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Research Article

Phytoplasma Associated with White-backed Planthopper on Rice Plants in Sidrap Regency, South Sulawesi

Saipul Abbas[1)]*, Ernawati Djaya[2)], Erwin Najamuddin[2)], Amelia Sebayang[2)], Ayyub Ar Rahman[2)], Aminah[2)], Hasbi[2)], Surianto Sipi[2)], Nur Fathurahman Ridwan[3)], Rini Ismayanti[2)], & Elisurya Ibrahim[2)]

[1)]*Faculty Agriculture, Lambung Mangkurat University*
Jln. Jend. A. Yani Km. 36 Banjarbaru, South Kalimantan 70714 Indonesia

[2)]*Research Center for Food Crops, National Research and Innovation Agency (BRIN)*
Jln. Raya Bogor Km. 46, Cibinong, Bogor, West Java, 16911 Indonesia

[3)]*Ecology Laboratory, Biology Program, Faculty of Mathematics and Natural Sciences, Universitas Negeri Yogyakarta*
Jln. Colombo No. 1 Yogyakarta, 55281 Indonesia

**Corresponding author. E-mail: saipul.abbas@ulm.ac.id*

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ABSTRACT

South Sulawesi is one of the largest rice production centers in Indonesia. Several important diseases of rice plants, such as those caused by viruses and phytoplasmas, can be transmitted by insect vectors, especially leafhoppers and planthoppers. Symptoms of diseases caused by viruses and phytoplasmas are quite diverse but visually similar and difficult to distinguish. This study aimed to analyze the presence of phytoplasma associated with white-backed planthopper which are commonly found in rice plantations. The research was done by conducting surveys and explorations of insect samples from six villages in Sidrap District. White-back planthoppers found on rice plantations showing symptoms of yellowing and stunted leaves were sampled for further analysis, including total DNA isolation of insects, standard PCR amplification for insect and Nested-PCR for phytoplasma identification, gene sequencing for both amplicons, and nucleotide analysis. The PCR with CO1 primer successfully amplified a 700 bp amplicon from insects, whereas nested-PCR using fP1/rP7 primers followed by m23SR/R16F2n amplified around 1800 bp and 1250 bp of supposedly 16S RNA gene of phytoplasma, respectively. Results of DNA sequencing analysis identified the insect samples as *Sogatella vibix* based on 83% homology using BLAST and Mega X Program. As for the phytoplasma, it leans towards the 16SrI group or *Candidatus phytoplasma asteris* (*Aster yellows phytoplasma*) with a homology percentage of 99%.

Keywords: *Aster yellows phytoplasma*; nested PCR; *Sogatella vibix*; rice; Sulawesi

INTRODUCTION

South Sulawesi Province is one of the largest rice production centers in Indonesia, with rice plantation of approximately 985,000 ha or contributing to about 9.5% of national rice plantations area (*Badan Pusat Statistik* [BPS], 2023). One of the most important challenges in rice production in the province is the presence of diseases caused by viruses, bacteria, and fungi. However, until now, in South Sulawesi, there have never been reports of rice diseases caused by phytoplasmas. Phytoplasmas are obligate prokaryotic, wall-less bacteria which are unculturable and multiply in plant phloem tissues and insect haemolymph (Firrao *et al*., 2004). They are pleomorphic, with varying sizes, ranging from 200 to 800 nm in length and possess small genomes of about 680–1600 kb (Valarmathi *et al*. 2013). Phytoplasmas have been reported causing more than 600 plant diseases worldwide (Bertaccini *et al*., 2014).

Two rice diseases are caused by phytoplasmas, namely: Rice yellow dwarf disease (RYD), cause by Rice yellow dwarf phytoplasma (RYDP) and Rice orange leaf disease (ROL), cause by Rice orange

leaf phytoplasma (ROLP) (Li *et al*., 2015). Rice yellow dwarf disease is a serious problem in many Asian countries (Nakashima & Murata, 1993). The symptoms of RYD disease are pale yellow leaves, less tillers, drying and decaying leaves (Valarmathi *et al*., 2013). Similarly, ROL presence has been reported only in Asia, including Thailand, Malaysia, Indonesia, and the Philippines. ROL-infected plants express symptoms of orange leaves, which later, roll inward and desiccate. Infected plants die in 2−3 weeks after the infection (Hibino & Cabauatan, 1987).

The 16SrI "Candidatus Phytoplasma asteris" group was demonstrated to contain the ROLP isolates from the Philippines and India. The ROLP isolate was subsequently classified in the aster yellows subgroup (Ca. Phytoplasma sp. AY 16S-group, AY-sg) phytoplasma (Jung *et al*., 2003). Mature rice plants infected with ROLP displayed stunting as well as the formation of a golden or orange leaves that begins from the tip continuing downward, followed by an inward rolling of leaves and eventually leading to leaf senescence. The symptoms vary depending on the plant's conditions and stages, such as yellow to golden or yellow to orange discoloration on the leaves of infected plants. The number of tillers can be slightly to severely reduced, plant height may be slightly to severely stunted, and growth habits may range from erect to spreading. Plant growth and tiller reduction have a negative impact on yield. When the plant is badly infected at the seedling stage, it is often deadly (Ling, 1972).

ROLP is transmitted by different leafhopper species (Hemiptera: Cicadellidae): *Nephotettix cincticeps*, *N. virescens*, *N. nigropictus*, and *N. malayanus* (Jung *et al*., 2003). While RYDP are transmitted by *N. cincticeps*, *N. virescens*, and *N. nigropictus* (Nakashima & Murata, 1993). In addition, the phytoplasmas can also be transmitted by planthoppers (Hemiptera: Delphacidae) (Bertaccini *et al*., 2014; Kumari *et al*., 2019). Both phytoplasmas are transmitted in a persistent-propagative manner by their insect vectors. The transmission mechanism involves acquisition of the phytoplasma from an infected plant by its vector, the pathogen movement and multiplication within the insect vector's body, and inoculated into other plants (Hogenhout *et al*., 2008; Huang *et al*., 2020).

One of the most important rice diseases in Sidrap Regency is rice tungro disease, caused by Rice tungro viruses (Abbas *et al*., 2020). The viruses share the same vectors as the rice phytoplasmas, namely: *Nephotettix cincticeps*, *N. virescens*, *N. nigropictus*, and *N. malayanus* (Nesmi, 2016). Recently, more plants showing orange-yellow discolored leaves but after using PCR techniques, the tungro viruses were not detected in the symptomatic plant samples (Rosida *et al*., 2023), thus, the symptoms were suspected to be caused by phytoplasma. Therefore, the objectives of this study were to: 1) detect the presence of phytoplasma in the white-back planthopper collected from symptomatic rice plants in the field, and 2) confirm the identification of the white-backed planthopper using a molecular technique.

MATERIALS AND METHODS

Study Site

The study was conducted in four districts, six villages in Sidrap Regency, South Sulawesi, Indonesia, namely: Baranti District (Panreng, Tangkoli, and Tonronge Rijang villages), Pancarijang District (Kedidi Village), Maritengae District (Tanete Village), and Watangpulu District (Carawali Village) (Figure 1). The study took place in September 2021.

Disease Incidence

Symptomatic plants showing yellowing symptoms in 0.5 ha rice plantations each village (Panreng, Tangkoli, Tonronge Rijang, Kedidi, Tanete, and Carawali) was observed to determine disease incidence. The disease incidence was calculated by formula from Zadoks and Schein (1979).

$$ID = \frac{n}{N} \times 100\%$$

ID= Incidence of disease; n= number of infected plants; N= number of observed plants.

Insect Sample

Sampling was performed by using a purposive sampling method by selecting six rice plantations that showed yellowing symptoms of disease and the presence of insects. From each location, 30 net swings were taken to collect the insect samples. The insect samples were then stored in tubes containing 70% alcohol. Under a light microscope, all samples

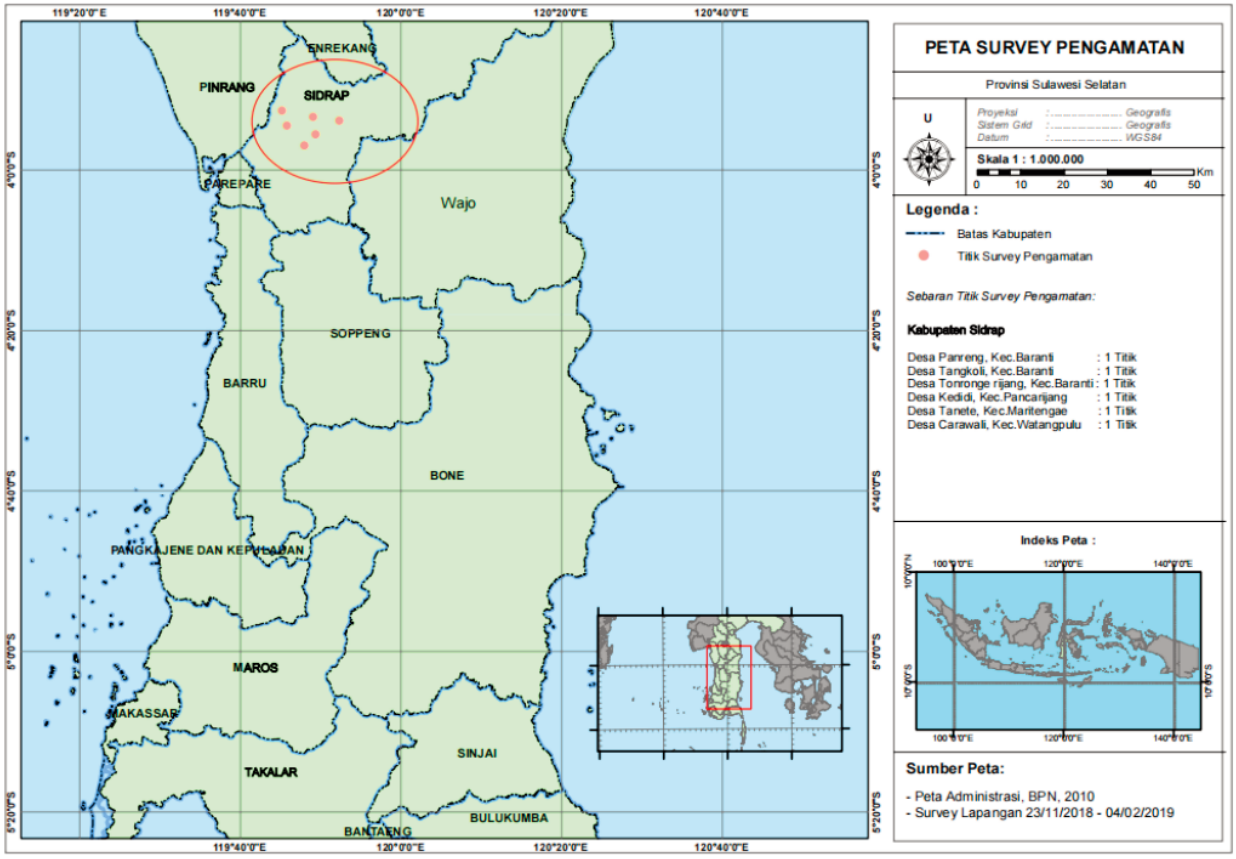

Figure 1. Locations of insect sampling in several sub-districts of Sidrap Regency, South Sulawesi (in Indonesian version)

were counted and grouped into families using the taxonomic key from the Training on Rice Arthropod Biodiversity and Taxonomy (International Rice Research Institute, 2011).

Molecular Identification of the Insect Samples

Insect DNA isolation. Isolation of the insect sample's DNA was performed in Microbiology Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Negeri Yogyakarta from 2021 to 2022. Total DNA extraction from insect samples followed the protocol of the CTAB Method (Doyle & Doyle, 1990) with slight modifications. The insect samples were washed with sterilized water to remove contaminants. Then, the insects were macerated with a CTAB buffer containing a detergent that would help to break down the cell membrane and separate lipid and protein from the DNA. The homogenized mixture was shaken with 80% alcohol to separate the DNA from other cell components, then was centrifuged at 12.000 rpm for 20 minutes. The supernatant containing the insect DNA was collected and used for PCR analysis.

Insect DNA amplification. The PCR reaction was performed using a total of 30 µl consisting of 2 µl of template DNA, 1 µl each of COX1 primers, namely the LCO1490 primer (5'-GGTCAACAA ATCATAAAGATATTGG-3') and the HCO2198 primer (5'-TAAACTTCAGGGTGACCAAAAAA TCA-3') (Ramaiah *et al.*, 2023), 21 µl of DH_2O, and 5 µl of Ready Mix (MyTaqTM HS RedMix). The PCR cycle begins with an initial stage of denaturation at 94 °C for 2 minutes, followed by 35 cycles at 94 °C for 30 seconds, annealing for 60 seconds at 47 °C, extension for 50 seconds at 72 °C, and 10 minutes for the last cycle at 72 °C in a Bio-Rad T100 TM Thermal Cycler (PCR machine).

Molecular Detection of Phytoplasma in Vector's Body

Phytoplasma DNA amplification. The template DNA used in this procedure was the total insect DNA isolated as described before. DNA amplification was carried out in the Microbiology Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Negeri Yogyakarta, from 2021

to 2022. Phytoplasma DNA amplification was performed using a polymerase chain reaction (PCR) method with a universal phytoplasma primer pair, P1 (5'-AAG AGT TTG ATC CTG GCT CAG GAT T-3') (Deng & Hiruki, 1991) / P7 (5'-CGT CCT TCA TCG GCT CTT-3'). The total reaction volume of 10 µl consisted of 10 pmol primers concentration (consists of each primer, forward and reverse, at a volume of 0.5 µl), 3 µl of DH_2O, 1 µl of DNA sample, and 5 µl of Ready Mix (MyTaqTM HS RedMix). The PCR comprised of three stages, including an initial denaturation at 94 °C for 2 minutes followed by 35 cycles at 94 °C for 30 seconds, annealing for 30 seconds at 53 °C, and extension for 1 minute, and 10 minutes for the final cycle at 72 °C using a PCR machine (Bio-Rad T100 TM Thermal Cycler).

Phytoplasma DNA amplification with Nested PCR. The DNA from PCR amplification was then used as a DNA template to amplify phytoplasma using the second primary pair, namely R16F2n (5'-GAA ACG ACT GCT AAG ACT GG-3') (Gundersen & Lee, 1996) / R16R2 (5'-TGA CGG GCG GTG TGT ACA AAC CCC G-3') (Lee *et al*., 1994). The PCR reaction was performed on a volume of 30 µl consisting of 2 µl of template DNA, 1 µl of forward primer (R16F2n), 1 µl of reverse primer (R16R2), 21 µl of free water nuclease, and 5 µl of Ready Mix (MyTaqTM HS RedMix). The PCR cycle begins with an initial stage of denaturation at 94 °C for 2 minutes followed by 35 cycles at 94 °C for 30 seconds, annealing for 30 seconds at 50 °C, extension for 30 seconds at 72 °C, and 10 minutes for the last cycle at 72 °C.

Electrophoresis. The PCR amplification product was then run on an electrophoresis device (PowerPacTM Basic) with an agarose composition of 0.30 grams dissolved with 30 ml of TBE 1x until homogeneous then heated using at microwave for 5 minutes. One µl Green safe was added to the agarose solution as DNA stain. PCR product was inserted 4 µl to each well, and markers of 100 bp and 1 kb each 5 µl, the electrophoresis was run at 50 volts for 50 minutes (Abbas *et al*., 2023). The results of the electrophoresis were visualized using an ultraviolet (UV) transilluminator (Bio Rad 2000) and the formed DNA band were photographed using a digital camera.

DNA Base Sequencing

The amplified products that tested positive for insect DNA and phytoplasma using the Nested PCR were then analyzed using the sequencing gene. A total of 35 µl of PCR product and 15 µl of each of the reverse and forward primers were sent to the PT Genetika Science Laboratory in Jakarta. The nucleotide analysis was performed on a web server with the BLAST (GenBank NCBI) program for identification and the MEGA X program for tree construction.

RESULTS AND DISCUSSION

Disease Incidence

Observations of disease incidence of rice plants were carried out in six villages, namely Panreng, Tangkoli, Tonronge Rijang, Kedidi, Tanete, and Carawali villages in Sidrap Regency at different planting ages, ranging from plants age of 10 to 80 days after planting (DAP). Disease symptoms, including yellowing and dwarf were found in the field (Figure 2). At first glance, the symptoms seemed to be similar to symptoms of tungro disease. Disease incidences varied in those villages, ranging from 10–55%, while white-backed planthopper were only found in three villages: Tangkoli, Tonronge Rijang, and Carawali. Carawali Village was where the highest disease incidence occurred (55%) (Table 1).

Based on Table 1, the population of white-black planthoppers that was successfully counted and identified ranged from 5 to 21 individuals. Our analysis revealed that crop seasons and pesticide management practices significantly influenced the population dynamics of white-backed planthoppers. Specifically, non-selective compounds were observed to decrease the population of white-backed planthoppers, while selective compounds were associated with an increase in their population (Nakata *et al*., 2019).

During the insect survey, white-black planthoppers were found to have the highest population among all planthopper species in six Sidrap villages. This finding led to their selection as a focal species for molecular identification of phytoplasmas, insects, and their interactions. In-depth research on *Sogatella* spp. as phytoplasama vectors on Sulawesi Island had not been reported. Additionally, according to Wulandari *et al*. (2020), the Sogatella genus population in West Java was associated with Phytoplasma and acted as a vector for diseases in carrot plants.

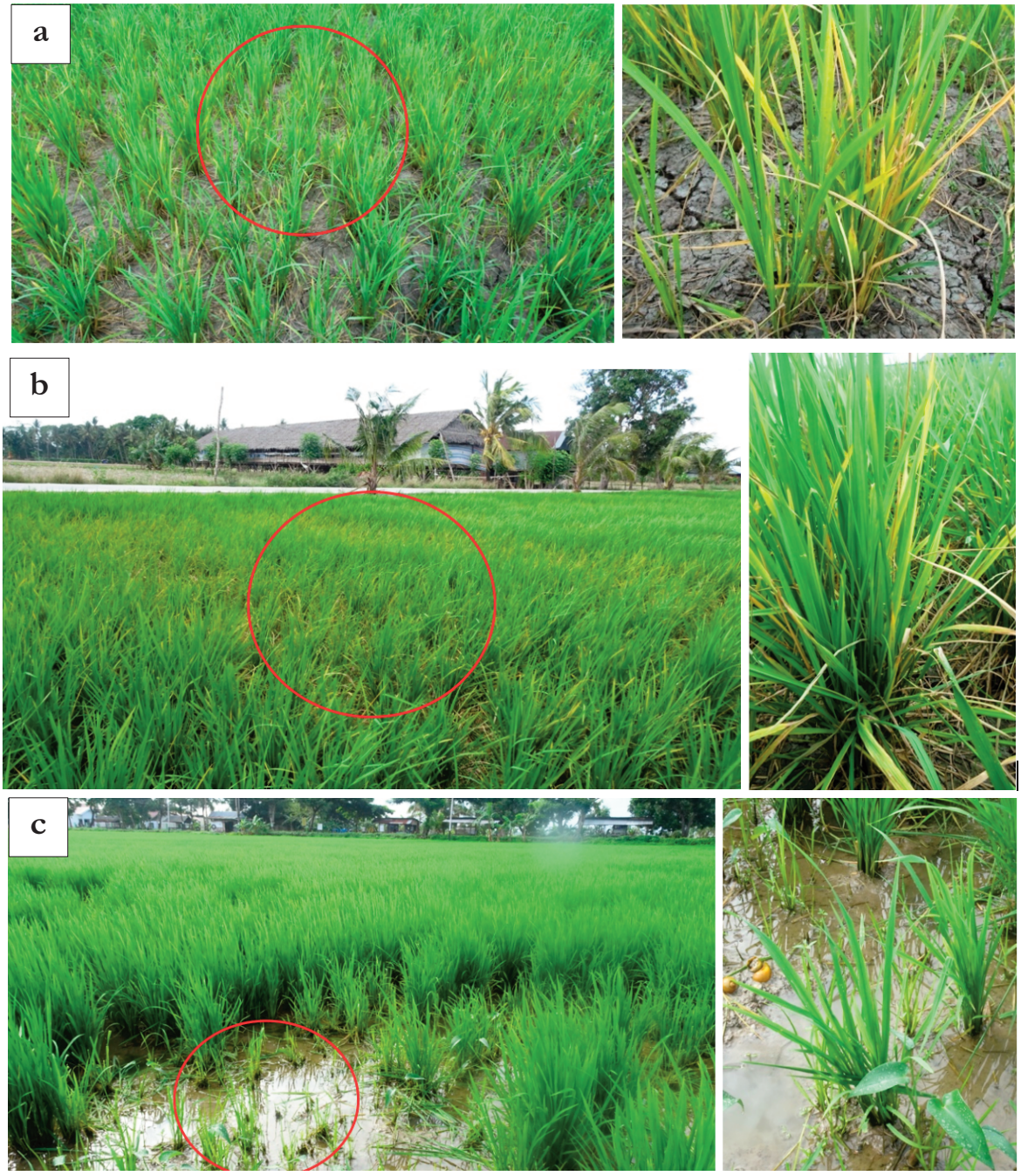

Figure 2. Appearance of symptoms on rice plants found in Carawali Village (a), Tonronge Rijang (b), Panreng (c), Sidrap Regency

Insect Morphological and Molecular Identification

Insect specimens (Table 1) collected from yellowing rice plants were morphologically identified as *Sogatella* spp. (Hemiptera: Delphacidae) based on morphological features under the microscope examination. Following measurements with a microscope, molecular techniques were used to evaluate at the species level (Figure 3). Sogatella was a relatively small and slender planthopper with white longitudinal stripe extending from the vertex posteriorly onto the mesonotum (Asche & Wilson, 1990). It differed from other delphacid genera by having the dorsal margin of the pygofer diaphragm forming a broad U-shape, a compressed and twisted aedeagus with two rows of spines; phallotreme was located subapically on the left side and parameres diverged, tapering to apex and distally bifurcated in most

Table 1. Disease incidence and insect vector population in six villages of Sidrap Regency

Villages	Rice Varieties	Age (DAP)	Population of WBP	DI (%)	Symptoms
Panreng	Situ Begendit	80	15	15	Dwarf
Tangkoli	Inpari 7	70	5	10	Yellowing
Tonronge Rijang	Inpari 4	70	21	15	Yellowing, dwarf
Kedidi	Ciherang	14	15	16	Yellowing, dwarf
Tanete	Ciherang	10	20	15	Yellowing, dwarf
Carawali	Inpari 4	60	5	55	Yellowing, dwarf

Notes: DAP= Days after planting, DI= Disease incidence, WBP= White-backed planthopper

Figure 3. White-backed planthopper: dorsal section (a), ventral section (b)

species (Mariani & Marino de Remes Lenicov, 2018). There are 15 species belonging to Genus *Sogatella* (Mariani & Marino de Remes Lenicov, 2018), thus, to confirm the species of the insect, a molecular procedure was performed. PCR analysis of insect samples showed a DNA band of 700 bp on the electrophoresis gel (Figure 4). Furthermore, insect DNA sequencing was performed to confirm the species of the insect samples. The constructed phylogenetic tree showed that the insect sample with the WPP code of Sidrap's had a kinship with *Sogatella vibix* (HAUPT) (GenBank database MK694718) with a boostrap value of 100% similarity (Figure 5). The isolate MK694718_*Sogatella vibix* has the highest homology percentage value of 83% (Table 2).

According to Asche and Wilson, (1990), *Sogatella vibix* was reportedly distributed throughout Asia, Europe, North Africa, mainland Australia, and the islands of Papua New Guinea. Ammar (1977) raised *S. vibix* in Egypt on wheat for eight successive generations while subjected to experimental conditions; the cultures had originally come from rice and other Gramineae. In the Middle East, *S. vibix* was the vector of virus that cause maize rough dwarf disease. Because of their function as viral disease vectors or the consequences of their feeding and oviposition, some *Sogatella* species, including *S. furcifera*, *S. kolophon*, and *S. vibix*, were recognized as serious pests of wheat, maize, and rice in Asia as well as Central and South America (Wilson & Claridge, 1991). These findings also indicated that *S. vibix* has a presence in Indonesia, particularly on the island of Sulawesi, and was one of the phytoplasma vectors in rice plants in Indonesia.

Molecular Detection of Phytoplasma

The PCR amplification region using fP1/rP7 (1800 bp) covered almost the entire 16S rRNA gene, the intergenic spacer (16S-23S spacer region), and a small portion of the base of the 23S rRNA gene. The size of the 1800 amplicon was the first PCR result with the fP1/rP7 primer pair and nested-PCR around 1250 bp (Figure 4b) (Constable *et al.*, 2007; Gibb *et al.*, 1999).

The results of the phylogenetic tree analysis showed that insect sample coded WPP16S of Sidrap origin contained DNA closely related to several phytoplasmas, with a bootstrap value of about 95%, including DQ381534_*Dogfennel yellows phytoplasma*, GQ365729_*Aster yellows phytoplasma*, FN825683_*Matricaria chamomilla phytoplasma*, and AF487779.1 *Maize bushy stunt phytoplasma* (Figure 6). The boostrap value of 98% was sufficient to confirm that our insect sample contained a phytoplasma (Nei & Kumar, 2000).

The results of the homology percentage analysis showed that the sample with the WPP16S code had the highest homology percentage value of 99%

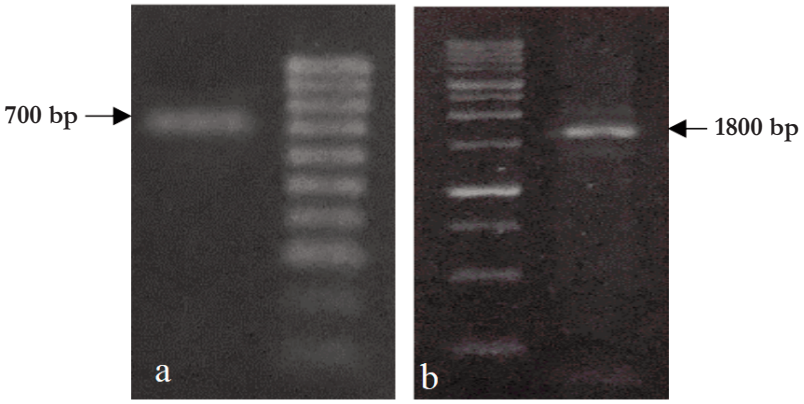

Figure 4. Visualization of DNA tape by PCR and Gel Electrophoresis methods. (a) Insect DNA using COX1 primer, and Marker DNA 100bp, (b) Phytoplasma DNA using primary pairs fP1/rP7 and m23SR/R16F2n (nested), and Marker DNA 1 kb.

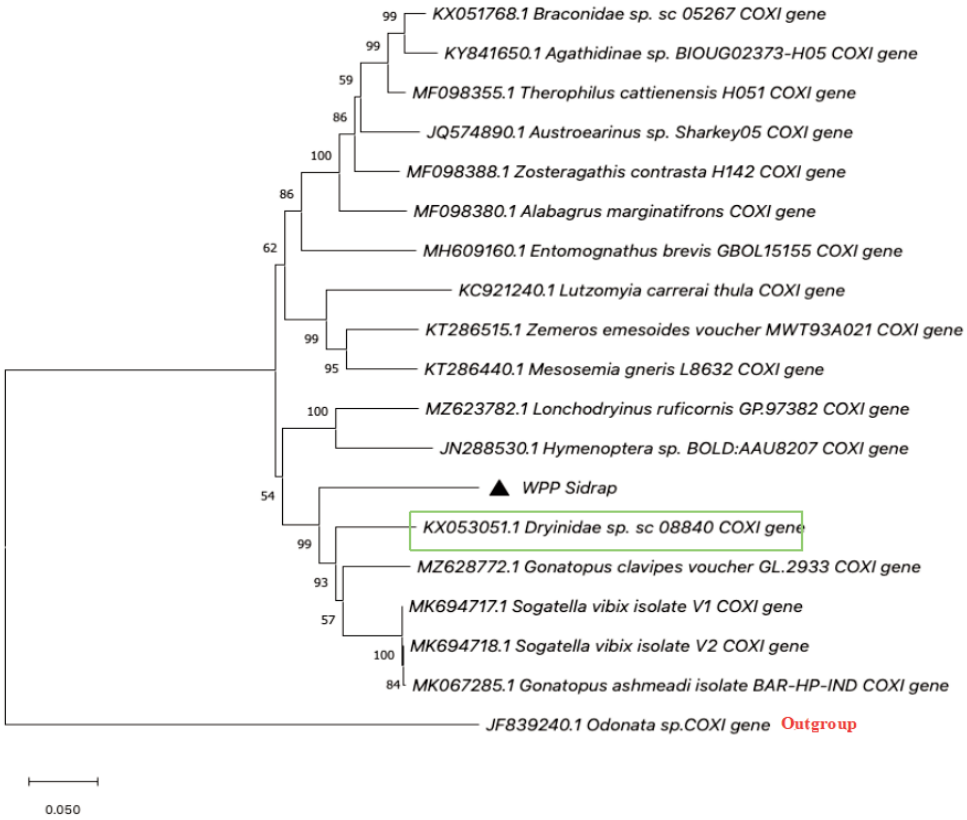

Figure 5. Insect DNA phylogenetic tree using the Mega X program with the Neighbor -Joining method with 1000X Boostrap

(Table 3) among all isolates available in the NCBI database, based on the BLAST method. However, upon examining the host relationships of this phytoplasma (Table 3), it was found that the closest host species of the rice plant is the corn plant. Thus, it can be said that the sample of insects analyzed was identified closest type to *Aster yellows phytoplasma* species. According to the report by Wulandari *et al.* (2020), phytoplasma infection from *Cicadulina bipunctata* from Bogor showed the highest homology with group 16SrI phytoplasma, namely *Ca. Asteris phytoplasma*. The findings of this study aligned with those reported in Wulandari *et al.*'s study.

Table 2. Percentage of DNA sequence homology between insect sample and NCBI phytoplasma database

No.	Identity	Homology (%)							
		1	2	3	4	5	6	7	8
1	WPP_Sidrap	ID							
2	MK694718_*Sogatella vibix*	83	ID						
3	MK067285_*Gonatopus ashmeadi*	82	100	ID					
4	KX053051_*Dryinidae* sp.	81	90	90	ID				
5	MZ628772_*Gonatopus clavipes*	81	91	91	91	ID			
6	MF098355_*Therophilus cattienensis*	77	81	81	81	81	ID		
7	KX051768_*Braconidae* sp.	74	79	79	79	79	80	ID	
8	KY841650_*Agathidinae* sp.	74	78	78	78	78	79	95	ID
9	MZ623782_*Lonchodryinus ruficornis*	75	81	81	81	81	82	80	78
10	MH609160_*Entomognathus brevis*	76	81	81	81	81	81	85	83

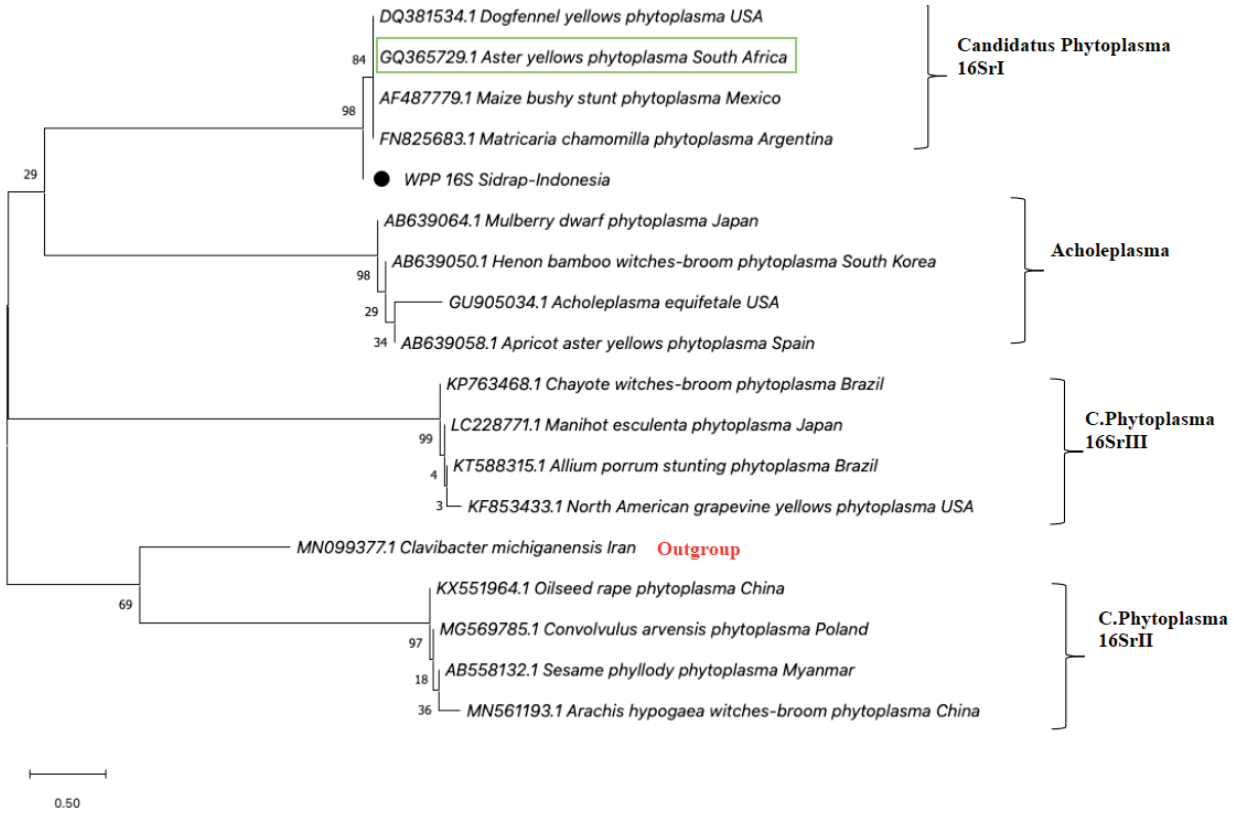

Figure 6. Phylogenetic reconstruction tree of phytoplasma use method Neighbor Joining with 1000x boostrap

Table 3. Percentage of DNA sequence homology between phytoplasma sample and NCBI phytoplasma database

No.	Identity	Homology (%)			
		1	2	3	4
1	WPP 16S_Sidrap	ID			
2	DQ381534.1_*Dogfennel yellows_phytoplasma* - USA	99.3263	ID		
3	GQ365729_*Aster yellows phytoplasma* - South Africa	99.3263	100	ID	
4	FN825683.1_*Matricaria_chamomilla_phytoplasma* - Argentina	99.2300	99.9038	99.9038	ID
5	AF487779.1_*Maize_bushy_stunt_phytoplasma* - Mexico	99.2300	99.9038	99.9038	99.8075

Nucleotide Composition

The results of the analysis of nucleotide composition at 20 phytoplasma sequencies showed that the average composition of nitrogenous bases was almost completely similar (Figure 7, Table 4, Table 5). This suggests that the phytoplasma samples obtained had identical relationships between the 19 sequences obtained from the GenBank database. This was evidenced by the presence of a conserved sequence site of 1057 out of a residual value of 1077.

Figure 7. Multiple sequence alignment using Clustal Omega (1.2.4)

Table 4. Nucleotide Composition of phytoplasma sample and NCBI phytoplasma database

No.	Identity	T	C	A	G	Total
1	WPP 16S Sidrap	25	20	30	25	1073
2	DQ381534.1 *Dogfennel yellows phytoplasma*	25	20	30	26	1077
3	GQ365729.1 *Aster yellows phytoplasma*	25	20	30	26	1076
4	FN825683.1 *Matricaria chamomilla phytoplasma*	25	20	30	26	1077
5	AF487779.1 *Maize bushy stunt phytoplasma*	25	20	30	26	1077
6	MG569785.1 *Convolvulus arvensis phytoplasma*	24	19	29	28	1191
7	AB558132.1 *Sesame phyllody phytoplasma*	24	19	29	28	1192
8	KX551964.1 *Oilseed rape phytoplasma*	24	19	29	28	1192
9	MN561193.1 *Arachis hypogaea witches-broom phytoplasma*	24	20	28	28	1194
10	KT588315.1 *Allium porrum stunting phytoplasma*	36	14	35	15	1192
11	KP763468.1 *Chayote witches-broom phytoplasma*	36	14	35	15	1192
12	LC228771.1 *Manihot esculenta phytoplasma*	36	14	35	15	1192
13	KF853433.1 *North American grapevine yellows phytoplasma*	37	14	34	15	1192
14	GU905034.1 *Acholeplasma equifetale*	20	19	31	30	1882
15	AB639058.1 *Apricot aster yellows phytoplasma*	22	18	33	26	1912
16	AB639064.1 *Mulberry dwarf phytoplasma*	22	18	33	26	1912
17	AB639050.1 *Henon bamboo witches-broom phytoplasma*	23	18	33	26	1914
18	MN099377.1 *Clavibacter michiganensis*	20	24	24	32	1276
	Average	26	18	31	25	1323

Table 5. Phytoplasma taxa in the analysis of homology and phylogenetic trees (NCBI)

Genus	Species	Host	Accession	Gene	Size (bp)	Origin Country
Candidatus Phytoplasma 16SrI	*Dogfennel yellows phytoplasma*	*Eupatorium capillifolium*	DQ381534	16S rRNA	1830	USA
	Aster yellows phytoplasma	Grape vine	GQ365729	16S rRNA	1765	South Africa
	Matricaria chamomilla phytoplasma	*Matricaria chamomilla*	FN825683	16S rRNA	1830	Argentina
	Maize bushy stunt phytoplasma	Maize	AF487779	16S rRNA	1830	Mexico
Candidatus Phytoplasma 16SrII	*Convolvulus arvensis phytoplasma*	*Convolvulus arvensis*	MG569785	16S rRNA	1582	Poland
	Sesame phyllody phytoplasma	*Sesame phyllody*	AB558132	16S rRNA	1842	Myanmar
	Oilseed rape phytoplasma	Oilseed rape	KX551964	16S rRNA	1830	China
	Arachis hypogaea witches-broom phytoplasma	Peanut	MN561193	16S rRNA	1248	China
Candidatus Phytoplasma 16SrIII	*Allium porrum stunting phytoplasma*	*Fragaria ananassa*	KT588315	rpL15	1692	Brazil
	Chayote witches-broom phytoplasma	*Sechium edule*	KP763468	rpL15	1685	Brazil
	Manihot esculenta phytoplasma	*Manihot esculenta*	LC228771	secY	1651	Japan
	North American grapevine yellows phytoplasma	*Vitis vinifera*	KF853433	rpL15	1682	USA
Acholeplasma	*Acholeplasma equifetale*	horses	GU905034	23S rRNA	2893	USA
	Apricot aster yellows phytoplasma	*Prunus armeniaca*	AB639058	16S rRNA	5831	Spain
	Mulberry dwarf phytoplasma	*Morus bombycis*	AB639064	16S rRNA	5830	Japan
	Henon bamboo witches-broom phytoplasma	*Phyllostachys nigra*	AB639050	16S rRNA	5814	South Korea
Clavibacter	*Clavibacter michiganensis*	Potato	MN099377	16S rRNA	1276	Iran

The results of the nitrogenous base composition analysis in the sequences indicated that the average occurrence of the thymine base (T) was 25%, the cytosine base (C) was 20%, the adenine base (A) was 20%, and the guanine base (G) was 26%. The multiple sequencing alignment results showed a dominant area consisting of 1057 conserved values, with a variable site value of 20 out of 1077. Additionally, the parsimony variable value was 5, and the singleton variable value was 15 out of 1077 nitrogenous bases. The singleton variable principle distinguishes between two types of bases, where one type of the repeated bases appears more than once. In contrast, parsimony involves several bases, with at least two of them appearing twice (Table 4).

These results provide important information about the genetic composition of the phytoplasma, which can be used for further analysis and characterization. Understanding the nitrogenous base composition enables researchers to gain insights into the genetic structure and evolution of the phytoplasma, as well as its relationship with other microorganisms. This information is valuable for developing new diagnostic and treatment methods for plant diseases caused by phytoplasmas.

CONCLUSION

The insect's DNA was successfully amplified to a 700 bp sequence using CO1 primers and PCR, whereas the phytoplasma was successfully amplified to a 1800 bp sequence by Nested-PCR using the fP1/rP7 primers followed by m23SR/R16F2n, with the second primer set producing an amplicon of roughly 1250 bp. Based on BLAST and Mega X analysis, the insect samples were identified as *Sogatella vibix* species with a 83% homology percentage. With a homology percentage of 99%, the phytoplasma showed a stronger association with the *Candidatus phytoplasma asteris* (*Aster yellows phytoplasma* group) or 16SrI group. These strains of phytoplasma, which were classified as Plant Quarantine Organisms (PQOs) A1 and were under observation to avoid their infiltrations into Indonesian territory.

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