THESIS TITLE : DETECTION AND DIFFERENTIATION OF PHYTOPLASMA ASSOCIATED WITH SUGARCANE WHITE LEAF DISEASE IN SUGARCANE AND INSECT VECTOR USING MOLECULAR TECHNIQUES

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ABSTRACT

The Polymerase Chain Reaction (PCR) method was developed to detect phytoplasma associated with white leaf disease in sugarcane, other plants and insect vector *Matsumuratettix hiroglyphicus* (Matsumura). The primer sets used were from three phytoplasmas DNA regions including a conserved region in the 16s rRNA (Universal primers: U 1, U 2, specific primers MLO 1, MLO X, P 1), a specific region in the 23s rRNA (specific primers MLO 7, MLO Y, P 2) and intergenic spacer region (specific primer MLO 3). Optimal concentration of magnesium chloride in the reaction was 4 mM for primers MLO X and MLO Y and 1.5 mM for other primer sets. Each PCR cycle consisted of 60s denaturation at 94 ^{o}C (92 ^{o}C for primers MLO X, MLO Y and primers P 1, P 2), 30s annealing at 60 ^{o}C (68 ^{o}C for primers P 1, P 2) and 90s extension

at 72 °C. Thirty PCR cycles were carried out in plants and thirty-seven or forty PCR cycles were used in insect vector depended on the set of primers.

Detection of phytoplasma that cause white leaf disease in sugarcane, brachiaria grass, bermuda grass, crowfoot grass; and yellow leaf disease in carpet grass, gold beard grass, panicum grass, dayflower and citronella grass; chlorosis symptoms in sugarcane and wire bush and grassy shoot symptom in sugarcane was carried out using PCR. Universal primers amplified a 1.35 kb DNA fragment from phytoplasma sugarcane with white leaf and grassy shoot symptoms and other plants with symptoms of white leaf and yellow leaf disease. No band was obtained from healthy sugarcane and from sugarcane and wire bush with chlorosis symptoms. Two DNA fragments of 654 bp and 720 bp were amplified by using specific primers MLO1 and MLO3 from carpet grass, gold beard grass, dayflower, and panicum grass showing yellow leaf symptoms. But a DNA fragment of 654 bp was amplified in sugarcane with grassy shoot symptom and sugarcane, brachiaria grass, bermuda grass, citronella grass and crowfoot grass with white leaf symptoms. PCR amplification with specific primers MLO1 and MLO 7 of DNA from sugarcane with grassy shoot symptom and sugarcane, brachiaria grass, bermuda grass, and crowfoot grass with white leaf symptoms resulted in a 810 bp fragment. Nested PCR performed in insect vector *Matsumuratettix hiroglyphicus* (Matsumura) resulted in 654 bp and 218 bp fragments by using primer sets of U 1, MLO 7; primer MLO 1, MLO 3 and primer sets MLO X, MLO Y; primer P 1, P 2, respectively.

RFLP patterns of phytoplasma infected sugarcane and other plants were studied by digestion of PCR product that amplified by using Universal primers with 15 restriction endonucleases: *Msp* I, *Rsa* I, *Alu* I, *Acc* I, *Hpa* I, *Hpa* II, *Dra* I, *Xba* I, *Bgl* I, *Eco*R I, *Kpn* I, *Sal* I, *Bam*H I, *Hind* III and *Taq* I . Restriction digestion of PCR product with *Msp* I and *Hpa* II showed different banding patterns in sugarcane with grassy shoot symptom compared to sugarcane and weeds showing white leaf symptom. Digestion of PCR products with *Taq* I showed different banding patterns in sugarcane with white leaf disease compared to brachiaria grass, bermuda grass and crowfoot grass showing

white leaf symptoms. No different banding pattern was obtained in sugarcane, brachiaria grass, bermuda grass and crowfoot grass with white leaf symptoms compared to sugarcane with grassy shoot symptom by digestion of PCR product that amplified with specific primers MLO 1, MLO 3.

The genetic relationships of phytoplasma in sugarcane with grassy shoot symptom; sugarcane, brachiaria grass, bermuda grass with white leaf symptoms and the insect vector *Matsumuratettix hiroglyphicus* (Matsumura) were investigated by sequencing of DNA fragments between 16s rRNA and 23s rRNA in plants, and 16s rRNA and intergenic spacer region in insect vector. Phytoplasmas in sugarcane with grassy shoot and white leaf symptoms were closely related and clustered in the same group. Phytoplasma in brachiaria grass, crowfoot and bermuda grass with white leaf symptoms were clustered in another group. The phytoplasma in insect vector *Matsumuratettix hiroglyphicus* (Matsumura) was closely related with phytoplasma associated with sugarcane white leaf.