PEMANFAATAN BAKTERI RIZOSFER UNTUK PROTEKSI CABAI TERHADAP INFEKSI GANDA VIRUS

TRI ASMIRA DAMAYANTI
NISA R. MUBARIK
HENDRA PARDEDE
TRIAS KATERINA

(Hayati, 2007 Vol.14, hal 105-109 & J. ISSAAS, 2008 Vol.14, hal 92-100)

DEPARTEMEN PROTEKSI TANAMAN
FAKULTAS PERTANIAN
INSTITUT PERTANIAN BOGOR

PLANT VIRUS

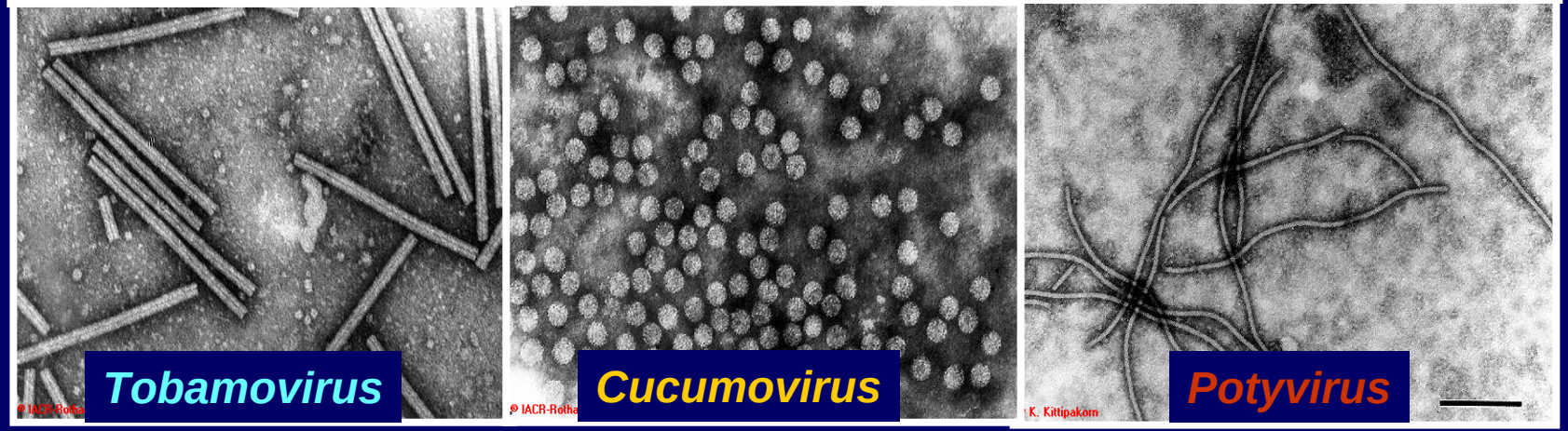
- Submicroscopic particles
- Contain RNA or DNA (ss or ds)
- Nucleic acid protected by coat protein form virion
- Do not have organelle cells
- Obligate parasite (only living in live cells)
- Mainly replicate in viroplasm/cytoplasm

Symptoms of virus infection

Healthy ChiVMV Mix CMV TMV

SYMPTOM AND VIRAL PARTICLES

Tobacco Mosaic Virus *Cucumber Mosaic Virus* *Chilli Veinal Mottle Virus*



[Particle Source; CPC 2005]

SYMPTOM OF MIX INFECTION
TMV & ChiVMV

Management of virus diseases:

- Resistant varieties
- Cultural practices
- Eradication of vectors
- Genetically engineered crops
- Cross protection

↓

Root colonizing bacteria ?

↓

Objectives: To utilize the potential PGPR isolates to protect hot pepper against multiple infection of virus

Root colonizing bacteria - *Rhizobacteria*

- Abundantly present in rhizosphere
- Live from plant root secretion
- Stimulate plant growth,

referred as :

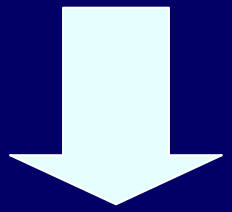
Plant-Growth Promoting Rhizobacteria (PGPR)

The roles of PGPR

- Nitrogen fixation
- Promoting plant growth
- Protecting plants from infection by pathogen (antibiosis, ISR etc)

Large-scale application of PGPR reduce the use of chemical fertilizer and pesticides; and increase crop yield

HOW IS PGPR SUPPRESS THE DISEASE?



Induced Systemic resistance (ISR)

ISR → an increased resistance to disease that develops systemically throughout plants after appropriate stimulation (Hammerschmidt and Kuc, 1995)

PGPR as stimulant

⬇

Seed treatment, Soil drench, Foliar spray, Combination

⬇

Challenge inoculation of pathogens

⬇

Elicits Plant's defense response

⬇

Decrease disease incidence, severity, symptom expressions

MECHANISMS

- Alters host physiology and metabolic responses, fortifies plant cell wall strength
- Antibiosis
- Increased SA ⟶ PRs gene, chitinase etc
- Increased Jasmonate Acid and ethylene, peroxidase, phytoalexin, enhance ability to lignify
- Siderophores (pyoverdine, pyoceline, SA)
- Competition for iron

METHODS

RHIZOBACTERIA ISOLATION

1 g

Rhiszosfer soil

100 ml 0.85% NaCl

Vortex 15 min

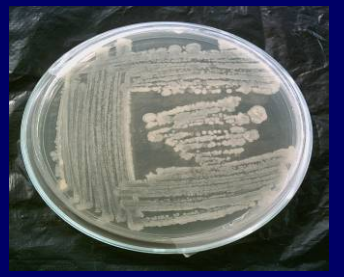
24hr shaking

Heating at 60 °C, 10 min
Shaking 24 hr

0,1 ml

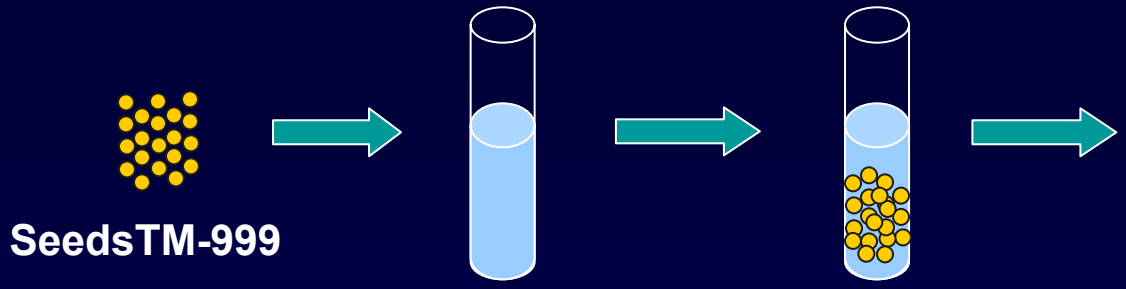
Pour on TSA

Single Colonies

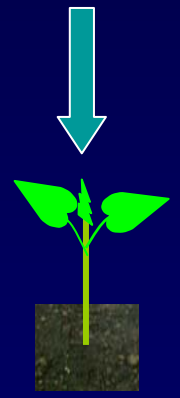


Streak on TSA

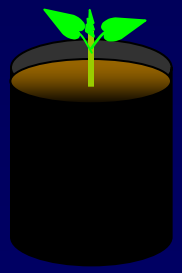
➢ Seed treatment and cultivation



Seedling + a drop bact. suspension



2 Weeks after



Pot

Sterile Soil : cow-dung = 1:1

Placed in greenhouse

Methods:

Evaluation of plant growth characteristics

- Plant height at 1 day prior- and at 2, and 4 weeks post-virus inoculation (wpi)
- Number of Leaves
- Number of flowers and fruits
- Plant fresh weight

2 WPI = 6 WAP

Method : Viral Inoculation

Infected leaves

Grind +
bufer fosfat pH 7
[1:10 (b/v)]

=

Inoculum

Inoculation

Plants at 4 WAP

Observation

Methods: Disease assessments

1. **Disease incidence (%)**

$$I = \Sigma \frac{n}{N} \times 100\%$$

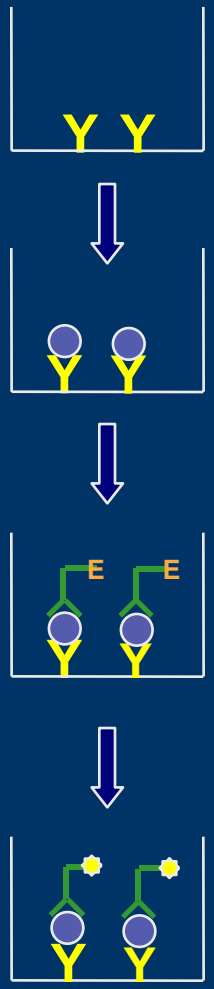
I = disease incidence (%)
n = number of infected plants
N = total number of inoculated plants

2. Disease severity rating made at 2 wpi and 4 wpi.
   It is performed with mock inoculated plants as standard.

Disease severity rating scales
- 0 = no symptom
- 2 = leaves with mild mosaic symptom
- 4 = leaves with severe mosaic symptoms
- 6 = leaves with mosaic and deformation
- 8 = leaves with severe mosaic, deformation and yellowing along veins
- 10 = leaves with severe mosaic, deformation, yellowing along veins and abrupt growth reduction

3. Detection of Viral Protein by ELISA

ELISA (*Enzyme Linked-Immunosorbent Assay*)



Coating 1 st AB

Washing 4-8 times

Antigen bound to the 1st AB

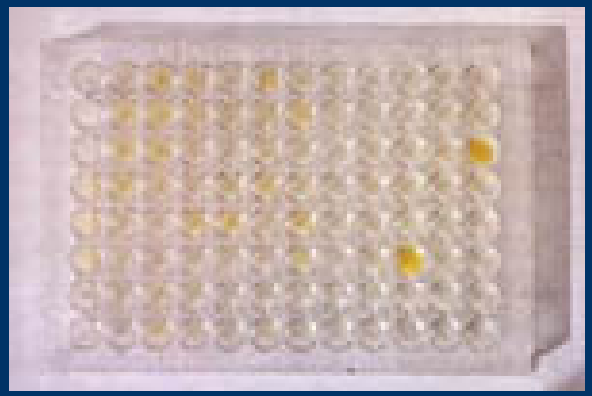
Washing 4-8 times

2nd AB conjugated with enzyme

Washing 4-8 times

Substrates addition (yellow)

ELISA Reader at OD 405 nm



4. PEROXIDASE ENZYME ACTIVITY

Measured by Spectrophotometer at 470 nm wavelength; every 30 seconds for 3 minutes

5. ETHYLANE PRODUCTION

Gas Chromatography methods at Balai Besar Pasca-Panen Cimanggu, Bogor

Samples measured at 5 days post viral inoculation

Hasil dan Pembahasan

RHIZOBACTERIA ISOLATES

55 isolates obtained; 17 are gram positive and 38 are gram negative (14 isolates are pathogenic, 5 isolates were unable to re-cultured)

↓

36 isolates were tested for inducing seed germination

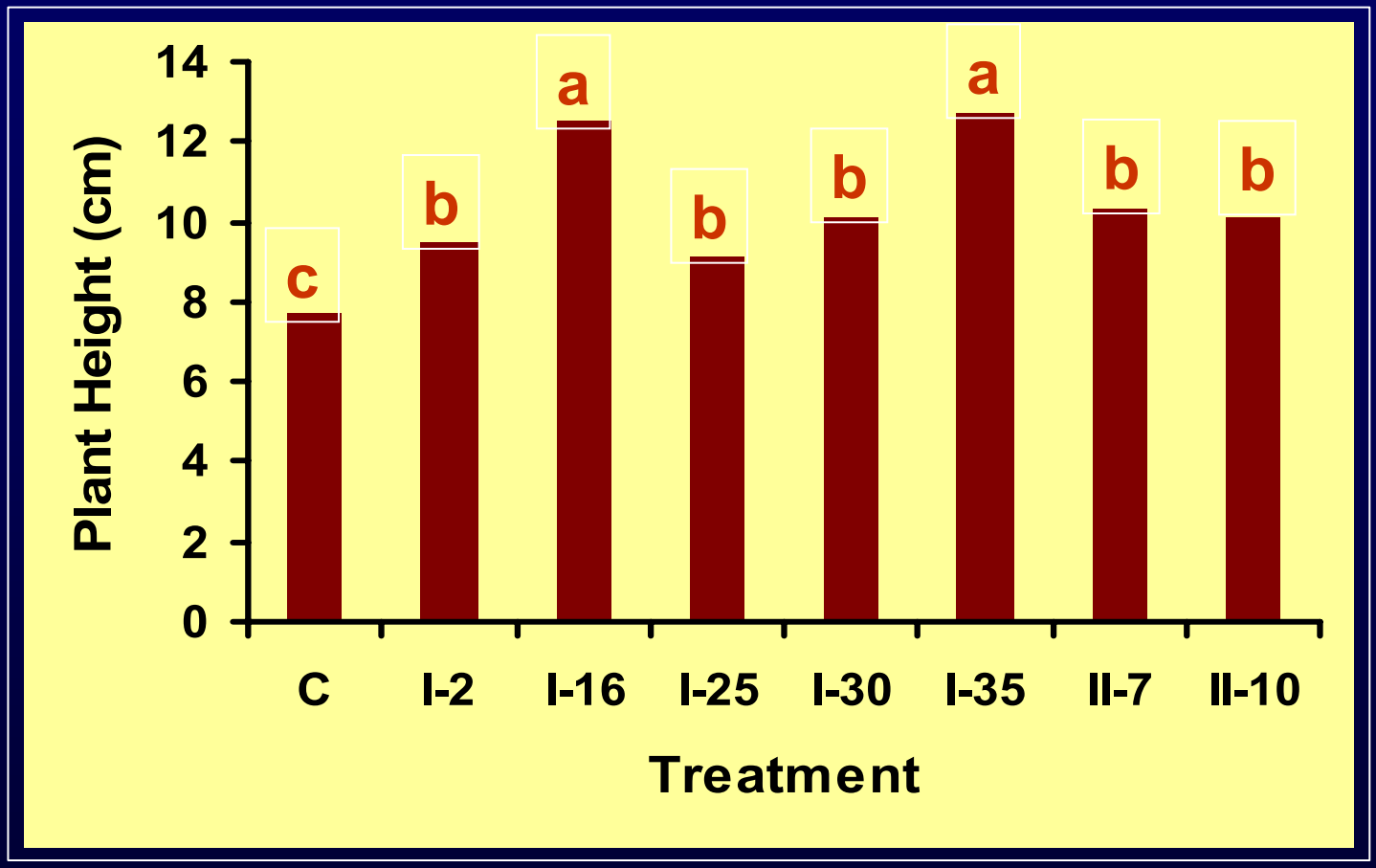
↓

Bacterized-seeds showed comparable germination rate, but better seedling vigor and fitness than untreated control

↓

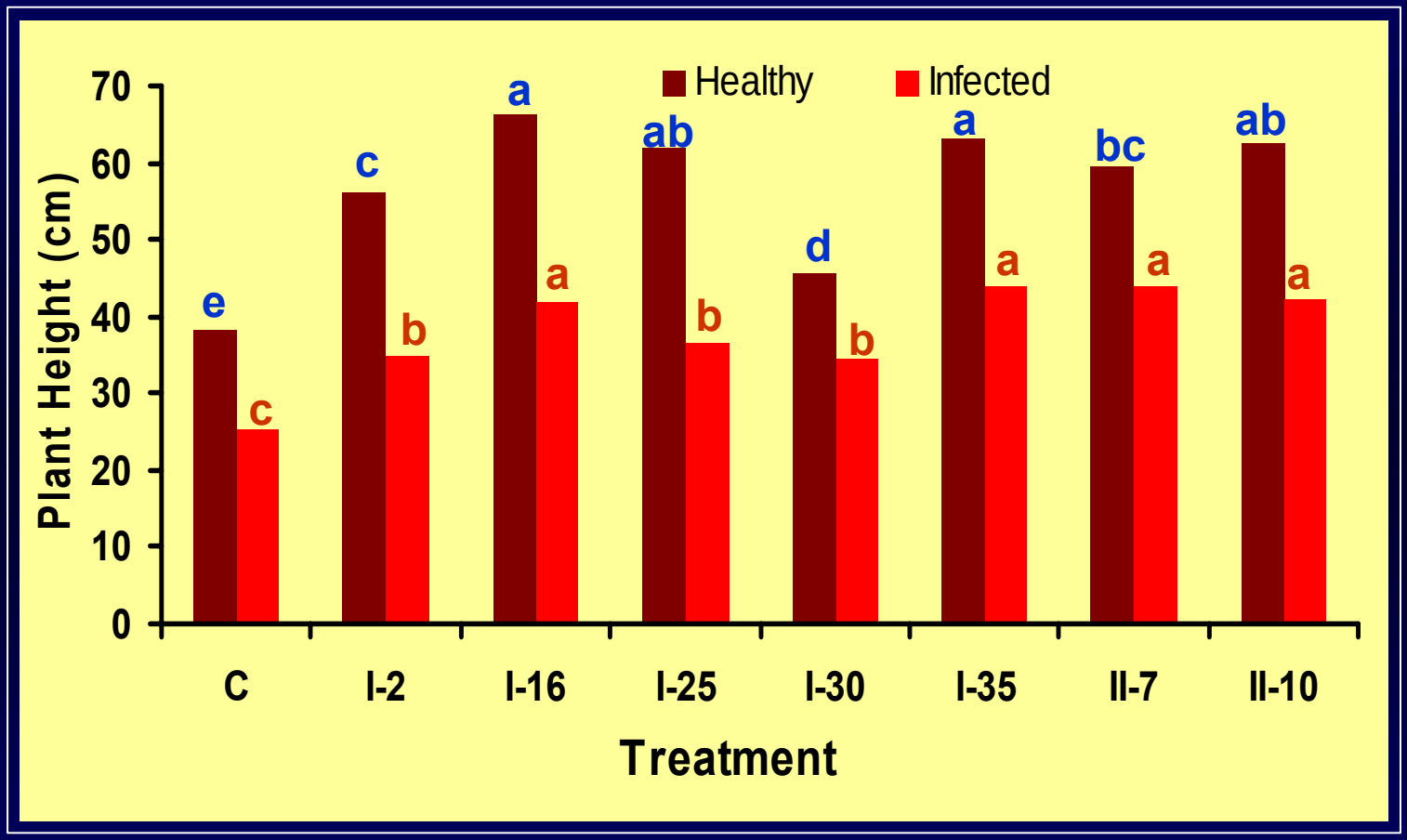
7 isolates were evaluated their ability to protect pepper against 3 viruses

Tinggi Tanaman 1 HSI



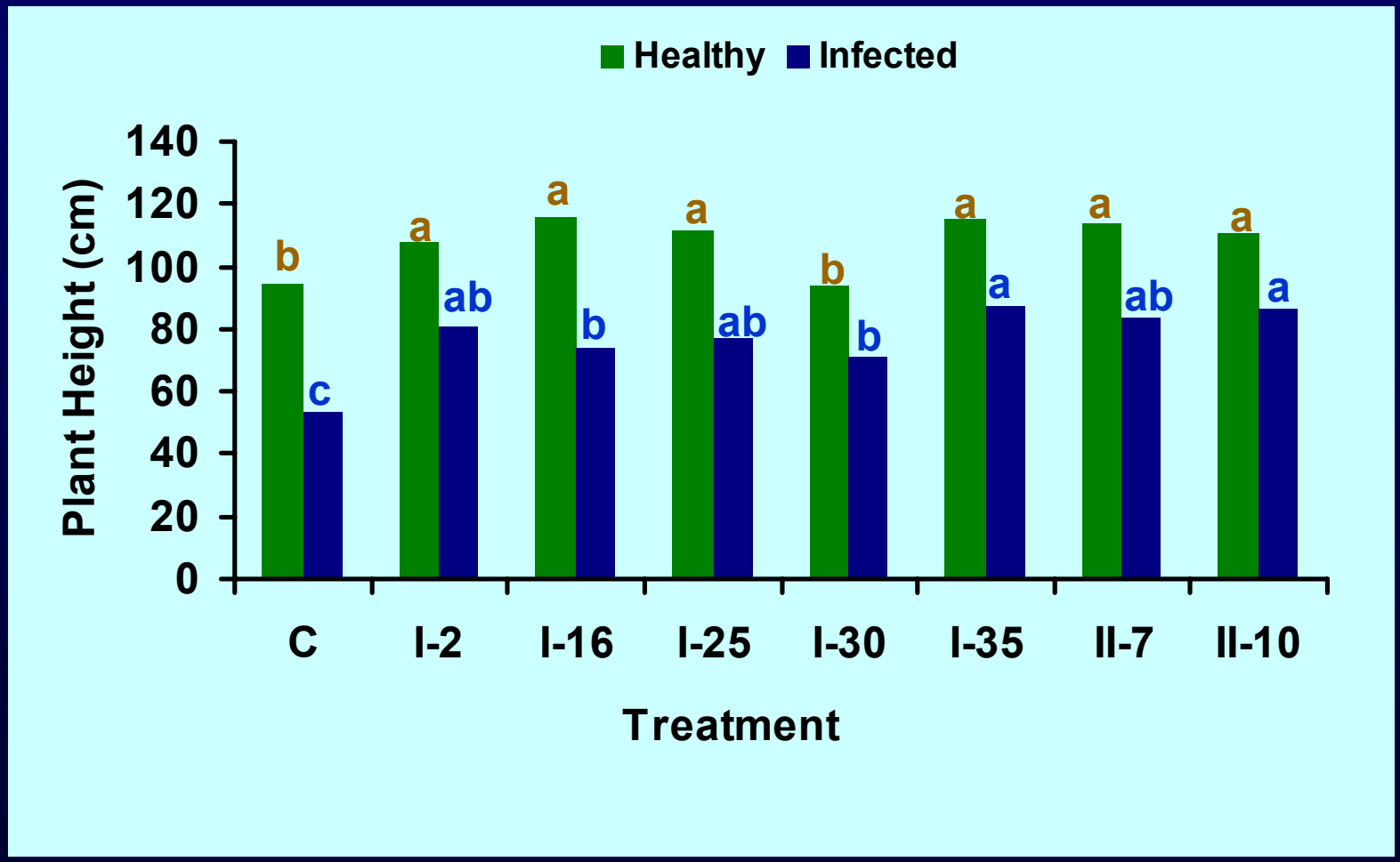
HSI – Hari Sebelum Inokulasi Virus

Tinggi Tanaman Pada 8 MST



MST – Minggu Setelah Tanam

TINGGI TANAMAN PADA 12 MST



TANAMAN SEHAT

Pembibitan

12 MST

TANAMAN SEHAT

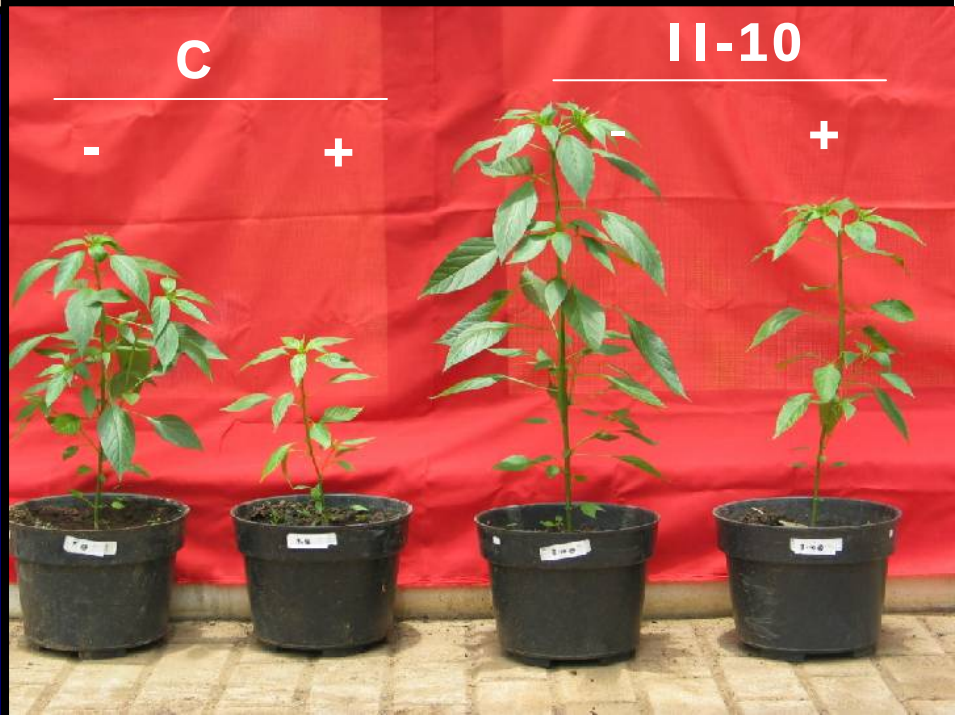
Pembibitan

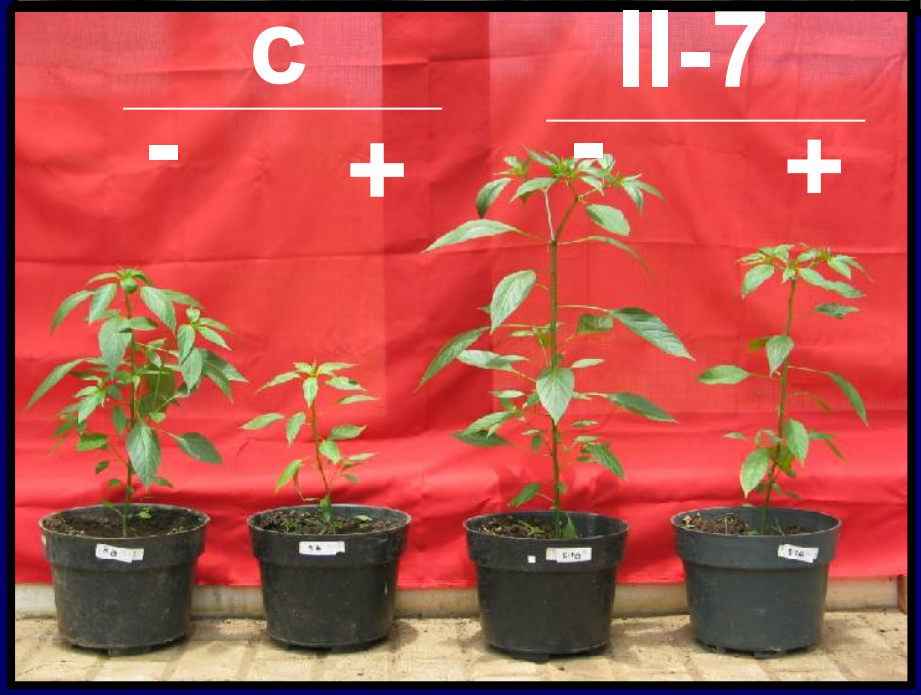
8 MST

c I-16 I-35 I-35 I-16 c

6 MST

+ : Sakit
- : Sehat





6 WAP

+ : Sakit
- : Sehat

8 MST

- + + -

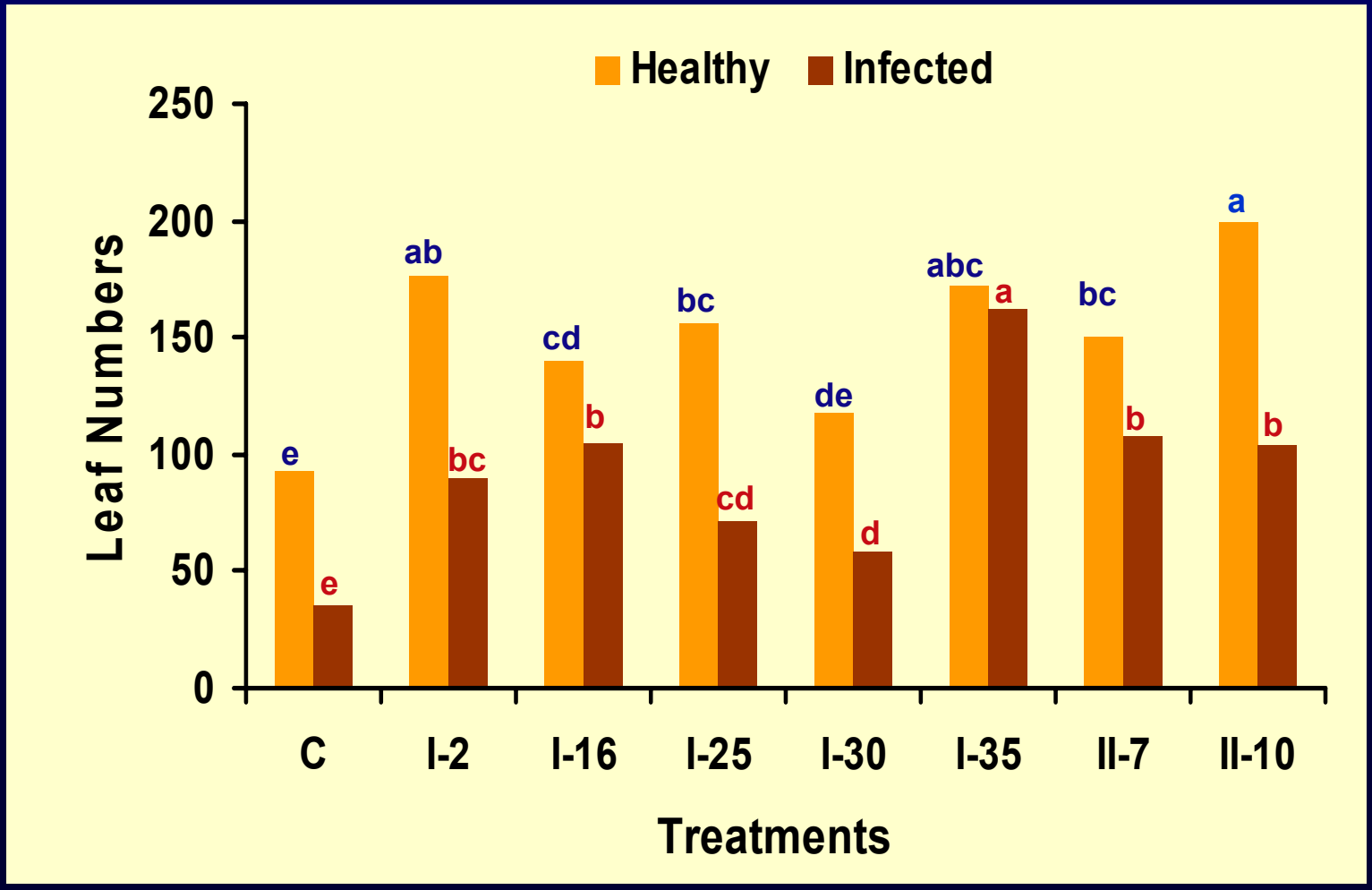
I-35 c

- + - +

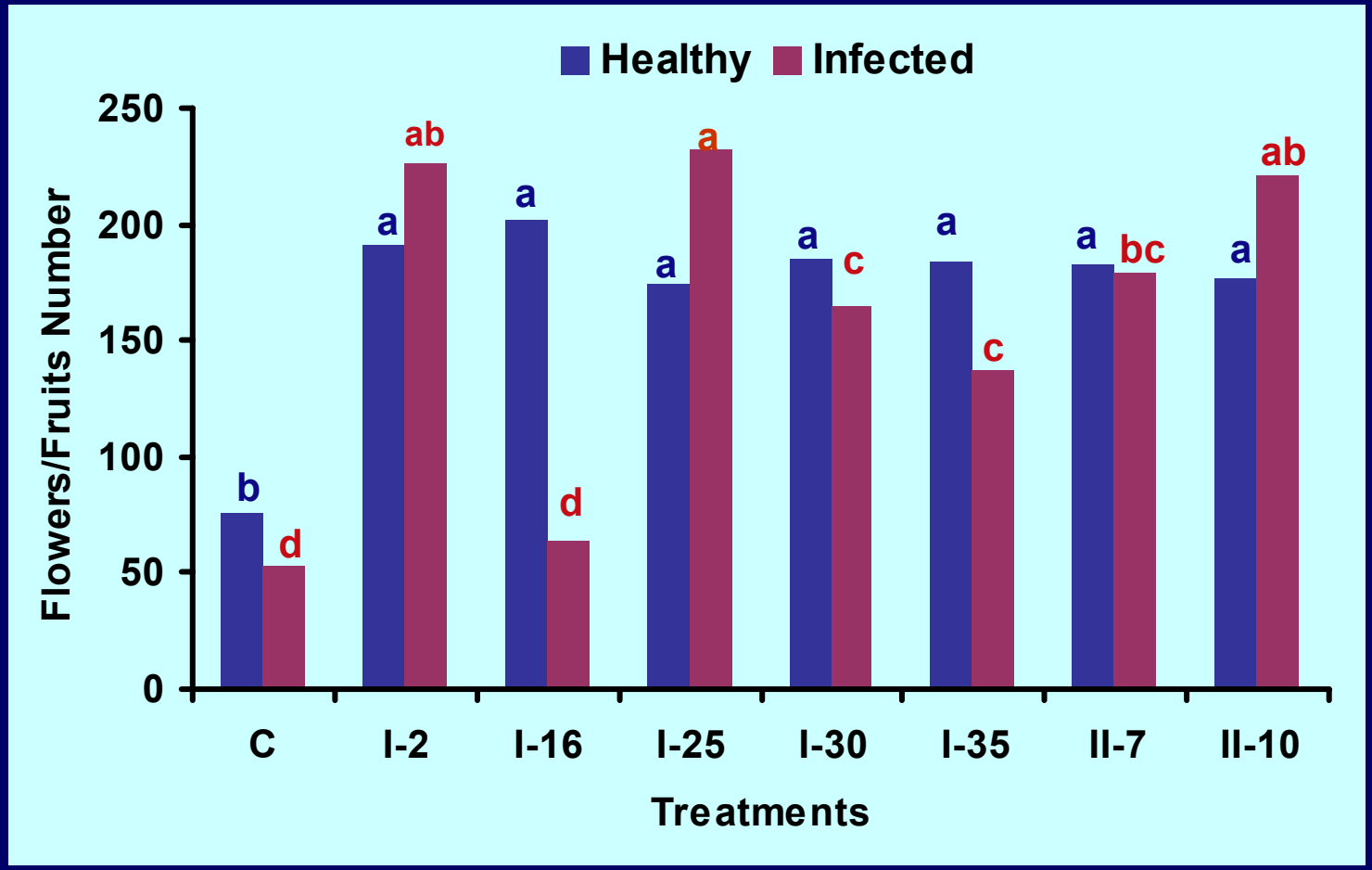
I-16 c

+ : Sakit ; - : Sehat

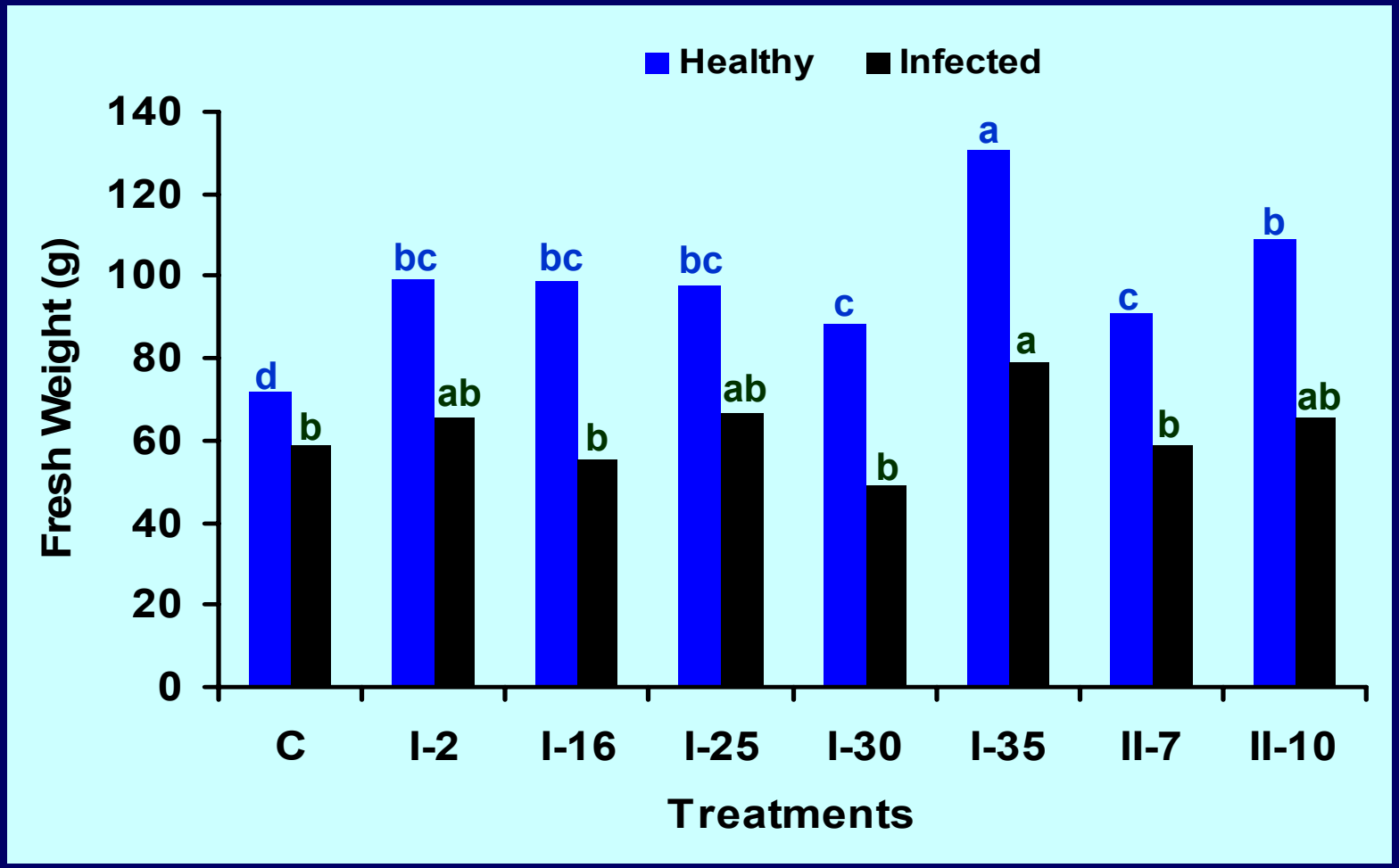
Jumlah Daun


Healthy
Infected
Leaf Numbers
250
200
150
100
50
0
e
e
ab
bc
cd
b
bc
cd
de
d
abc
a
bc
b
a
b
C
I-2
I-16
I-25
I-30
I-35
II-7
II-10
Treatments

Jumlah Bunga/Buah



Bobot Basah


Healthy
Infected
Fresh Weight (g)
140
120
100
80
60
40
20
0
d
b
bc
ab
bc
b
bc
ab
c
b
a
a
c
b
b
ab
C
I-2
I-16
I-25
I-30
I-35
II-7
II-10
Treatments

Disease Assessments

A. Nilai Absorban ELISA

	TMV		ChiVMV	
Treatment	**2 MSI**	**4 MSI**	**2 MSI**	**4 MSI**
C (-)	0.195	0.195	0.061	0.061
C (+)	**1.624**	**1.895**	**0.326**	**0.472**
I.2	**1.889**	**1.942**	**0.187**	**0.386**
I.16	**2.198**	**1.765**	0.080	0.074
I.25	**2.100**	**1.769**	**0.221**	**0.410**
I.30	**1.810**	**1.712**	**0.234**	**0.360**
I.35	**2.039**	**1.751**	0.089	0.065
II.7	**1.955**	**1.660**	**0.195**	**0.358**
II.10	**1.925**	**1.770**	**0.187**	**0.341**

- **Positive if EAV = 2 x C (-) (Orange type)**

B. Disease Incidence and Severity

Treatment	Disease Incidence (%)	Disease Severity
C (-)		
C (+)	100	5.60 a
I.2	100	2.00 bc
I.16	100	1.67 c
I.25	100	3.33 b
I.30	100	2.67 bc
I.35	100	1.67 c
II.7	100	3.33 b
II.10	100	2.67 bc

C. PEROXIDASE ENZYME ACTIVITY AND ETHYLENE PRODUCTION

Treatments	PO. Enzyme Activity (U/mg/min)		Ethylene Prod. (umol/gr)	
	Healthy	Infected	Healthy	Infected
C	0.76	3.42	0.14	0.16
I - 2	3.30	3.50	0.19	0.19
I - 16	2.55	5.70	0.10	0.18
I - 25	5.40	3.60	0.12	0.16
I - 30	4.20	6.60	0.32	0.20
I - 35	4.04	7.74	0.25	0.33
II - 7	4.10	9.40	0.08	0.16
II - 10	2.70	1.40	0.02	0.30

Identification of Rhizobacteria

Morphological Characters & 16S rRNA sequences

Code	Species	Accession No.
I – 2	*Bacillus cereus*	AB288105
I - 25	*B. cereus*	AB288105
I - 35	*B. cereus*	AB288105
I – 16	*Brevibacterium sanguinis*	AB288106
I – 30*	*B. macerans*	-
II -7	*Acinetobacter sp* II -7	AB288107
II –10	*Stenotrophomonas sp* II-10	AB288108

* Based on morphological characters & Microbact-Kit test only

CONCLUSIONS

- All tested isolates could enhance plant growth characters and could suppress the severity, even infected by viruses

- *Bacillus cereus* (I-35) and *Stenotrophomonas* sp II- 10 were the most potential PGPR which able to protect hot pepper against multiple infection of virus

ACKNOWLEDGEMENTS

- SEAMEO BIOTROP & ITSF
- PHK B Dept. of Plant Protection, IPB
- Prof. Tetsuro Okuno & Dr. Kazuyuki Mise
- Mr. Edi Supardi - Teknisi


TERIMAKASIH