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Original Article

Morphological and molecular characteristics of *Liriomyza* sp. (Diptera: Agromyzidae) on onion plants (*Allium cepa* L.) in Bali

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Abstract

Liriomyza sp. leafminer fly is an important pest of shallot crops that spread widely in Southeast Asia and Pacific regions such as Indonesia, China, Japan, Korea, Thailand, Malaysia, Singapore, Taiwan and Vietnam. This study aims to identify the type of *Liriomyza* species attacking onion plants in Bali, Indonesia through morphological and molecular characterization. Onion leaf samples showing symptoms of *Liriomyza* attack, taken from the study site, were subsequently cultured in the laboratory. Adult flies obtained from breeding were then identified based on their morphological characteristics. While the molecular characteristics were identified through several steps: DNA isolation, DNA electrophoresis, PCR chain reaction, then put back into the re-electrophoresis box, and finally sequencing method was applied. The results showed that the *Liriomyza* species was found to have a morphological characteristic such that it has a simple color pattern on a simple abdomen with no connecting line between the segments and the next segment, dark grey or near black scutellum. These characteristics refer to the species *L. chinensis*. GenBank data tracking results through identification of squamous alignment results indicate that the *Liriomyza* COI nucleic acid found showed the highest homology with *L. chinensis* (EF104701.1), *L. chinensis* (AB721340.1) and *L. chinensis* (AB721343.1) with identical rate of 99%. Based on the results then *Liriomyza* species found attacking onion planting on the island of Bali is *L. chinensis*.

Keywords: Morphological and molecular characteristics, *L. chinensis*, Onion

Introduction

Leafminer fly is one of the main pest onion crops in Southeast Asia and the Pacific regions such as Indonesia, China, Japan, Korea, Thailand, Malaysia, Singapore, Taiwan and Vietnam. Four species of leafminer pests were originally found by in Indonesia, namely *L. huidobrensis*, *L. trifolii*, *L. sativa* and *L. chinensis* (Winasa et al., 2002). According to Tran (2009), the number of species increased from four to five namely *L. sativae*, *L. huidobrensis*, *L. chinensis*, *L. bryoniae*, and *L. horticola*. Meanwhile, Nonci and Muis (2011) reported seven *Liriomyza* species that attack vegetable crops in Indonesia, namely *L. sativae*, *L. huidobrensis*, *L. bryoniae*, *L. strigata*, *L. trifolii*, *L. brasicae*, and *L. cicerina*. Previous Supartha et al. (2005) reported three leafminer species that attacked vegetable crops in Bali, Lombok, and Sumbawa namely *L. huidobrensis*, *L. sativae* and *Phytomyza horticola*. Wahyuni et al. (2017) also reported the type *Liriomyza* spp. with previous reports in Bali and Nusa Tenggara.

L. chinensis leafminer fly~~ers~~ include the first monofag pests found in Indonesia to attack red onions in Klampok Village, Brebes Regency (Central Java) in early August 2000 (Rauf and Shepard, 2001). The granular fly is a cosmopolitan insect, which in recent years has become an important pest in *Allium* spp. in Southeast Asian countries (Tran and Takagi, 2007) including Indonesia (Rauf et al., 2000). The existence of *L. chinensis* in Indonesia is designated as a quarantine organism of A2 quarantine plants according to Regulation of the Minister of Agriculture No. 93 / Permentan / OT.160 / 12/2011 on the Type of Quarantine Plant Pest Organism. It is important to know about the presence of these pests on the onion plants in Bali through the identification of morphological and molecular characteristics. Identification is a way of classifying an organism to a certain status whether it is a taxon or based on the economic losses it causes. For the purpose of identification of morphology of insect pests, it was performed through tracing the morphological characteristics of the antenna, wing vein characteristics, caput, thorax, limb color, scutellum color and abdominal tergite pattern and its attack on onion leaf (Shiao et al., 1991; Shiao, 2004). According to Malipatil et al. (2004) corroborating the morphological identification results can be done by identification of molecular characteristics.

Identifying the molecular characteristics of insect pests is done by looking at similarities of mitochondrial DNA (mtDNA), to trace the phylogenetic relations of the species. Phylogenetic analysis can represent a process of biological evolution of a species, from a species of simple organism to more complexity through the accumulation of change from generation to generation. To study genetic variation and genetic differentiation between species of organisms can be done by looking at differences in the number of polymorphic bases of a gene locus of each organism based on its DNA sequence (Cavalli-Sforza, 1997). Knowing the barcoding of DNA gradually is an effective tool for identifying species within a wide range of taxonomic groups. A genetic diagnosis using mtCOI and DNA16s can differentiate specifically among *Liriomyza* species. Detection by using Polymerase Chain Reaction (PCR) with specific primers based on mitochondrial cytochrome oxidase I (COI) is capable of producing rapid and specific detection (Yu et al., 2005).

This study focuses on identifying *Liriomyza's* morphological and molecular characteristics as well as connecting the phylogenetics of specimens of onion plant origin in the onion cultivation centers in Bali. Specifically this study is intended to describe the morphological and molecular characteristics of *Liriomyza* found in Bali, and state the phylogenetic relationship of *Liriomyza* found in several locations of onion plantations in Bali.

Material and Methods

Onion leaf samples showing symptoms of Liriomyza attack were taken from the study site, and then cultured in the laboratory (Supartha, 1998). Flies adult obtained from breeding were then identified based on the morphological characteristics at Integrated Pest Management Laboratory (IPMLab), Faculty of Agriculture, Udayana University, with reference to Shiao et al. (1991) and Shiao (2004). The observed morphological characters were the color of scutellum, the color pattern of abdominal targite, the shape of the antenna, and the color of the limbs.

The next step for the purpose of molecular analysis was the preservation of flies in pure ethanol. Each pest was stored in a freezer -20^{0}C until the specimen was required for isolation. Molecular fly identification was done through several steps: DNA isolation, DNA electrophoresis, PCR chain reaction, re-electrophoresis, and finally the sequencing method. Molecular identification of flies was done in Lab Genetics, Genetics Science, Indonesia.

DNA was visualized using horizontal electrophoresis using 1% agarose gel. After electrophoresis, the DNA was visualized in the UV transilluminator. A good PCR product was followed by a sequencing stage. DNA sequencing was performed to determine the order of nucleotides in the cox1 region. The results of the sequencing were put in NCBI's Basic Local Alignment Search Tool (BLAST) to see homology with nearby species. The phylogenetic tree was obtained by using neighbor-joining method.

Results and Discussion

Characteristics of *Liriomyza* morphology at Bedugul and Kintamani Bali

The *Liriomyza* flies that attack onion plantings on the island of Bali are characterized by morphological characters; a simple abdominal targite color pattern, absence of connecting line between segments and the next segment (Figure 1) and dark grey or near-black scutellum (Figure 2).

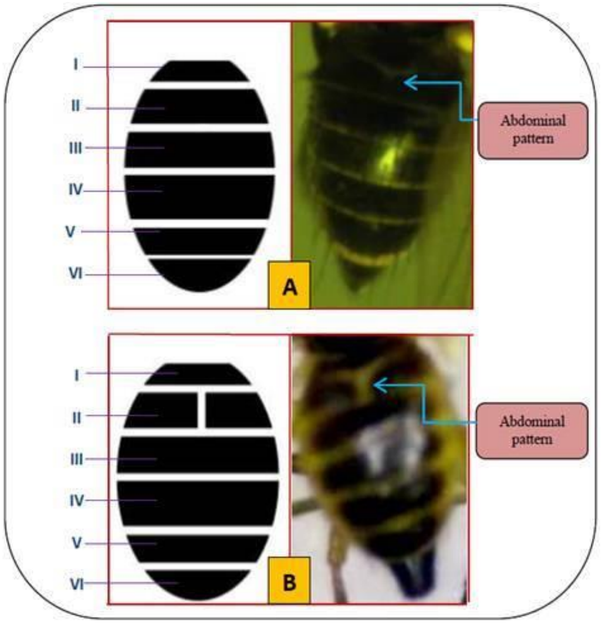

Figure 1. The morphological character of the color pattern is obtained on the abdomen of *Liriomyza* obtained from the study site, (A) Simple color pattern of abdominal targite *L. chinensis* (I-VI) with no connecting line between the segment and the next segment, (B) color patter of abdominal targite of *L. huidobrensis* (I-VI) has a connecting line between

Figure 2. Character of *Liriomyza's* scutellum morphology obtained from the study site (A) Imago *L. chinensis* adult is dark grey or close to black and (B) Imago *L. huidobrensis* adult is yellow

These morphological characteristics correspond to the characteristics of *L. chinensis* proposed by Shiao (2004) which state that the main morphological character for differentiating *Liriomyza* species are the color of the scutellum and the color pattern of the abdominal targite. Other features of *Liriomyza* found in some onion planting sites on the island of Bali are yellow, femur and tibia shiny yellow heads (Figure 3), average wingspan of 1.3-2.0 mm (Figure 4) and hollow larvae form starts from the tip of the leaf and then entering the center near the petiole (Figure 5).

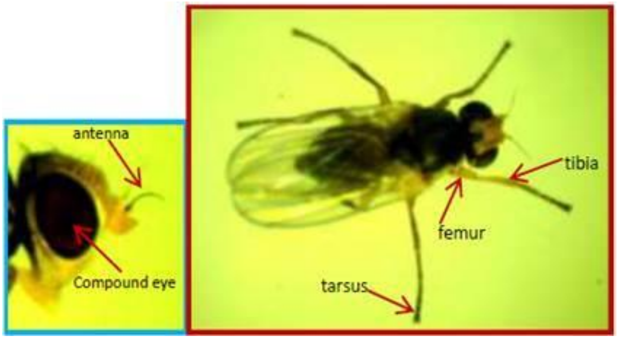

Figure3. Patterns of the head, antenna, compound eyes, femur, tibia and tarsus of *Liriomyza* obtained from the study site, with the characteristics of a yellow head,

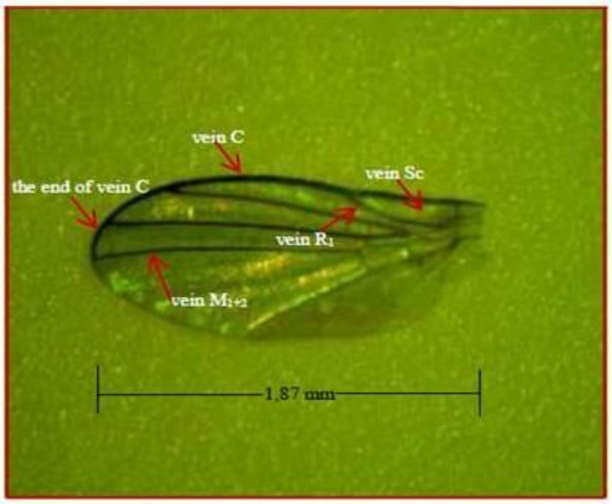

Figure 4. Right wings of female *Liriomyza* adult imago obtained from the study site, the average wing length is 1.3-2.0 mm

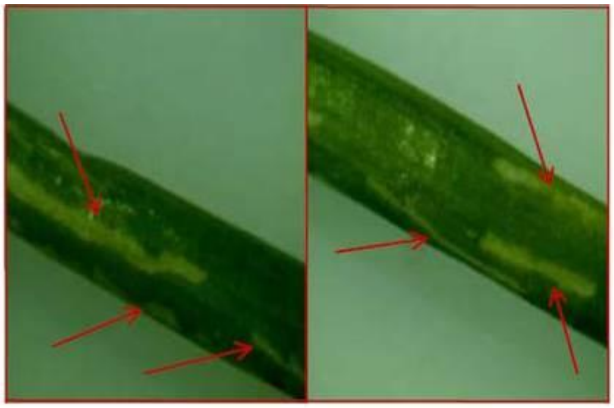

Figure 5. *Liriomyza* larvae (red arrow) on the red onion leaf, obtained from the research sites with the hollow larva form starting from the tip of the leaf and then entering the center near the petiole

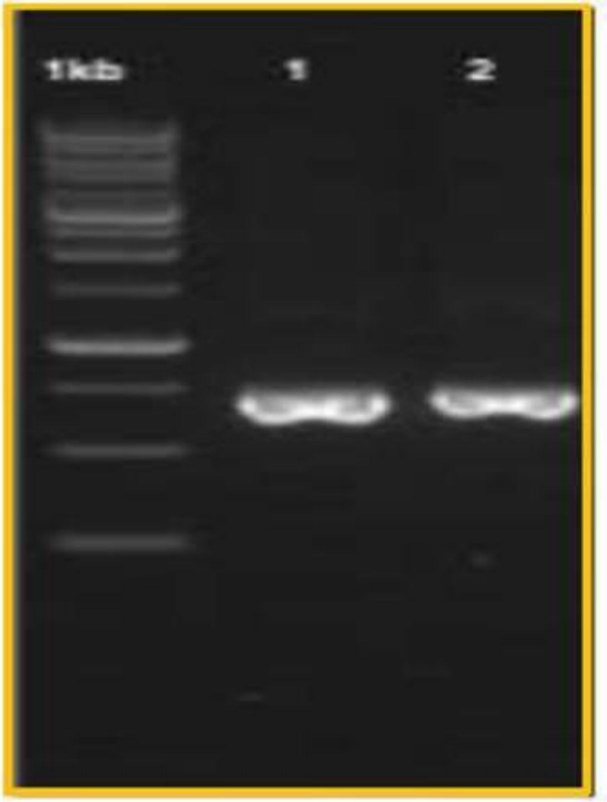

Figure 6. Result of DNA amplification,
(1) *Liriomyza* location Bedugul Bali and
(2) *Liriomyza* location Kintamani Bali, with PCR method using COI primer pair

Liriomyza molecular characteristics at Bedugul and Kintamani Bali

DNA isolation was performed to obtain the DNA template in the amplification process. The PCR process was successfully amplified as shown through electrophoresis results in figure 6. The electrophoresis results showed the presence of clear and thick DNA bands as evidence of successful amplification. The PCR results were desequenced to determine the sequence of nucleotides.

The results of *Liriomyza's* sequencing based on the COI genes were searched for homology in GenBank using BLAST software. Some *Liriomyza* species nucleic acid sequence data were found in GenBank data that had similarities to *Liriomyza* found at Bedugul and Kintamani Bali sites. The BLAST result obtained 100 data from GenBank which had identical level of ≥ 87% as shown in Figures 7 and 8. *Liriomyza's* sequence data in GenBank with similarity to sequence data obtained from Bedugul and Kintamani Bali sites were *L. chinensis* (EF104701.1), *L. chinensis* (AB721340.1) and *L. chinensis* (AB721343.1) with an identical rate of 99%. Whereas when compared with *Liriomyza* sp. (KR691019.1), *Liriomyza* sp. (KM864539.1) and *Liriomyza* sp. (KY831514.1) with an identical rate of 88%, and when compared with *L. huidobrensis* (JN570505.1), *L. huidobrensis* (FJ435888.1) and *L. huidobrensis* (EU219615.1) with an identical rate of 87%

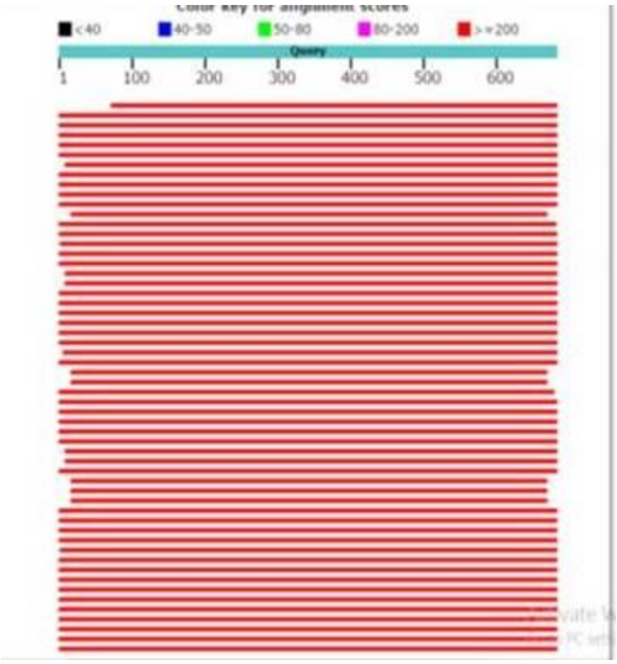

Figure 7. The similarity level of COI gene (682 bp) from *Liriomyza* location Bedugul Bali, with GenBank NCBI data (www.ncbi.nlm.nih.gov)

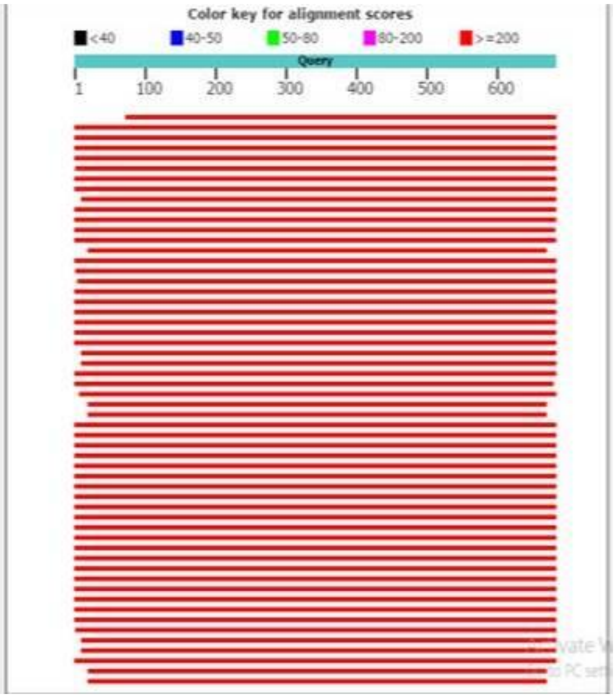

Figure 8. The similarity level of COI gene (684 bp) from *Liriomyza* Kintamani Bali location, with GenBank NCBI data (www.ncbi.nlm.nih.gov)

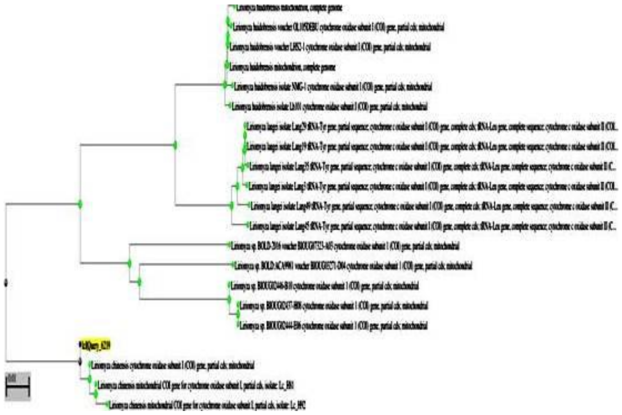

Figure 9. Phylogenetic tree of COI *Liriomyza* gene from Bedugul Bali location (IclQuery_6219), compared with data from NCBI with the closest resemblance and comparison (outsider) (www.ncbi.nlm.nih.gov)

The results of sequence alignment indicates that the *Liriomyza* COI nucleic acid from the Bedugul and Kintamani Bali sites showed the highest homology with *L. chinensis* (EF104701.1), *L. chinensis* (AB721340.1) and *L. chinensis* (AB721343.1) with identical levels of 99%. The genetic characteristics of *Liriomyza* from Bedugul and Kintamani Bali, based on homologous levels of COI gene DNA sequences and phylogenetic analyzes, have proximity of genetic characters with *L. chinensis* present in GenBank data, as indicated by the phylogenetic tree presented in ffigures 9 and 10. Genetic proximity based on the *Liriomyza* COI gene sequence from the Bedugul and Kintamani Bali sites, with existing data on GenBank, was believed to be supported by similar environmental and host plant factors. This is supported by the statement of Samie and Zaki (2011) stating that the analysis based on COI gene sequences on some insects shows similarities from several locations that have similar environmental and host plant conditions.

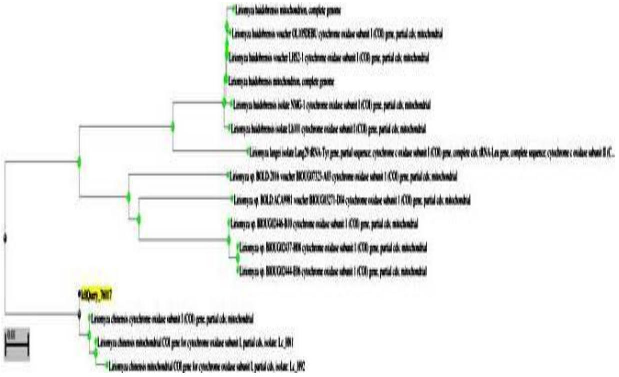

Figure 10. Phylogenetic tree of COI *Liriomyza* gene from Kintamani Bali location (IclQuery_76017), compared with data from NCBI with the closest resemblance and comparison (outsider) (www.ncbi.nlm.nih.gov)

Conclusion

Morphological characteristic of *Liriomyza* sp. found on this research showes a simple color pattern on a simple abdomen with no connecting line between the segments and the next segment, dark grey or near black scutellum. These characteristics refer to the species *L. chinensis*. GenBank data tracking results through identification of squamous alignment results indicate that the *Liriomyza* COI nucleic acid found showed the highest homology with *L. chinensis* (EF104701.1), *L. chinensis* (AB721340.1) and *L. chinensis* (AB721343.1) with identical rate of 99%. Based on the results then *Liriomyza* species found attacking onion plant on the island of Bali is *L. chinensis*.

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