Kajian Sifat Bioekologi dan Biomolekuler Virus Mosaik Bengkuang di Indonesia

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Utilize

1. COSMETICS
2. SALADS
3. SNACK
4. PICKLES
5. Leaves - soil covers at plantations
6. Fertilizer - form rhizobium to bind Nitrogen
7. Seed – botanical insecticide

SYMPTOMS

Mosaic

Leaf green vein-banding

Leaf malformation

Bean malformation

Objective

- Karakterisasi bioekologi dan biomolekuler penyebab mosaik pada bengkuang
- Respon beberapa kultivar bengkuang terhadap infeksi virus

Informasi fundamental sebagai landasan untuk penentuan strategi pengendalian

INCIDENCE in YAMBEAN FIELDS at BOGOR (WEST JAVA)

Location	Plant Age (WAP)	Infected Plant (n)	Total Plants (N)	Incidence (%)
Babakan Raya	4	301	2126	14.16
Cibeureum 1*	16	56	60	93.33
Cibeureum 2	12	6627	8138	81.43
Cifor	8	1344	5051	26.61
Situgede	20	4610	4610	100.00

WAP - Week after Planting
* Plants for seed production

INCIDENCE in YAMBEAN FIELDS at PREMBUN (CENTRAL JAVA)

Location	Plant Age (WAP)	Infected Plant (n)	Total Plants (N)	Incidence (%)
Kedung Bulus 1	16	2.500	2.500	100
Kedung Bulus 2	8	1.440	7.200	20
Kedung Bulus 3	12	788	2.250	35
Mulyo Sri	10	6.000	6.000	100
Sembir Kadipaten	14	2.304	3.840	60

BIOLOGICAL CHARACTERS

1. TRANSMISSION MODES

a. Mechanically transmitted

b. Insect Vector (Aphids)

A. craccivora, Koch.

A. glycines, Mats.

A. gossypii, Glov.

Aphid free virus

Overnight in Taro

New nymph

A. craccivora → Cowpea

A. glycines → Soybean

A. gossypii → Hot pepper

Virus Transmission

Aphid

Starving 1 hr

Acquisition feeding

Inoculation feeding

Observation until 30 dpi

Inocula

Healthy yam bean

Treatment	Incubation period (dpi)	Incidence (n/N)
Control*	0	0/10
A. craccivora	12-17	10/10
A. glycines	14-28	7/10
A. gossypii	13-17	10/10

* Inoculated by virus free aphid

Healthy

Inocula

Transmission by *A. craccivora*

Healthy

Inocula

Transmission by *A. gossypii*

Healthy

Inocula

Transmission by *A. glycines*

c. Seed detection

100 seeds/ location

Seedling

Young leaf sampling

Dot blot

Positive samples

Reconfirmed by ELISA

Source	Absorbance Value ELISA**	Result
Bufer	0,227	
Healthy	0,324	
Infected leaves	0,506	
West Java 1	0,487	+
West Java 2*	0,690	+
West Java 3	0,622	+
East Java	0,556	+

* Seed taken from infected plants

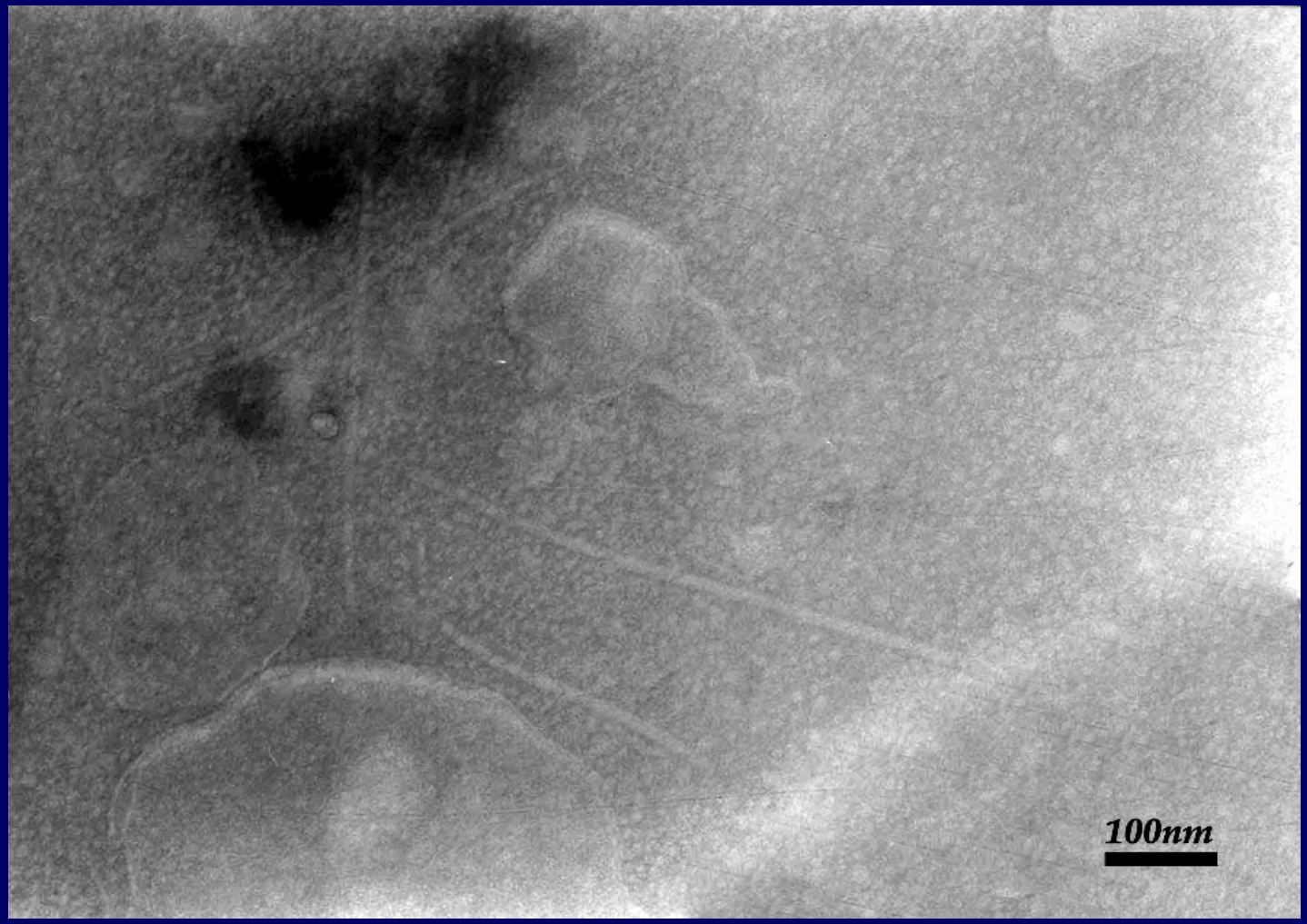
** DAS-ELISA using BCMV antisera (DSMZ)
Positively if AVE 1.5 x healthy

2. SEROLOGYCAL TEST

VIRUS GROUP	ANTISERA	AVE*				
		Buffer	Positive control	Negative Control	Sample	Results
Tobamovirus	TMV	0,184	0,517	0,185	0,195	-
Cucumovirus	CMV	0,196	0,347	0,188	0,199	-
Comovirus	SqMV	0,135	0,447	0,266	0,355	-
Potyvirus	ChiVMV	0,184	1,738	0,193	0,191	-
	ZYMV	0,150	1,543	0,193	0,328	+
	PVY	0,124	0,372	0,136	0,127	-
	WMV-2	0,176	2,429	0,276	1,086	+
	PRSV-W	0,152	0,978	0,371	0,243	-
	PStV	0,263	4.400	0,268	0,834	+
	TuMV	0,169	0,613	0,389	0,392	-
	General Potyvirus	0,138	2,356	0,141	2,359	+

*AVE = absorbance value ELISA

3. Virus Particles



Electron micrograph of “YbMV” particles from infected Leaf (leaf dip method), negatively stained with PTA

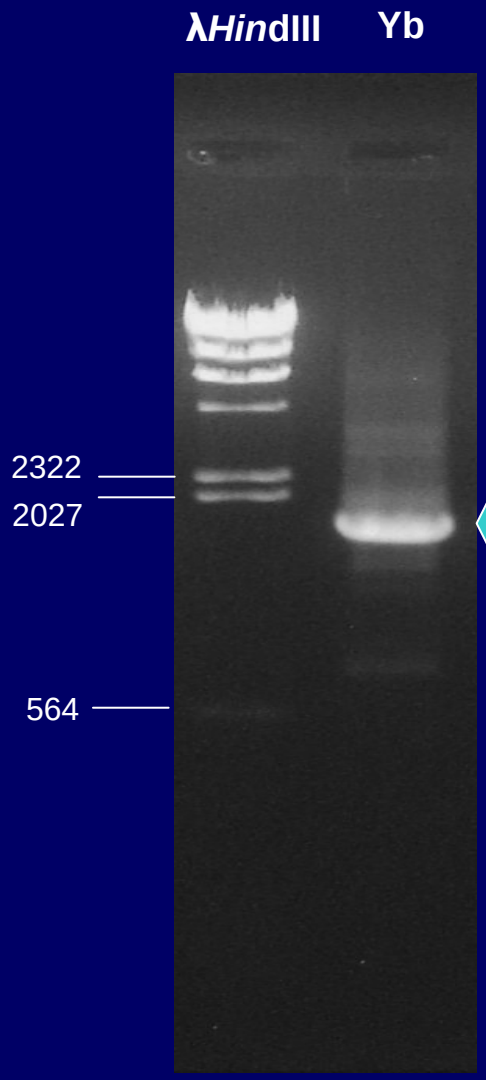
4. HOST RANGE TEST

Plant Species	Incubation periods (day)	Symptom	ELISA test
Solanaceae			
Capsicum annuum	-	-	-
Lycopersicon esculentum	13-15	CS	+
Nicotiana tabacum	-	-	-
Leguminoseae			
Vigna unguiculata	21-23	CS	+
Phaseolus vulgaris	10-12	MS	+
Arachis hypogea	-	-	-
Cucurbitaceae			
Cucumis sativus	-	-	-
Cucumis melo	-	-	-
Chenopodiaceae			
C. amaranticolor	-	-	-
Compositae			
Ageratum conyzoides	-	-	-
Amaranthaceae			
Gomphrena globosa	-	Lt	+

* CS= Chlorosis systemic; MS = mosaic systemic; Lt = latent infection

MOLECULAR DETECTION

Potyvirus Genome Structure



Infected leaves

Total RNA Extraction
(Qiagen kit)

cDNA (M4T) ← RT-PCR

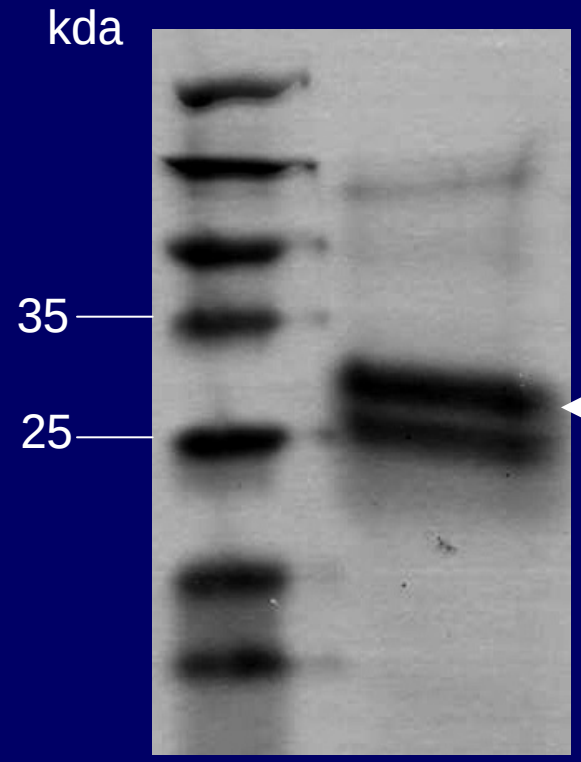
~1.7 kb → DNA isolated from gel

DNA Purification

Bulk Sequencing

Seq. analysis
(DNASIS software for Mac. System)

PROTEIN ANALYSIS
SDS-PAGE



Infected leaves

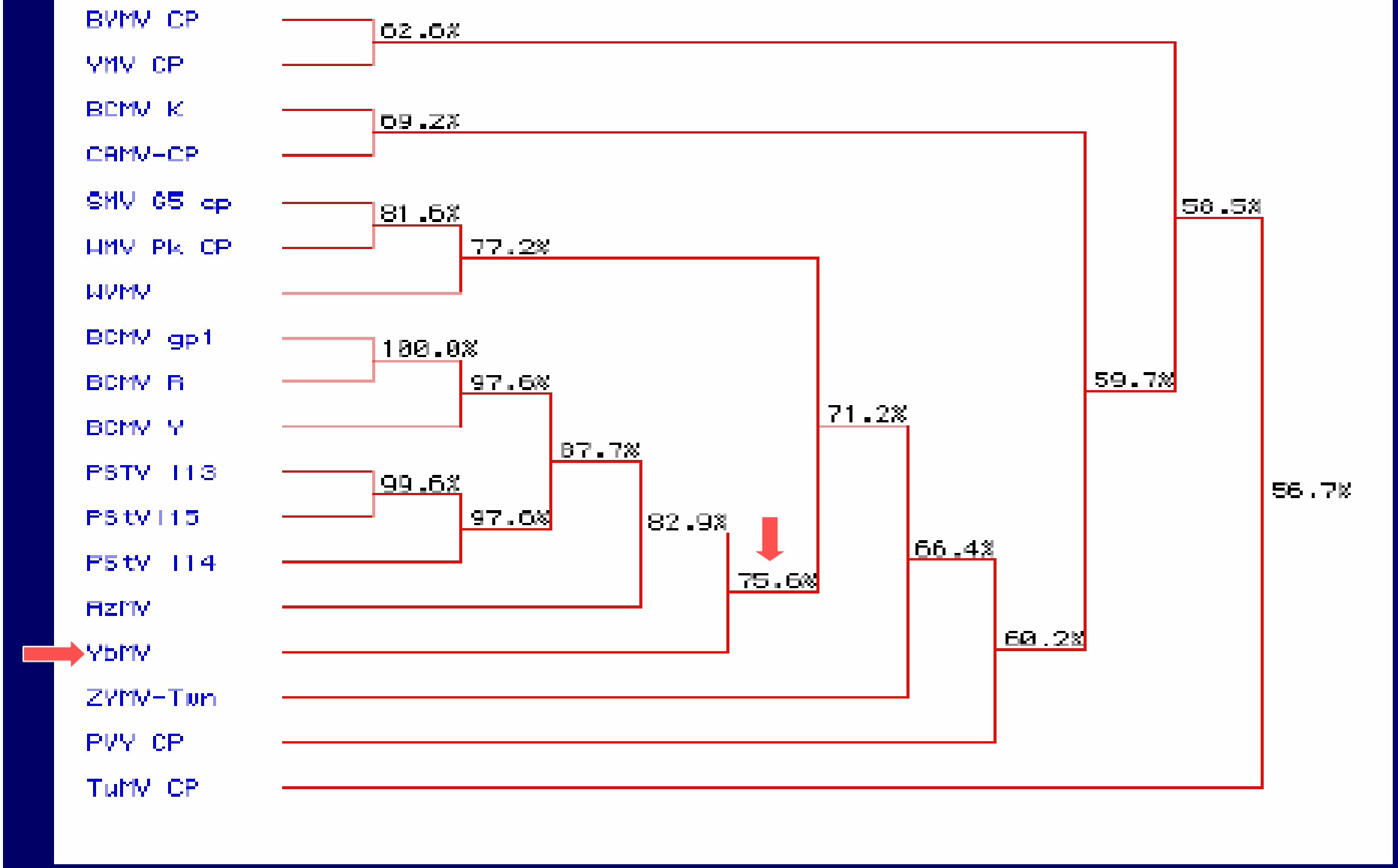
↓

Viral Purification

↓

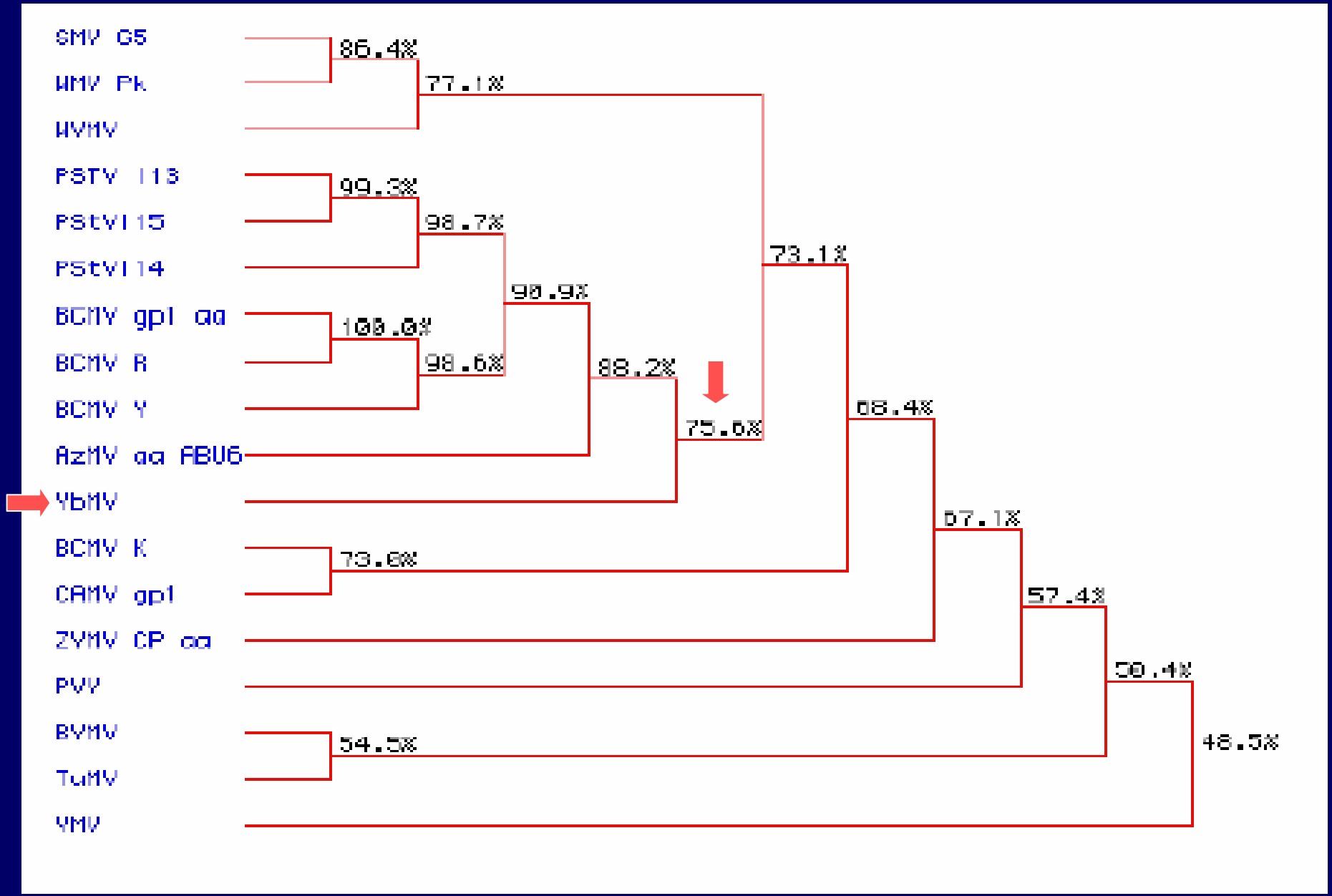
Virion

CP nucleotide alignment (75.6%)



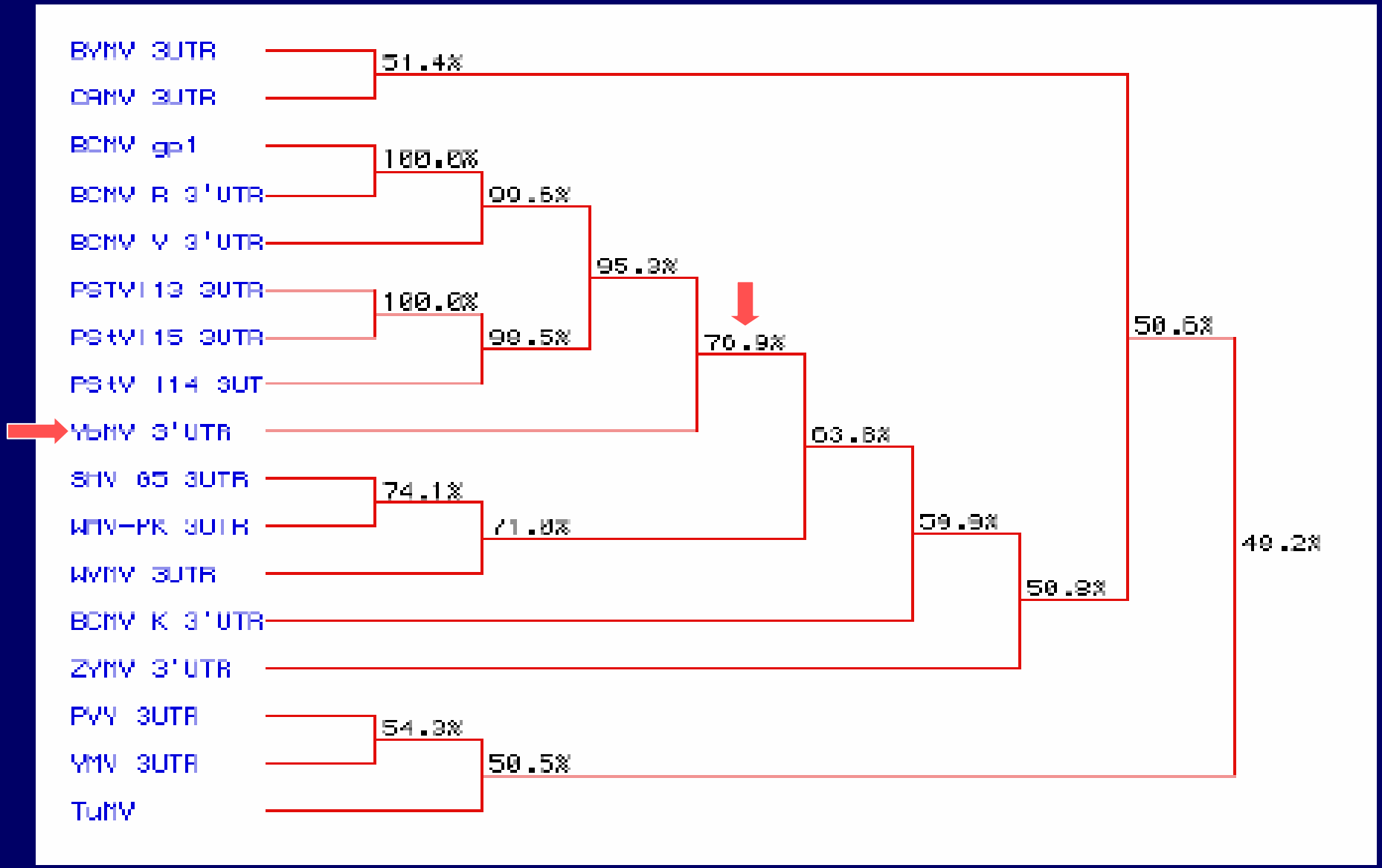
Seq. aligned together against “YbMV”

CP Amino Acid Alignment (75.6%)



Seq. aligned together against “YbMV”

3’-UTR nucleotide Alignment (76.9%)



Seq. aligned together against “YbMV”

Seq. identities of CPs and 3'UTR between YbMV and other potyviruses

Virus	Coat Proteins		3' UTR	Accession no.
	Amino acid	Nucleotide		
AzMV	75.1	73.7	75.1	ABU60100
BCMV-gp1	76.0	74.6	75.8	NC003397
K	69.2	49.1	56.6	AY864314
R	76.0	51.7	75.4	AJ312437
Y	75.7	52.1	74.7	AJ312438
NL1	75.7	75.4	76.7	AY968604
BYMV	49.8	60.1	52.0	NC003492
CabMV	64.3	65.8	48.0	NC004013
PStV I13	76.8	77.4	78.6	AJ132156
I14	76.4	77.2	78.2	AJ132157
I15	76.4	77.2	78.7	AJ132158
PVY	54.6	59.4	43.7	NC001616
SMV	74.3	72.3	59.8	AY294044
TuMV	47.1	56.4	44.6	NC002509
WMV	68.8	70.8	61.8	AB218280
WVMV	72.1	72.8	63.4	NC007216
YMV	46.3	57.1	52.4	NC004752
ZYMV	68.8	66.1	51.6	NC003224

Seq. aligment was compared one by one with "YbMV"

Species demarcation in Potyvirus

Shukla & Ward (1989) & Frenkel (1989) similarity between strain of a potyvirus in CP nucleotide sequences showed over 90% and 3'UTR 83-99% homology

Adam *et al* (2005) reviewed from many published papers; demarcation of CP Nucleotide identity optimal was 76-77% and CP amino acid identity over 79.6% In same species

"YbMV" CP nucleotide, amino acid, 3'UTR (77.4%, 76.8% and 78.6%) closely with BCMV strain peanut stripe

New strain of BCMV (BCMV strain yam bean) and/or new potyvirus proposed as

YbMV

Response 5 Yam-bean cultivars Against Viral Infection

No.	Cultivar	Incidence	Severity	NAE	Response
1.	Jawa Tengah	18/25	1.7778c	0.9666b	+
2.	Hideung	15/25	2.6923b	1.7990a	++
3.	Porselen	18/25	2.7222b	1.9983a	++
4.	Paris	12/25	3.6364a	2.2070a	+++
5.	Kapas	13/25	2.3077bc	2.1517a	+++

NAE = ELISA absorbance value

CONCLUSION

1. The mosaic disease on yam bean caused by a Potyvirus, with proposed name Yam bean Mosaic Virus (YbMV) based on nucleotide sequences and biological characters

2. YbMV transmitted mechanically, through seed, and at least via 3 species of aphids

3. Long bean, tomato, *P.vulgaris* and *G. globosa* able to infected by virus

4. Response of yambean cultivars against viral infection varied, cultivar from Central Java considered relative resistant than others.

Acknowledgement

1. Competition Research fund B of Department of Plant Protection, BAU from Directorate of Higher Education (Partially)

2. Fundamental research grant from Directorate of Higher Education

3. Prof. Dr. Tetsuro Okuno and Dr. K. Mise, Laboratory of Plant Pathology, Graduate School of Agriculture, Kyoto University for research facilities, suggestions and technical supports

4. Ms Desmiarti, Ms. Siti Nurlaelah and Mr. Edi Supardi for for their invaluable supports


Thank you