

**PROTECTION OF HOT PEPPER AGAINST MULTIPLE
INFECTION OF VIRUS BY UTILIZING PLANT GROWTH
PROMOTING RHIZOBACTERIA (PGPR)**



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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

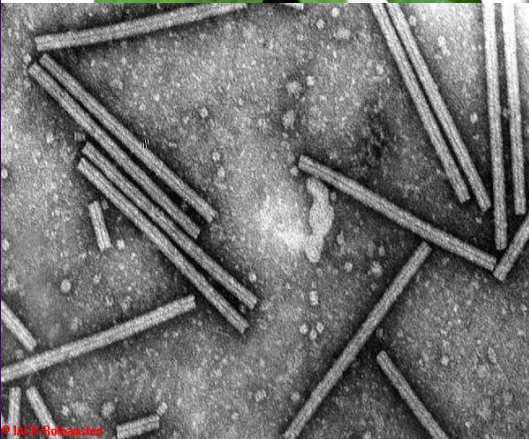
Mix

CMV

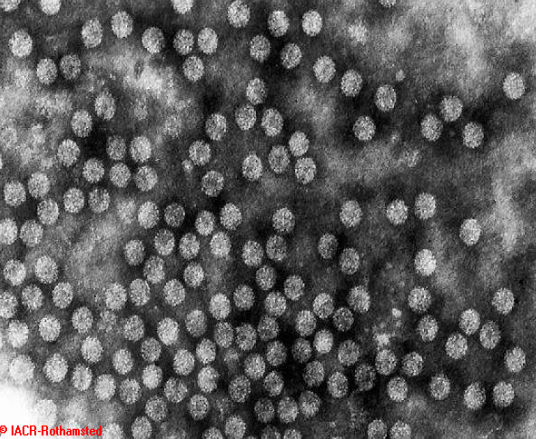
TMV

SYMPTOM AND VIRAL PARTICLES

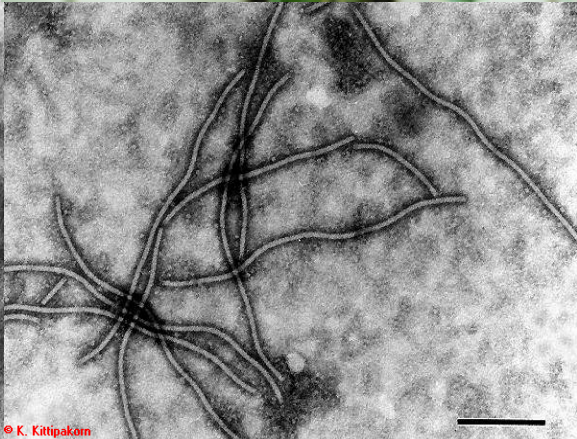
TMV



CMV



ChiVMV



[Particle Source; CPC 2003]

SYMPTOM OF MIX INFECTION



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 :

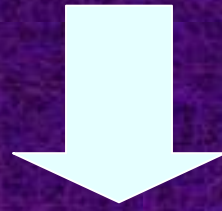
Plant-Growth Promoting Rhizobacteria (PGPR)

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



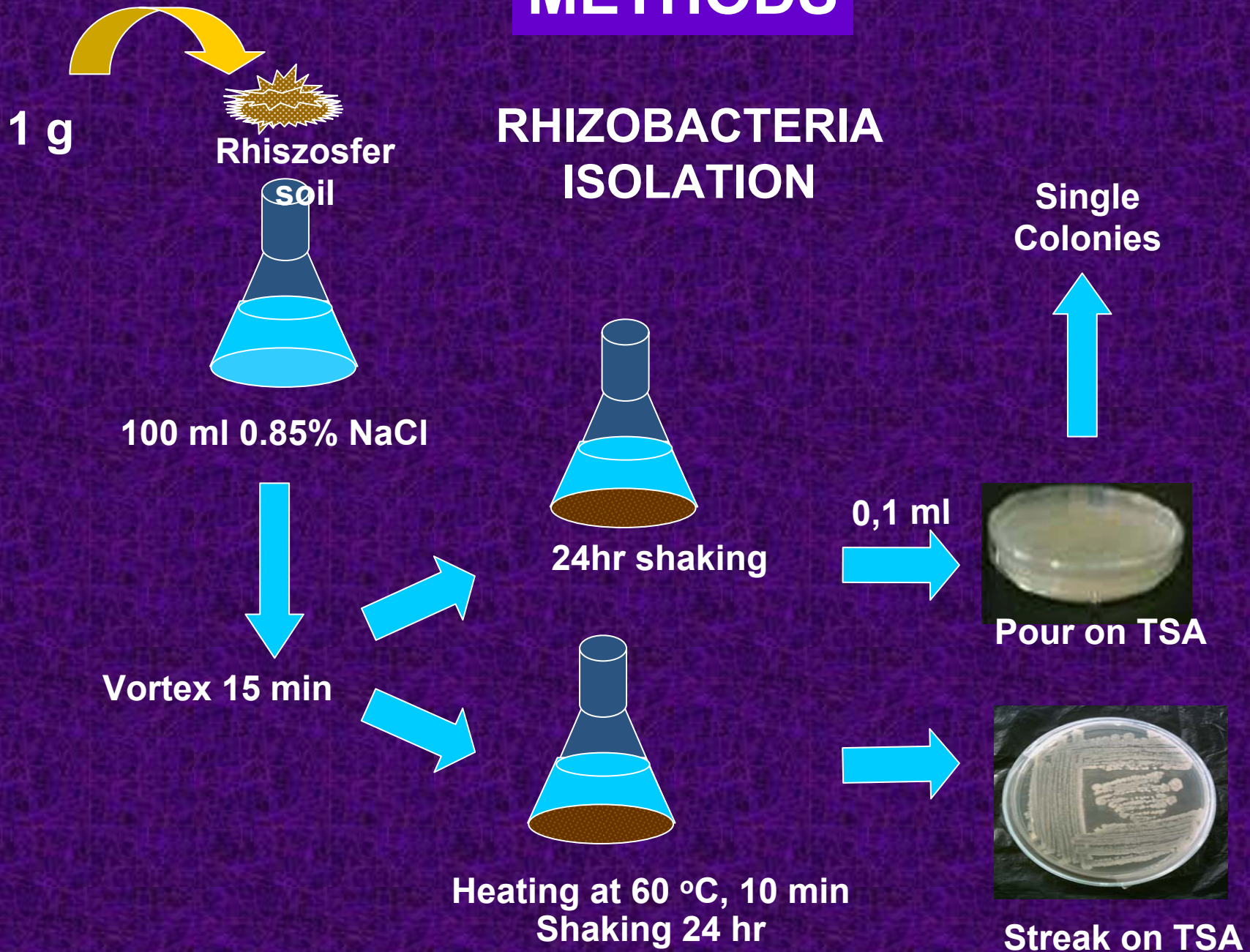
**Decrease disease incidence, severity, symptom
expressions**

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



➤ Seed treatment and cultivation


SeedsTM-999



Bacteria Suspension
 1×10^9 cfu/ml



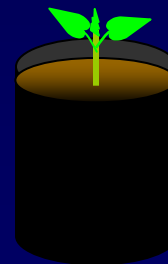
Ssoaked for 3 hr



Seedling + a drop
bact. suspension



2 Weeks after



Pot

Sterile Soil : cow-dung =
1:1



Placed in greenhouse

Methods:

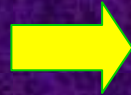
Evaluation of plant growth characteristics

- ❖ 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



Observation



Plants at 4 WAP

Methods: Disease assessments

1. Disease incidence (%)

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

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

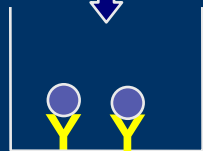
ELISA (Enzyme Linked-Immunosorbent Assay)



Coating 1st AB



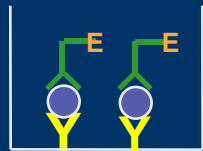
Washing 4-8 times



Antigen bound to the 1st AB



Washing 4-8 times



2nd AB conjugated with enzyme



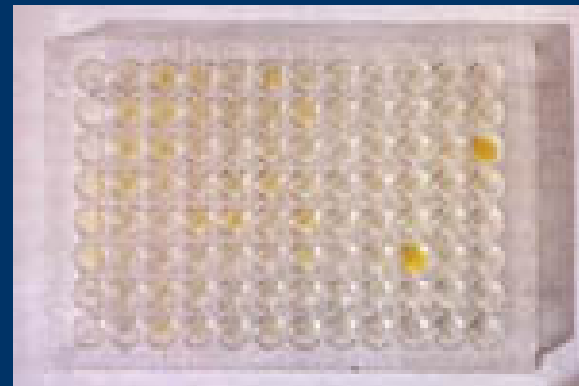
Washing 4-8 times



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. ETHYLENE PRODUCTION



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

Samples measured at 5 days post viral inoculation

RESULTS

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

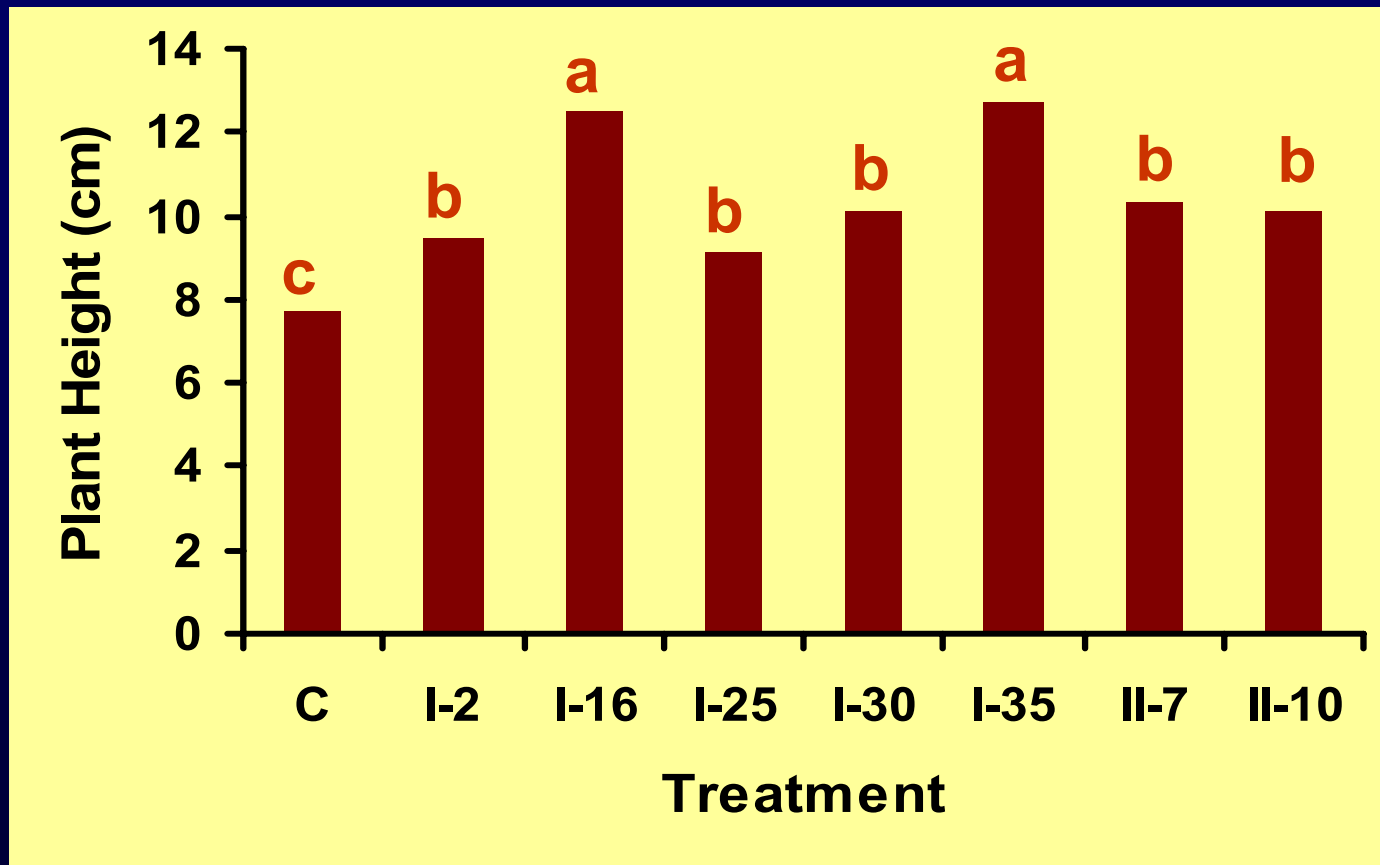


Bacterized-seeds showed **comparable germination rate**, but better seedling vigor and fitness than untreated control



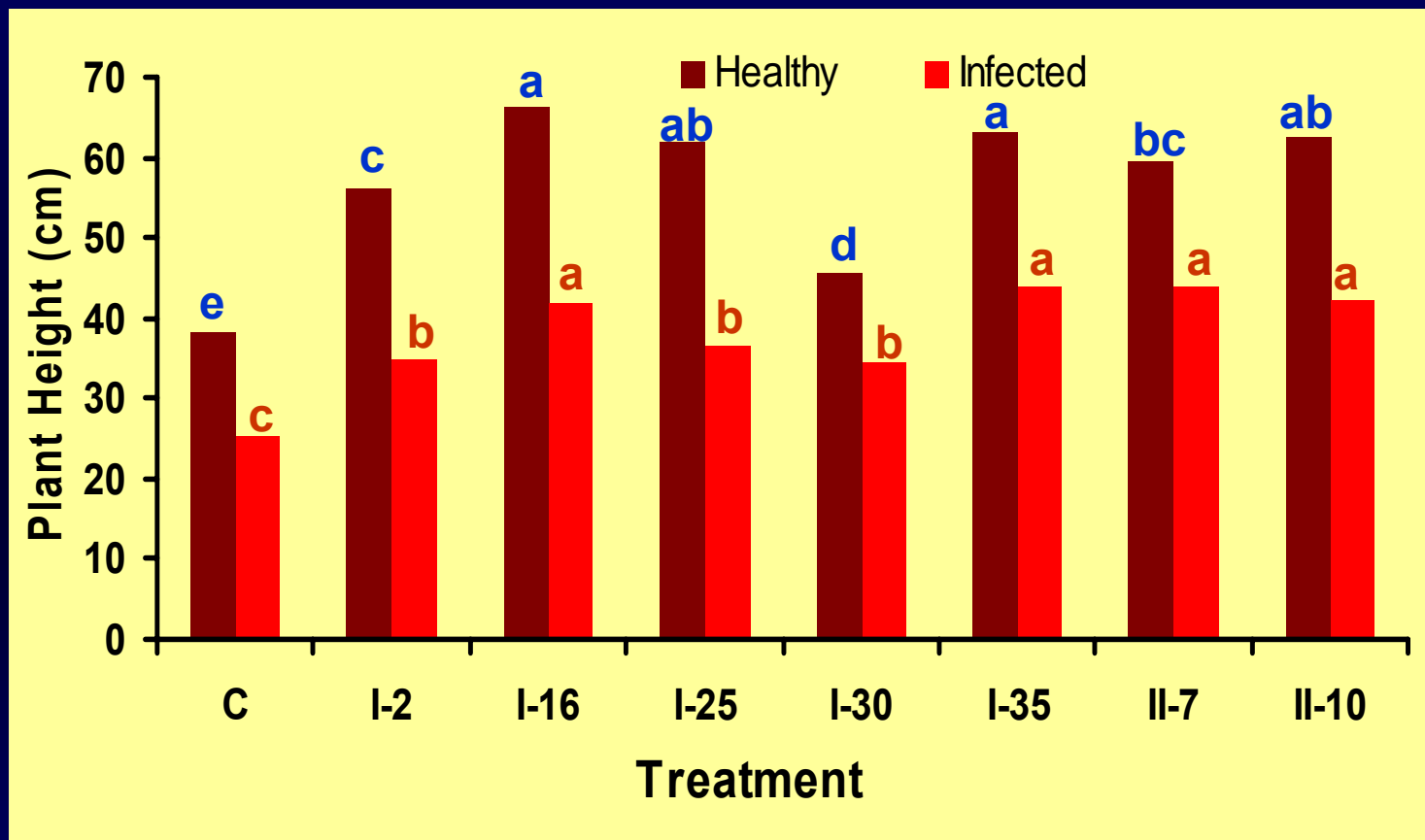
7 isolates were evaluated their ability to protect pepper against 3 viruses

Plant Height at 1 dbi



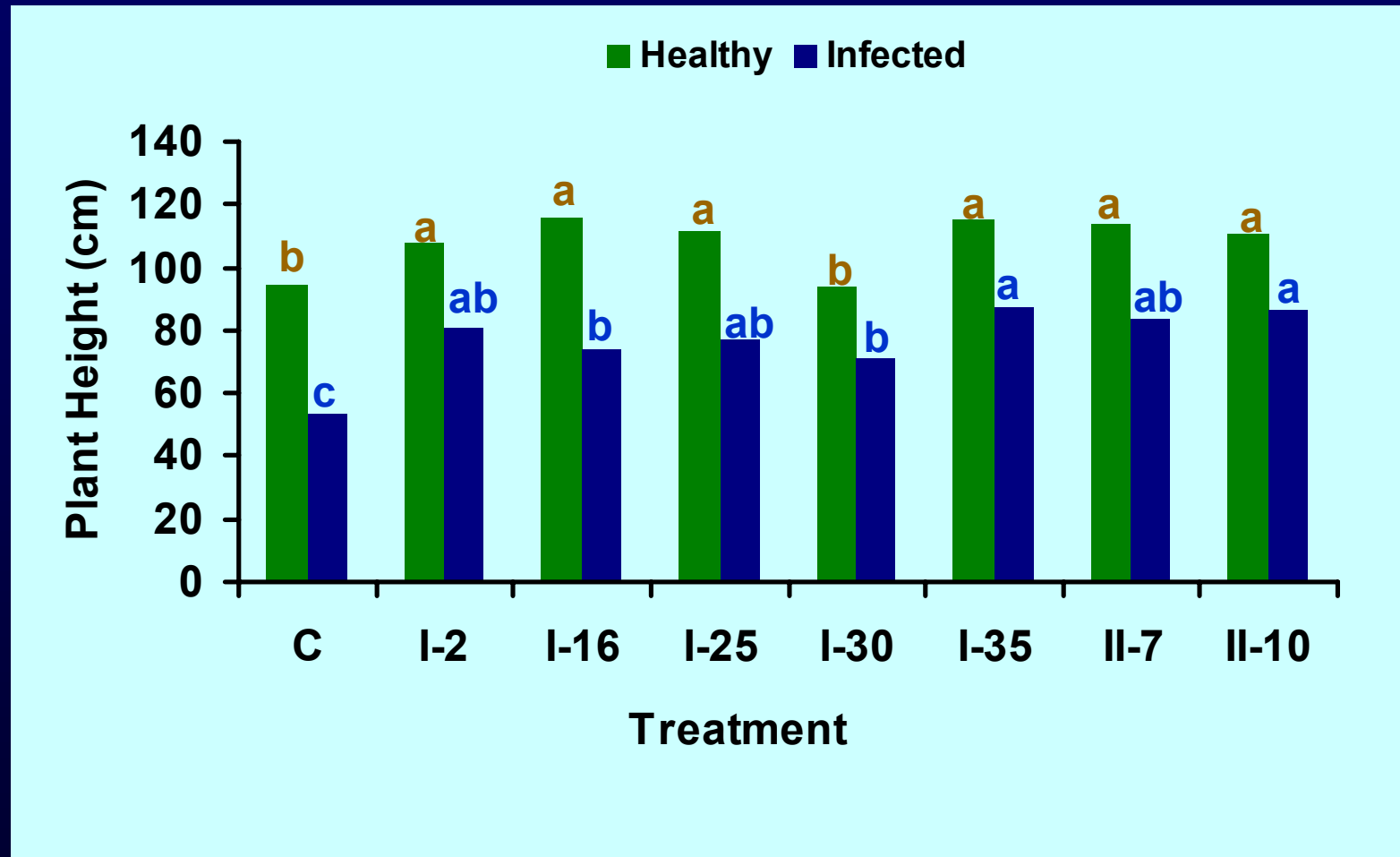
dbi – day before inoculation

Plant Height at 8 WAP



WAP- week after planting

Plant Height at 12 WAP



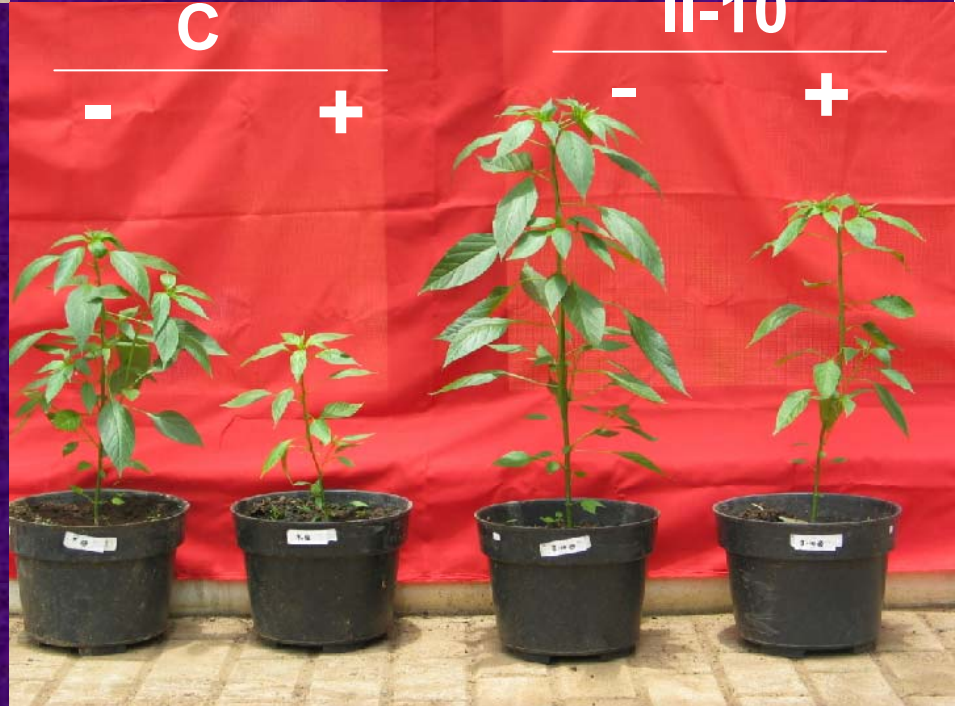


HEALTHY

SEEDLING



12 WAP



HEALTHY PLANTS

Seedling

8 WAP



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

8 WAP



- + + -

I-35

c

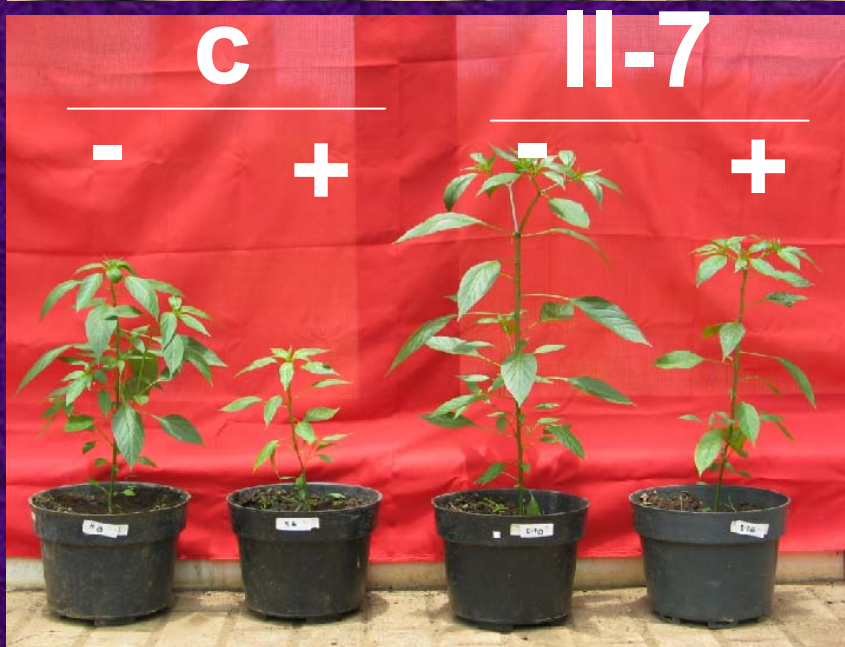
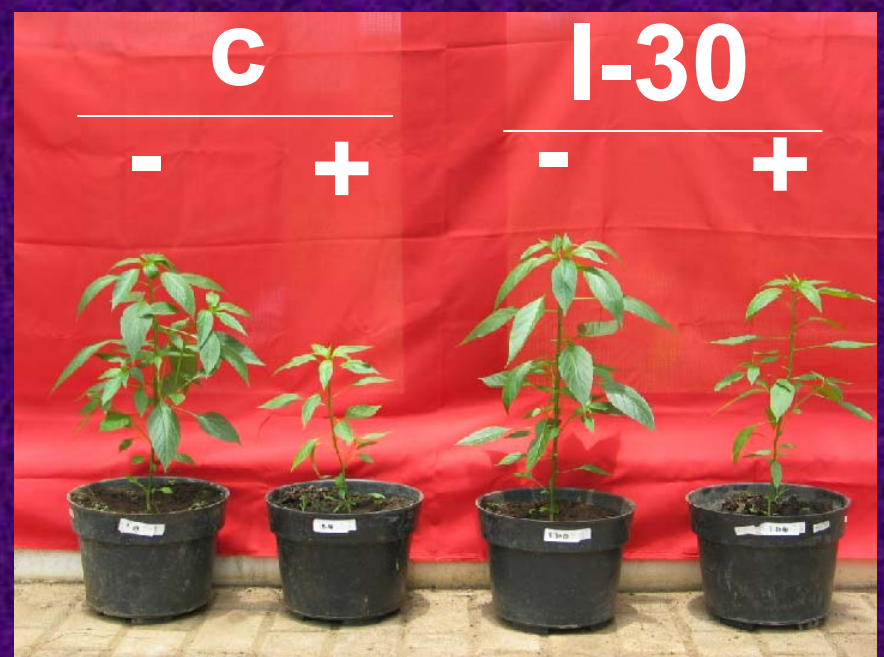


- + - +

I-16

c

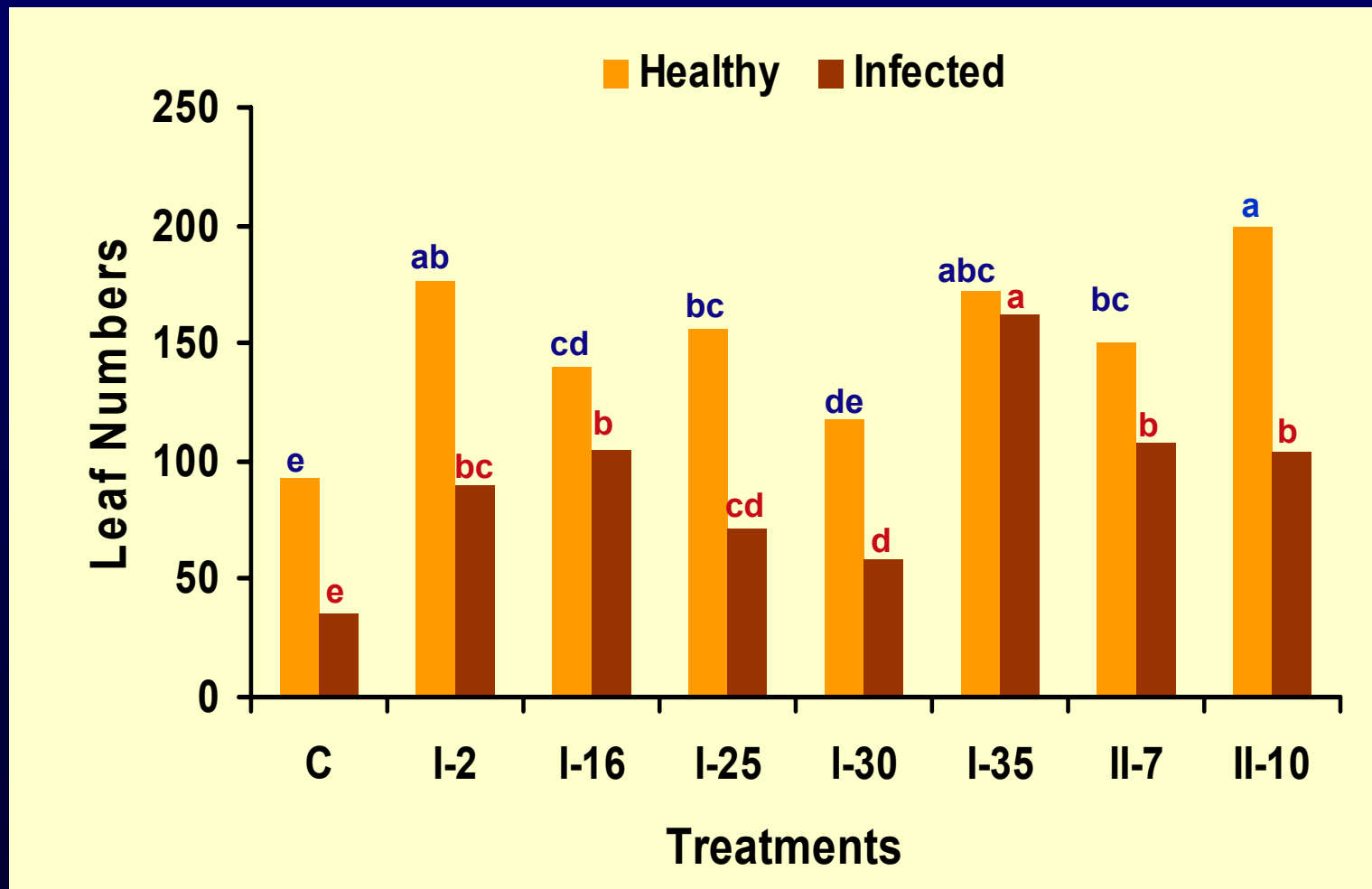
+ : Infected; - : Healthy



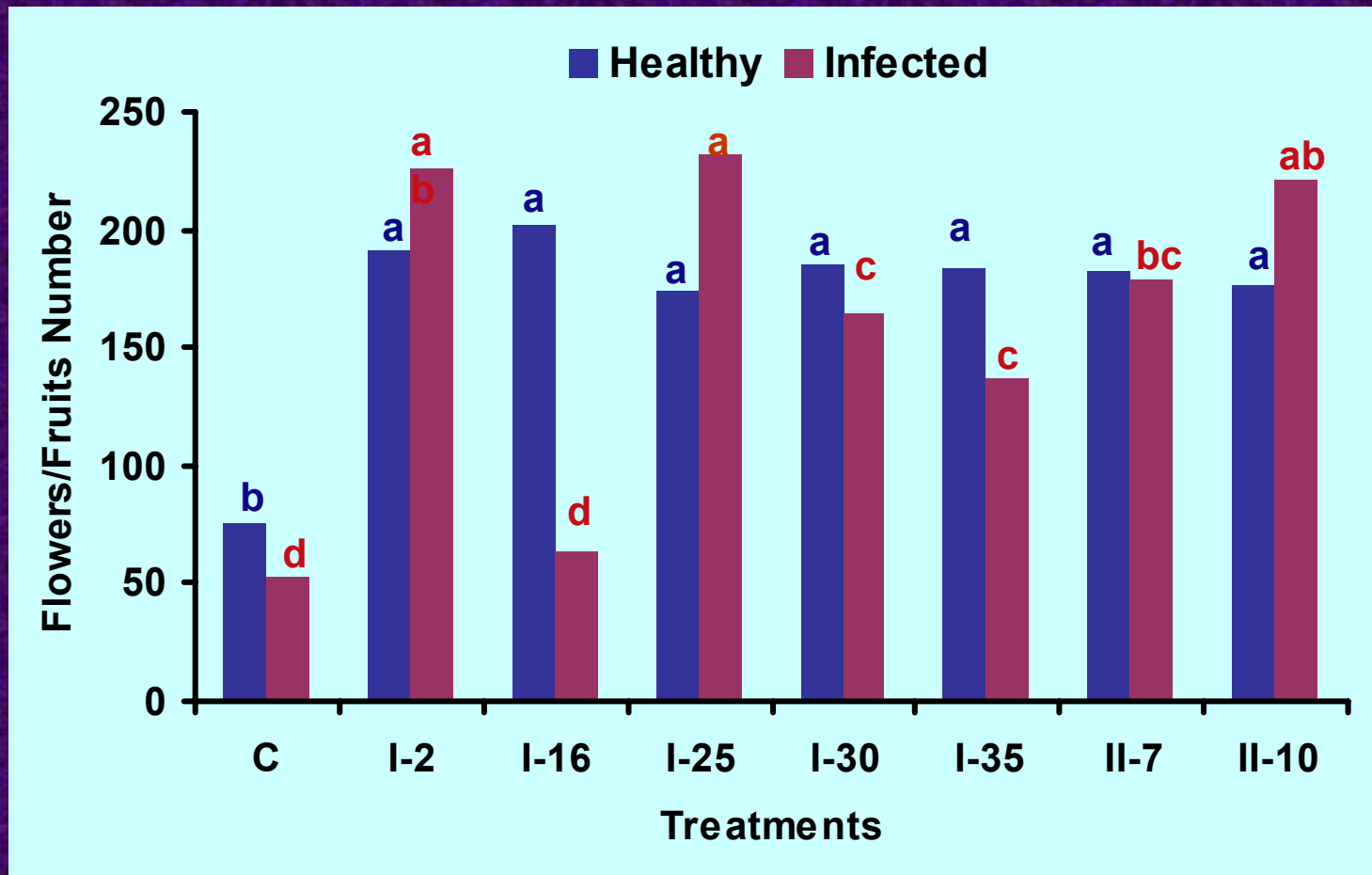
6 WAP

+ : Infected
- : Healthy

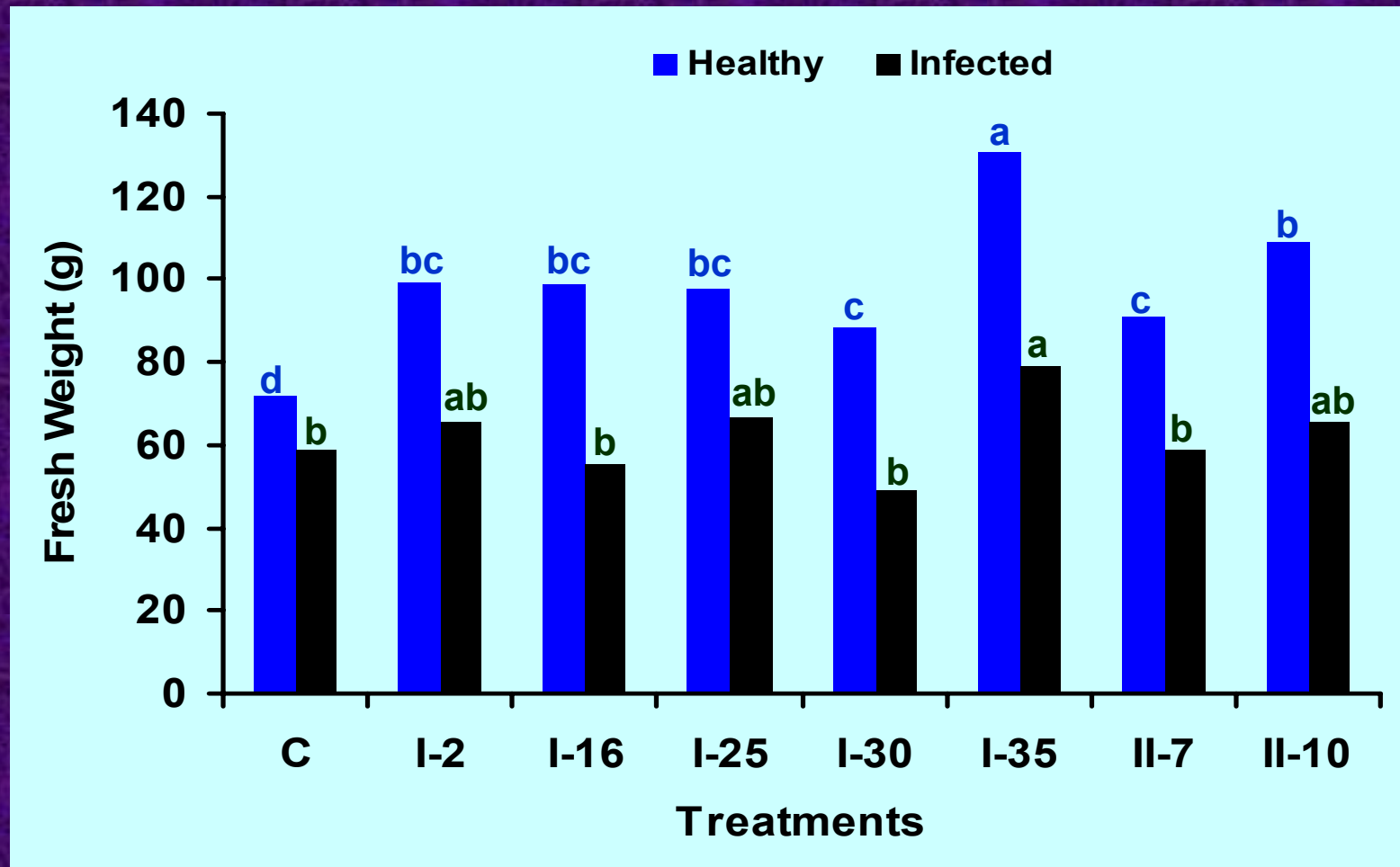
Leaf Numbers



Flowers/Fruit Numbers



Fresh Weight



Disease Assessments

A. Viral Protein Accumulation

Treatment	ELISA Absorbance Value					
	TMV		CMV		ChiVMV	
	2 wpi	4 wpi	2 wpi	4 wpi	2 wpi	4 wpi
C (-)	0,195	0,195	0,093	0,093	0,061	0,061
C (+)	1,624	1,895	0,096	0,100	0,326	0,072
I.2	1,889	1,942	0,071	0,069	0,187	0,386
I.16	2,198	1,765	0,069	0,068	0,080	0,074
I.25	2,100	1,769	0,071	0,066	0,221	0,410
I.30	1,810	1,712	0,077	0,074	0,234	0,360
I.35	2,039	1,751	0,091	0,089	0,089	0,065
II.7	1,955	1,660	0,095	0,095	0,195	0,358
II.10	1,925	1,770	0,095	0,102	0,187	0,341

- Positive if EAV = 1.5 x C (-) (Orange type)

B. Disease Incidence and Severity

Treatment	Disease	Disease
	Incidence (%)	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	<i>Bacillus cereus</i>	AB288105
I - 25	<i>B. cereus</i>	AB288105
I - 35	<i>B. cereus</i>	AB288105
I – 16	<i>Brevibacterium sanguinis</i>	AB288106
I – 30*	<i>B. macerans</i>	-
II -7	<i>Acinetobacter sp II -7</i>	AB288107
II –10	<i>Stenotrophomonas sp II-10</i>	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

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THANK YOU

