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Molecular Identification of viruses infecting Sugarcane in Nanning and Preparation of Antiserum against *Sorghum mosaic virus*

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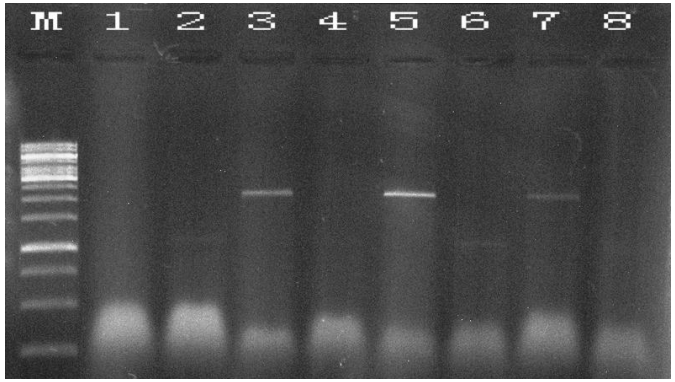
- Why…
- Investigate the viral diseases of sugarcane in Nanning region
- Virus diversity
- Setup a fast, simple, economic diagnosis method.
- Screen and evaluate resistant cultivars.



1. Molecular Identification of viruses infecting Sugarcane in Nanning region

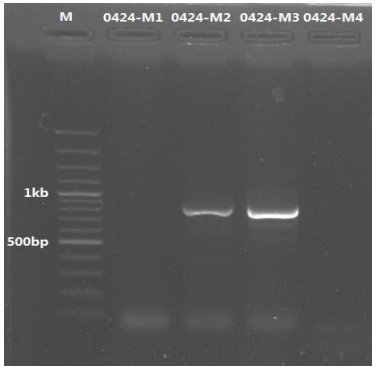
Table 1. Primers design of virus infected sugarcane

Virus	Type	Taxonomic status	Primers	References
Potyviridae	+ssRNA	*Potyvirus*	degenerate primers	Chen J. et al., 2001
Sugarcane yellow leaf virus, ScYLV	+ssRNA	*Luteoviridae*	YLCPF, YLCPR	DQ768748, AY236971
Sugarcane fiji disease virus， SFDV	dsRNA	*Fijivirus*	FF, FR	McQualter et al., 2004
Maize dwarf mosaic virus，*MDMV*	+ssRNA	*Potyvirus*	D2F, D2R	AM110758
Sugarcane chlorotic streak virus, SCSV	dsDNA	*Badnavirus*	CS1F, CS1R	NC_001634, U13988
Sugarcane streak virus，SSV	ssDNA	*Mastrevirus*	S1F, S1R	Shamloul et al., 2001
Sugareane baeilliform virus，SCBV	dsDNA	*Badnavirus*	B1F, B1R	AJ277091, M89923



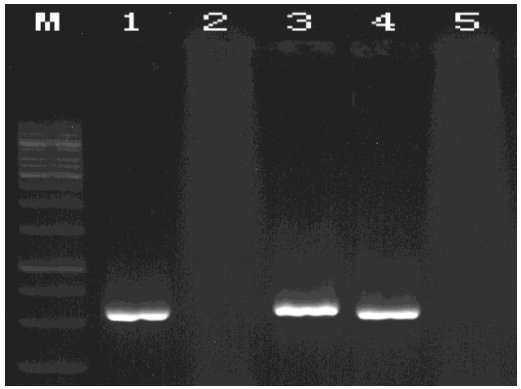
1.8kb

A



800bp

B



600bp

C

Sorghum mosaic virus (SrMV)
Sugarcane yellow leaf virus (ScYLV)
Sugarcane mosaic virus (SCMV)

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Fig. 1. RT-PCR amplification results of *Potyviridae* specific degenerate primers (A) and specific primers (B, C)

2. Phylogenetic analysis of the cp gene of Sugarcane yellow leaf virus

Genbank No.	Location	References
AM072750	Brazil	Abu Ahmad,Y., Rassaby, L., Royer, M.,et al.,2006
AF157029	Florida	Moonan,F., Molina,J. and Mirkov,T.E.,2000
AM072751	China	Abu Ahmad,Y., Rassaby, L., Royer, M.,et al.,2006
AY236971	India	Gaur,R.K., Rao,G.P., Singh,M.
GU190159	China	Gao,S.J., Wang,Q.N., Chen,R.K.,et al.
AJ249447	USA	Smith,G.R., Borg,Z., Lockhart,B.E., et al.,2000
AM072753	Peru	Abu Ahmad,Y., Rassaby, L., Royer, M.,et al.,2006
AM072752	Peru	Abu Ahmad,Y., Rassaby, L., Royer, M.,et al.,2006
GU570007	Germany	Elsayed,A.I., Weig,A.R., Komor,E.
AM072756	Reunion	Abu Ahmad,Y., Rassaby, L., Royer, M.,et al.,2006
AM072755	Reunion	Abu Ahmad,Y., Rassaby, L., Royer, M.,et al.,2006
AM072754	Reunion	Abu Ahmad,Y., Rassaby, L., Royer, M.,et al.,2006
GU570008	Germany	Elsayed,A.I., Weig,A.R., Komor,E.
GU570006	Germany	Elsayed,A.I., Weig,A.R., Komor,E.
GU327735	China	Wang,M.Q. and Zhou,G.H.

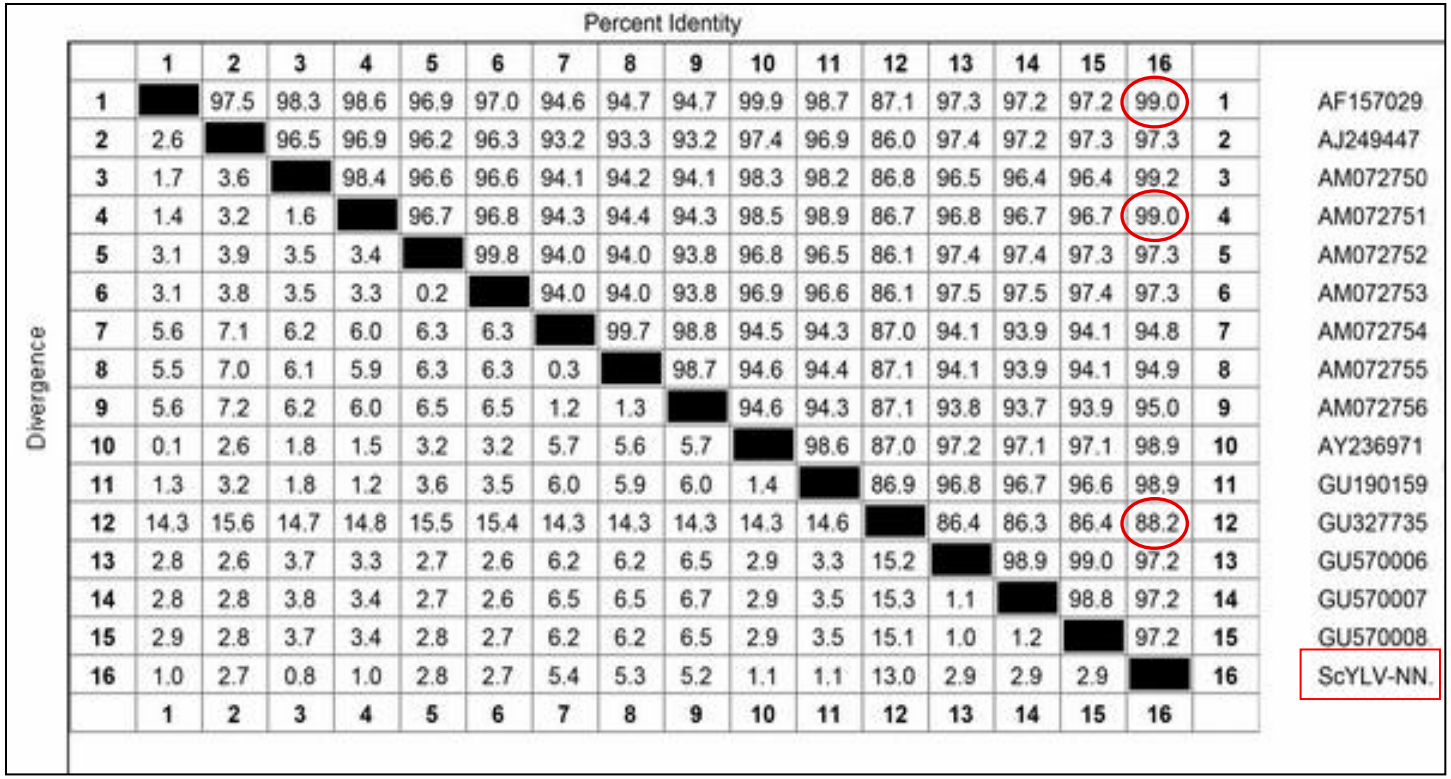


Fig.2. nucleotide sequence homology of cp gene of SYLV

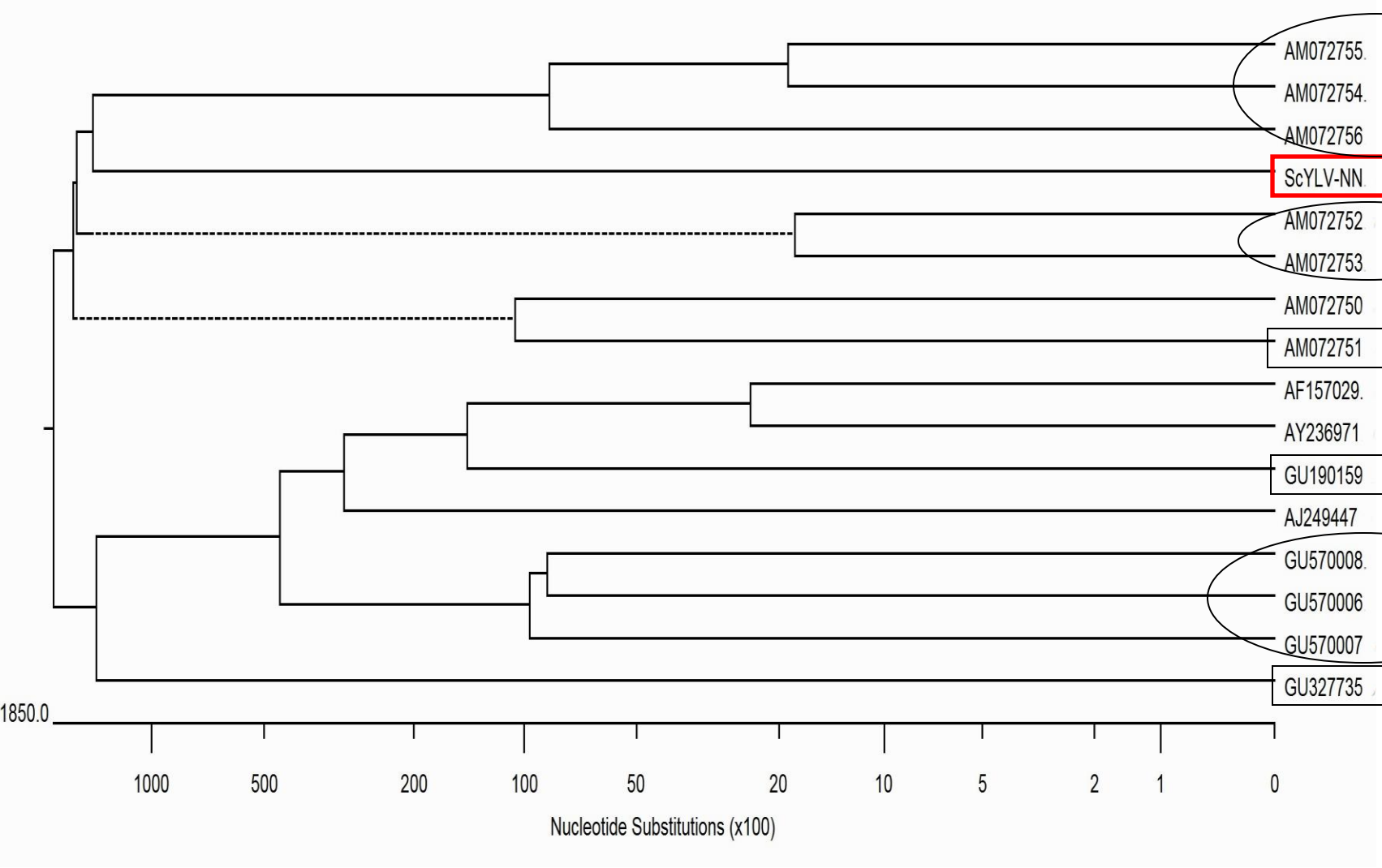


Fig.3. Phylogenetic tree of ScYLV

3. Genomic sequence analysis of the Nanning strain of the Sorghum mosaic virus

- 1). the strategy of cloning and sequencing of the genome of Sorghum mosaic virus

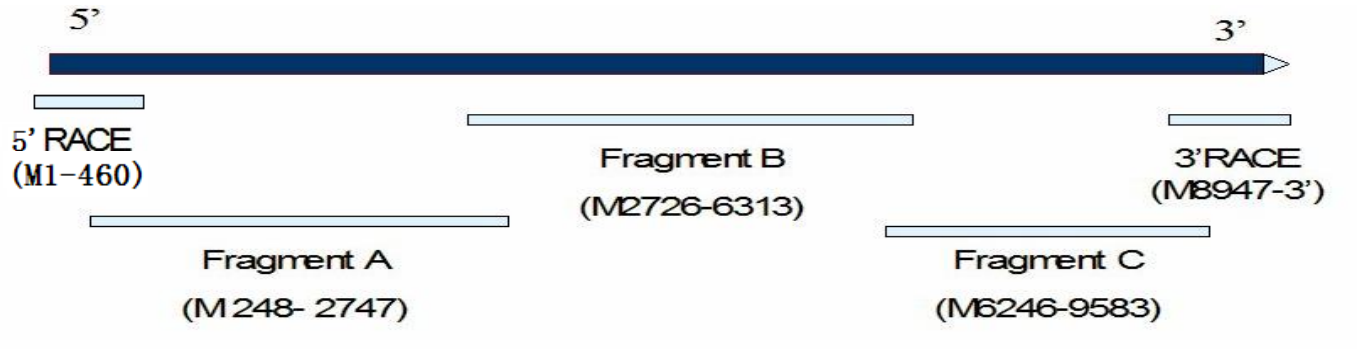
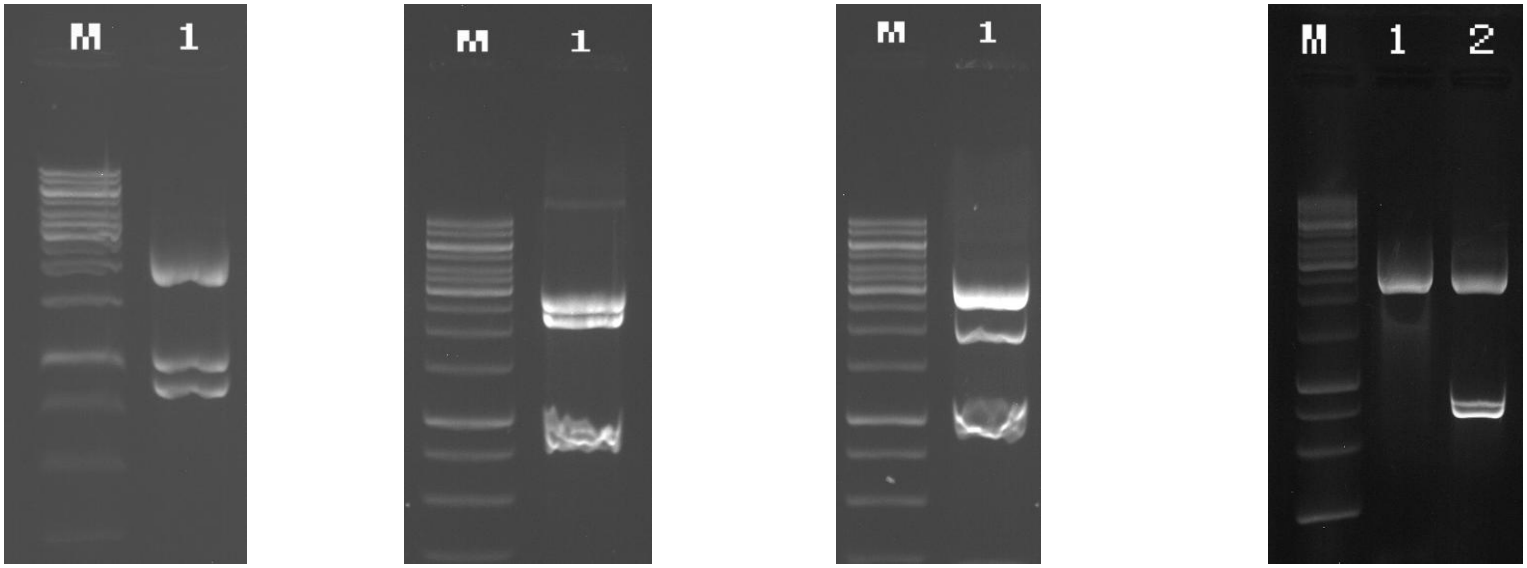


Fig. 4 the strategy of cloning and sequencing of the genome of Nanning strain of Sorghum mosaic virus (SrMV-NN)

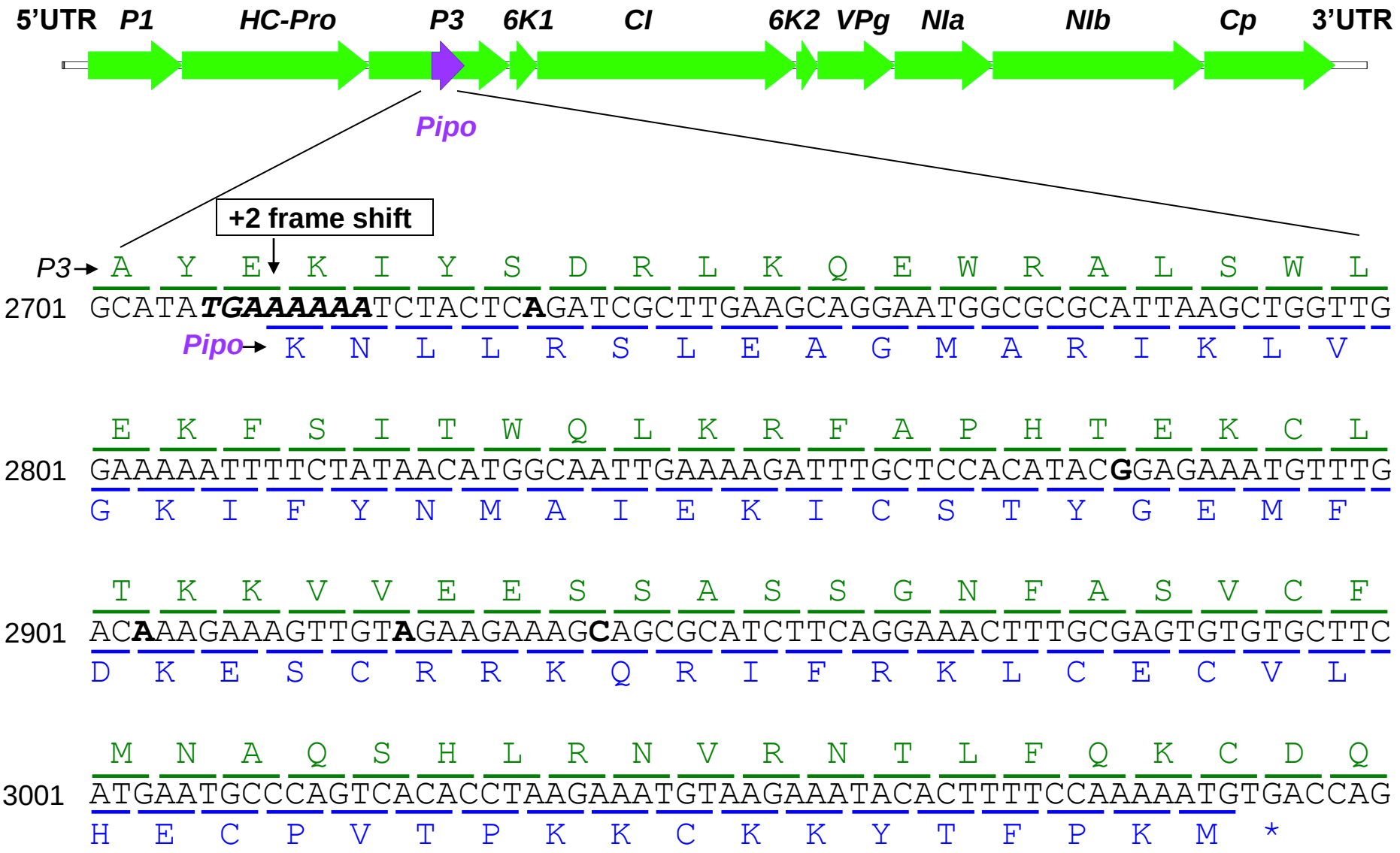
2). RT-PCR, cloning, identification



(A) M:Marker, 1: p-MA/BglⅡ (B) M:Marker, 1: p-MB/BglⅡ (C) M:Marker, 1: p-MC/BglⅡ (D) 1:pM3', 2: pM3'/BglⅡ, M:Marker,

Fig. 5. Digestion analysis of recombinant plasmid of pJET-MA, pJET-MB, pJET-MC and pJET-M3'

3). A new small embedded ORF *pipo*???



4). Phylogenetic analysis based on the whole genome of SrMV

Table 3-3 Genes were used in genetic diversity analysis of SrMV

Genbank No.	Location	References
AJ310197	Zhejiang Xiaoshan, China	Chen,J,et al.,2002
NC_004035	Zhejiang Xiaoshan, China	Chen,J,et al.,2002
AJ310198	Zhejiang Yuhang, China	Chen,J,et al.,2002
U57358	Texas, USA	Yang, Z N,et al.,1997

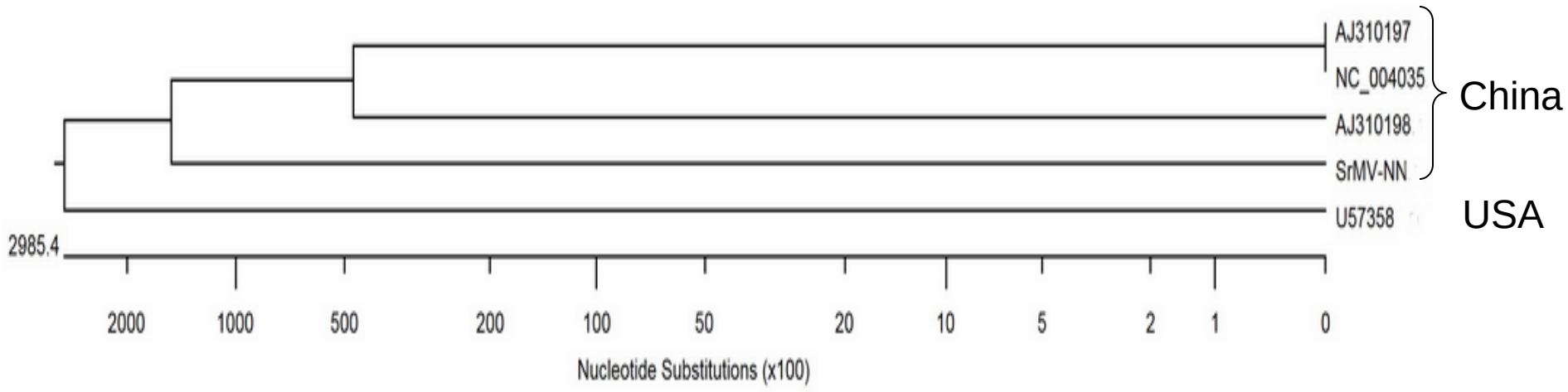
Percent Identity

Divergence

	1	2	3	4	5	
1		95.8	100.0	95.8	80.7	1
2	4.4		95.8	94.8	80.8	2
3	0.0	4.4		95.8	80.7	3
4	4.4	5.5	4.4		80.9	4
5	22.8	22.6	22.8	22.6		5
	1	2	3	4	5	

AJ310197 Xiaoshan
AJ310198 Yuhang
NC_004035 Xiaoshan
SrMV-NN Nanning
U57358 Texas

Fig.3-8 SrMV nucleotide sequence homology



Phylogenetic tree inferred from parsimony analysis of polyprotein of SrMV isolates

4. Preparation of Antiserum against Sorghum mosaic virus

SrMV CP gene expression by prokaryotic expressing system

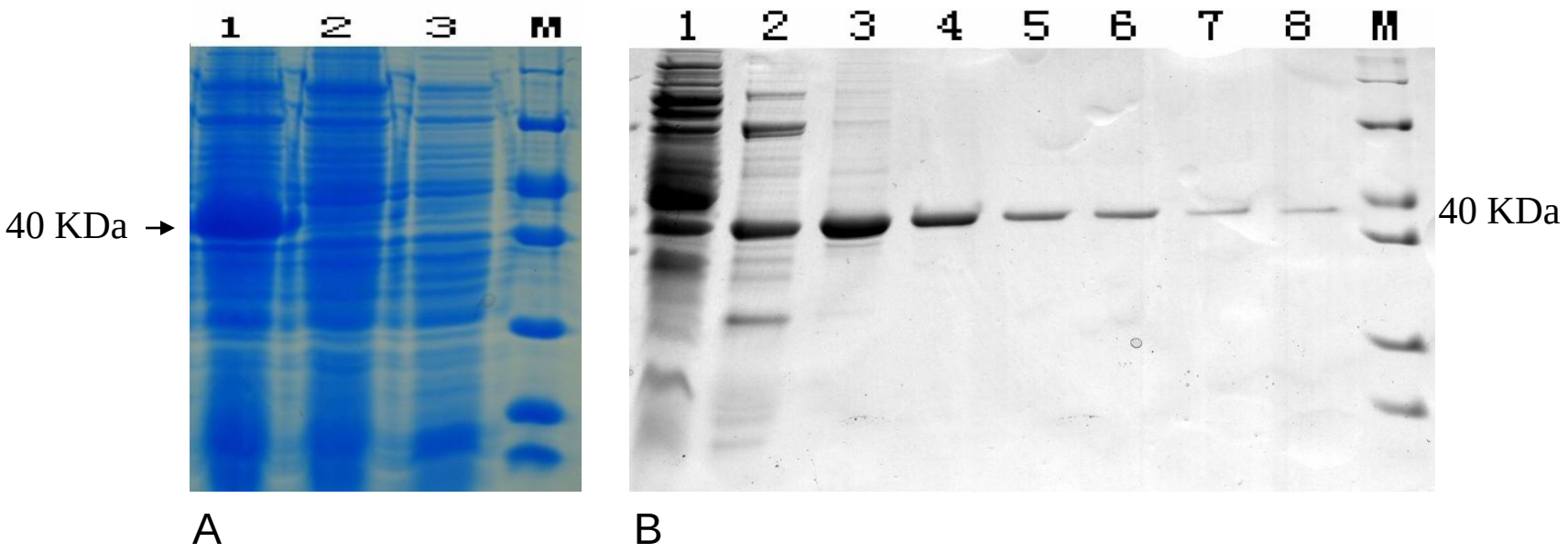


Fig. 8. A. Western blot of recombinant SCMV-SC coat protein inducibly expressed in *E. coli*. M： Marker. 1: protein from *E. coli* containing recombinant plasmid pET30a-SrMVCP, induced 2: uninduced. 3: protein from *E. coli* containing original plasmid pET30a.
B. purified SrMV-NN coat protein. M： Marker. 1, protein from *E. coli* containing recombinant plasmid pET30a-SrMVCP, induced. 2-8, recovery protein by different washing times.

Detection of SrMV in plant material by Immunoblot

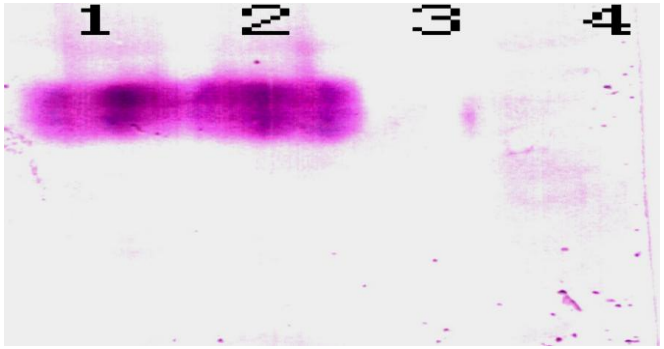


Fig. 9. Immunoblot analysis of non-infected (3-4) and SYLV-infected (I-2) sugarcane with anti-CP antiserum.

Acknowledgements

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- **Yongmei Liao** — **Professor**