence alignments of SP1 and 23 other begomoviruses found in Asia, Africa and Europe.

HYVMV–[UK1], but 66–75% with JHBVs as well as JTBVs (Table 1). Other begomovirsues from the rest of Asia and Africa compared poorly with SP1. When individual encoded proteins were compared, the highest amino acid sequence similarity was between SP1 and HYVMV–[UK1] with 86%CP, 85% V2, 96% C1, 79% C2 and 66% C3. Generally, among the JHTBVs, the CP was the most conserved region followed by C1 and V2 in that order (Table 1) while the IR was found to be most diverse.

DNA β sequence determination and analysis

The complete satellite DNA of SP1 was determined and found to be a deletion mutant with 426 nucleotides (AB182262). The molecule had a satellite–conserved region (SCR), typical of the geminiviridae family containing a TAATATTAC motif in the stem loop. Except for the deletion of 918 nts (364–1282) including the C1 gene and adenine– (A) rich region, the molecule was closely related (97%) to that of honeysuckle yellow vein mosaic disease (HYVMDA, AJ316140). Phylogenetic analysis of the aligned molecules from Japan and rest of Asia generated a tree with two clusters. The one, in which Sapporo–Ass (SP1 DNA β) fell, contained Japanese molecules while the other those from the rest of Asia (Fig. 5).

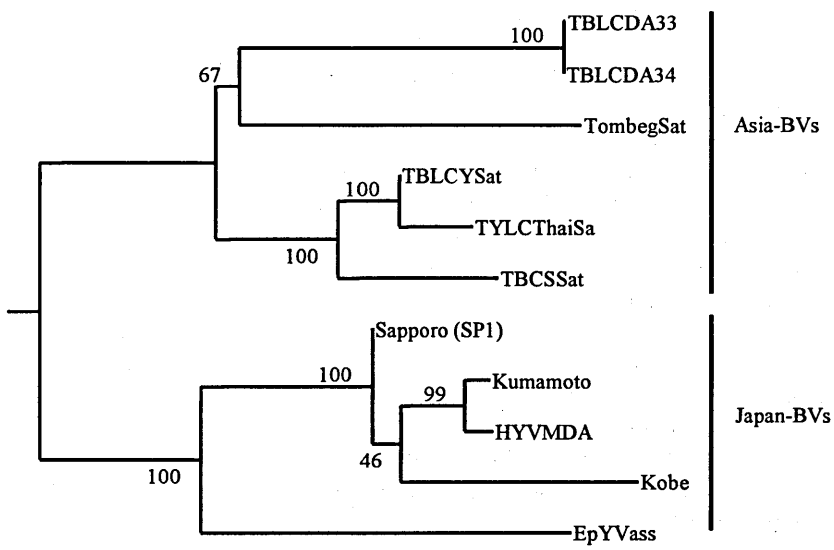

Fig. 5. Phylogenetic tree generated from alignment of Sapporo–Ass and 10 other begomoviruses found in Japan and the rest of Asia.

Recombination in SP1 DNA–A sequence

SP1 had several significant as well as insignificant recombination events with a major one (287–1081 nt) starting from the core region of V2 spanning the entire CP to the C–terminal of C3. In this event SP1 sequence formed part of two sequences with TbLCYV appearing as major parent while the minor parent was unknown. Two other events, one (1146–1828 nt) within the C1 while the other, (1698–2311) starting at core of C1 and cov-

ering almost the entire C4 were also identified. Both events involved a group of viruses HYVMV–[KG5Tob], HYVMV–[OT1], TbLCKoV–[KK] and HYVMV–[HY12]. The last event, (1847–225) covering part of C1, entire C4 and the IR region involved HYVMV–[FK1], EpYVV, TbLCJV with HYVMV–[UK1] as a major parent.

DISCUSSION

In this study, we have described the characterization of a novel distinct begomovirus variant isolated from honeysuckle in Sapporo, Japan. Sequence analysis has clearly demonstrated that SP1 is included in Japanese geminiviruses obtained from tobacco, tomato and honeysuckles. Phylogenetic analysis of SP1 sequences indicated that Japanese begomoviruses have evolved on their own and are distantly related even to those found in next–door China even though they may be found infecting the same host. It is surprising therefore to find HYVMV–[UK1] from the UK to be closely related to SP1 and other Japanese honeysuckle and tobacco, tomato begomoviruses. This can be explained by the fact that ornamental honeysuckle (*Lonicera japonica* var. *aureo-reticulata*) now naturalized in the UK (Mansoor *et al.*, 2003) originated from Japan. That the ornamental plant infected with the virus is more beautiful than the healthy one may have significantly contributed to the importation and subsequent adoption of the plant by the UK.

The DNA β deletion mutant constantly found in association with the DNA–A of SP1 was 97% identical to HYVMDA only that a big chunk (918 nts) had been ripped off the middle (from position 364 to position 1283) of the HYVMDA sequence leaving the deletion mutant with only 426 nts. The part deleted comprised βC1 and the A–rich region. This is the first report of a deletion mutant whose A–rich region has been deleted. Briddon *et al.* (2003) after analyzing a big number of DNA β as well as deletion mutants found that the SCR and A–rich region were maintained in all DNA β deletion mutants analyzed. DNA β is dependant on the helper begomovirus for its replication and is responsible for the accumulation of the helper virus to levels normally found in symptomatic plants, implying that it is involved either in replication, systemic movement or the suppression of a host defense mechanism (Saunders *et al.*, 2000).

In conclusion, sequence analyses have clearly demonstrated that SP1 is a new previously unrecognized begomovirus. Following guidelines on geminivirus classification and nomenclature (Fauquet *et al.*, 2000; Fauquet and Stanley, 2003), we propose the name, HYVMV–[SP1] for SP1.

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