Bulletin of Insectology ***64*** *(Supplement): S197-S198, 2011*
ISSN 1721-8861

Evaluation of the efficiency of various treatments used for sugarcane white leaf phytoplasma control

Chananat KAEWMANEE, Yupa HANBOONSONG
Entomology Section, Department of Plant science and Agricultural resources, Faculty of Agriculture, Khon Kaen University, Mitraparb Rd., Khon Kaen 40002, Thailand

Abstract

The objective of this study was to evaluate and compare the efficiency of various treatments used for sugarcane white leaf phytoplasma control and quantify the amount of phytoplasma after using different treatments. Different treatments were used with cane stalks to attempt to reduce the phytoplasma concentration as follows; control (no treatment), dual hot water treatment, hot water followed by cold water, and hot water followed by tetracycline HCl 500 ppm. The results showed that the control had the highest percentage of sugarcane white leaf phytoplasma at 100%, followed by the use of hot water, followed by cold water at 95.24%, dual hot water at 90.48% and hot water+tetracycline HCl 500 ppm at 71.43%. Moreover, the control had the highest quantification of the amount of SCWL phytoplasma at 158.71 copies/sample, followed by the use of hot water+cold water, dual hot water and hot water+tetracycline HCl with the amount of phytoplasma at 31.50, 10.99 and $3.72 \cdot 10^{-2}$ copies/sample, respectively. The control without any treatment had the highest cane germination at 77.5%, followed by the use of hot water followed by cold water, hot water followed by tetracycline HCl, and dual hot water with germination rates at 42.5%, 5% and 2.5% respectively. In conclusion, soaking cane stalks in hot water, followed by tetracycline HCl can reduce the percentage of SCWL phytoplasma more than other treatments whereas the cane germination rate is comparatively low.

Key words: sugarcane white leaf phytoplasma, quantification of phytoplasma, hot water, tetracycline.

Introduction

Sugarcane white leaf disease (SCWL) is a major disease of sugarcane production in Thailand, associated with phytoplasmas which transmitted to the plant by the leafhopper *Matsumuratettix hiroglyphicus* (Matsumura) and *Yamatotettix flavovittatus* Matsumura (Hanboonsong *et al*., 2002; 2006). Phytoplasma infected sugarcane shows the white leaf symptoms due to loss of chlorophyll. So far there is no effective methods for controlling this disease. In order to reduce or kill the phytoplasma pathogen, various treatments such as hot water, cold water and antibiotic have been recommended for cane stock treatment before planting (Moutia and Dookun, 1999). However, the quantification of the amount of pathogen reduction from the cane stalk after the treatments has not been studied. Therefore, the objective of this study was to evaluate and compare the efficiency of various treatments used for SCWL phytoplasma control and quantify the amount of SCWL phytoplasma pathogen after using different treatments.

Materials and methods

Different treatments were used with SCWL phytoplasma infected cane stalk (Khon Kaen 3 variety) to attempt to reduce or kill the SCWL phytoplasma as follows 1). Control (no treatment) 2). Treatment with hot water at 55°C for 10 min, followed by hot water at 50°C for 2 hrs. 3). Treatment with hot water at 50°C for 2 hrs., followed by cold water at 5°C for 2 hrs. 4). Soaking in hot water at 50°C for 2 hrs. followed by soaking in Tetracycline HCl 500 ppm for 2 hrs. After the treatment was completed the cane stalks were divided into 2 sets. The first set was planted into the soil to test for cane germination.

The second set was used for extraction of sugarcane DNA and detection of SCWL phytoplasma by using nested PCR with a SCWL phytoplasma 16s rRNA and 23S rRNA specific primers according to the protocol of Hanboonsong *et al*. (2006). The quantification of SCWL phytoplasma from each treatment used was also performed by real-time PCR technique.

Results

Sugarcane germination

Different treatments of hot water, cold water and antibiotic used on cane stalks at different times and temperature negatively affected cane germination. The results showed that the control without any treatment had the highest cane germination at 77.5% but they germinated to show the white leaf symptom. Next highest was from the use of hot water followed by cold water, then hot water followed by tetracycline HCl 500 ppm, and dual hot water with germination rates at 42.5%, 5% and 2.5%, respectively (figure 1). Cane stalks soaked in hot water followed by cold water germinated to show a pale green leaf but soaking cane stalks in dual hot water and hot water followed by tetracycline HCl 500 ppm germinated stalks showed a green leaf. Dual hot water treatment of cane stalks greatly reduced the germination as compared to the control.

Quantitative of sugarcane white leaf phytoplasma

The result of PCR showed that the control had the highest percent of sugarcane white leaf (SCWL) phytoplasma at 100%, followed by the use hot water followed by cold water at 95.24%, dual hot water at 90.48% and hot water followed by tetracycline HCl 500 ppm at 71.43% (table 1). Hot water followed by tetracycline HCl

500 ppm can reduce percentage of SCWL phytoplasma more than dual hot water and hot water followed by cold water at 28.57%, 9.52% and 4.76%, respectively (table 1). Moreover, the results of real-time PCR showed that the control had the highest quantification of the amount of SCWL phytoplasma at 158.71 copies/sample, followed by the use of hot water followed by cold water, dual hot water and hot water followed by tetracycline HCl with the amount of SCWL phytoplasma at 31.50, 10.99 and $3.72 \cdot 10^{-2}$ copies/sample, respectively (table 1, figure 1).

After 7 months, the shoots were cut for detection of SCWL phytoplasma. The result showed the shoots of cane stalks which were soaked in dual hot water had the highest SCWL phytoplasma at 100% followed by the use of hot water followed by tetracycline HCl 500 ppm and hot water followed by cold water, with SCWL phytoplasma rates at 75% and 69.23% respectively. But the cane stalks of the control without any treatment died within 2 months.

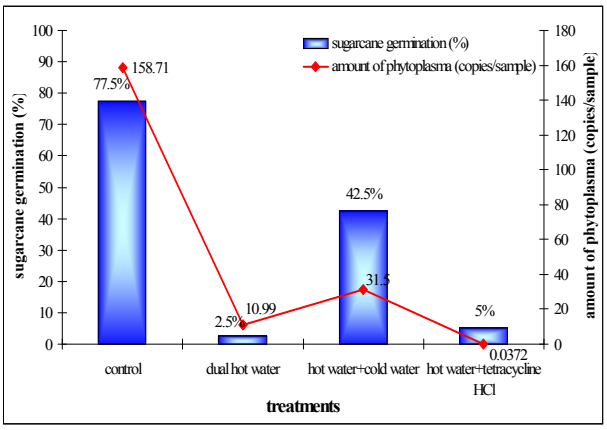

Figure 1. Germination (%) of cane stalks and amount of phytoplasma after different treatments.
(In colour at www.bulletinofinsectology.org)

Table 1. Percentage of sugarcane white leaf phytoplasma in sugarcane buds from different treatments by PCR technique and the amount of phytoplasma by real-time PCR technique.

Treatments	SCWL phytoplasma (%)	Decreasing SCWL phytoplasma		Amount of phytoplasma (copies/sample)	
		T/F[1]	%	mean	range
Control	100	21/20	-	158.71	$2.44 \cdot 10^{-8}$ - 2832
Dual hot water	90.48	21/19	9.52	10.99	$1.69 \cdot 10^{-6}$ - 135.7
Hot water+old water	95.24	21/20	4.76	31.50	$8.99 \cdot 10^{-8}$ - 279.3
Hot water+tetracycline HCL 500 ppm	71.43	21/16	28.57	$3.72 \cdot 10^{-2}$	$1.86 \cdot 10^{-9}$ - 0.1803

[1] T = Total number of cane stalk, F = Number of cane stalk that found the sugarcane white leaf phytoplasma.

Discussion

Sugarcane white leaf phytoplasma spreads through sugarcane stocks and is transmitted plant to plant by the insect vectors (Hanboonsong *et al*., 2002; Marcone, 2002). Using phytoplasma free stock through tissue culture method is currently applied by the sugarcane farmers in Thailand. It helps to reduce the disease occurrence ,however, it is an expensive approach and there is insufficient tissue culture seedling for distribution during the planting season. Alternative treatments such as thermotherapy and antibiotic were introduced to sugarcane stocks for controlling sugarcane grassy shoot phytoplasma and showed positive results (Pliansinchai *et al*., 1997). In the case of sugarcane white leaf (SCWL) phytoplasma, our results showed that soaking cane stalks in hot water at 50°C for 2 hrs followed by soaking in antibiotic tetracycline HCl for 2 hrs can reduce SCWL phytoplasma up to 28.57% more than using only hot water or with cold water. In addition the quantification of the amount of SCWL phytoplasma found that heat treatment can dramatically reduce the SCWL phytoplasma in comparison with non heat treatment. However, the cane germination after heat treatment is comparatively low. It is noticed that after 7 months of planting treated canes, the SCWL phytoplasma can be detected in all canes of heat treatment alone but only from 75% of treated cane with antibiotic. Further studies will investigate the appropriate timing for treatment use in order to increase the germination rate.

Acknowledgements

I would like to thank the Royal Golden Jubilee Ph.D. program for supporting the research under the RGJ-Ph.D. grant no. PHD/0296/2550.

References

HANBOONSONG Y., CHOOSAI C., PANYIM S., DAMARK S., 2002.- Transovarial transmission of sugarcane white leaf phytoplasma in the insect vector *Matsumuratettix hiroglyphicus* (Matsumura).- *Insect Molecular Biology*, 11: 97-103.

HANBOONSONG Y., RITTHISON W., CHOOSAI C., SIRITHORN P., 2006.- Transmission of sugarcane white leaf phytoplasma by *Yamatotettix flavovittatus*, a new leafhopper vector.- *Journal of Economic Entomology*, 99*:* 1531-1537.

MARCONE C. 2002.- Phytoplasma diseases of sugarcane.- *Sugar Tech*, 4(3/4): 79-85.

MOUTIA M., DOOKUN A., 1999.- Evaluation of surface sterilization and hot water treatments on bacterial contaminants in bud culture of sugarcane.- *Explication Agricolture*, 35: 265-274.

PLIANSINCHAI U., SURINTHU N., PRAMMANEE S., THANAWON T., YIMSAARD M., 1997.- Etiology and symptomatology of grassy shoot syndrome of sugarcane and heat therapy. [online] From: http://kucon.lib.ku.ac.th/Fulltext/KC3501048.pdf.

Corresponding author: Yupa HANBOONSONG (e-mail: yupa_han@kku.ac.th), Entomology, Department of Plant science and Agricultural resources, Faculty of Agriculture, Khon Kaen University, Mitraparb Rd., Khon Kaen 40002, Thailand.