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Short communication

Detection of cardamom mosaic virus-related sequences in plant genomes

T Jebasingh[1]*, S Backiyarani[2], C Manohari[1] and R Usha[1,]

[1]Department of Plant Biotechnology, School of Biotechnology, Madurai Kamaraj University, Madurai 625 021, India

[2]National Research Center for Banana, Thogamali Road, Thayanur Post, Trichy 620 102, India

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Cardamom mosaic virus (CdMV)-related sequences have been found in cardamom plant genome. Integrated DNA was sequenced and was found identical to the 3′ end of NIb, the RNA dependent RNA polymerase gene of CdMV. This is the first report on the integration of potyviral partial NIb sequence into a plant genome. These CdMV-related integrated DNA sequences were also detected in a few other plant genomes.

Keywords: Cardamom mosaic virus, genome integration, RNA dependent RNA polymerase, Potyvirus

Until recently, one of the basic tenets of virology was that plant viruses, unlike animal and bacterial viruses, did not integrate into the host genome. Observations over the past few years have broken this tenet and now an increasing number of integrated plant viral sequences in plant genomes are being reported. Generally, the sequences from various DNA viruses were found to be integrated into the host genome. The sequences of single-stranded DNA of geminiviruses were also reported to be the part of their host plant genome[1-3]. However, the viruses with RNA genome known to integrate a DNA version of their genome into the host chromosomal DNA were the only retroviruses. In this case, the RNA genome was reverse-transcribed and the resultant DNA was inserted into the host DNA by a virus-encoded integrase. DNAs of pararetroviruses have also been found in the host nucleus as either being integrated into the host DNA or remain there in the form of episomes[4-9]. Integrants of three pararetroviruses, banana streak virus (BSV), tobacco vein clearing virus (TVCV) and petunia vein clearing virus (PVCV), were reported to generate episomal infections in certain hybrid plant hosts in response to stress. In 1989, Tanne *et al*[10] reported the integration of the potyvirus, potato virus Y (PVY), sequences in several grapevine varieties. Dot blot analysis of nucleic acid extracts also reacted with a potyviral probe[10]. PVY-homologous sequences were also found in tobacco DNA, albeit in a rearranged form[11].

*Author for correspondence:
Tel: +972-54-2523483; Fax: +972-8-934-9181.
E-mail: jebasinghs@gmail.com

Cardamom mosaic virus (CdMV) sequences in cardamom were reported while screening transgenic cardamom plants for NIb gene of CdMV by dot blot analysis. The wild type cardamom DNA also showed positive signal with the full-length NIb probe. To investigate the origin of the positive signal, a number of probes made from various portions of NIb and dot blot analyses revealed that wild type cardamom DNA hybridized with the 3′ end of the NIb probe. In the present work, the genomic DNA from '*Greengold*' variety of cardamom, maintained in tissue culture, was extracted by a modified Dellaporta method[12]. A 0.2 kb fragment corresponding to the 3′ end of NIb was amplified from cardamom genomic DNA with NIbFL.for (5′-GTTTACGGGGATCCA TGTCAA-3′) and NIbERI (5′-GGATCCTATTGTC TTGGTGCTGTTGGC-3′) primers at an annealing temperature of 50°C. The amplification was reproducible with a number of cardamom plants using a fresh set of primers and PCR reagents, thus ruling out any PCR contamination. The amplicon also showed a positive signal in a Southern blot probed with the 0.182 kb corresponding to the 3′ end of NIb (Fig. 1A). The amplicon was cloned in pXcmKn12 vector[13] and was sequenced using M13 forward primer. The 182 bp sequence was found to be100% identical to the 3′ end of the NIb gene of CdMV. The nucleotide sequence was translated to amino acid sequence and was subjected to a motif search using http://www.expasy.com. However, no functional motifs were found. In a BLAST search using www.ebi.ac.uk, the amino acid sequence showed

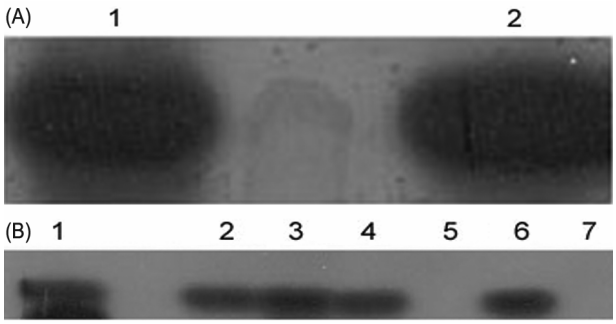

Fig. 1 (A & B)—A. Southern analysis of PCR product from cardamom with 0.182 kb NIb probe (lane 1, amplification from cardamom; lane 2, probe); B. Southern analysis of PCR products from various plants with 0.182 kb NIb probe (lane 1, probe; lane 2, cardamom; lane 3, soybean; lane 4, rice; lane 5, tomato; lane 6, *N. tabacum*; lane 7, banana).

100% identity to the C-terminal end of the NIb protein of CdMV, 46.9% (in 49 amino acids overlap) with maclura mosaic virus, 38.7% (in 49 amino acids overlap) with narcissus latent virus and 49.2% (in 59 amino acids overlap) with alphinia mosaic virus. Apart from these macluraviruses, a plant protein namely OSJNBb0048E02.11 and an N-acetyl transferase present in the *Japonica* variety of *Oryza sativa* showed 33.4% identity in 45 amino acids overlap.

In order to screen other plants for the presence of the NIb fragment, DNA extracted from soybean, rice, tomato, banana and *Nicotiana tabacum* were subjected to PCR as described earlier for cardamom DNA[14]. Except tomato and banana, all the other plants showed nearly 0.2 kb amplicon that hybridized with the NIb-specific 0.182 kb probe in a Southern blot (Fig. 1B). The region upstream to this 3′ end fragment was absent in all the above plants as revealed through PCR with NIbEF1 and NIbFG.rev primers. The dot blot analysis also did not show any signal in an autoradiogram with a probe synthesized using NIbEF1 and NIbFG.rev.

For the presence of an RNA viral sequence in a host genome, there could be two possibilities. In the first possibility, the viral sequence could have recombined with the RNA of a retrotransposable element, perhaps *via* template switching by the reverse transcriptase or the viral replicase, leading to the integration of the chimeric DNA into the host genome. The second possibility could be that the viral sequences were originated from a plant genome. In the present case, the second one could be the possible hypothesis since the partial NIb sequence was also found in soybean, rice and tobacco (Fig. 1B). In the case of flaviviruses, about two thirds of the viral genome was found integrated into the mosquito genome[15]. But most of the flavi-like sequences were small and dispersed, indicating a series of recombination events. No indication of transposition was reported for the integration of flaviviruses. Potyviruses and flaviviruses share a common replication pathway. A positive strand of a viral RNA is translated into a polyprotein and then processed to produce mature viral proteins. Integration of PVY genome was due to recombination between a viral RNA and a retrotransposable element with the product subsequently inserted into the host genome[10]. RNA recombination was reported to occur with viral RNAs of the same or related viruses likely *via* involvement of the viral replicase[16]. However, non-replicative viral RNA recombination was demonstrated[17], indicating that joining of RNA pieces may occur in the absence of the viral replicase. Processing of ribosomal and transfer RNAs as well as splicing of primary transcripts are, in fact, a form of RNA recombination. Indeed, cases of recombination between viral and host RNAs have been reported[18-20].

The potyviral insertion may be regarded as a 'native transgene' and may play a role in the development of diversity among the widely spread potyviruses. If the phenomenon is of a general nature, then horizontal carryover of a sequence from one plant species to another could account partially for this property. In summary, we have reported for the first time the presence of CdMV-related sequences in cardamom and other plant genomes.

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References

1 Ashby M K, Warry A, Berjarano E R, Khashoggi A, Burrell M *et al*, Analysis of multiple copies of geminiviral DNA in the genome of four closely related *Nicotiana* species suggest a unique integration event, *Plant Mol Biol*, 35 (1997) 313-321.

2 Bejarano E R, Khashoggi A, Witty M & Lichtenstein C, Integration of multiple repeats of geminiviral DNA into the nuclear genome of tobacco during evolution, *Proc Natl Acad Sci, USA*, 93 (1996) 759-764.

3 Harper G, Osuji J O, Heslop-Harrison J S & Hull R, Integration of banana streak badnavirus into the *Musa* genome: Molecular and cytogenetic evidence, *Virology*, 255 (1999) 207-213.

4 Jakowitsch J, Mette M F, van der Winden J & Matzke A J M, Integrated pararetroviral sequences define a unique class of dispersed repetitive DNA in plants, *Proc Natl Acad Sci, USA*, 96 (1999) 13241-13246.

5 Lockhart B E, Menke J, Dahal G & Olszewski N E, Characterization and genomic analysis of tobacco vein clearing virus, a plant pararetrovirus that is transmitted vertically and related to sequences integrated in the host genome, *J Gen Virol*, 81 (2000) 1579-1585.

6 Mette M F, Kanno T, Aufsatz W, Jakowitsch J, van der Winden J *et al*, Endogenous viral sequences and their potential contribution to heritable virus resistance in plants, *EMBO J*, 21 (2002) 461-469.

7 Ndowora T, Dahal G, LaFleur D, Harper G & Hull R, Evidence that badnavirus infection in *Musa* can originate from integrated pararetroviral sequences, *Virology*, 255 (1999) 214-220.

8 Richert-Poggeler K R, Noreen F, Schwartzacher T, Harper G & Hohn T, Induction of infectious petunia vein clearing (pararetro) virus from endogenous provirus in petunia, *EMBO J*, 22 (20003) 4836-4845.

9 Staginnus C, Gregor W, Mette M F, Teo C H, Borroto-Fernandez E G *et al*, Endogenous pararetroviral sequences in tomato (*Solanum lycopersicum*) and related species, *BMC Plant Biol*, 7 (2007) 24.

10 Tanne E, Naveh L & Sela I, Serological and molecular evidence for the complexity of the leafroll disease of grapevine, *Plant Pathol*, 38 (1989) 183-189.

11 Tanne E & Sela I, Occurrence of a DNA sequence of a non-retro RNA virus in a host plant genome and its expression: Evidence for recombination between viral and host RNAs, *Virology*, 332 (2005) 614-622.

12 Echevarria-Machado I, Sanchez-Cach L A, Hernandez-Zepeda C, Rivera-Madrid R & Moreno-Valenzuela O A, A simple and efficient method for isolation of DNA in high mucilaginous plant tissues, *Mol Biotechnol*, 31 (2005) 129-135.

13 Cha J, Bishai W & Chandrasegaran S, New vectors for direct cloning of PCR products, *Gene*, 136 (1993) 369-370.

14 Porebski S, Beiley L G & Baum B R, Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components, *Plant Mol Biol Rep*, 15 (1997) 8-15.

15 Crochu S, Cook S, Attoui H, Charrel R N, de Chesse R *et al*, Sequences of flavivirus-related RNA viruses persist in DNA form integrated in the genome of *Aedes* spp. mosquitoes, *J Gen Virol*, 85 (2004) 1971-1980.

16 Nagy P D & Simon A E, New insights into the mechanisms of RNA recombination, *Virology*, 235 (1997) 1-9.

17 Gallei A, Pankraz A, Thiel, H J & Becher P, RNA recombination *in vivo* in the absence of viral replication, *J Virol*, 78 (2004) 6271-6281.

18 Charini W A, Todd S, Gutman G A & Semler B L, Transduction of a human RNA sequence by poliovirus, *J Virol*, 68 (1994) 6547-6552.

19 Meyers G, Rumenapf T & Thiel H-J, Ubiquitin in a togavirus, *Nature (Lond)*, 341 (1989) 491.

20 Monroe S S & Schlesinger S, RNAs from two independently isolated defective interfering particles of Sindbis virus contain a cellular tRNA sequence at their 5′ end, *Proc Natl Acad Sci, USA*, 80 (1983) 3279-3283.