Partial epitope mapping of *Alfalfa mosaic virus* and the effect of coat protein gene mutation on aphid transmission

Hossain Massumi*[1,2,3], Phil Jones[1] and Nigle Hague[2]

[1]Department of Plant Pathology, IACR Rothamsted, Harpenden, Herts. AL5 2JQ, UK [2]Deptartment of Agriculture, The University of Reading, P.O. Box: 236, Reading, RG6 2AT UK [3]Department of Plant Pathology, Faculty of Agriculture, Shahid Bahonar University of Kerman, Kerman, I.R. Iran

Abstract

Epitope mapping with seventy-one overlapping octapeptides representing the whole sequence of Alfalfa mosaic virus (AMV) coat protein (CP) (strain S) was done using monoclonal antibodies. In total, five monoclonal antibodies identified 5 epitopes, at different sites along the amino acid sequence of AMV coat protein. Four MAbs each reacted with a single epitope, while one MAb bonded with peptides on two widely separated parts of the coat protein. A full length DNA copy of RNA 3 of AMV strain S in a pBS plasmid was used as a template for mutation and transcription. Both before and after modification, the RNA 3 of this strain was inoculated to Nicotiana tabacum transgenic P12 plants and AMV particles were purified. Strain S of AMV produced systemic symptoms in N. tabacum transgenic P12 plants. It was suggested that MAb-2 was effective in blocking insect transmission of AMV. Epitope-2 which play an important role in transmission is recognized by MAb-2. To further investigate the interaction between epitope-2 and transmission of AMV mutation of the amino acids of epitope-2 was made in the appropriate coding regions of the coat protein of AMV strain S. These changes were (i) a phenylalanine to tyrosine and (ii) asparagine to glutamine. The mutation of asparagine to glutamine had no effect on the transmission of AMV by M. persicae, but the mutation of phenylalanine to tyrosine gave a significant reduction (P = 0.02) in aphid transmissibility. This indicated that the phenylalanine residue at position 67 of the AMV coat protein was involved in transmission.

***Keywords**: Alfalfa mosaic virus; Coat protein; Epitope mapping; Transmission; Mutation.*

Correspondence to:* **Hossain Massumi, Ph.D.
Tel: +98 341 3222042; Fax: +98 341 3222043
E-mail: masoomi@mail.uk.ac.ir

*Present address: Department of Plant Pathology, Faculty of Agriculture, Shahid Bahonar University of Kerman, P.O. Box: 76169-133, Kerman, I.R. Iran

INTRODUCTION

Two different strategies for non-persistent transmission of plant viruses are recognized, the capsid strategy and the helper strategy (Pirone and Blanc, 1996). In the capsid strategy, virions, without indication of a helper factor, interact directly with their vectors. This is the case with *Cucumber mosaic virus* (CMV) where the capsid protein alone mediates virus/vector interaction (Perry *et al*.,1994) and probably with *Alfalfa mosaic virus* (AMV) and the Carlaviruses as well, since these viruses can be transmitted by their vector aphids after purification (Pirone and Megahed, 1966; Weber and Hampton, 1980). In the case of helper strategy, a viral protein called helper component (HC) is acquired along with virus particles as the aphid feeds on plant sap, but the role of this protein in transmission is unknown (Gray, 1996).

AMV, a member of the family *Bromoviridae*, has three genomic and one subgenomic RNA molecules. RNAs1 and 2 of this virus encodes the replicase proteins P1 (126 kDa) and P2 (90 kDa) whereas RNA 3 encode the movement protein P3 (32 kDa) and the viral coat protein P4 (24 kDa) (Van Dun *et al.,* 1987 and Taschner *et al.*, 1994). The CP is translated from a subgenomic messenger, RNA4 which is homologous

to the 3´ terminal 881 nucleotides of RNA3 (Langereis *et al*., 1986). AMV is transmitted by a number of different aphids in a non-persistent fashion (Garran and Gibbs, 1982).

The diagnostic use of synthetic peptides as antigens continues to be an extremely attractive proposition because their production is highly controllable and their use is theoretically safe. Synthetic peptides are increasingly used in immunology and molecular biology to characterize antigenic sites (epitopes) on the surface of proteins (Jemmerson and Paterson, 1986). Therefore, the easiest method of mapping epitopes on virus particle proteins includes testing the reactivity of antibodies with synthetic peptides.

Peptides have been synthesized directly onto the surface of both polyethylene and polypropylene pins (Wang *et al*., 1993 and Geysen, *et al*., 1984), on activated flat membrane sheets made up of cellulose or cotton which can be repeatedly probed and blotted (Wang and Laursen, 1992; Rinnova *et al*., 1993). Different epitopes were localised on the coat protein of *Potato virus A* (PVA) (Andreeva *et al*., 1994), *Beet necrotic yellow vein virus* (BNYVV) (Commandeur *et al*., 1994), and *Zucchini yellow mosaic virus* (ZYMV) (Desbiez *et al*., 1997) by means of synthetic overlapping peptides.

The coat proteins (CP) of plant viruses are multifunctional and whilst some of their functions have been elucidated, the precise nature of many is undetermined. Recently site-directed mutagenesis has been used to determine some virus CP functions. This technique is used to determine the role of specific nucleotides (or amino acids), once an important region of a gene has been identified. The use of synthetic peptides, corresponding to the AMV coat protein, and epitope mapping has the potential to find epitopes or residues that effect the insect transmission of virus.

It was suggested (Massumi *et al.,* 2005) that MAb-2 was effective in blocking (inhibiting) insect transmission of AMV. In this paper we describe the epitope which play an important role in transmission and is recognized by MAb-2. It can be deduced that some of amino acids in this epitope may be important in blocking insect transmission. Therefore, the goal of this research was to test which amino acid could affect aphid transmissibility by site directed mutagenesis.

MATERIALS AND METHODS

Peptides: A complete set of 71 octapeptides, with 5 amino acid overlaps, corresponding to the 218 amino acid sequence of the AMV coat protein (RNA3) strain S was synthesized by Research Genetics, Inc. (2130 South Memorial Parkway Huntsville, Albama 35801) on a membrane sheet in an 8 x 12 array.

Preparations of monoclonal antibodies (MAbs): Purified AMV strain 425 was prepared in an equal volume of PBS for immunization. The method for cell fusion and maintenance were as previously described (Massumi *et al*., 2005). The results antisera which used in the tests were MAbs-1,2,6,7,10,11,13,17,22,24 and 28.

Multi spot peptide synthesis apparatus: An apparatus was designed to present an area of membrane isolating individual peptides for reaction with different antibodies in a standard 8 x 12 well microplate format. The device consisted of two templates between which the peptide membrane was sandwiched. Ninety-six holes were bored in each template in the format of a standard microtiter plate with a diameter of 6.3 mm the top template and 3 mm at the bottom. To prevent cross-talk between wells a seal was used around each. The two templates, membrane and silicon seal were clamped together by four screws, then placed above a microtiter plate (Nunc).

Immunoassays: The set of octapeptides coupled to the membrane was tested as described by Wang and Laursen (1992) with some modification. The membrane bearing the peptides was washed once in 50 ml of Tris-NaCl pH 7.5 and was then precoated with Tris-NaCl, 10% ovalbumin (v/w) and 1% Tween 20 (v/v) for 1h at room temperature to block non-specific protein binding site on the membrane. The membrane was washed twice (5 min each) with 0.05% Tween 20 (v/v) in Tris-NaCl and was incubated for 2h at room temperature with antisera diluted in Tween 20/Tris-NaCl containing 1% ovalbumin (v/w) (1:200 for MAb and 1:1000 PAb). The membrane was again washed twice with Tween 20 Tris-NaCl, after which it was incubated with gentle shaking for 2h at room temperature in 15 ml of IgM or IgG alkaline phosphatase conjugate (depending on the class and isotype of antibody) diluted in Tween 20/Tris-NaCl containing 1% ovalbumin (1:2000). The membrane was again washed as before with Tween 20/Tris-NaCl and then with Tris-NaCl to remove Tween 20. The membrane was mounted between the template and silicon seal in the peptide synthesis device, then the apparatus was bolted together and placed above a plastic microplate ELISA plate. 25 µ l of alkaline phosphate substrate was added, after 10 min a second 25 µl of substrate added and the reaction was allowed to continue for 10 more minutes. The

substrate solutions were expressed into the wells of the microplate with air pressure (using a syringe with o rings at the tip) and each well containing a reaction was washed with 50 µl of substrate buffer. Absorbance was measured in an ELISA reader (Dynex Technologies) at 450 nm. For control purposes the antibody step was omitted. After completion of the assay, bound antibody was removed from the membrane by sonication for 30 min in a pre-heated bath (60°C) containing disruption buffer. After sonication, the membrane was washed 3-4 times with Tris-NaCl. The membrane was allowed to dry in air prior to use again.

Plasmid preparation: A 3BS-(12,0) clone containing a full length copy of RNA3 of AMV strain S in a pBS plasmid (Dore *et al*., 1990) was kindly provided by Dr. Lothaire Pinck (Institut de Biologie Moleculaire des Plantes, Strasbourg, France).

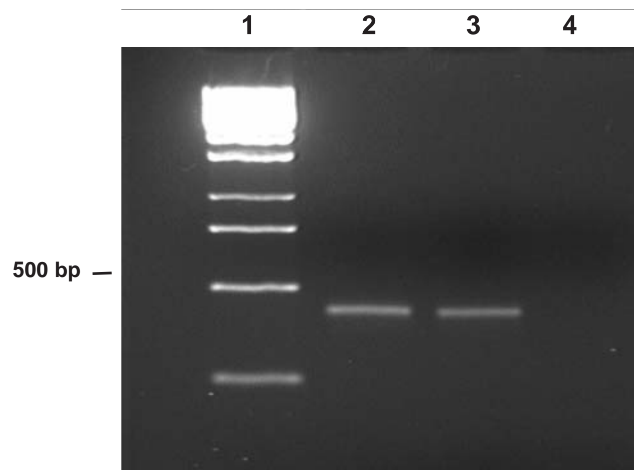

Figure 1. Site-directed mutageneised product of cloned cDNA3-S by PCR and fractionated by 2% agarose gel electrophoresis, lane1: 100 bp ladder, lane 2: PCR product after modification in DNA by mutation in amino acid Asp to Gln residue, lane 3: PCR product after modification in DNA by mutation in amino acid Phe to Tyr residue, lane 4: sterile distilled water negative control.

Construction of a genome-length cDNA plasmid containing mutation in the CP gene area of RNA-3: Modified DNA containing changes corresponding to amino acids 67 from Phe to Tyr and 68 from Asn to Gln in AMV-S CP was prepared by PCR. A PCR strategy (Tao and Lee, 1994) was used with the 3BS-(12,0) clone as a template and primers listed in Table 1. The PCR product was gel purified and cleaved with two restriction enzymes, *Sac*I and *Apa*I. Cleavages were at nt position 1401 and 1810 in the RNA-3 strain S genome, respectively. Appropriate DNA fragments (PCR product; 409 bp) (Fig. 1) including the mutated coat protein gene were ligated into plasmid containing genomic-length 3BS-(12,0) clone, to obtain clones 3BSN-(12,0) and 3BSF-(12,0) with mutation in Asn and Phe amino acid residues, respectively. The plasmids containing ligated circular double-strand clones were transformed in the *E. coli* strain JM 109 cells and recovered. The mutations were confirmed by sequencing of recoverd plasmids (Applied Biosystems 373 (STRETCH) (Figs. 2 and 3).

***In vitro* transcription:** The 3BS-(12,0) clone and two modified clones; 3BSF-(12,0) and 3BSN-(12,0) were linearized by *Pst*I restriction enzyme. The protruding 3′ end generated by *Pst*I was removed with T4 DNA polymerase as described by the manufacturer (Life

Table 1. Oligonucleotide primers used for mutation of the AMV coat protein gene region of RNA3-S.

Mutation No.	Primers[a]	Amino acids and codon		Amino acid position in the coat protein
		Wild-type	Change	
1	5′ TCTGAGCTCTTTT**CAG**GGGCTCGGCGT 3′ (1398-1424)	Asn(AAC)	Gln (CAG)	68
	3′ TCGTCCCGGGGAGGCGTCAAACT 5′ (1806-1828)			
2	5′ TCTGAGCTCT**TAC**AACGGGCTCGGCGT 3′ (1839-1424)	Phe (TTT)	Tyr (TAC)	67
	3′ TCGTCCCGGGGAGGCGTCAAACT 5′ (1806-1828)			

[a]Primers are complimentary to the AMV RNA 3 cDNA strand and the nucleotide position is according to Ravelonandro *et al.* (1984). Mutated codons are indicated in bold. Sequences of the restriction enzyme *Sac*I and *Apa*I sites introduced during mutation are underlined.

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 51 TTCTTCACAAAAAAAAGCTGGTGGGAAAGCTGGTAAACCTACTAAACTTC 100    1
    |||||||||||||||||||||||||||||||||||||||||||||||||
 59 TTCTTCACAAAAAAAAGCTGGTGGGAAAGCTGGTAAACCTACTAAACTTT 108    2
              .         .         .         .         .
101 CTCAAAACTATGCTGCTTTACGCAAAGCTCAACTGCCGAAACCTCCGGCG 150    3
    ||||||||||||||||||||||||||||||||||||||||||||||||||
109 CTCAAAACTATGCTGCTTTACGCAAAGCTCAACTGCCGAAACCTCCGGCG 158    4
              .         .         .         .         .
151 TTAAAATTCCCGGTTGCAAAACCAACAAATACTATACTGCCACAAACGGG 200    5
    ||||||||||||||||||||||||||||||||||||||||||||||||||
159 TTAAAATTCCCGGTTGCAAAACCAACAAATACTATACTGCCACAAACGGG 208    6
              .         .         .         .         .
201 CTGTGTTTGGCAAACCCTCGGGACCCCTCTGATTCTGAGCTCTTACAACG 250    7
    ||||||||||||||||||||||||||||||||||||||||||||  ||||
209 CTGTGTTTGGCAAACCCTCGGGACCCCTCTGATTCTGAGCTCTTTTAACG 258    8
              .         .         .         .         .
251 GGCTCGGCGTGAAATTCCTCTACAGTTTCCTGAAGGATTTCACGGGACCT 300    9
    ||||||||||||||||||||||||||||||||||||||||||||||||||
259 GGCTCGGCGTGAAATTCCTCTACAGTTTCCTGAAGGATTTCACGGGACCT 308   10
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Figure 2. Comparison of the nucleotide sequences of cDNA-3 from 3BS-(12,0) clone and 3BSF-(12,0) mutant clone. The even numbers in the right (bold type) represents 3BS-(12,0) clone and the odd numbers 3BSF-(12,0) mutant clone. Nucleotides are numbered from the 5′ end of the cDNA-3 which represents part of the coat protein gene. The mutated codon is indicated in bold.

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    ||||||||||||||||||||||||||||||||||||||||||||||||||
102 AAACTTTCTCAAAACTATGCTGCTTTACGCAAAGCTCAACTGCCGAAACC 151    2
              .         .         .         .         .
156 TCCGGCGTTAAAATTCCCGGTTGCAAAACCAACAAATACTATACTGCCAC 205    3
    ||||||||||||||||||||||||||||||||||||||||||||||||||
152 TCCGGCGTTAAAATTCCCGGTTGCAAAACCAACAAATACTATACTGCCAC 201    4
              .         .         .         .         .
206 AAACGGGCTGTGTTTGGCAAACCCTCGGGACCCCTCTGATTCTGAGCTCT 255    5
    ||||||||||||||||||||||||||||||||||||||||||||||||||
202 AAACGGGCTGTGTTTGGCAAACCCTCGGGACCCCTCTGATTCTGAGCTCT 251    6
              .         .         .         .         .
256 TTTCAGGGGCTCGGCGTGAAATTCCTCTACAGTTTCCTGAAGGATTTCAC 305    7
    ||| | ||||||||||||||||||||||||||||||||||||||||||||
252 TTTAACGGGCTCGGCGTGAAATTCCTCTACAGTTTCCTGAAGGATTTCAC 301    8
              .         .         .         .         .
306 GGGACCTCGGATCCCCAAAAAGGATCTGATTTACAGGATGGTGTTTTCTA 355    9
    ||||||||||||||||||||||||||||||||||||||||||||||||||
302 GGGACCTCGGATCCCCAAAAAGGATCTGATTTACAGGATGGTGTTTTCTA 351   10
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Figure 3. Comparison of the nucleotide sequences of cDNA-3 from 3BS-(12,0) clone and 3BSN-(12,0) mutant clone. The even numbers in the right (bold type) represents 3BS-(12,0) clone and the odd numbers 3BSN-(12,0) mutant clone. Nucleotides are numbered from the 5′ end of the cDNA-3 which represents part of the coat protein gene. The mutated codon is indicated in bold type.

Technology: 18005-017). Capped transcripts from the linearized plasmid templates containing the T7 promoter were synthesized with T7 RNA polymerase in a 20 µl reaction according to the supplier's instructions (Boehringer Mannheim).

Infection of transgenic *Nicotiana tabacum* P12 plants with clones: Seeds of transgenic P12 plants were kindly provided by Professor J. Bol (Leiden University). Seeds were germinated on Murashige and Skoog (MS) medium as described by Van Dun *et al*. (1988). *In vitro* transcribed RNA3 transcripts were diluted in ratio 1:3 (v:v) with 0.1 M phosphate buffer and leaf of transgenic P12 tobacco plants were mechanically inoculated with it. Inoculation was done with 500 ng transcript per half leaf and symptoms scored 7 to 10 days after inoculation. The inoculation was also done with wild type RNA3. Control seedlings were inoculated with 0.1 M phosphate buffer. The progeny viruses (Phe to Tyr, Asn to Gln and wild type) derived from RNA transcriptase were extracted and purified from the infected leaves as described by Van Vloten - Doting and Jaspars (1972) and RNAs extracted from these particles were analysed by agarose gel electrophoresis (Fig. 4).

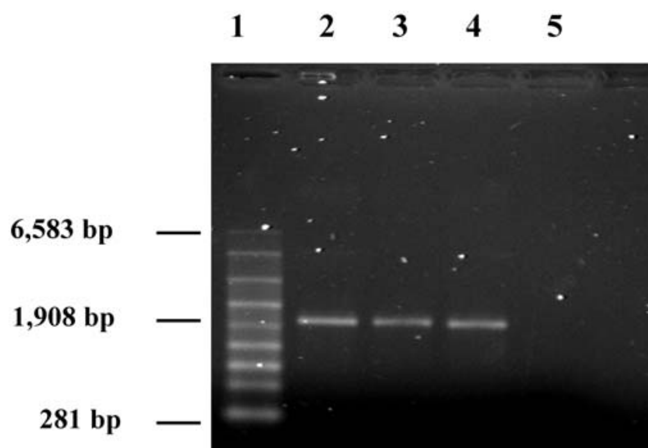

Figure 4. Denaturing agarose gel electrophoresis of *in vitro* transcribed RNA. The RNA was denatured in formaldehyde/formamide sample buffer and one microlitter of it was electrophoresed through a 0.7% (w/v) agarose gel and stained with 0.5 µg ml^{-1} ethidium bromide. Lane 1: Ladder (Promega RNA marker, Cat. No. G3191), lane 2: RNA from 3BS-(12,0) clone, lane 3: RNA from 3BSN-(12,0) clone (mutation in Asp to Gln), lane 4: RNA from 3BSF-(12,0) clone (mutation in Phe to Tyr), lane 5: negative control having sterile distilled water.

Virus acquisition and transmission by aphids: Transmission of modified and unmodified AMV was done by the membrane feeding technique (Pirone,

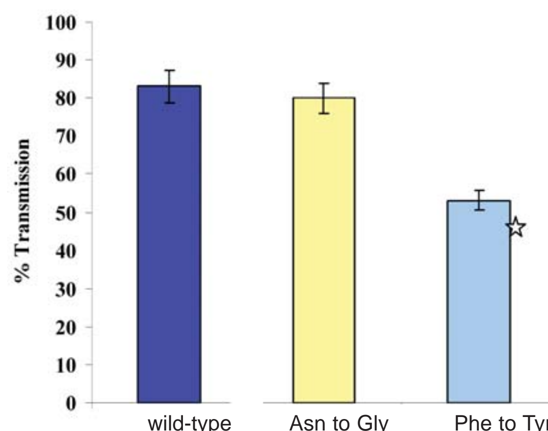

Figure 5. Comparison aphid transmissibility rate of two mutant AMV having alterations from amino acid Phe to Tyr and Asn to Gln in wild-type AMV in transmission studies.

1964) using *M. persicae*. Two population of modified AMV particles (Phe to Tyr and Asn to Gln) and one population of unmodified virus were used. Thirthy transgenic *N. tabacum* P12 plants were used for each experiment. Each plant was fed by 10 aphids. The effect of infection by AMV in transgenic P12 plants was tested by ELISA (Clark and Adams, 1977). The result of the transmission in each mutant was directly compared with the wild type virus.

Statistical analysis: The results of the virus acquisition and transmission tests were analysed using a generalized linear model (Genstat Committee, 1993) with a binomial error and logit link. Figure 5 was predicted from the model.

RESULTS

Reactivity of synthetic octapeptides: Interpretation of the results obtained with MAbs was in most instances straightforward, because background values were low and uniform. The background level was defined as the mean absorbancy for those peptides giving a uniformly low value (Geysen *et al*., 1987a). Therefore, in each scan the mean background reaction of all octapeptides, except the highest, was calculated (Pereira *et al*., 1994). The positive binding was calculated by multiplying the mean by a factor of two. MAbs 1, 2, 17, 24 and 28 reacted with different peptides, but MAbs 6,7,10,11,12,13 and 22 did not react with any. The epitopes recognized by five of the MAbs were identified (Table 2).

Table 2. Octapeptide sequences that reacted with monoclonal antibodies (MAbs).

Epitope	MAb	Octapeptide
1 (7-14)[a] (13-20)	MAb-1	KAGGKAGK GKPTKRSQ
2 (61-68)	MAb-2	PLSLSSFN
3 (133-140)	MAb-24	HGAFHANE
4 (103-110) (127-134)	MAb-28	PMQEFPHG SHAGTFCL
5 (184-191) (187-194)	MAb-17	SYRFNEVW FNEVWVER

[a] Numbers in parentheses denote position in amino acid sequence counting from the N-terminus.

Mapping of the epitope, which blocks transmission of AMV, by MAb: MAb-2 was effective in blocking transmission as described before (Massumi *et al*., 2005). This antibody localised epitope-2 (residues 61-68; peptide 21) (Table 2). Therefore, it seems that this epitope was effective in blocking transmission.

Analysis of pBS plasmid: Plasmids pBS containing a full length DNA copy of the RNA 3 of the strain S transformed in *E. coli* was the plasmids were recovered from *E. coli* after propagation of component cells on media. The viral DNA (clone) ligated in the plasmid was digested with *Pst*I and *EcoR*I separately or together and then analysed by electrophoresis in 0.70% agarose gels. As shown in figure 6 the plasmid is about 3200 bp consisting of inserted DNA at about 2200 bp.

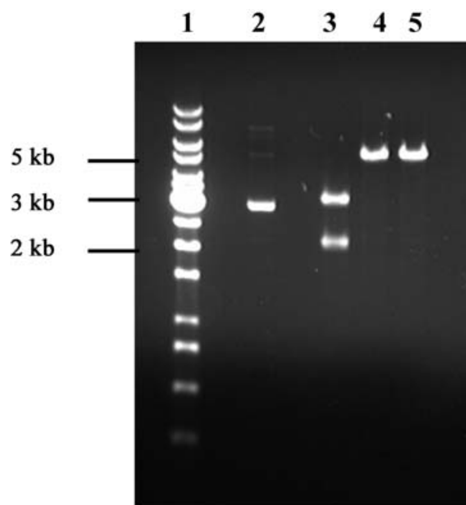

Figure 6. Restriction digestion of plasmid isolated from clone 3 BS-(12,2) carrying 3´ modified complete cDNA of RNA 3 of AMV strain S. Lane 1: 1 Kb ladder (MBIl fermentase), lane 2: uncut plasmid, lane 3: plasmid digested with *Pst*I and *EcoR*I, lane 4: plasmid digested with *Pst*I and lane 5: plasmid with *EcoR*I.

Transcription of DNA copy of RNA3 of AMV strain S: The position of a mutation in the coat protein gene of clones 3BSN-(12,0) and 3BSF-(12,0) is shown in figure 7. 3BS-(12,0) clone and two mutants, 3BSN-(12,0) and 3BSF-(12,0) were *in vitro* transcribted. The RNA products were used for inoculation of the transgenic tobacco P12 plant. Prior to transcription, plasmids linearized with *Pst*I were treated by T4 DNA polymerase to remove the 3´ protruding end. This treatment enhanced the amount of RNA transcripts produced by T7 polymerase and prevented the synthesis of RNA products of larger molecular weight (Dore *et al.,* 1990).

Infection of transgenic tobacco P12 plants, symptoms and purified progeny virus: RNA 3 transcripts of the clones 3BS-(12,0)(wild-type) and the two mutant clone 3BSN-(12,0) and BSF-(12,0) were inoculated to transgenic P12 plants which were transformed with the AMV replicas P1 and P2 genes. Inoculation of the transgenic P12 plants with all three different RNA transcripts led to systemic symptoms. Plants inoculated with wild-type RNA 3 transcripts began to develop typical systemic symptoms such as mosaic on the leaves in 7-10 days. Those plants inoculated with RNA 3 mutant (Asn to Gln) transcripts developed mosaic sometimes without any other typical symptoms such as vein clearing, but the leaves of transgenic P12 plants inoculated with RNA 3 mutant (Phe to Tyr) transcripts, continued to develop typical mosaic and vein clearing symptoms in 7-9 days. The symptoms in infected plants with different mutant transcripts or wild-type began to disappear by 10-11 days post inoculation. Infected plants had no symptoms after two weeks and were indistinguishable from healthy plants. The effect of the inoculation in a systemically infected or symptomless leaf was determined by indirect ELISA using antiserum to AMV. The progeny of AMV derived from RNA transcriptase, with and without mutation, were purified from inoculated leaves. RNAs extracted from these particles were analysed by agarose gel electrophoresis and virus particles were also observed under the electron microscope (Jeol 1200 EX) (Fig. 8).

Effect of the Phe to Tyr and Asn to Gln mutation in aphid transmissibility of AMV: Mutated infectious

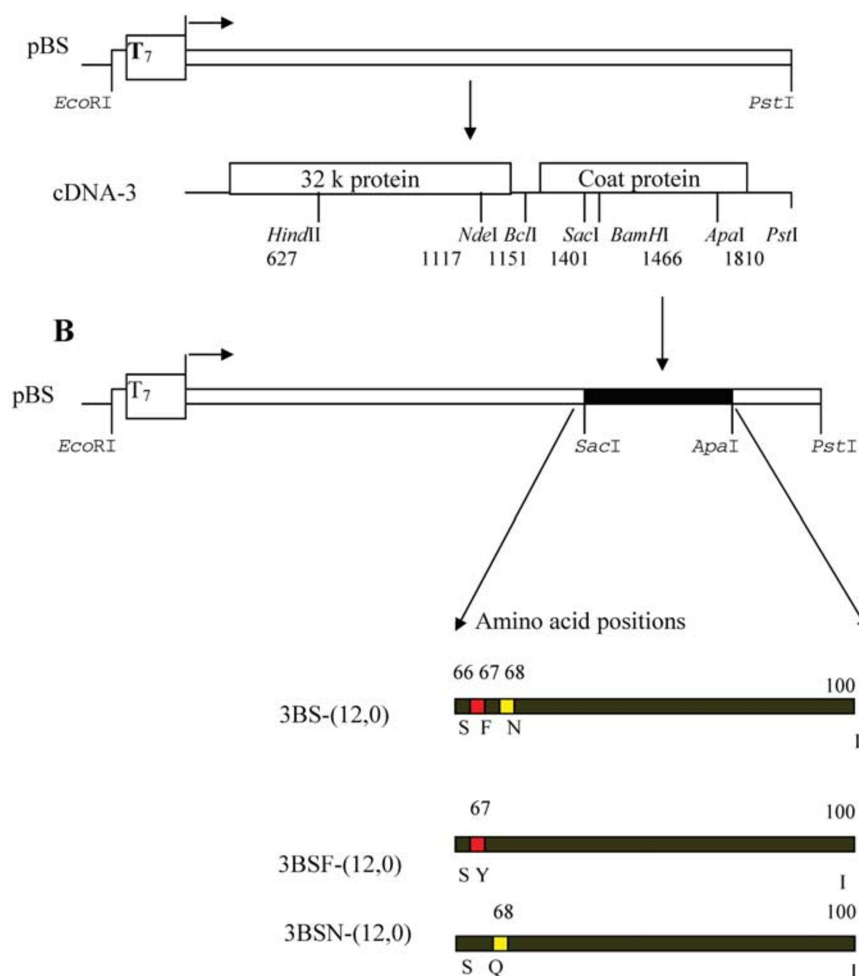

Figure 7. Schematic representation of the strategy for site-directed mutagenesis of the coat protein gene of AMV. (A) pBS plasmid containing a full length DNA copy of RNA 3. Only the cDNA is shown. The arrow on the T7 RNA polymerase promoter denotes the direction of transcription from *EcoRI* digested plasmids. Number at the bottom of cDNA-3 represents the positions of the respective restriction enzyme recognition sites using RESearch 1.0 (John Antoniw program, 1995) with respect to the RNA-3 (strain S). (B) The area of mutation containing the *SacI* to *ApaI* is indicated by black box. N- and C-terminal amino acids (66 and 100, respectively) of the coat protein are shown according to Ravelonandro *et al*., (1984). Numbers 67 and 68 represent the respective amino acid substitutions from the mutant clones designated 3BSF-(12,0) and 3BSN-(12,0). Relevant amino acids shown in single-letter code are, F, Phe; I, Ile; N, Asn; Q, Gln; S, Ser; Y, Tyr.

RNA transcripts were made to determine whether transmissibility of AMV could be blocked or inhibited by changing the two amino acids; Phe and Asn of the AMV coat protein in the S strain. The aphid transmissibility rate of the mutated AMV in Phe to Tyr was found to be 53% (16/30 test plants infected) (Fig. 5). The percentage transmissibility rate of original non-mutated AMV wild type was 83 % (25/30 test plants infected). This rate was nearly similar to that obtained by aphids fed on mutated AMV in Asn to Gln (24/30 test plants infected; 80% transmission). Therefore, this mutation (Asn to Gln) had drastically reduced aphid transmissibility. There was a significant difference in transmission rate between Phe to Tyr mutated AMV and wild type (P = 0.02).

DISCUSSION

The epitope mapping technique employed here is a very powerful method for localization of different epitopes on the coat protein. It was shown that the reactions of the synthetic octapeptides with monoclonal antibodies enabled the identification of five epitopes on the coat protein of AMV (strain S). A similar result has also been reported for epitopes of other virus coat proteins which were analysed by means of synthetic peptides as well as mono- and polyclonal antibodies (Torrance, 1992; Pereira *et al*., 1994; Commandeur *et al.,* 1994 and Andreeva *et al*., 1994).

Geysen *et al*. (1988) reported that, amino acid residues outside an epitope, may presumably either

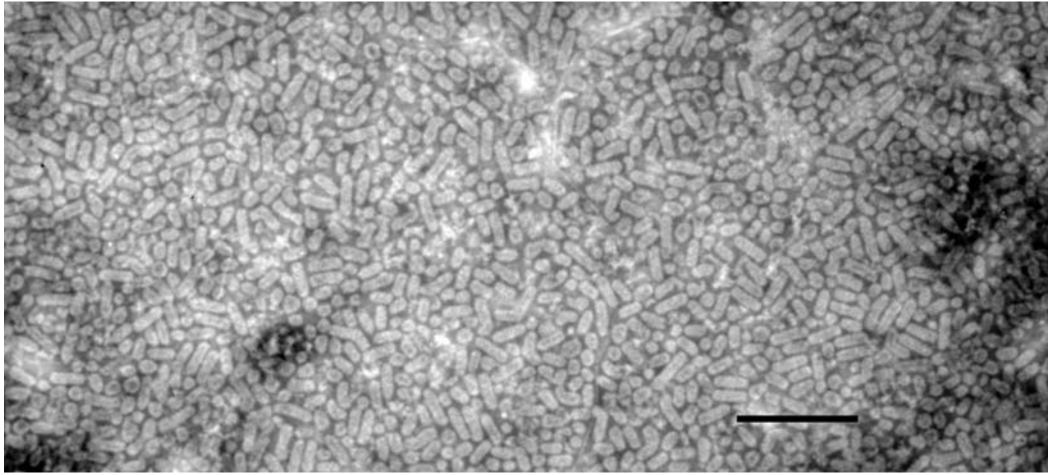

Figure 8. The virus particles of AMV strain S, visualised by negative stain in the electron microscope (Jeol 1200 EX). Bar represents 100 nm.

interfere with the folding of the epitope's amino acid chain or may influence the binding of an antibody due to their physical or physico-chemical properties, such as charge and hydrophilicity or –hydrophobic forces. A number of MAbs (MAbs-6, 7, 10,11,12,13 and 22) which had reacted with AMV coat protein in an immunoblotting test (Massumi *et al*., 2005), did not react with overlapping peptides on the surface of the membrane sheet. This might be due to the lack of supportive amino acid residues in the vicinity of the epitope or to conformational change which may be induced by the support matrix (Geysen *et al*., 1987b). Another likely possibility is that these MAbs may require more than eight amino acid residues to produce significant binding (Pereira de Carvalho, 1993).

MAb-1 reacted with two octapeptides in epitope 1. They were KAGGKAGK (amino acid residues 7-14; peptide 3) and GKPTKRSQ (residues 13-20; peptide 5). The strongest reaction was with octapeptide GKPTKRSQ ($A_{405\ nm}$ = 0.6 with the mean background reaction of 0.188 ± 0.007). MAb-17 reacted with two consecutive octapeptides SYRFNEVW (peptide 62) and FNEVWVER (peptide 63) representing residues 184-191 and 187-194, respectively. The strongest reaction was with FNEVWVER ($A_{405\ nm}$ = 0.32 with the mean background reaction of 0.062 ± 0.005) which seems to play a key role in formation of epitope 5 (Table 2). In contrast MAb-2 reacted only with octapeptide PLSLSSFN ($A_{405\ nm}$ = 0.6 with the mean background reaction of 0.205 ± 0.005). This octapeptide (residues 61-68; peptide 21) also reacted with PAbs (data not shown) and is evidently an important part of epitope 2 (Table 2). In addition, MAb-24 reacted with HGAFHANE ($A_{405\ nm}$ = 0.33 with the mean background reaction of 0.076 ± 0.005) (peptide 45) representing residues 133-140. This residue also reacted with PAb which is apparently an important constituent of epitope 3. Andreeva *et al*. (1994) reported that MAb A5B6 reacted with two octapeptides, in the pepscan test, in different parts of the coat protein of potato virus A. The result obtained from the experiments performed with MAb-28 was different from the other MAbs, because of the discontinuity of its reactivity with the overlapping octapeptides. It had reacted highly with peptide 43 (PMQEFPHG; residues 127-134) ($A_{405\ nm}$ = 0.24 with the mean background reaction of 0.048 ± 0.003) and also reacted weakly with peptide 35 (SHAGTFCL, residues 103-110) ($A_{405\ nm}$ = 0.1) in two different parts of the coat protein. One possible explanation is that the epitope is in two parts which are brought together by folding of the polypeptide chain but each of which has some reactivity on its own.

It has been known for a long time that virus coat proteins are involved in vector transmission of many viruses (see Harrison and Robinson, 1988; Gray, 1996 for reviews). The present result confirm that coat protein determines aphid transmissibility of AMV. It was concluded that genomic RNA 3 which encodes viral coat protein and one other protein (the movement protein, P3) determines the ability of AMV to be transmitted by aphids.

RNA1 and RNA2 of AMV encode proteins P1 and P2, respectively, both of which have a putative role in viral RNA replication. Tobacco plants were transformed with DNA copies of RNA1 and RNA2 which resulted in transgenic P12 plants. Therefore, transgenic P12 plants were able to replicate RNA3 (Taschner *et al.,* 1991).

The influence of amino acids in positions 67 and

68 of the AMV coat protein on transmission by *M. persicae* was tested. The two mutations were introduced into the coat protein gene and had different effects on AMV transmission; mutant 3BSN-(12,0) was transmitted with the same frequency as wild–type virus (Fig. 5), whereas mutant 3BSF-(12,0) was transmitted with a lower frequency. The data obtained in aphid transmissibility study indicates that in mutant 3BSN-(12,0) the amino acid Asn, at position 68 in the coat protein, is not involved in transmission. Whereas, in mutant 3BSF-(12,0) amino acid Phe, at position 67 in the coat protein, is involved in AMV transmission. Thus, it seems reasonable to conclude that a Phe residue at the epitope-2 may be critical for aphid transmissibility.

Perry *et al*. (1994) comparing the predicted amino acid sequences of the coat proteins of different strains of CMV suggested that either a single amino acid or possibly a few amino acids may be involved in aphid transmission of CMV. It can also be assumed that Phe is not the only amino acid which is important in AMV transmission, because substitution of this amino acid only resulted in reduction and not transmission blocking. Further explanation for transmission blocking by alteration of other amino acids situated within the epitope-2 or other parts of the AMV CP.

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