

Chapter 4

Phytoplasma Diseases of Industrial Crops



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Abstract Phytoplasmas are associated with diseases in several hundred plant species, including many economically important industrial crops like sugarcane, sugar beet, cassava, and cotton. A number of phytoplasma diseases are associated with sugarcane. Originally restricted to Asian countries, they are spreading rapidly to newer locations with the help of infected seed material and leafhopper vectors. In cassava, the two phytoplasma diseases causing serious yield losses are cassava frog skin in Latin America and cassava witches' broom in Asia. Because of unreliable and nonspecific symptoms, the identification and characterization of the phytoplasmas associated with these and other industrial crops at an early stage of plant growth are challenging. Here the progress made in understanding biology, economic importance, symptomatology, diagnosis, epidemiology, and control of phytoplasmas infecting sugarcane, sugar beet, cassava, and cotton crops are summarized.

Keywords Sugarcane · Sugarbeet · Cassava · Cotton · Identification · Epidemiology · Genetic diversity

4.1 Introduction

Phytoplasmas infect various industrial crops where they cause serious economic losses. This chapter presents the historical background, geographical distribution, economic loss, characterization, genetic diversity, transmission, and management aspects of phytoplasma disease of sugarcane, sugar beet, cassava, and cotton crops in the world.

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4.2 Cassava or Manioca

Cassava (*Manihot esculenta* Crantz) is the most important energy and food source in tropical regions of the world. Native to South America, cassava was domesticated 5000 years ago and has been cultivated extensively since. Today, it is a staple food in the diet of 17.8 million people worldwide (FAOSTAT 2015) as well as being an industrial crop with high potential in the socio-economic development of the regions where it is produced. Cassava is primarily a vegetatively propagated crop and prone to several biotic and abiotic stresses among which the phytoplasma diseases are a potential threat to food security for millions of people (Jackson 2014) since they cause important yield losses wherever cassava is grown. Phytoplasma diseases are economically important in Asia, Latin America, and the Caribbean. The most important cassava diseases associated with phytoplasma infection are cassava frog skin disease (CFSD) reported in 1971 in Colombia (Pineda et al. 1983) and cassava witches' broom (CBW) discovered in 2010 in Southeast Asia (Alvarez et al. 2013). These pathogens are spread through cuttings and also spread by insect vectors. Losses caused by cassava phytoplasma diseases vary greatly depending on the region, local environmental conditions, and cassava variety. Existing reports have documented losses in commercial crop yield of up to 90% in production areas (Alvarez et al. 2009).

Cassava Frog Skin Disease The origin of cassava frog skin disease (CFSD) is most probably the Amazon region of Colombia, Peru, or Brazil. It was described for the first time in Colombia in 1971, in the cassava-growing region of Quilcacé, Cauca Department, and is considered to be one of the most problematic diseases for cassava cultivation that affects the production of roots (Pineda et al. 1983). CFSD has been reported in Colombia (Alvarez et al. 2009), Costa Rica (Pardo et al. 2014), Paraguay (Cardozo et al. 2016), Brazil (Santos de Oliveira et al. 2014), Panama, Peru, and Venezuela (Pineda et al. 1983; Chaparro-Martínez and Trujillo-Pinto 2001). CFSD affects root production and can cause severe economic loss. In Colombia and Costa Rica, yield losses of more than 90% have been reported. Different groups and subgroups of phytoplasmas associated with CFSD were reported. The CFSD-associated phytoplasmas were identified as group 16SrIII-L by restriction fragment length polymorphism (RFLP) (Fig. 4.1a) and sequence analyses of amplified rDNA products in Colombia (Alvarez et al. 2009). A 16SrIII-A phytoplasma (de Souza et al. 2014), and a phytoplasma affiliated to subgroup 16SrIII-L were also described (Santos de Oliveira et al. 2014), the latter subgroup was also reported in Paraguay and Costa Rica (Pardo et al. 2014) associated with symptoms similar to those reported by Alvarez et al. (2009). A quantitative PCR was developed, and a TaqMan probe was designed for the phytoplasma detection in field material based on the *rp* gene (16SrIII-L phytoplasma) (Fig. 4.1b) enabling the increase of detection sensitivity from 100- to 1000-fold than that obtained from PCR (Alvarez et al. 2010).

The disease causes deep lesions, decreased diameter, and increased woodiness in the roots. Symptoms consist of small longitudinal fissures distributed throughout the root. As roots increase in diameter, the fissures tend to heal, giving the injuries a

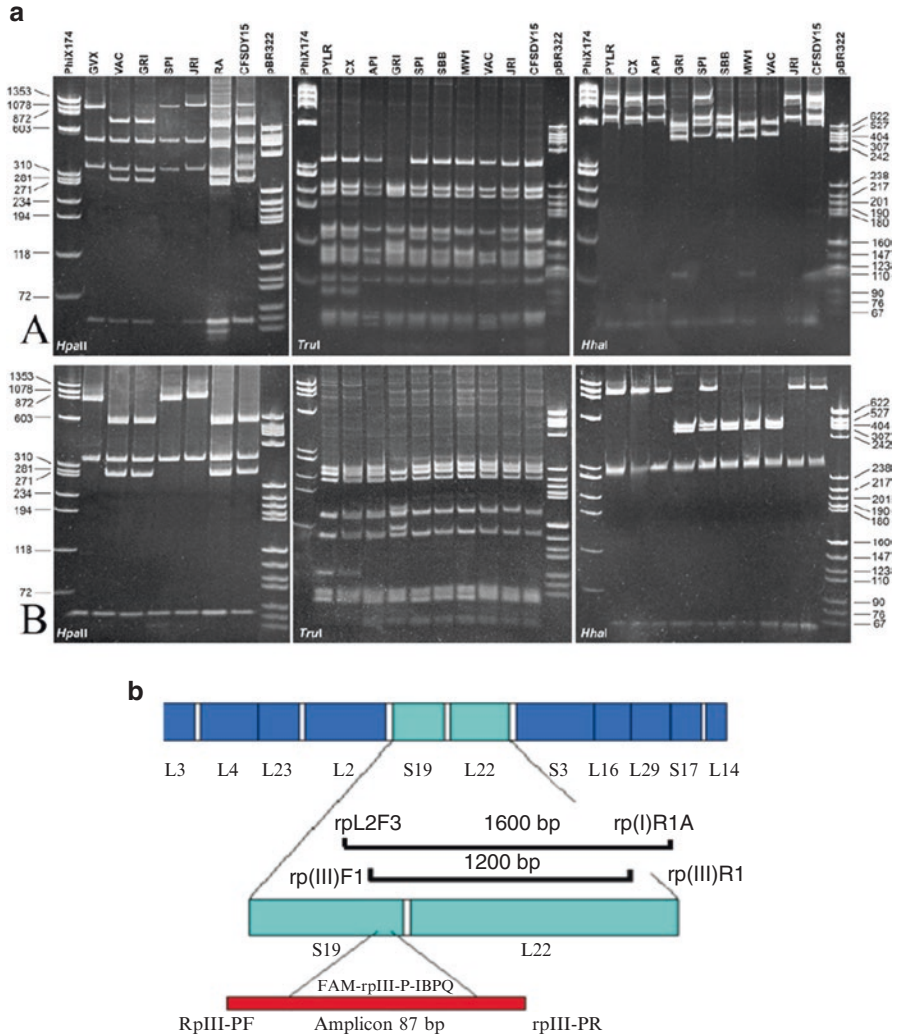


Fig. 4.1 (a) Polyacrylamide gels showing the restriction fragment length polymorphism profiles of 16S rDNA amplified by PCR with **A**, primers P1/P7, and **B**, in nested PCR with primers R16F2n/R16R2 from representative phytoplasma strains and cassava frog skin disease phytoplasma strain CFSDY15 (from Alvarez et al. 2009, with permission of APS). (b) *rp* gene region from which a TaqMan probe and primers for qPCR of CFSD phytoplasmas were designed

lip form. Symptoms in the roots are a woody aspect and a thickened peel that is cork-like, fragile, and opaque. The peel also presents liplike slits, creating a netlike or honeycomb pattern (Alvarez et al. 2009). The expression of CFSD symptoms is influenced by temperature and host genotype. Depending on the severity of symptoms, the depth and number of lesions increase until the root becomes deformed (Pineda et al. 1983; Alvarez et al. 2003). The symptoms on the aerial parts of the



Fig. 4.2 Characteristic symptoms of cassava frog skin disease in Colombia (**a, b, c, and d**); symptoms of CFSD in Costa Rica (**e**), in Paraguay (**f**), and in Brazil (**g**); healthy cassava roots (**h**)

plant are uncommon and mostly observed when the plants are harvested (Fig. 4.2). In Paraguay, symptoms including longitudinal liplike fissures on roots and a thick cork-like appearance on root peel and the diseased cassava roots were observed. The disease roots contained very low starch content (Cardozo et al. 2016). Disease symptoms observed in Paraguay were less severe than those observed in Costa Rica, where phytoplasma group 16SrIII-L was associated greater disease severity. The disease is exponentially propagated through stem cuttings. Since stems of diseased plants are thicker than those of healthy ones, diseased plants are selected for propagation, creating a demand for disease-free planting materials to prevent the dissemination of disease (Pardo et al. 2014). Most cassava varieties infected with CFSD express no leaf or stem symptoms. Molecular tests carried out on plants of cassava and periwinkle after dodder transmission trials confirmed the presence of phytoplasmas of group 16SrIII. Graft transmission could transfer phytoplasmas from infected to healthy cassava plants (CIAT 2005), and CFSD is transmitted by vectors of families of hemiptera, such as Cicadellidae and Delphacidae (Mejía et al. 2011). Insects in the *Scaphytopius* genus, in particular *S. fuliginosus* (Osborn), (Granada 1979), and *S. marginelineatus* (Stål) were shown to carry the phytoplasmas associated with CFSD (CIAT 2003, 2005). Recently, it has been demonstrated that *S. marginelineatus* is able to acquire the CFSD phytoplasma from infected plants and successfully transmit it to healthy plants (Alvarez and Betancourth 2016). Further

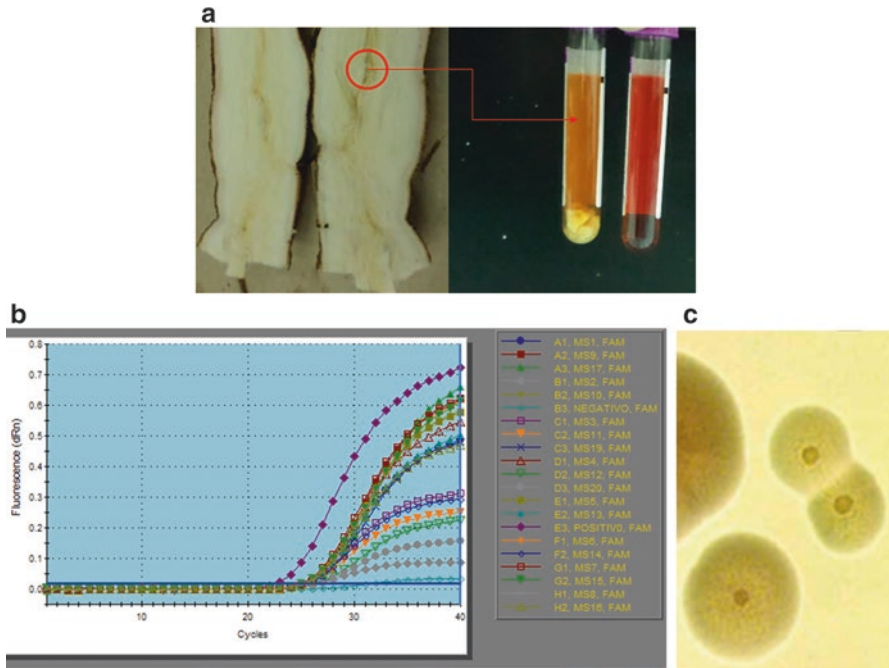


Fig. 4.3 On the left, symptoms in vascular tissue: the red circle indicates the vascular beams of phloem, and on the right the tube on the left shows symptomatic root fragments deposited in the liquid medium 8 days after sowing, and on the right, control tube (a). TaqMan quantitative PCR with 16SrIII-specific primers detecting phytoplasma DNA from colonies grown in solid medium (b). Phytoplasma colonies under the optical microscope grown in solid medium in an atmosphere of 95% N₂ and 5% CO₂ (magnification 40 X) (c)

studies are required, to determine the epidemiological significance of this insect as a vector of the disease in field transmission trials.

In Colombia, isolation of a phytoplasma associated with CFSD was accomplished using a method described by Contaldo et al. (2012). Fragments of roots, petioles, stems, leaves, and embryos from diseased cassava plants were used as a source for phytoplasma isolation in liquid medium, and colonies were observed in the solid medium; the presence of phytoplasmas was verified using qPCR (Fig. 4.3). Other molecular tests showed 450-bp bands with primers M1/M2, polymorphic patterns referable to those of group 16SrIII, and sequences with 99% homology with CFSD phytoplasma from both media (Betancourth et al. 2014). Pathogenicity was proven using stem injection of phytoplasma cells; five clones (CM 2952, Col 1, Col 896, Bra 184, SM 909-25) exhibited severe CFSD and typical root symptoms 6 months after inoculation (Alvarez et al. 2017).

The disease is managed mainly by using cuttings from healthy-looking plants that bear normal roots as propagation material. Improved quarantine procedures are required to prevent long-range disease spread. A heat treatment method has been

developed and implemented in Colombia for the mass propagation of cassava planting material and subsequently applied in Brazil, Costa Rica, and Paraguay. Thermal chamber systems reportedly raised the efficiency of production to 90% in cassava compared with traditional farming systems and increased the availability of stalks of commercial genotypes for farmers.

Cassava Witches' Broom Disease Another important phytoplasma disease associated with cassava is the witches' broom (CWB) that is present in diverse tropical regions, mainly in Central and South America, including Cuba (Arocha et al. 2008) and Brazil (Lozano 1992). In Brazil, this disease was described in the 1940s in the southeast region (Silberschmidt and Campos 1944); later, it was observed in the central (Kitajima and Costa 1971), northeast (Mariano et al. 1991), and northern regions (Lozano et al. 1981; Lozano 1992). Molecular analysis using specific primers provided evidence that the phytoplasma was affiliated with group 16SrIII (X disease). Based on the phylogeny, virtual RFLP patterns, and similarity coefficient calculations, the phytoplasma was classified as a member of subgroup 16SrIII-B (Flôres et al. 2013).

In Cuba, cassava plants have been associated with disease symptoms that include leaf yellowing, small fruits, and stunting, and the presence of '*Ca. P. asteris*' has been reported (Arocha et al. 2009b). In Brazil, in areas of the northeast region, CWB was present in 85% of the fields and caused losses up to 70% (Fukuda et al. 1990). The disease also provokes yield losses of cuttings for planting, considering that affected plants display reduced size and excessive bud burst (EMBRAPA 2012). Recently, the disease was observed in fields located in the state of São Paulo (SP), southeastern Brazil. The symptoms included stunting, shoot proliferation, foliar chlorosis, and leaf malformation (Flôres et al. 2013). In Southeast Asia, CWB disease was observed in 2010 with incidence levels of 32% (Graziosi et al. 2016). Aster yellows phytoplasmas have been detected, and RFLP analyses of nested PCR-amplified fragments from Vietnamese and Cambodian CWB phytoplasmas indicated the presence of differentiable strains all related to the 16SrI group (Alvarez et al. 2013). The major symptoms are small yellow leaves, short internodes, sprout proliferation, stem vascular necrosis, and/or stunting (Fig. 4.4) in Vietnam. Stakes produce only a few dwarf and weak spindly sprouts that never reach a normal size, and, when the affected cassava is uprooted, the roots are thinner and smaller, with rough-textured skins and drastically reduced starch content (Alvarez et al. 2012). In 2010, the disease was observed in Quang Ngai, Dong Nai, and Yen Bai provinces of Vietnam, where more than 60,000 ha were affected, with crop losses as high as 80%, and reductions in yield and starch content reaching 30% (Alvarez et al. 2013; Hoat et al. 2015). In Thailand, cassava witches' broom disease was first reported in 2008. It spread rapidly and is now widely distributed across the region. Affected plants show bunches of shoots with short internodes, small yellowish leaves at the top of the plants, brown vascular tissues, and poor storage root development (Jackson 2014). Similarly, many cassava farms in Cambodia were affected in 2012, with losses of up to 50% (Alvarez et al. 2014). Currently, cassava witches' broom is spreading in Southeast Asia (Cambodia, China, Indonesia, Laos, the Philippines,



Fig. 4.4 Symptoms observed in cassava plants with witches' broom phytoplasma disease in Asia. (a, b, and c) Characteristic symptoms of CWB disease in Cambodia (a, b, c), phytoplasma disease in cassava in Thailand (d, e) and in Vietnam (f)

Thailand, and Vietnam) (Jackson 2014). In Wallis and Futuna Islands in the South Pacific, phytoplasmas found in cassava plants with symptoms associated with CWB disease have 100% identity with the '*Ca. P. aurantifolia*' (16SrII) (Davis et al. 2005). CWB has no known insect vector, but its spread is known to be mediated by the use of planting material obtained from infected stems. Key aspects of disease etiology, epidemiology, and control remain to be investigated (Graziosi et al. 2016).

During the crop growth, it is recommended to remove plants with phytoplasma symptoms as soon as they are seen and to destroy them by burning (Jackson 2014). Restricting the movement of cassava planting stakes, especially from infected areas, and restricting the movement of related species such as *Jatropha* spp. are also the best way to minimize disease incidence. Varietal resistance also exists but is not a significant management practice (Alvarez et al. 2012). Vector control could constitute an effective way of managing this disease and slowing its spread in fields. Also, heat therapy is successfully used to control phytoplasma diseases in cassava. Abiotic

and biotic resistance inducers could carry ample potential for phytoplasma control in cassava. As a systemic pathogen, phytoplasmas can modulate plant hormones and down-regulate plant defenses, thus opening an avenue to external application of phytohormones to prime defense systems and induce resistance (Graziosi et al. 2016). Also, an accurate and rapid detection is a key component of disease management strategies, and a loop-mediated isothermal amplification (LAMP) assay has been developed to allow specific detection of CWB phytoplasmas from field-collected samples (Vu et al. 2016).

Cassava Phytoplasma Antholysis Jayasinghe et al. (1983) observed antholysis in some experimental clones of cassava in southwestern Colombia in 1981. This disease with no economic importance occurs in Brazil, the Caribbean, Central America, Colombia, and Venezuela (Frison and Feliu 1991). It was associated with the presence of phytoplasmas, and dissemination is reported mainly by vegetative propagation (Jayasinghe et al. 1984). The first observed symptoms were virescence followed by phyllody in the inflorescence.

Infected inflorescences commonly exhibit a very swollen gynophore and develop internodes in the floral receptacle, a phenomenon known as apostasis. Furthermore, elongation of the receptacle occurs above the insertion of the pistil, with the development of sprouts. Flower fertility is lost, resulting in nonfunctional flowers that abort prematurely. Transmission is 100% by stakes. Under greenhouse conditions, symptoms of antholysis appear within 1 month from planting. No vector transmission is known. The disease is reduced by selecting stakes from healthy plants. Varietal resistance also exists. Treatment with penicillin (500 to 1,000 ppm) did not reduce symptoms, whereas tetracycline reduced antholysis by 90%. This sensitivity and detection by Dienes' stain allowed to associate the disease to phytoplasma presence (Jayasinghe et al. 1983). Recently in Argentina disease symptoms associated with phytoplasmas were observed in a cassava field in the Misiones province. Typical symptoms of "superbrotamiento" are shown in Fig. 4.5. Stem cuttings from a diseased cassava plant generated new plants exhibiting the disease symptoms (H. Ceballos, 2017 unpublished data).

Cassava Phytoplasma Disease in Africa In Uganda, symptoms of leaf yellowing, chlorosis, shortening of internodes, and stunting were observed in cassava fields in Kawanda. The 16S rDNA sequence and restriction profile comparison obtained after RFLP assays of PCR amplicons identified the associated phytoplasma as a strain of '*Ca. P. aurantifolia*' (16SrII group) (Arocha et al. 2009a). In Côte d'Ivoire, cassava production is second to yams, and it is widely used as a typical food side dish called "attieké." Moreover, cassava-based flour or starch is widely used by private companies, and this has made cassava one of the most important Ivorian industrial crops. Cassava could become an alternative income source for women in the south littoral of the coconut-growing area of Grand-Lahou. Since the Côte d'Ivoire lethal yellowing phytoplasma (CILY, 16SrXXII-B, '*Ca. P. palmicola*'-related strains) devastated more than 400 hectares of coconut plantations in this area (Arocha Rosete et al. 2017). However, Kra et al. (2017) reported phytoplasmas of



Fig. 4.5 Cassava “superbrotamiento” disease with symptoms of multiple branching (a), healthy plants (left) and diseased plants (right) (b), healthy cassava stems (left) vs diseased cassava stems (right) (c)

16SrXXII-B subgroup affecting cassava in Côte d’Ivoire. Symptoms of leaf curling and yellowing were observed in cassava orchards located in the coconut-growing villages in Grand-Lahou, which are currently affected by CILY.

4.3 Cotton

A few reports are available on phytoplasma disease associated with cotton (*Gossypium hirsutum* L.) crops. Symptomatology is characterized by floral abnormalities, virescence, phyllody, and shoot proliferation. Disease symptoms also include little leaf, leaf yellowing, shortening of internodes, and stunting (Cousin et al. 1969; Kumar et al. 2010). The first report of the cotton phytoplasma disease (termed as “stenosis”) was published by Uppal et al. (1944); in India and association with phytoplasmas (MLO at the time) was reported by Capoor et al. (1972). Recently, ‘*Ca. P. asteris*’ was reported in cotton from Delhi, India (Kumar et al. 2010), while ‘*Ca. P. aurantifolia*’-related strains (16SrII-C, 16SrII-F) were detected in Mali and Burkina Faso (Martini et al. 2007; Marzachi et al. 2009).

4.4 Sugarbeet

Sugarbeet (*Beta vulgaris* L. subsp. *vulgaris* var. *altissima*) is a biennial, sugar-producing tuber crop grown in different parts of the world. It is an alternative crop to sugarcane and sweet sorghum for sugar production and contributes about 40% of total sugar production globally (Leilah et al. 2005). Sugarbeet cultivation in different parts of the world has been threatened from time to time by various pernicious plant pathogens. A phytoplasma disease of sugarbeets with symptoms of stunted growth with numerous small, narrow leaves (Fig. 4.6) and reduced tuber size was



Fig. 4.6 Little leaf symptoms in sugar beet associated with the presence of peanut witches' broom phytoplasma in India. (Courtesy of L. Rajendran)

observed during 2008 in Tamil Nadu, India (Rasu et al. 2011). Phytoplasma association of peanut witches' broom group (16SrII) was confirmed using PCR (Thilagavathi et al. 2011). In 1990 in Italy, a rosette disease of sugarbeet was associated with the presence of phytoplasmas by transmission electron microscopy (Canova et al. 1990). Later, Mumford et al. (2000) recorded unusual symptoms in sugar beet in 1999 in Hungary where symptoms included a pineapple-shaped crown, along with stunted, chlorotic, and necrotic leaves and petioles.

The disease incidence was recorded up to 60%, and the analyses indicate phylogenetic relatedness with aster yellows (16SrI) phytoplasmas. The observed symptoms were similar to the "low sugar disease," a condition recorded in France, associated with a phytoplasma of the "stolbur" group vectored by the leafhopper *Pentastiridius beieri* (Wagner) (Munchembled et al. 1999). Salehi and Izadpanah (2005) identified a strain of peanut witches' broom phytoplasma (16SrII) associated with witches' broom disease of sugarbeet from Chahgeer region in Abarque (Yazd Province of Iran) during 1998–2000. The associated agent was transmitted from sugar beet to sugarbeet, periwinkle, and eggplant and from periwinkle to sugarbeet via dodder and from periwinkle to periwinkle and from eggplant to eggplant, ornamental eggplant, and tomato by grafting. PCR using universal phytoplasma primer pairs consistently amplified segments of the expected size from the symptomatic sugar beet samples. Phylogenetic and putative restriction site analyses and similarity values showed that sugarbeet witches' broom phytoplasma was closest to members of peanut witches' broom phytoplasma group (16SrII).

The presence of a yellow wilt disease of sugar beet reported in Chile may cause 100% yield loss and seemed to have disappeared from 2001 to 2012 (IANSAGRO 2012). The evidence for the presence of phytoplasmas in sugarbeet was by Hepp and Sandoval (1999); later a phytoplasma belonging to ribosomal group 16SrIII was

identified (Castro et al. 2000). Fiore et al. (2015) detected the sporadic occurrence of the disease and associated with it the presence of 16SrIII-J phytoplasmas. These phytoplasmas are present in different weed species and crops of agronomic interest in Chile and are transmitted by the leafhopper *Paratanus exitiosus* (Beamer) (Hepp and Vargas 2002; González et al. 2010, 2011; Longone et al. 2011; Fiore et al. 2012).

The sugarbeet disease called the “basses richesses” syndrome of sugar beet (SBR) was first described in 1991 in eastern France (Richard-Molard et al. 1995) and then repeatedly appeared in epidemic forms from 1991 to 1992 and from 1996 to 1998. It caused loss of root sugar content with dramatic economic consequences in 1992, with income loss nearly 50% over 1000 ha. SBR symptoms appear in late summer; affected plants showed new shoots with small, narrow, chlorotic leaves; and old leaves are yellow and necrotic. A brownish discoloration of vascular tissues, seen after cutting the tap root, is the most characteristic symptom of plants affected by SBR. Epidemiological studies in sugarbeet plots affected by SBR have shown that *P. beieri* can be infected by “stolbur” phytoplasma and may transmit it to sugar beet (Gatineau et al. 2001). The etiology of the disease remains unclear. Despite the association with a “stolbur” phytoplasma, several observations and studies indicated that this phytoplasma did not play a major etiological role in the disease. Preliminary microscopic observations of affected roots suggested that another phloem-limited organism (a bacterium-like organism: BLO) was involved. Further experiments confirmed that a BLO, related to ‘*Ca. Phlomobacter fragariae*’ (agent of marginal chlorosis in strawberry), was present in symptomatic sugarbeet and could experimentally be associated with disease symptoms. Also, it was observed that *P. beieri* was an effective vector of this BLO. Further, Bressan et al. (2007) confirmed that the syndrome “basses richesses” of sugarbeet in France is associated with two phloem-restricted uncultured bacteria: a “stolbur” phytoplasma and γ -proteobacteria. The vector of proteobacteria is *P. leporinus* (Hemiptera, Cixiidae), formerly shown to transmit both the prokaryotes. The role of *P. leporinus* and two other planthopper species, *Cixius wagneri* China and *Hyalesthes obsoletus* Signoret, in spreading the two pathogens to sugarbeet were compared and quantified. Because of its abundance and high infection rates with proteobacterium, *P. leporinus* was confirmed to be the dominant vector of SBR disease. Symptoms associated with the two prokaryotes were similar, but “stolbur” was associated with a stronger reduction in taproot biomass and sugar content than proteobacteria. Other plant pathogenic phloem-restricted bacteria are proteobacteria. Marginal chlorosis of strawberry and syndrome “basses richesses” (SBR) of sugarbeet are associated with two related γ -3 proteobacteria in the Arsenophonus clade, i.e., ‘*C. Phlomobacter fragariae*’ and SBR proteobacterium (SBRpr), transmitted, respectively, by *C. wagneri* and *P. leporinus* (Danet et al. 2002; Gatineau et al. 2002; Sémétey et al. 2007b). SBR can also be associated with a “stolbur” phytoplasma, which is also transmitted by *P. leporinus* and causes no differentiable symptoms in affected sugarbeets (Gatineau et al. 2001, 2002; Sémétey et al. 2007a). Experiments showed that *P. beieri* could transmit “stolbur” to periwinkle and sugarbeet.

4.5 Sugarcane

The phytoplasma diseases of sugarcane are more widespread than previously known and are of significant economic importance. 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 (SCLY), 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. SCWL, SCGS, and SCLY are the most important as causing significant economic losses to sugarcane yield and sugar recovery in Asian countries. These diseases have been spreading rapidly to newer locations by the use of infected propagation material and by leafhopper vectors. Both are associated with a specific phytoplasma that is a member of the rice yellow dwarf group (16SrXI) and appears to infect only sugarcane (Yadav et al. 2013); the SCWL and SCGS phytoplasmas could be differentiated by RFLP analysis of 16S ribosomal DNA using suitable restriction endonucleases. Sugarcane green grassy shoot is a recently discovered phytoplasma disease of sugarcane in Thailand. The SCLY disease has been reported in several Asian, African, American, and Australian countries and is associated with phytoplasmas in 16SrI, 16SrIII, 16SrXI, and 16SrXII groups. Ramu stunt disease of sugarcane (SCRS) is known to occur in Papua New Guinea. Moreover, mixed infections of SCGS+SCWL and SCGS+SCYL and/or SCYLV+SCLYP are reported from India and Thailand associated with serious yield decline in quality and quantity (Rao et al. 2012). Since the reported phytoplasma diseases are regularly emerging and re-emerging, hence it would be important to detect and manage them at an early stage of sugarcane growth to avoid further spread and significant losses.

Grassy Shoot SCGS was first reported in 1949 in India (Chona 1958), and then it has been recorded in most of the sugarcane-growing states of India and also in Thailand and Vietnam (Wongkaew et al. 1997; Sdoodee et al. 1999; Sdoodee 2001; Nasare et al. 2007; Viswanathan and Rao 2011; Rao et al. 2012; Hoat et al. 2012; Yadav et al. 2017). 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; Ariyaratna et al. 2007). 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 and narrow, reduced in size, and have a soft texture (Fig. 4.7a). Each stalk that is produced from the affected stool shows shortened internodes and the development of side shoots from the bottom to the top (Fig. 4.7b). 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 perennial grass (Fig. 4.7c) (Rao et al. 2008).

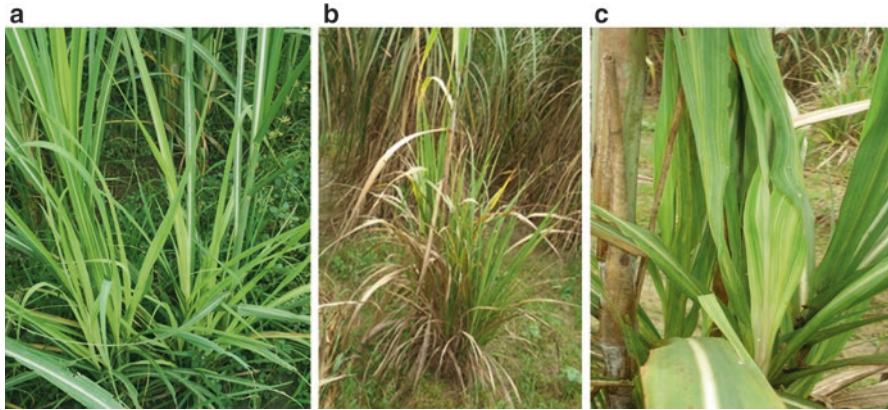


Fig. 4.7 Symptoms of sugarcane grassy shoot disease: grassy shoot like chlorotic leaves of the affected clump of variety CoS 767 (a), grassy shoot symptoms with no tiller at 6 months of the affected clump (b), chlorotic leaves emerging in ratoon crops (c)

DAPI (4'-6-diamidino-2-phenylindole) stains are commonly used for quick and inexpensive phytoplasma detection using fluorescence and immunofluorescence microscopy (Viswanathan 2000). The phloem cells of the infected material showed a strong fluorescence, brighter than the typical of nuclei of parenchymal cells when stained using DAPI (Yadav et al. 2013). Universal phytoplasma-specific primer pairs were mostly used in nested PCR assays that successfully detect the SCGS phytoplasma in sugarcane and its reported leafhopper vectors (Viswanathan et al. 2005; Srivastava et al. 2006; Rao et al. 2014; Tiwari et al. 2017). Multilocus genes such as *secA*, *secY*, *poC*, *gyrA*, *gyrB*, and *dnaB* were also utilized for characterization of SCGS phytoplasmas (Nasare et al. 2007; Manimekalai et al. 2015, 2016; Rao et al. 2014; Kumar et al. 2017). Nucleotide sequence analysis of the 16S rRNA genes revealed that the SCGS phytoplasma affecting sugarcane crops in India is very closely related to RYD phytoplasma group (16SrXI group). Although there were significant variations in symptomatology and in the genetics of the detected phytoplasmas, no correlation could be established between symptoms and phytoplasma strain (Nasare et al. 2007; Viswanathan et al. 2011; Yadav et al. 2017). So far three phytoplasma subgroups 16SrXI-B, 16SrXI-D, and 16SrXI-F were found associated with SCGS disease (Rao et al. 2014, 2017; Zhang et al. 2016; Yadav et al. 2017) in India and China (Fig. 4.8). Nasare et al. (2007) concluded that the 16S rRNA gene and 16S–23S rRNA spacer region sequence identity among the SCGS-associated phytoplasma strains in India are more than 99%, and these results are confirmed by other studies (Viswanathan et al. 2011). Srivastava et al. (2006) demonstrated that the leafhopper *Deltocephalus vulgaris* was the vector of SCGS; later Rao et al. (2014) demonstrated *Exitianus indicus* as a putative vector for SCGS phytoplasma. Recently, Tiwari et al. (2017) reported two additional leafhopper vectors *Maiestas portica* (Melichar) and *Cofana unimaculata*.



Fig. 4.8 Neighbor-joining tree showing the evolutionary relationship of representative phytoplasma strains of 16SrXI and 16SrXIV groups from different sugarcane cultivation regions

Deterioration in yields of many promising varieties of sugarcane by SCGS has been reported (Dhumal 2001; Viswanathan and Rao 2011; Tiwari et al. 2012; Gogoi et al. 2017). If diseased setts are used for planting, the germination percentage is reduced of 30–60% (Madan et al. 1981; Dhumal 1983); Bachchav et al. (1979) have reported 40–90% loss in sugarcane yield due to SCGS. The affected canes have very poor milling quality; the juice shows a reduction in brix, pol, CCS percentage, and purity but increases in the invert sugar (Dhumal and Nimbalkar 1983; Usmani and Rao 1991; Rao et al. 2000; Gogoi et al. 2017). Dhumal and Nimbalkar (1982) reported that the SCGS-infected canes had greater quantities of phosphorus, potassium, iron, copper, and zinc; on the contrary the contents of nitrogen, magnesium, manganese, and silica were lower. The reduced nitrogen content adversely affects growth parameters, photosynthesis efficiency, and carbohydrate and chloro-

phyll contents, leading to altered morphology and physiology of disease cane plants. The high content of phosphorus may be responsible for stunted growth, chlorotic leaves, premature and profuse tillering, and stimulation of invertase activity, which adversely affects the juice quality. The higher content of potassium, copper, and zinc induces higher auxin concentration, resulting in premature sprouting of buds and profuse tillering, and influences the accumulation of reducing sugars by stimulating the invertase activity that deteriorates the juice quality. The reduction in silica content in diseased cane leaves may be responsible for soft and papery leaves and reduction in disease resistance, making the host more prone to other pathogens (Dhumal 2001). The infected leaves showed reduction in the contents of magnesium and manganese resulting into loss of chlorophylls (Usmani and Rao 1991), reduced photosynthetic efficiency (Rao et al. 1992), and inhibition of sucrose-synthesizing enzymes like sucrose synthetase and carboxylating enzymes like PEPCase and RuBPCase (Dhumal and Nimbalkar 1983; Dhumal and Hedge 1984). The lower activity of sucrose synthase and sucrose P synthase may be attributed to the lower contents of magnesium and manganese which are acting as cofactors in these reactions (Madan et al. 1981, Dhumal and Nimbalkar 1983). Studies on photosynthetic enzymes in diseased plants indicate that the phytoplasma has adverse effects on carboxylating enzymes like PEPCase, RuBPCase, and pyruvate Pi kinase. A similar trend was reported in the activity of NADP malic (decarboxylating) enzyme, while the activities of NADP-malate dehydrogenase, aspartate amino transferase, and alanine amino transferase were highly stimulated as compared to healthy plants. Leaf photorespiration was also reported higher in SCGS-affected leaves (Dhumal and Nimbalkar 1983a, b).

White Leaf Sugarcane white leaf (SCWL) is one of the most destructive sugarcane diseases in Sri Lanka and Thailand. It was first described in 1954 in the Lampang province in the northern part of Thailand (Mangelsdorf 1962), and 4 years later it was also 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 diseases of sugarcane. Currently, it seems present in all areas of Asian Countries (Thailand, Sri Lanka, Bangladesh, Vietnam, China, Nepal) where the crop is grown (Rishi and Chen 1989; Sarindu and Clark 1993; Nakashima et al. 1994, 1996; Wongkaew et al. 1997; Hoat et al. 2013). SCWL was also recorded in 1986 in Japan, in the Tanegashima island, but later it 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, chlorotic, and smaller than those of healthy plants, with a soft texture (Fig. 4.9). Severely diseased plants fail to produce millable canes. SCWL phytoplasma strains are very closely related to the SCGS agents and phylogenetically related to other phytoplasmas in grasses such as RYD and SGS (Seemüller et al. 1994) but could be assigned to a different subgroup by RFLP analyses of 16S rDNA (Lee et al. 1998). SCWL is transmitted by the leafhopper *Matsumuratettix hiroglyphicus* Matsumura (Matsumoto et al. 1968). The minimum acquisition and inoculation feeding periods are 3 h and



Fig. 4.9 Symptoms of sugarcane white leaf disease: field view of SCWL disease in ratoon crops of variety in Sri Lanka (a); sugarcane clump showing grassy shoot and white leaf symptoms (b); total chlorotic leaves of a sugarcane variety (c) (Courtesy of S. Thushari)

30 min, respectively (Chen 1978). The incubation period of SCWL phytoplasma in the insect vector is 25–35 days while on the host plant is 70–90 days (Matsumoto et al. 1968). Lee and Chen (1972) reported the optimum temperature for vector transmission is 25 °C. According to the studies of Chen (1978), female adults seem to be more efficient than the males in the transmission of this disease. *M. hiroglyphicus* is widely distributed in central and southern parts of Taiwan, Sri Lanka, and Thailand. The vector population is particularly abundant from July through October, and then it declines rapidly and remains low until April; six overlapping generations may occur in a year (Yang and Pan 1979). Disease incidence is correlated with the

population trend of the vector in the field. 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 in sandy soils. Sugarcane and *Saccharum spontaneum* are the preferred known plant hosts of SCWL. Up to 100% incidence of SCWL has been recorded resulting in complete yield loss. Serious recent epidemics of SCWL have been recorded from Udon Thoni in 2000 and Burirum Districts in 2002 (Kusalwong et al. 2002). With PCR assays, phytoplasma DNAs were detected in SCWL diseased plants collected from Thailand (Nakashima et al. 1996; Wongkaew et al. 1997; Sdoodee et al. 1999; Hanboonsong et al. 2002, 2006) and in insect vectors *M. hiroglyphicus* and *Yamatotettix flavovittatus* (Nakashima et al. 1994; Hanboonsong et al. 2006). The transmission efficiency of *M. hiroglyphicus* (55%) was higher than that of *Y. flavovittatus* (45%). These two species peak at different times of the year and therefore complement each other in the transmission of SCWL disease; therefore their management requires the control of both insects (Hanboonsong et al. 2006; Kaewmanee and Hanboonsong 2011). Eight other leafhopper species *Balclutha rubrostriata* (Melichar), *Bhatia olivacea* (Melichar), *Exitianus indicus* Distant, *Macrosteles strifrons* Anufriew, *Recilia distincta* (Motschulsky), *Recilia dorsalis* (Motschulsky), *Thaia oryzivora* Ghauri, and *Xestocephalus* sp. were reported as putative vectors of SCWL in northeastern Thailand (Hanboonsong et al. 2006). The disease is spread through stem cuttings from healthy-looking and latently infected plants (Cronjé et al. 1998; Tran-Nguyen et al. 2000). Antibodies against SCWL phytoplasma had been generated (Sarindu and Clark 1993), but universal antigenic targets for the different phytoplasma strains need to be developed to avoid false negatives. Cultural practices, disease awareness, and farmer understanding remain as the major factors for successful planting of sugarcane with minimum losses from phytoplasma.

Weed grasses have been suggested to be a reservoir of SCWL but no molecular evidence is available to prove this hypothesis. The fact that SCWL occurs mainly in Asia and not in other sugarcane-growing countries in the world strongly suggests that quarantine barriers should be reinforced to prevent its spread and restrict its movement to other areas. Manimekalai et al. (2010) confirmed that the phytoplasma associated with arecanut palm (*Areca catechu*) has 99% identity with sugarcane white leaf and coconut root wilt disease phytoplasmas (16SrXI) and 98% identity with Bermudagrass white leaf phytoplasma (16SrXIV). The phylogenetic analysis confirmed the clustering of the yellow leaf disease phytoplasma of arecanut palms with 16SXI and 16SXIV groups. This indicates very close relationships of arecanut palm phytoplasma with SCWL and other related Bermudagrass phytoplasma and suggests that they could be a threat for the possibility of transfer and harbor this phytoplasma as alternative and collateral hosts. Use of resistant clones to control the SCWL disease is limited due to the lack of varieties combining high yield with disease resistance. Planting disease-free cuttings, rouging of diseased plants, and the prohibition of ratooning in infected fields are, therefore, recommended to control the disease. In Thailand, the disease is under control in infected areas by the routine use

of healthy planting material, hot water treatment of cuttings for 2 h at 50°C, micro-propagation of disease-free plantlets, strict quarantine regulations, and various soil amendments (Chen and Kusalwong 2000; Kaewmanee and Hanboonsong 2011).

Yellow Leaf Sugarcane yellow leaf syndrome (SCYLS), characterized by a yellowing of the midrib and lamina, was first reported in the 1960s from East Africa (Rogers 1969) and later from Hawaii (Schenck 2001), South Africa (Cronjé et al. 1998), and Cuba (Peralta et al. 1999). It is now widely distributed in most sugarcane-growing countries from all continents. Losses from 30% to over 60% of susceptible varieties have been reported (Comstock et al. 1994, 1998; Arocha 2000). Symptoms of yellow leaf have been attributed to many causes, both biotic and abiotic, but the biotic agents are luteovirus or phytoplasmas in Hawaii, Brazil, Australia, South Africa, Cuba, the USA, and Mauritius (Vega et al. 1997; Cronjé et al. 1998; Matsuoka and Meneghin 2000; Arocha et al. 1999; Scagliusi and Lockhart 2000; Aljanabi et al. 2001; Rott et al. 2008). Phytoplasmas have been consistently associated with SCYLS, but latent infections also occur (Bailey et al. 1996; Cronjé et al. 1998; Arocha 2000; Aljanabi et al. 2001). Phytoplasma infection was reported associated with YLS of sugarcane in Africa, and a phytoplasma member of the X-disease group (16SrIII) was detected (Cronjé et al. 1998). It was also reported in Cuba, India, and Australia (Viswanathan 1997; Arocha et al. 1999; Tran-Nguyen et al. 2000). Parmessur et al. (2002) reported *Saccharosydne saccharivora* as the most abundant leafhopper species found in Cuban sugarcane plantations and responsible for SCYL transmission. In some cases, mixed infection of both viruses and phytoplasmas has been observed (Gaur et al. 2008; Rao et al. 2017). The major symptoms consist of yellowing of leaves with a bright yellow midrib, often when the rest of the lamina is still green (Fig. 4.10). Guerra and Cano (2005) detected YLS phytoplasma using DAPI staining. 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 SCYL in nine African countries, although the plants were symptomatically similar (Cronjé et al. 1998, 1999; Aljanabi et al. 2001). The prevalent agent is a member of the X-disease group which showed a sequence identity of 98.8% with the X-disease phytoplasma. Detection and molecular characterization of AY phytoplasmas (subgroup 16SrI-A) from SCYL-diseased sugarcane plants from Cuba were also confirmed (Arocha et al. 1999). In Australia, a great genetic diversity among SCYL phytoplasmas was determined by RFLP and sequence analyses of PCR-amplified 16S rDNA (Tran-Nguyen et al. 2000). Yellow leaf syndrome (YLS) of sugarcane is a widely distributed disease syndrome in many sugarcane producing countries of the world and causes significant losses in yield and quality (Lockhart and Cronje 2000; Viswanathan et al. 2011). Losses of over 60% are reported in highly susceptible varieties (Arocha 2000). A strain of SCYL belonging to 16SrXII group (“stolbur”) was shown to be associated with sugarcane leaf yellows in India (Gaur et al. 2008; Viswanathan et al. 2008). Recently, a 16SrI-B subgroup phytoplasma has been confirmed associated with sugarcane leaf yellows from two commercial sugarcane varieties from Lucknow, Uttar Pradesh, India (Kumar et al. 2015). The major symptoms associated



Fig. 4.10 Symptoms associated with sugarcane leaf yellows phytoplasmas

with the disease were midrib yellowing and irregular yellow patches on leaf lamina. These findings indicated a great phenotype and genetic diversity of phytoplasmas associated with leaf yellows disease. Peralta et al. (1999) observed histopathological alterations in SCYLS sugarcane leaves such as chloroplast disorganization, starch accumulation, and increasing number of mitochondria; biochemical alterations like a decrease in amylase activity, alterations of juice quality, and an increase in invertase activity were also reported. Higher levels of sucrose (Peralta et al. 1999; Arocha 2000) have also been found, which may be influenced by a proportional increase in some non-sugar carbohydrates. SCYLS incidence in different commercial cultivars in India was also reported as being responsible for the 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 SCYLS infection alters the contents and composition of polysaccharides, phenols, and polyamines in the juice of infected plants (cv. Cuba 120-78) in Cuba. The disease was associated with an increase in the concentration of reducing sugars, glucose index, and glycoproteins recovered in juice, whereas the amount of sucrose decreases. Sugarcane juice obtained from both healthy and SCYLS-affected Cuba 120-78 cultivars of sugarcane contained putrescine (PUT), cadaverine (CAD), spermidine, and spermine (SPM) as free and macromolecule 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 with an increase in total PA fraction. Arginase and ornithine decarboxylase activities, responsible for the synthesis of PUT, were higher in SCYLS juice than in those obtained from

healthy plants. CAD and SPM presumably conjugated mostly to chlorogenic, syringic, and ferulic acids in juice from SCYLS plants. Many methods have been developed for the generic and specific detection of SCYL phytoplasmas, either based on nested PCR or NAH assays (Arocha et al. 2004a, b; Rott et al. 2008). Nucleic acid hybridization assay has been established for the generic detection of phytoplasmas (Harrison et al. 1994; Kirkpatrick and Smart 1995; Arocha et al. 2004a). 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 X-disease group was shown to be present in Mauritius. This group previously described in sugarcane from South Africa was detected in both sugarcane *Sorghum verticilliflorum* and the planthopper *Perkinsiella saccharicida* in Mauritius. The presence of a closely related phytoplasma in the planthopper *P. saccharicida* indicates the possible involvement of the delphacid in the transmission of SCYLS phytoplasma (Joomun et al. 2007).

Green Grassy Shoot Disease Sugarcane green grassy shoot (SCGGS) is a recently discovered phytoplasma disease 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 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. The disease could be transmitted through the canes with transmission percentages up to 15–100% (Pliansinchai and Suchato 1995). The highest percentage of infection was obtained when the basal stalk of the affected canes was planted (Pliansinchai et al. 1998). Sett transmission plays a major role in the disease spread, and insect vectors involved in transmission have not yet been identified.

Ramu Stunt Ramu stunt disease (RStD) of sugarcane was first observed in the late 1980s in the Ramu Valley of Papua New Guinea (PNG) causing severe crop losses in commercial sugarcane varieties (Eastwood 1990). The cultivar Ragnar proved to be highly susceptible. Since that time, the replacement of susceptible cultivars with resistant ones has kept the disease under control. The RStD disease is restricted to Papua New Guinea (Braithwaite 2010). The most common symptom of the disease is pronounced stunting. Leaves show a yellow mottled striping with short, erect, and stiff texture. In some cultivars, excessive tillering and grassy shoot appearance are also present. Affected plants die within 1 year after the appearance of the first symptoms. Kunita et al. (1994) and Cronjé et al. (1999) reported experimental transmission of RStD agent by the leafhopper *Eumetopina flavipes* Muir. A SCWL-related phytoplasma was identified by sequence analysis of 16S/23S rDNA spacer region and RFLP analysis of PCR-amplified 16S rDNA sequences that showed a sequence identity of 95.98% with the SCWL agent. It caused up to 40% loss in total sugarcane production (Suma and Jones 2000). This disease is a major quarantine threat particularly to the neighboring 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 airborne insect vector, *E. flavipes* (Kunita 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 and stunting. 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). Ramu stunt is transmitted mainly through infected cuttings, and it has been suggested that its transmission also involve *E. flavipes*. The main commercial control used in PNG crops is the planting of resistant varieties and the destruction of infected crops. Control of the leafhopper may be another effective control strategy. In PNG, due to the widespread distribution and persistence of *E. flavipes* across multiple wild and cultivated hosts, management effort should focus on the planting of new, resistant varieties and vigilant surveillance for new outbreaks of the disease (Magarey et al. 2002; Magarey 2008).

For SCGS and SGWL diseases, 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. Parmessur et al. (2002) reported the elimination of sugarcane yellows phytoplasma by regenerating plantlets from callus derived from young leaf rolls. Since the alternative/reservoir plants harboring the SCGS phytoplasma are not confirmed yet, understanding the host range of leafhoppers as well as other potential insect vector insects is also desirable for planning sustainable management strategies for SCGS disease. In Australia, phytoplasmas related to SCWL disease were observed in weeds growing near sugarcane fields (Blanche et al. 2003).

4.6 Conclusion and Perspectives

The phytoplasma diseases of industrial crops are widespread and of significant economic importance. SCWL and SCGS diseases seem to occur only in the Southeast Asian regions, and their agents have never been identified in plants other than sugarcane; they also seem to have strict insect vector specificity. In contrast, SCYL disease occurs in all the continents and is associated with a number of different phytoplasmas. Although some of these diseases have attracted significant research attention, many of their associated phytoplasmas are only partially characterized, and many research gaps still need to be addressed. The limited progress in research and management of diseases of these crops is at least partly due to the nature of cultivation of the crop. A number of sugarcane and cassava diseases including those associated with phytoplasmas have in the past been disseminated through the 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 and cassava phytoplasmas, it is necessary for countries to reinforce their inspection and quarantine facilities by acquiring molecular diagnostic tools. The use of resistant clones is of limited value; with the advancement of molecular detection methods, phytoplasmas can be detected in crops at an early stage resulting in timely disease management. No single approach can provide effective and long-lasting management of these phytoplasma diseases considering also the large extension of these crop cultivations. Judicious integration of phytoplasma-free planting material, appropriate cultural practices, and resistant clones can provide ideal management of phytoplasma associated diseases. International movement of phytoplasma-free germplasm as cryopreserved stocks could be a way to decrease inadvertent dispersal of phytoplasmas (Wang and Valkonen 2008; Wang et al. 2009).

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