Coat protein-mediated resistance against an Indian isolate of the *Cucumber mosaic virus* subgroup IB in *Nicotiana benthamiana*

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Coat protein (CP)-mediated resistance against an Indian isolate of the *Cucumber mosaic virus* (CMV) subgroup IB was demonstrated in transgenic lines of *Nicotiana benthamiana* through *Agrobacterium tumefaciens*-mediated transformation. Out of the fourteen independently transformed lines developed, two lines were tested for resistance against CMV by challenge inoculations. The transgenic lines exhibiting complete resistance remained symptomless throughout life and showed reduced or no virus accumulation in their systemic leaves after virus challenge. These lines also showed virus resistance against two closely related strains of CMV. This is the first report of CP-mediated transgenic resistance against a CMV subgroup IB member isolated from India.

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1. Introduction

Cucumber mosaic virus (CMV), the type species of the genus *Cucumovirus* of the family Bromoviridae, is an important plant pathogen worldwide, which infects many crops and causes yield losses. The particles are polyhedral virions which encapsidate three linear, plus sense and single-stranded genomic RNAs. The CMV genome encodes five proteins which are distributed on three genomic RNAs (RNA 1, 2 and 3). The coat protein (CP) of CMV is encoded by RNA 3 but is expressed from the subgenomic RNA 4 (Palukaitis and Garcia-Arenal 2003). The CP is required for host range, encapsidation (Suzuki *et al* 1991), systemic virus movement (Canto *et al* 1997) and aphid transmission (Ng *et al* 2000).

Cuozzo *et al* (1988) first demonstrated engineered resistance against CMV utilizing the CP gene. Since then, many examples of CP-mediated resistance in varying degrees have been described, with different constructs in different hosts (Beachy *et al* 1990; Fitchen and Beachy *et al* 1993; Palukaitis and Garcia-Arenal 2003). Different mechanisms also appear to be responsible for protection, depending on the virus group or the viral transgene studied (Loesh-Fries *et al* 1987; Lomonossoff 1995). Resistance could be mediated either by the protein encoded by the transgene (protein-mediated) or by the transcript produced from the transgene (RNA-mediated), also known as post-transcriptional gene silencing (PTGS), or both (Varma *et al* 2002).

In this report, we demonstrate virus resistance in transgenic *N. benthamiana* model plants expressing the CP gene of CMV isolated from *Amaranthus* (CMV-A) in India and characterized as a member of the subgroup IB (Srivastava *et al* 2004). Though there are several reports from different parts of the world demonstrating transgenic resistance utilizing the CP gene from CMV subgroup IA or II, a report from a subgroup IB strain is still awaited (Palukaitis and Garcia-Arenal 2003). The prevalence of CMV IB infection has been noted in several crop species in India such as banana (Srivastava *et al* 1995), tomato (GenBank Acc. EF710773), black pepper (Bhat *et al* 2004), *Amaranthus*

Keywords. Closely related strains; coat protein; *Cucumber mosaic virus*; transgenic lines; virus resistance

Abbreviations used: CMV, *Cucumber mosaic virus*; CP, coat protein; DAS-ELISA, double antibody sandwich-enzyme linked immunosorbent assay; dpi, days post-inoculation; SDS-PAGE, sodium dodecyl sulphate–polyacrylamide gel electrophoresis

(Srivastava *et al* 2004), hanbane (Samad *et al* 2004) and vanilla (Madhubala *et al* 2005). Therefore, development of transgenic plants resistant to CMV IB seems essential. This is the first report from India which demonstrates transgenic resistance against a CMV subgroup IB member in *N. benthamiana* experimental plants.

2. Materials and methods

2.1 *Construct preparation and transformation of* N. benthamiana

The 657 bp CP gene of CMV-A was introduced in between the CaMV 35S promoter and the NOS terminator of the binary vector (pRoK2), which also has the *NPTII* marker gene for kanamycin resistance. The construct (pACPR2) was mobilized into *Agrobacterium tumefaciens* LBA4404 through triparental mating using a pRK2013 helper plasmid. One positive conjugant (pACPR2.5) was chosen for transformation of *N. benthamiana* leaf discs following a procedure based on Horsch *et al* (1985) and McCormick (1991).

The transformed leaf discs were regenerated on selective Murashige–Skoog (MS) medium containing Gamborg B5 vitamins, antibiotics (500 mg/l cefotaxime and 100 mg/l kanamycin) and hormones (1 mg/l zeatin and 0.1 mg/l indole acetic acid [IAA]) in a growth chamber. Shoots regenerating from the explants were subsequently transferred to MS medium containing 0.1 mg/l each of zeatin and IAA for shoot elongation and, finally, to a medium containing antibiotics, indole butyric acid [IBA] 0.05 mg/l but no cytokinin for root formation. The putative transgenic plants were finally planted in pots and grown further in a glasshouse.

2.2 *PCR, Southern and northern blot analysis for transgene in T0 plants*

The total genomic DNA was extracted from 1 g leaf tissue of *N. benthamiana* as described by Dellaporta *et al* (1983). To verify the presence of the gene in T0 plants, polymerase chain reaction (PCR) was carried out using the total DNA isolated from each plant and the CP-specific primers of CMV-A (Srivastava *et al* 2004). The PCR products were electrophoresed with DNA standard in 1% agarose gel. Integration and copy number of the constructs in the T0 plants were confirmed by Southern blotting of plant genomic DNA (15 μg) digested with *Hin*dIII. The blotted DNA/bands were hybridized with a probe prepared from ~350 bp sequences located at the 3′ end of the CMV CP gene so that the exact copy number could be determined.

To determine the transcription of the CP gene introduced into the *N. benthamiana* plants, total RNA was extracted from 1 g leaf tissue of selected transgenic plants following the method of MacDonald *et al* (1987). The RNA was assayed for the presence of the transgene transcript by using a DNA probe for the CMV CP gene. The DNA or RNA to be transferred was electrophoresed on 1% agarose gels according to standard procedures described (Sambrook *et al* 1989) and blotted on a nylon membrane. The probes used for hybridization were prepared according to the random primer labelling method (Fienberg and Vogelstein 1983). Prehybrization and hybridization were carried out at 42°C with 50% formamide and the blots were washed as instructed by the manufacturers.

Northern hybridization of the inoculated and systemic leaves of selected progeny plants was also performed to determine the level of RNA accumulated in them after virus infection. For this, the probe used to hybridize with the total RNA was prepared from ~ 250 bp sequences conserved in the 3′ end of all four CMV RNA species.

2.3 *Western blot immunoassay and ELISA for protein analysis*

To determine the protein levels in transgenic plants, SDS-PAGE was performed using 12% polyacrylamide gel for resolving total proteins. Western blots were made by transferring the protein onto a nitrocellulose membrane in a mini-transblot apparatus (Bio-Rad). Expression of the gene in plants at the protein level was determined by using antiserum to CMV (PVAS 242a, ATCC, USA). Roughly, 20 μg of total soluble protein from each sample was loaded every time and the accumulation of CP in uninoculated, inoculated and upper leaves was determined.

Simultaneously, double antibody sandwich-ELISA (DAS-ELISA) was performed to determine the relative level of CP accumulation in transgenic progeny lines in their inoculated or upper leaves as against the control (untransformed) diseased plant. Antiserum specific to CMV (primary antibody) was used at a dilution of 1:500 and alkaline phosphates conjugate (secondary antibody) at 1:1000.

2.4 *Challenge inoculations of transgenic plants for virus resistance*

Transgenic plants at 4–6-leaf stage were challenged with 1: 10 inoculum (crude extract) prepared from CMV-infected tobacco leaves macerated in 0.1 M sodium phosphate buffer, pH 7.0 containing 1% sodium sulphite (1 ml buffer/100 mg tissue). Inoculated plants were observed daily for 15–20 days for the development of symptoms and compared with the control (untransformed challenged). Plants that did not develop any symptoms were checked by back inoculation tests to detect latent infection, if any.

3. Results

3.1 *Chimeric construct and regeneration of transgenic plants*

The prepared construct (pACPR2) contained the CMV-A CP gene (GenBank Acc. AF198622) located between the T-DNA borders of the binary plasmid pROK2 (figure 1). Restriction digestion of the construct in *E. coli* (pACPR2) used for triparental mating and subsequently *Agrobacterium* conjugant (pACPR2.5) with appropriate restriction enzymes revealed the presence of an ~650 bp CMV CP gene (data not shown).

Transformation of *N. benthamiana* resulted in direct shoot initiation from a large number of leaf explants (about 60%) after 4 weeks. Out of the 68 putatively transformed shoots obtained, 33 independent shoots from 14 lines designated as A–N lines, which represented at least one transformation event each, were finally established in the glasshouse. Acclimatized putative transformed plants grew to maturity and produced normal flowers. However, in several plants, low or no seed setting was observed, probably because of interference by the transgene.

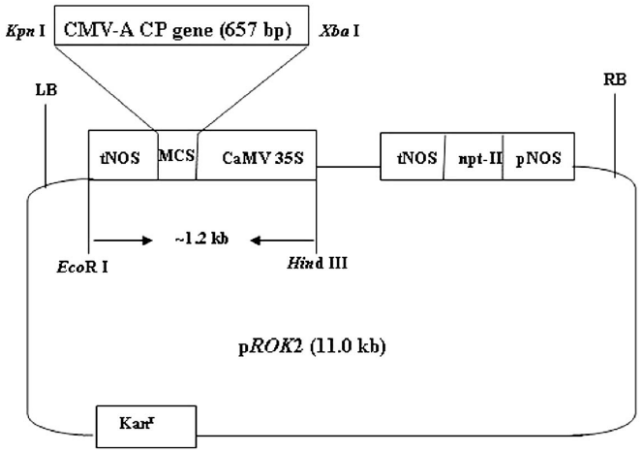

Figure 1. Diagrammatic representation of a construct showing the *Cucumber mosaic virus* (CMV)-A coat protein (CP) gene (GenBank Acc. AF198622) in between the CaMV 35S promoter and NOS terminator of the p*ROK*2 binary vector.

3.2 *Analysis of T0 transgenic plants*

PCR analysis of primary transformants (T0) using CMV CP-specific primers revealed the presence of the CP gene in ~84% of plants (10/12), whereas the untransformed *N. benthamiana* was scored PCR negative (figure 2).

Southern hybridization of the electrophoresed genomic DNA (~10 μg) extracted from five randomly selected transformed plants when digested with *Hind* III and allowed to hybridize with a probe prepared from ~ 350 bp fragment from the 3′ end of cloned CMV-A CP showed positive signals, indicating incorporation of the CMV CP gene into the genome of the lines screened (L1, A2, M1, J3 and K2) (figure 3).

Northern blot analysis using a CMV-A CP probe detected a single mRNA band of ~1.0 kb in the transformed A2, J3 and M1 lines (figure 4) but not in the untransformed *N. benthamiana* taken as a negative control. The levels of

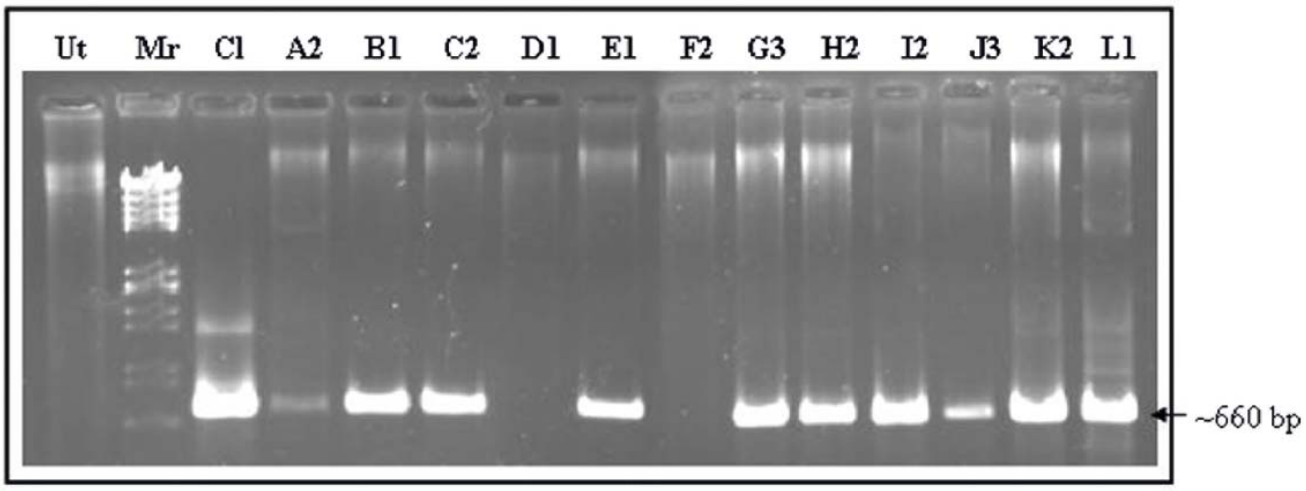

Figure 2. Polymerase chain reaction (PCR) analysis of T0 generation transgenic lines using *Cucumber mosaic virus* (CMV) coat protein (CP)-specific primers showing an amplicon of the expected size (~650 bp) in A2, B1, C2, E1, G3, H2, I2, J3, K2 and L1 lines but not in D1 and F2 lines. Lanes Ut, Mr and Cl are: untransformed healthy (negative control), λ DNA *Eco*RI-*Hin*dIII double digest marker (Genei, India) and pACP7 clone (positive control), respectively.

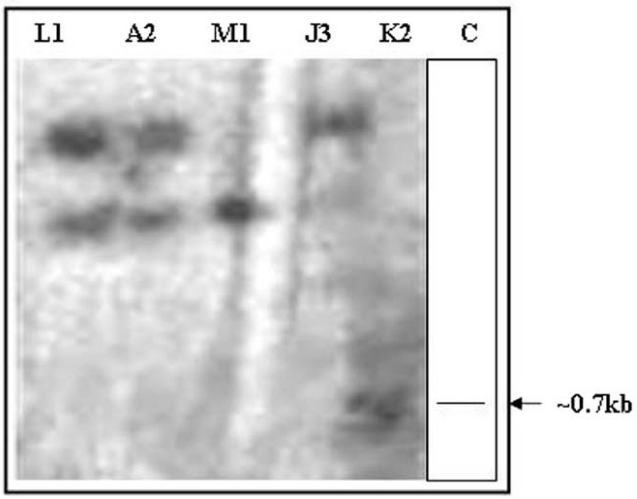

Figure 3. Southern hybridization test of T0 lines with a probe of ~350 bp fragment corresponding to the 3′ end of the *Cucumber mosaic virus* (CMV) coat protein (CP) showing integration of the CMV CP gene. Lanes L1, A2, M1, J3 and K2: selected transgenic lines and C: diagrammatic representation of the position of the probed CMV CP gene used as a positive control.

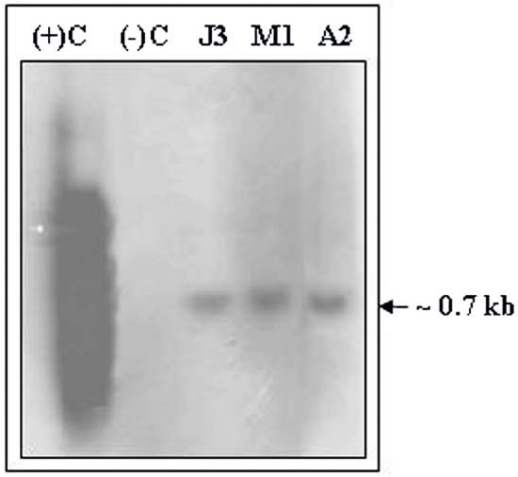

Figure 4. Northern blot hybridization of the *Cucumber mosaic virus* (CMV) coat protein (CP) gene transcripts in some of the transgenic plants probed with a [P^{32}]-dCTP labelled CMV CP gene. Lane 1: untransformed inoculated with CMV-A (positive control); Lane 2: untransformed healthy (negative control); J3, M1, A2 are transgenic lines showing transcripts of the expected size.

CP mRNA did not differ significantly between the various transgenic plants.

Western blot immunoassay using antiserum to CMV (PVAS 242a, ATCC, USA) detected a protein of 24 kDa in all the tested transformed plants (figure 5), confirming the *in vivo* translation of CP mRNA in the leaves of T0 plants.

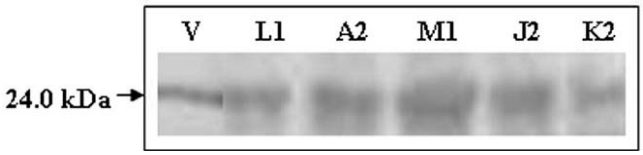

Figure 5. Western blot immunoassay of selected transgenic lines expressing the *Cucumber mosaic virus* (CMV) coat protein (CP). Lanes L1, A2, M1, J2, K2 are transgenic lines showing the presence of a 24 kDa protein as compared with V: CMV purified virus.

3.3 *Evaluation of virus resistance in T1 transgenic lines*

To analyse the degree of resistance against CMV infection the J3 and M1 lines (of two independent events), which showed one copy number of CP in their genome and scored maximum seed setting in the T0 generation, were selected for screening. The T0 seedling progeny from both lines were self-fertilized and ~50 seeds from each line were aseptically germinated on MS medium containing kanamycin (100 mg/l) to analyse the percentage survival of the plants. The results indicated that the progeny of both the lines segregated with a ratio of ~3:1, thus suggesting that a single-copy gene was carried by each.

In the T1 generation, 32 and 29 plants of the J3 and M1 lines, respectively, which survived on kanamycin, were challenged for resistance to CMV infection. Challenge inoculation of 7–8-week-old progenies at a 1:10 concentration showed chlorosis in 2–4 days in the inoculated leaves of almost all the non-transformed control plants and mosaic symptoms. Transgenic lines of the J3 and M1 lines, which expressed the CP, did not show any symptoms on the inoculated leaf in the first week after inoculation. Broadly, the development of response in both the lines could be categorized into three: fully susceptible, fully resistant and intermediate. Plants showing intermediate responses were those that consisted of symptomatic and asymptomatic plants in varying proportions (table 1). As evident, 17–22% of the plants in both the lines showed full resistance, whereas 25–27% of the plants turned out to be fully susceptible after two weeks of inoculation. Intermediate responses were seen in 55–56% of the plants, which either developed very mild mosaic symptoms at a later stage or accumulated higher levels of CMV on ELISA but remained symptomless.

One progeny from the J3 line (J3-2) and three from the M1 line (M1-4, M1-7 and M1-21), which showed full resistance to CMV infection on the basis of naked eye examination (no symptoms in the inoculated leaf), were assessed for virus accumulation through western blot immunoassays using the uninoculated, inoculated and upper leaf protein of each plant line, collected at different time intervals. The upper leaf only of three progeny lines – M1-13, M1-16 and M1-23 – that showed chlorotic symptoms on their inoculated leaf

Table 1. Percentage of J3 and M1 progenies (T1 generation) showing resistance or susceptibility to *Cucumber mosaic virus* (CMV) A infection at 25 days post inoculation

Response of plants to CMV infection *	Transgenic line			
	J3 line		M1 line	
	No. of plants	% Resistance / susceptibility	No. of plants	% Resistance / susceptibility
Fully resistant	5/29	17.24	7/32	21.80
Fully susceptible	8/29	27.50	8/32	25.00
Intermediate response #	16/29	55.17	18/32	56.25

* Symptoms produced in all the transgenic plants were delayed by 10–15 dpi even in plants showing complete susceptibility as compared with the non-transformed control where clear mosaic appeared by 7–8 dpi when inoculated at 1:10 dilution, and within 3–5 days at a still higher concentration of 1:5 or 1:1.

Plants showing intermediate response were those that developed very mild to moderate mosaic symptoms in later stages throughout their life cycle or those plants that accumulated high levels of CMV but remained symptomless.

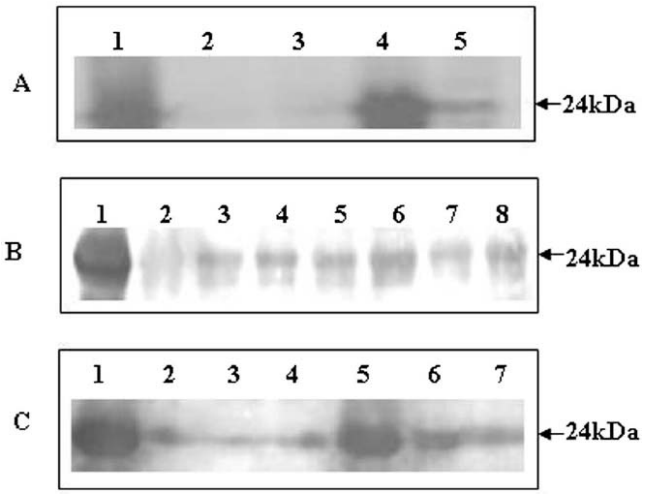

Figure 6. Western blot immunoassay of T0 progeny lines: J3 **(A)** and M1 (**B** and **C**) from uninoculated, inoculated and systemic leaves showing the level of virus accumulation. **(A)** Lane 1: inoculated healthy (positive control); Lane 2: uninoculated healthy (negative control); Lane 3: uninoculated leaf of J3-2; Lane 4: inoculated leaf of J3-2 at 7 dpi; and Lane 5: upper leaf of J3-2 at 21 dpi. **(B)** Lane 1: inoculated untransformed (positive control); Lane 2: uninoculated healthy (negative control); Lanes 3–5: uninoculated leaf of M1-4, M1-7 and M1-21; and Lanes 6–8: inoculated leaf of the same lines at 7 dpi. **(C)** Virus accumulation in the upper leaf at 21 dpi: Lane 1: inoculated untransformed (positive control); Lanes 2–4: same as in **(B)** of M1-4, M1-7 and M1-21; and Lanes 5–7: M1-13, M1-16 and M1-23.

(but no systemic symptoms) were also assessed. The results revealed that although there was an increase in the levels of virus accumulation in the inoculated leaves of plants (figure 6A, lane 4 and figure 6B, lanes 6–8), the systemic leaves showed significantly lower levels of virus accumulation (figure 6A, lane 5 and figure 6C, lanes 2–4). The level of virus accumulation in the upper leaves was found to be ~2–3 times lower as compared with diseased ones (lane 1 in figure 6A, B and C). Progenies of M1 whose upper systemic leaves only were evaluated, showed higher virus accumulation (compared with lines that showed significantly lower levels) but virus accumulation in them was still lower than in the susceptible control (figure 6C, lanes 5–7). Of these, M1-13 produced systemic mosaic after a long incubation period but M1-16 and M1-23 remained symptomless.

DAS-ELISA of the upper leaves at 30 days post-inoculation (dpi) (figure 7) correlated well with the findings of the western blot analysis. Virus accumulation in the lines was found to be ~2.5 times (J3-2 and J3-3) and ~3.0 times (M1-4, M1-7 and M1-21) lower than in the control. M1-16, which showed comparatively higher accumulation on western analysis, showed about half the amount of virus accumulation detected in the control. Back inoculation tests of J3-2 and M1-7 resistant lines failed to produce symptoms even after two months of inoculation.

The line that was the most highly resistant, J3-2, which also produced more seeds than the M1-7 line, was selected for further evaluation of resistance in its T2 and T3 generations. Ten aseptically grown and kanamycin-resistant plants from self-fertilized seeds obtained from T1 of J3-2 were mechanically inoculated at 1:10 w/v dilution of infected leaf tissue and observed for symptom development. Of the 10 inoculated plants, one died during establishment and from the remaining 9 plants, only one (J3-2-1) produced visible symptoms after 14 dpi, whereas the rest remained symptomless throughout their life cycle, grew to maturity and set seeds. DAS-ELISA conducted on the upper leaf in all plants (figure 8) showed reduced virus accumulation as compared with the control, with the highest reduction observed (~3 times lower than the diseased control) in lines J3-2-2 and J3-2-6. Failure to produce symptoms through back inoculation tests further confirmed that these transgenic lines were highly resistant.

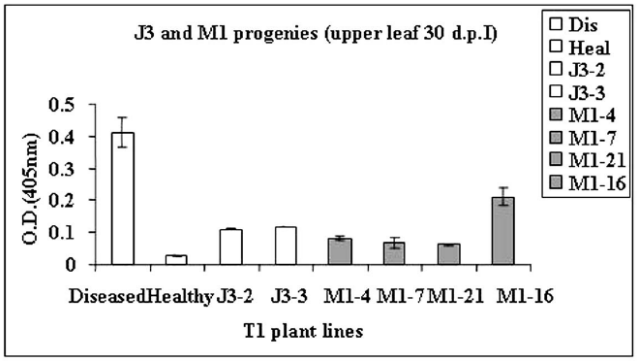

Figure 7. Double antibody sandwich-enzyme linked immunosorbent assay (DAS-ELISA) of upper leaves of T1 generation of *N. benthamiana* J3 (J3-2 and J3-3, open bars) and M1 (M1-4, M1-7, M1-21 and M1-16, closed bars) lines showing virus accumulation levels at 30 dpi. Plants were challenged with inoculum diluted ten times prepared from the leaves of tobacco infected with *Cucumber mosaic virus* (CMV)-A. Bar lines on each histogram indicate the standard error.

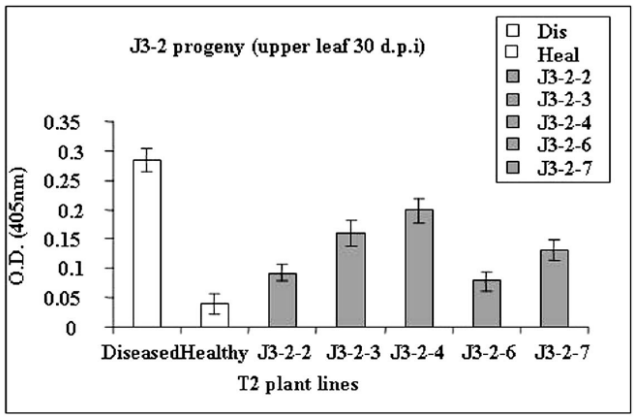

Figure 8. Double antibody sandwich-enzyme linked immunosorbent assay (DAS-ELISA) of upper leaf of T2 generation of the J3-2 transgenic line showing different levels of virus accumulation at 30 dpi. Plants were challenged with inoculum diluted ten times prepared from leaves of tobacco infected with *Cucumber mosaic virus* (CMV)-A. Bar lines on each histogram indicate the standard error.

Northern blot analysis of the inoculated and systemic leaves at 8 dpi to determine the accumulation of CMV RNAs revealed that the mRNA could be detected in both the lines (J3-2-2 and J3-2-6) in their inoculated leaves (figure 9A). However, the level of transcript accumulation in their upper leaves (figure 9B) was again much lower (~2–2.5 times lower than the diseased) and correlated with the ELISA results. Despite differences in the virus accumulation levels, no phenotypic differences could be identified between the two lines. In the T3 generation, a noticeable observation, apart from reduced viral RNA accumulation, was the detection

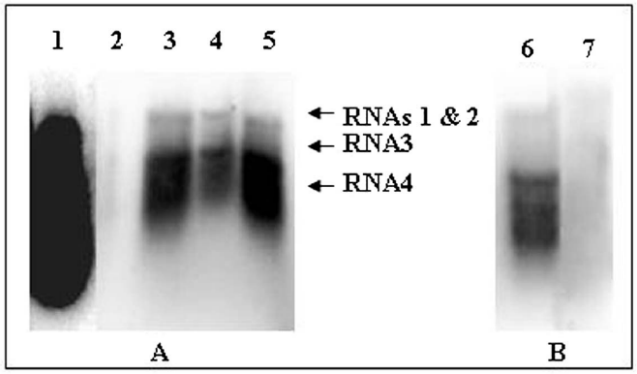

Figure 9. Northern blot hybridization of T2 generation *N. benthamiana* J3-2 (J3-2-2 and J3-2-6) lines showing accumulation of *Cucumber mosaic virus* (CMV) RNAs in the **(A)** inoculated and **(B)** upper leaves. The blot was hybridized with an α-P^{32} labelled probe prepared from conserved sequences at the 3′ end of all four RNA species of CMV. Lane 1: inoculated untransformed; Lane 2: healthy control; Lanes 3 and 6: J3-2-2 plant line; Lanes 4 and 7: J3-2-6 plant line and Lane 5: inoculated leaf of the J3-2-7 line.

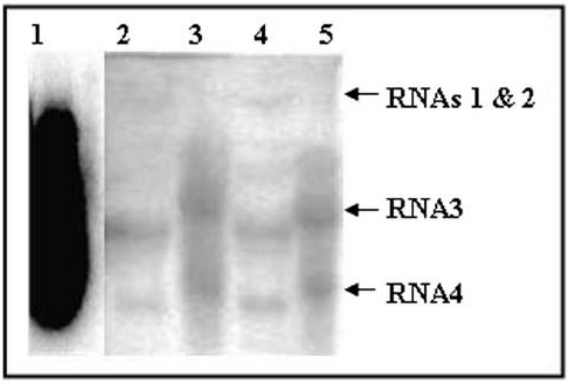

Figure 10. Northern blot hybridization of T3 generation *N. benthamiana* J3-2-2 and J3-2-6 (J3-2-2-2 and J3-2-6-4) lines showing accumulation of *Cucumber mosaic virus* (CMV) RNAs in the inoculated and upper leaves. Lane 1: inoculated untransformed; Lanes 2 and 4: RNA accumulation in the inoculated and upper leaves of the J3-2-2-2 line, respectively; Lanes 3 and 5: RNA accumulation in the inoculated and upper leaves of the J3-2-6-4 line, respectively.

of higher levels of CMV RNAs 3 and 4, respectively, than RNAs 1 and 2 in the inoculated as well as upper leaves of resistant plant lines at 25 dpi (figure 10). In contrast, the non-transformed control showed higher accumulation of all four CMV RNA species.

3.4 *Resistance against closely related strains*

Resistance was also evaluated against closely related strains CMV-D and CMV-H (possessing 98% sequence identity with

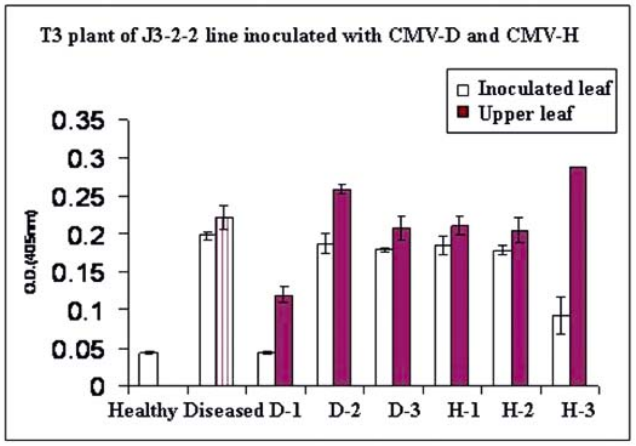

Figure 11. Double antibody sandwich-enzyme linked immunosorbent assay (DAS-ELISA) of inoculated (open bars) and upper leaves (closed bars) of T3 generation *N. benthamiana* J3-2-2 lines showing virus accumulation levels at 25 dpi. Three plants each were inoculated with either *Cucumber mosaic virus* (CMV)-D (D-1 to D-3) or CMV-H (H-1 to H-3). Bar lines on each histogram indicate the standard error.

CMV-A) of subgroup IB (Srivastava and Raj 2004). Tests were conducted by taking three plants of the J3-2-2 progeny line for each virus strain. These plants were designated as D-1 to D-3 and H-1 to H-3, depending on the virus strain with which they were inoculated. Results indicated that 66% of the plants in each case were able to protect themselves from infection by both the CMV-D and CMV-H strains even after one month post-inoculation (figure 11). However, there was not much difference in virus accumulation levels between the inoculated or upper leaves except in two plants (D-2 and H-3), where the difference was comparatively higher.

4. Discussion

CP genes have been shown to be effective in preventing infection or reducing disease caused by homologous and closely related viruses (Gonsalves and Slightom 1993). Resistance has been observed against both the infecting virus particles as well as viral RNA. In this study, the resistance shown by transgenic plants to CMV indicated that it was protein-mediated rather than RNA-mediated because CP was detected at low levels by ELISA in the upper leaves of transgenic plants after challenge inoculations.

The T0-transformed *N. benthamiana* plants analysed by PCR, Southern, northern and western analyses confirmed that the CMV CP had successfully integrated into the plant genome and was functioning as expected. However, incorporation of the CP gene in various transgenic lines as determined by Southern analysis did not show any apparent correlation with the RNA accumulation levels. Evaluation of resistance in different plant lines to CMV infection in the T1 generation as determined by virus accumulation levels at different times (dpi) and from different locations of leaf (inoculated leaf and/or upper leaf) indicated that it ranged from near-complete resistance to none. Primarily, all resistant transgenic plants (>50%) exhibited delayed symptom development as reported in a number of studies (Nakajima *et al* 1993; Gielen *et al* 1996) though a few strongly resistant plants were also obtained where no symptoms developed even as late as 30 dpi.

Significantly reduced virus accumulation levels in the systemic leaves of T1 plants compared with inoculated leaves were further supported by low virus levels detected in the upper leaves by ELISA at 30 dpi. Okuno *et al* (1993) also reported higher virus accumulation in the inoculated leaves of transgenic plants expressing the CP gene than in the upper leaves. Resistance was also shown by plants that remained symptomless throughout their life cycle, although they accumulated virus at high levels in their upper leaves (but less than the control).

As observed in T1 progenies, northern hybridization and/or ELISA of the T2- and T3-generation progenies of line J3-2 also showed greatly reduced virus accumulation levels in the upper leaves or a delay in disease development. Similar observations have been recorded earlier by Powell-Abel *et al* (1986). Higher CP levels in inoculated transgenic plants have presumably affected the uncoating of the virus particle at later stages. According to Reimann-Philipp (1998), a reduced rate of virus accumulation in inoculated leaves and slower systemic spread are frequently observed in transgenic CP-accumulating plants owing to slower replication rates or interference with local or systemic virus transport. Higher accumulation of CMV in inoculated leaves but no systemic spread may be due to interference with either entry into the phloem or vascular long-distance transport as suggested earlier by Taliansky and Garcia-Arenal (1995).

The limited tests conducted showed that resistance could be achieved not only against homologous (CMV-A) infection but also against the related strains of CMV (CMV-D and CMV-H) of the same subgroup 1B. However, ELISA showed higher virus accumulation in the upper leaves compared with inoculated leaves in all plants. Though virus accumulation levels in all the plants tested were comparable with the untransformed control, no systemic symptoms were visible even at 45 dpi. Xue *et al* (1994) also observed similar findings in transgenic tomato lines containing the CP gene of the CMV-WL strain, a member of subgroup II. R0 plants that accumulated detectable levels of CMV-WL CP were found to be resistant to another strain of CMV-China (a member of subgroup I). Progeny from R0 plants tested for resistance to infection by CMV-WL and CMV-China showed a high level of resistance to systemic infection by both the strains and

virus could not be recovered from asymptomatic inoculated plants.

The main objective of this study was to determine whether the CP-mediated resistance (MR) strategy could be applied for raising resistant transgenic plants utilizing the CP gene of the CMV strain isolated from India. Evaluation of the transgenic *N. benthamiana* J3 line up to three generations showed that resistance could be achieved both against the homologous as well as two other closely related strains of CMV. The novelty of the work is that the CP of a subgroup IB isolate of CMV has been used to generate transgenic plants for the first time in India to demonstrate resistance in these plants against homologous and related CMV strains of subgroup IB. Therefore, the strategy may be applied to commercially important crops such as tomato, chilli and brinjal in which CMV causes drastic reduction of yield and quality (Tomlinson 1987).

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