Analysis of Phyllody Disease Caused by Phytoplasmas in Sesame and Richardia Plants

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Abstract

Sesame phyllody (SP) caused by phloem-limiting bacteria, phytoplasmas, is primarily distributed in the tropics. The characteristic symptoms of this disease consist of malformation of the floral organs which appear as green leaf-like structures. In Northeast Thailand, phyllody symptoms on a rubiaceous plant, *Richardia* sp. (RP) were observed in addition to SP. Phylogenetically the RP phytoplasmas are close to the SP phytoplasmas based on 16S rDNA data. Hybridization assay using the chromosomal DNA fragment of the SP phytoplasmas, SP28, as a DNA probe showed that the phytoplasmas were distributed not only in flowers showing symptoms but also in symptomless leaves and stems in phyllody-diseased sesame and Richardia plants. Sesame plants inoculated with the udo dwarf phytoplasmas which are different from the SP phytoplasmas phylogenetically showed witches' broom-like symptoms, but not phyllody symptoms. These results suggest that the phyllody symptoms of sesame plants may be due to an inhibition of the morphogenesis of the floral organs by metabolites synthesized by phytoplasmas, rather than an interruption of phloem flow by phytoplasmas.

Additional key words: phytoplasma, mycoplasmalike organism, distribution, udo dwarf

Introduction

Sesame phyllody (SP) occurs in many tropical countries of Africa and Asia including Thailand[3, 7, 13]. The disease is caused by phytoplasmas (forma: mycoplasma-like organisms [MLOs])[7, 13]. In Northeast Thailand, phyllody symptoms on a rubiaceous plant, Richardia sp. (RP), and SP were observed in fields with upland crops[12, 13]. DNA probes developed for the SP phytoplasmas hybridized with the DNA of the RP phytoplasmas. 16S rDNA analysis revealed that the SP phytoplasmas are clearly related to the RP phytoplasmas phylogenetically. On the other hand, the studies on the vector insect and host range, as well as the symptoms caused by the SP phytoplasmas in India indicated that the SP phytoplasmas are similar to the sunhemp or crotalaria witches' broom (SUNHP) phytoplasmas [15]. SUNHP disease is also ubiquitous in Northeast Thailand[11, 12]. Our studies conducted in Thailand reveled that the SUNHP phytoplasmas were closely related to the SP phytoplasmas phylogenetically[11, 12]. Recently sequence analysis of the 16S rDNA of phytoplasmas has revealed that the SUNHP phytoplasmas formed one clade within at least 13 phylogenic clades of phytoplasmas[6]. These results indicated that the SP phytoplasmas were closely related to the RP and SUNHP phytoplasmas phylogenetically. On the other hand, udo dwarf (UD) phytoplasmas, which are far from the SUNHP phytoplasmas phylogenetically[16], are known to infect sesame plants[1].

So far it has not been possible to culture phytoplasmas in vitro. Therefore, there is a lack of information about phytoplasmas including their pathogenicity. As a first step toward the elucidation of the pathogenicity of the SP phytoplasmas, it is necessary to analyze the symptoms and to clarify the relationship between the symptoms and the distribution of the SP phytoplasmas in plants.

In the present study, we analyzed the phyllody symptoms and the distribution of phytoplasmas in phyllody-infected sesame and Richardia plants. We also examined the symptoms in sesame plants infected with the UD phytoplasmas.

Materials and Methods

Plant materials: Specimens of sesame plants and *Richardia* sp. with phyllody were collected in a sesame field of Khon Kaen University, Khon Kaen, Thailand[11, 12]. The presence of phytoplasmas in the plant tissues was confirmed by electron microscopy.

Protein profile analysis: Total proteins were extracted from leaves and flowers of healthy and phyllody diseased sesame plants, and were separated by electrophoresis using a 10 % polyacrylamide gel containing sodium dodecyl sulfate (SDS-PAGE)[8]. The proteins in the gel were stained with a silver staining kit (DAIICHI; Daiichi Pure chemicals, Tokyo, Japan). A SDS-PAGE Molecular Weight Marker Kit (Amersham, Aylesbury, USA) was used as a molecular marker for the electrophoresis analysis.

Hybridization assay: DNA extraction and hybridization assay using the DNA probe SP28 of the SP phytoplasmas were performed as described previously[11].

Inoculation test: Leafhoppers *Scleroracus flavopictus* infected with the Nagano isolate of the udo (Aralia cordata) dwarf (UD) phytoplasmas[1, 16] were supplied by the National Agricultural Research Center, Tsukuba, Japan. Sesame plants incubated with the infected leafhoppers for one week were maintained in a greenhouse.

Results

1) Symptoms of phyllody in sesame plants

In Northeast Thailand, phyllody disease is

ubiquitous in sesame fields (Fig. 1A). The symptoms of the disease in this area were very similar to the description of the sesame phyllody symptoms occurred in other areas[7]. The most characteristic symptoms consist of the transformation of flowers into deep green leaf-like structures (Figs. 1 and 2). The typical appearance of the infected sesame plants was compared to that of a healthy plant (Fig. 1B). The flowers and fruits of the plants mainly showed symptoms. The symptoms of the flowers on the upper part of the diseased plants were more severe than those of the lower flowers. Three phylloid flowers appeared at the axillae of the diseased plants whereas one normal flower appeared at the axillae of the healthy plants. The infected inflorescence on the upper part of the plants consisted of short leaves closely arranged on a stem with very short internodes. Leaves on the lower part of the plants, stem and root did not show any visible symptoms. The phylloid flowers became symmetrical (Fig. 2). Sepals and petals changed to green leaf-like structures with thick veins. Most of the stamens retained their normal shape, but some anthers of the flowers became green leaf-like structures. The diseased flower had six stamens whereas the normal flower had five stamens. The pistils were transformed into a pseudosyncarpous ovary by their fusion at the margins. Inside the ovary, instead of ovules, there were small petiole-like outgrowths which later grew and burst through the wall of the false ovary producing small shoots (Fig. 1B). These shoots continued to growth and they produced more phylloid flowers at the axillae. Thus phyllody-infected plants were sterile, resulting in total loss of yield.

Microscopic analysis showed that the phylloid flowers contained mesophyll tissue which differentiated into palisade tissue and spongy tissue between the epidermis and developed vascular bundles similar to those of leaves, whereas uniform cells and small vascular bundles were observed between the epidermis of healthy flowers(data not shown). SDS-PAGE analysis showed that the protein profile on the diseased flowers was very similar to that of leaves of healthy or diseased plants (Fig. 3). A thick band corresponding to a large subunit of ribulose-1,5-

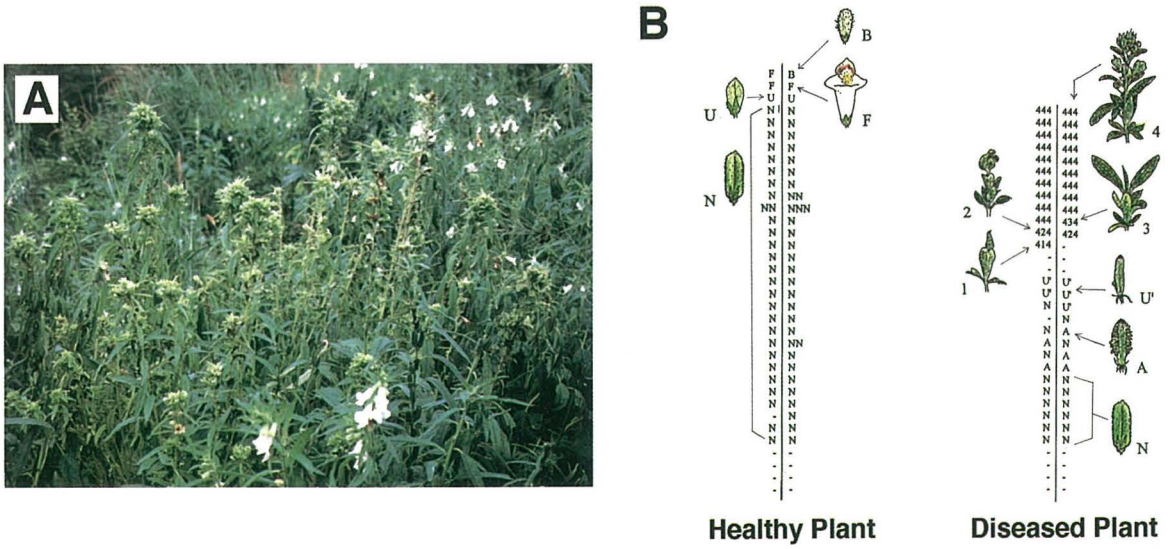

Fig. 1. Phyllody disease of sesame. (A) Sesame plants with phyllody disease in Luei, Northeast Thailand. (B) Diagrams of shoot style of healthy (left) and sesame plants with phyllody disease (right). B, bud; F, flower; U, unripe fruit; N, normal fruit; A, Abnormal fruit; U', abnormal unripe fruit; 1-4, phyllody flowers.Phyllody disease of sesame. (A) Sesame plants with phyllody disease in Luei, Northeast Thailand. (B) Diagrams of shoot style of healthy (left) and sesame plants with phyllody disease (right). B, bud; F, flower; U, unripe fruit; N, normal fruit; A, Abnormal fruit; U', abnormal unripe fruit; 1-4, phyllody flowers.

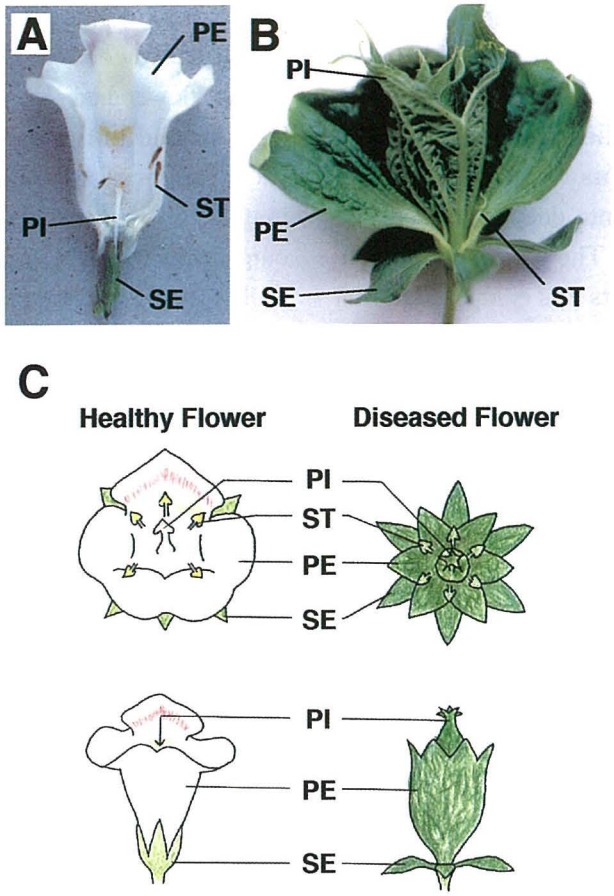

Fig. 2. Phyllody symptoms of sesame plants. (A) Healthy sesame flower. (B) Diseased sesame flower. (C) Diagrams of healthy and phyllody sesame flowers. PI, pistil; ST, stamen; PE, petal; SE, sepal.

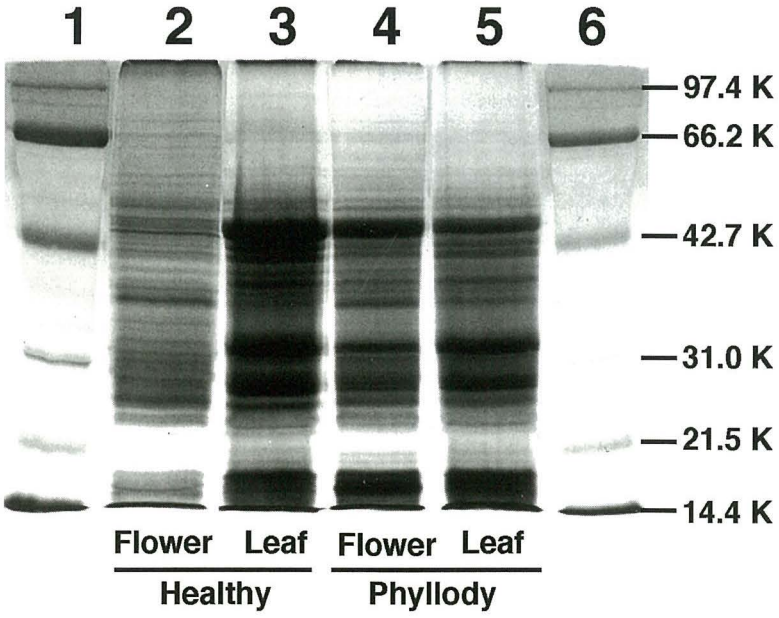

Fig. 3. SDS-PAGE analysis of total proteins of healthy and sesame plants with phyllody disease. Lane 2, flowers of healthy sesame plants; lane 3, leaves of healthy sesame plants; lane 4, flowers of diseased sesame plants, and lane 5, leaves of diseased sesame plants. Lanes 1 and 6 are molecular marker sets. The gel was stained with a silver.

bisphosphate carboxylase/oxygenase (Rubisco; ca. 50 K) which is characteristic of chloroplasts was observed in the proteins of the phylloid flowers, while, the Rubisco band was not observed in healthy flowers. Some proteins showed the same mobility as that of specific proteins of the flowers with phyllody disease (Fig. 3). Proteins of the pathogens and the pathogenesis-related proteins originating from the plants were not identified by this analysis.

2) Distribution of phytoplasmas in the sesame plants with phyllody disease

Four sesame plants with mild phyllody symptoms (Fig. 4) were collected in a sesame field in Khon Kaen, Thailand. Hybridization assay using the DNA probe SP28 showed that the phytoplasmas were distributed in the flowers with phyllody symptoms as well as in symptomless leaves and stems in the diseased plants (Fig. 4). No hybridization signals were observed in the tissues of a healthy sesame plant. The titer of the pathogens was not always high in the phylloid flowers of the diseased plants.

3) Symptoms and distribution of phytoplasmas in Richardia plants

Phyllody symptoms on Richardia plants were observed in fields with sesame plants affected with phyllody disease in Khon Kaen, Thailand (Fig. 5A). All of the floral organs turned green, and showed leaf-like structures (Fig. 5B). Leaves, stem and roots of the diseased plants appeared normal in most cases. However, dwarf and witches' broom symptoms were also observed in severe cases.

We collected four Richardia plants which showed phyllody symptoms in flowers and no visible symptoms in leaves, stems and roots in a sesame field in Khon Kaen, Thailand. Dot hybridization assay using the DNA probe SP28 showed that the phytoplasmas were distributed in flowers with phyllody symptoms as well as in symptomless leaves and stems in the four Richardia plants (Fig. 5C).

4) Inoculation test of udo dwarf phytoplasmas to sesame plants

Sesame plants inoculated with UD phytoplasmas using leafhoppers *Scleroracus flavopictus* showed witches' broom-like symptoms, but not phyllody symptoms, about one month after the inoculation (Fig. 6).

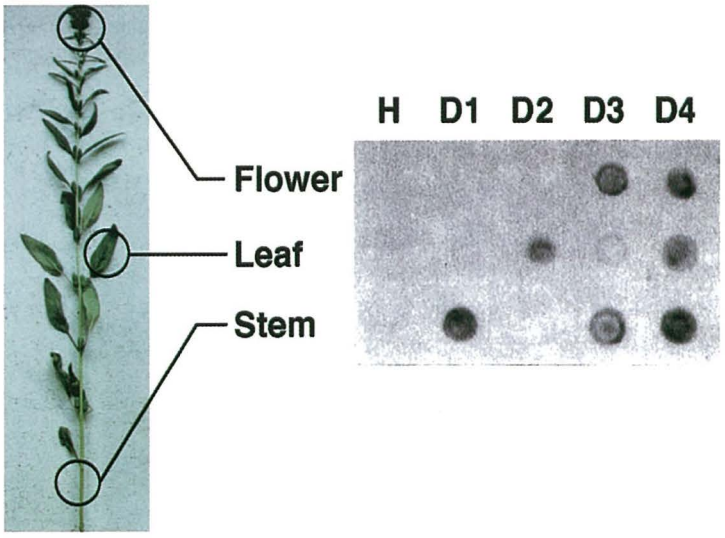

Fig. 4. Distribution of DNA of phytoplasmas in diseased sesame plants. DNA extracted from flowers, leaves, and stems of a healthy plant (H) and four diseased plants (D1-D4) were spotted onto a nylon membrane and hybridized with the DNA probe SP28.

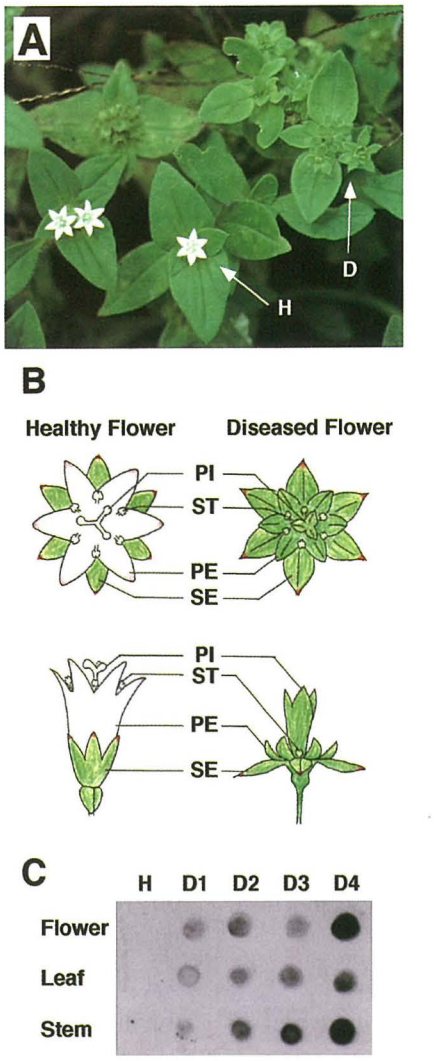

Fig. 5. Phyllody symptoms of Richardia plants and distribution of phytoplasmas in plants. A: Healthy flowers (H) and flowers of Richardia plants with phyllody disease (D). B: Diagrams of healthy flowers and Richardia flowers with phyllody. PI, pistil; ST, stamen; PE, petal; SE, sepal. C: Distribution of DNA of phytoplasmas in Richardia plants. DNA extracted from flowers, leaves, and stems of a healthy plant (H) and four diseased plants (D1-D4) were spotted onto a nylon membrane and hybridized with a DNA probe SP28.

Fig. 6. Symptoms in sesame plants after inoculation of the udo dwarf phytoplasmas using leafhoppers *Scleroracus flavopictus*.

Discussion

In this study, we examined phyllody disease caused by phytoplasmas in sesame and Richardia plants in Northeast Thailand. The phyllody symptoms were more severe in the flowers on the upper part of the diseased plants than on the lower part (Fig. 1). On the top of the plants, very severe phylloid flowers appeared (Fig. 1B). In the most severe case a phylloid flower contained an ovule transformed into a shoot containing phylloid flowers at the axillae. The difference in the severity of the symptoms in flowers and fruits may reflect the time of infection of SP phytoplasmas: when the plants were infected with phytoplasmas, the meristems of the upper flowers may be forming floral organs, and the meristems of the lower flowers may have already formed floral organs.

The morphological studies and protein profile analysis showed that the diseased floral organs resembled leaf structures in sesame plants (Figs. 2, 3). The floral organs of Richardia plants also showed leaf-like structures (Fig. 5). Hybridization assay using the cloned chromosomal DNA fragment SP28 as a DNA probe showed that the phytoplasmas were distributed not only in the flowers showing phyllody symptoms but also in symptomless leaves and stems in sesame and Richardia plants (Figs. 4, 5). In some diseased plants, it was difficult to detect phytoplasmas in phylloid flowers (Fig. 4). We reported earlier that the amounts of rice yellow dwarf phytoplasmas and sugarcane white leaf phytoplasmas were not correlated with the severity of the chlorosis symptoms in the infected tissues[9, 10]. Apparently, phytoplasma concentration in newly emerging leaves with severe chlorosis was not always high. In these two cases as well in the phyllody disease case in sesame and Richardia plants, it is possible that symptom-inducing metabolites synthesized by the phytoplasmas may be translocated through the phloem elements from other parts of plants to the meristems. On the other hand, the highest concentration of pathogens was observed in the regions of infected plants where symptoms were most severe in strawberry plants infected with clover phyllody spiroplasmas[4] and peach and chokecherry infected with eastern X-phytoplasmas [5], suggesting that these phytoplasmas may colonize locally and synthesize metabolites which induce localized symptoms. Thus, the mechanisms of symptom expression by phytoplasmas may not be uniform.

Sequence analysis of the 16S rRNA gene of 12 Japanese phytoplasmas isolates transmitted by *Scleroracus flavopictus* indicated that the sequence of the UD phytoplasmas was the same as that of the others but different from that of the SUNHP group[16]. We showed that the sesame plants inoculated with the UD phytoplasmas did not display phyllody symptoms (Fig. 6). These results also indicated that the phyllody symptoms of sesame may be caused by an inhibition of morphogenesis of the floral organs by metabolites synthesized by the SP phytoplasmas, rather than by a physical interruption of phloem flow by the phytoplasmas. As the SP phytoplasmas are known to cause witches' broom symptoms but do not cause phyllody in some kinds of plants such as sunhemp[15], the putative compounds associated with phyllody symptoms in sesame and Richardia plants may not affect the floral morphogenesis in some plant species.

Recently in molecular genetic studies using *Arabidopsis thaliana* and snapdragon, some genes associated with floral organ identity have been identified[2, 14]. In the *apetala2 apetala3 agamous* triple mutants of *A. thaliana*, lacking all three activities, all the floral organs developed essentially as leaves[2]. In the *leafy* mutants of *A. thaliana* and *floricaula* mutants of snapdragon, the flowers were replaced by shoots[14]. The SP phytoplasmas may affect the expression of such genes in sesame and Richardia plants. Detailed studies are needed to elucidate the mechanisms of phyllody symptoms expression.

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ゴマ及びRichardia植物においてファイトプラズマにより引き起こされる葉化病の解析

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摘　要

篩部局在性細菌であるファイトプラズマによって引き起こされるゴマ葉化病（フィロディー）(SP) は，熱帯の多くの地域において発生している。この病害の特徴は花の各器官が緑色の葉状の器官に変わること（葉化症状）である。東北タイにおいては，ゴマ以外にも*Richardia*属の雑草においてもこの葉化症状が見られる。ファイトプラズマの16S rDNAの解析から，葉化症状のRichardiaで発生しているファイトプラズマは，SPファイトプラズマと系統発生的に極めて近いことが明らかになっている。SP28をプローブとして用いたハイブリダイゼーションにより，ゴマ及びRichardiaにおいて，ファイトプラズマは症状が現れている花だけでなく，症状の見られない葉や茎からも検出されることが明らかになった。一方SPファイトプラズマとは系統発生的に離れているウド萎縮病ファイトプラズマを接種したゴマにおいてはてんぐ巣症状は見られたが，葉化症状は出現しなかった。よって，ゴマの葉化症状はファイトプラズマが篩部を物理的に閉塞するためではなく，SPファイトプラズマが分泌する化学物質がゴマの花の器官形成を特異的に阻害するためであると示唆された。

キーワード：ファイトプラズマ，マイコプラズマ様微生物，分布，ウド萎縮病