



Research article

Potential of sugarcane tolerant to white leaf disease to increase profitability in Thai sugarcane production

Varinthon Jarnkoon^{a,d,†}, Lawan Kladsuwan^a, Peeraya Klomsa-ard^a, Klanarong Sriroth^a, Somchai Anusonpornperm^b, Tanee Sreewongchai^c, Ratchadawan Ngoenklan^{d,†,*}

^a Mitr Phol Sugarcane and Research Center, Mitr Phol Group, Chaiphaphum 36110, Thailand

^b Department of Soil Science, Faculty of Agriculture, Kasetsart University, Bangkok 10900, Thailand

^c Department of Agronomy, Faculty of Agriculture, Kasetsart University, Bangkok 10900, Thailand

^d Department of Entomology, Faculty of Agriculture, Kasetsart University, Bangkok 10900, Thailand

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Abstract

Importance of the work: Sugarcane tolerant to sugarcane white leaf disease (SCWL) has the potential to greatly reduce crop losses and enhance sugarcane production profitability in Thailand.

Objectives: To evaluate the potential of SCWL-tolerant sugarcane varieties compared to Khon Kaen 3 (KK3) sugarcane, which is preferred by farmers.

Materials and Methods: Real-time polymerase chain reaction targeting the secA gene was used to quantify the phytoplasma causing SCWL in sugarcane. Electron microscopy techniques were used to confirm the presence of the phytoplasma in phloem tissues. Finally, the impact of SCWL on sugarcane yield, Commercial Cane Sugar and the incidence of SCWL symptoms were evaluated after harvest.

Results: At 7 d after phytoplasma inoculation of KK3, MPT16-110, MPT01-956, MPT04-204 and MPT12-618 by *Matsumuratettix hiroglyphicus* (Matsumura), MPT12-618 consistently maintained the lowest infection level throughout the year, while KK3 had the highest infection. At 12 mth, MPT12-618 had a significantly higher sugarcane yield than the other varieties. Varieties showed no significant differences in sweetness. However, MPT16-110 was the sweetest (9.90 ± 0.17), followed by MPT12-618 (9.60 ± 0.54), MPT04-204 (9.28 ± 0.33), MPT01-956 (9.21 ± 0.29), and KK3 (8.70 ± 0.31). Electron microscopy techniques showed that KK3 had thinner sieve plates in its phloem tissue compared to the MPT varieties. However, the MPT varieties had fewer SCWL symptoms (10–15%) during the tillering phase than KK3 (25%).

Main finding: Compared to KK3, sugarcane variety MPT12-618 had lower levels of phytoplasma infection from 2 mth to 12 mth and a lower incidence of SCWL symptoms in the tillering phase of the first ratoon.

† Equal contribution.

* Corresponding author

E-mail address: fagrrwn@ku.ac.th (R. Ngoenklan)

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Introduction

In Thailand, sugarcane white leaf (SCWL) disease, caused by phytoplasmas, has been considered the most important disease of sugarcane in Thailand since its initial detection in North Thailand in Kao Ka district, Lampang Province, during 1962–1964 (Chen, 1974; Kusolwong, 1980). This SCWL disease has caused major yield losses of up to 50%, impacting the country's sugar industry by an estimated USD 30–40 million annually (Wongkaew, 2012; Hanboonsong et al., 2017). The northeastern region, which has over 700,000 ha of sugarcane plantations, has reported severe outbreaks of SCWL disease, affecting more than 90% of the Khon Kaen 3 (KK3) sugarcane variety (Sukyai et al., 2016; Jantasorn et al., 2020; Khumla et al., 2022; Lohmaneeratana et al., 2024).

The phytoplasma (obligate intracellular parasites of the plant phloem tissue) are transmitted through infected seed material and by phloem-feeding leafhopper vectors (Weintraub and Beanland, 2006; Wongkaew et al., 2014). In the northeastern region, the leafhoppers *Matsumuratettix hiroglyphicus* (Matsumura) and *Yamatotettix flavovittatus* (Matsumura) from the family Cicadellidae are known phytoplasma vectors that transmit SCWL, particularly in Udon Thani, Khon Kaen, and Mahasarakham provinces (Hanboonsong et al., 2002; Hanboonsong et al., 2006; Wongkaew, 2012; Rattanabunta and Hanboonsong, 2015). As reservoirs for phytoplasma-associated SCWL, pathogens remain viable in the digestive system, hemolymph and salivary glands for generations and are passed directly to offspring through transovarial transmission in insect vectors (Hanboonsong et al., 2002). In general, SCWL symptoms consist of abnormal tillering, side shoots on the upper part of infected stalks and, most characteristically, bleached white or striped leaf blades (Nithya et al., 2023). However, the severity and symptom variance depend on soil fertility, temperature, cane sett quality, cultural practice and the amount of phloem-colonized phytoplasma (Wongkaew, 2012).

In Thailand, controlling of SCWL disease has mainly involved physical or chemical treatment of the cane setts, including hot water and tetracycline treatments, and disease-free plant production through tissue culture (Wongkaew, 2012). While initial treatments with hot water and tetracycline on KK3 cane stalks reduced phytoplasma levels, long-term efficacy was limited, with infection rates exceeding 70% after 7 mth of germination (Kaewmanee and Hanboonsong, 2011). Hot water treatment is particularly suitable for small-scale farmers due to the capacity of treatment devices, which typically process

between 1,000 and 1,500 sugarcane setts per batch at a cost of USD 3.47 (Sukcharoenvipharat et al., 2023). Accordingly, the strategy of control through disease-free plants was one of the most effective methods for controlling SCWL phytoplasma during 1996–1999 (Wongkaew, 1999).

Sugarcane cultivar KK3 was released in 1938 and now accounts for 85% of Thailand's sugarcane production (Khumla et al., 2022). Its susceptibility to SCWL has been confirmed based on secA gene quantification using reverse transcription quantitative polymerase chain reaction (Ratchaseema et al., 2021). Thailand's standard sugarcane breeding programs evaluate cultivars based on multiple criteria: cane and sugar yield, Commercial Cane Sugar (CCS, a measure of the sucrose that is commercially obtainable from cane), yield components (millable cane number, stalk length and stalk weight) and disease resistance (red rot, wilt and smut) assessed through artificial inoculation and ratooning ability. These criteria led to the dominance of six cultivars in 2018/19, with KK3 accounting for 85.83% of production, followed by LK92-11 (8.87%), U-Thong5 (1.65%), K95-84 (0.54%), U-Thong12 (0.45%) and K88-92 (0.43%) (Khumla et al., 2022).

Despite studies of diverse sugarcane varieties with yield performance comparable to KK3 across various production environments, such as Kps94-13 (Klomsa-ard et al., 2013), Kps01-12, TBy28-0941 (Palachai et al., 2019), UT13 (Khonghintaing et al., 2020), KK07-478 and RT2004-085 (Bunphan et al., 2021), and KPK98-51 (Wongtamee et al., 2022), a critical gap remains regarding their tolerance to SCWL. Several studies have evaluated sugarcane cultivars for SCWL disease tolerance, emphasizing the major impact of cultivar characteristics on the insect vector's survival and feeding behavior. For example, one study by Sunthorn and Hanboonsong (2018) examined insect vector feeding preference and survival on six different sugarcane cultivars: Khon Kaen 3, Q229, UT17, UT13, *Saccharum spontaneum* (ThS98-185) and *Erianthus* spp. (ThE10-6). Their results demonstrated that insects had longer feeding durations and higher feeding frequencies on Khon Kaen 3 than on ThE10-6 and Q229, which have hard stems and are less preferred by the insect vector. Worachai and Hanboonsong (2018) demonstrated that sugarcane cultivars had varying levels of tolerance to the leafhopper vector and the SCWL phytoplasma. By assessing insect survival, reproduction and phytoplasma levels within the plant, their study evaluated four commercial cultivars (Q229, KPS 01-12, UT15 and Khon Kaen 3) under greenhouse conditions. Sugarcane cultivar Q229 had the highest tolerance, with the lowest insect survival rate (37.5%), net reproductive rate ($R_0=8.57$),

number of eggs per female (18–24) and the lowest number of SCWL phytoplasma cells in plant DNA (363.42 ± 34.55 cells/ μ g). In contrast, Khon Kaen 3 was the most susceptible, with the highest insect survival rate (80%), net reproductive rate ($R_0=19.87$), number of eggs per female (46–50), and the highest number of SCWL phytoplasma cells in plant DNA ($1,018.11 \pm 32.63$ cells/ μ g).

In addition to greenhouse studies, a field trial was conducted in Sri Lanka to evaluate the performance of 50 sugarcane varieties in a region with severe SWCL disease incidence. The evaluation encompassed cane yield components and sugar yield. SWCL disease incidence was monitored monthly in the plant crop for 6 mth and bimonthly in the first ratoon crop for 4 mth. Based on the results, the varieties SL 89 309, SL 92 5588 and SL 95 4225 had higher cane yield components and sugar yield, while having lower SWCL disease incidence (less than 16%) than the susceptible standard, Co 775 (Wickramasinghe et al., 2019).

Therefore, screening for tolerant or resistant sugarcane cultivars to SCWL based solely on physiological characteristics has proven to be insufficient, as evidenced by the adverse results obtained with a sett treatment. Even though the infection was present, symptoms of SCWL disease are sometimes not observed under unsuitable environmental conditions (Zhang et al., 2020; Lohmaneeratana et al., 2024). Recently, to achieve the desired results, many procedures have been performed, including loop-mediated isothermal amplification assays (Quoc et al., 2021), UVA-derived RGB imagery (Amarasingam et al., 2022) and micro-X-ray fluorescent spectroscopy (Saengmanee et al., 2024).

Plant tissue culture techniques have been used to generate plants with new genetic traits, including disease resistance (Tegen, 2016). This is achieved by selecting cells in a culture that can survive exposure to toxins produced by harmful organisms, even if the cells have had no prior toxin exposure (Brettell and Ingram, 1979). In one study, tissue culture had a substantial role in producing sugarcane yellow leaf virus- and sugarcane yellows phytoplasma-free healthy sugarcane plants (Parmessur et al., 2002). To confirm that pathogens persisted in sugarcane or cultured plantlets, real-time polymerase chain reaction assay (RT-PCR) and nested PCR have been developed to detect very low concentrations of the phytoplasma causing SCWL, using sequences of the non-coding 16S rRNA gene and the 16S-23S rRNA intergenic spacer region (16-23S ISR) and primers that enable amplification of these regions from all phytoplasmas based on PCR (Wongkaew and Fletcher, 2004; Wongwarat et al., 2011). Additionally, a semi-nested PCR assay using the *secA* gene has been developed, which resulted in phytoplasma clustering similar to that observed using

a 16-23S ISR-23S rRNA gene contig or the 16S rRNA gene alone in a single or nested PCR approach (Dickinson and Hodgetts, 2013; Hodgetts et al., 2008). However, there have been few studies that have used the PCR technique to assess white leaf disease resistance in diverse sugarcane cultivars.

Therefore, the current research aimed to evaluate the potential of SCWL-tolerant sugarcane by: 1) diagnosing the amount of the SCWL phytoplasma in sugarcane using the real-time PCR technique targeting the *secA* gene; 2) observing the presence of SCWL phytoplasma within sugarcane tissues using transmission electron microscopy (TEM); (3) evaluating performance for yield components; and 4) observing the incidence of white leaf symptoms on the first ratoon during the tillering phase.

Materials and methods

Plant material and growth conditions

Sugarcane variety KK3, known to be susceptible to SCWL, served as the reference variety. Four germplasm clones (MPT12-618, MPT04-204, MPT16-110 and MPT01-956) were compared to KK3 to evaluate their potential for SCWL tolerance. All the germplasm clones of sugarcane (*Saccharum officinarum* L.) were obtained from the collection of the Mitr Phol Sugarcane and Research Center, Phu Khiao district, Chaiyaphum province, Thailand.

The four MPT varieties were developed by breeders based on a selection stage, followed by a clonal stage (2nd selection), where 100–300 plants per station were established in a lattice design with 1.50 m $\times$ 0.50 m spacing and a row length of 5–8 m. Clonal selection was implemented on both plant and first ratoon crops, with a selection intensity of 15–10% to identify promising genotypes for yield evaluation in larger farmer plots (Khumla et al., 2022). This process was undertaken under greenhouse conditions with a temperature range of 30–32°C and a relative humidity (RH) range of 65–80%.

To produce phytoplasma-free plantlets under greenhouse conditions, the phytoplasma causing SCWL was detected initially in the rooting stage of plantlets aged 2 mth using nested PCR (Zhang et al., 2020). Subsequently, these phytoplasma-free plantlets were planted in 38 cm diameter pots. The plants were watered weekly using a drip system (2 L/pot/time) for 2 hr each time, twice a week. Additionally, a NPK 16-16-8 fertilizer was added at a rate of 312.50 kg/ha through the automatic drip irrigation system.

Establishing colony of Matsumuratettix hiroglyphicus for phytoplasma inoculation into healthy sugarcane plants

M. hiroglyphicus, the insect vector for phytoplasma inoculation, was collected using light traps (from 1800 hours to 2000 hours) and sweep nets in sugarcane fields located in Phu Wiang district, Khon Kaen province, Thailand. Colony formation of *M. hiroglyphicus* was established by feeding them on phytoplasma-free sugarcane plants in a cylindrical plastic container (15 cm diameter, 75 cm height) covering 1 plant/pot under controlled greenhouse conditions (25–29°C, 70–80% RH and natural lighting) for 8 wk. Once the colony had reached the 2nd generation (F2), the insects were tested randomly for phytoplasma using nested PCR to obtain disease-free vectors.

The disease-free vectors (F2) were starved for 4 hr prior to generating SCWL-infected vectors. Subsequently, they were introduced into a cylindrical plastic container and allowed to feed on SCWL-infected KK3 sugarcane aged 12 wk (1 plant/pot) for 48 hr to achieve an 80% feeding success rate. Finally, the SCWL-infected vectors were reinspected, and the results were determined using nested PCR.

The leafhoppers were left for 7 d to transmit SCWL. A completely randomized design was used applied to inoculate disease-free sugarcane plants with the phytoplasma in a controlled greenhouse environment. Five treatments were investigated: KK3 and four Mitr Phol varieties. Each treatment consisted of four pots with five replicates, totaling 25 experimental units per treatment. SCWL-infected vectors (five insects) were released into a cylindrical plastic container placed phytoplasma-free sugarcane plants aged 2 mth (1 plant/pot) for each of the sugarcane varieties: KK3, MPT12-618, MPT04-204, MPT16-110 and MPT01-956.

The sugarcane plants inoculated with phytoplasma were grown in a greenhouse under controlled conditions (31.10–42.02°C, 75–82% RH). The plants were drip-irrigated twice weekly (2 L/pot/time for 2 hr) and fertilized with a NPK 16-16-8 fertilizer at a rate of 312.50 kg/ha using an automatic watering system.

At 1 mth post-inoculation, leaf samples from the sugarcane plants in each pot and treatment were collected. Nested PCR was used to quantify phytoplasma, ensuring selection of only SCWL-infected plants in each treatment. Subsequently, the selected infected plants in each treatment were grown in greenhouse conditions and fertilized with a NPK 16-16-8 fertilizer at a rate of 312.50 kg/ha through an automatic drip irrigation system when the sugarcane reached age 3 mth.

Phytoplasma genomic DNA extraction

In each treatment, a leaf was sampled from the third fully unfolded leaf from the top of each sugarcane stalk within each pot, resulting in a total of 20 leaf samples from 5 replications per treatment. Then, each leaf sample was cut into 10 circular pieces that were used to extract DNA, according to Bermúdez-Guzmán (2016) by being placed in a 1.50 mL Eppendorf tube. Then, 720 µL of extraction buffer were added to the sample and ground thoroughly using a Mini-Beadbeater-16 (BioSpec Products; Bartlesville, OK, USA). The tubes were incubated at 60°C for 60 min and mixed by inversion every 5 min. After incubation, the clear supernatant (500 µL) was transferred into a new centrifuge tube and mixed with 225 µL of 5M potassium acetate. The mixture was gently mixed and centrifuged at 14,000 rpm for 5 min using a refrigerated centrifuge at 4°C (Centurion Scientific; Chichester, UK). The clear supernatant (500 µL) was collected in a new tube. An equal amount of phenol chloroform was added, mixed using a Vortex Mixer KMC-1300V (VISION SCIENTIFIC Co. Ltd; Gyeongsan, Korea), and centrifuged at 14,000 rpm for 5 min. The upper clear phase (500 µL) was transferred to a new reaction tube.

The DNA pellet was collected based on adding 500 µL of cold isopropanol, mixing by vortexing, followed by centrifuging at 14,000 rpm for 5 minutes. Then, the pellet was washed with 70% ethanol (300 µL), decanted from the ethanol and left to air dry. The DNA was stored by resuspending in 50 µL of Tris-ethylenediaminetetraacetic acid buffer and stored at -20°C. DNA quantification was performed using a NanoDrop™ 2000c Spectrophotometer (Thermo Fisher Scientific; Waltham, MA, USA).

Cloning of target and reference genes using bacterial plasmids

The extracted DNA solutions were used as templates for amplification based on PCR of the target phytoplasma gene *secA* and the reference sugarcane gene 18S SGC. Two primer sets were generated and used to amplify target DNA fragments (*SecA*for: 5'-GTTTTATATGGATGCTAATCGTTTT-3', *SecA*rev: 5'-CTATTGTTCTTCCTGTAAATTGATC-3' and 18S SGCf: 5'-CCTTAGGCGTCAAGGAACAC-3', 18S SGCr: 5'-GCGTTCAA AACTCGATGGT-3') (Ratchaseema et al., 2021).

The PCR reactions were performed using 1 µL of 200 ng/µL genomic DNA template in a 20 µL total reaction volume containing 124x Green Master Mix (Biotechrabbit; Berlin, Germany),

and 1 μ M of each primer was added. The PCR condition was set up with a pre-denaturing step at 95°C for 2 min, followed by 35 cycles of denaturation 95°C for 30 s, annealing at 55.50°C (secA) or 59.50°C (18S SGC) for 30 s and at 72°C for 45 s, with a final extension at 72°C for 5 min. The target DNA fragment size of the secA gene was 210 bp and for the 18S SGC gene was 201 bp.

The DNA fragments of the secA and 18S SGC genes were purified using a GenepHlow™ Gel/PCR Kit (Geneaid Biotech; New Taipei, Taiwan), according to the method specified. The DNA fragments were ligated with an RBC TA cloning vector (RBC Bioscience; New Taipei, Taiwan) to create RBC-secA and RBC-18S SGC plasmids.

The plasmids were introduced into *Escherichia coli* Top10 competent cells (Thermo Fisher Scientific; Waltham, MA, USA) and subsequently incubated overnight in solid lactose broth medium (Himedia; Mumbai, India) containing 50 mg/mL ampicillin at 37°C. Colonies were selected on the medium and the plasmids were extracted using a Biotechrabbit™ GenUP Plasmid Kit (Biotechrabbit; Berlin, Germany). Then, the plasmid concentration was measured using a NanoDrop™ 2000c Spectrophotometer (Thermo Fisher Scientific; Waltham, MA, USA.) and the plasmid solution was stored at -20°C.

Quantification of the sugarcane white leaf phytoplasma

Absolute quantification real-time PCR (qPCR) was used to quantify the amount of the phytoplasma secA gene in the sugarcane following inoculation with *M. hiroglyphicus* at 4 mth, 6 mth, 8 mth, 10 mth or 12 mth. The reaction was performed using a Rotor-Gene Q Real-Time PCR System (Qiagen; Hilden, Germany). The amount of the secA gene in the phytoplasma was finally analyzed and quantified using the Q-Rex software (Qiagen; Hilden, Germany).

Effect of sugarcane white leaf disease on yield component performance and Commercial Cane Sugar in sugarcane varieties

At age 12 mth, the primary and secondary shoots of the sugarcane were harvested from each pot within each treatment and within each replication. The average number of these shoots was used as the representative value for that pot to ensure 20 data samples from the 20 experimental units (4 pots per treatment $\times$ 5 replications).

The performance for yield components, consisting of the number of internodes, stalk length (measure in centimeters),

stalk diameter (centimeters), stalk weight (kilograms), number of stalks per clump and sugarcane sweetness (CCS), was determined at the Mittr Phol Sugarcane and Research Center Co., Ltd. Chemical Laboratory, Chaityaphum, Thailand. Briefly, the length of a sugarcane stalk was measured from the base to the topmost leaf sheath. The diameter was measured at the widest part of a stalk at mid internode. The weight of a sugarcane stalk was directly weighed using scales. The sugarcane juice was extracted from the stalk and assessed for its sweetness using a VariRef refractometer (in °Brix) (Schmidt+Haensch; Berlin, Germany.) and a Saccharomat V sugar polarimeter (in Pol; Schmidt+Haensch; Berlin, Germany). The calculation of CCS involves the use of Brix, Pol and fiber content values.

Effects of sugarcane white leaf disease on sugarcane phloem tissue

Microscopy techniques, consisting of scanning electron microscopy (SEM; SU-8020, Hitachi; Tokyo, Japan) and transmission electron microscopy (TEM; HT-7700; Hitachi, Tokyo, Japan), were performed using leaf cross-sections to observe the presence of SCWL phytoplasma in phloem tissue. These microscopy techniques were conducted at the Kasetsart Agricultural and Agro-Industrial Product Improvement Institute (KAPI), Bangkok, Thailand.

Examination of sugarcane white leaf disease incidence in symptomatic sugarcane

A qualitative approach was applied, focusing on the presence or absence of SCWL symptoms in the first ratoon. SCWL incidence was evaluated in tillering aged 3 mth by visually inspecting each pot for the presence of white or white-green leaves in each treatment.

Statistical analysis

A one-way analysis of variance followed by Tukey's honestly significant difference *post-hoc* test was used to determine differences in: 1) mean phytoplasma amounts among the five sugarcane varieties at each of the five time points (4 mth, 6 mth, 8 mth, 10 mth and 12 mth); 2) mean performance for yield components (the number of internodes per stalk, stalk length, stalk diameter, stalk weight, number of stalks per clump and CCS at age 12 mth); and 3) differences in sieve plate thickness among the five sugarcane varieties.

A χ^2 test of independence was applied to assess whether there was a significant difference in the percentage of SCWL incidence between KK3 (the reference variety) and the four Mitr Phol varieties (the tested varieties), according to Bem et al. (2019). Results were presented as mean $\pm$ SD values unless otherwise stated.

Statistical significance was determined at $p < 0.05$ using the jamovi software (Version 2.3.6; Sydney, NSW, Australia), which was retrieved from <https://www.jamovi.org>.

Results

Quantification of sugarcane white leaf disease phytoplasma

After phytoplasma inoculation with SCWL-infected vectors for 7 d, sugarcane leaves from each MPT variety were collected, and genomic DNA was extracted to determine the phytoplasma quantity using qRT-PCR at ages 4 mth, 6 mth, 8 mth, 10 mth and 12 mth for comparison with KK3 as the reference variety. At age 4 mth, the phytoplasma was detected in all sugarcane varieties. The mean amounts of phytoplasma detected in all four Mitr Phol sugarcane varieties were significantly lower than that detected in KK3 ($13.10 \pm 0.33 \times 10^3$ copies/ng plant) DNA. Among the Mitr Phol varieties, MPT12-618 had the significantly lowest amount of infection ($0.13 \pm 0.01 \times 10^3$ copies/ng plant DNA) compared to MPT04-204 ($1.57 \pm 0.04 \times 10^3$ copies/ng plant DNA), MPT16-110 ($2.76 \pm 0.22 \times 10^3$ copies/ng plant DNA) and MPT01-956 ($4.60 \pm 0.24 \times 10^3$ copies/ng plant DNA) (Table 1).

At 6 mth, 8 mth, 10 mth and 12 mth, the phytoplasma was still present in higher quantities in KK3 than in the MPT varieties (Fig. 1). The phytoplasma quantity sharply increased

from 4.42×10^3 (at 8 mth) to 55.60×10^3 (at 10 mth) to 282.57×10^3 copies/ng plant DNA (at 12 mth) in MPT16-110 and from 10.50×10^3 (8 mth) to 61.60×10^3 (at 10 mth) to 686.76×10^3 copies/ng plant DNA (at 12 mth) in MPT01-956. In contrast, there was a slight increase in MPT04-204 and MPT12-168 at 12 mth. These results suggested that MPT12-618 had the least amount of infection, while MPT 01-956 had the highest number of infections across all age groups for which data were available. (Table 1 and Fig. 1).

Effect of sugarcane white leaf disease on yield component performance and Commercial Cane Sugar in sugarcane varieties

Following inoculation with the phytoplasma from insect vectors at age 12 mth, the sugarcane plants were harvested in a greenhouse environment to assess their performance based on yield components. The number of internodes differed significantly among sugarcane varieties ($F = 5.40$, $df = 4$, $p < 0.001$). MPT16-110, MPT01-956, MPT12-618 and KK3 exhibited similar mean number of internodes ($23.10 \pm 0.48 - 24.60 \pm 3.29$), all significantly higher than that of MPT04-204 (21.70 ± 2.74) (Table 2).

Highly significant variations were observed in the sugarcane stalk length among the studied varieties ($F = 25.32$, $df = 4$, $p < 0.001$). MPT01-956 had the significantly longest stalk length (248 ± 3.28 cm), while there were no significant differences in stalk length among KK3 (183.46 ± 7.25 cm), MPT16-110 (180.64 ± 6.19), MPT04-204 (168.20 ± 6.18) and MPT12-618 (202.94 ± 7.24 cm). Although the stalk diameter did not vary significantly between varieties ($F = 0.36$, $df = 4$, $p = 0.839$), MPT12-618 had significantly heavier stalks, averaging 1.70 ± 0.05 kg, compared to KK3 and the other MPT varieties ($F = 9.89$, $df = 4$, $p < 0.001$), as shown in Table 2.

Table 1 Phytoplasma quantification in sugarcane varieties at various ages using absolute quantification real-time polymerase chain reaction technique after inoculation with sugarcane white leaf disease-infected vectors

Sugarcane variety	N	Mean number of phytoplasma $\pm$ SD (cells/ μ g of plant DNA $\times 10^3$)				
		4 mth	6 mth	8 mth	10 mth	12 mth
KK3	20	13.10 ± 0.33^a	14.60 ± 1.73^a	17.50 ± 1.51^a	$1,180.00 \pm 44.61^a$	$22,000.00 \pm 292.99^a$
MPT16-110	20	2.76 ± 0.22^c	3.26 ± 0.21^c	4.42 ± 0.42^c	55.60 ± 0.56^b	282.57 ± 10.76^c
MPT01-956	20	4.60 ± 0.24^b	5.55 ± 0.30^b	10.50 ± 0.35^b	61.60 ± 1.15^b	686.76 ± 10.24^b
MPT04-204	20	1.57 ± 0.04^d	2.13 ± 0.12^d	4.23 ± 0.07^c	5.60 ± 0.14^c	14.60 ± 0.64^d
MPT12-618	20	0.13 ± 0.01^e	0.17 ± 0.01^e	0.72 ± 0.01^d	0.76 ± 0.01^c	1.70 ± 0.23^d
F value		1,133	1,010	1,699	13,259	109,562
(p Value)		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

Different superscript letters indicate significant differences in phytoplasma levels among sugarcane varieties at each time point of quantification, as determined by analysis of variance and Tukey's honestly significant difference *post-hoc* test at $p < 0.05$.

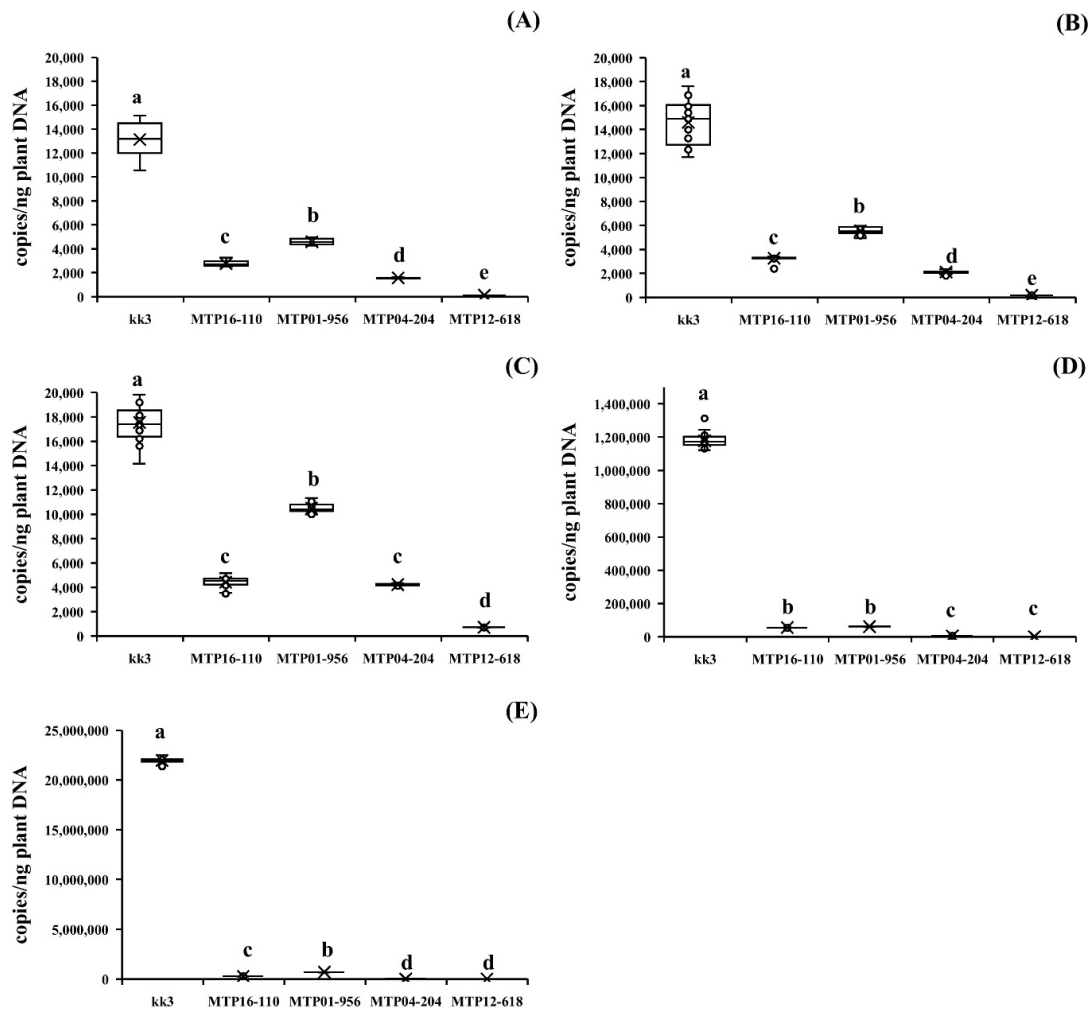


Fig. 1 Phytoplasma levels (based on absolute quantification real-time polymerase chain reaction) with significant differences at different time points post-inoculation with sugarcane white leaf (SCWL)-infected vectors: (A) 4 mth; (B) 6 mth; (C) 8 mth; (D) 10 mth; (E) 12 mth

Table 2 Performance for yield components and Commercial Sugar Cane of sugarcane varieties infected with sugarcane white leaf disease phytoplasma by insect vectors, measured at 12 mth

Sugarcane variety	Mean $\pm$ SE (95% Confidence interval)					
	Internodes/stalk	Stalk length (cm)	Stalk diameter (cm)	Stalk weight (kg)	Stalks /clump	Sweetness (CCS)
KK3	23.10 $\pm$ 0.51 ^a	183.46 $\pm$ 7.25 ^b	2.52 $\pm$ 0.04	1.52 $\pm$ 0.05 ^b	5.50 $\pm$ 0.11 ^a	8.70 $\pm$ 0.31
	(22.07–24.05)	(169.26–197.66)	(2.44–2.60)	(1.43–1.61)	(5.28–5.72)	(8.08–9.32)
MPT16-110	23.10 $\pm$ 0.48 ^a	180.64 $\pm$ 6.19 ^b	2.51 $\pm$ 0.04	1.42 $\pm$ 0.04 ^b	4.30 $\pm$ 0.11 ^c	9.90 $\pm$ 0.17
	(22.18–24.06)	(168.51–192.77)	(2.42–2.60)	(1.35–1.49)	(4.09–4.51)	(8.53–10.66)
MPT01-956	23.20 $\pm$ 0.36 ^a	247.78 $\pm$ 3.28 ^a	2.49 $\pm$ 0.04	1.49 $\pm$ 0.04 ^b	4.35 $\pm$ 0.11 ^c	9.21 $\pm$ 0.29
	(22.51–23.89)	(241.35–254.21)	(2.40–2.58)	(1.40–1.57)	(4.14–4.56)	(8.65–9.77)
MPT04-204	21.70 $\pm$ 0.39 ^b	168.20 $\pm$ 6.18 ^b	2.50 $\pm$ 0.04	1.35 $\pm$ 0.03 ^b	4.20 $\pm$ 0.09 ^c	9.28 $\pm$ 0.33
	(20.94–22.46)	(156.08–180.32)	(2.41–2.59)	(1.29–1.41)	(4.02–4.38)	(8.63–9.94)
MPT12-618	24.60 $\pm$ 0.47 ^a	202.94 $\pm$ 7.24 ^b	2.56 $\pm$ 0.06	1.70 $\pm$ 0.05 ^a	4.75 $\pm$ 0.10 ^b	9.60 $\pm$ 0.54
	(23.69–25.51)	(188.75–217.13)	(2.44–2.68)	(1.60–1.80)	(4.56–4.94)	(9.57–10.23)
F value	5.40	25.32	0.36	9.89	26.20	1.64
(p Value)	< 0.001	< 0.001	0.839	< 0.001	< 0.001	0.202

Different superscript letters indicate significant differences between the sugarcane varieties according to analysis of variance and Tukey's honestly significant difference *post-hoc* test at $p < 0.05$.

The KK3 variety had a significantly higher mean number of stalks per clump (5.50 ± 0.11), followed by MPT12-618, MPT01-956, MPT16-110, and MPT04-204 (4.75 ± 0.10 stalks/clump, 4.35 ± 0.11 stalks/clump, 4.30 ± 0.11 stalks/clump and 4.20 ± 0.09 stalks/clump, respectively ($F = 26.2$, $df = 4$, $p < 0.001$), as shown in Table 2.

There were no significant differences in sweetness among the varieties ($F = 1.64$, $df = 4$, $p = 0.202$) with mean values ranged from 8.70 ± 0.31 (KK3) to 9.90 ± 0.17 CCS (MPT16-110) (Table 2).

Effects of sugarcane white leaf disease on sugarcane phloem tissue

At 12 mth after inoculation with the SCWL phytoplasma by the insect vectors, the phenotypic characteristics of the phloem in sugarcane were studied using TEM to reveal phenotypic changes of the sieve tube cells in the phloem. Among the sugarcane varieties, KK3 had a significantly thinner sieve plate, measuring $0.152 \pm 0.02 \mu\text{m}$ ($F = 13.50$, $df = 4$, $p < 0.001$). In comparison, the sieve plate thicknesses in the other sugarcane varieties were: $0.278 \pm 0.06 \mu\text{m}$ in MPT01-956, $0.264 \pm 0.05 \mu\text{m}$ in MPT16-110, $0.262 \pm 0.04 \mu\text{m}$ in MPT12-618 and $0.233 \pm 0.05 \mu\text{m}$ in MPT04-204. The KK3 variety had a high amount of phytoplasma within the phloem sieve elements, while there were low amounts of phytoplasma in the four MPT varieties (Figs. 2 and 3).

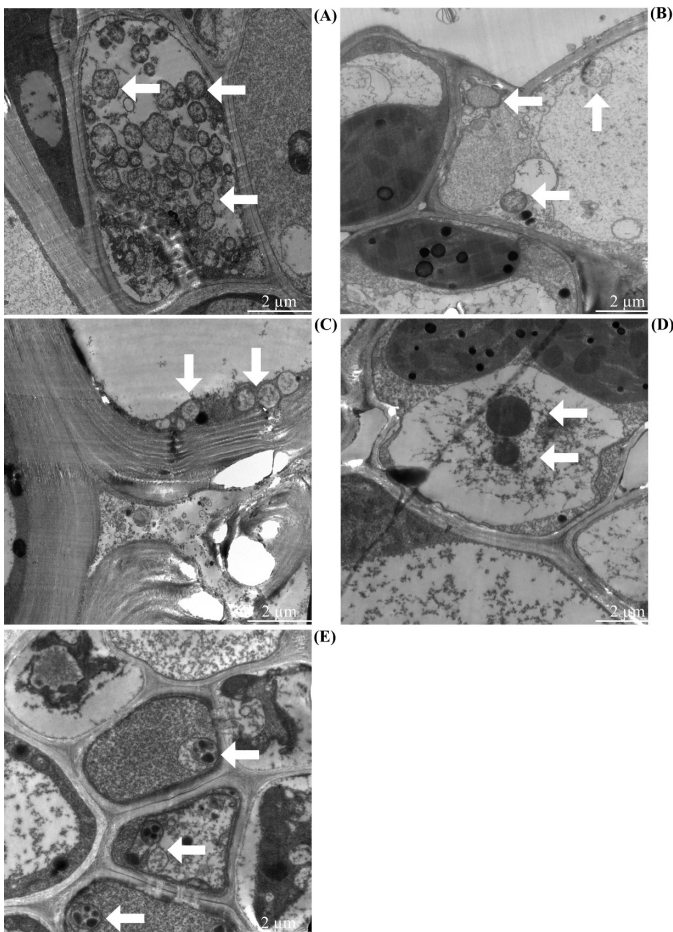


Fig. 2 Transmission electron microscope images ($3,000\times$ magnification) show phytoplasma (arrows) inside the phloem sieve elements from phytoplasma-infected sugarcane varieties: (A) KK3; (B) MPT16-110; (C) MPT01-956; (D) MPT04-204; (E) MPT12-618

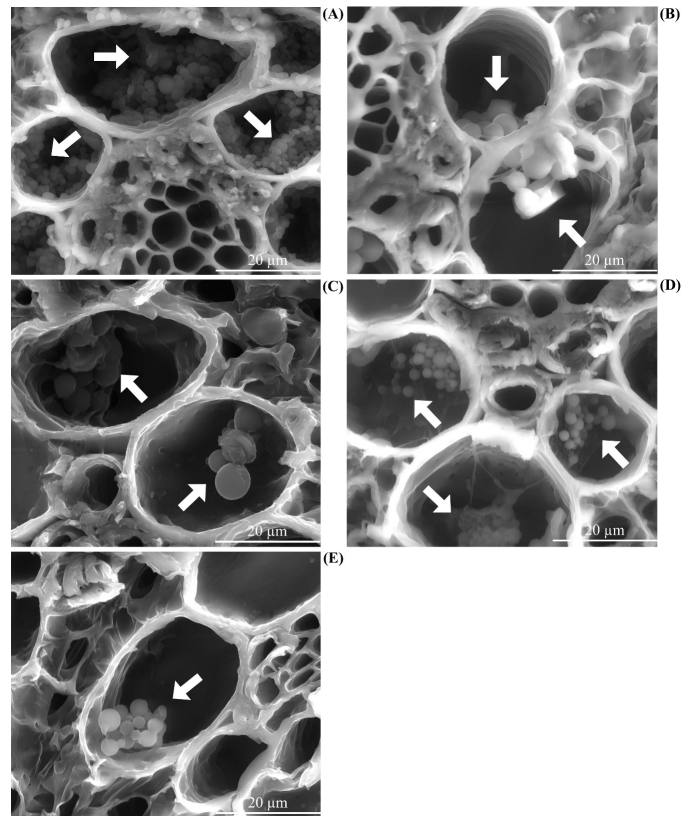


Fig. 3 Scanning electron microscope images ($5000\times$ magnification) show phytoplasma (arrows) inside the phloem sieve elements from phytoplasma-infected sugarcane varieties: (A) KK3; (B) MPT16-110; (C) MPT01-956; (D) MPT04-204; (E) MPT12-618

Examination of sugarcane white leaf disease incidence in symptomatic sugarcane

The SCWL investigation at the germination and establishment stages revealed that KK3 sugarcane had the highest percentage of pots with symptoms of white leaf disease (25%), compared to MPT16-110 (15%), MPT01-956 (10%), MPT08-204 (10%) and MPT12-618 (10%). However, of the 14 pots showing SCWL symptoms, the proportion of positive pots (percentage within rows of SCWL) was not significantly different among the sugarcane varieties ($\chi^2 = 2.82$, $df = 4$, $p = 0.588$; Table 3).

Discussion

An inoculation period of 7 d was applied to inoculate the SCWL phytoplasma into the healthy sugarcane samples, which was longer than 5 d used by Hanboonsong et al., (2005). The adjustment in the current study was based on preliminary experiments, which indicated a low infection rate of 50–60% when using the shorter period of 5 d. Thus, the inoculation period was extended to 7 d to optimize the infection rate to 80%, aligning with the same incubation period of phytoplasma in the salivary glands of insect vectors when the insects had been feeding on SCWL-infected plants for 24 hr (Roddee and Hanboonsong, 2018). After 1 mth of inoculation using insect vectors, the current study excluded sugarcane plants that did not become infected.

Since phytoplasma has been reported to completely infest the sugarcane stalk by day 35 post-infection (Roddee et al., 2018), the phytoplasma quantification was designed for 4 mth, 6 mth, 8 mth, 10 mth and 12 mth.

Based on the current results, the phytoplasma levels increased steadily in all varieties from month 4 to month 8, with a subsequent rapid increase from month 10 to month 12. Notably, MPT12-618 consistently had the lowest phytoplasma levels. These findings were consistent with the typical SCWL disease progression during the germination and early growth stages. Sugarcane variety KK3 had a higher incidence of symptomatic plants (25%) than the Mitr Phol varieties (10–15%). These results suggested a correlation between initial phytoplasma levels at 12 mth and subsequent SCWL symptom severity upon germination. This aligned with the strong correlation between phytoplasma concentration and sugarcane white leaf disease severity, where the asymptomatic samples had less than 1,000 copies, while symptomatic samples were in the range 1,000–10,000,000 copies (Sakuanrungrsirikul et al., 2021). At 12 mth, KK3 had a significantly higher phytoplasma level (22,000,000 copies/ng plant DNA), resulting in a 25% incidence of SCWL. In contrast, the other four MPT varieties had lower phytoplasma levels in the range 1,700–686,760 copies/ng plant DNA, leading to a 10–15% incidence of SCWL white leaf. However, while there was visually observation of white leaves, the percentage of white leaves per clump was not quantified. It was possible that low-level phytoplasma infections resulted in asymptomatic sugarcane (Saengmanee et al., 2024).

Table 3 Contingency table showing proportion (%) of sugarcane white leaf (SCWL) incidence in KK3 (referenced variety) compared to Mitr Phol varieties (tested varieties)

Sugarcane exhibiting SCWL symptoms		Sugarcane variety					Total
		KK3	MPT16-110	MPT01-956	MPT08-204	MPT12-618	
No Symptoms	Observed	15	17	18	18	18	86.00
	Expected	17.20	17.20	17.20	17.20	17.20	86.00
	% Within row	17.40	19.80	20.90	20.90	20.90	100.00
	% Within column	75.00	85.00	90.00	90.00	90.00	86.00
SCWL	Observed	5	3	2	2	2	14.00
	Expected	2.80	2.80	2.80	2.80	2.80	14.00
	% Within row	35.70	21.40	14.30	14.30	14.30	100.00
	% Within column	25.00	15.00	10.00	10.00	10.00	14.00
Total	Observed	20	20	20	20	20	100
	Expected	20.00	20.00	20.00	20.00	20.00	100.00
	% Within row	20.00	20.00	20.00	20.00	20.00	100.00
	% Within column	100.00	100.00	100.00	100.00	100.00	100.00

Proportion of positive pots (% within row of SCWL) not significantly different among sugarcane varieties ($\chi^2 = 2.82$, $df = 4$, $p = 0.588$).

The current results clearly demonstrated that MPT12-618, with the lowest phytoplasma levels, had significantly longer and heavier stalks than KK3. The TEM and SEM analyses further confirmed these findings. At 12 mth, the KK3 plants had thinner sieve plates and a greater phytoplasma load in the phloem tissue than the four Mitr Phol varieties. These observations aligned with other research that reported the high phytoplasma levels in sugarcane had significantly impacted plant health, leading to reduced cane length, diminished chlorophyll content and structural damage to chloroplasts (Saengmanee et al., 2024). In addition, sugarcane varieties with higher phytoplasma infection tend to have fewer internodes, shorter stalks and narrower diameters, leading to a lower stalk weight (Zhang et al., 2020). This was evident in KK3, which had the highest level of phytoplasma infection and had a lower stalk weight than the other Mitr Phol sugarcane varieties.

Although there were no significant difference in CCS levels among the four MPT and the KK3 varieties, Lohmaneeratana et al. (2024) showed that an abundance of phytoplasma in SCWL canes suppressed photosynthesis, porphyrin and chlorophyll metabolism, reasonably contributing to the blockage of sucrose transport and resulting in a low sugar content in canes.

In conclusion, MPT12-618 demonstrated potential as a genetic resource for breeding white leaf disease-tolerant varieties, with lower levels of phytoplasma infection, higher stalk weight and a lower incidence of SCWL symptoms after harvest than KK3 as a reference. To confirm this potential, the disease incidence and yield potential in tolerant genotypes should be examined in terms of the tolerance mechanism. Likewise, in the study by Leetanasaksakul et al. (2024), four genotypes (K88-92, K95-84, UT1 and KK3) were used to identify the proteins involved in the defense mechanism against SCWL phytoplasma. That study showed that two potential candidate protein biomarkers (peptidyl-prolyl cis-trans isomerase and 9-cis-epoxycarotenoid dioxygenase) were detected only in UT1. These proteins are associated with reduced susceptibility to SCWLD phytoplasma.

The key limitation of this study was conducting the experiments in greenhouse conditions that were not identical to real-world farming environments. Factors, such as fluctuating temperatures, rainfall patterns, and humidity, as well as the presence of other pests and diseases, could significantly impact plant growth and disease resistance. Therefore, comprehensive yield assessments under real-world farming conditions are crucial to accurately evaluate the tolerance and suitability of MPT12-618 for practical applications.

Conflict of Interest

The authors confirm that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. All authors contributed to the study design, data collection and analysis, decision to publish and preparation of the manuscript.

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