Genome homologies among geminiviruses infecting *Vigna*, cassava, *Acalypha*, *Croton* and *Vernonia*

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***Acalypha indica*, *Croton sparsiflorus* and *Vernonia cinerea*, that grow around *Vigna radiata* and *Vigna mungo* fields and exhibit viral symptoms, were tested whether they serve as the reservoirs of *Vigna* yellow mosaic virus (YMV), a geminivirus. DNA A of Indian cassava mosaic virus (ICMV) was used as a general probe and DNA B of ICMV and YMV were used as specific probe. ICMV DNA A hybridized intensely to DNA from infected *V. radiata*, *V. mungo*, *A. indica*, *C. sparsiflorus* and *V. cinerea*, indicating that all these plants harboured whitefly-transmitted geminiviruses. The presence of single-stranded virion DNA and double-stranded replicative form in these plants was characterized by non-denaturing Southern analysis and by nuclease-sensitivity analyses. DNA B of ICMV, a specific probe, hybridized only to DNA from infected cassava and *Acalypha* suggesting that the geminiviruses that infected cassava and *Acalypha* are related. DNA B of YMV hybridized only to the DNA from infected *V. mungo* and *V. radiata* but not to the DNA of any of the weeds. Our results show that geminiviruses that infect the weeds *A. indica*, *C. sparsiflorus* and *V. cinerea* are not related to *Vigna* YMV and do not serve as reservoirs of *V. mungo* YMV.**

THE yellow mosaic disease in *Vigna radiata* (L.) Wilczek (mungbean) and *Vigna mungo* (L.) Hepper (blackgram) is a serious problem since all known varieties of these plants are susceptible to this disease. The causal agent of the yellow mosaic disease was found to be transmitted by the whitefly, *Bemisia tabaci* Genn. and not by mechanical means[1]. Infection at an early stage causes a very severe loss in yield[2]. The virus causing the yellow mosaic disease was first characterized by Honda *et al.*[3] as a geminivirus and was named as mungbean yellow mosaic virus (MYMV). It is a whitefly-transmitted, bipartite geminivirus that infects primarily *V. radiata* and *V. mungo*. A Thailand isolate of MYMV (TMYMV) has been sequenced and its similarity to other whitefly-transmitted geminiviruses has been established[4].

Field epidemiology of geminiviruses is a complex phenomenon. The insect vectors such as whiteflies have a highly evolved relationship with a specific group of geminiviruses and play a key role in the transmission of the viruses between plants[5]. Whiteflies have been shown to carry the virions in a transmittable form for three days in male flies and ten days in female flies[6].

V. radiata and *V. mungo* are cultivated for one season of three months (June to August) in Southern India. Mungbean yellow mosaic virus is not seed-borne[2]. This raises an important question regarding the survival of the viruses during the remaining nine months of non-cropping season and their subsequent transmission to the crops in the next cropping season. Gilbertson *et at.*[7] performed dot-blot hybridization analysis of a number of weeds in Dominican Republic that grew around bean fields and exhibited golden mosaic symptoms. General geminivirus probes and probes specific to the Dominican Republic strain of bean golden mosaic virus (BGMV-DR) were used in the analysis. While a majority of the tested weeds harboured geminiviruses in general, only one weed *Rhynchosia minima* (L.) Dc. was found to harbour the BGMV-DR. Their findings suggested that *R. minima* may serve as a 'weed reservoir' for BGMV-DR. On similar lines, we selected for analysis four weeds, *Acalypha indica* L., *Croton sparsiflorus* Morong, *Sida acuta* Burm., and *Vernonia cinerea* Less. that exhibited yellow mosaic or yellow vein-mosaic symptoms among the weeds that grew around *V. radiata* and *V. mungo* fields. We investigated the nature of the viral genomes in all these weeds to test whether these weeds could possibly serve as reservoirs of the *V. mungo* YMV during the off-season period.

Many methods have been used to evaluate the relatedness of geminiviruses. Serological studies using ELISA[8] revealed that the Indian cassava mosaic virus (ICMV) is related to *Acalypha* yellow mosaic virus (AYMV). Immunosorbent electron microscopic[9] analysis revealed the relationship between African cassava mosaic virus (ACMV) and BGMV. Nucleic acid hybridization studies using general probes and specific probes were employed to evaluate the homologies between three strains of BGMV and bean dwarf mosaic virus (BDMV)[7].

In the present study, we performed nucleic acid hybridization studies to evaluate the genome homologies among geminiviruses that infect *Vigna*, *Cassava*, *Acalypha*, *Croton*, *Sida* and *Vernonia*.

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Materials and methods

Virus-infected plants

Infected leaves of *Acalypha indica* L. and *Sida acuta* Burm. showing yellow mosaic symptoms were collected from Madurai Agricultural College and Research Institute. Infected leaves of *Croton sparsiflorus* Morong and *Vernonia cinerea* Less. with yellow vein mosaic symptoms and *Vigna radiata* (L.) Wilczek cv Co5 and *Vigna mungo* (L.) Hepper cv. Co5 with yellow mosaic symptoms were collected from the National Pulses Research Centre (NPRC), Vamban, Tamil Nadu. Infected leaves of *Manihot utilissima* Pohl (cassava) showing mosaic symptoms were collected from cultivated fields in Salem, Tamil Nadu. The leaves were washed in sterile distilled water, blotted dry and stored at –70°C. *V. radiata* and *V. mungo* plants, germinated and grown under tissue culture conditions, were used as uninfected controls.

DNA extraction

DNA was extracted as described by Covey and Hull[10]. The frozen leaves were ground to fine powder using liquid nitrogen in a prechilled mortar with pestle. The frozen powder was transferred to 30 ml screw capped polypropylene tubes. For each gram of frozen powder, 1 ml of extraction buffer [1% (w/v) of triisopropylnaphthalenesulphonic acid, 6% (w/v) of *p*-aminosalicylic acid, 50 mM Tris-Cl (pH 8.3), 6% (v/v) phenol and 1% (v/v) β-mercaptoethanol] was added and mixed well. Two volumes of neutral phenol/chloroform were added, mixed and centrifuged at 2000 *g*. The aqueous phase was extracted twice with neutral phenol/chloroform. Ethanol precipitation of DNA was performed as described by Sambrook *et al.*[11]. Precipitated DNA was dried in a speedvac system and dissolved in TE buffer (1 mM Tris-Cl and 0.1 mM EDTA, pH 8.0). DNA used for different slot-blot and Southern-blot hybridizations was derived from the same set of infected plant tissues.

Southern hybridization

DNA concentration was determined by using Hoechst dye 33258 in a Hoefer DNA fluorometer (TKO 100) as per the recommendations of the supplier (Hoefer Scientific Instruments, USA). One microgram of total DNA from each of the tissues was run on 1% (w/v) agarose gel in TNE buffer (40 mM Tris, 20 mM sodium acetate and 2 mM EDTA, pH adjusted to 7.5 with acetic acid). After ethidium bromide staining (0.5 μg/ml) the DNA was transferred to zeta-probe nylon membrane (Bio-rad Laboratories, USA) following alkali-denaturation and neutralization[11] and DNA was fixed by UV-crosslinking at 0.12 J/cm^2 in a Hoefer UV-crosslinker.

For performing non-denaturing Southern analysis, the gel was not treated with NaOH prior to transfer to nitrocellulose membrane (Hybond-C, Amersham, UK). DNA was fixed by baking the membrane at 80°C for 2 h under vacuum. Other conditions were the same as used for denatured Southern transfer. Under these conditions, only single-stranded DNA binds to nitrocellulose membrane.

Nucleic acid hybridization analysis was performed by the method of Southern[12]. Hybridization with [α-^{32}P]dCTP-labelled probe was carried out for about 16 h and post hybridization washes were done three times with 2 × SSC and 0.1% (w/v) sodium dodecyl sulphate (SDS) at 65°C (each wash for 30 min). Probe DNA was radiolabelled with [α-^{32}P]dCTP (specific activity, 11.1 × 10^{13} Bq/mmole, BRIT, Bombay) using a random primer labelling kit supplied by Boehringer Mannheim, Germany. Konica X-ray films were used for autoradiography.

Sensitivity to nucleases

The nature of the nucleic acids was determined on the basis of their sensitivity to S1 nuclease, exonuclease III, deoxyribonuclease I (DNase I) or RNase A (Type II-A). S1 nuclease (Pharmacia, Sweden) digestion was performed for 30 min at 37°C with 1.5 units/μg DNA in 50 mM sodium acetate (pH 4.6), 280 mM NaCl and 4.5 mM $ZnSO_4$. Exonuclease III (Pharmacia, Sweden) treatment was done for 30 min at 37°C with 5 units/μg DNA in 66 mM Tris-Cl (pH 6.8) and 0.66 mM $MgCl_2$. DNase I (Promega, USA) digestion was performed for 30 min at 37°C with 1 unit/μg DNA in 40 mM Tris-Cl (pH 7.9), 10 mM NaCl, 6 mM $MgCl_2$ and 10 mM $CaCl_2$. RNase A (Type II-A, Sigma, USA) treatment was done for 10 min at 37°C with heat-treated RNase A (0.25 unit/μg of DNA) in 10 mM Tris-Cl and 1 mM EDTA (pH 8.0). All the reactions were stopped by adding 0.1 volume of 0.1 M EDTA (pH 8.0).

Slot-blot hybridization

Slot-blot analysis was performed using the method of Robinson *et al.*[13]. Total DNA extracted from the tissues (1 μg, 0.1 μg, 0.01 μg) was denatured with NaOH at a final concentration of 0.1 N for 10 min at room temperature and neutralized by adding 0.1 volume of 3 M sodium acetate, pH 5.0. The nitrocellulose membrane (Gibco BRL, USA) was presoaked first in water and then in 20 × SSC and blotted dry. Samples were blotted on the membrane using a BRL manifold. The membrane was baked in a vacuum oven at 80°C for 2 h for fixing the DNA.

Plasmids used for probe preparation

Plasmids with DNA A (pICM18) and DNA B (pICM15) of Indian cassava mosaic virus (J. Stanley and V. G. Malathi, unpublished) were kindly provided by Dr John Stanley, John Innes Institute, UK. Plasmid pICM18 has the full-length DNA A (2700 bp) as a *Hin*dIII fragment in the vector pIC20R and the plasmid pICM15 has the full-length DNA B (2700 bp) as a *Bam*HI fragment in the vector pIC20R.

Since the sizes of ICMV DNA A and ICMV DNA B are of 2.7 kb and the vector DNA size is also 2.7 kb, it was not possible to purify the insert from the vector DNA. The plasmids pICM18 and pICM15 were digested with *Hin*dIII and *Bam*HI respectively, before probe preparation by random primer labelling. Though the probe carried the vector as well, the vector did not interfere with the analysis by cross hybridization with either the plant DNA or the viral DNA.

The plasmid pKA1 containing 1.6 kb fragment and pKA4 containing 1.1 kb fragment, which together constitute the DNA B genome of *Vigna mungo* yellow mosaic virus were cloned as *Xba*I fragments in the vector pOK12 (2.1 kb) in our laboratory (Karthikeyan *et al.*, unpublished). These two clones (pKA1 and pKA4) were digested with *Xba*I and the viral DNA fragments (1.6 kb and 1.1 kb) were gel-purified and used for probe preparation.

Results and discussion

Detection of geminiviral DNA

DNA A of all whitefly-transmitted geminiviruses shares a high degree of sequence homology in the coat protein gene[14]. Therefore, DNA A of ICMV was used as a general probe to detect the presence of whitefly-transmitted geminiviruses in *Vigna*, cassava and four weeds exhibiting symptoms of geminivirus infection. DNA B of ICMV and *V. mungo* YMV were used as specific probes since the geminiviruses exhibit a high degree of sequence divergence in their B DNAs.

DNA was extracted from the tissue culture-grown, uninfected *V. radiata* and *V. mungo* seedlings and field-collected, infected plants of *V. radiata*, *V. mungo*, cassava, *A. indica*, *C. sparsiflorus*, *S. acuta* and *V. cinerea* and subjected to slot-blot hybridization analysis with ICMV DNA A as the probe (Figure 1). Intense hybridization was seen in slots that contained DNA from infected *V. radiata*, *V. mungo*, cassava, *A. indica*, *C. sparsiflorus* and *V. cinerea*. Hybridization was not seen in the slots that contained control DNA from uninfected *V. radiata* and *V. mungo* plants and DNA from *S. acuta* that exhibited yellow mosaic symptoms.

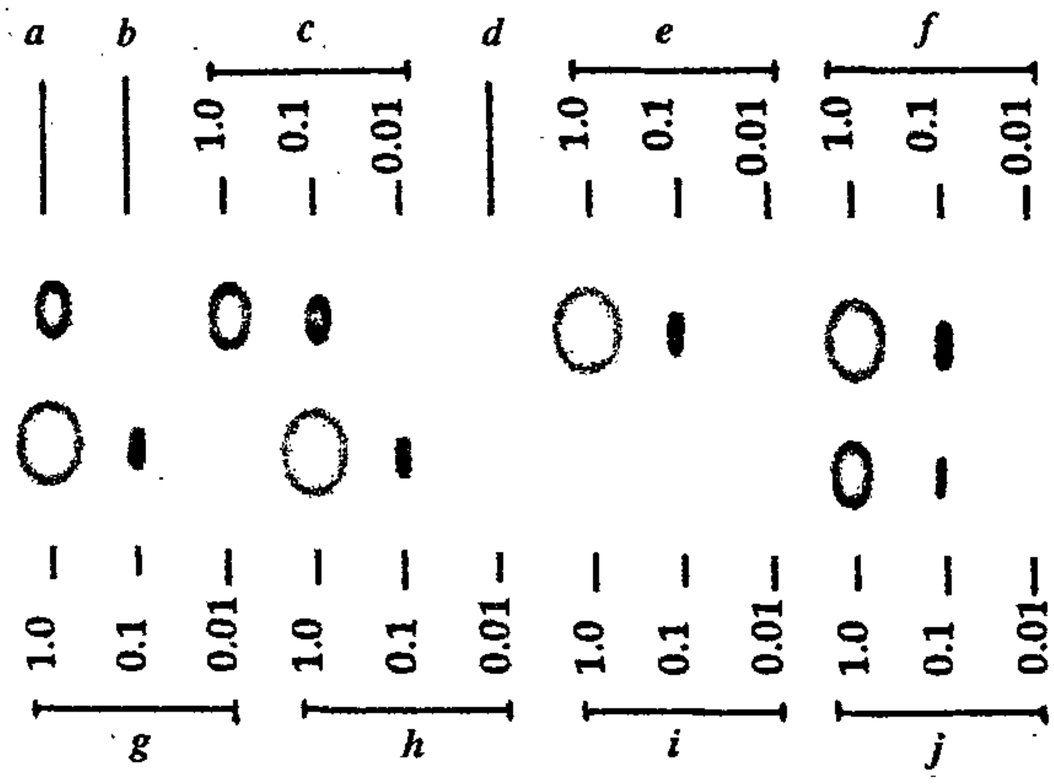

Figure 1. Detection of geminiviral DNA in infected plants. Slot-blot hybridization analysis was done with [α-^{32}P]dCTP-labelled ICMV DNA A as the probe (the plasmid pICM18 with ICMV DNA A as a *Hin*dIII fragment in pIC20R was digested with *Hin*dIII and labelled by random primer labelling). Slots: ***a***, pICM18 digested with *Hin*dIII (300 pg); ***b***, DNA from uninfected *V. radiata* (1 µg); ***c***, DNA from infected *V. radiata*; ***d***, DNA from uninfected *V. mungo* (1 µg); ***e***, DNA from infected *V. mungo*; ***f***, DNA from infected cassava; ***g***, DNA from infected *A. indica*; ***h***, DNA from infected *C. sparsiflorus*; ***i***, DNA from infected *S. acuta* and ***j***, DNA from infected *V. cinerea*. DNA from infected plants was added at three concentrations 1 µg, 0.1 µg and 0.01 µg to three adjacent slots. Signal at slot A is due to hybridization with viral (ICMV DNA A) as well as vector sequences.

These results indicated that infected *V. radiata*, *V. mungo*, cassava, *A. indica*, *C. sparsiflorus* and *V. cinerea* harbour whitefly-transmitted geminiviruses. Geminivirus infection was either low or absent in the *S. acuta* leaves that exhibited yellow mosaic symptoms.

Characterization of nucleic acids

The nucleic acids extracted from infected *Vigna*, cassava and the weeds were further characterized to ascertain whether they share the features of the genomes of the geminiviruses. Nucleic acids extracted from infected leaves of cassava, *A. indica*, *C. sparsiflorus*, *S. acuta* and *V. cinerea* were subjected to Southern hybridization analysis with ICMV DNA A as the probe (Figure 2). Intense hybridization was seen in the 1.8 kb region in the lanes with DNA from infected cassava, *A. indica*, *C. sparsiflorus* and *V. cinerea*. Hybridization was also seen in the 2.7 kb region in the lanes that contained DNA from infected cassava and *A. indica*. The signal in the 2.7 kb region was weak in the lane with the DNA from infected *C. sparsiflorus* and was very weak in the lane with the DNA from infected *V. cinerea*. Both 2.7 kb and 1.8 kb signals were not found in the lane with the DNA from *S. acuta*. The smear below the 1.8 kb band in lanes 2 and 3 in Figure 2 may be due to degradation of nucleic acids.

The nature of the nucleic acids from the infected

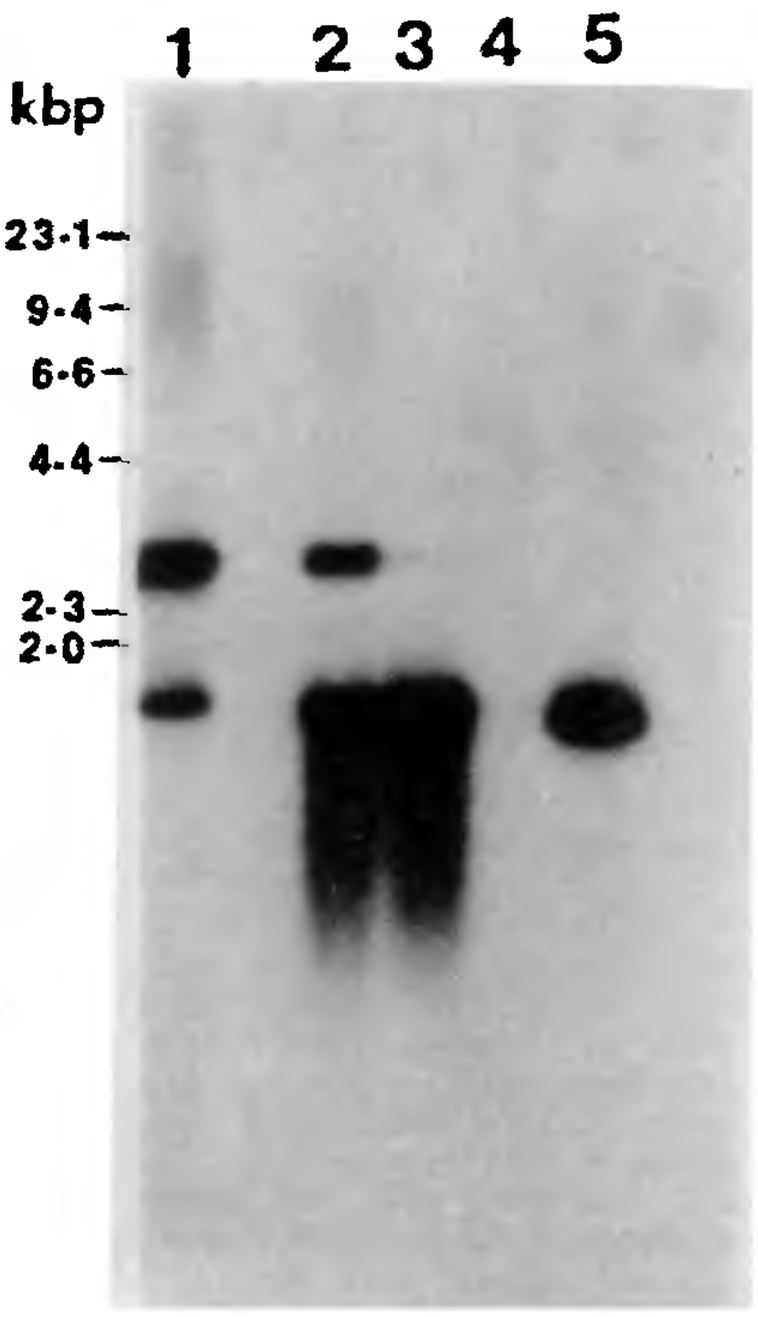

Figure 2. Southern hybridization analysis of DNA extracted from infected weeds. The blot was probed with [α-^{32}P]dCTP-labelled ICMV DNA A in pICM18. Each lane contained 1 µg of total DNA extracted from infected weeds: (1) Cassava; (2), *A. indica*; (3), *C. sparsiflorus*; (4), *S. acuta*; and (5), *V. cinerea*.

weeds showing hybridization in the 2.7 kb and 1.8 kb regions was analysed further by treating with S1 nuclease, exonuclease III, DNase I or RNase A and subjecting to Southern analysis with ICMV DNA A as the probe (Figure 3). The 2.7 kb band was resistant to S1 nuclease, exonuclease III and RNase A, but was digested by DNase I, suggesting that it represents a double-stranded, circular DNA, corresponding to the geminiviral replicative form[15]. The 1.8 kb band was resistant to exonuclease III and RNase A, but was digested by S1 nuclease and DNase I in the lanes that contained DNA of infected *A. indica*, *C. sparsiflorus* and *V. cinerea*, suggesting that it represents the single-stranded, virion DNA. The similar 1.8 kb band in the lane with the DNA from cassava was resistant to exonuclease III, RNase A and S1 nuclease (Figure 3) and it did not bind to nitrocellulose membrane under non-denaturing Southern analysis with ICMV DNA A as the probe (data not shown), indicating that it is not the single-stranded virion DNA, but represents another form of double-stranded DNA. The relative amounts of single-stranded (1.8 kb) and double-stranded (2.7 kb) forms were different in DNA preparations from different infected plants. We generally find higher levels of replicative forms (ds DNA) in young leaves and higher levels of virion DNA (ss DNA) in older leaves (unpublished).

Non-denaturing Southern analysis was performed to confirm whether the 1.8 kb band represents single-stranded virion DNA. Only single-stranded DNA will bind to nitrocellulose membrane if the DNA in the gel is not denatured with NaOH. Intense hybridization was

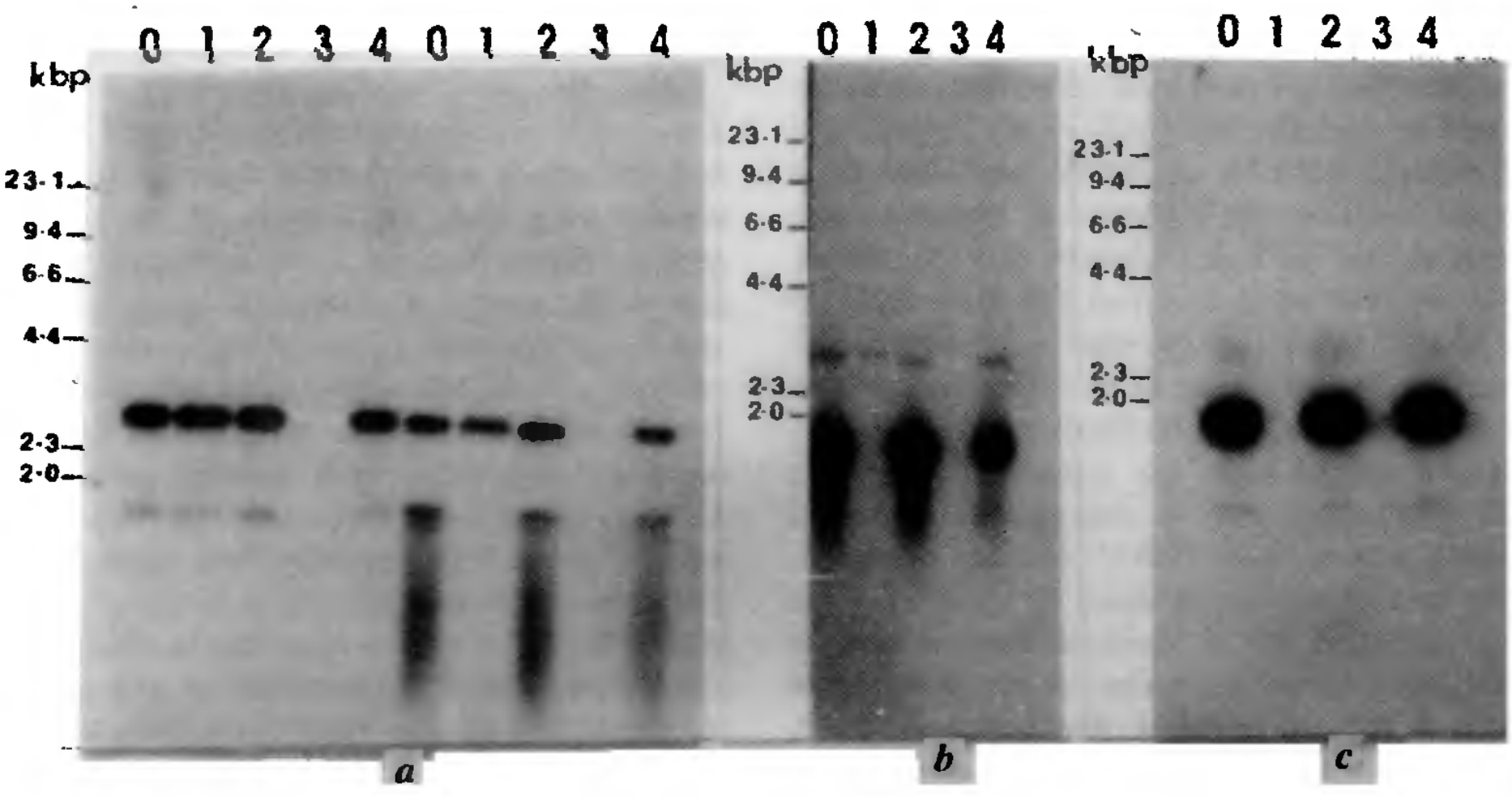

Figure 3. Nuclease-sensitivity analyses of nucleic acids extracted from infected weeds. Southern blots were probed with [α-^{32}P]dCTP-labelled ICMV DNA A in pICM18. One µg of total DNA was loaded in each lane. Lanes: 0, untreated; 1, S1 nuclease-treated; 2, exonuclease III-treated; 3, DNase I-treated; 4, RNase A-treated. *a*, Nuclease sensitivity analysis of samples from infected cassava (left half of the panel *a*) and *A. indica* (right half of the panel *a*). *b*, Analysis of samples from infected *C. sparsiflorus*. *c*, Analysis of samples from infected *V. cinerea*.

seen at the 1.8 kb region in the lanes that contained DNA from infected *A. indica*, *C. sparsiflorus* and *V. cinerea* using ICMV DNA A as the probe (Figure 4). Hybridization was not observed in the 2.7 kb region corresponding to the double-stranded replicative form and in the lane that contained cloned, linearized ICMV DNA A (lane 1). These results prove that the 1.8 kb band in the DNA from the weeds is a single-stranded, virion DNA molecule.

The presence of both single-stranded virion DNA and double-stranded replicative form, and hybridization to ICMV DNA A confirm that infected *A. indica*, *C. sparsiflorus* and *V. cinerea* harbour whitefly-transmitted geminiviruses.

Identification of geminiviral DNA of weeds

Gilbertson *et al.*[7] observed that certain weeds may play an important role in the epidemiology of geminiviruses by acting as reservoirs of the virus during the non-cropping seasons. They found that DNA from many weeds growing around the bean fields and exhibiting golden mosaic symptoms showed positive hybridization to general geminivirus probes (DNA A of BGMV or a mixture of DNA A and B). However, DNA from only one weed, *Rhynchosia minima* was found to hybridize to a specific probe (DNA B of BGMV-DR). Therefore, Gilbertson *et al.*[7] concluded that *R. minima* may act as a reservoir of BGMV-DR.

We investigated whether any of the four weeds that we studied could serve as weed reservoirs of ICMV or *V. mungo* YMV. DNA B of ICMV and *V. mungo* YMV were used as specific probes to identify whether the weeds harbour geminiviruses that are homologous to ICMV or YMV.

DNA was extracted from infected weeds and subjected to slot-blot hybridization analysis, using ICMV DNA B as the probe (Figure 5). DNA B sequences are highly specific and hybridization is expected to occur only when the viruses are same or closely related[14]. Intense hybridization was seen in the slots that contained DNA from infected cassava and *A. indica*. Hybridization was not seen in the slots with DNA from *V. radiata*, *V. mungo*, *C. sparsiflorus*, *S. acuta* and *V. cinerea*.

Southern hybridization analysis of DNA from infected leaves of cassava, *A. indica*, *C. sparsiflorus*, *S. acuta* and *V. cinerea* was performed with ICMV DNA B as the probe to confirm the relationship between the geminiviruses of cassava and *A. indica* (Figure 6). Hybridization was seen in the lanes with DNA from infected cassava and *A. indica* in both 2.7 kb and 1.8 kb regions. These results strongly suggest that the geminivirus that infects *A. indica* is closely related to ICMV. It has earlier been reported[8] that ICMV is serologically related to *Acalypha* yellow mosaic virus

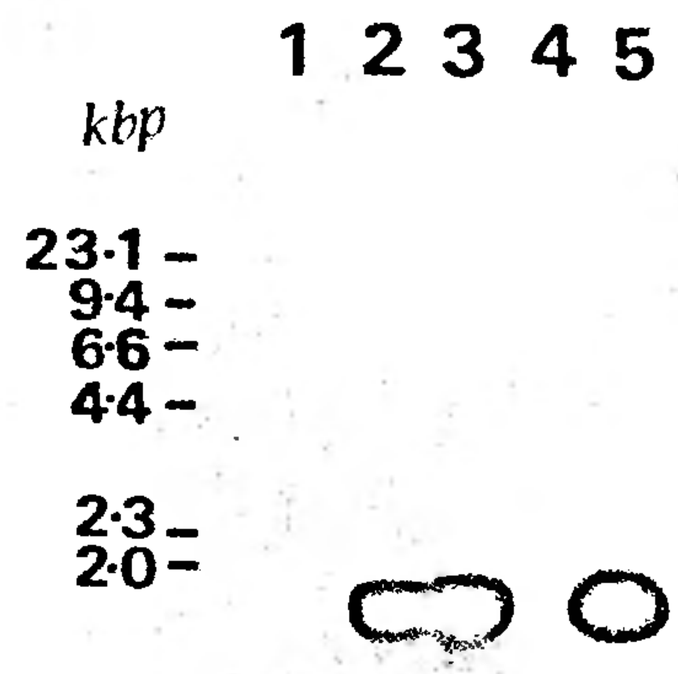

Figure 4. Non-denaturing Southern blot analysis of DNA from infected weeds. The blot was probed with [α-^{32}P]dCTP-labelled ICMV DNA A in pICM18. Lane 1, pICM18 digested with *Hin*dIII (contains 2.7 kb ICMV DNA A in 2.7 kb pIC20R vector, 500 pg). Each other lane contained 1 µg of total DNA from the infected weeds. (2), *A. indica*; (3), *C. sparsiflorus*; (4), *S. acuta*; and (5), *V. cinerea*.

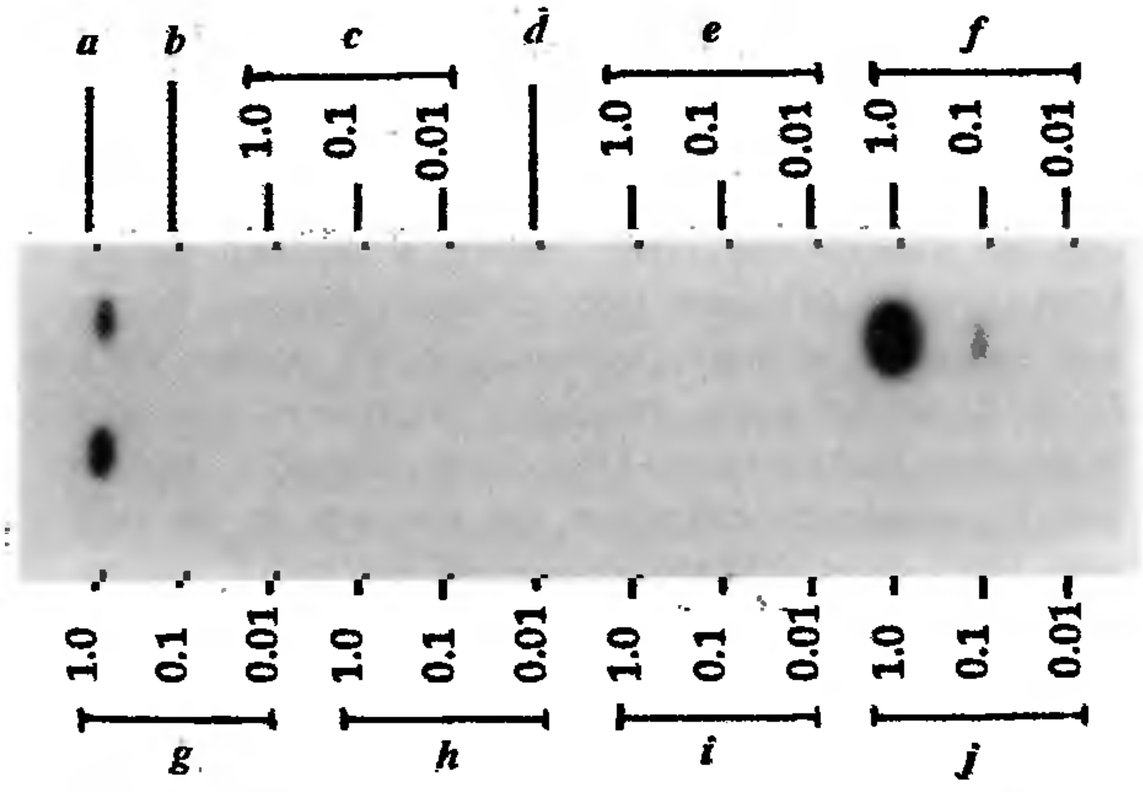

Figure 5. Relatedness of geminiviruses of weeds to ICMV. Slot-blot hybridization analysis with [α-^{32}P]dCTP-labelled ICMV DNA B as the probe (the plasmid pICM15 with ICMV DNA B as a *Bam*HI fragment in pIC20R was digested with *Bam*HI and labelled by random primer labelling). Slots: ***a***, pICM18 (ICMV DNA A) digested with *Hin*dIII (300 pg); ***b***, DNA from uninfected *V. radiata* (1 µg); ***c***, DNA from infected *V. radiata*; ***d***, DNA from uninfected *V. mungo* (1 µg); ***e***, DNA from infected *V. mungo*; ***f***, DNA from infected cassava; ***g***, DNA from infected *A. indica*; ***h***, DNA from infected *C. sparsiflorus*; ***i***, DNA from infected *S. acuta* and ***j***, DNA from infected *V. cinerea*. DNA from infected plants was added at three concentrations 1 µg, 0.1 µg and 0.01 µg to three adjacent slots. Signal at slot A is due to hybridization with vector sequences and common region of ICMV DNA A in pICM18.

(AYMV) and to *Croton* yellow vein mosaic virus (CYVMV). Hybridization analysis with ICMV DNA B reveals that ICMV is related to AYMV but not to CYVMV.

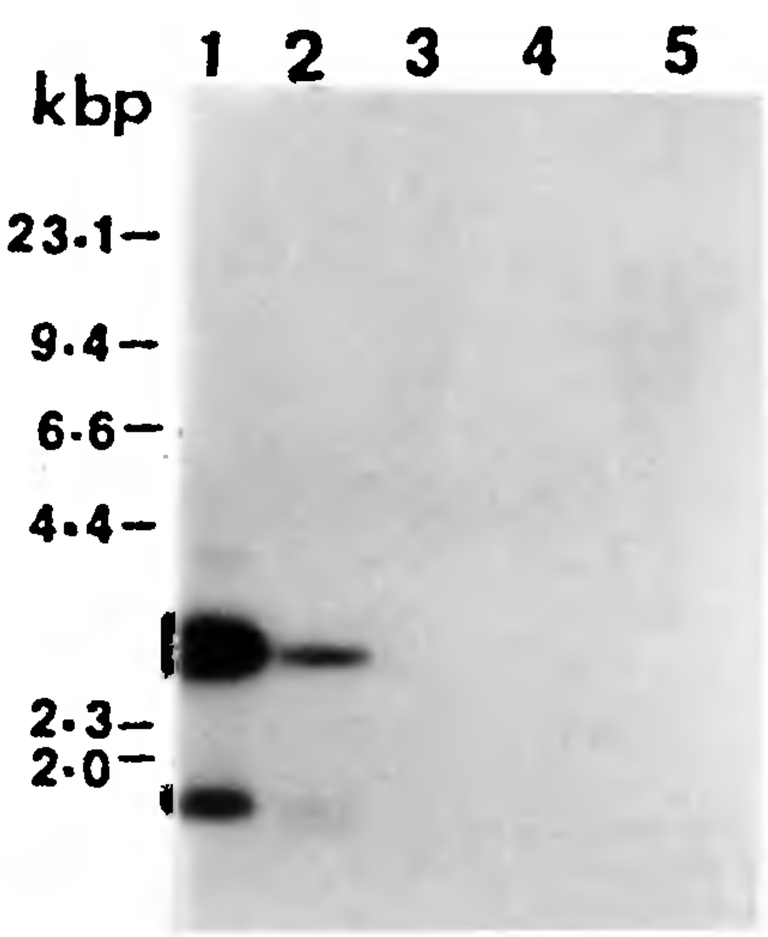

Figure 6. Determination of relatedness of weed geminiviruses to ICMV by Southern hybridization. The blot was probed with [α-^{32}P] dCTP-labelled ICMV DNA B in pICM15. Each lane contained 1 µg of total DNA from infected plants: (1), cassava; (2), *A. indica*; (3), *C. sparsiflorus*; (4), *S. acuta*; and (5) *V. cinerea*.

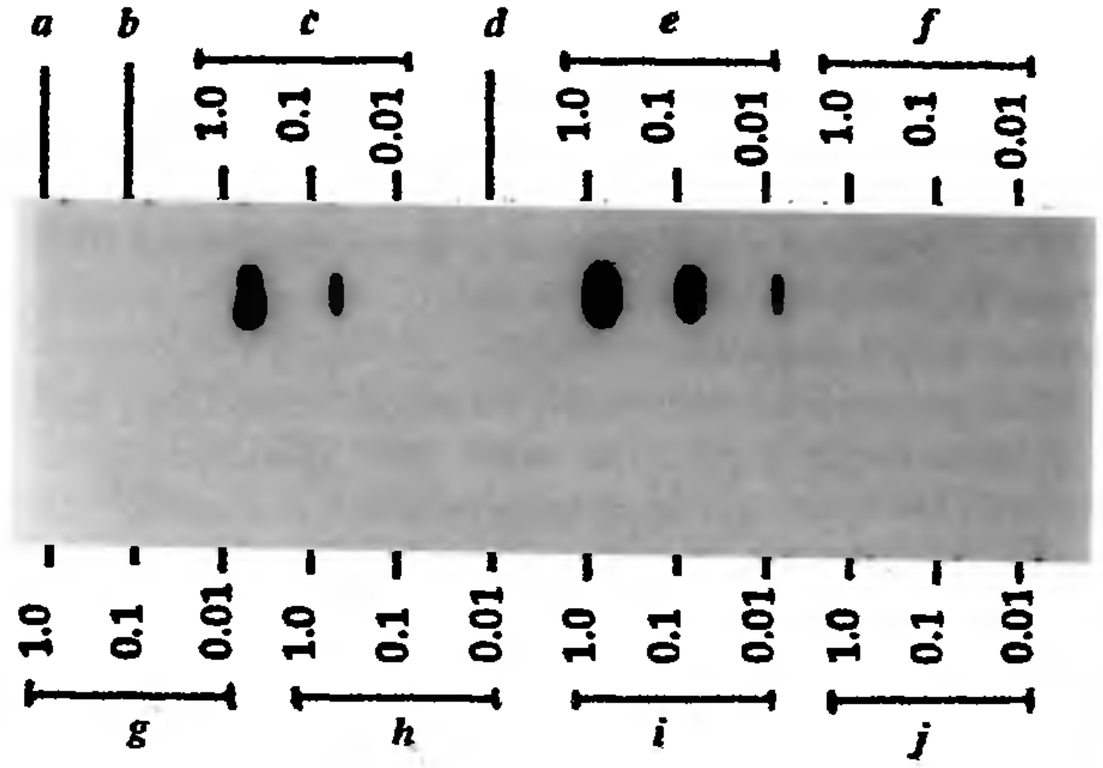

Figure 7. Relationship between YMV of *V. mungo* and geminiviruses of weeds. Slot-blot hybridization analysis with [α-^{32}P]dCTP-labelled DNA B fragments of *V. mungo* YMV as probe (1.6 kb *Xba*I fragment of pKA1 and 1.1 kb *Xba*I fragment of pKA4 which constitute the DNA B genome of YMV were gel-purified and labelled by random primer labelling) Slots: *a*, pICM18 digested with *Hin*dIII (300 pg); *b*, DNA from uninfected *V. radiata* (1 µg); *c*, DNA from infected *V. radiata*; *d*, DNA from uninfected *V. mungo* (1 µg); *e*, DNA from infected *V. mungo*; *f*, DNA from infected cassava; *g*, DNA from infected *A. indica*; *h*, DNA from infected *C. sparsiflorus*; *i*, DNA from infected *S. acuta* and *j*, DNA from infected *V. cinerea*. DNA from infected plants was added at three concentrations 1 µg, 0.1 µg and 0.01 µg to three adjacent slots.

To evaluate the relationship between *V. mungo* YMV and the viruses that infect weeds, a slot-blot having DNA samples extracted from different infected tissues was subjected to hybridization with *V. mungo* YMV DNA B as the probe. Intense hybridization was seen in the slots that contained DNA from infected *V. radiata* and *V. mungo*. Hybridization was not seen in the slots with DNA from infected cassava, *A. indica*, *C. sparsiflorus*, *S. acuta* and *V. cinerea* and in the slots with DNA from uninfected controls of *V. radiata* and *V. mungo*. These results suggest that the geminiviruses infecting cassava, *A. indica*, *C. sparsiflorus* and *V. cinerea* are not related to *V. mungo* YMV. Through serological studies[16] it was suggested earlier that the Thailand mungbean YMV (TMYMV) is related to BGMV, ACMV and tobacco leaf curl virus (TLCV). Our nucleic acid hybridization experiments suggest that *V. mungo* YMV and ICMV are not related to each other.

On the basis of whitefly-transmission studies[17,18] in Uttar Pradesh, India, it was reported that the weeds *Brachiaria ramosa*, *Eclipta alba*, *Xanthium strumarium* and *Cosmos bipinnatus* may serve as weed reservoirs of mungbean YMV. These weeds do not grow around *V. mungo* and *V. radiata* fields in the experimental stations from where we collected the infected plants. Therefore, we could not test by DNA hybridization whether these plants serve as weed reservoirs.

Harrison *et al.*[19] reported seven different whitefly-transmitted geminiviruses from India based on serological relationships. These are bhendi yellow vein mosaic virus (BYVMV), *Croton* yellow vein mosaic virus (CYVMV), *Dolichos* yellow mosaic virus (DYMV), horsegram yellow mosaic virus (HYMV), ICMV, tomato leaf curl virus (TomLCV) and *Malvastrum* yellow vein mosaic virus (MYVMV). Although serological relationship among these geminiviruses has been established, DNA hybridization studies were not performed to evaluate homologies between these geminiviruses. We have confirmed in this paper the single-stranded virion forms and double-stranded replicative forms of geminiviruses in infected *A. indica* and *C. sparsiflorus*. Besides, we are reporting in this paper the characterization of *Vernonia* yellow vein mosaic virus, which is a whitefly-transmitted geminivirus. It is not related to ICMV and *V. mungo* YMV.

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RESEARCH COMMUNICATIONS

Partially oxidized ferrocenyl complexes for nonlinear optics

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Second-order nonlinearity (β) in a series of partially oxidized bisferrocenes linked through conducting –C=N– linkages has been measured and found to vary linearly with the redox potential difference between the ferrocene moiety and the oxidant.

DESIGNING organic molecules having large optical nonlinearities has been the topic of much current research[1,2]. Organometallic complexes prepared along these lines have been found to exhibit large second-order nonlinearities[3–5]. Indeed, they offer a variety of tunable parameters unmatched in the realm of organic chemistry such as low energy electronic transitions, variable oxidation states of the metal atom, and electronic and steric properties of the ligand for optimizing molecular hyperpolarizabilities. The recent discovery of large second-order nonlinearity (β) in certain mixed valence compounds[6] with a tunable intervalence charge transfer transition suggests that there may be considerable potential for the discovery of large optical nonlinearities in mixed valent organometallic molecules. The largest second harmonic generation efficiency ($220 \times U$) to date for an organometallic compound came from a series of ferrocene-based conjugated salts[7]. But their microscopic nonlinearities have not been measured. Here we report first hyperpolarizability (β) in a series of oxidized bisferrocenes linked through various conducting organic spacers, measured by the recently developed hyper-Rayleigh scattering technique (HRS)[8–10].

Ferrocene carboxaldehyde has been linked to aromatic amines and diamines to generate mononuclear functionalized ferrocenes and the corresponding bisferrocenes. These molecules are then oxidized using I_2, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) and 7,7′,8,8′-tetracyanoquinodimethane (TCNQ) to give the products indicated in Figure 1. Compounds 1a–3d have been characterized[11] by various spectroscopic and analytical techniques and their β values measured in acetonitrile with 1064 nm radiation at low solute concentrations (10^{-5}–10^{-6} M) by the HRS technique (Table 1). Since these molecules are ionic in nature, the HRS technique is specially suited for β measurements on them. The error on these hyperpolarizabilities is ±8%. The wavelength of peak absorption in these molecules in the visible region is also shown in Table 1. For compounds absorbing appreciably in the green (~ 532 nm) corrections were made as suggested by Laidlaw *et al.*[9] and corrected values of β are listed.