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# Recent Biotechnological Approaches in Diagnosis and Management of Sugarcane Phytoplasma Diseases

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## ABSTRACT

Phytoplasmas have been reported to be associated with two major sugarcane diseases viz., grassy shoot disease (GSD) and white leaf disease (WLD). Sugarcane yellow leaf syndrome is a new syndrome of sugarcane caused by a luteovirus and a phytoplasma. Sugarcane green grassy shoot (SCGGS) and ramu stunt are also associated with sugarcane from Thailand and Papua New Guinea, respectively. GSD and WLD are causing significant economic losses to sugarcane yield and sugar recovery in Asian countries. Both these phytoplasmas have been spreading very rapidly to newer locations with the help of infected seed material and leafhopper vectors. Hence it would be important to diagnose and manage these phytoplasmas at an early stage of sugarcane growth to avoid further spread and significant losses caused by them. Because of unreliable and unspecific symptoms, the identification and characterization of the associated phytoplasma at an early stage of plant growth is problematic and unreliable. The introduction of molecular genetic methods into plant mycoplasmology about 15 years ago greatly improved the diagnosis of phytoplasma infections in plant and insect hosts. PCR offers several advantages over other methods including versatility, relative simplicity, specificity and high sensitivity, which can be increased by a two-step PCR (nested PCR). It has also become possible to differentiate, characterize and classify the phytoplasmas on a phylogenetic basis, using mainly sequence analysis of ribosomal DNA (rDNA). The biotechnological and molecular assays developed in recent years have been utilized at a large scale to characterize these pathogens at an early stage of growth and for screening planting seed materials for possible elimination and management of theses phytoplasma at an early stage of their growth and propagation. We summarize research being conducted on characterization and management of these phytoplasma by novel biotechnological and molecular approaches.

**Keywords:** characterization, detection, PCR assays, grassy shoot disease, white leaf disease
**Abbreviations:** **PCR**, polymerase chain reaction; **RFLP**, restriction fragment length polymorphism; **SCGS**, sugarcane grassy shoot; **SCWL**, white leaf disease; **YLS**, yellow leaf syndrome

## CONTENTS



## INTRODUCTION

Phytoplasmas are phytopathogenic prokaryotes that were formerly named as mycoplasma-like organism (MLOs) and were discovered in Japan in 1967 (Doi *et al.* 1967). They were observed in ultrathin sections of plant phloem tissue and named MLOs because they physically resembled mycoplasmas. Later, these microorganisms were renamed phytoplasmas in 1994 at the 10th Congress of the International Organization of Mycoplasmology (1993). Phytoplasmas are wall-less pleomorphic bacteria with sizes ranging from 200 to 800 nm, which could survive and multiply only in plant phloem or insect haemolymph. They are strictly host-dependent, but could multiply in insect vectors and also survive in their eggs. Phylogenetic studies have shown that the common ancestor of phytoplasmas is *Acholeplasma laidlawii*. Also, phytoplasmas and acholeplasmas use the conventional UGG codon for tryptophan (Trp) and retain UGA as a stop codon, while most other mollicutes use UGA as a Trp codon. In infected plants, phytoplasmas live mainly or perhaps only, in the sieve cells of phloem tissue. The sieve cells are highly specialized cells that are living but do not contain nuclei when they are mature. Infection of a plant by a phytoplasma often results in disease. Symptoms that are characteristic of phytoplasma diseases include yellowing and reduced size of leaves, stunting of the plant, and proliferation of axillary buds. Such growth from axillary buds often results in a witches' broom appearance. Other

Received: 25 June, 2011. Accepted: 22 February, 2012.

Invited Review

symptoms may be dieback (death) of terminal portions of branches on trees and other woody plants, poor growth of roots, and bunchy growth at ends of branches. Being mollicutes, phytoplasmas lack cell walls and instead are bound by a triple layered membrane. The cell membranes of all phytoplasmas studied so far usually contain a single immunodominant protein (of unknown function) that makes up the majority of the protein content of the cell membrane (Christensen *et al*. 2004).

The typical phytoplasma exhibits a pleiomorphic or filamentous shape and is < 1 μm in diameter. Like other prokaryotes, DNA is free in the cytoplasm. Phytoplasmas cannot be cultured *in vitro* and thus create a major drawback in their diagnosis and characterization. Due to the lack of molecular characterization of phytoplasmas in earlier days, their taxonomy was mainly based on symptoms, host-parasite interactions, host range and insect vector. Microscopic methods including transmission electron microscopy (TEM) and light microscopy have been used to detect phytoplasmas, but most sensitive is the DAPI (DNA-specific-6-diaminido-2-phenylindole) fluorescence microscopy technique (Deeley *et al*. 1979). However, these techniques require tissue fixation. Recently bio-imaging methods requiring sensitive, specific and non-toxic fluorescent dyes and the use of confocal or multiphoton microscopy have allowed identification of phytoplasmas in living tissues (Christensen *et al*. 2004). The pathogen identification relied for more than two decades on DAPI staining or electron microscopy detection. However, in the last 20 years, the applications of DNA-based technology allowed to distinguish different molecular clusters inside these prokaryotes. The Phytoplasma Working Team of the International Research Project for Comparative Mycoplasmology (IRPCM) adopted the trivial name 'phytoplasma' to identify the prokaryotes belonging to this group. The '*Candidatus* Phytoplasma' genus has been proposed and adopted in order to start formal classification of these prokaryotes. Up to date satisfaction of Koch's postulates has not been achieved, but indirect proofs, such as phytoplasma and symptoms elimination after tetracycline treatments as well as pathological investigations based on graft and insect vector transmissions, confirmed that they are the causal agents of many yellows and decline plant diseases worldwide.

The genomes of four phytoplasmas have been fully sequenced. These include *Ca.* Phytoplasma asteris, strains Aster Yellows Witches Broom and Onion Yellows, *Ca.* Phytoplasma australiense and *Ca*. Phytoplasma mali (Oshima *et al*. 2004; Bai *et al*. 2006; Tran-Nguyen *et al*. 2008; Kube *et al*. 2008, 2011). Phytoplasmas have very small genomes estimated to range from 530 Kb to 1350 Kb with a low G+C content between 24 and 33 mol% which is thought to be the threshold for a viable genome. The value of 530 Kb which is known to occur in the Bermuda grass white leaf phytoplasma '*Candidatus* Phytoplasma cynodontis' represents the smallest known genomes of any living organism. The small genome size associated with phytoplasmas is the result of reductive evolution from *Bacillus/Clostridium* ancestors. Phytoplasmas have lost 75% or more of their original genes which is why they can no longer survive outside of insects or plant phloem. Some phytoplasmas contain extrachromosomal DNAs such as plasmids and many phytoplasmas contain two rRNA operons.

Phytoplasma genomes contain large numbers of transposon and insertion sequences. They also contain a unique family of repetitive extragenic palindromes (REPs) called PhREPS whose role is unknown, though it is theorised that the stem loop structures play a role in transcription termination or genome stability. Before molecular techniques were developed, the diagnosis of phytoplasma diseases was difficult because they could not be cultured. Thus classical diagnostic techniques, such as observation of symptoms, were used. Molecular diagnostic techniques for the detection of phytoplasma began to emerge in the 1980s and included ELISA-based methods. In the early 1990s, PCR-based methods were developed that were far more sensitive than those that used ELISA, and RFLP analysis allowed the accurate identification of different strains and species of phytoplasma. More recently, techniques have been developed that allow assessment of the level of infection. Both qPCR and bioimaging have been shown to be effective methods of quantifying the titre of phytoplasmas within the plant (Christensen *et al*. 2004).

A consensus for naming novel phytoplasmas was recommended by the IRPCM Phytoplasma/Spiroplasma Working Team-Phytoplasma Taxonomy Group that a '*Candidatus* Phytoplasma' species description should refer to a single, unique 16S rRNA gene sequence that has <97.5% similarity to that of any previously described '*Ca*. Phytoplasma' species. However, because of the highly conserved nature of the 16S rDNA and of the not uncommon presence of 16S rDNA interoperon sequence heterogeneity, the classification based on '*Candidatus*' or on 16S ribosomal group does not always provide the molecular distinction necessary for phytoplasma strain characterization. Moreover, some additional tools for phylogenetic analyses and finer strain differentiation of phytoplasmas such as rp, secY, tuf, groEL genes, and the 16S-23S rRNA intergenic spacer region sequences have been used as supplementary tools selecting those providing the most useful and reliable taxonomic information in combination with 16SrDNA .The nucleic acid techniques based on polymerase chain reaction (PCR) procedures developed in the last 20 years are now used routinely and are adequate for detecting phytoplasmas. RFLP analyses of 16S rDNA nested PCR products from 34 representative phytoplasma strains with 17 restriction enzymes was used by Lee *et al*. in 1998 to differentiate various phytoplasmas by their distinct RFLP patterns. Based on similarity coefficients derived from RFLP analyses, the 34 phytoplasma strains were differentiated into 14 major groups (termed 16Sr groups) and 32 sub-groups. The phytoplasma 16Sr groups has been shown to be consistent with the phylogenetic groups (clades) defined by phylogenetic analysis of near-full-length 16S rRNA gene sequences, indicating that the RFLP-based groups are phylogenetically valid. The approach using RFLP analyses of PCR amplified 16S rDNA provides a simple, reliable and rapid means for differentiation and identification of known phytoplasmas (Duduk and Bertaccini 2011).

## PHYTOPLASMAS INFECTING SUGARCANE

Phytoplasmas infecting sugarcane (*Saccharum* spp. hybrids) are reported to be associated with several diseases including sugarcane grassy shoot (SCGS), sugarcane white leaf (SCWL), sugarcane green grassy shoot (SCGGS), sugarcane leaf yellows and Ramu stunt. These diseases cause more or less similar symptoms but differ in the identity of the associated phytoplasmas, vector relationship and geographic distribution.

### Grassy shoot

SCGS is one of the most important diseases of sugarcane in Indian subcontinent. It was first observed near Belapur in the Ahmadnagar district of Bombay (India) in 1949 (Chona 1958). Similar disease was also reported from other parts of the country and described under different names such as "new chlorotic disease", "yellowing disease", "albino disease", "bunchy disease" or "leafy tuft" (Marcone *et al.* 2001). Studies by Rane and Dakshindas (1962) showed that grassy shoot, yellowing and albino symptoms are associated with the same disease and subsequently the term "grassy shoot" was accepted as common name. SCGS has been recorded in most sugarcane-growing areas of India and is known to occur also in Thailand (Wongkaew *et al.* 1997; Sdoodee *et al.* 1999; Sdoodee 2001). Symptoms similar to those of SCGS have been observed in Bangladesh, Malaysia, Nepal, Pakistan, Sri Lanka and Sudan (Rishi and Chen 1989; Viswanathan 1997, 2001; Rao *et al*. 2003; Ariyaratana *et al*. 2007).

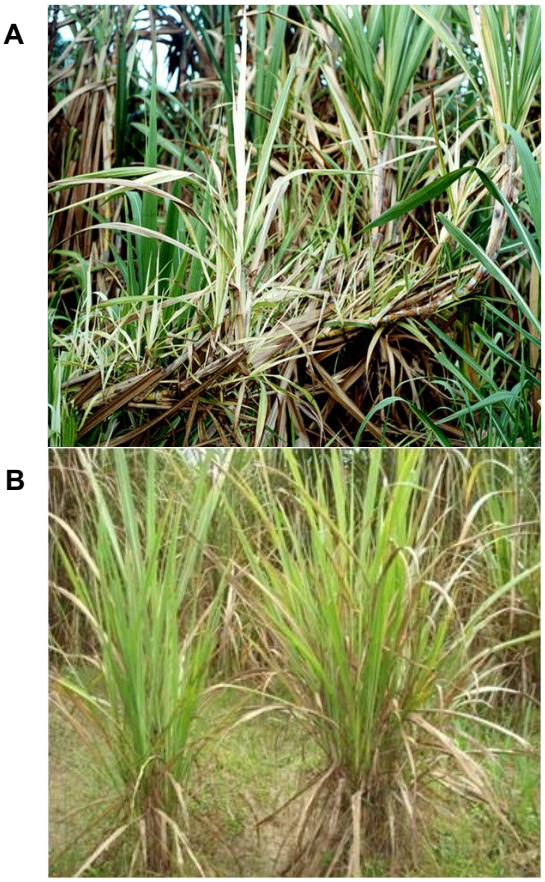


**Fig. 1** (**A**) Symptoms of SCGS on sugarcane variety CoS 91269 showing bud sprouting on standing stalks with narrow and chlorotic leaves with soft texture (**B**) variety CoS 8432 at Ucchani, Karnal, Haryana showing many short tillers with no stalk formation, no chlorosis was observed in infected clump.

SCGS disease is characterized by the production of a large number of thin, slender, adventitious tillers from the base of the affected stools. This profuse growth gives rise to a dense or crowded bunch of tillers bearing pale yellow or chlorotic leaves which remain thin, narrow, reduced in size and have a soft texture (**Fig. 1**). Each stalk that is produced from the affected stool shows shortened internodes and the development of side shoots from the bottom to the top. Affected plants do not produce millable canes. The disease is particularly pronounced in the ratoon crop where the clusters of slender tillers with reduced leaves usually growing erect give the appearance of a field full of perennial grass, and from which it has derived its popular name "grassy shoots".

One leaf hopper species, *Deltocephalus vulgaris*, has been found to transmit the SCGS phytoplasma successfully to sugarcane (Singh *et al.* 2002). This was further confirmed by nested PCR analysis of infected leafhopper, *D. vulgaris*, in India (Srivastava *et al*. 2006).

The SCGS-phytoplasma was detected in sugarcane by ELISA and immunofluorecence (Viswanathan 1997, 2000). Universal phytoplasma-specific primer pairs P1 and P7 were used for nested PCR assays that successfully detect the SCGS phytoplasma in sugarcane and its reported leafhopper vector *Deltocephalus vulgaris* (Viswanathan *et al*. 2005; Srivastava *et al.* 2006). Since the diseased plants exhibit various phenotypic symptoms under field conditions, diagnosis of the disease becomes difficult. Hence, a rapid method for enrichment and isolation of SCGS phytoplasma from the infected plants was developed. Differential filtration approach was used to isolate and enrich the SCGS phytoplasma and its genomic DNA that was detected by PCR analysis for phytoplasmal 16S rDNA. Ratio of pathogen to host plant DNA was found in the order of $10^3$ and $10^5$ in infected tissue and enriched fraction respectively, offering 148-fold increase in sensitivity for their detection (Prabhu *et al.* 2008).

Nucleotide sequence analysis of 16S rRNA genes revealed that SCGS phytoplasma affecting sugarcane crops in India is very closely related to the SCWL agent and is, thus, a member of the RYD phytoplasma group. SCGS and SCWL phytoplasmas shared a 16S rDNA sequence similarity which varied from of 97.5 to 98.8%. Of the phytoplasmas that cluster in other phylogenetic groups, those most closely related to SCGS phytoplasma are the BGWL (= '*Candidatus* Phytoplasma cynodontis') and brachiaria grass white leaf (BraWL) agents, which share 97.3 and 97.1% 16S rDNA sequence similarity, respectively (Rao *et al.* 2008). More distantly related to SCGS agent, are the sorghum grassy shoot (SGS), RYD (= '*Candidatus* Phytoplasma oryzae') and leafhopper (*Psammotettix caphalothes*)-borne (BVK) phytoplasmas sharing 16S rDNA sequence similarity values of 96.4-97.8, 95-96.1 and 95-96%, respectively. These phytoplasmas are members of the RYD phytoplasma group as well (Jung *et al*. 2003; Marcone *et al*. 2004). By showing divergences of 5-5.7 and 4-5%, respectively, cirsium phyllody (CirP) and galactia little leaf (GaLL) phytoplasmas are most distantly related to the SCGS agent in the RYD group (**Fig. 2**). Phytoplasmas from the remaining phylogenetic groups differ from the SCGS agent in more than 6% of 16S rDNA positions (Lee *et al*. 1998; Seemuller *et al.* 1998; Schneider *et al*. 1999; Lee *et al*. 2000; Tran-Nguyen *et al.* 2000; Blanchi *et al*. 2003; Jung *et al.* 2003; Marcone *et al.* 2004).

Nasare *et al.* (2007) analysed 198 sugarcane plant samples exhibiting grassy shoot symptoms in India tested positive for phytoplasma through PCR amplification of 16S rRNA gene and 16S-23S rRNA SR using primers specific for phytoplasmas and concluded that the sequence homology in the present SCGS-causing phytoplasma in India is more than 99%, and their homology with SCWL and BGWL is from 98 to 99%. Therefore, it can be concluded that SCGS, SCWL, SGS, and BGWL belong to the same species-level taxa (Rao *et al*. 2008). In another study, the phylogenetic relationships of SCGS phytoplasma strains to each other and to the most related phytoplasmas were examined, by sequencing both 16S rRNA gene and 16S/23S rRNA spacer region. The phylogenetic relationships of SCGS phytoplasma isolates among themselves and related phytoplasmas based on 16S rRNA gene sequences showed 100% identity. No variation among 16S rRNA gene sequences of all the 18 SGGS isolates of India were observed. Although there were significant variations in phenotypic expression of SCGS phytoplasmas on sugarcane, no genotypic variations could be established (Viswanathan *et al.* 2011).

Further studies are needed to locate other genes in phytoplasma genome which determine variations in phenotypic expression of the disease, such information is totally lacking and further characterization of SCGS-phytoplasma need to be done based on their pathogenecity genes.

## White leaf

SCWL is one of the most destructive sugarcane diseases in Thailand. It was first observed in 1954 in the Lumpang province in the northern part of Thailand (Mangelsdorf 1962). Only four years later, SCWL was discovered in Taiwan (Ling 1962). In Thailand, the disease subsequently spread to all important sugarcane-growing areas in the north, northeast and east, resulting in one of the most lethal disease of sugarcane. Currently, it seems to be present in all areas where the crop is grown (Rishi and Chen 1989; Sarindu and Clark 1993; Nakashima *et al.* 1994, 1996; Wongkaew *et al.* 1997; Rao and Ford 2001). In Taiwan, SCWL spread from the Pingtung district into all sugarcane-growing areas except Chifu, Taichung and Yaemei districts. However, due to intensive control campaigns, the disease occurs sporadically only in areas such as Pingtung, Chishan, Yuching and Wushulin districts (Rishi and Chen 1989). SCWL was also recorded in 1986 in Japan, in the Tanega-

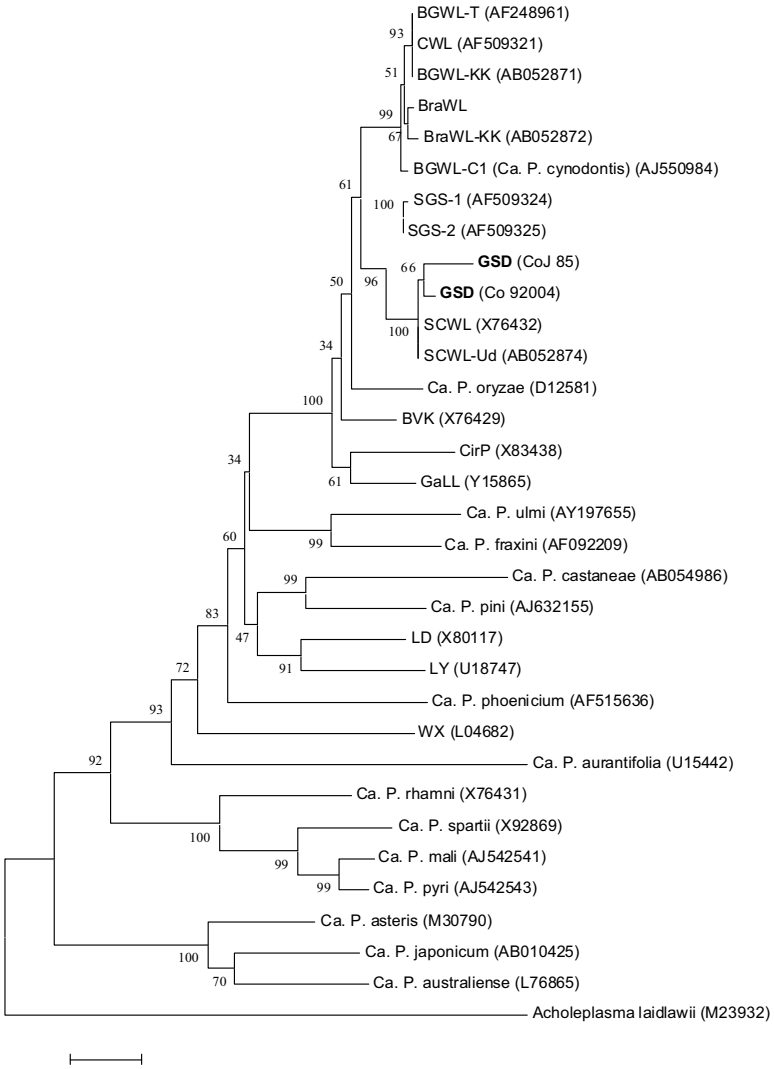


**Fig. 2** Position of the sugarcane grassy (SCGS) phytoplasma in a phylogenetic tree of 16S rDNA sequences, constructed by using the neighbor-joining method. Bar represents a phylogenetic distance of 1%. *Acholeplasma laidlawii* was used as the outgroup. GenBank accession numbers and bootstrap values are shown in parentheses and on branches, respectively. Other abbreviations are as follows: *Ca.* P. ulmi, '*Candidatus* Phytoplasma ulmi'; *Ca.* P. fraxini, '*Candidatus* Phytoplasma fraxini'; *Ca.* P. castaneae, '*Candidatus* Phytoplasma castaneae'; *Ca.* P. pini, '*Candidatus* Phytoplasma pini'; LD, coconut lethal disease; LY, coconut lethal yellowing; *Ca.* P. phoenicium, '*Candidatus* Phytoplasma phoenicium'; WX, western X-disease; *Ca.* P. aurantifolia, '*Candidatus* Phytoplasma aurantifolia'; *Ca.* P. rhamni, '*Candidatus* Phytoplasma rhamni'; *Ca.* P. spartii, '*Candidatus* Phytoplasma spartii'; *Ca.* P. mali, '*Candidatus* Phytoplasma mali'; *Ca.* P. pyri, '*Candidatus* Phytoplasma pyri'; *Ca.* P. asteris, '*Candidatus* Phytoplasma asteris'; *Ca.* P. japonicum, '*Candidatus* Phytoplasma japonicum'; *Ca.* P. australiense, '*Candidatus* Phytoplasma australiense'.

shima Island, but later disappeared (Nakashima and Murata 1993; Nakashima *et al.* 2001).

The most characteristic symptoms of SCWL are the presence of leaves with total chlorosis, proliferating tillers and pronounced stunting. The leaves are narrower and smaller than those of healthy plants, with a soft texture and borne on slender chlorotic shoots. Initially, a single white or cream line parallel to the midrib occurs, which can be seen from either side of the blade. Later, several straight white to light green to yellow stripes develops parallel to the midrib develop extending along the entire leaf length, but rarely onto the upper portion of the leaf sheath. The stripe wide ranges from narrow pin stripe to as broad as the leaf. A mottled pattern of normal or light green dot, spot, streak or patch islands may develop on a white background, varying in size and shape. If there are many green islands, the leaves will look green. As the disease develops, the plant vigour decreases. Others symptoms are stunted stalks, absence of side shoots on the upper part of infected stalks and abnormal tillering (**Fig. 3**).

Severely diseased plants do not produce millable canes. SCWL is naturally transmitted by the leafhopper *Matsumuratettix hiroglyphicus* Matsumura (Matsumoto *et al.* 1968). The minimum acquisition and inoculation feeding

**Fig. 3** Total chlorosis of sugarcane leaves caused by white leaf disease of sugarcane.

periods are 3 h and 30 min, respectively (Chen 1978). The incubation period of SCWL phytoplasma in the insect vector is 25-35 days while in the host plant is 70-90 days (Matsumoto *et al.* 1968). Transovarial transmission of SCWL phytoplasma is also reported (Hanbonsoong *et al.* 2002). Lee and Chen (1972) reported the optimum temperature for vector transmission at 25°C. According to the studies of Chen (1978), female adults seem to be more efficient than the males in transmission of SCWL disease. *M. hiroglyphicus* is widely distributed in central and southern parts of Taiwan and in Thailand. Sugarcane and *S. spontaneum* L. (wild cane) are the preferred hosts. In sugarcane fields, the vector population is particularly abundant from July to October. The population declines rapidly in December and remains low until April. Six generations may occur in a year, with overlapping between generations (Yang and Pan 1979). Disease incidence is correlated with the population trend of the vector in the field. Cuttings planted from July to October are more severely affected than those planted from December to March (Lee 1970). The females of *M. hiroglyphicus* usually lay their eggs in the soil to a depth of about 0.5 cm, but sometimes eggs are laid in the leaf sheath near the ground. Sandy soils are preferred for oviposition and this may be one of the reasons why the disease is often more severe on sandy soils (Rishi and Chen 1989).

The causal agent, the SCWL phytoplasma, is a member of the phylogenetic SCWL group, which includes other important phytoplasmas infecting plants of the Poaceae family, such as rice yellow dwarf (RYD), sugarcane grassy shoot (SCGS) and sorghum grassy shoot (SGS) phytoplasmas, as well as the strain BVK obtained from the leafhopper *Psammotettix cephalotes* in Germany (Wongkaew *et al.* 1997; Lee *et al.* 1998, 2000; Seemuller *et al.* 1998; Schneider *et al.* 1999; Marcone *et al.* 2001; Rao *et al.* 2008). The SCWL agent shows a 16S rDNA sequence similarity of 97.7 and 97.3% to RYD and BVK phytoplasmas, respectively. It is lacking the *Taq*I site following position 228 of the 16S rRNA gene, which is present in BVK and RYD phytoplasmas. Sequence analysis of a less conserved sequence, the region between the 16S and 23S rRNA genes (16S/23S rDNA spacer) of SCWL, RYD and BVK, resulted in a classification scheme similar to that based on full-length or nearly full-length 16S rDNA sequences. The SCWL phytoplasma was assigned to the same subclade of BVK and RYD agents (Kirkpatrick *et al.* 1994; Schneider *et al.* 1995). SCWL agent can be differentiated from BVK, RYD, SCGS and SGS phytoplasmas using RFLP analysis of PCR-amplified rDNA with *Alu*I, *Sau*3AI, *Hae*III, *Mse*I, *Taq*I and *Hin*fI restriction endonucleases (Nakashima *et al.* 1996; Lee *et al.* 1998; Tran-Nguyen *et al.* 2000; Marcone *et al.* 2001).

Disease incidence is correlated with planting date of cuttings and type of soil. In Taiwan, cuttings planted from July to October, when the vector is in abundance, are more severely affected than those planted from December to April when the vector population is low (Lee 1970). The vector has a preference for sandy soils for oviposition, which explains the severity of the disease in such soils. Weed grasses have been suggested to be a reservoir of SCWL. However, sequence analysis of SCWL phytoplasma 16S rDNA amplified from three different white leaf infected gramineous weeds as well as from sugar cane showed that each weed was infected by a different phytoplasma, which was also different from that of sugar cane. Transmission of SCWL phytoplasma by leafhoppers from infected sugar cane plants to weeds and from infected weeds to healthy sugar cane was not successful (Nakashima *et al*. 1994) suggesting the specificity of the phytoplasma to its host plant. Therefore, in order to determine the SCWL reservoir, more plant species need to be screened to identify the possible hosts of this phytoplasma, from which the insect vectors transmit the disease to sugar cane. The fact that SCWL occurs mainly in Asia and not in other sugar cane growing countries in the world, quarantine barriers should be reinforced to prevent its spread and restrict its movement to other areas.

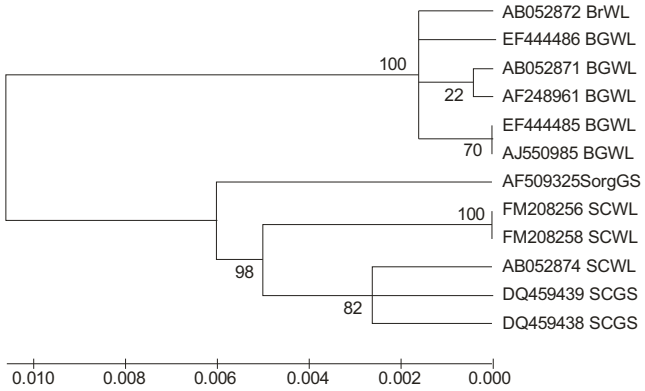


**Fig. 4** Phylogenetic tree constructed by using MEGA 4.0 showed relationship among different SCWL phytoplasmas and other closely related phytoplasmas.

SCWL can cause severe yield losses, particularly when the planting material is obtained from the infected sources, or when the disease transmission occurs during the early stages of the plant crop growth. Up to 100% incidence of SCWL has been recorded resulting in 100% yield loss. Serious recent epidemics of SCWL have been recorded from Udorn Thoni in 2000 and Burirum Districts in 2002 (Kusalwong *et al.* 2002).

With PCR assays, phytoplasma DNAs were detected in SCWL and SCGS diseased plants collected from Thailand (Nakashima *et al*. 1996; Wongkaew *et al*. 1997; Sdoodee *et al*. 1999; Hanbonsoong *et al.* 2002, 2006). Phytoplasmas DNA was also detected in insect vectors *M. hiroglyphicus* and *Yamatotettix flavovittatus* collected from sugarcane fields infected with SCWL in Thailand (Nakashima *et al*. 1994; Hanbonsoong *et al*. 2006).

Phylogenetic tree constructed by using sequences of WLD isolates and related sequences available in GenBank concluded that SCWL phytoplasma showed close association with SCGS phytoplasmas (DQ459439 and DQ459438) and with isolates of SGS phytoplasma (AF509325). However, *Brachiaria* grass white leaf phytoplasmas (BGWL) (AF248961; AJ550985; EF444486) also grouped closely to SCWL (**Fig. 4**). Thus it revealed that SCWL phytoplasma is very closely related to the SCGS agent and is, thus, a member of the rice yellow dwarf (RYD) phytoplasma group.

SCWL phytoplasma is phylogenetically related to other phytoplasmas in grasses such as RYD and SGS as well as to BVK strain obtained from leafhopper *Psammotettix cephalotes* (Seemüller *et al*. 1994). Despite this close relation, SCWL phytoplasma is distinct from RYD phytoplasma. RFLP analysis of PCR product of the 16S rDNA of SCWL phytoplasma and RYD phytoplasma with 15 different endonucleases showed similar pattern for both, but upon digestion of the same PCR product with *Taq*I and *Mse*I different profiles were observed (Lee *et al*. 1998). The 16S rDNA sequence analysis of SCWL phytoplasma showed 97.7 and 97.3% sequence similarity to RYD and BVK, respectively (Schneider *et al*. 1995; Lee *et al*. 1998, 2000; Rao *et al* 2008). The DNA-based detection methods of SCWL phytoplasmas and information about the genetic/phylogenetic characteristics of SCWL and other phytoplasmas may contribute to the promotion of research on the epidemiology and plant-microbe interaction of agent, and eventually to the development of methods of control of the SCWL disease.

Using nested-PCR with specific primers, a 210-bp amplified DNA fragment corresponding to phytoplasma associated with SCWL was detected from 12 species of leafhoppers *Balclutha rubrostriata* (Melichar), *Balclutha* sp., *Bhatia olivacea* (Melichar), *Exitianus indicus* Distant, *Macrosteles striifrons* Anufriew, *Matsumuratettix hiroglyphicus* (Matsumura), *Recilia distincta* (Motschulsky), *Recilia dorsalis* (Motschulsky), *Recilia* sp., *Thaia oryzivora* Ghauri, *Yamatotettix flavovittatus* Matsumura, and *Xesto-*

*cephalus* sp. in northeastern Thailand. The percentage of individual infection with phytoplasma varied from 5% in *B. olivacea* to 35% in *Xestocephalus* sp. The most abundant leafhopper species, i.e., *E. indicus*, *Y. flavovittatus*, and *M. hiroglyphicus* were used in transmission tests to determine their vector status for the SCWL transmission. Infected insects were reared on healthy plants and specific PCR followed by sequencing of the amplicons was used to determine whether the phytoplasma was transmitted to the plants. The results showed that both *Y. flavovittatus* and *M. hiroglyphicus*, but not *E. indicus*, can transmit sugarcane white leaf phytoplasma to healthy sugarcane plants. The transmission efficiency of *M. hiroglyphicus* (55%) was higher than that of *Y. flavovittatus* (45%). *Y. flavovittatus* was a newly discovered vector for SCWL, in addition to *M. hiroglyphicus* (Hanbonsoong *et al*. 2006). These two species peak at different times of the year and therefore complement each other in the transmission of the phytoplasma. Because there are no natural known alternative host plants for the SCWL, management of the disease will necessarily require the control of both *Y. flavovittatus* and *M. hiroglyphicus* (Hanboonsong *et al.* 2006; Kaewmanee and Hanboonsong 2011).

Manimekalai *et al.* (2010) found that phytoplasma associated with areca nut palm (*Areca catechu* L.) reduces the yield as much as 50% in South India. This phytoplasma has 99% nt identity with sugarcane white leaf phytoplasma FM208260, 16sr XI), coconut RWD phytoplasma (GQ850122, 16Sr XI) and 98% nt identity with bermuda grass white leaf phytoplasma (AJ550986, 16Sr XIV). The phylogenetic analysis revealed the clustering of YLD phytoplasma of areca palms with 16S XI and 16S XIV groups. However, the YLD phytoplasma is closely related to the 16S XI group. PhytoDB--group identifier tool (http://220.227.88.253/phytodb) showed YLD phytoplasma from India belongs to the 16Sr XI group. This indicates a very close relationships of areca palm phytoplasma with SCWLD and other related Bermuda grass phytoplasma and impose a threat for further which always indicate a possibility of transfer and harbor the of phytoplasma in these alternative and collateral hosts.

Use of resistant clones to control SCWL disease is limited due to the lack of varieties combining high yield with disease resistance. Planting disease free cutting, rouging of diseased plants and the prohibition of ratooning in infected fields are, therefore, recommended to control the disease. In Thailand, the disease is now under control in infected areas by the routine use of healthy nurseries, hot water treatment of cutting for 2 h at 50°C, micropropagation of disease-free plantlets, strict quarantine regulations and various soil amendments (Chen and Kusalwong 2000; Kaewmanee and Hanboonsong 2011).

## Leaf yellows

YLS was first reported in 1969, since then has been reported in most of sugarcane growing countries causing losses of more than 50% of susceptible varieties. The aetiology of the disease has been controversial; however, phytoplasmas have been consistently associated with YLS in Cuba, South Africa and Mauritius. Latent infections commonly occur in YLS affected cane plants, so it is difficult the diagnosis of the disease. Now, the disease has been reported to occur in USA, Australia, Brazil, Cuba and several African countries including South Africa and Mauritius (for reviews see Tran-Nguyen *et al.* 2000; Schenck 2001). In Australia and Brazil, the disease was most evident during cooler months (for review see Schenck 2001). A virus, member of the luteovirus group, has been found to be associated with the disease in many sugarcane-growing areas worldwide (Schenck 2001). However, during the last few years, phytoplasmas were detected in SCYLS affected sugarcane plants in several countries using electron microscope observations and PCR-based methods. In some cases, the affected plants were doubly infected with both viruses and phytoplasmas and latent infections have also been observed (Guar *et al.* 2008). Stress conditions seem to exacerbate the symptom expression incited by viral and phytoplasmal infections (Cronje *et al.* 1998). Symptoms consist of yellowing of leaves with a bright yellow midrib, often when the rest of the lamina is still green. Pink colouration may also occur as well as early drying of leaves from the edges (Gaur 2003) (**Fig. 5**). Guerra and Cano (2005) identified and detected YLS phytoplasma with DAPI (4-6 di-amino 2-phenylindol) staining (which is based on the binding of DAPI to the phytoplasma DNA, forming a fluorescent complex in the phloem cells) with °Brix values from 15-18 in infected sugarcane varieties. No fluorescence was observed in those plants without symptoms and °Brix values < 15. These results have shown DAPI staining to be an excellent technique to complement field diagnosis of YLS based on °Brix values. This technique is especially useful for the early detection of infection in apparently healthy plants.

**Fig. 5** (**A**) Yellowing of sugarcane leaves with prominent mid-rib yellowing. (**B**) Parallel yellow and chlorotic strips with pinkish discolouration of midrib of sugarcane.

Later studies employing sequence analysis of 16S/23S rDNA spacer region and RFLP analysis of PCR amplified 16S rDNA sequences revealed that two different phytoplasmas are associated with SCYLS in nine African countries, although the plants were symptomatically similar (Cronje *et al.* 1998, 1999; Aljanabi *et al.* 2001). The prevalent agent is a member of the X-disease group which showed a sequence similarity of 98.8% with the western X-disease phytoplasma. Detection and molecular characterization of phytoplasmas from SCYLS-diseased sugarcane plants have also been reported from Cuba (Arocha *et al.* 1999). In this case, an organism of the AY group, subgroup 16SrI-A, was identified on the basis sequence analysis of 16S/23S rDNA spacer region and RFLP analysis of PCR-amplified 16S rDNA sequences using *Alu*I, *Rsa*I and *Hae*III. In Australia, a great genetic diversity among phytoplasmas associated with YLS disease has been evidenced by RFLP and sequence analyses of PCR-amplified rDNA (Tran-Nguyen *et al.* 2000).

Peralta *et al.* (1999) observed histopathological alterations in SCYLS sugarcane leaves such as: chloroplast disorganization, starch accumulation and increasing number of mitochondria; and biochemical alterations like decreasing of amylase activity, alterations of juice quality and increasing of invertase activity. Higher levels of sucrose (Peralta *et al.* 1999; Arocha 2000) have been also found, which may be influenced by a proportional increase in the amount of non sugar carbohydrates that accumulate in high concentrations after the maturing and harvest optimal. These non sugar carbohydrates are responsible of the abnormal growth of the sucrose crystal so it becomes more fragile and may be lost and broken during the industrial centrifugation, affecting the subsequent juice quality (Arocha 2000). In Cuba, SCYLS agent is present in cane plants of all ages with the highest incidence (61%) in plants aged from 13-22 months, 19% in those from 1-6 months, and 13% in 3 month old plants (Arocha 2000). These results suggest that symptoms are not directly related to the age of the planting but probably depend on other factors such as seed health,

varietal susceptibility or the influence of the environment. These interactions require further study in the future. Symptomless phytoplasma infections in sugarcane occur widely, and the relatively long growth period of this crop allows infection to be carried through seasonal barriers and crop cycles.

The SCYLS infection reduced sugar yields by 38% (Peralta *et al.* 1999; Arocha 2000). SCYLS was first attributed to the effects of abiotic factors such as nutrient imbalances, water logging, environmental factors, cold stress, low soil fertility, or restricted root growth due to soil compaction (Scagliusi and Lockhart 2000; Schenck and Lehrer 2000). SCYLS incidence in different commercial cultivars in India were also reported which are responsible for reduction in sugarcane production and sugar recovery in India (Rao *et al.* 2000; Viswanathan 2002, 2004; Gaur *et al*. 2008).

Fontaniella *et al.* (2003) observed that YLS infection alters the contents and composition of polysaccharides, phenols and polyamines content in infected sugarcane (cv. 'Cuba 120-78') juice in Cuba. YLS was associated to an increase of the concentration of reducing sugars, glucose index, and glycoproteins recovered in juices whereas the amount of sucrose decreases. Sugarcane juices obtained from both healthy and YLS-affected Cuba 120-78 cultivars of sugarcane contained putrescine (PUT), cadaverine (CAD), spermidine and spermine (SPM) as free and macromolecules-conjugated compounds. Only CAD and SPM appeared as acid-soluble conjugates to small molecules whereas PUT and CAD are the major polyamines (PAs) conjugated to macromolecules, mainly to high molecular mass glycoproteins. The disease was associated to an increase in total PA fraction. Arginase and ornithine decarboxylase activities, responsible for the synthesis of PUT, were higher in YLS juices than in those obtained from healthy plants. CAD and SPM presumably conjugated mostly to chlorogenic, syringic and ferulic acids in juices from YLS plants.

Many methods have been developed for the generic and specific diagnosis of phytoplasmas causing SCYLS (Arocha *et al.* 2004a, 2004b) and PBT-like diseases, either based on nested PCR or NAH assays using radioactive or non radioactive alternatives. Both nested PCR and NAH assays have been validated which increases their reliability and feasibility for the diagnosis of phytoplasma associated with SCYLS (Arocha *et al.* 2004a, 2004b; Rott *et al*. 2008). NAH assay has been established for the generic diagnosis of phytoplasmas in Cuba. NAH assays have a number of advantages over PCR since they are easier to manipulate, more practical for analysing a high number of samples, and have the capacity to reduce the number of false positives (Tymon *et al.* 1998; Arocha *et al.* 2004a). Non-radioactive methods have sensitivity similar to radioactive ones (Harrison *et al.* 1994; Kirkpatrick and Smart 1995; Arocha *et al.* 2004a) and are safer for human health. Arocha *et al*. (2005) confirmed the vector status of the delphacid planthopper, *Saccharosydne saccharivora*, associated with SCYLS phytoplasma in Cuba.

A new strain of SCYLS agent belonging to the Western X group was shown to be present in Mauritius. This group previously described in sugarcane in South Africa was now identified in sugarcane as well as in *Sorghum verticilliflorum* and *Perkinsiella. saccharicida* in Mauritius. This adds to the diversity of strains of SCYLS phytoplasma previously detected in Mauritius. The presence of a closely related phytoplasma in the leafhopper *P. saccharicida* indicates the possible involvement of the Delphacid in the transmission of SCYLS phytoplasma and hence transmission studies are being carried out to establish its involvement in the spread of leaf yellows (Joomun *et al*. 2007).

## Green grassy shoot disease

Sugarcane green grassy shoot (SCGGS) is a newly discovered phytoplasmal disease of sugarcane. It has been observed in Thailand (Pliansinchai and Prammanee 2000). The symptoms are very similar to those of SCGS disease. However, in SCGGS-affected sugarcane plants the leaves do not become chlorotic. The result from PCR detection showed that SCGGS agent is genetically partly related to a phytoplasma infecting periwinkle and the SCWL phytoplasma (Pliansinchai and Prammanee 2000). Further study on the DNA sequence is required to characterize the phytoplasma associated with SCGGS disease of sugarcane.

The disease could be transmitted through the seed cane. The percentage of transmission was recorded up to 15-100% (Pliansinchai and Suchato 1995). Highest percentage of infection was obtained when basal stalk of the affacetd cane was planted (Pliansinchai *et al.* 1998). The disease was not transmitted mechanically (Pliansinchai and Suchato 1995). Sett transmission plays a major role in the disease spread. The insect vector involved in its transmission has not yet been identified.

## Ramu stunt

Ramu stunt was first observed in the late 1980s in the Ramu valley of Papua New Guinea where an outbreak of the disease caused devastating crop losses in commercial sugar cane varieties (Eastwood 1990). The disease is known only from mainland Papua New Guinea (Suma and Jones 2000; Magarey *et al.* 2002).

Varieties planted in commercial fields reacted differently to the disease at the time of the outbreak. Cultivar 'Ragnar' was the most susceptible, while 'Q 90' and 'Yasawa' were slightly susceptible and cultivar 'Q 107' highly resistant. In Papua New Guinea, Ramu stunt is a major disease of sugarcane. It caused up to 40% loss in total sugarcane production (Suma and Jones 2000). This disease is a major quarantine threat particularly to the neighbouring sugarcane industries in Australia and Indonesia. Ramu stunt is a very severe, rapidly spreading, systemic disease with a range of symptoms. The rapid spread is due to the air-borne insect vector, *Eumetopina flavipes* (Kuniata *et al*. 1994). The most striking effect of Ramu stunt is its ability to suddenly and rapidly reduce growth, seen as a shortening of internodes, hence the stunting. Typical symptoms start as short irregular streaks or flecks, pale to creamy green in colour and may resemble a mosaic. As symptoms develop, the streaks become yellowish-green in colour, continuous or interrupted and interspersed by apparently healthy green tissue. Streaks can vary from several mm in length to run the full length of the leaf blade and range in width from 2-5 mm or more. Leaves are short, stiff and erect and become senescent prematurely.

Diseased canes are thinner than healthy canes. Stools are severely stunted and there is progressive death of stalks. Diseased stools ratoon poorly. In the field, infection in a susceptible variety can lead to total ratoon failure. Root systems are severely reduced and stunted. Older roots collapse and become necrotic (Braithwaite 2010).

Due to the rapid spread of the disease, it was believed to be caused by a virus until Cronjé *et al*. (1999) confirmed an association with a phytoplasma. Ramu stunt is transmitted mainly through the plantation of infected cuttings. It has been suggested that transmission of the disease is by the insect vector *E. flavipes* Muri. Phytoplasma was detected in Ramu disease-infected sugar cane as well in samples of the putative vector, *E. flavipes* (Kuniata *et al*. 1994; Cronjé *et al*. 1999). Such leafhopper is particularly abundant in commercial sugar cane as well as in wild canes in Papua New Guinea.

Ramu stunt phytoplasma sequence analysis of 16S and 23S rDNA spacer region as well as RFLP analysis of PCR amplified 16S rDNA sequences with *Rsa*I and *Hae*III showed that this phytoplasma shares 95.98% homology with white leaf disease phytoplasma. The disease was controlled by including only resistant varieties and excluding the susceptible ones in the commercial fields. The disease seems to be restricted to Papua New Guinea (Braithwaite 2010).

The use of resistant varieties is the only control measure known. The main commercial control used in PNG crops is the planting of resistant varieties and the destruction of infected crops. The selection of disease-free planting material is an important control measure. Excellent control of Ramu stunt has been achieved by cultivation of resistant varieties. Screening trials conducted at Ramu Agri-Industries, Gusap, PNG reveal that only 25% of Australian commercial varieties exhibit Ramu stunt symptoms (Braithwaite 2010).

The island sugarcane planthopper, *Eumetopina flavipes* Muir (Hemiptera: Delphacidae) is the only known vector for Ramu stunt disease of sugarcane. Fortunately, this disease does not occur in Australia, but virus-free populations of this planthopper occur in the Torres Strait Islands (TS) and northern peninsula area (NPA) of Queensland. In order to mitigate the risk of E. flavipes and/or Ramu stunt disease invasion into Australia through the Torres Strait, and to contribute to the development of an integrated management program for *E. flavipes* in commercial sugarcane plantations at Ramu Sugar Ltd. – where Ramu stunt disease continues to impact upon production – project scientists sought a detailed understanding of the population ecology of *E. flavipes* populations throughout PNG and TS/NPA. They undertook the project through surveys, control trials, establishment of quarantine guidelines and cane grower education. The project team investigated three major aspects of *E. flavipes* population ecology in PNG and TS/NPA: (1) the relative effect of host plant type and distribution on the population structure and demographics of *E. flavipes* in both PNG and the TS/NPA; (2) the large-scale dispersal potential of *E. flavipes* from PNG into the TS/NPA; (3) the reproductive potential and life history characteristics of *E. flavipes*. The final goal of the project was to communicate project recommendations to out-growers at Ramu and to the Australian sugar industry. *E. flavipes* populations are widespread on a range of *Saccharum* host species in PNG, and that despite differing host plant occupancy rates and abundances between host types, *E. flavipes* is able to effectively use each of them. *E. flavipes* is able to survive on cut sugarcane; therefore, anthropogenic long-distance dispersal is also feasible. *E. flavipes* populations would increase in size 24-fold/generation from one single reproductive female, under standard environmental conditions. The hopper can survive large fluctuations in daily temperature and deteriorating host plant conditions. Therefore, colonisation following dispersal may be quick and populations may rapidly reach a threshold density that would prompt dispersal, thereby increasing population connectivity. Relative levels of population connectivity are still being tested using microsatellite DNA markers. In PNG, due to the widespread distribution and persistence of *E. flavipes* across multiple wild and cultivated hosts, management effort should be more focused instead on dealing with Ramu stunt disease – possibly via the planting of new, resistance varieties and vigilant surveillance for new outbreaks of the disease (Magarey *et al.* 2008).

## ALTERNATIVE HOSTS OF SUGARCANE PHYTOPLASMAS

Several weeds are reservoirs of important phytoplasmas causing serious diseases to important commercial crops and play an important role in spreading phytoplasmas and serve as natural alternative hosts (Blanche *et al*. 2003; Rao *et al.* 2007; Mall *et al*. 2010, 2011). Phytoplasmas cause diseases in several weeds and resulted in serious threat as a source of alternative natural host for the spread of phytoplasma pathogens to other economically important plants and thereby chances of causing severe losses. Early detection of these phytoplasmas associated with diseases of weed crops is very important to check the possibility of further spread of phytoplasmas diseases to other commercial crops.

Different weed species have been reported as being phytoplasma hosts (Seemüller *et al*. 1998; Blanche *et al*. 2003; Marcone *et al*. 2004). There are concerns that insect vectors could transmit phytoplasmas from weed species, which could act as alternative hosts. In Australia, phytoplasmas related to SCWL disease were observed in weeds growing near sugarcane fields (Blanche *et al*. 2003). The presence of a very closely related strain of a sugarcane phytoplasma in a weed species is also cause for concern as weeds could act as reservoirs for the phytoplasmas. More thorough investigation of phytoplasmas in grasses is necessary to clarify the role of weeds in harbouring phytoplasmas (Joomun *et al*. 2007).

Of the phytoplasmas that cluster in other phylogenetic groups, those most closely related to SCGS phytoplasma are the BGWL (='*Candidatus* Phytoplasma cynodontis') and *Brachiaria* grass white leaf (BraWL) agents, which share 97.3 and 97.1% 16S rDNA sequence similarity, respectively. Phytoplasma association with Bermuda grass in India has been recently proved to be a strain of '*Candidatus* Phytoplasma cynodontis' on the basis of sequence analysis of the amplified 16S rDNA and 16S/23S rDNA spacer region sequences and identified as member of 16SrXIV group (Rao *et al*. 2007). This phytoplasma has also close relationships with the SCWL pathogen (Marcone and Rao 2008).

Manimekalai *et al*. (2010) found that phytoplasma associated with YLD affected areca nut palm has 99% nt identity with sugarcane white leaf phytoplasma FM208260, 16sr XI) and 98% nt identity with bermuda grass white leaf phytoplasma (AJ550986, 16Sr XIV). This indicates a close relationships of areca palm phytoplasma with SCWLD and Bermuda grass phytoplasma which always indicate a possibility of transfer and harbour the of phytoplasma in these alternative and collateral hosts.

Hence, the weeds and other plants identified positive for phytoplasmas in and around sugarcane fields may also serve as natural reservoir of phytoplasmas to sugarcane (Joomun *et al*. 2007). Thus there is maximum possibility of secondary spread of these phytoplasmas from weeds to sugarcane plant and vice-versa through potential leaf hopper vectors and may significantly affect sugarcane crop (Mall *et al*. 2007). The sequencing of these phytoplasma from sugarcane, weed and leafhopper would further confirm the taxonomy and thus explaining their phylogeny as closely related strains in sugarcane weed species as well as leafhopper which would explain the real cause of weeds and insects harbouring phytoplasmas in sugarcane fields and role of leafhopper in secondary spread of sugarcane phytoplasma in nature.

## MANAGEMENT APPROACH

The identification of phytoplasma diseases are mostly relied on the identification of symptoms but sometimes it is too difficult to identify the diseased plant because absence of peculiar symptoms in affected cane stalk. Hence in these cases, the identification and management of phytoplasma disease are very difficult. A number of sugarcane diseases including those caused by phytoplasma have in the past been disseminated from their country of origin to other geographical areas through exchange of germplasm. Although movement of germplasm has been beneficial and is desirable, the potential risk of introducing new diseases should be considered. In order to prevent the spread of sugarcane phytoplasmas, it is necessary for countries to reinforce their inspection and quarantine facilities by acquiring biotechnological and molecular diagnostic tools to be able to identify these diseases that could be harmful to their industry. All important clones should be systematically indexed to reduce any risks.

It has already proven that it is difficult to control phytoplasmas by thermotherapy and chemotherapy of cuttings alone or by chemical control of vectors. The use of resistant clones is of limited values. Now, with advancement of biotechnology and molecular biology methods, we can easily identify the phytoplasma in sugarcane at early stage and therefore successful prevention would be taken at early

stage to prevent the disease for further spread. Side by side it is very important to restrict the movement of phytoplasma outside South East Asia where most of the diseases like SCGS, SCWL and SCGGS are not reported through following quarantine regulations. No single approach can provide effective and long lasting management of sugarcane phytoplasmas. Judicious integration of phytoplasma free seed-cane, appropriate cultural practices and resistant clones can only provide ideal management of phytoplasma associated disease of sugarcane (Rao 2006).

The cultivation of resistant varieties in commercial fields has contributed in the control of Ramu stunt in Papua New Guinea (Suma and Pais 1996). For grassy shoot and white leaf diseases which are transmitted by cane setts, treatment of cuttings with moist hot air at 54°C for 4 h and hot water treatment at 54°C for 2 h are recommended, respectively (Friso and Putter 1993). Leaf yellows can successfully be eliminated by tissue culture technique. Parmesurr *et al.* (2002) reported the elimination of sugarcane yellows phytoplasma by regenerating plantlets from callus derived from young leaf rolls. As number of phytoplasmas has recently being identified in graminaceous weeds (Tran-Nguyen *et al.* 2000; Rao and Ford 2001; Joomun *et al.* 2007; Mall *et al.* 2011), their elimination from sugarcane field is essential as they could act as reservoir for phytoplasma diseases.

A novel approach based on cryotherapy was developed in for efficient elimination of phytoplasmas and production of pathogen-free plant stocks. Cryotherapy has been used for production of pathogen-free (phytoplasma and viruses ) plants of *Prunus* (Brison *et al*. 1997), banana (Helliot *et al*. 2002), grapevine (Wang *et al*. 2003) potato (Wang *et al*. 2006) and sweet potato (Wang and Valkonen 2008; Feng *et al*. 2011). Thus, cryotherapy provides an alternative method for elimination of obligate parasites such as phytoplasmas and virus from plants. The advantage of this technique is that it can be simultaneously used for production of pathogen-free plants and for long-term storage of plant germplasm. Possibly, international movement of germplasm as cryopreserved stocks could be a mean to decrease inadvertent dispersal of viruses and phytoplasmas (Wang and Valkonen 2008; Wang *et al*. 2009).

## CONCLUSION

The phytoplasmal diseases of sugarcane are more widespread than previously known and are of considerable economic importance. SCWL and SCGS diseases seem to occur only in the south-east Asian region and not in other sugarcane growing areas of the world. Both are caused by a single phytoplasma type. The SCWL and SCGS agents have never been identified in plants other than sugarcane and seem to have strict insect vector specificity. In contrast, SCYLS disease occurs in all the continents and is associated with distinctly different phytoplasmas which are not specific pathogens. The presence of a closely related phytoplasma in the leafhopper indicates the possible involvement of the leafhopper in the transmission of sugarcane and hence transmission studies are being carried out to establish its involvement in the spread of sugarcane diseases. Phytoplasmas cannot be cultured *in vitro* and thus create a major drawback in their diagnosis and characterization. Due to the lack of biotechnological and molecular characterization of phytoplasmas in earlier days, their taxonomy was mainly based on symptoms, DAPI fluorescence microscopy technique, host range, Electron Microscopy and insect vector. However, in the last 15 years, the applications of DNA-based technology allowed to distinguish different molecular clusters inside these prokaryotes. Moreover, the presence of sugarcane related phytoplasmas in the weed species also warrants further investigations. The sequencing of these phytoplasmas from sugarcanes, weeds and leafhoppers would further confirm and elucidate their taxonomic, phylogenetic and epidemiological relationships. The current information about phytoplasma types associated with sugarcane diseases worldwide is likely to change with future research. For instance, there is still very little known about the occurrence of phytoplasmas in Ramu stunt and green grassy shoot diseased sugarcane plants. Disease control practices as for other sugarcane disease should be adopted for good management of phytoplasma disease. Theses include introduction of disease–free germplasm, cultivation of resistant varieties, disease avoidance strategies, and use of disease free planting area.

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