



# **WELCOME TO BOGOR AGRICULTURAL UNIVERSITY**



# **A MOSAIC DISEASE INFECTING YAMBEAN; MOLECULAR DETECTION AND TRANSMISSION**



**Tri Asmira Damayanti\* and Siti Nurlaelah\***

**\*Department of Plant Protection,  
Bogor Agricultural University  
Bogor, Indonesia**



## 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



Leaf green vein-banding



Mosaic



**Bean malformation**



**Leaf malformation**

## INCIDENCE in YAMBEAN FIELDS at BOGOR (WEST JAVA)

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

WAP - Week after Planting

\* Plants for seed production

## INCIDENCE in YAMBEAN FIELDS at PREMBUN (CENTRAL JAVA)

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

# BIOLOGICAL CHARACTERS

## 1. TRANSMISSION MODES

- a. Mechanically transmitted
- b. Insect Vector (Aphids)



*A. craccivora*, Koch.

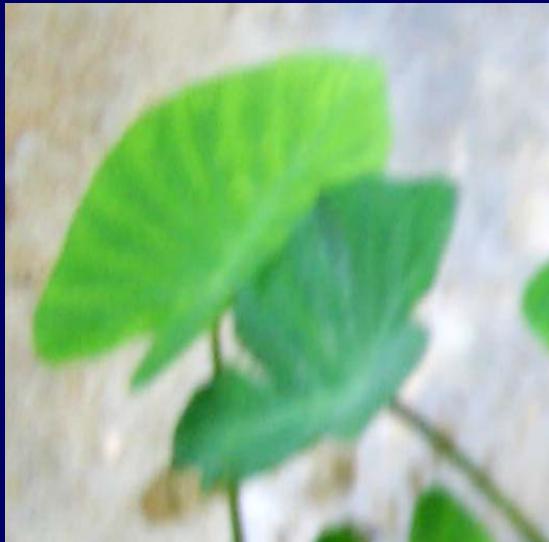


*A. glycines*, Mats.



*A. gossypii*, Glov.

## Aphid free virus



↓  
Overnight in Taro

↓  
New  
nymph

*A. craccivora*

Cowpea



*A. glycines*

Soybean

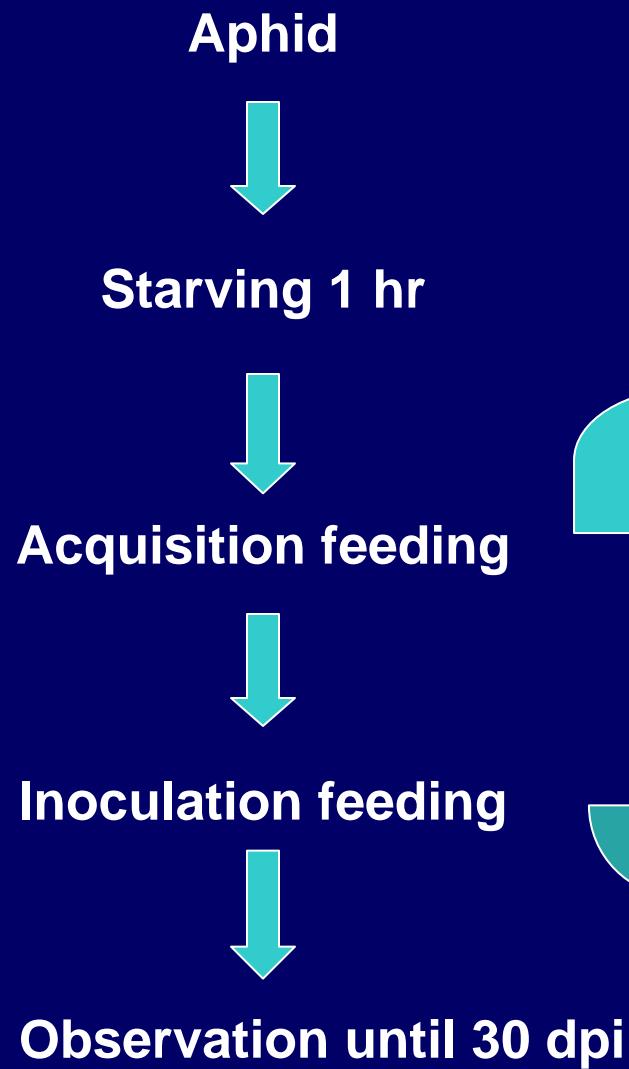


*A. gossypii*

Hot pepper



# Virus Transmission



Inocula



Healthy yam bean

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

\* Inoculated by virus free aphid

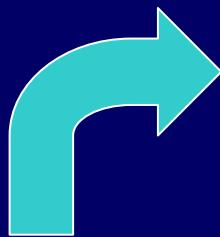
**Healthy**



**Inocula**



**Transmission by *A. craccivora***



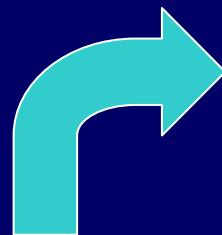
**Healthy**



**Inocula**



**Transmission by *A. gossypii***



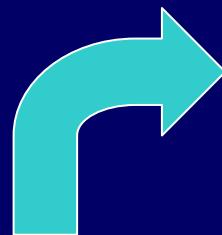
**Healthy**



**Inocula**



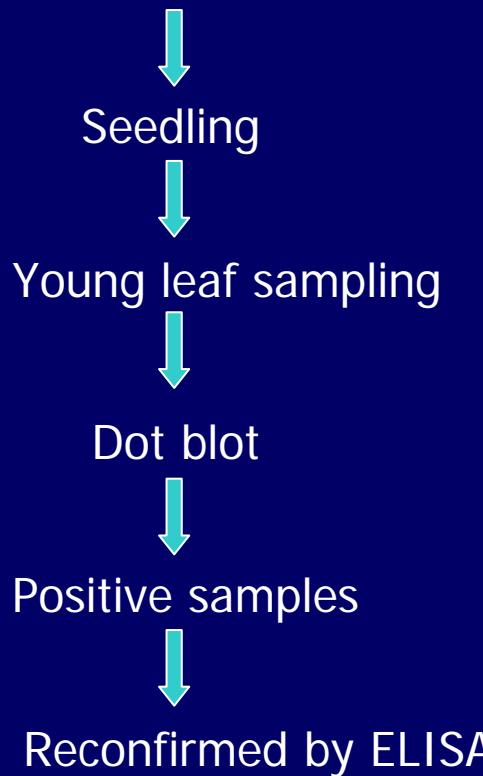
Transmission by *A. glycines*



## c. Seed detection



100 seeds/  
location



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)

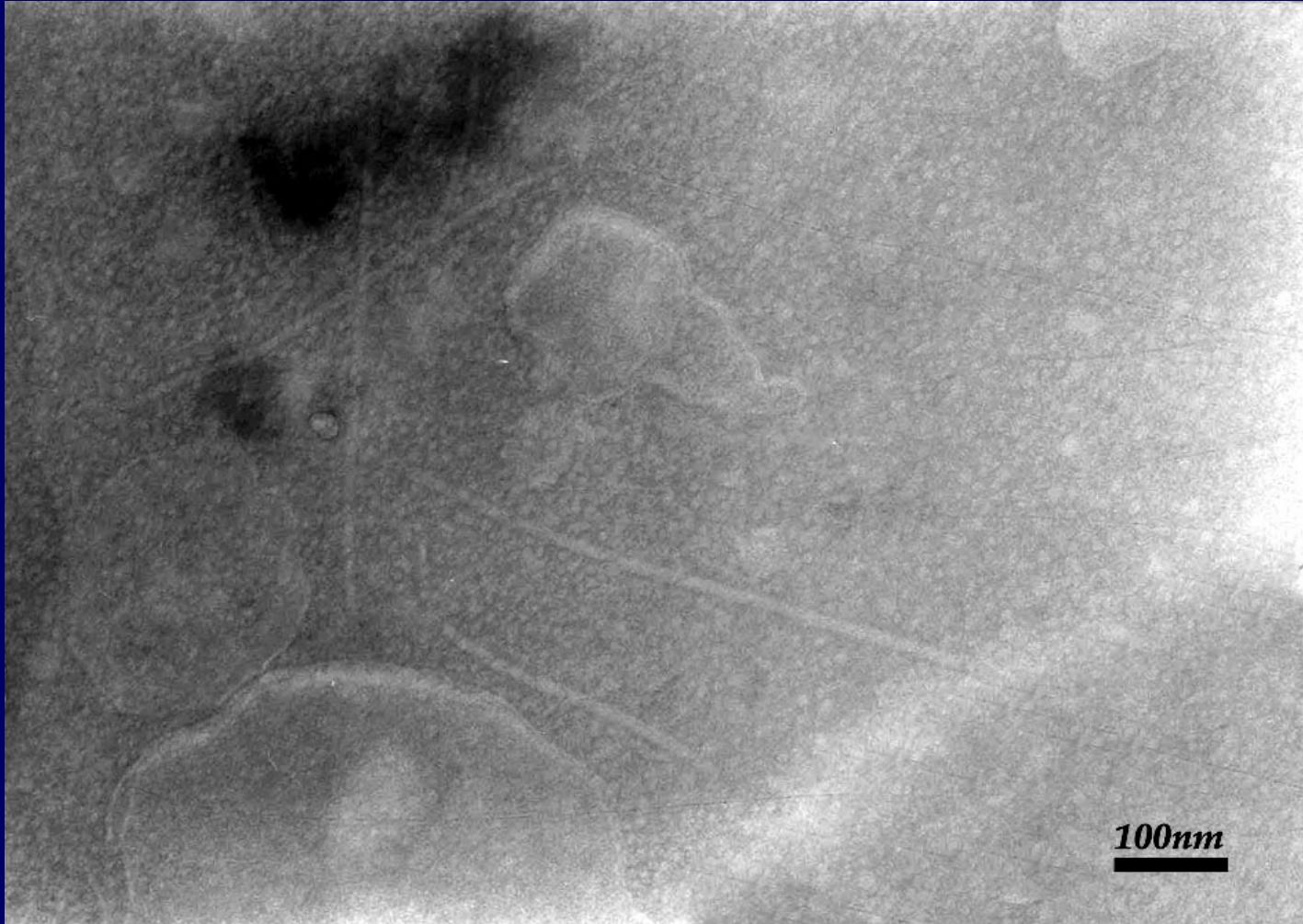
Positively if AVE 1.5 x healthy

## 2. SEROLOGICAL TEST

VIRUS GROUP	ANTISERA	AVE*				Results
		Buffer	Positive control	Negative Control	Sample	
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

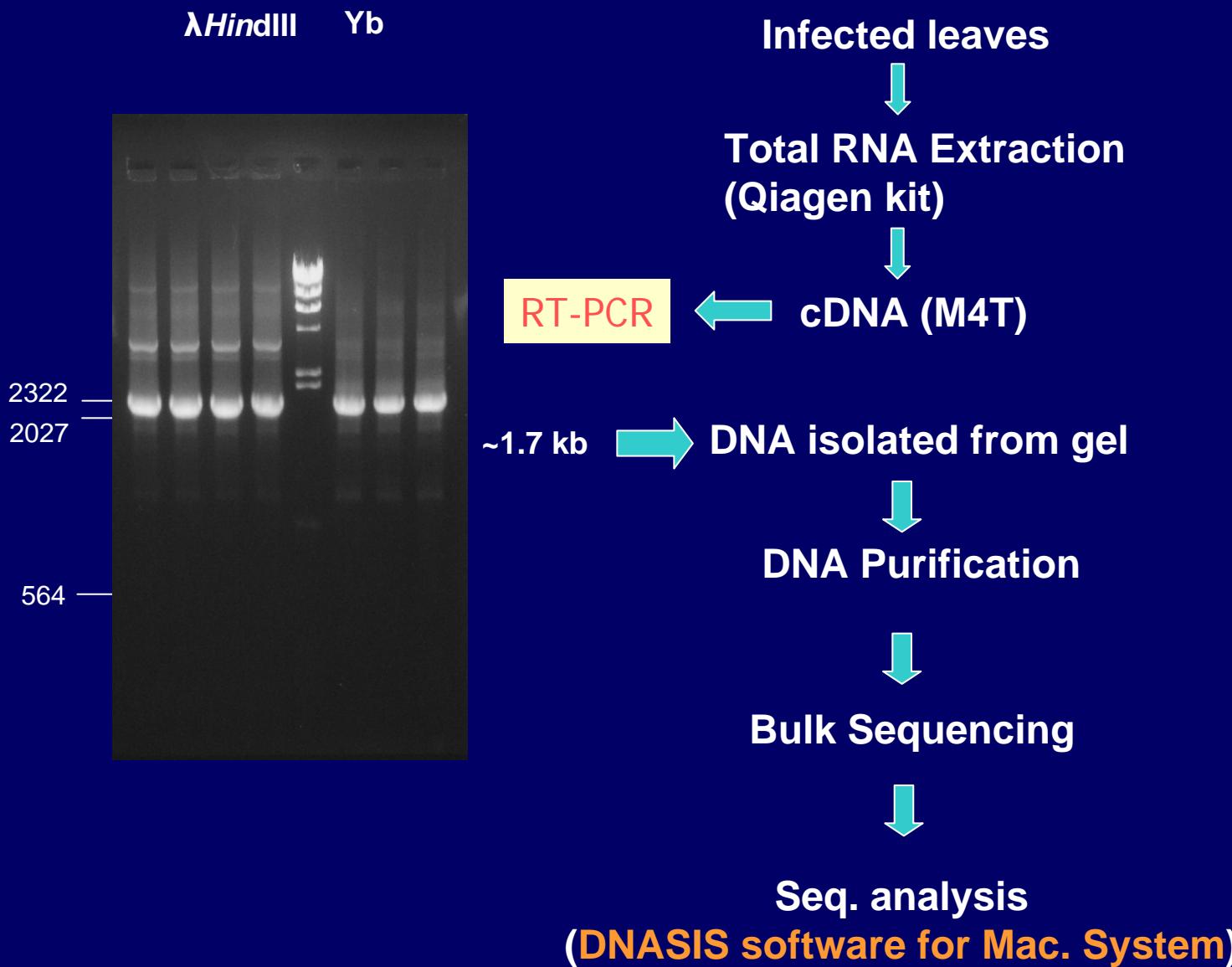
Plant Species	Incubation periods (day)	Symptom	ELISA test
<b><u>Solanaceae</u></b>			
<i>Capsicum annuum</i>	-	-	-
<i>Lycopersicon esculentum</i>	13-15	CS	+
<i>Nicotiana tabacum</i>	-	-	-
<b><u>Leguminosae</u></b>			
<i>Vigna unguiculata</i>	21-23	CS	+
<i>Phaseolus vulgaris</i>	10-12	MS	+
<i>Arachis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

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

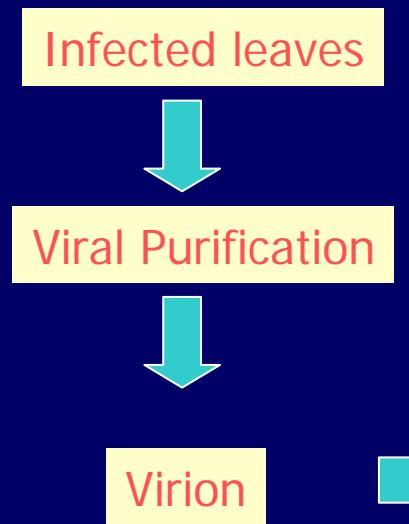
# MOLECULAR DETECTION

## Potyvirus Genome Structure



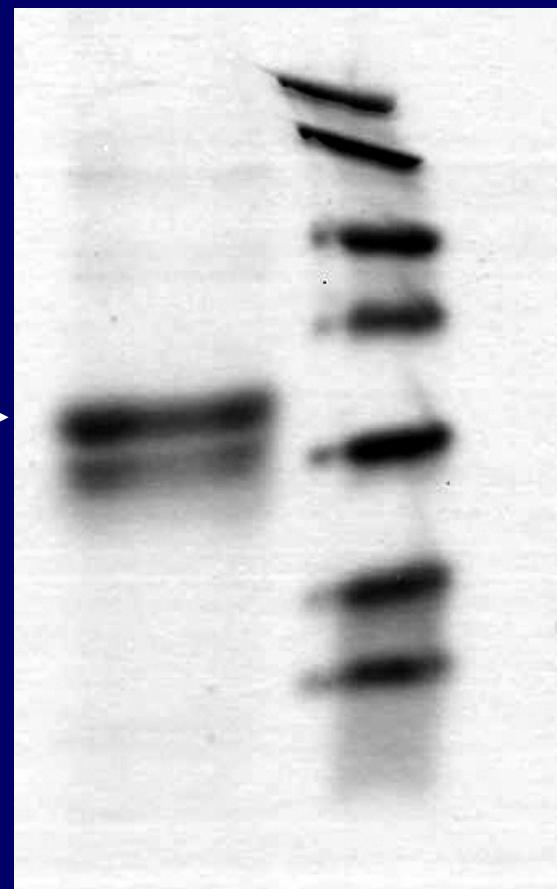


## PROTEIN ANALYSIS BY SDS-PAGE

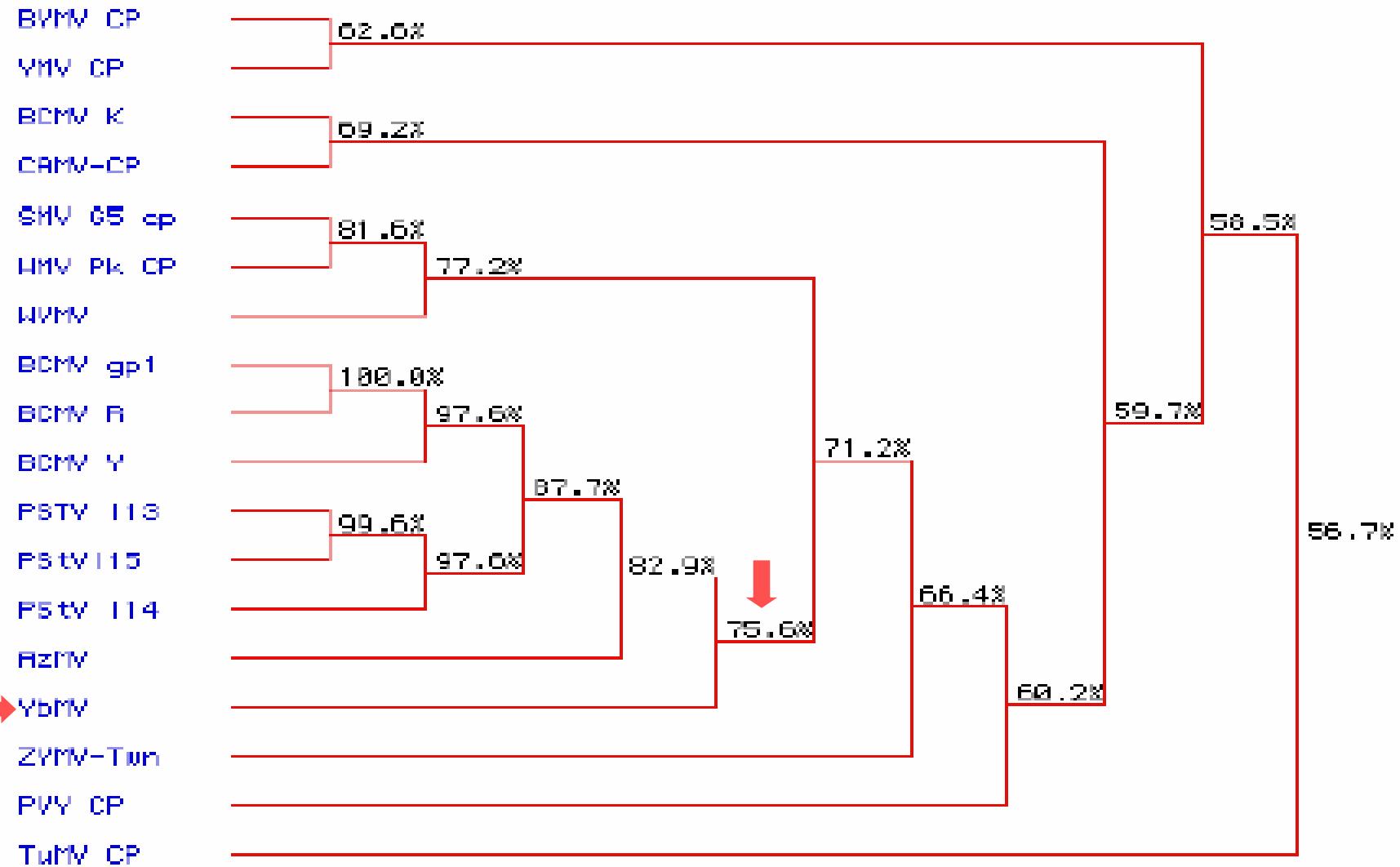


Kda

30

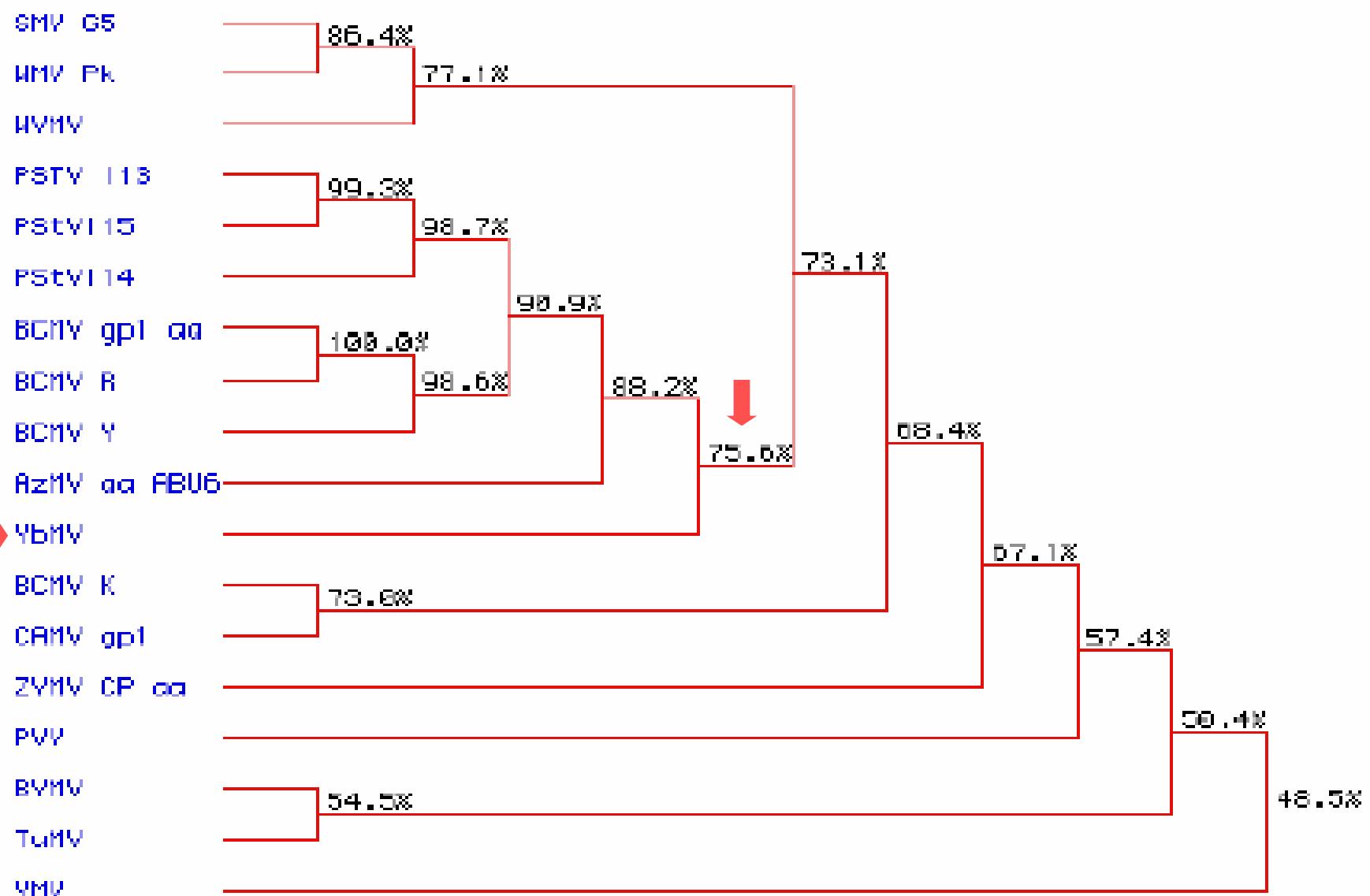


## CP nucleotide alignment (75.6%)



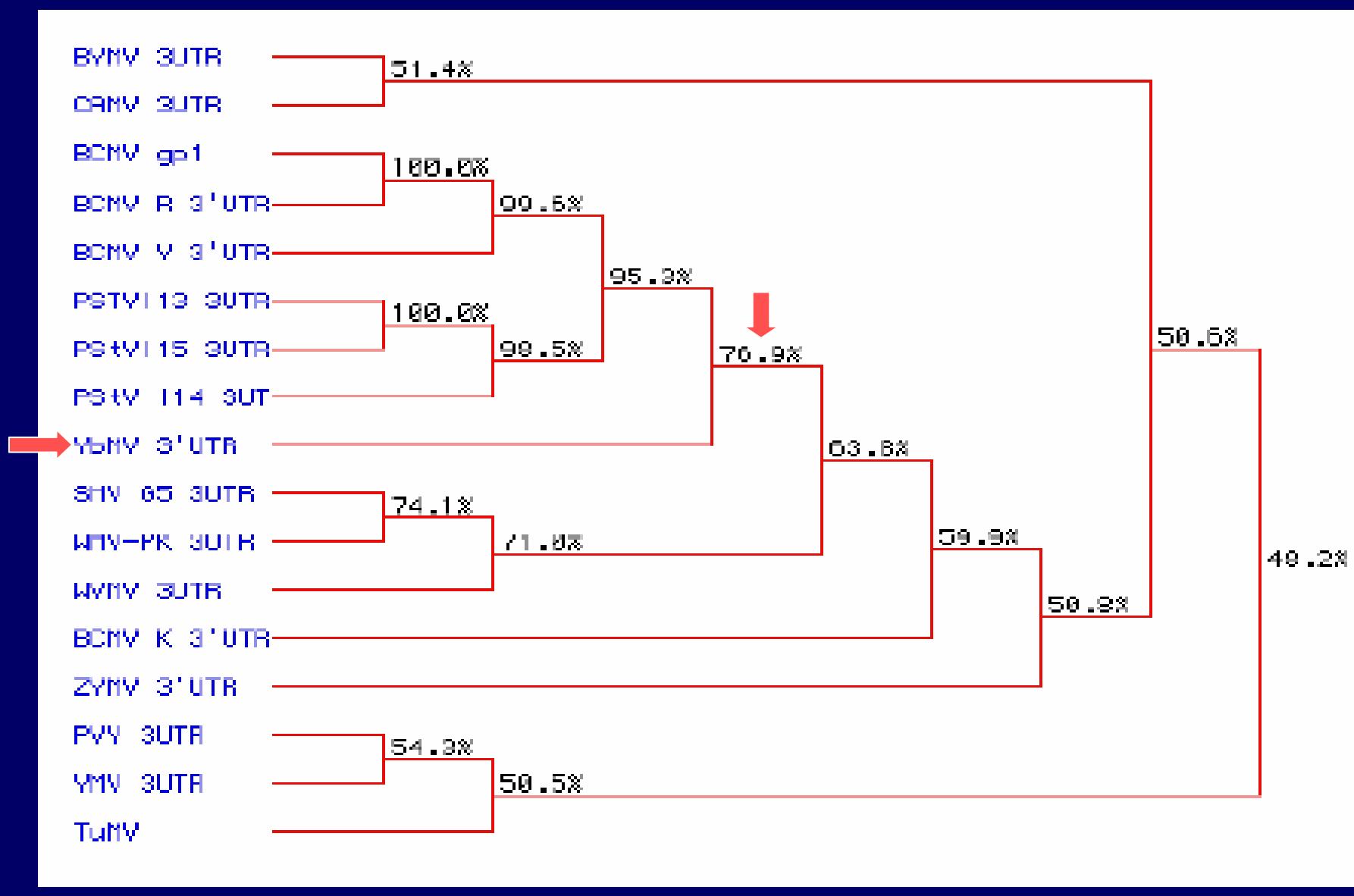
Seq. aligned together against "YbMV"

## 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. alignment 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**

# Acknowledgement

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Thank you

# Genome of Yambean Mosaic Virus Indonesian Isolate (9667 b)

