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Expression of psbA gene in watermelon infected with Cucumis melo amalgavirus 1 that treated with Effective microorganism-1

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Abstract

Watermelon (*Citrullus lanatus*) is an important horticultural crop and is susceptible to various viral infections, particularly *Cucumis melo amalgavirus 1* (CmAV1), a persistent virus associated with reduced growth and physiological stress. The aim of this study was to determine the effect of effective microorganism-1 (EM-1) on the expression of the psbA gene, which encodes the D1 protein of Photosystem II, in watermelon infected with CmAV1. EM-1 was applied before and after viral inoculation, and leaf samples were analyzed using RNA sequencing (RNA-seq using technique HTS) and bioinformatics. The results showed differences in psbA expression among treatments and between two hybrids, Sultan and Lord. The highest mapped reads were observed in T4 (Sultan) and T9 (Lord), indicating improved gene expression in EM-1-treated plants. Pairwise identity was consistently high (99.2–99.5%), with complete coverage of the reference sequence. These findings suggest that EM-1 can moderate viral stress, improve photosynthetic gene expression, and potentially enhance plant performance under CmAV1 infection.

Keywords: Watermelon, CmAV1, psbA gene, Effective microorganism-1, Photosynthesis, HTS

I. Introduction

Watermelon *(Citrullus lanatus)* is a most important cucurbit crop worldwide, valued for its nutritional quality, fruit sweetness, and economic importance. However, watermelon production is frequently limited by viral diseases, which can reduce vegetative growth, yield, and fruit quality (Sabanadzovic et al., 2009; Hull, 2014). Viral infections disrupt plant physiological and metabolic processes, often leading to chlorosis, mosaic symptoms, and impaired photosynthetic efficiency.

Among the emerging viruses affecting cucurbits, *Cucumis melo amalgavirus 1* (CmAV1), a member of the family Amalgaviridae, has recently been reported in several cucurbit crops. CmAV1 is associated with persistent infections that are often asymptomatic, yet may reduce plant vigor and productivity over time (Park et al., 2011; Nibert et al., 2016). Although CmAV1 was detected in most samples, its molecular impact on watermelon plant cells remains poorly understood, which may be attributed to its persistent and typically asymptomatic infection strategy (Zhan et al., 2019; Kavalappara et al., 2022).

Photosynthesis is one of the primary physiological processes affected by viral infection. Viruses can impair chloroplast structure and Photosystem II (PSII) function, reducing electron transport and generating reactive oxygen species (ROS) (Bhattacharyya and Chakraborty, 2018; Zhao et al., 2016). The psbA gene encodes the D1 protein, a core component of PSII, which plays a key role in photochemical reactions and in the PSII repair cycle under stress. The D1 protein is highly susceptible to damage and undergoes rapid turnover, making its regulation essential for maintaining photosynthetic efficiency (Nelson and Yocum, 2006; Komenda et al., 2012). Disruption of psbA expression or D1 protein function can result in photoinhibition, oxidative stress, and decreased plant productivity.

Sustainable approaches such as microbial biostimulants have gained attention for enhancing plant growth and stress tolerance. Effective Microorganisms (EM-1) is a consortium of beneficial bacteria and fungi that can improve soil fertility, nutrient uptake, plant metabolism, and systemic resistance against pathogens (Higa and Parr, 1994; Vessey, 2003). Studies have shown that microbial biostimulants can modulate gene expression related to photosynthesis, nitrogen metabolism, and stress response, suggesting a potential role in

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maintaining psbA function under stress conditions (Bolton, 2009; Rouphael and Colla, 2020; Khalid et al., 2025).

Given the importance of PSII function and the potential of EM-1 to enhance plant resilience, this study aimed to investigate the effect of EM-1 on psbA gene expression in two watermelon hybrids, Sultan and Lord, under CmAV1 infection. Understanding these interactions can provide insights into the physiological and molecular mechanisms underlying plant defense and the application of microbial biostimulants in crop protection.

II. Materials and Methods

Virus isolation and plant preparation

Leaf samples from watermelon plants exhibiting mosaic, chlorosis, and stunted growth were collected from infected fields. The samples were placed in sterile plastic bags, transported to the laboratory, and homogenized to prepare viral inoculum. Mechanical inoculation was applied by using carborundum to healthy seedlings to establish experimental infection. Seeds of two watermelon hybrids, Sultan and Lord Hybrids were planted in a growth chamber under controlled conditions: 16-hour light / 8-hour dark photoperiod, 60–70% relative humidity, and 28 ± 2°C. Seedlings were grown until the 3–4 true leaf stage before EM-1 treatment and viral inoculation (Figure 1).

Experimental design

The experiment was conducted using a Randomized Complete Block Design (RCBD) with three replicates per treatment. Each replicate contained nine plants. Treatments were applied as shown in Table 1. Samples were selected randomly and send to the company for sequencing using High-Throughput Sequencing (HTS refers to sequencing technologies that can **rapidly read millions of DNA or RNA fragments** simultaneously).

Table 1. Treatments chosen for RNA sequencing analyses

Hybrid	No.	Description	Treatment	Plants number
sultan	1	negative control (no virus, no EM1)	T1	9
	2	Plant treated with the EM1	T2	9
	3	Plant treated with virus	T3	9
	4	Plant first treated with the virus, thenEM1	T4	9
	5	Plant first treated with EM1, then infected with the virus	T5	9
lord	6	negative control (no virus, no EM1)	T6	9
	7	Plant treated with the EM1	T7	9
	8	Plant treated with virus	T8	9
	9	Plant first treated with the virus, then EM1	T9	9
	10	Plant first treated with EM1, then infected with the virus	T10	9

EM-1 activation

EM-1 fertilizer was set by mixing 1 liter of EM-1 stock solution with 1-liter molasses as an energy source. The volume was completed to 20 liters with RO water, mixed thoroughly, and incubated at room temperature for ten days before application.

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RNA extraction, library preparation, and High-throughput sequencing

Leaf samples (0.5 × 0.5 cm) from each treatment were placed in RNA later solution, labeled, and shipped to JS-Link, South Korea, for RNA sequencing. RNA extraction was performed using standard protocols. Library preparation used TruSeq Total RNA kits (Illumina, San Diego, CA, USA), and sequencing was performed on NovaSeq X (2 × 101 PE reads). Raw reads were trimmed using Trimmomatic v0.40 and BBDuk in Geneious Prime to obtain high-quality reads.

Mapping and bioinformatics analysis

RNA-seq reads were mapped to *Citrullus lanatus* plastid, complete genome (gene bank accession number: KY430688 - psbA gene) using Geneious RNA mapper with medium-low sensitivity. Contigs were assembled and consensus sequences were confirmed via BLAST analysis. Read counts were used to assess expression levels.

III. Results

The expression of psbA varied among treatments and hybrids. In the Sultan hybrid, mapped reads ranged from 2,522,040 (T3) to 12,149,606 (T4). In the Lord hybrid, mapped reads ranged from 2,957,821 (T6) to 10,029,783 (T9). Pairwise identity ranged from 99.2% to 99.5%, with all samples showing 100% reference coverage (Table 2, Figure 1).

The results in Table (2) demonstrate a clear treatment-dependent effect on psbA gene expression across both hybrids (Sultan and Lord), reflecting differences in physiological responses to viral infection and fertilizer application. In both hybrids, the negative controls (T1 and T6) exhibited baseline read counts, while fertilizer-only treatments (T2 and T7) showed a noticeable increase in psbA reads, suggesting enhanced photosynthetic activity and chloroplast function under nutrient supplementation. In contrast, virus-infected plants (T3 and T8) displayed reduced read counts relative to fertilizer treatments, indicating that viral infection negatively affects psbA expression, likely due to stress-induced impairment of photosystem II.

Interestingly, the highest psbA expression levels were observed in treatments where plants were first infected with the virus and subsequently treated with fertilizer (T4 and T9), with read counts reaching 12,149,606 and 10,029,783, respectively. This pattern suggests a potential compensatory or recovery response, where fertilizer application may mitigate viral stress and stimulate photosynthetic gene expression. Similarly, treatments involving fertilizer application prior to viral infection (T5 and T10) also showed elevated psbA levels compared to virus-only treatments, indicating a possible priming effect that enhances plant tolerance to subsequent infection.

Despite these quantitative differences in read counts, the pairwise identity values remained consistently high (99.2–99.5%) across all treatments, with 100% similarity to the reference sequence, confirming that the observed variation is not due to sequence divergence but rather differential gene expression. Overall, these findings highlight the critical role of nutrient management in modulating plant responses to viral stress and suggest that fertilizer application, particularly post-infection, can partially restore photosynthetic efficiency in infected plants.

EM-1 treatment before or after infection (T4, T5,T9,T10) regularly led to higher psbA expression in both hybrids, suggesting a protective effect on PSII function, while simultaneous or untreated samples had lower read counts, showing viral interference with photosynthetic gene transcription.

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Table 2. Assembled reads of psbA gene in two watermelon hybrids

Hybrid	Treatment	Reads / psbA gene	Pairwise identity (%)	Reference seq (%)
Sultan	T1	2,644,580	99.3	100
	T2	4,023,391	99.4	100
	T3	2,522,040	99.3	100
	T4	12,149,606	99.5	100
	T5	5,250,376	99.2	100
Lord	T6	2,957,821	99.4	100
	T7	5,616,242	99.4	100
	T8	2,976,740	99.3	100
	T9	10,029,783	99.4	100
	T10	6,819,992	99.4	100

The Figure 1 shows complete coverage of the psbA gene in all treatments, with differences in read depth reflecting variation in expression among EM-1 treatments and viral infection conditions.

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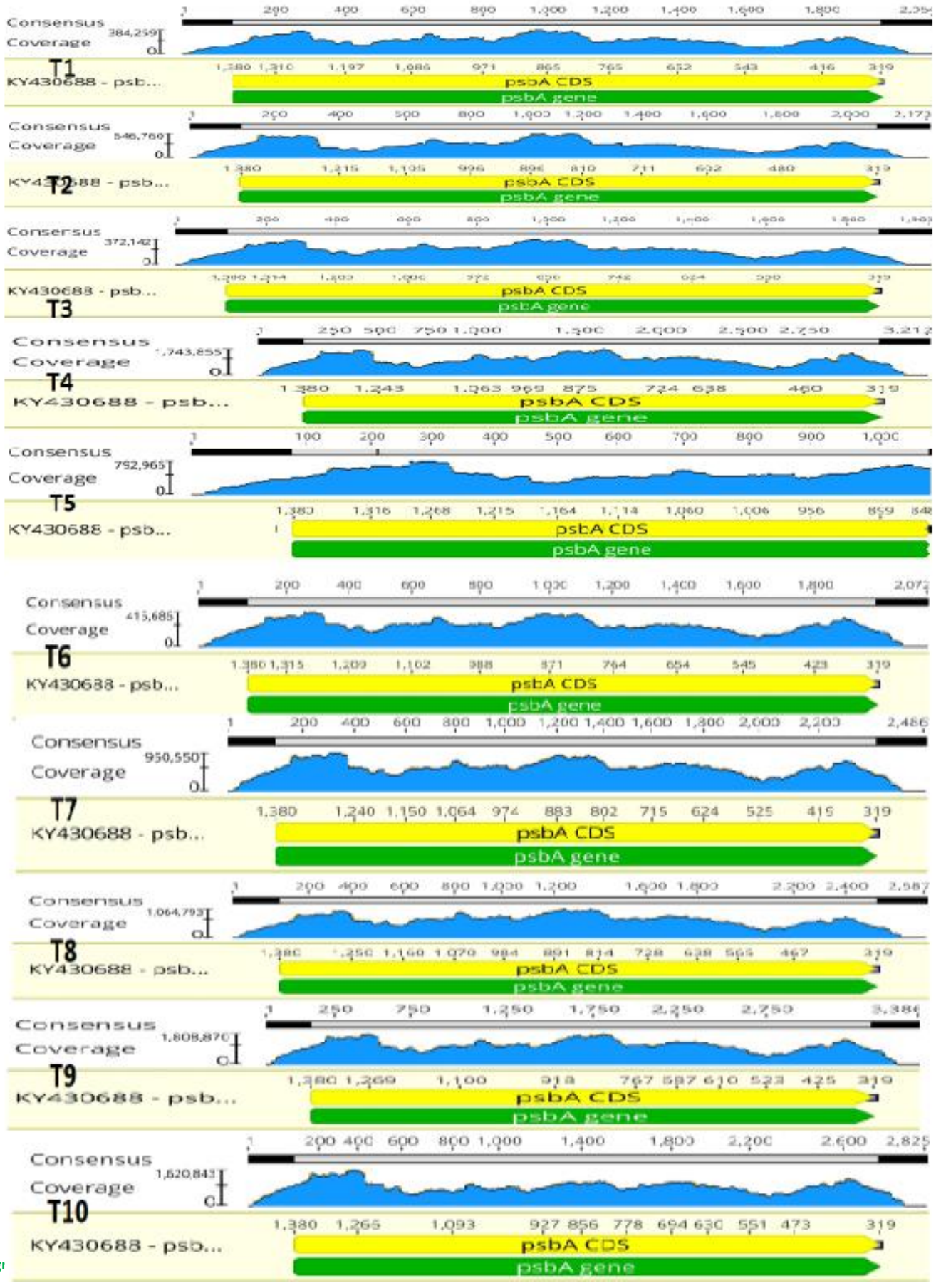
Figure 1 The mapping of treatments (T1-T10) against psbA gene



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IV. Discussion

Application of EM-1 before (T4, T9) and after (T5, T10) CmAV1 infection strongly enhanced psbA gene expression in both watermelon hybrids. The D1 protein encoded by psbA is a core component of Photosystem II (PSII) and is essential for photochemical electron transport and the PSII repair cycle under stress conditions (Nelson and Yocum, 2006). In contrast, CmAV1 infection alone significantly reduced psbA transcription, likely because of impaired electron transport, excessive generation of reactive oxygen species (ROS), and disruption of chloroplast homeostasis (Zhao et al., 2016; Bhattacharyya and Chakraborty, 2018). This suppression of psbA compromises PSII efficiency, leading to photoinhibition and reduced photosynthetic capacity, which can ultimately affect plant growth and productivity.

Pre-treatment with EM-1 (T4, T9) likely primes the photosynthetic machinery for enhanced stress tolerance by upregulating defense-related pathways, improving the stability of the D1 protein, and preparing PSII for potential viral stress. Post-treatment with EM-1 (T5, T10), applied after viral challenge, appears to mitigate ongoing stress by stabilizing D1 protein turnover, supporting PSII repair, and reducing oxidative damage during active infection (Komenda et al., 2012; Zhao et al., 2016). EM-1 enhances nutrient uptake, modulates antioxidant metabolism, and induces systemic resistance, which collectively contribute to the maintenance of photosynthetic efficiency under viral stress (Higa and Parr, 1994; Khalid et al., 2025; Vessey, 2003).

Recent studies have demonstrated that microbial biostimulants can directly influence the expression of chloroplast-encoded genes, including psbA, thereby improving PSII stability and facilitating ROS scavenging under biotic stress conditions (Vass, 2012; García et al., 2019). Such modulation may involve enhanced signaling through plant hormones, increased synthesis of protective proteins, and activation of antioxidant enzymes, which together reduce photodamage and maintain efficient energy conversion. Furthermore, high pairwise identity (99.3–99.5%) and full sequence coverage in our transcriptome analysis indicate that the observed differences in mapped reads primarily reflect transcriptional regulation rather than viral-induced sequence variation or mutations (Bolton, 2009).

Collectively, these findings indicate that EM-1 is highly effective in preserving photosynthetic gene expression, stabilizing PSII function, and enhancing plant resilience under CmAV1 infection. This highlights the potential of EM-1 as a microbial biostimulant for improving the physiological performance and viral tolerance of watermelon, supporting its application as a sustainable strategy for crop protection and productivity improvement (Rouphael and Colla, 2020; Khalid et al., 2025). The dual effect of EM-1—both priming before infection and mitigating stress after infection—emphasizes its versatility in integrated disease management programs and its role in maintaining chloroplast integrity under biotic stress.

Disclosure statement

The authors did not disclose any potential conflicts of interest.

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