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Expression of Glutamine Synthetase Leaf Isozyme (GS2) gene in eggplants infected with Eggplant mild leaf mottle virus that treated with Effective microorganism-1

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Abstract

Eggplant has been recognized as an important host for a number of viruses, particularly a new emerging virus known as Eggplant mild leaf mottle virus (EMLMV). The purpose of the study was to determine how effective microorganism-1 (EM-1) affects the expression of the Glutamine Synthetase Leaf Isozyme (GS2) gene in eggplants infected with EMLMV. Effective microorganisms applied before (BE2), within (WE2) and after (AE2) artificial infection with EMLMV and the treatments analyzed using RNA seq and bioinformatics. The symptoms of AE2-treated plants developed after 15 days, and when EM-1 was given, the bulk of the virus effect was reduced, and the injured plants recovered. While the symptoms of BE2 were less severe than those of AE2, they did subside after seven days, and the plants recovered completely. In the case of WE2, there were no symptoms, and the plant appeared healthy. The mapping to the GS2 gene sequence revealed a higher number of assembled reads in the AE2 and BE2 treatments (30,748 and 29,061 respectively), whereas WE2 produced less mapped reads (18,136). The negative control CN received 10,653 reads, whereas the positive gained the fewest assembled reads (6,446 reads).

Keywords : *Eggplant, Eggplant mild leaf mottle virus, GS2 gene , Effective microorganism-1.*

I. Introduction

Eggplant and its close wild relatives are resistant to a variety of diseases (Daunay, 2008; Syfert et al., 2016). In cultivated aubergine, resistance introgression breeding from related species has been limited till recently (Daunay, 2008). While the majority of Solanaceae crop plants are from the New World (tomato, potato, and pepper), aubergine and its wild relatives come from the Old World. The ancestor of aubergine was most likely East African and moved to Asia via the Middle East, either naturally or as a result of human migrations (Weese and Bohs 2010). The lengthy co-evolution of aubergine and its Old World diseases may explain why specific resistance genes are absent from New World solanaceous crops (Hirakawa et al., 2014). Eggplant, like tomato and pepper, is an autogamous diploid species with 12 chromosomes and a genomic size of around 1.1 Gb (Arumuganathan and Earle, 1991). Despite its agricultural importance, aubergine genetics and genomics are less well documented than other key solanaceous crops. The recent publication of the first sequenced draft genome of eggplant (Hirakawa et al., 2014) and the lowering cost of high-throughput sequencing methods (HTS) make it easier to create high-resolution maps for this species. Since 1998, a new disease with mosaic symptoms that caused by Eggplant mild leaf mottle virus has been observed in Iraq. It was first identified in eggplant fields south of Baghdad. The symptoms were clearly visible on the fruit and leaves, resulting in fruit deformation and leaf mottling. In the tested aubergine fields, the infection ratio ranged from 1 to 80% (Khafajah et al., 2022). Plant glutamate metabolism (GM) is essential to the metabolism of amino acids, coordinates vital metabolic processes, and plays important roles in plant defense against pathogens. These functions fall under three main categories: cellular redox regulation, tricarboxylic acid cycle-dependent energy reprogramming, and nitrogen transport via the glutamine synthetase and

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glutamine-oxoglutarate aminotransferase cycle. The host GM is significantly changed during contacts with pathogens, resulting in either a metabolic state known as "endurance," which preserves cell viability, or an opposing metabolic state known as "evasion," which promotes cell death. While evasion-related reconfigurations lead to resistance to biotrophic infections but encourage necrotroph infection, endurance-natured modulations appear to produce resistance to necrotrophic pathogens and vulnerability to biotrophs. However, in order to take advantage of the plant GM, pathogens have developed tactics including hemibiotrophy, toxin release, and selective amino acid use. When taken as a whole, changes in the host GM in response to various pathogenic situations seem to work in two opposed ways: either supporting the current defense strategy to eventually form an effective resistance response, or being used by the pathogen to encourage and aid infection. It has been discovered that using both pure and mixed cultures of microorganisms can digest phenol, making them a safer option. In the case of mixed substrates, Crossed inhibition and substrate competition could negatively impact the growth of microorganisms and rates of biodegradation (Allsop et al., 1993; Bouchez et al., 1995; Wang and Loh 1999). When the combined substrates are connected structurally, phenomena like interference Repression is catabolized during enzymatic induction. mechanisms, rivalry for the active locations of enzymes, and the co-substrates' toxicity could take place. In these situations, the concerned Microbial consortia's activities have an advantage control individual microbes since it has accelerating the rate of biodegradation and elimination effectiveness of combined pollutants (Yuan et al., 2000; Gianfreda et al., 2006). According to Pohl *et al.* (2019), bio-stimulated eggplant roots were able to absorb more nutrients and successfully penetrate deeper into the soil. A recent analysis examined the critical role that host primary metabolism plays in plant defense mechanisms (Bolton 2009). However, there is currently a dearth of molecular information on the function of central C/N metabolism in plant defense systems against various pathogenic behaviors (Liu et al. 2010). **The present work reported the role of effective microorganism-1 (EM-1) in the expression of Glutamine Synthetase Leaf Isozyme (GS2) gene in eggplants infected with Eggplant mild leaf mottle virus.**

II. Materials and Methods

Virus isolation and plant breeding

Plant samples of eggplant exhibiting clear viral symptoms, including mosaic, mottling, and stunting, were collected from eggplant fields in the Jableh in Babylon and Al-Abbasiya in Najaf districts. The infected samples were placed in clean plastic bags and then transported to the laboratory for the preparation of a viral inoculum.

To provide eggplants for infection, the seeds were grown in a growth chamber under controlled environmental conditions, including a 16-hour light/8-hour dark period, 60–70% relative humidity, and a temperature of 30 ± 5°C (Figure.1).

Figure.1 The treatment of EMLMV-infected eggplants, before, with and after infection with EM-1.

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Field Experiment

The field experiment was carried out at the College of Agriculture - University of Kufa inside a plastic greenhouse with an area of about 200 m² (20 meters long × 10 meters wide). The experiment started on 12/1/2025 and continued until 8/5/2025. It included 5 replicates for each treatment, and the Randomized Complete Block Design (RCBD) was applied to reduce the impact of environmental factors and achieve accurate results that are statistically analyzable (Table1).

EM-1 activation

500 ml of EM-1 (mother culture produced in Taiwan and imported from United Arab Emirates) was taken, and 300 ml of molasses (date syrup) was added as an energy source for the bio-microbes. Water was then added gradually until the total volume reached 10 liters (i.e., by adding 9.2 liters of water). The mixture was thoroughly mixed and then transferred to an airtight container and placed at room temperature. The activation process took place for a full week, after which the fertilizer was ready for use.

Preparing Plants for the Experiment

This study was conducted in a greenhouse containing eggplant (the hybrid variety Barcelona). The plants were prepared for the experiment, and work began on January 5, 2025. The activated EM-1 was distributed to 30 plants at a rate of 50 ml per plant using a graduated container to ensure accuracy. On the same day, the viral inoculum was prepared using the mechanical inoculation method. This involved mixing the inoculum with a a Carborundum before applying the mixture on the treated leaves**.**

Sample Preparation

The selected leaves of each treatment were cut into small pieces measuring 0.5 cm x 0.5 cm. Each piece was placed in a 10 ml tube, completely filled with RNA lattice solution, labeled with a specific code, sealed with parafilm, and sent to JS-Link in South Korea for RNA sequencing (Table 1).

Table 1 Treatments chosen for RNA sequencing analyses

No	Treatment	Description	Plants number
1	AE2	Plant first treated with the virus, then fertilizer	5
2	BE2	Plant first treated with fertilizer, then infected with the virus	5
3	WE2	Plant treated with both the virus and fertilizer simultaneously	5
4	CN	Healthy plant (negative control sample)	5
5	CP	Plant infected only with the virus (positive control sample)	5

High-throughput sequencing

The library was prepared within JS-Link company, Republic of Korea, utilizing TruSeq total RNA library preparation kits (Illumina, San Diego, CA, USA). The RNA sample's quality was assessed using a 2100 Expert Bioanalyzer manufactured by Agilent Technologies (Santa Clara, CA, USA). Subsequently, the RNA sample was sequenced using NovaSeq X with 2 × 101 PE reads to obtain the total RNA sequence. The raw RNA reads were subjected to Trimmomatic v 0.40 and then BBDuk tool in the Geneious prime to trim low-quality reads and produce clean and high-quality reads.

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Map to reference

Geneious RNA mappers (medium-low sensitivity) were used to map RNAseq data to the reference sequences of Mi1 genes. Contig was generated and the consensus sequence was extracted and then analyzed by Blast search to confirm identity.

III. Results

The symptoms of AE2-treated plants appeared after 15 days, and when EM-1 was administered, the majority of the virus effect was reduced, and the damaged plants recovered. While the symptoms of BE2 were not as severe as those of AE2, they did diminish after seven days and the plants had fully recovered. In the instance of WE2, there were no symptoms, and the plant appeared to be healthy. The mapping against GS2 gene sequence showed higher number of assembled reads in the treatments of AE2 and BE2 (30,748 and 29,061), while WE2 obtained lower mapped reads with only 18,136. Negative control CN got 10,653 reads, while the positive obtained lowest assembled reads with 6,446 reads. The coverage was complete over all treatments (Table 2; **Figure 2**).

Table 2 The assembled reads of the five treatments against the GS2 gene.

Gene ID	Function	CP	CN	AE2	BE2	WE2
GS2	Glutamine Synthetase Leaf Isozyme (GS2)	6,446	10,653	30,748	29,061	18,136

Figure.2 The mapping of the five treatments data against GS2 gene showing complete coverage and variable assembled reads over the gene sequence**.**

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III. Discussion

Different invasion tactics have been created by the dynamic co-evolutionary struggle of phytopathogens and plants over the host's nutritional resources. While necrotrophs favor dying tissues, biotrophic pathogens require living cells to provide resources for their growth and reproduction. Conversely, plants have developed complex strategies to deny nourishment to pathogens. A resistant plant can use a variety of defense mechanisms to stop the spread of pathogens when it detects the presence of harmful bacteria in a timely manner. These tactics include *de novo* synthesis of different anti-microbial chemicals (e.g., pathogenesis associated proteins or phytoalexins) and the construction of physical barriers (e.g., reactive oxygen species [ROS]-dependent cross linking of structural proteins in the cell wall). Through transcriptional profiling investigations, the regulation of genes governing these well-established defense systems has been thoroughly investigated, particularly on incompatible plant-pathogen interactions. These gene expression investigations frequently reveal significant changes in the transcriptional levels of genes encoding core metabolism-related pathways, indicating potential functions for host central carbon/nitrogen (C/N) metabolism in connection to defensive mechanisms. Numerous investigations have confirmed the connection between plant-pathogen interactions and primary metabolism (Berger et al. 2007). Recent research has reexamined the critical role that host primary metabolism plays in plant defense mechanisms (Bolton 2009). However, there is currently a dearth of molecular information on the function of central C/N metabolism in plant defense systems against various pathogenic behaviors (Liu et al. 2010). In both greenhouse and field experiments, a culture mix of three nitrogen-fixing bacteria species of the genus *Azospirillum* (*A. brasilienses* N040, *A. brasilienses* SP7, and *A. ipoferum* MRB16) and one strain of cyanobacteria (*Anabena oryzae* Fritsch) was used as a biofertilizer mixture to address the issue of the yield reduction caused by the viral satellite-mediated protection. In a greenhouse and field trial, the fruit production of protected plants treated with biofertilizer combinations rose by 48% and 40%, respectively, as compared to untreated plants inoculated with the protective viral strain alone(Dashti et al., 2007). Here, we looked at the GS2 gene's transcriptional response to the application of microorganisms, which revealed a significant impact both before and after the addition of EM1. Additionally, there is a significant reduction in the severity of the symptoms of EMLMV. **After being infected with WMV, squash plants injected with bacterial strains showed notable decreases in disease severity in pot studies. In particular, foliar sprays of *B. mycoides* (87%), *B. thuringiensis* (73%), *Paenibacillus glucanolyticus* (73%), *Niallia circulans* (70%), *B. paramycoides* (65%), and *Pseudomonas indica* (65%) reduced the severity of the disease. Furthermore, plants treated with *B. mycoides* exhibited higher shoot dry weight and plant height, suggesting improved growth performance in comparison to infected controls (Bashandy *et al*., 2025).** Among the microbial biostimulants that have effectively activated plant systemic resistance mechanisms are bacteria that promote plant growth. These biostimulants enhance nutrient uptake while fortifying the plant's natural defenses against viral diseases. Recent studies have shown that some PGPR strains can become systemically resistant in a range of crops, reducing the effects of infections caused by viruses such as *Cucumber mosaic virus* (CMV) and *Tobacco mosaic virus* (TMV) (Khalid *et al*., 2025).

Disclosure statement

The authors did not disclose any potential conflicts of interest.

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