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First Report of Stem Fly *Melanagromyza sojae* (Zehntner) Infesting Black Gram (*Vigna mungo* L.) in India

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ABSTRACT: The most severe pest, *Melanagromyza sojae* (Zehntner), severely damages crops at the seedling stage. The samples of black gram stem fly infestation came from the experimental plot set up at the Entomology Farm, B. A. College of Agriculture, Anand Agricultural University, Anand. The adult stem fly punctures the tissues of leaves with its ovipositor to lay its eggs. The first set of seedling leaves are favoured locations for oviposition and result in widespread tunnelling in young plants. Infested plants grow yellowish, stunted, and in severe situations, the pest completely destroys the crop as a result of the maggot feeding inside the stem after the egg hatches. If the injured plant lives, the damage will eventually show up in the older plants. When a plant is severely attacked, an infected leaf first hangs down before the plant begins to wilt and the leaves start to fall. The yield could be diminished if the stems crack. Finding an insect's species merely based on its molecular makeup is fairly challenging. In order to identify insect species, it is crucial to use a morphological key in addition to the molecular base. The species authentication and generation of DNA barcode for stem fly *M. sojae* infesting black gram was carried out through molecular characterization. Phylogeny analysis of stem fly AAU voucher specimen with the sequence of *M. sojae* obtained from NCBI database revealed the significant similarity with one specimen of *M. sojae viz*. NCBI accession no. MF441483.The identification and verification of the specimen as a stem fly, *M. sojae* infesting black gram, appears to be supported by the detailed molecular characterization of Cytochrome Oxidase subunit I (COXI). In India, this is the first report citing *M. sojae* as stem fly infesting black gram.

Keywords: Stem fly, Black gram, *Melanagromyza sojae,* tunneling and COXI.

INTRODUCTION

Black gram is one of the most significant pulse crops in India and is said to have originated there. Black gram is a high-protein food. It is mostly grown in Andhra Pradesh, Bihar, Madhya Pradesh, Maharashtra, Uttar Pradesh, West Bengal, Punjab, Haryana, Tamil Nadu, and Karnataka. In 2018-19, Gujarat produced 73560 tonnes of black gram with a yield of 669 kg/ha from an area of 1,09,960 hectares. Gujarat's key black gram producing districts are Sabarkantha, Panchmahal, Dahod, Vadodara, Mehsana, and Bharuch. It is also grown in Rajkot, Surendranagar, and Junagadh (Anonymous, 2019). Many factors contribute to black gram's low productivity. The insect-pest is one of the principal constraints in agricultural production, with the stem fly, *Melanagromyza sojae* (Zehntner), being the most severe pest, inflicting substantial damage to the crop's seedling stage. In India, 60 insect species have been identified as attacking black gram at various stages of development (Lal and Sachan 1987). Yield loss due to stem fly varies depending on location and plant growth stage. *M. sojae* produced 100% infestation and 33.84% stem tunnelling in soybean in Pantnagar, Uttarakhand, according to Gaur *et al*. (2015). In India, it has been documented in Delhi (Singh *et al*., 1979), Karnataka (Jayappa *et al*., 2002), Madhya Pradesh (Singh and Singh 1990), Maharashtra (Taware *et al*., 2001) and Rajasthan (Meena and Sharma, 2006) in soybean while in leguminous crops from Uttar Pradesh (Singh, 1982) and in mung bean from Uttarakhand (Srivastva and Sehgal 2002). There is a scarcity of data on the DNA sequencing of *M. sojae* infesting black gram, particularly in Gujarat. As a result, the current research was done with the goal of establishing a morphological and genetic basis for identifying the stem fly infesting black gram.

MATERIALS AND METHODS

M. sojae was found infesting black gram at the Entomology Farm, B. A. College of Agriculture, Anand Agricultural University, Anand (Gujarat), despite the fact that the plot was not protected by any plant protection measures. The plants that have been assaulted are showing signs of withering. At the plant's collar, the stems appear cracked. Twenty randomly selected plants were plucked from the plot and brought into the laboratory for stem fly identification. Each plant's stem was dissected using a knife and pupa (e) present in the stem was collected and emerged to adult in the laboratory. Ten adult stem fly infesting black gram specimens were collected and stored in 70% alcohol. Further, collected specimens were sent toDr. K. J. David, Senior Scientist, Division of Insect Systematics, Indian Council of Agricultural Research-National Bureau of Agricultural Insect Resources, Bangalore-560024, Karnataka. Adult stem flies were also preserved at -20 °C until DNA was isolated. QIAGEN DN easy blood and tissue kit (Qiagen) was used to extract DNA from the entire body according to the manufacturer's recommendations. The COXI (cytochrome oxidase subunit I) gene was amplified using the usual PCR technique. Forward primer (LCO 1490 5'-GGTCAACAAATCATAAAGATATTGG-3') and reverse primer (HCO 2198 5'-TAAACTTCAGGGTGACCAAAAAATCA-3') were used for amplification. The ABI 3500 Genetic Analyzer (Applied Biosystem, USA) was used to sequence the amplified products. Using a BLAST search at NCBI, the retrieved consensus sequences were utilized to identify species. The COXI sequences obtained in this investigation were aligned in MEGA6 using the CLUSTALW programme. MEGA6 software was used to infer evolutionary connections (Tamura *et al*., 2013). The phylogenetic tree was built using the Neighbour joining technique. This work on DNA barcoding was done at Anand Agricultural University's AICRP on Biological Control of Crop Pests.

RESULTS AND DISCUSSION

Nature of damage of *M. sojae*: The unifoliate or early trifoliate leaf stage is when the insect invasion starts. The initial pair of leaves of bean seedlings are preferred locations for oviposition by the adult stem fly (Fig. 1) and this causes widespread tunnelling in young plants. Infested plants turn yellowish, stunted, and in extreme situations, the pest may completely destroy the bean harvest because the larvae eat inside the stem (Fig. 2). Before the presence of stem flies is confirmed, the symptoms of a stem flies attack resemble those of a cut off of the food supply or root rot disease. In severe infestations, infected leaves hang down at first before wilting and sometimes dropping. The stems are brittle and have a limited yield (Fig. 3). The yield loss varies depending on the location and stage of plant growth at the time of infection.

Egg: Eggs are inserted in punctures in the leaf tissues, either in fully opened trifoliate leaves at the base of the leaf lamina close to the petiole or in unifoliate leaves if the plant only has two leaves. The upper surface of the leaves had several feeding punctures made in them. The egg is 0.15 ± 0.01 mm wider and 0.34 ± 0.02 mm longer than usual. The egg is translucent and somewhat white.

Maggot: The maggot emerges and immediately begins to tunnel through the mesophyll tissue into the next vein before disappearing downward in the leaf and finally tunneling through the petiole to reach the stem. Maggots tunnel through the stem to the junction of the root and shoot. The initial tunnel is widened as it digs deeper into the thicker tap root before turning around and moving upward into the pith. It consumes the xylem and phloem tissues to reach the epidermis, where it creates a hole that it seals with debris and pupates.

Pupa: The pupa is 2.75 mm long and 1.00 mm broad and it is golden yellow in colour. Pupa is always found in the pith tunnel, frequently at the level of young plants' unifoliate leaves, and generally close to the fly escape hole, where it appears as a dark depression.

Adult: The fly has damp, disintegrated wings and very little coloration on its legs and abdomen. For the first 30 minutes, the body wall and legs gradually become darker and harder. The fly quickly acquires its shiny black coloring and starts looking for host plants.

Molecular characterization of *M. sojae*

COXI amplification. The COXI gene amplified fragments were examined using 1.5% agarose gel electrophoresis. The single amplicon of COXI gene of size 650 bp was visualized during the analysis (Fig. 4). The consensus sequence COXI gene of black gram stem fly has been deposited in NCBI GenBank database (NCBI accession no. OK564405)

>Blackgram stem fly, AAU-Anand COXI gene partial cds: mitochondrial with GenBank accession No. OK564405 released on 25-10-2021

AAAGATATTGGAACTTTATATTTTATATTTGGA
GCTTGAGCTGGAATAGTTGGAACTTCATTAAGA
ATTCTAATTCGAGCTGAATTAGGACATCCTGGT
GCTTTAATTGGTGATGACCAAATTTATAATGTA
ATTGTAACTGCTCATGCATTTATTATAATTTTTT
TTATAGTTATACCTATCATAATTGGAGGATTTG
GTAATTGATTAGTACCATTAATATTAGGGGCAC
CAGATATAGCATTTCCTCGAATAAATAATATAA
GTTTTTGACTTTTACCTCCTGCATTAACTTTATT
ATTAGTAAGTAGAATAGTAGAAAACGGAGCTG
GAACTGGATGAACAGTTTACCCTCCTTTATCTT
CAGTAATTGCTCATGGAGGGGCATCTGTCGATT
TAGCTATTTTTTCTCTTCATTTAGCTGGTATCTC
ATCTATTTTAGGAGCAGTAAATTTTATTACTAC
TGTAATTAATATACGATCAACTGGAATTACATT
TGATCGAATACCATTATTTGTTTGATCTGTATTT
ATTACAGCATTTTTATTATTACTTTCTTTACCTG
TATTAGCTGGTGCAATCACTATACTATTAACAG
ATCGAAATTTTAATACATCATTTTTTGACCCTG
CGGGAGGAGGAGATCCTATTTTATATCAACATT
TATTTTGATTTTTTG

Sequence analysis and retrieval from NCBI database. When the consensus sequence of COXI gene of stem fly specimen was checked for homology using NCBI-BLAST finder, the sequence showed

resemblance to various COXI gene of dipteran insects in NCBI database. The identification report of the specimen based on morphological keys was received from ICAR-National Bureau of Agricultural Insect Resources (ICAR-NBAIR), Bengaluru, India and it was described as *Melanagromyza sojae*. Further, the species authentication through NCBI-BLAST program was carried out by limiting the query coverage to *Melanagromyza* sp. The three specimens of *Melanogromyza* sp. of NCBI database with accession nos. KR661203, KR655154 and KR645071 showed the similarity ranging from 87.84 % to 87.99 %. Further, the species *Melanagromyza obtuse* was the major species of *Melanagromyza* showing the similarity ranging from 87.12 to 88.04%. (NCBI accession no. KY833742, KY843749).

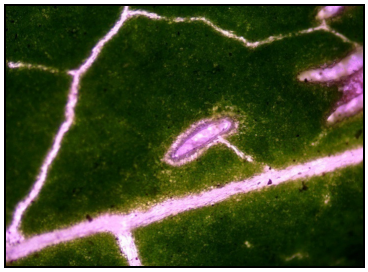
Based on the morphological identification report of the specimen as *M. sojae*, the available sequences of COXI gene of *M. sojae* were retrieved from NCBI database to compare with our isolated sequence. There were ten COXI gene sequences of *M. sojae* found in NCBI GenBank database (MK490675, MK490676, MF441480, MF441481, MF441482, MF441483, MF441484, MF441485, MF441486 and MF441487). All these sequences were submitted as the first report of soybean stem fly, *M. sojae* in Bolivia, South America. Further, the phylogenetic analysis of black gram stem fly specimen sequence obtained in the present study with the sequences of *M. sojae* obtained from NCBI GenBank was carried out. Neighbor-Joining method was used to construct evolutionary tree. The sequence of *M. sojae* from the present study shared the clade with the sequence of NCBI database isolate (GenbAnk accession no. MF441483) (Fig. 4). However, significant variations were noticed in COXI sequence of black gram stem fly specimen and the NCBI database isolates in CLUSTALW sequence alignment programme. The findings of molecular characterization and phylogeny studies comprehensively supports the morphological identification of the specimen as the first report of stem fly, *M. sojae* infesting black gram in India. Further, study clearly shows the availability of DNA tools to facilitate and complement traditional taxonomic studies. This combination serves as model that can be applied as integrated approach in taxonomy for the fast-track identification, authentication of insect pests.

(A) Mating

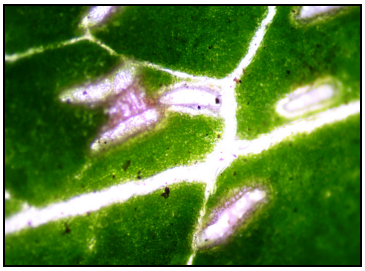
(B) Egg laying

(C) Most preferred stage of crop for oviposition

(D) Pin prink appearance on leaf



(E) Egg in between two epidermal layers



(F) Entry of early instar maggots in leaf vein

Fig. 1. Ovipositional behavior of *M. sojae* infesting black gram.

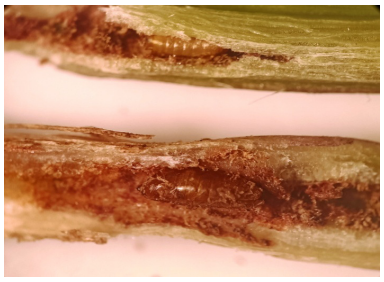
(A) Maggot inside the stem

(B) Maggot under microscope

(C) Pupa

(D) Adult

Fig. 2. Different life-stages of *M. sojae* infesting black gram.



(A) Stem tunneling

(B) Infested plant get easily detach

(C) Damage on collar region of the stem

(D) Tunneling inside the branches

(E) Severe infestation leads to death of seedlings

Fig. 3. Nature of damage caused by *M. sojae* in black gram.

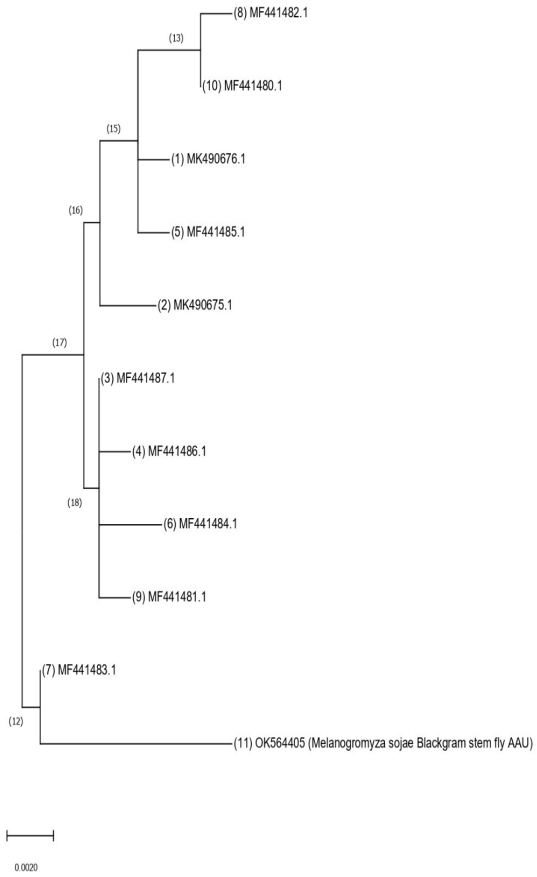


Fig. 4. Phylogenetic tree constructed for *Melanagromyza sojae* of present study and species retrieved from Genbank by Neighbor-Joining method.

CONCLUSIONS

The findings of morphological and molecular characterization studies confirms the species as *M. sojae* and this is the first confirmed report of the stem fly, *Melanagromyza sojae* (Zehntner) (Diptera: Agromyzidae) infesting black gram in Gujarat, India. The extensive molecular characterization of Cytochrome Oxidase subunit I (COXI) apparently supports the identification and authentication of the specimen as stem fly, *M. sojae*. In India, this is the first report citing *M. sojae* as stem fly infesting black gram. Present days, *Melanagromyza sojae* is becoming a major pest of several bean crops. Its management should start shortly after germination since, being a dipteran pest, it can cause significant damage to crops before the stems of the plants harden. Therefore, future management approaches should concentrate on the insect's aforementioned vulnerable point.

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Conflict of Interest. None.

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