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Rapid generation of probe for a ss DNA plant virus directly from total DNA of infected tissue

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A quick procedure for generating radiolabelled probe for ss DNA viruses using total DNA from infected leaves has been described. The method works well for detection of individual geminiviruses and can be used for establishing relationships amongst different geminiviruses affecting plants.

SINGLE-stranded DNA (ss DNA) containing plant viruses which belong to geminivirus group are often difficult to isolate due to their fragile nature and low concentration in plant tissue[1]. In such cases, generation of immunoprobes or nucleic acid probes poses a problem. We have prepared a viral genome-specific radiolabelled probe directly from the total DNA obtained from leaves of *Acalypha indica* affected by a putative geminivirus[2].

The genome of known geminiviruses is an ss DNA. The infected tissue also contains several replicative double-stranded forms (ds DNA), one of which is covalently closed in only one strand[3–6]. The basis of our approach was that ss DNA present in the infected tissue will be specifically labelled by primer extension if the total DNA from infected leaves is not melted for strand separation prior to labelling. Moreover, one of the replicative forms which has discontinuity in one strand will also be labelled simultaneously while most of the ds DNA of the host would not.

Total DNA (nucleic acids were earlier treated with excess RNAse) from plants was prepared by a method described earlier, with minor modifications[6]. Labelling by primer extension was done using random primers (Pharmacia, Sweden), AMV reverse transcriptase (Gibco BRL, USA) and total DNA isolated from leaves. Briefly the 50 μl reaction mixture contained 5 μg of unheated

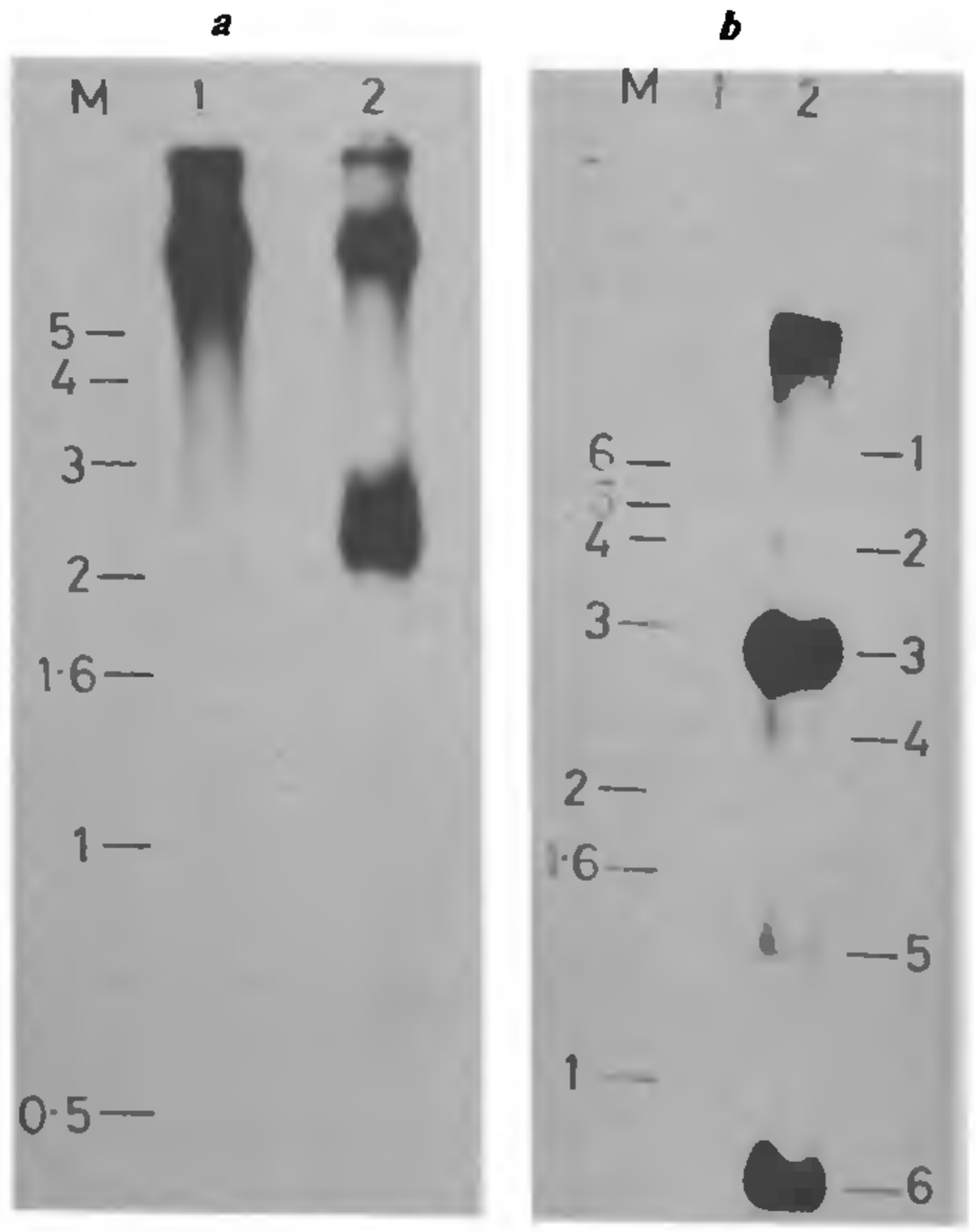

Figure 1. *a*, Autoradiograph showing separation of labelled DNA molecules generated from total healthy leaf DNA (HL-DNA) and total diseased leaf DNA (DL-DNA) on 1.2% agarose gel. *b*, HL-DNA and DL-DNA were electrophoresed on 1.2% agarose gel, transferred on NC membrane and probed with the product of DL-DNA obtained by primer extension using random primers, reverse transcriptase, α(-^{32}P) dGTP.
M = Position of BRL 1kb ladder, 1 = HL-DNA; 2 = DL-DNA.

Figure 2. Scanning electron micrographs of planktonic foraminifera. *a* to *f* from Hundung North and *g* to *l* from Mova. *a*, *Globorutaloida calcarata* (spiral view, × 170, P/VPL-S3-1), *b*, *G. calcarata* (umbilical view, × 148, P/VPL-S3-2), *c*, *G. calcarata* (keel view, × 97, P/VPL-S3-3); *d*, *Globorutaloida limeiana* (spiral view, × 115, P/VPL-S3-5), *e*, *G. limeiana* (umbilical view, × 101, P/VPL-S3-6); *f*, *G. limeiana* (keel view, × 129, P/VPL-S3-7), *g*, *Abathomphalus mayaroensis* (spiral view, × 114, P/VPL-S12-1), *h*, *A. mayaroensis* (umbilical view, × 117, P/VPL-S12-1), *i*, *A. mayaroensis* (keel view, × 119, P/VPL-S12-1), *j*, *Rosita contusa* (spiral view, × 121, P/VPL-S4-1), *k*, *R. contusa* (umbilical view, × 139, P/VPL-S4-2), *l*, *R. contusa* (keel view, × 122, P/VPL-S4-3)

DNA, 25 μg of random primers, 100 mM Tris-Cl (pH 8.0), 8 mM $MgCl_2$, 1% 2-mercaptoethanol, 1 mM each of dATP, dCTP, dTTP, 20μCi of 5'-(α-^{32}P)dGTP and 5 units of AMV reverse transcriptase. The reaction mixture was incubated at 42 C for 1.5 h. The incorporation of label was checked by electrophoresis of the labelled DNA in 1.2% agarose gel followed by autoradiography. At least three additional forms of DNA were efficiently labelled in the diseased sample (DL-DNA) as compared to its healthy counterpart (HL- DNA) which only showed the labelling of a high molecular weight DNA (Figure 1,*a*, lanes 1 and 2).

Unlabelled HL-DNA and DL-DNA electrophoresed in 1.2% agarose gel and transferred on nitrocellulose membrane were probed with the labelled mixture prepared from total DL-DNA. No band was observed in HL-DNA lane (Figure 1,*b*, lane 1) while in the DL-DNA lane six different bands marked 1–6 on right side (Figure 1, *b*, lane 2) were apparent. These bands, by analogy to DNA forms present in tomato golden mosaic virus-infected tissue are the various forms of ds- and ss-DNA present in *Acalypha* leaves affected by the virus[7]. The absence of high molecular weight band in healthy tissue may be due to its inefficient transfer to the nitrocellulose membrane.

The approach has worked well in the case of two other geminiviruses affecting mung bean and tomato during our recent observation. The strategy would help quick identification of different topological forms of viral DNA and their transcripts present in the infected tissue. It will also be useful in differentiation of other geminiviruses occurring in tropical countries without going through the difficult process of virus purification.

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Cloning and use of low copy sequence genomic DNA for RFLP analysis of somaclones in mustard (*Brassica juncea* (L.) Czern and Coss)

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***Brassica juncea* (L.) Czern and Coss genomic DNA fragments of 0.5 to 2.5 kb size, obtained by Pst I digestion were cloned into the pUC18 plasmid vector. Screening of the partial library by dot blot hybridization using labelled total genomic DNA as probe revealed that about 45% of the clones carry low copy number DNA sequences. Using some of these clones as probes, DNA polymorphism could be detected among somaclones of *B. juncea* cv. Varuna.**

RESTRICTION fragment length polymorphism (RFLP) analysis is used for tagging genes controlling complex quantitative traits, varietal identification and establishing phylogenetic relationship. Successful application of the technique, however, requires a large number of low copy DNA sequences, used as probes in Southern hybridization. To meet this need, partial libraries of random low copy genomic DNA sequences have been constructed in several plant species[1–4]. The methylation-sensitive Pst I restriction enzyme has played a key role in this context. Pst I can specifically access the low copy sequences which are generally un- or undermethylated. Figdore *et al.*[5] reported that more than 75% of Pst I-generated clones carry low copy sequences in the diploid *Brassica species — B. oleracea* and *B. campestris*. In this communication, we report construction of a partial Pst I genomic library of low copy sequence genomic DNA and its application in RFLP analysis of somaclones in the alloploid *Brassica juncea* (L.) Czern and Coss.

Genomic DNA isolated from 4-day-old etiolated seedlings of *B. juncea* cv. Varuna, following Dellaporta *et al.*[6], was purified by CsCl density gradient centrifugation, digested with Pst I and electrophoresed on a 1% agarose gel. DNA fragments, 0.5 to 2.5 kb in size, were electroeluted from the gel and purified by phenol–chloroform extraction[7]. Though the DNA was completely digestable with methylation-insensitive enzymes such as Hind III and Msp I, it was observed that even after overnight digestion with Pst I, a major portion of genomic DNA remained uncut, suggesting that *B. juncea* genome is highly C-methylated. This resulted in very low yield (3.6 μg out of 40 μg of DNA digested) of desired fragments. Besides, five fluorescing bands seen (Figure 1) within the desired size range indicated that some repetitive DNA sequences are still present in the eluted fraction.