

# Efficacy hot tetracycline treatment to control sugarcane white leaf disease in Thailand

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# Sugarcane diseases in Thailand





Sugarcane white leaf disease at Khon Kaen Province in 1977 with Asst. Prof.Dr. Kasem Sooksathan

- Sugarcane white leaf (SCWL) disease is the most destructive diseases of sugarcane in Thailand.
- Infected sugarcane caused changing of green to white leaves due to the total loss of chlorophyll, stunted, declined and eventually killed off the entire plant.



SCWL of ratoon cane showing white leaf symptom on shoot



Maha Sarakham, 2016











Nakhon Ratchasima

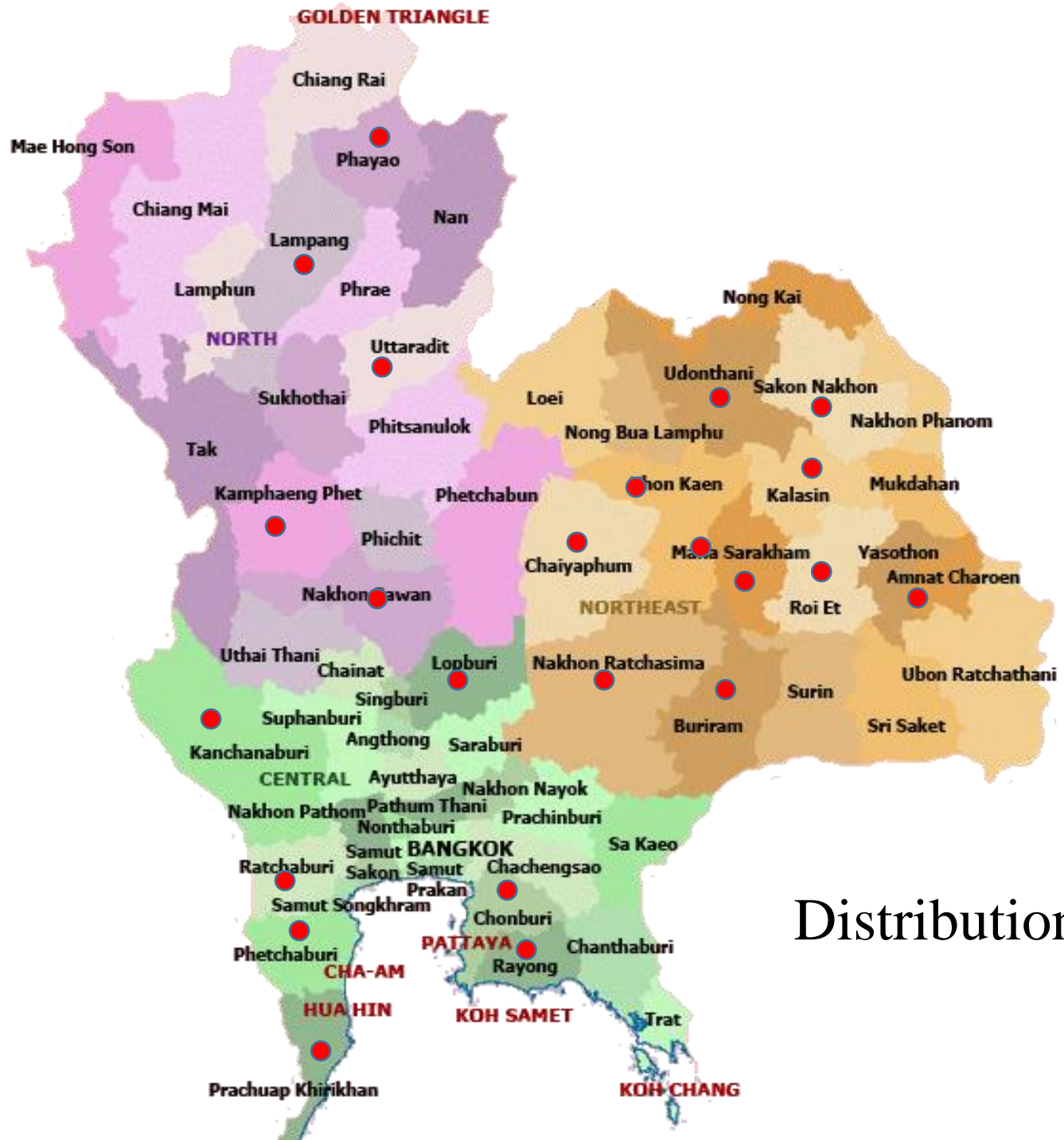
Sakon Nakhon



# SCWL on 1<sup>st</sup> year cane







Distribution Map of SCWL in Thailand

# SCWL-like symptoms



Chimera



# Fe deficiency in calcareous soil



# Sugarcane white leaf disease (SCWL)

- Pathogen: Phytoplasma subgroup 16SrXI-B
- Dissemination: Infected seed-cane (primary inoculum)  
Insect vectors (secondary infection)
- Favored conditions: sandy soil, drought (northeastern part of Thailand)
- Symptoms usually occur and easily observe on ratoon cane
- All varieties in Thailand are susceptible to SCWL
- Khon Kaen 3 is a susceptible and popular commercial variety

**Insect Vector  
(leaf hopper)**



*Matsumuratettix hiroglyphicus*



*Yamatotettix flavovittatus*



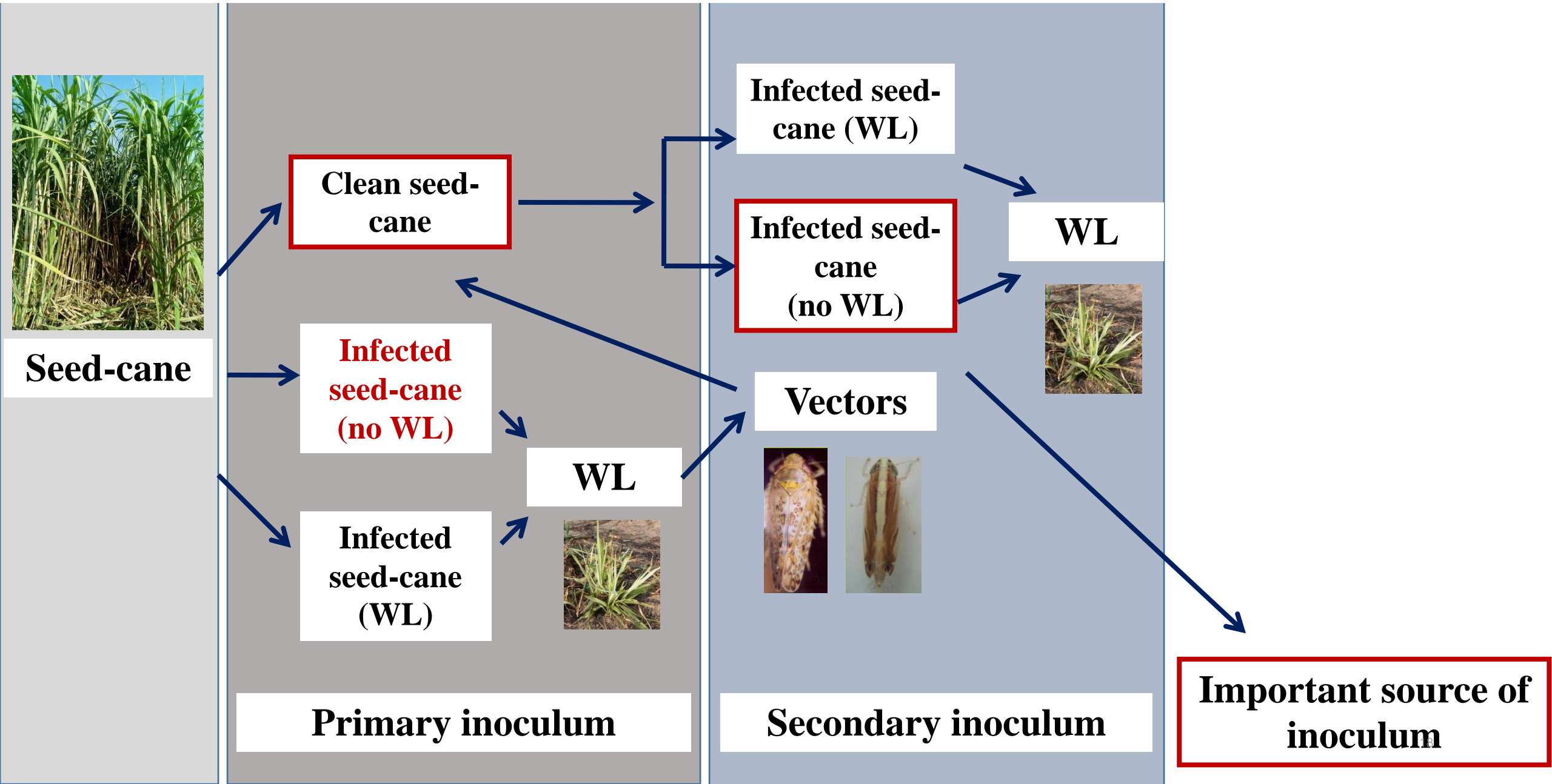


- Infected seed-cane is a major primary source of inoculum in the field
- Clean sugarcane seedling is an important strategy to control white leaf disease in Thailand



Udomsak, 2018

# Disease cycle of SCWL



# SCWL management in Thailand

- Eradicate: burning (Kusalwong and Ouvanich, 1993)
- Crop rotation (Kusalwong and Ouvanich, 1993)
- Planting time
- Control insect vectors
- Use clean seed-cane
  - Tissue culture
  - Hot water treatment: 50°C, 2 hr
  - Hot antibiotic treatment 500 ppm of Tetracycline HCl, 54 °C, 30 min (Sanoamung and Thaveechai, 1979)

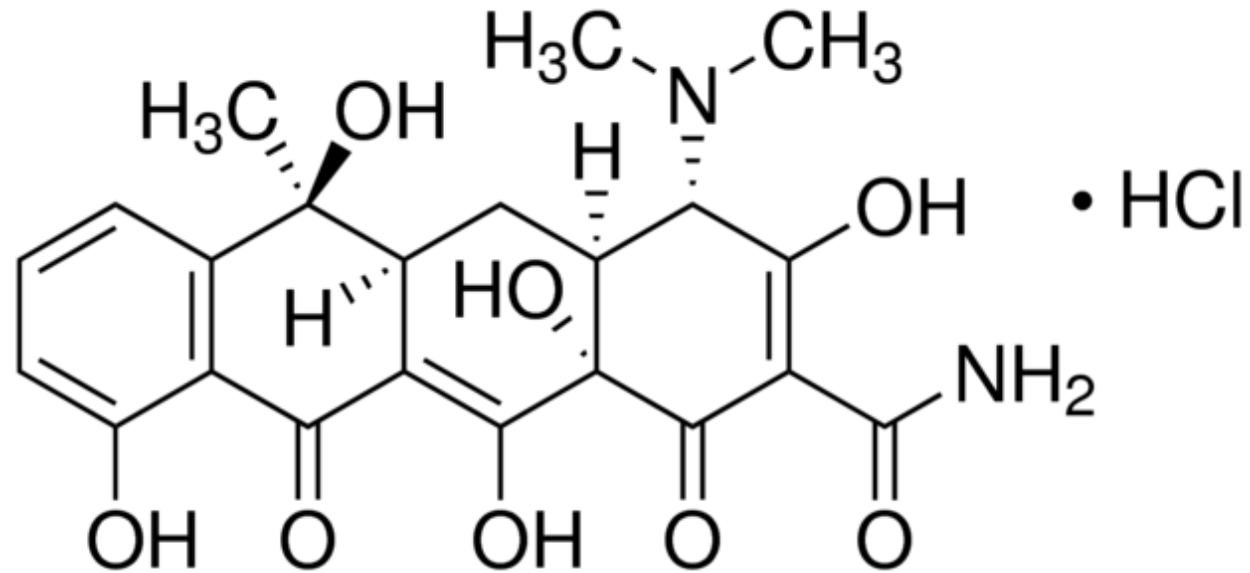
# Hot water treatment (50 °C, 2 hr.) with whole stalk



# Objectives

- To determine efficacy of hot tetracycline treatment for control SCWL in field experiment

# Tetracycline hydrochloride antibiotic



Tetracyclines is an antibiotic have been used to treat coconut lethal yellowing diseases of coconut, dwarf diseased of mulberry and white leaf disease of sugarcane.

Tetracyclines have a broad spectrum of antibiotic action.

Bacteriostatic activity against almost all medically relevant aerobic and anaerobic bacterial genera, both Gram-positive and Gram-negative,

# Classes of antibiotics/antibacterial agents and their modes of action on bacteria (Adopted from Lab notes week, 2013)

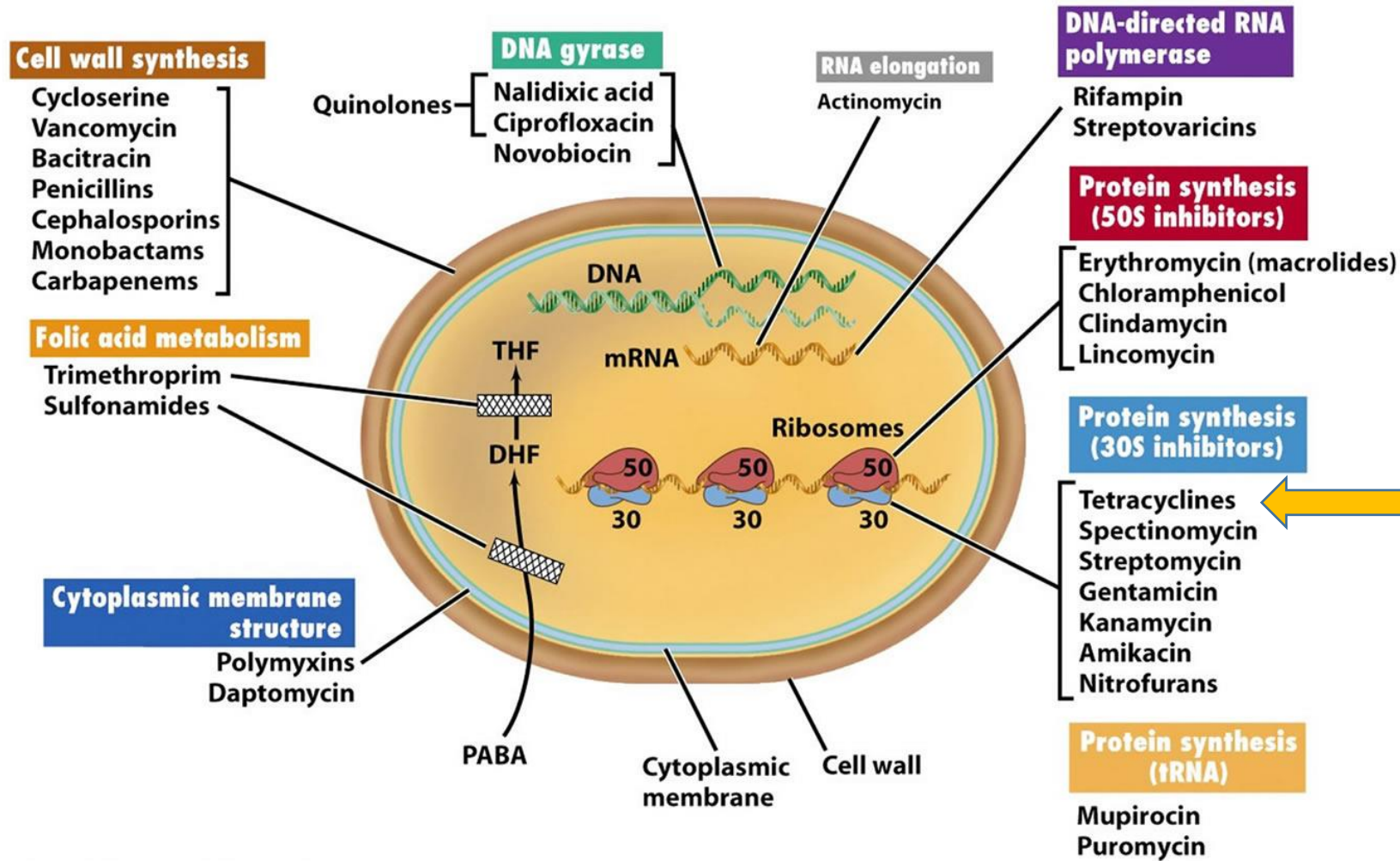
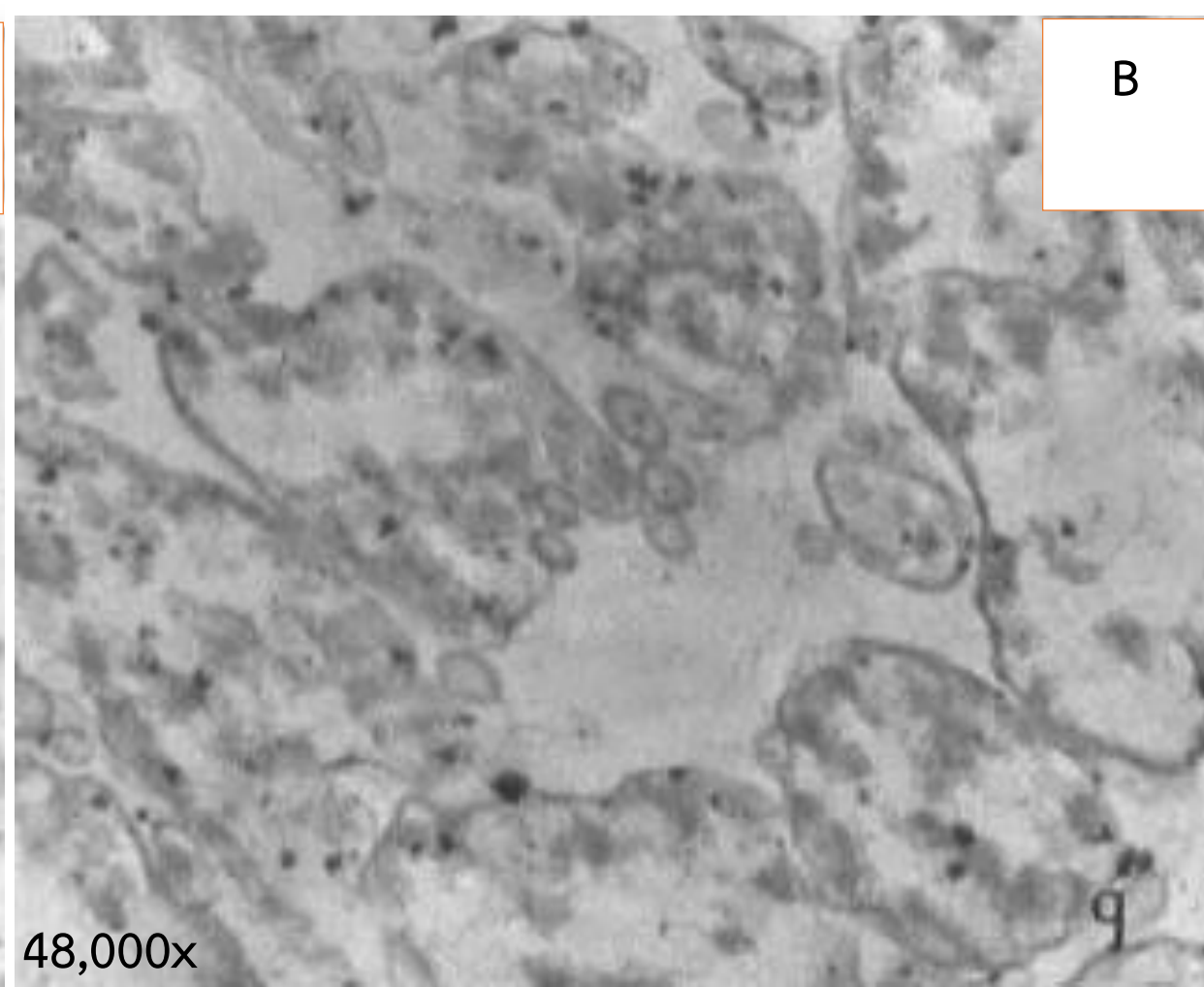
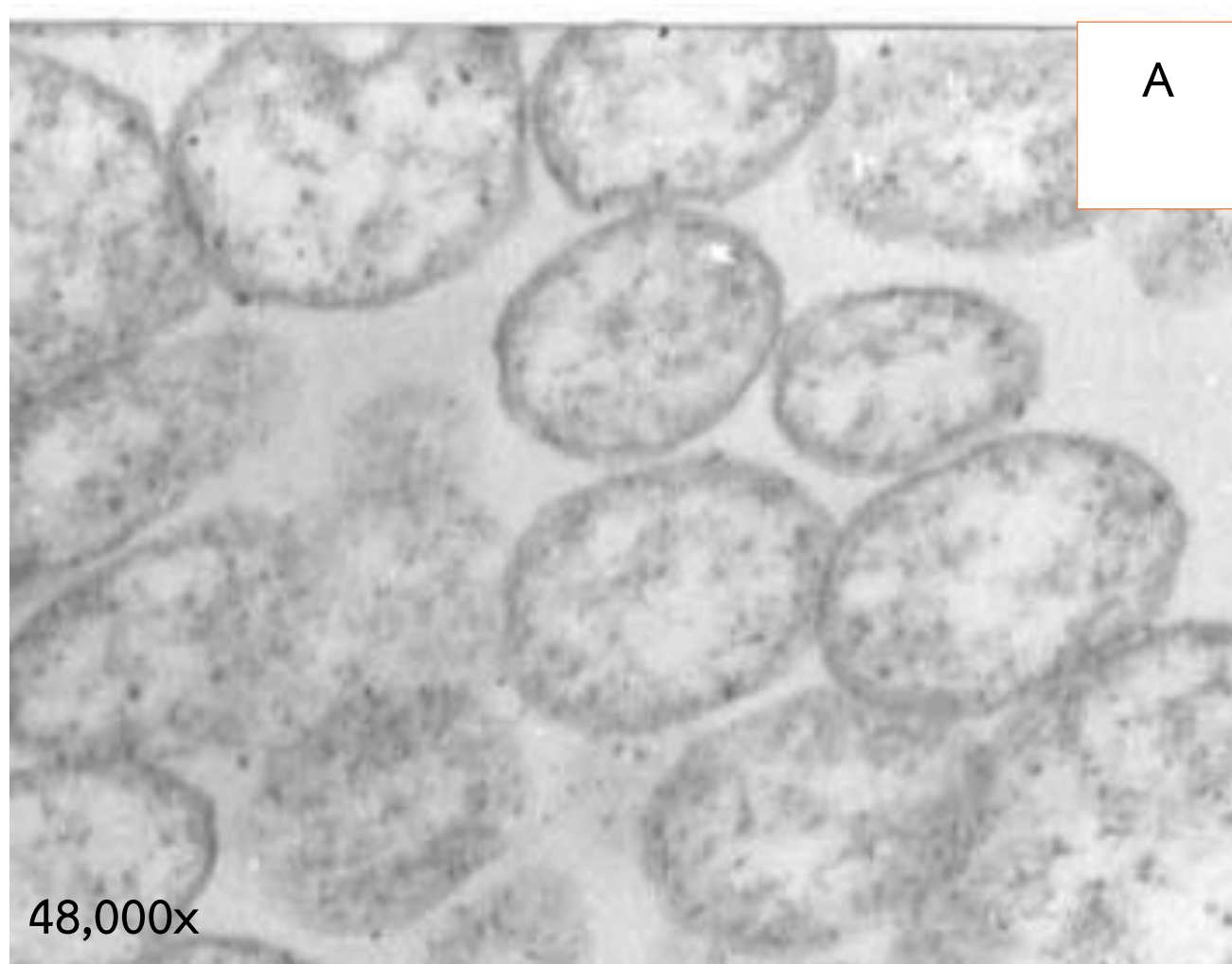


Figure 20-14 Brock Biology of Microorganisms 11/e  
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Sanoamung and Thaveechai, 1979

Ultrathin section of infected sugarcane cutting treated with 500 ppm tetracycline HCl 54°C, 30 min: phytosoma cell showed leaking of cell membrane and degradation of nuclear materials in cells



# I. Hot tetracycline treatment for control SCWL in field experiment



Cut cane stalk into a single bud setts

# Bud selection



Good bud



Incomplete buds

Put single-bud setts into nylon net bags



# Hot water treatment device

Handlebar

Temperature and pump controller

Pump



Maximum 1,000 buds/time

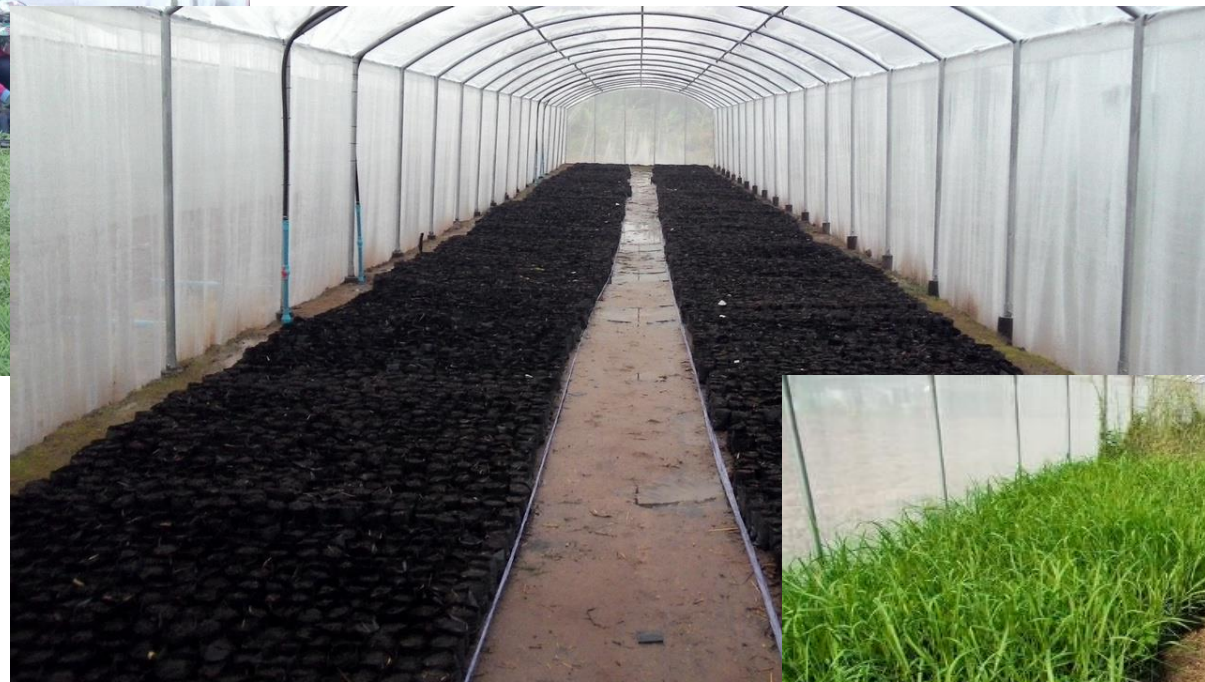
200 liter water tank

Heater 2,000 watt

Single-bud setts in nylon net bags were treated in tetracycline  
HCl 500 ppm at 54 °C, 30 min



Single bud setts were planted in a 3x6 inch HDPE bag and placed into the screen house for 30 days



# Results



Hot tetracycline 500ppm, 54°C ,30 min

71.25% germination



Untreated

54.84% germination

# Residue analysis of tetracycline in sugarcane seedling at 30 days after planting with LA-MS/MS

<b>Treatment</b>	<b>Results</b>		<b>LOD</b>	<b>Method</b>
1.Tetracycline HCl 500 ppm 54 °C, 30 min	Not detected	mg/kg	0.01	Journal of chromatography A, 987(2003) p.227-233 by LC-MS/MS
2.Control	Not detected	mg/kg	0.01	



# Seedling Transplanting at Kosumphisai district, Mahasarakham Province



# Hot tetracycline treatment sugar cane at 4 months at Kosumphisai district, Mahasarakham Province



# Detection of sugarcane white leaf disease in field experiment

<b>Age of sugarcane</b>	<b>White leaf symptoms</b>	<b>Phytoplasma detection</b>
2 months	-	-
4 months	-	-
6 months	-	-
8 months	-	+
10 months	-	+
Ratoon	+ (< 2%)	+

## II. Effect of growing media use for planting sugarcane seedling related to SCWL expression

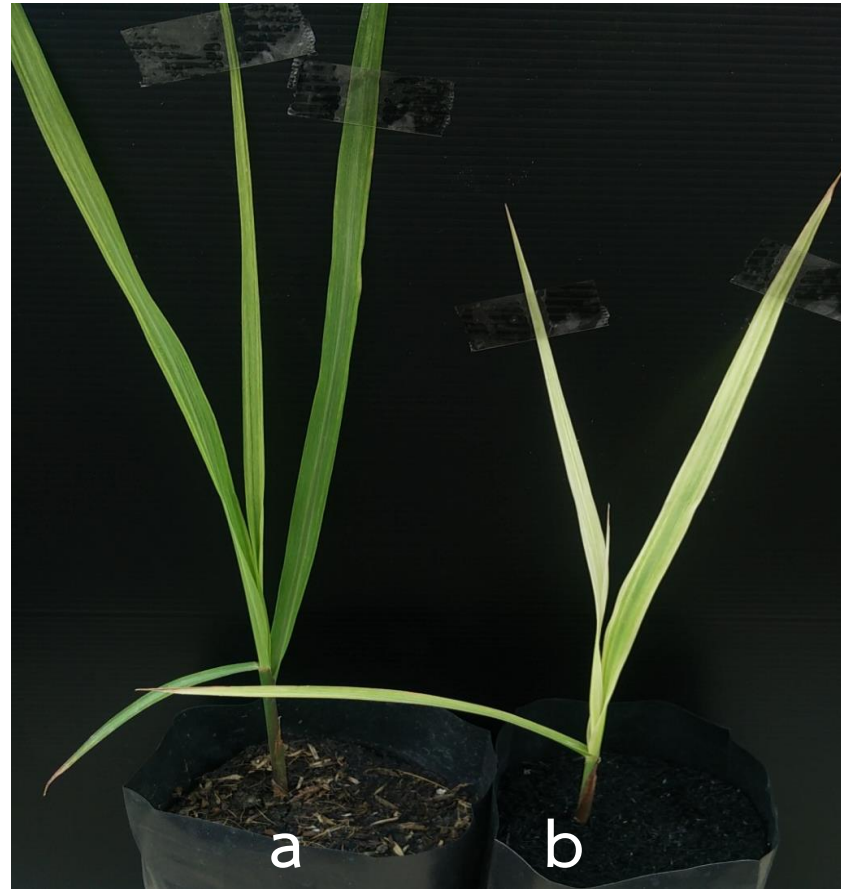
Sugarcane cultivar “Khon Kaen3”, a present popular commercial variety in Thailand, was used as plant material for this study. Mildly susceptible white leaf disease stalks were collected from sugarcane field at Kosumpisai District, Mahasarakham Province, Thailand.

Infected stalks were cut to single bud (length 7-10 cm.) and single bud setts from each stalk were divided into two sets for planted in soil and sterile black rice husk in a 3x6 inch HDPE bag. Sugarcane white leaf symptoms were observed and detected with nested PCR (Klinkong and Seemuller, 1997) at 21 days after planting.

# Efficacy of growing media on sugarcane white symptom expression

Sugarcane stalk no.	White leaf symptom (%)	
	Soil	black rice husk
1	0	0
2	100	100
3	40	100
4	50	100
5	100	100
6	100	100
7	100	100
8	75	33.33
9	25	100
Average	65.56	81.48

# Efficacy of growing media on sugarcane white symptom expression



Sugarcane seedlings from same infected cane stalk

A = soil

B = black rice husk

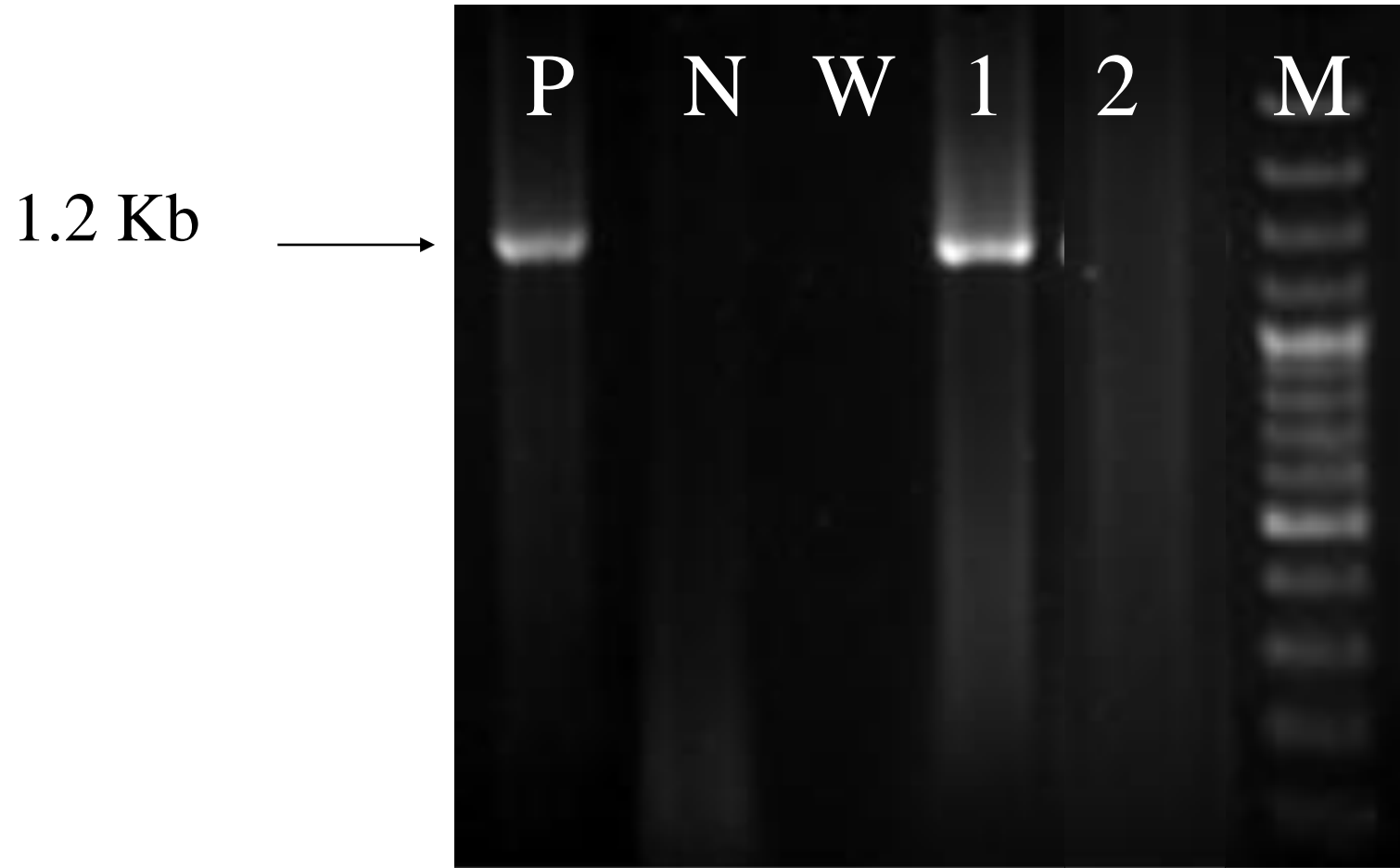
# Efficacy of growing media on sugarcane white disease expression of hot tetracycline treatment



Sugarcane seedlings from same infected cane stalk

A = treated with tetracycline HCl 500 ppm at 54 °C for 30 min

B = untreated.



Detection of phytoplasma by nested PCR (R16mF2/R1; R16F2/R2) from sugarcane seedling. M: 100 bp DNA ladder plus, 0.5 $\mu$ g/ $\mu$ l, Fermentas<sup>®</sup>; P: Positive control; N: Negative control (disease free sugarcane); W: Water control; lane 1: Untreated; lane 2: Treated with tetracycline HCl 500 ppm at 54 °C for 30 min.



# Conclusions

Tetracycline HCl 500 ppm at 54 °C for 30 min treatment can control sugarcane white leaf disease in infected cane stalks for 1<sup>st</sup> year of plantation.

This technique is suitable for Thai's farmer (small farmer) due to simple methods, low cost and short time to produce clean seed-cane.

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WANGKANAI



Thank you for  
your attention

