EXPLORATION OF RHIZOBACTERIA-MEDIATED GROWTH PROMOTION TO PROTECT PEPPER AGAINST *TOBACCO MOSAIC TOBAMOVIRUS*

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Background

1. Pepper is one of the most important horticulture crop
2. Virus diseases: *Cucumber Mosaic Virus* (CMV), *Chilli Veinal Mottle Virus* (ChiVMV), *Tobacco Mosaic Virus* (TMV), *Tomato Mosaic Virus* (ToMV), Geminivirus, etc

TMV (Tobamovirus, (+) strand RNA

Host : Solanaceae, Scrophulariaceae, Labiatae, Leguminosae, Chenopodiaceae, Alliaceae, and Cucurbitaceae

TMV cause severe damage to pepper in almost all planting areas. Yield loses up to 100%.

Phenotype Symptom

Healthy ChiVMV Mix CMV TMV

3. Management of virus diseases:

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

Alternative disease control (Biocontrol) ?

Rhizobacteria?

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 plant pathogenic organisms

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

Induced Systemic resistance (ISR)

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

Objectives

1. To explore rhizobacteria which promote plant growth
2. To evaluate their capacity as Plant Growth Promoting Rhizobacteria(PGPR)-Elicited Induced Systemic Resistance (ISR) to protect pepper against *Tobacco Mosaic Tobamovirus*

Research Location

Laboratory works:

Lab. Virology, Department of Plant Protection, IPB

Lab. Microbiology, Department of Biology, IPB

Green house experiments:

Green house of the Dept. of Plant Protection, IPB

(at Cikabayan, Darmaga)

Methods: Preparation of Rhizobacteria

1. Isolation of rhizobacteria from pepper rhizosphere (soil, rhizosphere, rhizoplane, root tissue)

Samples in 0.1 M $MgSO_4.7H_2O$ → Vigorous shaking ↓ Dilution plated on TSA medium → Single colony isolation& cryopreserved in 50% glycerol

2. Identification of rhizobacteria

(the presence of oxidase, gelatinase, nitrate reduction; other test using Microbact Kit)

3. Culturing and multiplication of rhizobacteria for seed treatment

Methods:
Treatments on Pepper Var. TM 999

Seed treatment
(soaked in rhizobacteria 10^9 CFU/ml for 2 hours)

⬇

Seed sowing on sterile growth media
(Soil : cow dung manure = 2 : 1)

⬇

Transplanting 2 week-old seedling
(into polybag containing sterile growth media)

⬇

Plant inoculation with sap containing TMV by mechanically inoculation (2 weeks post-transplanting)

⬇

Evaluation

Methods:

Evaluation of plant growth characteristics

- Rate of germination at 8, 11 and 14 days after seedling
- Plant height at 1 day prior- and at 2, and 4 weeks post-virus inoculation (wpi)
- Plant fresh weight
- Number of flowers and fruits

Methods: Disease assessment

1. Disease incidence (%)

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

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

Methods: Disease assessment

2. Specific 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

Research Design

Experiments are arranged in randomized complete design with 4 treatments, and 6 replications. Data analysis (mean of height, fresh weight, flowers and severity) was conducted using ANOVA (SAS 6.12) and then compared by Duncan's Multiple Range (DMRT)

The treatment;

1. Without rhizobacteria inoculated with TMV
2. With rhizobacteria inoculated with TMV
3. With rhizobacteria without TMV inoculation
4. Control (without rhizobacteria and TMV inoculation)

3. Detection of viral protein accumulation by DAS ELISA

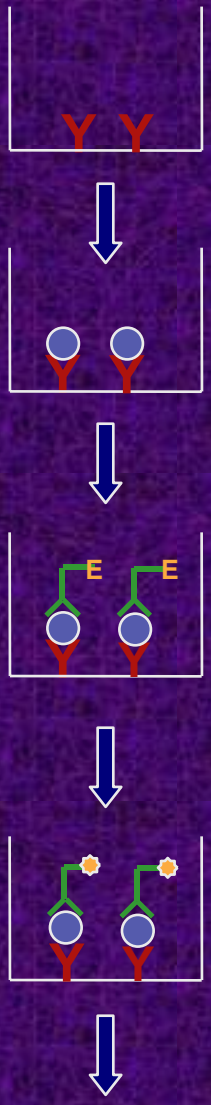
Coating Microtiter plates with
TMV antibody (AB) in Carbonate buffer

Three non-inoculated leaflets
are grinded with PBST buffer (1 : 10 = W : V)

Addition of 2nd AB conjugated with Enzyme

Substrates addition (PNP) in Alkaline phosphatase buffer

Positive result if Accumulation of protein is
At least 2 times of negative control



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. Extraction and quantification of Peroxidase enzyme activity after challenge inoculation

- It conducted at a week after virus inoculation

Expected Research Outcomes

Obtain potential PGPR-elicited ISR isolates which can be utilized to protect hot pepper against virus (TMV). It is offer an attractive in providing a natural, safe, effective, persistent and durable type of protection

RESULTS AND DISCUSSIONS

RHIZOBACTERIA ISOLATES

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

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36 isolates were tested for inducing seed germination

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

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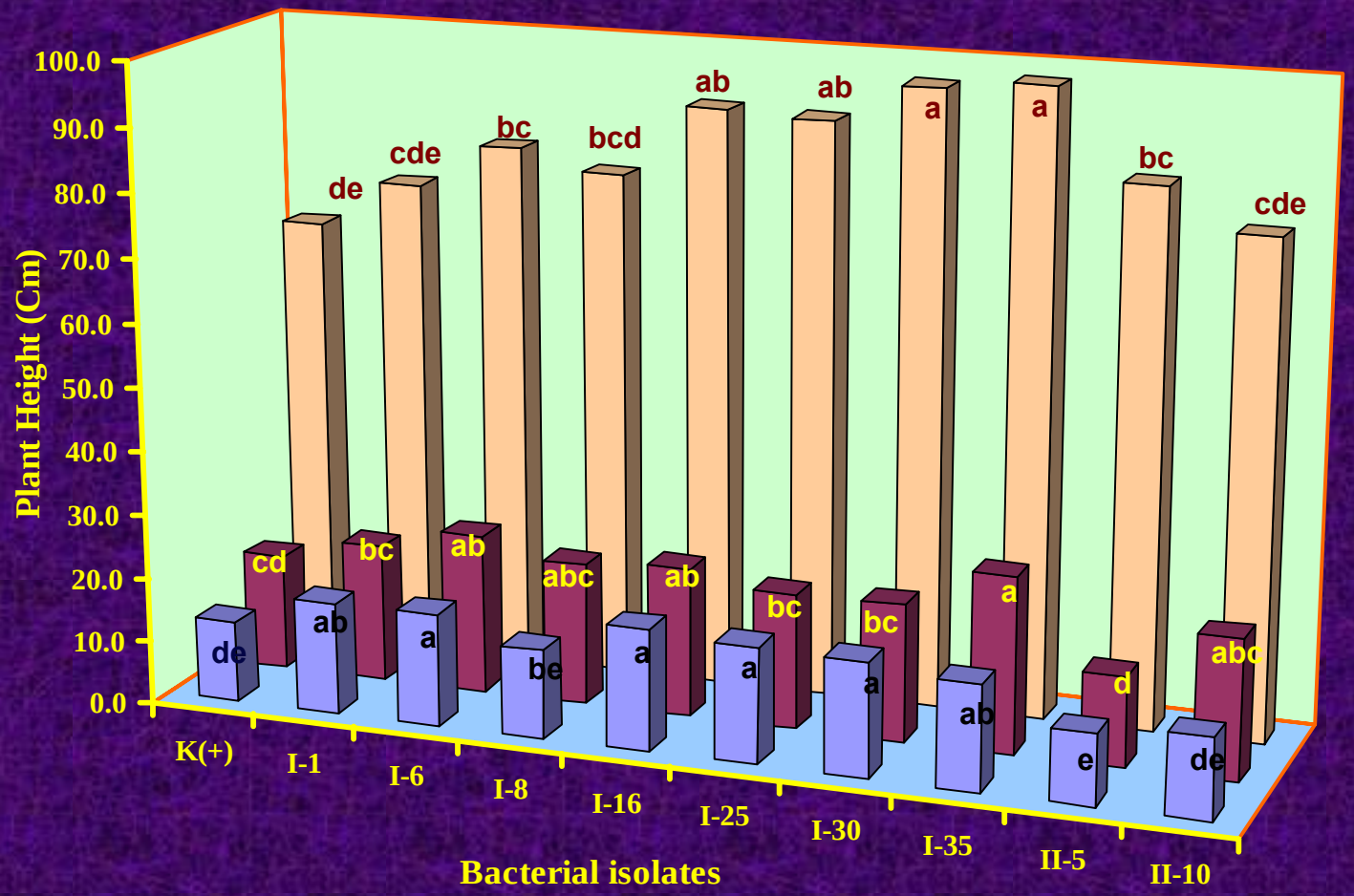
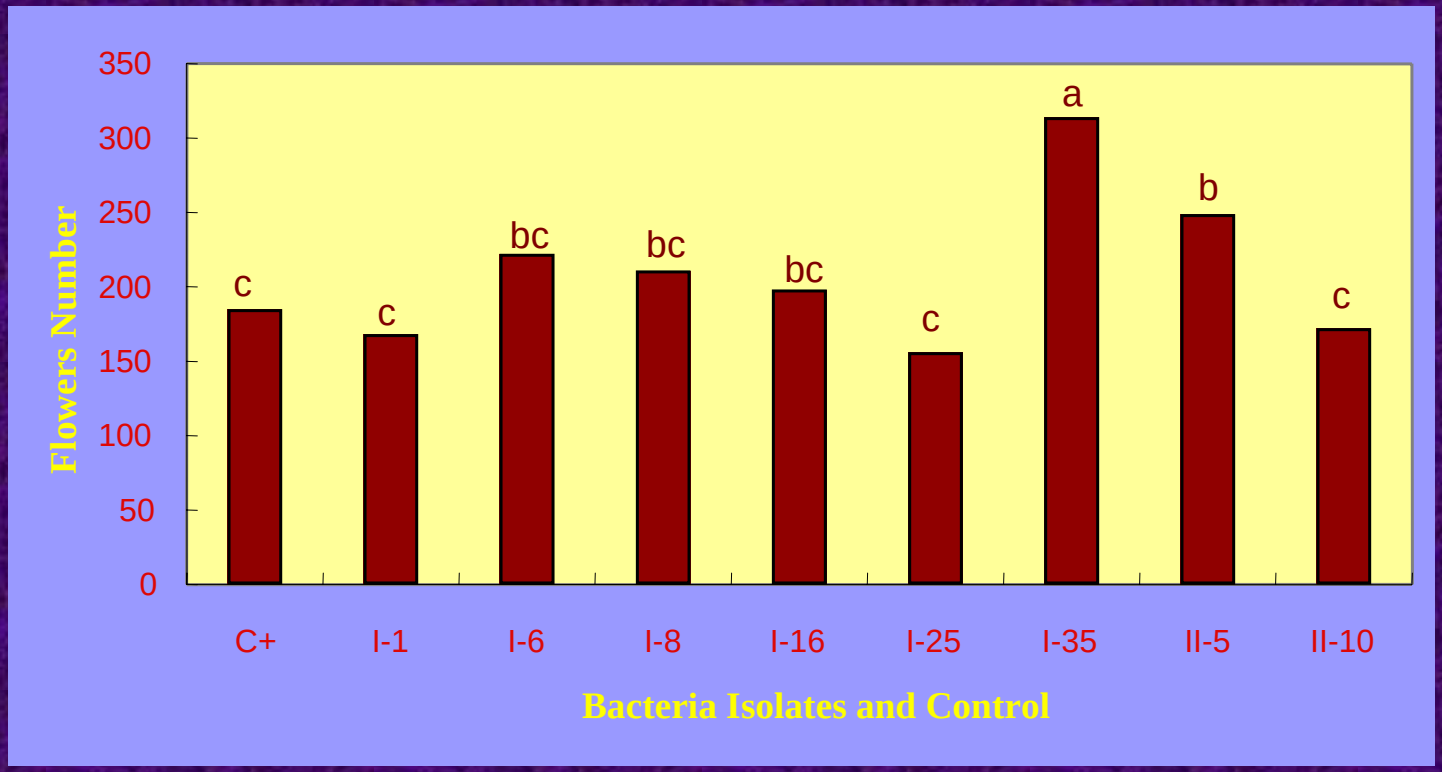
9 isolates were evaluated their ability to protect pepper against TMV

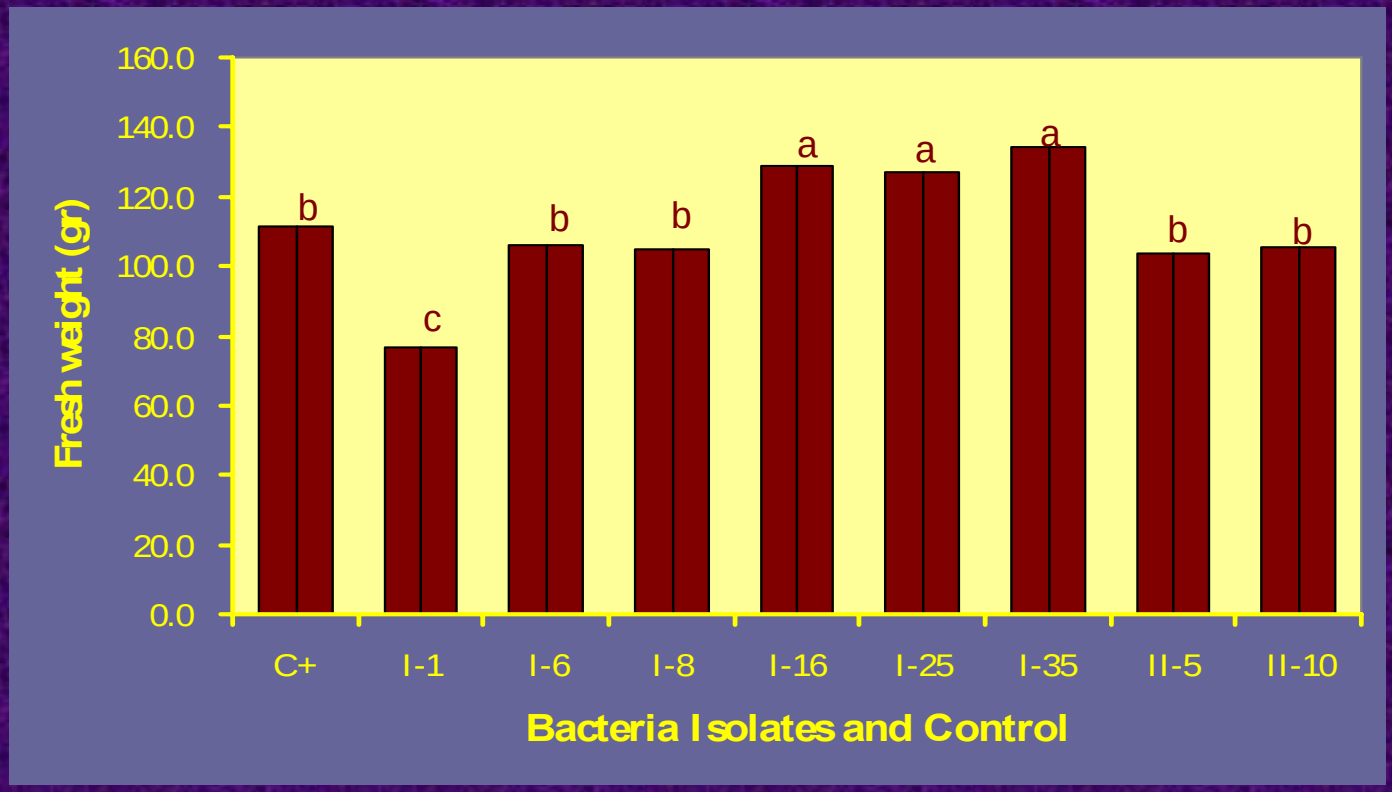
Plant Height of Bacterized-plants + TMV at 2 wpi, 4 wpi and 10 wpi



Number of Flowers



Fresh Weight

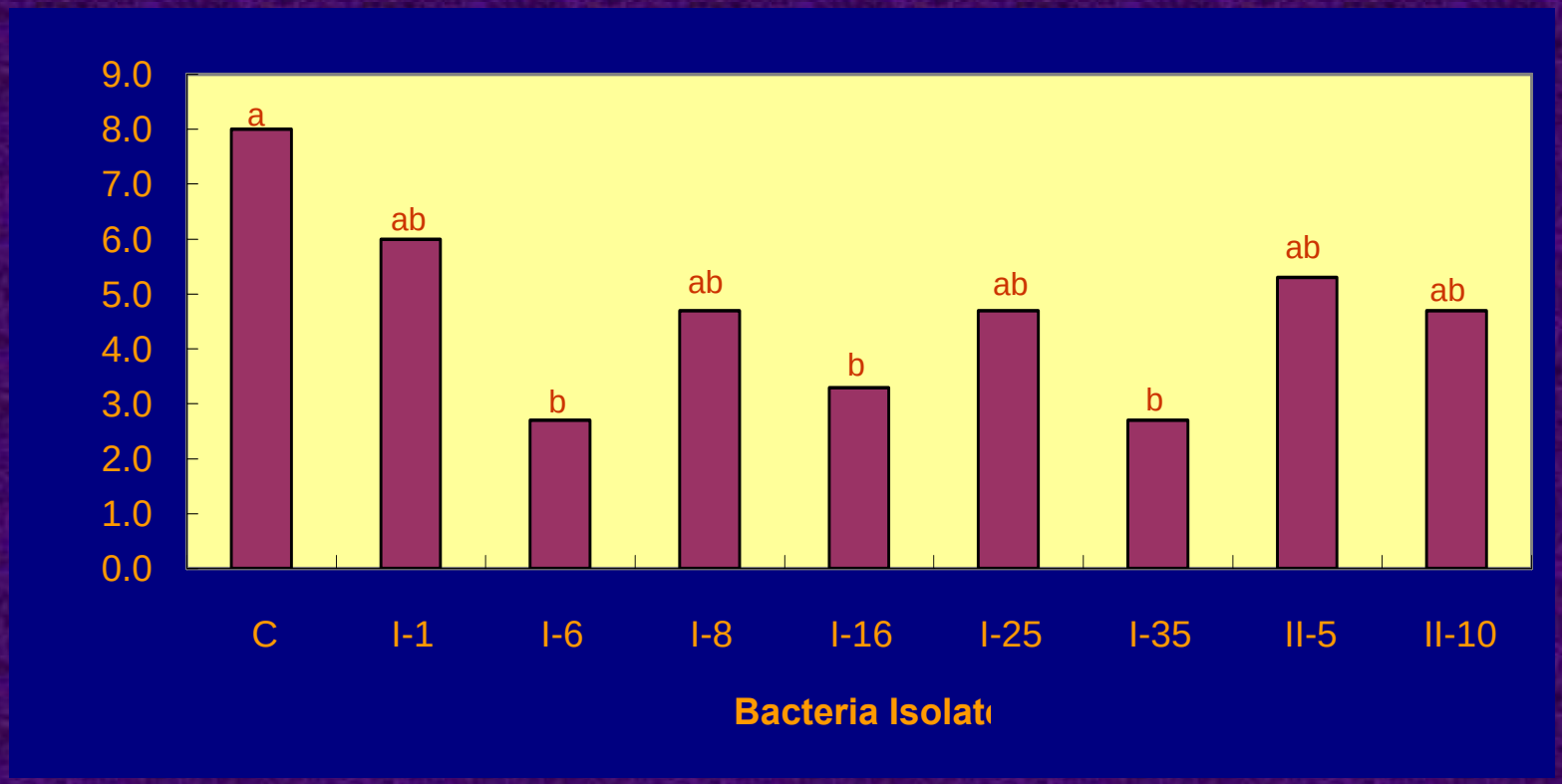


Protein Accumulation, Disease Incidence and Severity

Isolates	NAE 2 wpi	NAE 4 wpi	DI (%)	DS
Control (-)	0.309	0.337	0.0	0.0
Control (+)	2.283	1.527	100.0	8.0a
I-1	2.133	2.230	100.0	6.0ab
I-6	0.608	1.901	66.7	**2.7b**
I-8	2.202	1.546	100.0	4.7ab
I-16	2.005	1.774	66.7	**3.3b**
I-25	2.106	1.323	100.0	4.7ab
I-30	2.323	1.639	100.0	4.0ab
I-35	2.116	1.667	66.7	**2.7b**
II-5	2.282	2.029	100.0	5.3ab
II-10	2.235	1.578	100.0	4.7ab

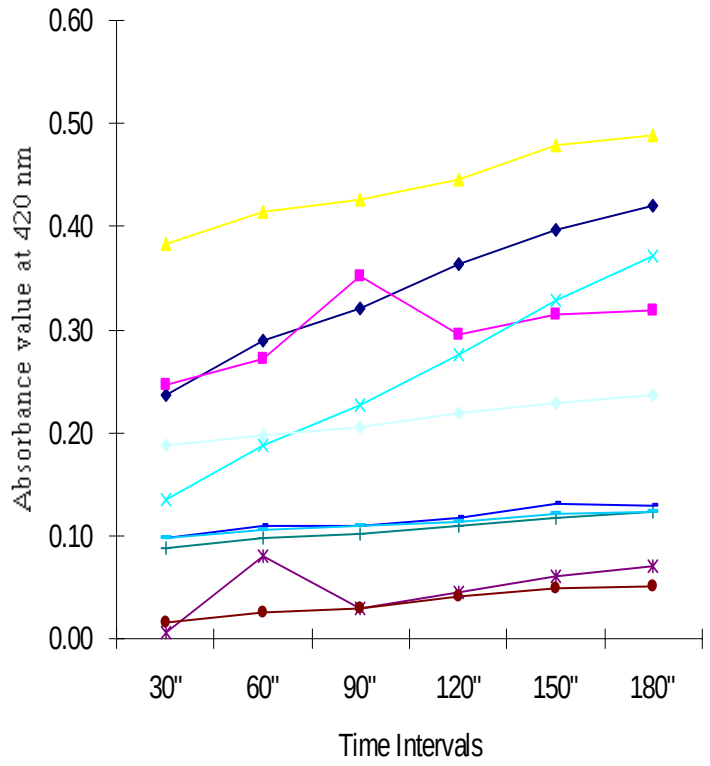
Wpi= weeks after viral inoculation; NAE= absorbance value of ELISA
DI= disease incidence; DS= disease severity

DISEASE SEVERITY

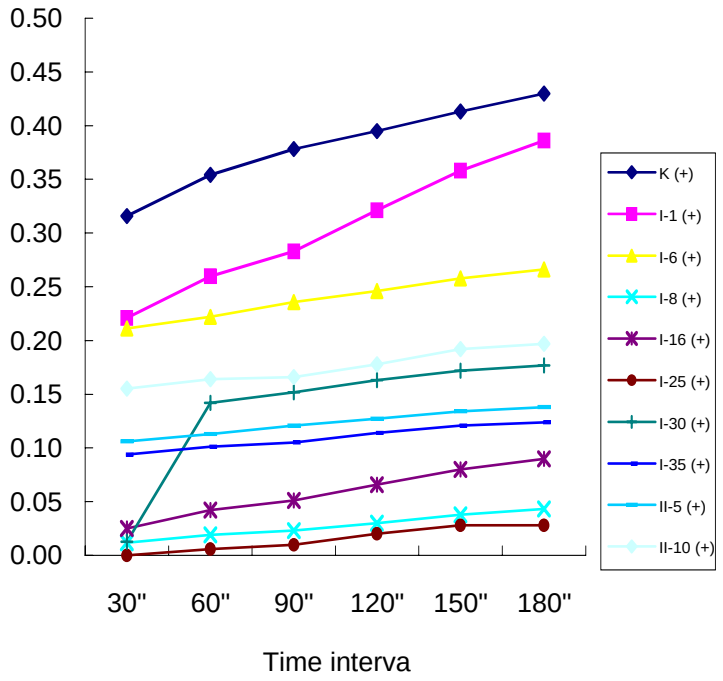


Peroxidase Enzyme Activity

NO TMV



TMV



Peroxidase Enzyme Activity

Treatment	Peroxidase activity (U/ml)		Spec.Perox.Activity (U/mg protein/min)	
	TMV (-)	TMV (+)	TMV (-)	TMV (+)
K	**6.60**	**3.80**	**5.47**	**2.89**
I-1	2.40	6.30	2.42	2.49
I-6	**3.70**	**2.20**	**3.01**	**1.62**
I-8	9.20	1.20	7.03	1.41
I-16	**2.30**	**2.40**	**1.34**	**1.81**
I-25	1.30	1.80	0.87	0.65
I-35	**1.20**	**1.80**	**1.02**	**1.41**
II-5	1.00	1.30	1.05	1.06
II-10	2.00	1.70	1.04	0.87


I-1


I-6


I-8


I-16


I-25


I-30


I-35


II-5


II-10

Plant growth of Bacterized-plants + TMV vs Untreated Control

A. I-1, B. I-6, C. I-8, D. I-16, E. I-25, F. I-30, G. I-35, H. II-5, I. II-10
Pot position from leaf-right : Healthy, TMV infected, Bacterized Healthy and Bacterized + TMV

On going Work

Identification of Potential Rhizobacteria Isolates

Using Microbact Kit and additional tests such catalase test, Growing on NaCl media etc.

I-6 : ***Acinetobacter* sp**

I-35 : ***Bacillus circulans***

I-16 : **on progress**

CONCLUSIONS

Isolates I-6, I-16, I-35 considerable as potential PGPR which capable to protect pepper against TMV. I-35 was consistently showed best characters during the experiment.

Future Plans

1. Evaluation of potential isolates to protect pepper against multiple disease in individual and combination formulation

2. Efficacy of potential isolates in field experiment, appropriate doses and formulation

3. Studies on induction of defense-related protein in hot pepper (defense mechanism)

ACKNOWLEDGEMENT

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THANK YOU

PGPR as stimulant

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Seed treatment, Soil drench
Foliar spray, Combination

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Challenge inoculation of pathogens
(Fungi, bacteria, virus etc)

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Elicits Plant's defense response

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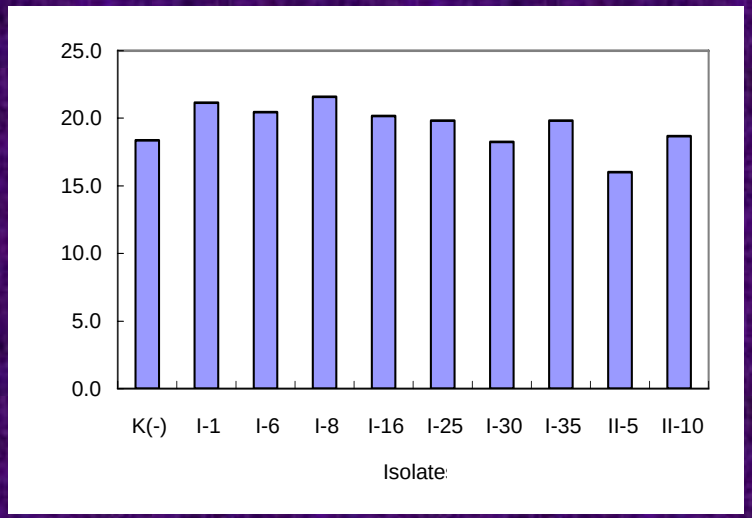
Decrease disease incidence, severity, symptom expression

- Increased Salicylic Acid → Pathogenesis related gene (PRs, chitinase etc)
- Increase Jasmonate Acid (MeJA) and ethylene, phytoalexins, enhance ability to lignify
- Siderophores (pyoverdin, pyochelin, SA)
- Competition for iron
- Alters host physiology and metabolic responses, fortifies plant cell wall strength
- Antibiosis

Plant Height at 28 and 42 DAP

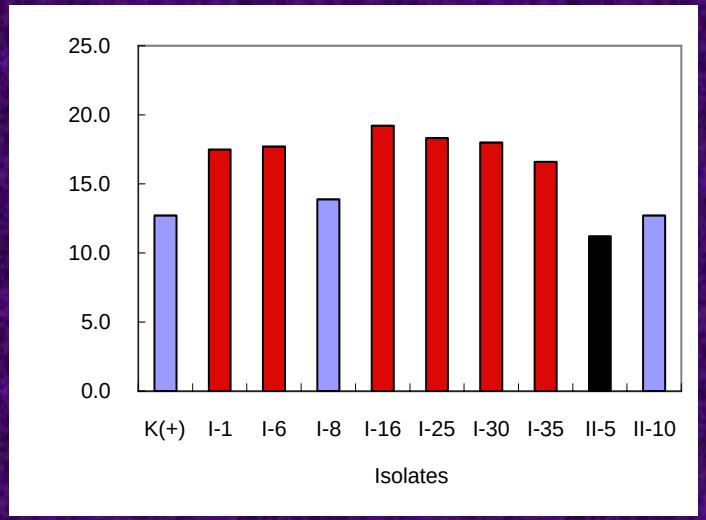
No TMV

A.

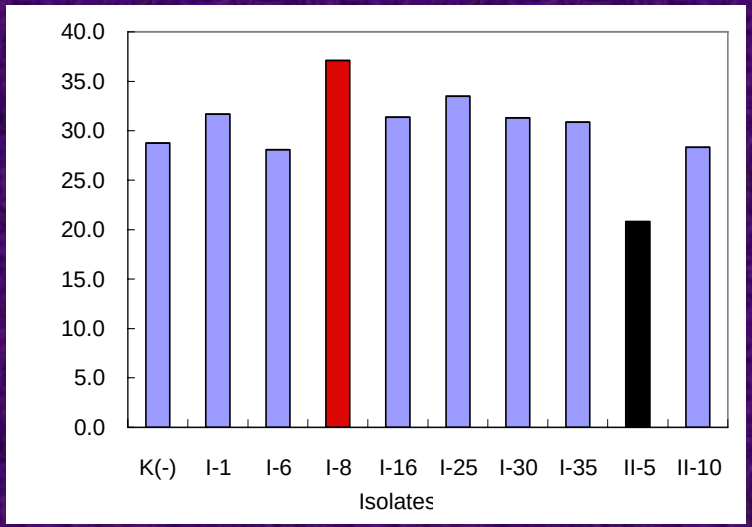


TMV

B.



C.



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