



Penelitian Strategis Internasional

**Kajian Sifat Bioekologi dan biomolekuler Penyebab
Outbreak Penyakit Kuning Pada Kacang Panjang di Jawa
Barat dan Jawa Tengah**

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Yellow Mosaic Disease Outbreak on 2008 Crop Season

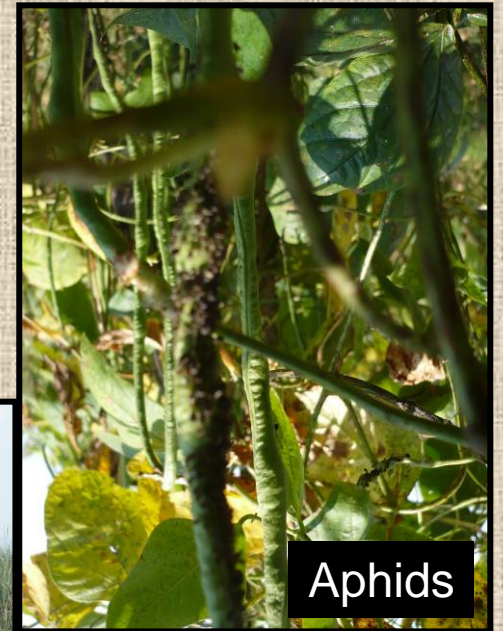


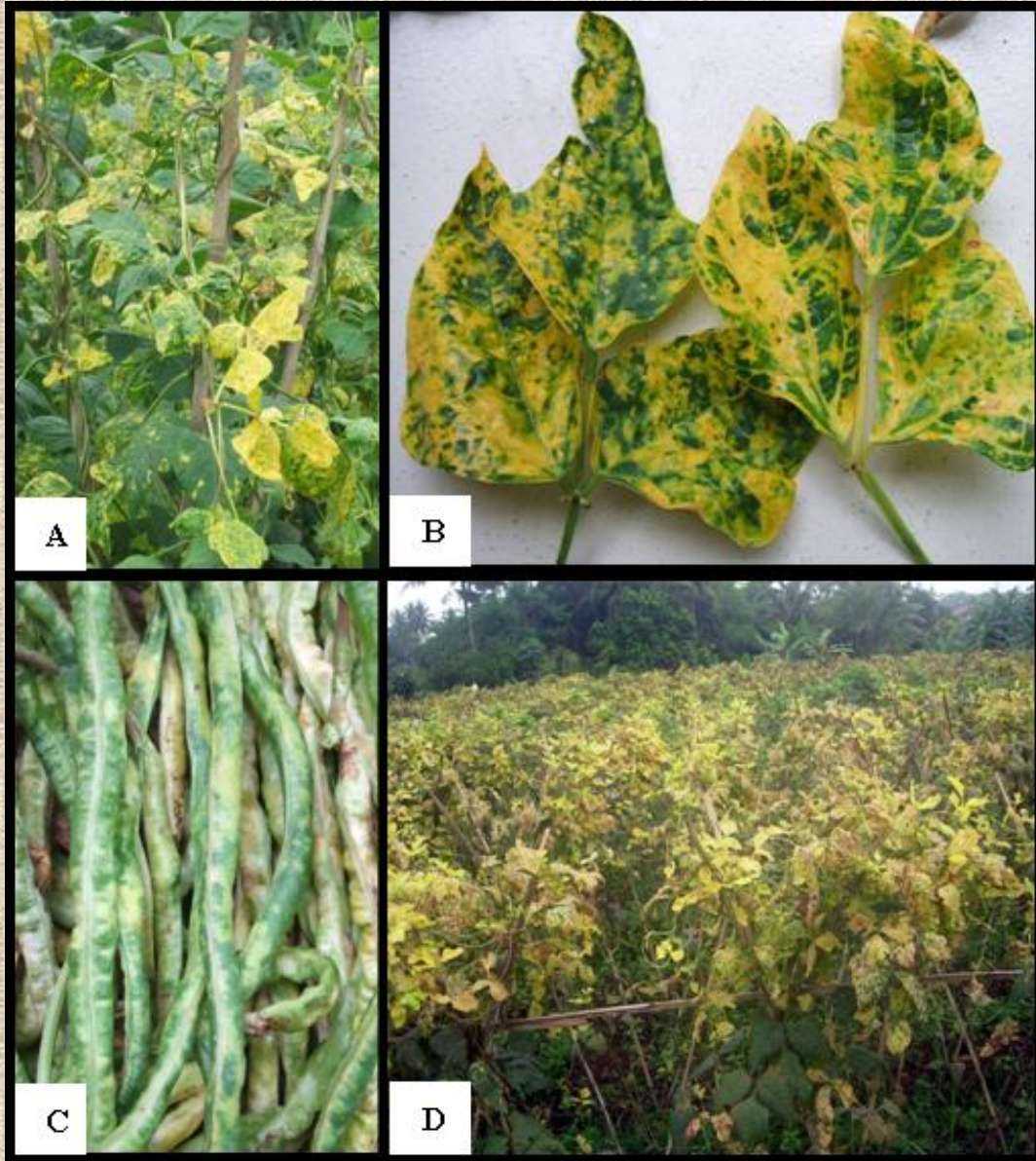
Bubulak, West Java



Aphids

Tegal & Pekalongan, Central Java





Main Symptom

- Yellow mosaic
- Leaf malformation
- Similar symptom on beans
- Distributed rapidly
- Aphid population usually high
- > Infected leaves totally turn yellow, subsequently dry up and dead
- **On 2008** incidence up to **80-100%** causing severe yield losses

Symptom development



Symptom in the same plants

Objectives

- To identify the causal of yellow mosaic disease on yard long bean (YLB)

Appropriate management strategies
To mitigate the disease



Materi Penelitian

I. Survei Lapang

II. Karakter Bio-ekologi

- > Deteksi serologi
- > Penularan (mekanis, serangga)
- > Uji Kisaran inang pada tanaman indikator
- > Pengamatan partikel virus

III. Karakter biomolekuler

- > Ekstraksi asam nukleat (RNA/DNA)
- > Konstruksi cDNA
- > RT-PCR
- > Cloning
- > Peruntan DNA & analisis genetik

IV. Respon Berbagai Kultivar terhadap infeksi virus

Work Outline

Field survey & sample collections



Serological test



Transmission



Indicator plants



Propagation



Nucleic acid extraction



cDNA construction



DNA amplification



Visualisation



Direct Sequencing



Genetic analysis

I. SURVEI

Incidence on 2009

Note;
> * in the greenhouse





| Location | Infected | Total Plants | Incidence (%) |
|---------------------|----------|--------------|---------------|
| WEST JAVA | | | |
| <u>Bogor</u> | | | |
| Bubulak | 30 | 3.000 | 1.0 |
| Cibatok | 395 | 1.400 | 28.2 |
| Leuwikopo | 193 | 1.200 | 16.1 |
| Cibeureum | 143 | 1.800 | 7.8 |
| CENTRAL JAVA | | | |
| Tegal | | | |
| Kedung Banteng 1 | | | |
| Ds Posong | 24.000 | 24.000 | 100 |
| Ds Tonggara 1 | 25.000 | 25.000 | 100 |
| Ds Tonggara 2 | 3.000 | 10.000 | 30 |
| Kedung Banteng 2 | 8.000 | 8.000 | 100 |
| Pangkah | 1.200 | 4.800 | 25 |
| Slawi 1 | | | |
| Ds Dukuh wringin | 560 | 2.800 | 20 |
| Ds Yamansari | 400 | 2.000 | 20 |
| Slawi 2 | 8.000 | 8.000 | 100 |
| Pekalongan | | | |
| Tirto | | | |
| Ds Silirejo | - | - | - |
| Ds Sidorejo | 20 | 450 | 4.4 |
| Ds Karanganyar * | 100 | 1.000 | 10 |

Incidence in Pantura

Incidence < dibandingkan
Di Jateng

| Location | Infected | Total Plants | Incidence (%) |
|------------------|----------|--------------|---------------|
| Karawang | 522 | 5.360 | 9.7 |
| Subang | | | |
| Ciasem | 25 | 6.000 | 0.4 |
| Ciasem Tengah | 79 | 2.499 | 3.2 |
| Pagaden | 10 | 15.668 | 0.06 |
| Indramayu | | | |
| Sukagumiwang | 169 | 12.064 | 1.4 |
| Jatibarang | 225 | 7.716 | 2.9 |
| Bongas | 15 | 1.440 | 1.1 |
| Cirebon | | | |
| Kaliwulu | 22 | 35.604 | 0.06 |
| Surabau | 241 | 6.020 | 4.0 |
| Dawuan | 192 | 11.952 | 1.6 |
| Babakan | 66 | 2.670 | 2.4 |





A. UJI SEROLOGI

| | Code | BCMV- PSt | BCMNV | BYMV | BLRV | BGMV | CaBMV | CMV | CPyMV | CPSMV | General Potyvirus |
|---|----------|--------------|-------|------|------|------|-------|-----|-------|-------|----------------------|
|  | Bblk-WJ | | | | | | | + | | | + |
|  | Cbrm-WJ | | | | | | | | | | + |
|  | Cbtk-WJ | | | | | | | | | | + |
|  | Leuwi-WJ | | | | | | | | | | + |

BCMV-bean common mosaic virus; BCMNV-bean common mosaic necrosis virus; BYMV-bean yellow mosaic virus; BLRV-Bean leaf roll virus; BGMV-bean golden mosaic virus; CaBMV-cowpea aphid-borne mosaic virus; CMV-cucumber mosaic Virus; CPyMV-cowpea yellow mosaic virus; CPSMV-cowpea severe mosaic virus.

Bblk-Bubulak; Cbrm-Cibeureum; Cbtk-Cibatok; Leuwi-Leuwikopo; WJ- west Java

Shading – negative; (+) - positive

| | Code | BCMV- PSt | BCMNV | BYMV | BLRV | BGMV | CaBMV | CMV | CPyMV | CPSMV | General Potyvirus |
|---|------------|--------------|-------|------|------|------|-------|-----|-------|-------|----------------------|
|  | DMG, WJ | | | | | | | | | | |
|  | TGL, CJ | | | | | | | | | | + |
|  | SIDO CJ | | | | | | | | | | + |
|  | KRN CJ | | | | | | | | + | * | |

DMG-Darmaga; TGL-Tegal; Sido-Sidorejo; KRN-Karanganyar; WJ-West Java; CJ-Central Java

*Weakly reacted

DETEKSI SAMPEL ASAL PANTURA

| RT-PCR | Karawang | Subang | Indramayu | Cirebon |
|--------------|----------|---------|-----------|---------|
| Potyvirus | (+)/(-) | (+)/(-) | (-) | (+)/(-) |
| CMV | (-) | (+) | (-) | (-) |
| Crinivirus* | +/- | +/- | +/- | +/- |
| Geminivirus* | ? | ? | ? | ? |
| Luteovirus* | ? | ? | ? | ? |

* Perlu deteksi lebih lanjut terhadap semua sampel uji

II. Mechanical Transmission

Bogor, West Java (WJ)



Bogor, WJ; Central Java (CJ)



Bogor, WJ & Tegal, CJ



Pekalongan, CJ



a. PENULARAN

| Penularan | Potyvirus | CMV | Lain-lain |
|--------------|-----------|-----|-----------|
| Mekanis* | (+) | (+) | (-) |
| Kutudaun* | (+) | (+) | (-) |
| Kutu kebul** | (-) | (-) | (+) |
| Benih* | (+) | (+) | (-) |

•* Sampel Bogor, Jawa Tengah

•** Sampel Cikabayan

BOGOR Isolate

b. Symptom on Indicator Plants

| HOST | Incubation period | Inoculated | Systemic | ELISA test Potyvirus | ELISA test CMV |
|----------------------------------|-------------------|------------|-----------|----------------------|----------------|
| Amaranthaceae | | | | | |
| <i>Gomphrena globosa</i> | 5-10 | Reddish LN | MM | + | + |
| Chenopodiaceae | | | | | |
| <i>Chenopodium amaranticolor</i> | 3-7 | LLN | - | + | + |
| Cucurbitaceae | | | | | |
| <i>Cucumis sativus</i> | 7 | Chlorosis | MM | - | + |
| Leguminosae | | | | | |
| <i>Vigna unguiculata</i> | 5-7 | DB spot | M, VC, UC | + | + |
| <i>V. radiata</i> | 3-7 | DB spot | Mosaic | + | + |
| <i>Pisum sativum</i> * | 7-10 | Chlorosis | yellowing | - | + |
| <i>Phaseolus vulgaris</i> | 7-10 | Chlorosis | Mosaic | + | + |
| Solanaceae | | | | | |
| <i>Capsicum annum</i> | 7-14 | - | MM | - | + |
| <i>Nicotiana tabacum</i> | 7-10 | - | MM | + | + |
| <i>Lycopersicon esculentum</i> | 7-14 | - | Chlorosis | - | + |
| <i>Physalis floridana</i> * | 5- 10 | Chlorosis | MC, VB | - | + |

LN - local necrotic; DB -dark brown; MM-mild mosaic; MC-mild chlorosis; VC-vein clearing; VB-vein Banding; UC-upward curl; LLN-lesio local necrotic; M-mosaic

* Majority infected leaves dried and dead

Symptoms



C. amaranticolor



G. globosa



L. esculentum

Symptoms



C. sativus

P. floridana

V. radiata

Symptoms

P. sativum

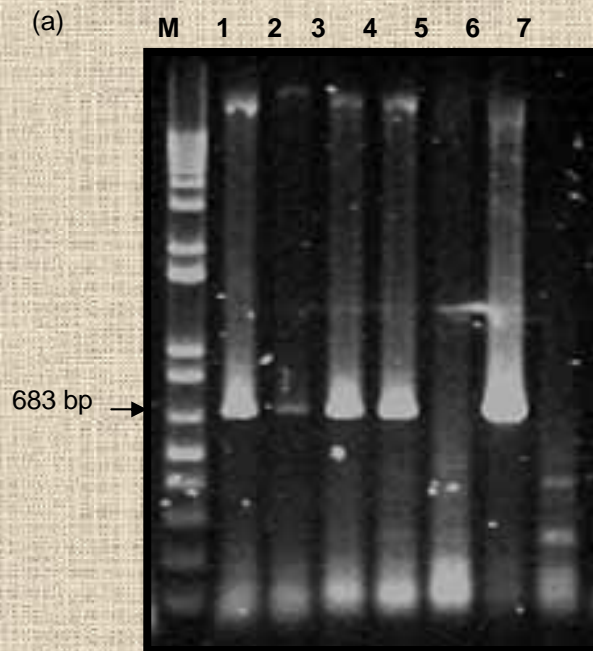


V. unguiculata

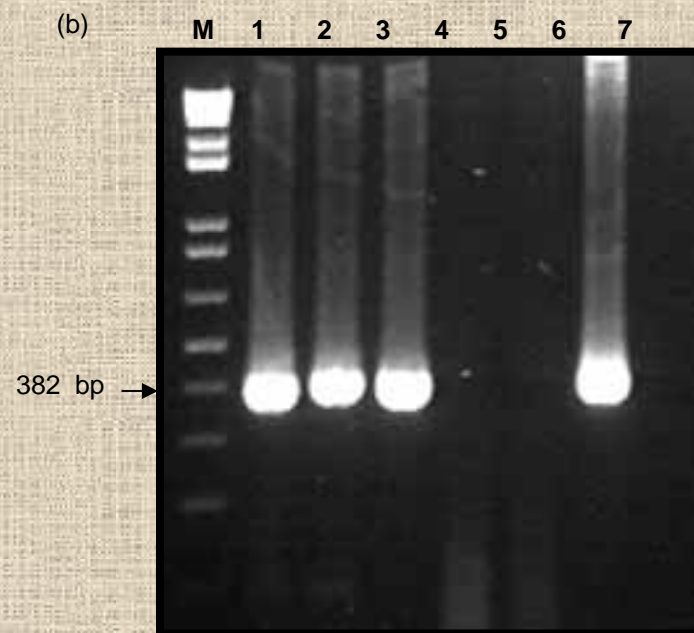
III. Molecular Detection

BUBULAK BOGOR ISOLATE

POTYVIRUS

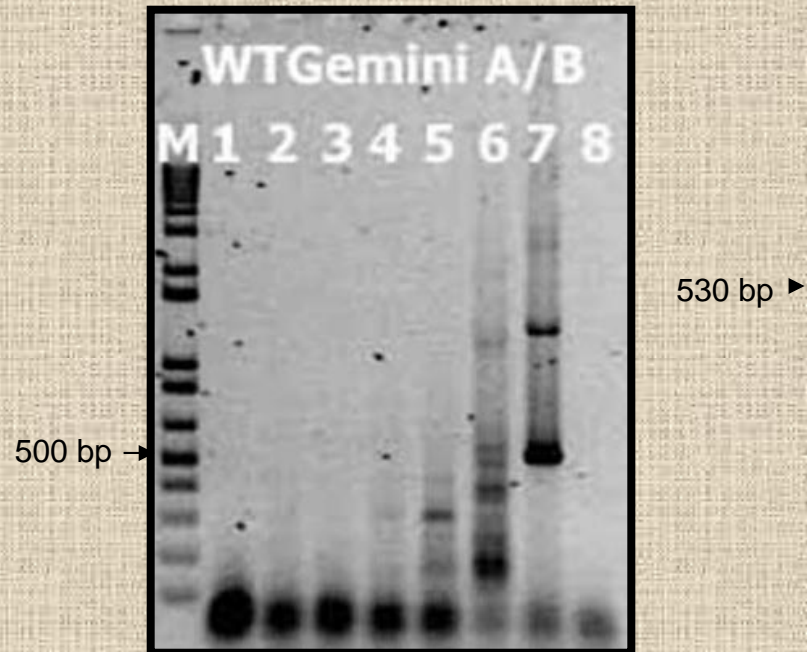


CMV subgroup Ib



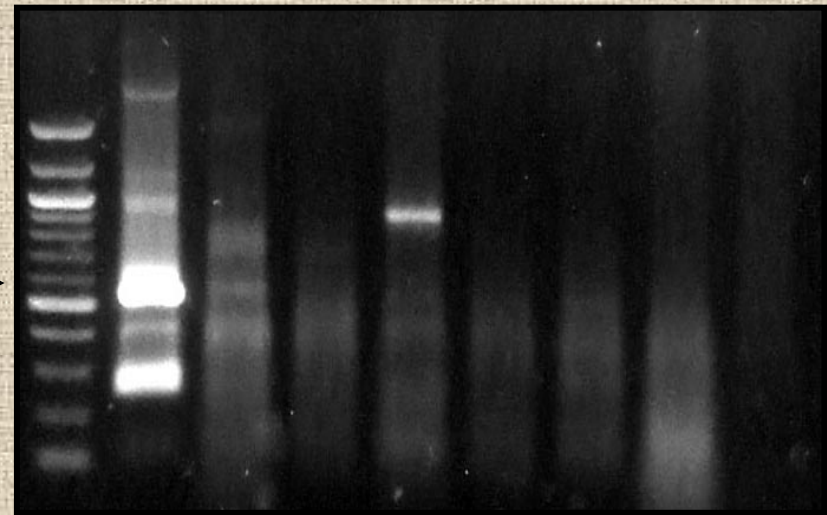
Lane 1-5 : samples from the field
Lane 6 : Positive control
Lane 7 : negative control

Geminivirus



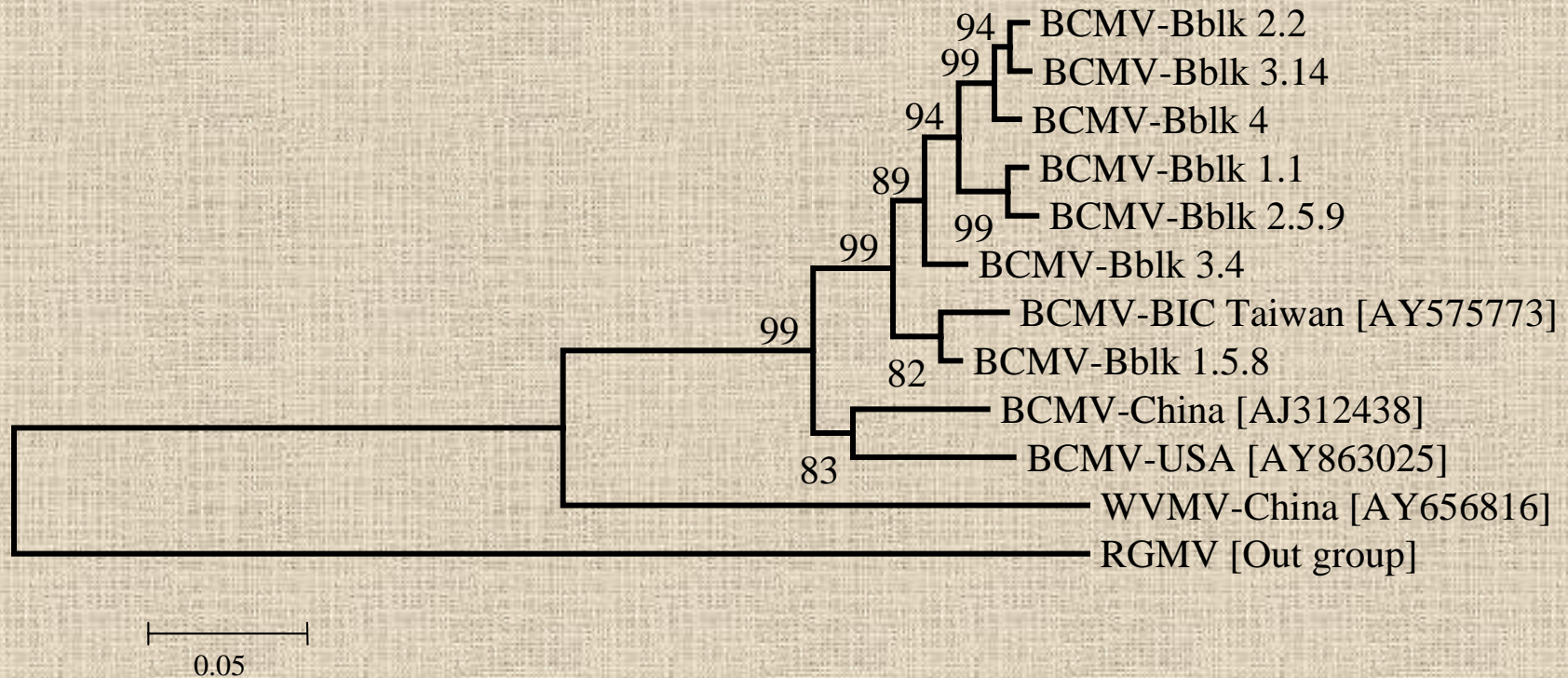
Lane 1-6 : samples
Lane 7 : positive control
Lane 8 : negative control

Luteovirus



Lane 1: Positive control
Lane 2-8 : samples

Phylogenetic analysis of CI gene



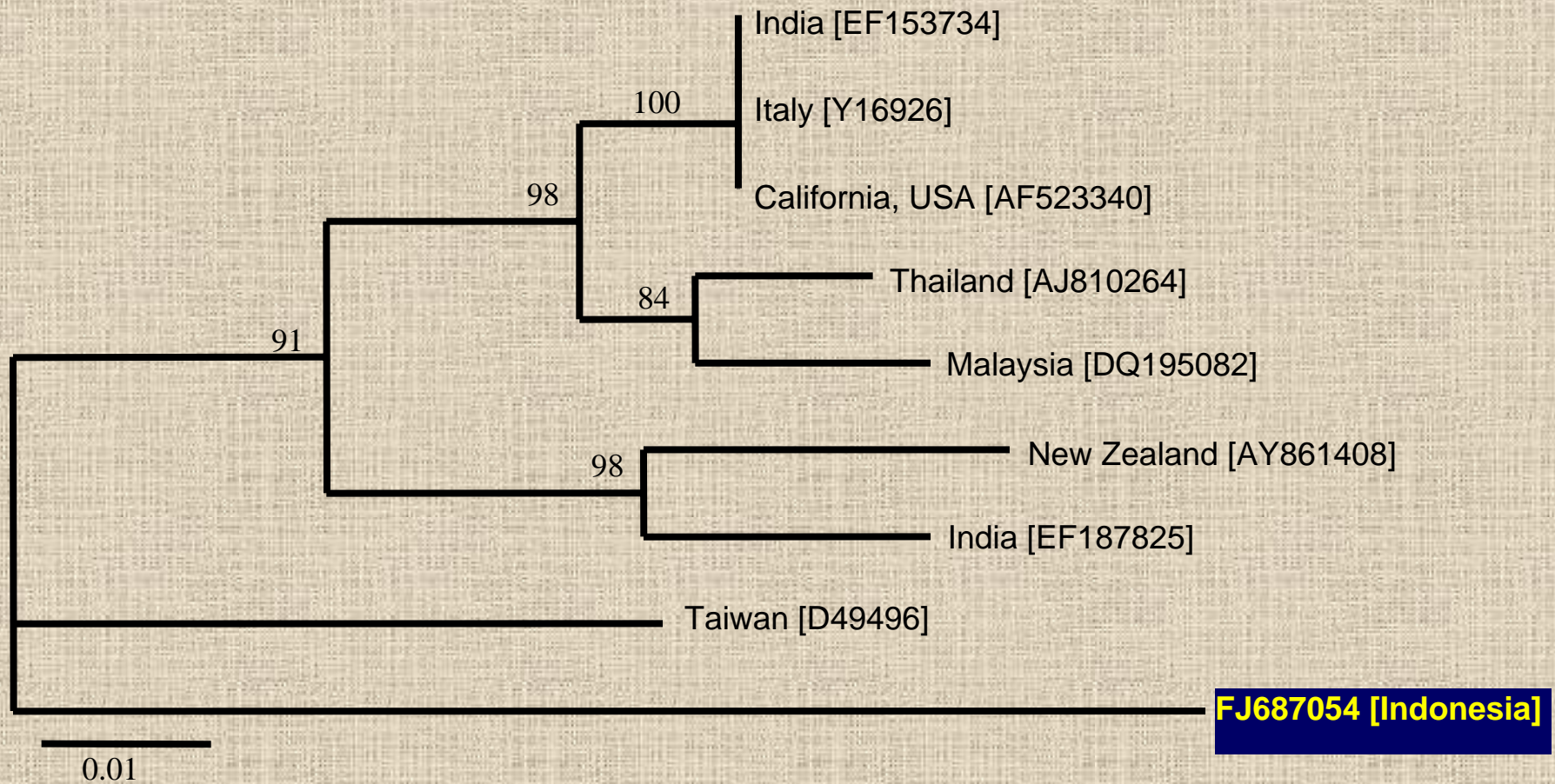
BCMV isolates from Bogor and their GenBank accession numbers are: Bubulak-1.1 (FJ653916), Bubulak-1.8 (FJ653917), Bubulak-2.2 (FJ653918), Bubulak-2.5 (FJ653919), Bubulak-2.9 (FJ653920), Bubulak-3.4 (FJ653921), Bubulak-3.14 (FJ653922), Bubulak-4.3 (FJ653923), Bubulak-4.4 (FJ653924), Bubulak-4.13 (FJ653925) and Bubulak-1.5 (FJ653926). CMV isolates is Bubulak 1.4 (FJ687054).

Comparison of Partial BCMV BIC Bblk isolate CP sequences

| Virus | Homology | | Accession no. |
|-----------------------|-----------|-----------|-----------------|
| | Nuc. | aa. | |
| AzMV | 93 | 94 | ABU60100 |
| BCMV – BIC Twn | 97 | 99 | AY575773 |
| - lybn | 80 | 86 | AB289438 |
| - BIC VN/BB2-5 | 81 | 88 | DQ925422 |
| - BIC VN/YB1 | 95 | 97 | DQ925424 |
| - I14 | 92 | 97 | AJ132157 |
| - PSt | 91 | 96 | X63559 |
| BYMV | 68 | 65 | NC003492 |
| CaBMV | 70 | 79 | NC004013 |
| SMV G5 | 77 | 83 | AY294044 |

AzMV-Azuki mosaic virus; *BCMV*- bean common mosaic virus; *BIC*- black eye cowpea; *BYMV*- bean yellow mosaic virus; *CaBMV* – cowpea aphid-borne mosaic virus; *SMV* – soybean Mosaic virus

Phylogenetic of Partial CMV CP gene



IV. Respon kultivar

Pengaruh infeksi virus terhadap pertumbuhan tanaman

| Kultivar Uji | Pengamatan pada minggu setelah inokulasi (MSI) | | | | |
|-----------------|--|----------------|----------------|----------------|---------------|
| | 0 MSI | 1 MSI | 3 MSI | 5 MSI | %Penghambatan |
| Bre Nero | 22.85acd* | 29.00f | 20.95e | 26.25de | 86.83a |
| Guma | 24.08abcd | 35.65de | 20.15e | 22.45e | 88.74a |
| Parade | 22.48cd | 32.40ef | 77.55b | 92.35b | 53.55c |
| Bapan | 26.00a | 47.70a | 53.00cd | 56.10c | 70.74b |
| Jaliteng | 23.58bcd | 39.35cd | 105.15a | 132.20a | 27.06d |
| Pilar | 24.08abcd | 40.25c | 47.40cd | 51.85c | 73.80b |
| Super Sainan | 21.98d | 29.90f | 38.70de | 44.45cd | 76.28b |
| Hijau Super | 25.43ab | 43.40bc | 57.60cd | 57.60c | 70.79b |
| Super Putih | 24.60abc | 48.30a | 51.25cd | 51.25c | 73.88b |
| Jangkis | 26.18a | 46.50ab | 66.05bc | 61.30c | 67.33b |

*Huruf yang sama pada kolom yang sama, tidak berbeda nyata secara statistik

Keparahan Penyakit dan Hasil Uji Serologi

| Kultivar | Keparahan | NAE** Potyvirus | NAE CMV |
|-----------------|---------------|-----------------------|------------------------|
| Bre Nero | 3.60 a* | 2.900 ± 0.034bc | 0.550 ± 0.025c |
| Guma | 3.50 a | 3.020 ± 0.008ab | 0.780 ± 0.089ab |
| Parade | 3.00 b | 2.290 ± 0.030e | 0.550 ± 0.008c |
| Bapan | 3.00 b | 3.040 ± 0.016a | 0.820 ± 0.052ab |
| Jaliteng | 2.25 c | 2.660 ± 0.045d | 0.670 ± 0.109bc |
| Pilar | 3.00 b | 3.050 ± 0.025a | 0.770 ± 0.083ab |
| Super Sainan | 3.00 b | 2.960 ± 0.092abc | 0.740 ± 0.014abc |
| Hijau Super | 3.00 b | 2.870 ± 0.049c | 0.820 ± 0.227ab |
| Super Putih | 3.00 b | 2.880 ± 0.061c | 0.870 ± 0.006ab |
| Jangkis | 2.95 b | 2.680 ± 0.033d | 0.880 ± 0.057a |
| K+ | | 1.450 ± 0.119f | 0.710 ± 0.040abc |
| K- | | 0.080 ± 0.003g | 0.150 ± 0.013d |

* Huruf yang sama pada kolom yang sama, tidak berbeda nyata secara statistik

** NAE, nilai absorbansi ELISA pada panjang gelombang 405 nm

Karanganyar Pekalongan, CJ

Bogor, WJ; Central Java (CJ)



???

Not transmitted mechanically
Transmitted by *B. tabaci* (?)
Negative against all tested antisera



Transmitted mechanically
Weakly positive against CPyMV

Conclusion

- YMD might distribute widely in Java
- YMD on yard long bean majority associated with **BCMV BIC**; *bean common mosaic virus* strain **black eye** either by single or multiple infection with CMV
- BCMV BIC/CMV Isolate Bogor has **narrow host range** and limited to legumes
- BCMV BIC & CMV isolate Bogor are closely and clustered with isolate from **Taiwan**
- CMV might considerate as first occurrence on yard long bean in Indonesia
- Kultivar Jaliteng asal Tegal menunjukkan respon toleran
- **Other viruses** might be involve causing yellow mosaic disease on yard long bean and further studies need to be address to get more information related with other viruses

Terimakasih