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# Disease Problems of Sugarcane in Vietnam, with Special Reference to Phytoplasma

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## ABSTRACT

Seventeen diseases of sugarcane have been found in Vietnam including 13 fungal, 2 bacterial and 2 phytoplasmal diseases. Among them, the most important diseases are smut, pineapple, red rot, sugarcane white leaf and sugarcane grassy shoot diseases. We applied nested PCR using universal primers for detection and characterization of phytoplasmas from infected tissues. PCR products of the expected size (1200 bp) were obtained from the 16S rRNA of the phytoplasma. The RFLP profiles indicated that all the samples were infected by the same pathogen. Nucleotide sequence analysis of the 16S rRNA genes revealed that the phytoplasma causing sugarcane grassy shoot disease in Vietnam is very similar to the phytoplasmas causing sugarcane grassy shoot disease in India sharing a sequence similarity of 99%. Phylogenetic trees showed that SCGS and SCWL strains collected from Vietnam and other reported SCGSs belong to the 16SrXI subgroup RYD (Rice Yellow Dwarf) 16S rRNA Group.

**Keywords:** phytoplasma, pineapple disease, red rot, SCGS, SCWL, smut
**Abbreviations: RFLP**, restriction fragment length polymorphism; **SCGS**, sugarcane grassy shoot; **SCWL**, sugarcane white leaf

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## INTRODUCTION

Sugarcane (*Saccharum officinarum* L.) is a high potential cash crop, widely grown throughout Vietnam mainly for raw sugar and industrial crystal/centrifugal sugar production. Cane is generally grown in the drier regions of the Mekong Delta area in the South, and at the Red River Delta area in the North. The annual sugarcane production in Vietnam reached 6.34 million tonnes in 1993 resulting in sugar production of 500,000 tonnes and reached 1 million tonnes of crystal/centrifugal sugar in 2000. At the moment, there are 42 sugarcane factories in total, including 14 in the South, 15 in the Highland and Center and 13 in the North. Currently, the commercial sugarcane milling capacity is around 13,500 tonnes per day, from 12 mill plants ranging in capacities from 500 to 2,000 tonnes per day. Domestic production targets at 12.7 million tonnes of sugarcane and 1.0-1.5 million tonnes of sugar by the year 2010, and 2 million tonnes of sugar in 2020 (VBN 2011).

However, the sugarcane industry in Vietnam is threatened by a wide range of insects and diseases. The most serious insects are white grub (*Heteronychus annulatus* Bates), sugarcane stem-borer (*Chilo terrenellus* Pagenstecher) and woolly aphid (*Ceratovacuna lanigera*). There are many important diseases, especially a devastating disease locally known as sugarcane grassy shoot disease (SCGS), which has occurred recently in many agro-ecological regions of the Nghe An Province in North-Central Vietnam since 2005. The disease causes severe loss in crops, reducing the sugar content, and the severity is multifold in ratoon crops. Another phytoplasmal disease of cane is sugarcane white leaf disease (SCWL), which was also detected in Vietnam by Man and his co-workers (Man *et al*. 2002). We have detected phytoplasmas associated with these diseases and further analyzed theirs relationship with other related phytoplasma strains.

Phytoplasmas are phloem-restricted pathogens belonging to the class Mollicutes that lack cell walls, have remarkably small (500-1350bp) AT-rich genomes (Razin *et al*. 1998), and are the causal agents of diseases in hundreds of

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Invited Mini-Review

**Table 1** List of diseases of sugarcane found in the North of Vietnam.

| Name | | Distribution |
|---|---|---|
| **Common name** | **Pathogen** | |
| Bacterial shoot rot | *Erwinia chrysanthemi* Burkholder | Thanh Hoa, Hoa Binh |
| Eye spot | *Helminthosporium sacchari* Butler | A |
| Fusarium sett and stem rot | *Fusarium moniliforme* Seldon | A |
| Leaf blight | *Nigrospora sphaerica* (Saccardo) Mason | A |
| Leaf scald | *Xanthomonas albilineans* | A |
| Leaf spot | *Septoria demidovae* Lavrov | A |
| Leaf streak | *Helminthosporium stenospilum* Drechsler | A |
| Pineapple disease | *Ceratocystis paradoxa* (Dade) Moreau | A |
| Red rot | *Collectotrichum falcatum* Went | A |
| Red spot of leaf sheath | *Cercospora vaginae* Krüger | A |
| Rhizoctonia sheath and shoot rot | *Rhizoctonia solani* Kühn | A |
| Ring spot | *Leptosphaeria sacchari* Breda de Haan | A |
| Rust | *Puccinia sacchari* Patel, Kamat & Padhye | A |
| Smut | *Sporisorium scitamineum* (Sydow) Piepenbring, Stoll & Oberwinkler | A |
| Sooty moulds | *Fumago vagans* Persoon | A |
| Sugarcane grassy shoot | SCGS phytoplasma | Nghe An |
| Sugarcane white leaf | SCWL phytoplasma | Dong Nai, Tuyen Quang, Ninh Binh, Thanh Hoa, Nghe An, Binh Duong |

**Note**: (A): The North Mountainous, the Red River Delta, the North Central Coast, the South Central Coast, the Central Highlands, the Southeastern Region and the Mekong Delta Regions.

plants (Corbett *et al*. 1971; McCoy *et al*. 1989; Ahrens and Seemüller 1992). It is believed that phytoplasmas are transmitted from plant to plant via propagation techniques, and by specific phloem-feeding insects including leafhoppers, planthoppers and psyllids (McCoy *et al*. 1989). SCGS is one of the most important diseases of sugarcane and was first detected in India (Chona 1958) and was later found in many other countries including Bangladesh, Malaysia, Nepal, Pakistan and Sri Lanka (Rishi and Chen 1989; Sdoodee *et al*. 1999; Viswanathan 2000; Singh *et al*. 2002a; Ariyarathna *et al*. 2007) and in Vietnam (unpublished data). SCGS exhibits different symptoms depending on the host genotype, location and environment (Chandrasene *et al*. 2003; Viswanathan *et al*. 2005; Srivastava *et al*. 2006; Ariyarathna *et al*. 2007; Nasare *et al*. 2007). It has been indicated that up to 60-80% or higher incidence of SCGS might result in heavy losses in cane yield and sucrose content (Rao *et al*. 2005; Srivastava *et al*. 2006). Sugarcane white leaf (SCWL) is another destructive sugarcane disease especially in Thailand, Sri Lanka, Taiwan and Japan (Ling 1962; Mangelsdorf 1962; Matsumoto *et al*. 1968; Phisitkul *et al*. 1989; Rishi and Chen 1989; Nakashima and Murata 1993; Sarindu and Clark 1993; Nakashima *et al.* 1994, 1996; Wongkaew *et al.* 1997; Kumarasinghe and Jones 2001; Nakashima *et al.* 2001; Rao and Ford 2001; Hanboonsong *et al*. 2002, 2006; Ariyarathna *et al*. 2007) and Vietnam (Man *et al*. 2002; unpublished data).

In this paper, a general review of the disease problems of sugarcane in Vietnam is provided. In addition, molecular tools for the detection and characterization of phytoplasmas associated with SCGS and SCWL are also discussed. The phylogenetic relationship of the SCGS phytoplasma with other closely related phytoplasmas including the SCWL agent at the 16S rRNA gene sequence level is examined.

According to the two surveys conducted by the Plant Protection Research Institute, Vietnam in 1967-1968 and 1977-1978, and recent studies conducted by our group, there are 17 diseases of sugarcane including 13 fungal, 2 bacterial and 2 phytoplasmal diseases (**Table 1**; Man *et al.* 2002; Plant Protection Research Institute 1975; 1999; Thanh 2008). In the following section, only important diseases for sugarcane growers and the industry in Vietnam are mentioned and discussed.

## DISEASES

### Smut

Sugarcane smut, caused by the fungus (*Sporisorium scitamineum* (Sydow) Piepenbring, Stoll & Oberwinkler), has been reported as one of the major factors reducing yield and sucrose content (Hoy 1986; Padmanaban *et al.* 1988), and can become a serious epidemic disease in susceptible cultivar–growing regions (Villalon 1982). In Vietnam, it is widely distributed in all sugarcane growing regions of Vietnam (**Table 1**). The typical symptoms of the disease are recognized by whip-like sorus-bearing structures, slender stalks, small narrow and short leaves. The disease forms from the terminal meristem or from lateral shoots of infected stalks which become covered with black spores (teliospores) (**Fig. 1**). It occurs in the dense cane plantations and the dry and high humid conditions that enable fungal spores to spread in the air. The disease incidence reached up to 45-50% in Ha Trung District, Thanh Hoa Province in the year 2006. The fungus invades plants at very early stages after planting and causes slow growth of the infected plants with short and thin internodes (unpublished data). It is believed that smut is transmitted by two ways: (*i*) air-borne spores enter the standing sugarcane through the buds; and (*ii*) spores in the soil or in irrigation water enter planted setts (Walker 1987).

The smut still remains a potentially serious disease as *S. scitamineum* has high variability (Xu *et al.* 2000). In Vietnam, fungicides are not efficient for control of smut as growers are often smallholders and the sugarcane-growing areas are in mountainous regions where spraying of pesticides is very limited. Therefore, use of resistant cultivars is the most effective method for management of the disease; however, it seems that most of the cultivated varieties are susceptible. Treatment of setts with hot water at 50°C for 3 h is a good method to eliminate the fungus, but this method is impractical for large-scale use. Instead, it is necessary to conduct field surveys more frequently to detect infected plants early, move them out of the field and burn them completely. An IPM model should be developed for management of smut disease such as the one described by Wada *et al*. (1999).

### Pineapple disease of sugarcane

*Ceratocystis paradoxa* (Dade) Moreau is the causal agent of pineapple disease of cane that occurs in all sugarcane-growing regions in Vietnam (**Table 1; Fig. 2**). It induces seed piece decay following planting and causes poor initial stands of sugarcane (Man 2007). The pathogen is a soil-borne fungus species affects sugarcane setts in the first weeks after planting and causes poor germination of buds and emergence of young shoots, especially if setts are excessively deep-planted and in wet or dry soil and low soil temperature conditions (Moutia and Saumtally 1999; Man

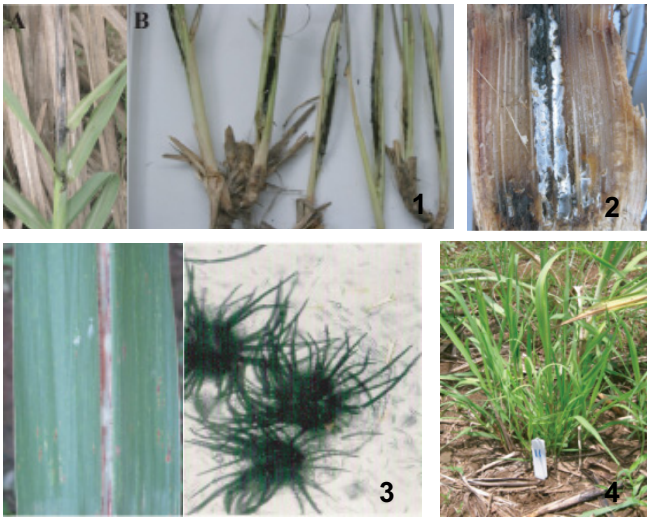


**Fig. 1** Typical symptom of sugarcane smut disease found in Vietnam. (**A**) Whip covered with black teliospores; (**B**) Teliospores inside the intected stems. **Fig. 2** Typical symptom of sugarcane pineapple disease found in Vietnam. **Fig. 3** Sugarcane red rot disease collected in Vietnam. (**A**) The typical symptom on the leaf; (**B**) Spores of *Colletotrichum falcatum* from the infected tissues. **Fig. 4** Typical symptom of sugarcane grassy shoot disease collected from Nghe An Province, Vietnam.

2007).

Use of resistant cultivars should be the easiest and most economic method for managing the disease. Disease-free setts should be used since the major source of disease is in the infected-plants and setts. Farmers usually treat setts with 2-3% lime solution for 12-24 hrs to eliminate the pathogen before growing. During storage, the cut sides of setts should be covered with lime or in combination with $CuSO_4$ solution to avoid the infection (Man 2007). Field surveys are recommended to detect, remove and destroy completely the infected plants and lime should be applied into the infected places prior to replacement with disease-free setts to reduce the disease inoculums in the field. Crop rotation between cane crops with soybean and/or peanut for several years may also interrupt the disease cycle and reduce its impact on plants. It is strongly recommend farmers to use dry and well-drained soils for planting cane. If necessary, several types of fungicide such as propiconazole and carbendazim can be used to inhibit the pathogen growth (unpublished data; Vijaya *et al.* 2007).

### Red rot

Red rot, caused by *Colletotrichum falcatum* Went, is a constraint for sugarcane production in Vietnam and usually found in the weakly growing-fields in all ecological regions. In the crops of the years 2003 and 2004, the disease severely damaged sugarcane in Quang Nam Province in Central part Vietnam. *C. falcatum* infected all parts of plant such as stalks, leaves, buds or roots; however, it was very hard to recognize the infected plants at the first stage of infection, because the symptom was inside the plant. There are different types of symptoms depending on the age of the stalk, time of infection and susceptibility of the cane genotype (**Table 1; Fig. 3**). The disease is sett-transmitted and the spores can be spread by wind, rain and invade into plants through wounding or the holes made by stem borers (Viswanathan and Samiyappan 2000; Man 2007; unpublished data).

It is recommended to treat setts with hot water at 50°C for 20 min or hot steam water at 45°C for 4 h for elimination the pathogen and increasing the germination capability (Singh 1973; Man 2007). In Vietnam, normally farmers cannot perform this method unless received support from sugarcane factories; however, its applicability is very limited due to the logistics of large scale of setts. To resolve this problem, crop rotation between cane and other plants such as peanut and soybean can be considered as easy and economic method for reduction the infection and its impacts. Importantly, old and infected leaves should be taken out of the field and burnt completely after harvesting to reduce the inoculums in the field (Thanh 2008). Biological control agents, such as *Trichoderma harzianum* or *T. viride*, have been used to manage the disease and enhance yield and shelf life (Singh *et al.* 2008, 2009). Viswanathan and Samiappan 2002, 2008 found that the plant growth-promoting rhizobacteria (PGPR) could induce systemic resistance against *C. falcatum* in susceptible varieties and suggested PGPR as a promising approach in managing the disease. Recent studies in India have also revealed systemic acquired resistance (SAR) mediated reduction in red rot intensity through biotic and abiotic elicitor molecules (Sundar *et al.* 2006, 2009).

## SUGARCANE GRASSY SHOOT AND SUGARCANE WHITE LEAF DISEASES

### Symptom analysis

There are some phytoplasmal diseases in sugarcane have been reported such as SCGS, SCWL and Ramu stunt, which are the most economically important factors in Asia and sugarcane yellow leaf syndrome (SCYLS) in Africa (Cronje *et al*. 1998; Aljanabi *et al*. 2001; Marcone 2002; Arocha *et al*. 2005; Rao *et al.* 2005).

SCGS has become the most important disease to both the farmers and sugar industry in Nghe An Province in north-central Vietnam since 2005, and reached epidemic levels from 2008 to 2010 affecting more than 6,577 ha. The SCGS symptom observed in Nghe An similar to those described previously in other countries (Chandrasene *et al*. 2003; Nasare *et al*. 2007). The infected plant is typically recognized by the presence of leaves that are thin, narrow, reduced in size, have a soft texture and are green, pale green, pale yellow or even chlorotic (**Fig. 4**). There is also production of a large number of thin, slender, adventitious tillers from the base. Each stalk produced from the infected stool shows shortened internodes with the development of side shoots from the bottom to the top. Infected plants do not produce millable canes, especially in the second and third ratoon crops. Man and his working group first detected SCWL in Dong Nai, Tuyen Quang, Ninh Binh and Thanh Hoa Provinces based on symptom analysis and using transmission electron microscopy (Man *et al.* 2002). The typical symptoms of SCWL in Vietnam is similar with that described by Kumarasinghe and Jones (2001), Hanboonsong *et al.* (2002), Marcone (2002), Chandrasene *et al.* (2003) and Hanboonsong *et al.* (2006). Recently, we have also used molecular tools for detection and characterization of a phytoplasma associated with SCWL in Nghe An and Binh Duong Province (unpublished data). The infected plant can be easily recognized as total chlorosis of the leaf, proliferation of tillers and pronounced stunting, with narrow and small leaves affecting the photosynthesis of the plant. Severely infected plants fail to flower, decline and do not produce millable canes.

### Detection and characterization of phytoplasmas

The detection and identification of SCGS phytoplasma is necessary for accurate disease management. Many methods, for example bright field, fluorescence, and electron microscopy observations and serological assays have been used for detection of phytoplasma. However, those methods do not attain pathogen classification and are not always sufficiently sensitive to detect phytoplasma infections, because phytoplasmas usually have uneven distribution in plant tissues. It was indicated that the FTA TM paper method may be a rapid and cost effective means of detecting sugarcane phytoplasmal diseases including SCWL and SCGS compared to the enrichment method (Chandrasene *et al*. 2003).

Polyclonal antisera were produced from partially purified SCGS phytoplasmas from infected sugarcane plants and ELISA techniques have been tested for the detection of SCGS phytoplasmas in infected sugarcane (Sarindu and Clark 1993; Viswanathan 1997). In Vietnam, the detection and characterization of the phytoplasmas diseases mainly just based on symptom and transmission electron microscopic (TEM) examination of ultrathin sections (Man *et al*. 2002). Therefore, a more efficient and reliable method for detecting the pathogen would be essential for indexing seed canes, disease monitoring/forecasting quarantine procedures and basic research on the behavior of the pathogens.

Nested PCR is currently the best choice for phytoplasma diagnosis because of its versatility, relative simplicity, specificity and high sensitivity. It has also become possible to differentiate, characterize and classify the phytoplasmas based on RFLP analysis (Sawayanagi *et al.* 1999; Jung *et al.* 2003; Lee *et al.* 2004; Marcone *et al.* 2004a, 2004b; Seemüller and Schneider 2004; Valiunas *et al.* 2006; Arocha *et al.* 2007), and a phylogenetic tree constructed from sequences of the amplified 16S rRNA genes (Lee *et al*. 1998; Seemüller *et al*. 1998; Lee *et al*. 2000). Regions of the rRNA operon, including the complete 16S rRNA, the 16S/23S rRNA spacer region a portion of 23S rDNA and other genes of some phytoplasmas have been sequenced (Seemüller *et al.* 1994; Gundersen *et al.* 1996; Hodgetts *et al.* 2008) for further classification of phytoplasma groups and subgroups.

We used nested PCR with primer pairs P1/P7 and R16F2n/R16R2 (Lee *et al*. 1998; Seemüller *et al*. 1998; Lee *et al*. 2000) to detect and characterize the causal pathogen of SCGS in Vietnam. Clear amplification of approx. 1200 bp bands was obtained from plants showing SCGS symptoms. Negative controls and healthy plants produced no amplification. The amplified PCR products were subjected to restriction enzyme digestions and the RFLP profiles indicated that the samples were infected by a single strain (unpublished data). The results from sequencing of the nested PCR products indicated that the phytoplasma found in Nghe An Province (SCGSNAVN) shares 99% similarity with the causal agent of SCGS in India (accession number AM262831). In another independent experiment, we applied the same method and detected a phytoplasma associated with the SCWL symptom collected from Binh Duong Province (SCWLBDVN) and the sequencing analysis showed that it is closely related to that of the phytoplasma strain causing SCWL disease in Thailand (accession number FM208260) (unpublished data). A phylogenetic tree was constructed with 43 sequences of 16S rRNAs from different phytoplasmal strains. The resulting tree reproduced the current taxonomic groups, the SCGSNAVN and SCWLBDVN sequence clustered with the 16Sr-XI group described as the Rice Yellow Dwarf (RYD) group by Lee *et al*. (1998). The phylogenetic position of SCGS and SCWL phytoplasmas and theirs relatedness to other closely related phytoplasmas, which infecting mainly gramineous plants, belong to the Rice Yellow Dwarf (RYD) or 16SrXI group (Lee *et al.* 1993, 1997; Wongkaew *et al*. 1997; Seemüller *et al*. 1998; Sdoodee *et al*. 1999; Lee *et al.* 2000; Tran-Nguyen *et al*. 2000; Hanboonsong *et al*. 2002; Jung *et al*. 2003; Marcone *et al*. 2004a, 2004b). Our results indicated that SCGS and SCWL phytoplasmas are phylogenetically closely related (99%) and are known to cause similar symptoms in infected sugarcane that are often indistinguishable (Wongkaew *et al*. 1997; Sdoodee *et al*. 1999; Marcone 2002). Several investigations have indicated that the 5′-end of the 16S rRNA gene of the rRNA operon is more informative for differentiating SCWL from SCGS (Wongkaew *et al*. 1997; Sdoodee *et al*. 1999).

16S rRNA gene-based RFLP analysis has been applied extensively for differentiation and classification of phytoplasmas strains into major 16Sr RFLP groups (Lee *et al*. 1993b; Gundersen *et al.* 1994; Seemüller *et al*. 1994; Schneider *et al.* 1995; Lee *et al*. 1998; Seemüller *et al*. 1998; Lee *et al*. 2000; Arocha *et al.* 2005). However, this method cannot achieve a fine differentiation into subgroups based on the rather limited number of variable markers present in the 16S rRNA gene (Martini *et al.* 2007). It has been proposed that each RFLP or phylogenetic group represents at least one phytoplasma species (Gundersen *et al*. 1994; Seemüller *et al.* 1998). Interestingly, in bacteria, ribosomal proteins (*rp*) are the genes that share a common history of evolution and carry a strong phylogenetic signal (Wolf *et al*. 2001; Daubin *et al*. 2002). Martini *et al.* (2007) reported that the *rp* gene-based phylogeny revealed more insights into the phylogenetic relationships among phytoplasma strains, and Hodgetts *et al.* (2008) have reported the use of a further gene, the *secA* gene for improved phylogenetic discrimination. With more variability than the 16S rRNA gene, the *rp* and *secA* gene sequences provide more phylogenetic markers useful for differentiation of genetically closely related but distinct ecological strains that are not readily separated on the basis of the highly conserved 16S rRNA gene (Martini *et al*. 2007; Hodgetts *et al*. 2008).

Recently, an alternative to PCR has been developed for phytoplasma diagnostics, the use of loop-mediated isothermal amplification (LAMP) (Tomlinson *et al.* 2010). This method is much quicker than PCR and does not require as many manual-handling steps as nested PCR so there is less potential for contamination of samples. The methods can be combined with a novel DNA extraction technique (Tomlinson *et al.* 2010) such that assays can be performed from start to finish in less than 1 hour. We have recently developed LAMP diagnostic primers for the 16SrXI and XIV group phytoplasmas, including SCGS and SCWL and are combining these methods into a rapid in-field real-time detection system for these phytoplasmas (Dickinson unpublished).

## Transmission of SCGS and SCWL

Insect vectors of the phytoplasmas are restricted to phloem-feeding leafhopper and planthopper members of the *Cicadellidae*, *Fulgoroidea* and *Psylloidea*, which transmit the phytoplasma in a persistent propagative manner (Srivastava *et al.* 2006). Successful transmission of the disease is dependent on acquisition of the phytoplasma by the vector during feeding (acquisition access period); passage of the phytoplasma from the insect gut through the haemocoel and passage across the salivary gland cell membranes (latent period); and inoculation of a healthy plant during subsequent feeding (inoculation access period) (Srivastava *et al.* 2006). There are no reports on mechanical or aphid transmission of phytoplasmas infecting sugarcane (Rishi and Chen 1989).

The vector(s) responsible for the natural spread of SCGS are still uncertain. There are reports on transmission of SCGS by three different species of aphids as well as by the fulgorid *Proutista moesta* Westwood (Edison *et al.* 1976; Rishi and Chen 1989). Another research group indicated that the leafhopper *Deltocephalus vulgaris* is responsible for transmission of the SCGS phytoplasma in India (Singh *et al*. 2002b; Srivastava *et al*. 2006). However, this species has not been recorded in Vietnam, therefore other insect species are probably responsible for transmission of SCGS in Vietnam. It is believed that SCWL phytoplasmas are transmitted by the leafhopper *Matsumuratettix hiroglyphicus* (Matsumura) (Matsumoto *et al.* 1968; Chen 1974; Maramorosch *et al*. 1975; Phisitkul *et al.* 1989; Hanboonsong *et al.* 2002) and by *Yamatotettix flavovittatus* (Hanboonsong *et al.* 2006). Interestingly, transovarial transmission of SCWL in the insect vector *M. hiroglyphicus* has been indicated (Hanboonsong *et al.* 2002). However, these insect species have not been found in Vietnam, therefore similar with that of the SCGS, other species are probably responsible for transmission of SCWL in Vietnam. Man *et al*. (2002) performed transmission experiments and reported that none of *Aphis sacchari*, *A. gosypii*, *Bemisia* sp. and *Myzus persicae* is vector of the SCWL (Man *et al*. 2002). Our recent attempts have failed in identification of insect

vector responsibility for transmission of SCGS and SCWL.

### Management of SCGS and SCWL

The identification of the reservoir of phytoplasma is important for the management of phytoplasmal disease (Hanboonsong *et al.* 2006). It is believed that several kinds of gramineous weeds in the cane-growing areas such as bermuda grass (*Cyanodon dactylon*), crowfoot grass (*Dactyloctenium aegyptium*) and brachiaria grass (*Brachiaria distachya*) can be infected with phytoplasmas and show the symptoms of white leaf disease (Rishi and Chen 1989; Nakashima *et al.* 1994). However, Wongkaew *et al*. (1997) performed DNA sequencing analysis and indicated that none of the phytoplasmas that infect the gramineous weeds was identical to that which causes SCWL disease suggesting that they are not a reservoir for phytoplasmas causing SCWL disease. Thus the reservoir of phytoplasma is either another as yet unidentified weed or the insect vector itself (Hanboonsong *et al.* 2006). In Vietnam, farmers usually burn all the plant debris and weed after harvesting that may help to eliminate any inoculums remaining in the field. During a season, they may use some herbicides for control weed in the field where weeds grow much faster than cane and may be the alternative hosts of any insects and pathogens.

Using pesticides for controlling insect vectors of phytoplasmal diseases are not the efficient way for management of diseases since even when frequently used, because pesticides cannot kill insect vectors before they contact plants, and transmit pathogens into healthy plant cells (Wally *et al.* 2004; Weintraub and Beanland 2006; Weintraub 2007). We treated cane stalks with hot water at 50°C for 3 h before growing. Interestingly, the results showed that the disease incidence in the treated treatment was 3.3% in comparison to that from the control (non-treatment) of 31.2% after 1 year of growing (unpublished data). This method can eliminate the pathogen; however, it is very hard and costly for farmers to apply it in Vietnam. Taken together, instead of directly use of hot water-treated setts for growing, we need to develop and establish nurseries of disease-free seedlings and supply for growers (Kumarasinghe, pers. comm.., 2011). In addition, using resistant/high tolerant varieties against SCGS and SCWL is the best choice for management of these diseases.

## CONCLUDING REMARKS

In Vietnam, there are several diseases considered as important ones for sugarcane growers including smut; pineapple, red rot disease along with SCGS and SCWL. We used molecular tool for detection and characterization of phytoplasmas associated with SCGS and SCWL; however, it is undistinguishable between 16SrRNA of the two phytoplasma strains. Using disease-free setts, resistant/high tolerant varieties in combination with hot water treatment wherever possible are the best methods for management of SCGS and SCWL as well as other diseases like smut, pineapple and red rot. It is also very important not to transfer diseased setts from infected areas to other areas to prevent the spread of the diseases. It is recommended to destroy completely the infected materials and rotate with other crops such as peanut or soybean but not rice or maize to interrupt the disease cycles. In addition, it is also good to remove and burn grasses such as bermuda grass, crowfoot grass and brachiaria grass that are infected with different phytoplasma strains but which are in the same group with SCGS and SCWL phytoplasmas. It is necessary to identify the insect vector for transmission of SCGS and SCWL in Vietnam; however, using pesticides for control of the vector(s) are not recommended, because sugarcane is planted in high mountainous areas, where water source is very limited.

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