



Article

Enhancing Tomato (*Solanum lycopersicum* L.) Resistance Against Bacterial Canker Disease (*Clavibacter michiganensis* ssp. *michiganensis*) via Seed Priming with β -Aminobutyric Acid (BABA)

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Abstract: Many stressors contribute to productivity and quality losses in agricultural production, ranging from the rising global population to shrinking agricultural lands. To boost yield and quality, plants must be protected from abiotic and biotic stressors. Seed priming is the process of boosting germination and seedling development by treating seeds with particular pre-treatments before germination. Seed priming is used to improve plant yield and germination. Plant defense elicitors stimulate the plant's natural immune system when administered externally, strengthening the plant and making it more resistant/tolerant to diseases. β -Aminobutyric Acid (BABA) is a plant defense elicitor, and in this study, the effect of BABA seed priming on *Clavibacter michiganensis* ssp. *michiganensis* (Cmm), which causes bacterial cancer in tomato (*Solanum lycopersicum* L.), was investigated. Tomato seeds were subjected to seed priming for 72 h with 12 mM BABA (BABA priming) or water (water priming) as the control group. Tomato seedlings that germinated normally were utilized as a positive control. When the plants reached the 3–4 leaf stage, they were infected with Cmm. According to the data, BABA priming was the most effective experimental group in reducing disease severity. Furthermore, it has been shown that the use of BABA as a spray or water-priming application gives better protection than the control treatment. To understand the molecular basis of this suppression, plant samples were obtained at two separate time points (0th and the 7th day), and transcriptional changes of essential plant immunity genes (*NPR1*, *PAL*, *PR1*, *WRKY70*, *WRKY33b*, *TPK1b*, and *PR5*) were studied. The qRT-PCR results showed that *NPR1* gene expression increased considerably with the BABA priming treatment compared to the control. BABA priming at the 0th hour enhanced *NPR1* gene expression by approximately five times. In addition, BABA priming increased *PR1* gene expression. Furthermore, foliar spraying of BABA (BABA priming+BABA-Sp) on seed-primed plants resulted in a nine-fold increase in *PR1* gene expression. At day 7, the BABA priming+Cmm treatment increased *PR5* gene expression. Along with the control of other genes, the molecular architecture of BABA seed priming has been attempted to be discovered. The application of BABA seed priming is expected to contribute to the literature and have favorable impacts on plant protection against Cmm.



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Keywords: β -Aminobutyric acid; Cmm; gene expression; plant immune system; seed priming; *Solanum lycopersicum* L.; plant defense elicitor

1. Introduction

The increasing global population causes the decline of agricultural land, and numerous stressors cause the loss of productivity and quality in agricultural production [1,2]. Plants are continually subjected to both biotic and abiotic stressors. To increase agricultural productivity, it is critical to protect plants from dangerous diseases and pathogens [2,3]. The germination and sowing of seeds under optimal conditions is very important as the first stage of plant production. Genetic traits, chemicals, irrigation, fertilization, drying methods, harvest time, and disease infection status all have an impact on seed quality and germination [3–6]. Seed-priming procedures enhance seed germination and development [7]. These applications consist of stimulating the seed through various means before planting to boost the germination ability of seeds.

Seed priming is an important agricultural practice. Priming approaches, such as hydropriming, osmopriming, chemo-priming, and bio-priming, enable seeds to germinate more quickly and frequently by improving the germination rate [8–13]. This approach also increases the resistance/tolerance of seeds to numerous stimuli (biotic or abiotic), making them less sensitive to potential detrimental effects during the germination process [7,14]. Priming compounds include sodium, potassium, magnesium, PEG 6000 (polyethylene glycol), PEG 8000, and hormones such as ethylene and gibberellic acid [15]. Seed priming is vital in plant cultivation, as it prepares the plant's immune system, increases the seed germination rate, and allows it to survive the obstacles that may develop during seed production [15,16].

Plant defense elicitors are substances that improve the resistance/tolerance of plants and make them more resilient to dangerous pathogens by boosting their natural immune system [17]. Plants have a complicated and effective defense mechanism that protects them against disease. This mechanism has two phases: Pattern-Triggered Immunity (PTI) and Effector-Molecule-Triggered Immunity (ETI). Pattern-Triggered Immunity (PTI) enables plants to recognize specific chemicals on pathogen surfaces via pattern recognition receptors (PRRs) and initiate an immune response. This identification initiates a cascade of cellular reactions in plants, allowing the plant to protect itself from pathogen attack. However, some infections can deactivate or decrease the PTI response by producing powerful chemicals. In these instances, plants may develop a reduced response known as the basal defense mechanism. Plants can activate the ETI response against effective pathogen compounds by employing specialized resistance (R) proteins. The ETI response serves to restrict the pathogen's impact on the plant by generating incompatible relationships. A wide range of defensive signals regulates plants' innate immune responses. Reactive oxygen intermediates (ROIs) and hormones such as jasmonic acid, ethylene, and salicylic acid are critical signaling molecules that regulate plant defense systems. Both biotic and abiotic stimuli can activate these defensive mechanisms [18,19].

β -aminobutyric acid (BABA) is a non-protein amino acid that supports plant defense mechanisms [20]. BABA was discovered in 1963 and has been proven to protect plants from a variety of plant diseases [15]. Several studies show that BABA is efficient against bacteria, viruses, nematodes, and arthropods in various plant species [16,21,22]. In *Arabidopsis thaliana*, BABA responds quickly and strongly to stress factors [15]. Further study has found that tomatoes, tobacco, and potatoes provide significant control against diseases such as late blights [23,24]. Thus, research using BABA has demonstrated that it can play an important role in priming activities [15,25–27]. For example, a study on beans revealed that BABA increases the plant's resistance against diseases through seed priming [28]. However, no systematic investigation of the effect of BABA on seed priming in different plants or against different diseases has been found.

Clavibacter michiganensis ssp. *michiganensis* (*Cmm*) is a Gram-positive seed-based bacterium that causes significant economic losses worldwide in the production of tomatoes (*Solanum lycopersicum* L.) [29–32]. This is one of the most devastating bacterial infections in tomatoes [30]. *Cmm* is typically detected on a plant's stem and leaves as leaks [33]. *Cmm* attacks the plant via wounded areas, colonizes the vasculature, and kills it by causing sores. Symptoms begin between six and eight weeks. It may make the *Cmm* pathogen more dangerous in hidden infections with no visible symptoms [34–36]. The severity of illness symptoms varies according to the virulence of *Cmm*, the age of the plant, and the level of humidity. It is challenging to manage the disease or other symptoms because there are no commercially marketed resistant tomato cultivars [37–39]. Thus, research on tomato protection against *Cmm* disease is essential [40].

Plant defense elicitors enhance the plant's natural immunity, improving its resistance and vigor against disease. Some research has been carried out with BABA, but the effect of seed priming has not been well understood [41]. As a result, our study assessed the role and benefits of BABA seed priming in plant defense against *Cmm*. In this study, we used BABA as a seed priming agent to (1) determine its effect on *Cmm* disease severity and lesion size in tomatoes and (2) to determine how these effects occur at the molecular level. The use of BABA seed priming has been found to slow the progression of *Cmm*-induced bacterial cancer disease and affect gene expression in plant immune systems. We believe the study's findings will contribute to the literature, enhance the frequency of seed priming treatments in agriculture, and boost production.

2. Materials and Methods

2.1. Plant Materials and Growth Conditions

This study used the H-2274 tomato cultivar (*Solanum lycopersicum* L.) obtained from the Bursa Seed Corporation. Surface sterilization was performed according to Bektas et al. [42]. Seeds were treated with a 1% *v/v* sodium hypochlorite (NaClO) solution for 10 min, followed by 70% ethyl alcohol for 5 min. Then, they were rinsed 3–4 times with sterile distilled water. After sterilization, 35 seeds were transferred into sterile 1.5 mL Eppendorf tubes. Seed germination was carried out on moistened sterile filter paper placed in Petri dishes under controlled conditions (25–27 °C) for 7 days. Uniformly germinated seedlings were transplanted into plastic pots containing a peat–perlite (2:1, *v/v*) growth substrate. Plants were grown in a controlled growth cabinet at 25 ± 2 °C under a 16/8 h light–dark photoperiod and 60–70% humidity until reaching an approximately 5-week vegetative stage, suitable for pathogen inoculation.

2.2. Synthetic Elisitor Preparation: Preparation of β -aminobutyric Acid (BABA), BABA Seed Priming, and Spraying Applications

This study used β -aminobutyric acid (BABA) (Sigma, California, USA) to prime seeds. The powdered BABA was concentrated to 12 mM with sterile distilled water and administered to the sterile seeds in the 1.5 mL Eppendorf tube, while the control group received distilled water. Seeds were stored in the dark at 24–26 °C for 72 h before germinating on moist filter paper in a Petri dish for 7 days at room temperature (25–27 °C). Tomato seedlings with 3–4 leaves were sorted into two groups. To apply the spray, a 2 mM concentration of powdered BABA was made with sterile distilled water, and 2 mM BABA spray (SP) was sprayed on the upper and lower surfaces of all plant leaves seven days and one day before *Cmm* application. The remaining half was sprayed with sterile distilled water (control). Table 1 lists the experimental design and label names. Tomato seedlings were supplied with liquid fertilizer (Gübretaş, NPK) as needed (in equal amounts for all experimental groups).

Table 1. Experimental design.

Seed Priming Application	YES			YES			NO		
Seed priming duration	72 h			72 h			0 h		
Application	12 mM BABA priming			Water priming			Normal germination (positive control)		
Application when the seedlings reach the 3–4 leaf stage (7 days and 1 day before <i>Cmm</i> application)	Distilled water spray			Distilled water spray			Distilled water spray		
<i>Cmm</i> applications (100 µL OD600:0.02)	2 mM BABA spray			2 mM BABA spray			2 mM BABA spray		
	- <i>Cmm</i>	+ <i>Cmm</i>	+ <i>Cmm</i>	- <i>Cmm</i>	+ <i>Cmm</i>	+ <i>Cmm</i>	- <i>Cmm</i>	+ <i>Cmm</i>	+ <i>Cmm</i>
Label	BABA priming- <i>Cmm</i> (BP- <i>Cmm</i>)	BABA priming+ <i>Cmm</i> (BP+ <i>Cmm</i>)	BABA priming+BABA- Sp+ <i>Cmm</i> (BP+BSP+ <i>Cmm</i>)	Water priming- <i>Cmm</i> (WP- <i>Cmm</i>)	Water priming+ <i>Cmm</i> (WP+ <i>Cmm</i>)	Water priming+BABA- Sp+ <i>Cmm</i> (WP+BSP+ <i>Cmm</i>)	Non-primed- <i>Cmm</i> (NP- <i>Cmm</i>)	Non-primed+ <i>Cmm</i> (NP+ <i>Cmm</i>)	Non-primed+BABA- Sp+ <i>Cmm</i> (NP+BSP+ <i>Cmm</i>)

2.3. Bacterial Strain, Inoculation, and Disease Measurement

Clavibacter michiganensis ssp. *michiganensis* (*Cmm*) was received from Dr. Ümit ÖZYILMAZ of Aydın Adnan Menderes University in Turkey. Bacterial growth was achieved using King's B medium (KB). Bacteria were dissolved in a 0.9% isotonic salt solution, and the bacterial solution was measured at OD₆₀₀:0.02 using a spectrophotometer. *Cmm* was applied 24 h following the second dosage of BABA spray. Tomato seedlings were injected with 100 µL of bacterial solution at the junction of the lower first petiole and stem. As a negative control, 100 µL of isotonic solution (0.9% NaCl) was added to the corresponding branch points of the control plants. Initially, each plant was covered with transparent plastic bags for 16 h, and then the plants were kept in controlled growth conditions at a room temperature of 26–28 °C and relative humidity of 60–70% under a 16/8 h light–dark regime for four weeks. Disease severity was measured beginning 8 days after inoculation and every 3 days thereafter. Disease severity was measured based on ocular assessment and wilting leaf stage. The 0–5 disease severity scale was used following Baysal et al. [43]. A score of 0 indicates no symptoms; 1: 1–10% wilted leaves; 2: 11–25% wilted leaves; 3: 26–49% wilted leaves; 4: 50–75% wilted leaves; and 5: greater than 75% wilted leaves or dead plants. After the plants were harvested, a ruler was used to measure the damage caused by *Cmm* to the tomato conduction bundles (lesion length) and plant height. The fresh weight was determined using a precision scale (Weightlab). The dry weights were measured after drying the plants in an oven (MiproLab) at 70 °C for 72 h.

2.4. Gene Expression Analyses

Plant tissue samples were taken at 2 different times. First, plant tissue samples were taken 24 h after the second BABA spray application, just before the *Cmm* application; samples were labeled as hour 0. A total of nine single leaves from three plants in each experimental group were rapidly ground in liquid nitrogen. The 2nd sampling was performed one week after the *Cmm* application, and samples were labeled as day 7. In the 2nd sampling, leaf samples were taken from 3 plants that had not been previously leaf-sampled and had not been injured and were quickly ground in liquid nitrogen. In the 2nd sampling, samples were also taken from negative control groups without *Cmm* treatment. Total RNA isolation was performed using the RNA from plant tissue samples collected at two distinct periods. The first plant tissue samples were collected 24 h following the second BABA spray treatment, shortly before the *Cmm* application; samples were identified as hour 0. Nine individual leaves from three plants in each experimental group were quickly ground in liquid nitrogen. The second sampling was performed at one week following the *Cmm* treatment, and the samples were labeled as day 7. In the second sampling, leaf samples were collected from three plants that had not previously been leaf sampled and had not been harmed, and they were immediately crushed in liquid nitrogen. In the second sampling, samples were gathered from negative control groups that did not receive *Cmm* treatment. RNA isolation was performed using a Mini-Preps Kit (Catalog No. BS82314, Bio Basic Inc., Markham, ON, Canada) according to the manufacturer's instructions. Gel electrophoresis (Thermo Fisher Scientific, Waltham, MA, USA) and Nano-400-A (Allsheng, Hangzhou, China) were used to determine the concentration and purity of total RNA. The amounts (ng/µL) of RNA collected in the nanodrop were estimated, and cDNA synthesis was carried out according to the RevertAid First Strand cDNA Synthesis Kit procedure (Catalog number: K1622, Thermo Fisher Scientific).

The experiments used PicoReal Real-Time PCR (real-time reverse transcription-quantitative PCR) from Thermo Scientific. The expression of several genes known to be important in tomato defense was measured at two distinct times (0th hour and 7th day). The genes and primer sequences utilized are shown in Table 2. Three biological replicates

and three technical replicates were used for each experimental group. For the data analysis, the mean threshold cycle values were determined for each gene of interest using three independent biological samples and standardized to *Actin*. Relative transcript levels were determined according to Livak and Schmittgen [44].

Table 2. Genes and primers used in the gene expression study.

Genes	Functions of Genes	Primer Sequences (5'-3')	Temperature (°C)	References
<i>PR1</i>	<i>Pathogenesis 1 gene</i>	F: GGATCGGACAACGTCCTTAC R: GCAACATCAAAAGGGAAATAAT	52	Molinari et al. [45]
<i>PR5</i>	<i>Pathogenesis 5 gene</i>	F: GCAACAACGTGTCATACACC R: AGACTCCACCACAATCACC	52	
<i>NPR1</i>	Non-expression of <i>PR1</i> gene	F: GGGAAAGATAGCAGCACG R: GTCCACACAAACACACACATC	52	Hu et al. [46]
<i>WRKY70</i>	<i>WRKY transcription factor 70</i>	F: CATGGATGAGAGAATCTGCA R: GATTTTCTTGGATTATTTGAAC	52	Atamian et al. [47]
<i>PAL</i>	Phenylalanine ammonialyase like gene	F: CGCTATGCTCTCCGAACATCTC R: ATTCACCGAGTTAATCTCCCTCTC	55	Chandrasekaran and Chun [48]
<i>WRKY33B</i>	<i>WRKY transcription factor 33b</i>	F: CCACAACAGTCTGAAATGGG R: CAGCAAAGCAATGACTCCAT	52	Zhou et al. [49]
<i>ACTIN</i>	<i>Actin gene</i>	F: TGTCCCTATTTACGAGGGTTATGC R: CAGTTAAATCACGACCAGCAAGAT	52	
<i>TPK1b</i>	<i>Tomato protein kinase 1b</i>	F: ATGGGGATATGTTTGAGTGCTAGAA R: GAACGTGTTCTCGTCGATCCACCCT	55	Ray et al. [50]

2.5. Statistical Analyses

Each treatment group was replicated three times with seven pots (plants) per replication. All data were analyzed using ANOVA, and means were separated using the LSD multiple range test ($p < 0.05$). Letters indicate significant differences ($p < 0.05$), whereas NS denotes non-significant differences. All statistical analyses were carried out using Statistix software V10 (Analytical Software, Tallahassee, FL, USA).

3. Results

3.1. Effect of BABA Seed Priming on *Cmm* Disease Severity and Progression

Tomato seeds were seed-primed with either BABA priming or water priming for 72 h in order to evaluate the effect of BABA seed priming on the severity of *Clavibacter michiganensis* ssp. *michiganensis* (*Cmm*) disease in plants. The effectiveness of BABA as a foliar spray were also studied. The first disease symptoms were found one week after *Cmm* inoculation, and disease severity (DS) was monitored from that point until the end of the trial. These observations were made every three days and recorded as DS1, DS2, DS3, DS4, DS5, and DS6. The latest disease severity (DS7) was recorded at harvest. An average of 26 days elapsed between *Cmm* treatment and the end of the experiment.

Based on the results, the BP+*Cmm* and BP+BSP+*Cmm* groups fared better than the other groups in the initial disease severity measurement (DS1) (Figures 1 and 3). In all treatment groups, BABA seed priming had the highest effect on disease severity and symptom reduction. Furthermore, water priming alone proved efficient in disease control. Spraying 2 mM BABA on BABA-primed seedlings did not provide any further protection against *Cmm*. The BP+BSP+*Cmm* and BP+*Cmm* treatments were similarly beneficial depending on disease severity, with BP+*Cmm* being more effective in the last four disease severity evaluations (Figures 1 and 3). During harvest, lesion length was assessed to determine *Cmm* damage to the plant, and the results are shown in Figure 2. The smallest lesion size was seen in the BP+BSP+*Cmm* and BP+*Cmm* treatment groups. The obtained results are consistent with the disease severity levels.

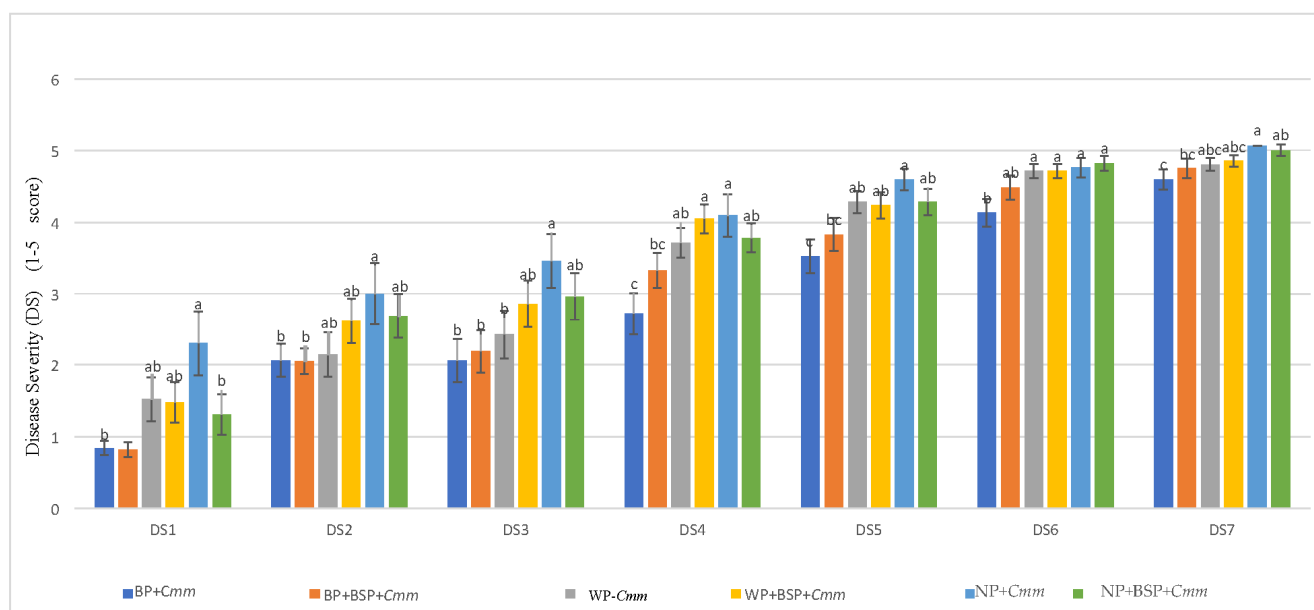


Figure 1. Effect of treatments on disease severity: *Cmm* source disease severity (DS) was taken every three days and analyzed as DS1, DS2, DS3, DS4, DS5, DS6, and DS7. Letters indicate significant differences between treatments at $p < 0.05$.

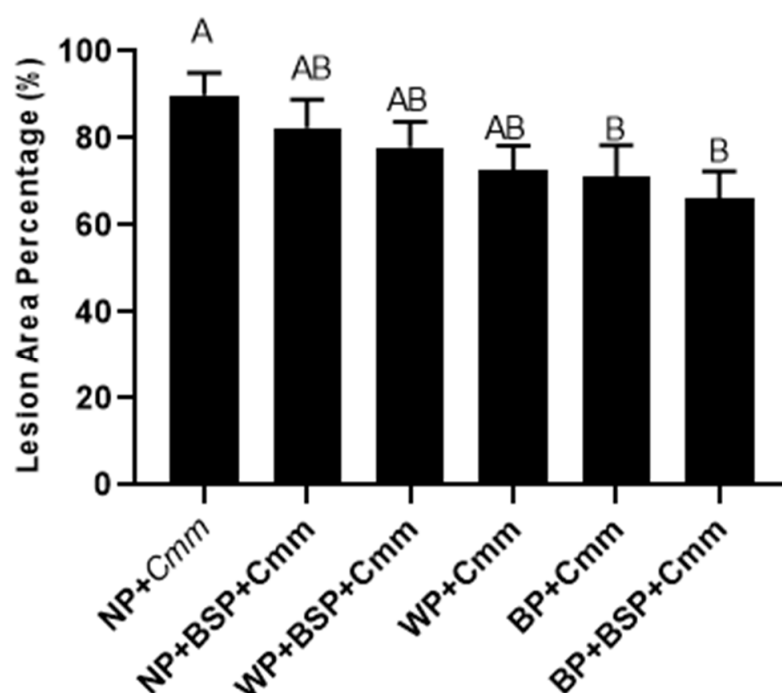


Figure 2. The effect of treatments on lesion area percentage. Letters indicate significant differences between treatments at $p < 0.05$.

Post-harvest, the fresh and dry weights of the green parts of plants were taken in the *Cmm*-inoculated BP+Cmm, BP+BSP+Cmm, WP+Cmm, WP+BSP+Cmm, NP+Cmm, and NP+BSP+Cmm groups, as well as the *Cmm*-free control groups (WP-Cmm, BP-Cmm, and non-normal germinations), and the fact that the water-priming treatment had a lower effect on *Cmm* than BP+Cmm implies that it is advantageous in raising plant biomass (Table 3).

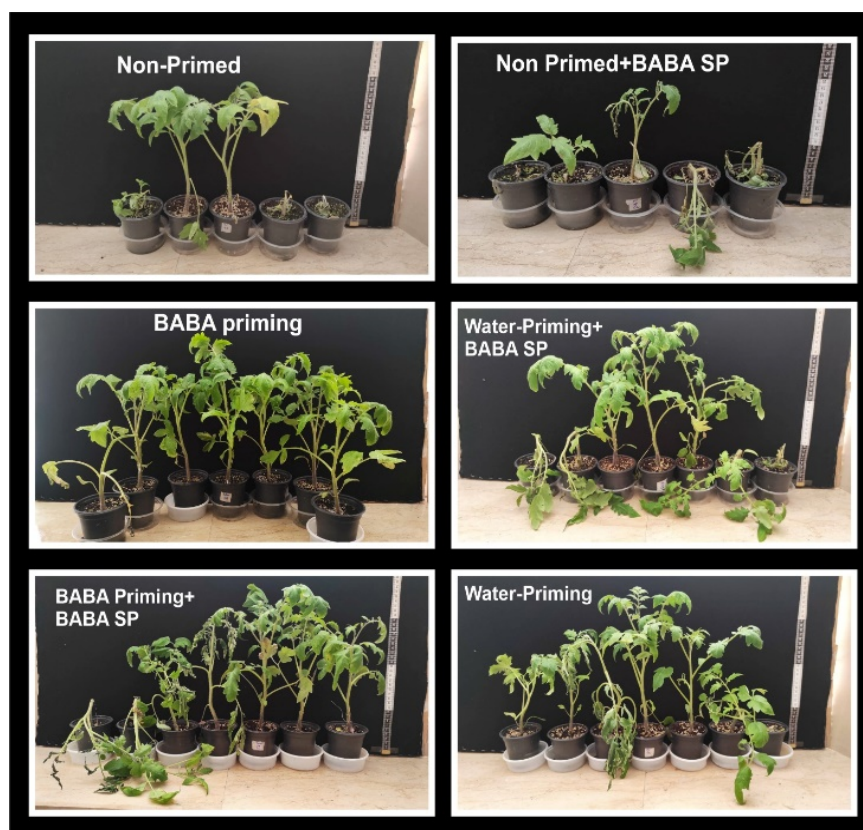


Figure 3. Effect of different priming treatments (BP, BP+BSP, WP, WP+BSP, NP, and NP+BSP) on *Cmm* infection.

Table 3. Plant fresh and dry weights of the groups: WP-*Cmm*, NP-*Cmm*, BP-*Cmm*, WP+*Cmm*, BP+BSP+*Cmm*, WP+BSP+*Cmm*, BP+*Cmm*, NP+BSP+*Cmm*, and NP+*Cmm*.

Application	Plant Fresh Weight (g)	Plant Dry Weight (g)
Water priming- <i>Cmm</i> (WP- <i>Cmm</i>)	31.14 ± 1.18 ^a	2.79 ± 0.18 ^a
Non-primed- <i>Cmm</i> (NP- <i>Cmm</i>)	26.34 ± 2.45 ^b	2.39 ± 0.28 ^a
BABA priming- <i>Cmm</i> (BP- <i>Cmm</i>)	16.89 ± 0.94 ^c	1.61 ± 0.12 ^b
Water priming+ <i>Cmm</i> (WP+ <i>Cmm</i>)	6.82 ± 0.95 ^d	0.93 ± 0.12 ^c
BABA priming+BABA-SP+ <i>Cmm</i> (BP+BSP+ <i>Cmm</i>)	6.79 ± 0.96 ^d	0.92 ± 0.11 ^c
Water priming+BABA-SP+ <i>Cmm</i> (WP+BSP+ <i>Cmm</i>)	6.40 ± 1.04 ^{de}	0.81 ± 0.12 ^{cd}
BABA priming+ <i>Cmm</i> (BP+ <i>Cmm</i>)	6.37 ± 0.90 ^{de}	0.75 ± 0.08 ^{cd}
Non-primed+BABA-SP+ <i>Cmm</i> (NP+BSP+ <i>Cmm</i>)	4.39 ± 1.02 ^{de}	0.63 ± 0.12 ^{cd}
Non-primed+ <i>Cmm</i> (NP+ <i>Cmm</i>)	3.72 ± 0.94 ^e	0.58 ± 0.11 ^d

Letters indicate significant differences between treatments at $p < 0.05$.

3.2. Gene Expression Analysis

According to the disease severity and lesion length data, the BABA priming (BP) application activated plant basal resistance while suppressing *Cmm* growth in the plant. To better understand the underlying molecular mechanisms of this protection, the expression levels of key defense-related genes were analyzed. The RT-qPCR results showed dynamic changes in *NPR1*, *PAL*, *PR1*, *WRKY70*, *WRKY33b*, *TPK1b*, and *PR5* gene expressions in different treatment groups at both hour 0 and day 7. These genes, which are known to play pivotal roles in the plant immune system, displayed significant transcriptional activation in the BABA-treated plants. Relative gene expression levels were calculated in comparison

to the positive control group. According to the results, *NPR1* gene expression was higher in the BABA priming (BP) and BABA priming+BABA-SP (BP+BSP) groups compared to the non-primed (NP) group at 0 hr. BABA priming (BP) enhanced *NPR1* gene expression about five-fold. *NPR1* was activated in BP+BSP, although only at a low level. At the same time, water priming alone increased *NPR1* gene expression (Figure 4A). The increase in *PR1* gene expression, which is the accumulation of SA in the cell, was positively influenced by both BP and BSP therapy, and the combination of the two resulted in increased *PR1* gene activity. Furthermore, in the WP+BSP and NP+BSP treatments, BABA spray administration increased *PR1* transcript levels (Figure 4B). *PR5*, like *PR1*, acts as a marker gene for the SA pathway. *PR5* gene expression was decreased in both the WP and WP+BSP groups. On day 7, the BP+Cmm treatment increased *PR5* gene expression (Figure 4C). The phenylpropanoid pathway is responsible for a variety of plant defensive biosyntheses. On day 0, the BP and BP+BSP groups had higher *PAL* gene expression than the control group. On day 7, the BABA priming treatment increased *PAL* gene expression, but there was no significant difference between the other groups (Figure 4D). Interestingly, the water priming (WP) treatment enhanced *PAL* gene expression activity thrice on day 7 (Figure 4D). Members of the *WRKY* transcription factor family are critical to the transcriptional modulation of plant immunological responses. We investigated the transcriptional regulation of two key members of this family: *WRKY33b* and *WRKY70*. No increase in *WRKY33b* gene expression was seen in either group at 0 h; however, on day 7, the water-priming treatment raised *WRKY33b* transcription levels (Figure 4E). When we looked at the relative gene expression of *WRKY70*, BP and WP+BSP, it was stimulated at similar rates at 0 h, but the BP+BSP treatment enhanced gene expression by 3.5-fold. On day 7, this stimulation subsided, and no increased expression of *WRKY70* was detected in either treatment group (Figure 4F).

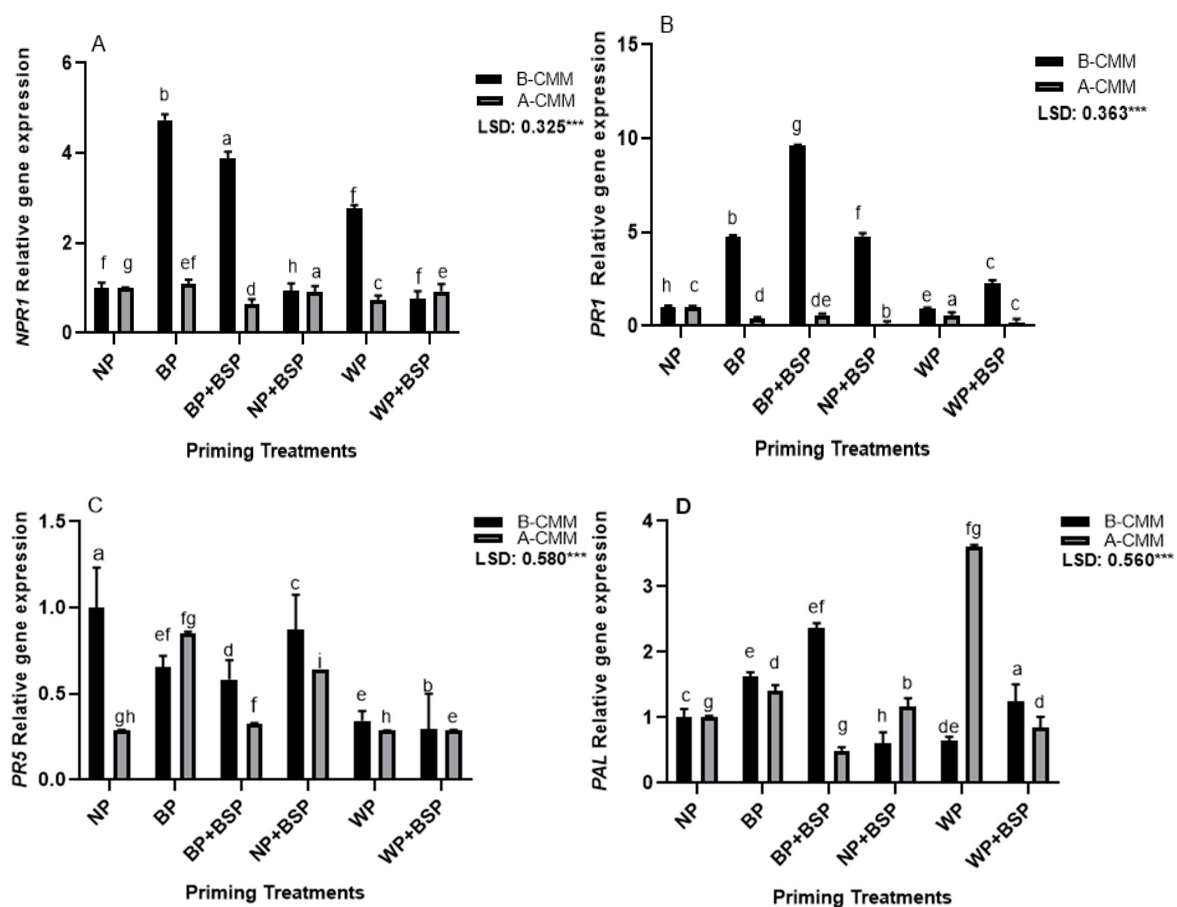


Figure 4. Cont.

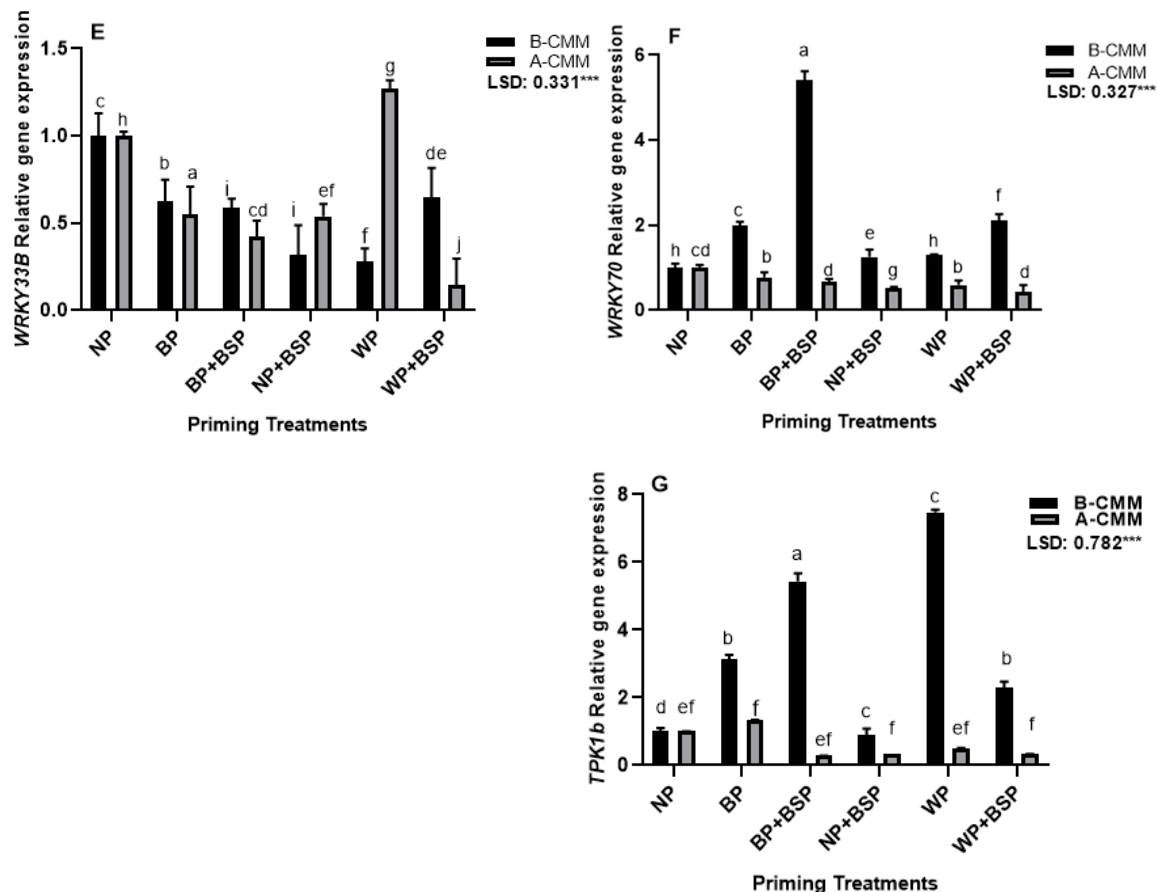


Figure 4. Effect of different priming strategies [BABA priming (BP), BABA priming+BABA-SP (BP+BSP), water priming (WP), water priming+BABA-SP (WP+BSP), non-primed (NP), non-primed+BABA-SP (NP+BSP)] on the expression of defense-related genes [*NPR1* (A), *PR1* (B), *PR5* (C), *PAL* (D), *WRKY33b* (E), *WRKY70* (F), and *TPK1b* (G)] before and after *Cmm* infection. A-CMM—after *Cmm* infection; B-CMM—before *Cmm* infection. *** $p < 0.001$ (LSD = 0.001). Letters indicate significant differences between treatment groups at $p < 0.05$.

The *TPK1* gene is a protein kinase that is known to be involved in the signaling pathway of the phytohormone ethylene (ET). *TPK1b* gene expression increased in the BP, BP+BSP, and WP experimental groups but not in the NP+BSP group (Figure 4G). All of these results demonstrated that the activation of practically all plant immune genes increased in the BABA-priming treatment group, while gene expression was reduced on day 7.

4. Discussion

4.1. Effect of BABA Seed Priming on *Cmm* Disease Severity and Progression

Clavibacter michiganensis ssp. *michiganensis* (*Cmm*) is a major bacterial pathogen that causes significant economic losses in tomato (*Solanum lycopersicum* L.) crops globally. Controlling the disease or other symptoms is challenging since there are no resistant commercial tomato varieties [37,39]. Therefore, investigations on the protection of tomato against the *Cmm* pathogen are vital [40].

When administered externally to a plant, plant defense elicitors stimulate the plant's natural immune system, strengthening it and making it more resistant to plant pests. β -Aminobutyric acid (BABA) is a plant defense elicitor. This study evaluated the effect of seed priming with BABA on *Cmm*, which causes bacterial canker disease in tomato. Previously, Worrall [51] demonstrated that seed priming with BABA provided resistance

to the fungus *Oidium neolycopersici*. Similarly, Anup et al. [52] additionally demonstrated that treating pearl millet seeds with *Pseudomonas fluorescens* and BABA elicitors protected the plant from the disease *Sclerospora graminicola*. The protective effect of BABA has been previously reported not only against fungi but also against bacterial pathogens. In this study, we further confirm its efficacy against bacteria. According to the findings of our study, the BP application remained effective until the end of the trial and resulted in decreased disease severity, even as the disease worsened over time based on disease severity. BP+Cmm performed particularly well in the last four disease severity measurements. It was also discovered that BP+BSP+Cmm and BP+Cmm had the smallest lesion size, and other parameters of the plants exposed to the disease showed considerable improvement. It is worth noting that water priming alone gave better protection than the positive control. Although many benefits of seed priming and water priming have been discovered in the literature, there are no studies on their effect on the Cmm pathogens [7]. However, it is possible that BABA seed priming and BABA spray treatments work via similar pathways or that the amount of BABA used was insufficient in terms of stimulation, which is why the 2 mM BABA spray did not provide additional protection against Cmm in our experiment. Baysal et al. [53] used a 5 mM BABA spray against the Cmm pathogen, resulting in a 54% reduction in disease development. The results reveal that BABA not only has a priming effect in the plant but is also beneficial in seed-priming applications and inhibits the harmful effects of Cmm in tomato. Interestingly, despite the reduced lesion size and disease severity in the BP+BSP+Cmm group, lower plant biomass was observed, likely due to the energy allocation toward defense responses, a common trade-off in BABA-induced resistance. To better understand this balance and its implications for plant health, further discussion and detailed analysis are warranted in future studies.

4.2. Gene Expression Analysis

According to disease severity and lesion length statistics, the BABA priming treatment activated plant basal resistance and suppressed Cmm infection. To understand the molecular substructure behind this suppression, transcriptional changes of *NPR1*, *PAL*, *PR1*, *WRKY70*, *WRKY33b*, *TPK1b*, and *PR5* genes, which play essential roles in the plant immune system, were examined using RT-qPCR in tomato leaves on two separate dates.

NPR1 is a gene that functions as a transcriptional cofactor and is essential for the induction of salicylic acid (SA)-related plant immunity [54]. This study examined the expression of the *NPR1* gene in response to the BABA seed-priming treatment. When compared to NP at 0 h, BP and BP+BSP increased *NPR1* gene expression. BP enhanced *NPR1* gene expression by about five-fold. At the same time, the water-priming treatment increased *NPR1* gene expression. According to studies in the literature, BABA's signaling route is not unique. Depending on the sort of stress given, BABA has been seen to influence plant defense through multiple pathways [15]. It has been stated that BABA treatment against bacteria and fungi works through the SA route and *NPR1* protein accumulation [15,55]. It was discovered that the BABA seed-priming application increased *NPR1* gene expression, resulting in active resistance against Cmm. The *NPR1* gene expression levels in all experimental groups were identical 7 days after Cmm administration. The Cmm infection may have weakened the plant's immune response, resulting in this decline.

PR1 proteins play a vital role in plant immunity, and SA buildup in the cell activates the *PR1* gene. *PR1* is an essential gene in the SA defense system [56]. Our investigation focused on the transcriptional gene expression of *PR1*. According to the findings, the BP+BSP group demonstrated the highest increase in *PR1* gene expression at the 0th hour. As a result, both the BP and BSP treatments had a favorable effect on increasing *PR1* gene expression, and combining the two resulted in higher *PR1* gene expression. The WP+BSP

and NP+BSP treatments also resulted in an increase in *PR1* transcript levels due to the BABA spray treatment. Previous research demonstrated that the external administration of BABA increased *PR1* gene expression, protecting the bacterial pathogen *Pseudomonas syringae* pv. *tomato* (*Pst*) DC3000. In a study conducted with mutant lines to examine the mechanism of *PR1* stimulation, it was shown that BABA-induced *PR1* stimulation is not only dependent on the SA route but also happens independently of the SA pathway [57]. Martínez-Aguilar et al. [58] sprayed BABA on the leaves of *Phaseolus vulgaris* L. (bean), and the effect of the treatment on the *Pseudomonas syringae* pv. *phaseolicola* NPS3121 (PspNP3121) bacterial strain was compared to the control group. According to their results, the BABA spray provided resistance against the pathogen through histone changes in the promoter–exon regions of defense-related genes such as *PR1*. On day seven of our study, the *PR1* gene's stimulation was stopped in all groups, and down-regulation took place. The suppression of the plant immune system by *Cmm* may be connected to this.

PR5, like *PR1*, is a marker gene for the SA pathway. According to the results of our study, the gene expression of *PR5* did not increase in any of the treatments when compared to the control group at the 0th hour. In their study with two different plant elicitors, Bektas [40] discovered that one of them activated the SA pathway by stimulating the *PR5* gene, whereas the other stimulated the *PR1* gene. This implies that the application's gene stimulation will be limited, and that protection can be supplied by stimulating only a few specific genes.

The phenylpropanoid pathway enables several types of biosynthesis associated with plant defense (SA biosynthesis, lignin biosynthesis, etc.) [59]. *Phenylalanine ammonia-lyase* (*PAL*) is an entry gene in this pathway. In this study, we investigated the influence of seed priming and BABA spray treatments on *PAL* gene expression. Cumplido-Najera et al. [60] treated plants with copper nanoparticles and potassium silicate, which were efficient against *Cmm* infection. According to their molecular-level findings, these applications enhanced *PAL* gene expression. Our findings indicate that the BP and BP+BSP groups provide the most effective protection against *Cmm*. The increased *PAL* gene expression in these experimental groups shows that *PAL* may play a role in *Cmm* protection. Surprisingly, water priming increased *PAL* gene expression three-fold on day 7. The data collected imply that the transcription control of plant defense system genes is influenced in a complicated manner.

Members of the *WRKY* transcription factor family serve a vital role in the transcriptional regulation of plant immune responses [61]. In this study, we looked at the transcriptional regulation of two key members of this family: *WRKY33b* and *WRKY70*. Previous research has shown that *WRKY33* plays a crucial role in plant resistance to necrotrophic fungal infections [62,63]. Zhou et al. [49] discovered that *WRKY33b* is also effective in abiotic stress tolerance. BABA treatment in beans produced histone changes on the *PvWRKY53* and *PvWRKY29* genes, providing resistance against *Pseudomonas syringae* pv. *phaseolicola* [28]. Bektas [40] studied DPMP and INA, two plant immunostimulatory elicitors, for *Cmm* protection. Based on their results, INA enhances plant defense against *Cmm* by stimulating the *WRKY33b* gene, while DPMP does not activate *WRKY33b* gene expression. In this experiment, no elevation in *WRKY33b* gene expression was detected in any group at 0 h. However, on day 7, the water-priming treatment was discovered to boost *WRKY33b* transcription levels.

The *WRKY70* transcription factor is an important gene for plant defense and induces basal resistance against the biotrophic pathogens *Erysiphe cichoracearum*, *Hyaloperonospora parasitica*, and *A. brassicicola* [46,64]. It has also been shown to increase sensitivity against necrotrophs [65]. When we examined the relative gene expression of *WRKY70* in our study, we observed that the BP+BSP treatment increased gene expression by 3.5-fold at the 0th

hour. On day 7, this stimulation disappeared again, and no increase in *WRKY70* expression was found in either treatment group.

The *TPK1* gene is a protein kinase that is known to be involved in the signaling pathway of the phytohormone ethylene (ET) [66]. *TPK1* gene expression was found to be induced by mechanical injury, pathogen infection, and oxidative stress [67]. In this study, we looked at the transcriptional regulation of *TPK1b*. The results showed that the experimental groups of BP, BP+BSP, WP, and WP+BSP increased expression at the 0th hour. This shows that priming alone enhances *TPK1b* gene expression, and it was also discovered that the BABA spray treatment led to *TPK1b* gene stimulation in the primed groups.

BABA has been known since 1963, but studies into its effects on plants have lately intensified. Studies have demonstrated that external spray or root administration of BABA protects plants and boosts immunity against numerous diseases in different plants [15,28]. BABA's impacts have even been referred to as BP. Priming refers to the preparation of the plant against diseases and does not involve pre-germination applications of the seeds.

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Abbreviations

<i>PR1</i>	<i>Pathogenesis-Related 1 gene</i>
<i>PR5</i>	<i>Pathogenesis-Related 5 gene</i>
<i>NPR1</i>	<i>Nonexpressor of the PR1 gene</i>
<i>WRKY70</i>	<i>WRKY Transcription Factor 70</i>
<i>WRKY33B</i>	<i>WRKY Transcription Factor 33b</i>
<i>TPK1b</i>	<i>Tomato Protein Kinase 1b</i>
BABA	Beta-Aminobutyric Acid
LSD	Least Significant Difference
INA	2,6-Dichloroisonicotinic acid
DPMP	2,4-Dichloro-6-[(E)-[(3-Methoxyphenyl)imino]methyl]phenol
JA	Jasmonic Acid
SA	Salicylic Acid
NPK	Nitrogen, Phosphorus, and Potassium
DS	Disease Severity
WP	Water Priming
BP	BABA Priming

NP Non-primed
BSP BABA Spray Priming

References

- Jisha, K.; Vijayakumari, K.; Puthur, J.T. Seed priming for abiotic stress tolerance: An overview. *Acta Physiol. Plant.* **2013**, *35*, 1381–1396. [\[CrossRef\]](#)
- Sher, A.; Sarwar, T.; Nawaz, A.; Ijaz, M.; Sattar, A.; Ahmad, S. *Methods of Seed Priming. Priming and Pretreatment of Seeds and Seedlings: Implication in Plant Stress Tolerance and Enhancing Productivity in Crop Plants*; Springer: Singapore, 2019; pp. 1–10.
- Kaya, G. Tohum Uygulamaları (Priming)'nın Tohum Yağ Asitleri Kompozisyonuna Etkisi ve Tohum Kalitesi ile İlişkisi. *Tarla Bitk. Merk. Araştırma Enstitüsü Derg.* **2008**, *17*, 53–62.
- McDonald, M. Seed deterioration: Physiology, repair and assessment. *Seed Sci. Technol.* **1999**, *27*, 177–237.
- Karakurt, H.; Aslantaş, R.; Eşitken, A. Tohum çimlenmesi ve bitki büyümesi üzerinde etkili olan çevresel faktörler ve bazı ön uygulamalar. *Uludağ Üniversitesi Ziraat Fakültesi Derg.* **2010**, *24*, 115–128.
- Gökçöl, A.; Duman, İ. Kapari tohumlarının çimlenmesinin iyileştirilmesinde farklı tohum uygulamalarının etkisinin belirlenmesi. *J. Agric. Fac. Ege Univ.* **2018**, *55*, 71–80.
- Zulfiqar, F. Effect of seed priming on horticultural crops. *Sci. Hortic.* **2021**, *286*, 8. [\[CrossRef\]](#)
- Heydecker, W.; Gibbins, B. Attempts to synchronise seed germination. *Acta Hortic.* **1978**, *72*, 79–92. [\[CrossRef\]](#)
- Jones, J.D.; Dangl, J.L. The plant immune system. *Nature* **2006**, *444*, 323–329. [\[CrossRef\]](#)
- Mostek, A.; Börner, A.; Weidner, S. Comparative proteomic analysis of β -aminobutyric acid-mediated alleviation of salt stress in barley. *Plant Physiol. Biochem.* **2016**, *99*, 150–161. [\[CrossRef\]](#)
- Uddin, S.; Ullah, S.; Nafees, M. Effect of seed priming on growth and performance of *Vigna radiata* L. under induced drought stress. *J. Agric. Food Res.* **2021**, *4*, 100140. [\[CrossRef\]](#)
- Yokotani, N.; Hasegawa, Y.; Sato, M.; Hirakawa, H.; Kouzai, Y.; Nishizawa, Y.; Yamamoto, E.; Naito, Y.; Isobe, S. Transcriptome Analysis of *Clavibacter Michiganensis* Subsp. *Michiganensis*-Infected Tomatoes: A Role of Salicylic Acid in the Host Response. *BMC Plant Biol.* **2021**, *21*, 476. [\[CrossRef\]](#) [\[PubMed\]](#)
- Lee SangHyeob, L.S.; Hong JongChan, H.J.; Jeon WoongBae, J.W.; Chung YoungSoo, C.Y.; Sung SoonKee, S.S.; Choi Doil, C.D.; Joung YoungHee, J.Y.; Oh BoungJun, O.B. The salicylic acid-induced protection of non-climacteric unripe pepper fruit against *Colletotrichum gloeosporioides* is similar to the resistance of ripe fruit. *Plant Cell Rep.* **2009**, *28*, 1573–1580.
- Özkaynak, E.; Orhan, Y.; Kargın, İ.; Tuncel, M. Biber ve domates tohumlarında organik priming uygulamaları. *Black Sea J. Agric.* **2020**, *3*, 301–307.
- Cohen, Y.; Vaknin, M.; Mauch-Mani, B. BABA-induced resistance: Milestones along a 55-year journey. *Phytoparasitica* **2016**, *44*, 513–538. [\[CrossRef\]](#)
- Jakab, G.; Manrique, A.; Zimmerli, L.; Métraux, J.-P.; Mauch-Mani, B. Molecular characterization of a novel lipase-like pathogen-inducible gene family of *Arabidopsis*. *Plant Physiol.* **2003**, *132*, 2230–2239. [\[CrossRef\]](#) [\[PubMed\]](#)
- Bektas, Y.; Eulgem, T. Synthetic Plant Defense Elicitors. *Front. Plant Sci.* **2015**, *5*, 804. [\[CrossRef\]](#)
- Weralupitiya, C.; Eccersall, S.; Meisrimler, C.-N. Shared signals, different fates: Calcium and ROS in plant PRR and NLR immunity. *Cell Rep.* **2024**, *43*, 114910. [\[CrossRef\]](#)
- Wang, D.; Wei, L.; Liu, T.; Ma, J.; Huang, K.; Guo, H.; Huang, Y.; Zhang, L.; Zhao, J.; Tsuda, K. Suppression of ETI by PTI priming to balance plant growth and defense through an MPK3/MPK6-WRKYs-PP2Cs module. *Mol. Plant* **2023**, *16*, 903–918. [\[CrossRef\]](#)
- Zimmerli, L.; Hou, B.H.; Tsai, C.H.; Jakab, G.; Mauch-Mani, B.; Somerville, S. The xenobiotic β -aminobutyric acid enhances *Arabidopsis* thermotolerance. *Plant J.* **2008**, *53*, 144–156. [\[CrossRef\]](#)
- Kim, Y.C.; Kim, Y.H.; Lee, Y.H.; Lee, S.W.; Chae, Y.-S.; Kang, H.-K.; Yun, B.-W.; Hong, J.K. β -Amino-n-butyric acid regulates seedling growth and disease resistance of kimchi cabbage. *Plant Pathol. J.* **2013**, *29*, 305. [\[CrossRef\]](#)
- Bacelli, I.; Mauch-Mani, B. Beta-aminobutyric acid priming of plant defense: The role of ABA and other hormones. *Plant Mol. Biol.* **2016**, *91*, 703–711. [\[CrossRef\]](#) [\[PubMed\]](#)
- Cohen, Y.R. β -aminobutyric acid-induced resistance against plant pathogens. *Plant Dis.* **2002**, *86*, 448–457. [\[CrossRef\]](#) [\[PubMed\]](#)
- Luna, E.; Beardon, E.; Ravnskov, S.; Scholes, J.; Ton, J. Optimizing chemically induced resistance in tomato against *Botrytis cinerea*. *Plant Dis.* **2016**, *100*, 704–710. [\[CrossRef\]](#) [\[PubMed\]](#)
- Du, B.; Luo, H.; He, L.; Zhang, L.; Liu, Y.; Mo, Z.; Pan, S.; Tian, H.; Duan, M.; Tang, X. Rice seed priming with sodium selenate: Effects on germination, seedling growth, and biochemical attributes. *Sci. Rep.* **2019**, *9*, 4311. [\[CrossRef\]](#)
- Jisha, K.; Puthur, J.T. Seed priming with BABA (β -amino butyric acid): A cost-effective method of abiotic stress tolerance in *Vigna radiata* (L.) Wilczek. *Protoplasma* **2016**, *253*, 277–289. [\[CrossRef\]](#)
- Kulak, M.; Jorrín-Novo, J.V.; Romero-Rodriguez, M.C.; Yildirim, E.D.; Gul, F.; Karaman, S. Seed priming with salicylic acid on plant growth and essential oil composition in basil (*Ocimum basilicum* L.) plants grown under water stress conditions. *Ind. Crop. Prod.* **2021**, *161*, 113235. [\[CrossRef\]](#)

28. Martínez-Aguilar, K.; Hernández-Chávez, J.L.; Alvarez-Venegas, R. Priming of seeds with INA and its transgenerational effect in common bean (*Phaseolus vulgaris* L.) plants. *Plant Sci.* **2021**, *305*, 110834. [\[CrossRef\]](#)
29. Chang, R.; Ries, S.; Pataky, J. Reductions in yield of processing tomatoes and incidence of bacterial canker. *Plant Dis.* **1992**, *76*, 85–89. [\[CrossRef\]](#)
30. Nandi, M.; Macdonald, J.; Liu, P.; Weselowski, B.; Yuan, Z.C. *Clavibacter michiganensis* ssp. *michiganensis*: Bacterial canker of tomato, molecular interactions and disease management. *Mol. Plant Pathol.* **2018**, *19*, 2036–2050. [\[CrossRef\]](#)
31. Poysa, V. Evaluation of tomato breeding lines resistant to bacterial canker. *Can. J. Plant Pathol.* **1993**, *15*, 301–304. [\[CrossRef\]](#)
32. Chalupowicz, L.; Barash, I.; Reuven, M.; Dror, O.; Sharabani, G.; Gartemann, K.H.; Eichenlaub, R.; Sessa, G.; Manulis-Sasson, S. Differential contribution of *Clavibacter michiganensis* ssp. *michiganensis* virulence factors to systemic and local infection in tomato. *Mol. Plant Pathol.* **2017**, *18*, 336–346. [\[CrossRef\]](#)
33. Holtsmark, I.; Mantzilas, D.; Eijnsink, V.; Brurberg, M. The tomato pathogen *Clavibacter michiganensis* ssp. *michiganensis*: Producer of several antimicrobial substances. *J. Appl. Microbiol.* **2007**, *102*, 416–423. [\[CrossRef\]](#) [\[PubMed\]](#)
34. Eichenlaub, R.; Gartemann, K.-H. The *Clavibacter michiganensis* subspecies: Molecular investigation of gram-positive bacterial plant pathogens. *Annu. Rev. Phytopathol.* **2011**, *49*, 445–464. [\[CrossRef\]](#) [\[PubMed\]](#)
35. Sharabani, G.; Manulis-Sasson, S.; Borenstein, M.; Shulhani, R.; Lofthouse, M.; Chalupowicz, L.; Shtienberg, D. The significance of guttation in the secondary spread of *Clavibacter michiganensis* subsp. *michiganensis* in tomato greenhouses. *Plant Pathol.* **2013**, *62*, 578–586. [\[CrossRef\]](#)
36. Vega, D.; Romero, A.M. Survival of *Clavibacter michiganensis* subsp. *michiganensis* in tomato debris under greenhouse conditions. *Plant Pathol.* **2016**, *65*, 545–550. [\[CrossRef\]](#)
37. Yuqing, W.; Zhang, Y.; Zhipeng, G.; Wencai, Y. Breeding for resistance to tomato bacterial diseases in China: Challenges and prospects. *Hortic. Plant J.* **2018**, *4*, 193–207.
38. Mohammad, H.B.; Lee, S.E.; Kim, M.S. Refining a Pathogen's Genome to Know the Enemy Better for the Winning Battles. *Proteomics* **2019**, *19*, 1900005. [\[CrossRef\]](#)
39. Mohd Nadzir, M.; Vieira Lelis, F.; Thapa, B.; Ali, A.; Visser, R.; van Heusden, A.; Van der Wolf, J. Development of an in vitro protocol to screen *Clavibacter michiganensis* subsp. *michiganensis* pathogenicity in different *Solanum* species. *Plant Pathol.* **2019**, *68*, 42–48. [\[CrossRef\]](#)
40. Bektas, Y. The synthetic elicitors 2, 6-dichloro-isonicotinic acid (INA) and 2, 4-dichloro-6-[(E)-(3-methoxyphenyl) imino] methyl] phenol (DPMP) enhances tomato resistance against bacterial canker disease with different molecular mechanisms. *Physiol. Mol. Plant Pathol.* **2021**, *116*, 101740. [\[CrossRef\]](#)
41. Abbasi, S.; Sadeghi, A.; Omidvari, M.; Tahan, V. The stimulators and responsive genes to induce systemic resistance against pathogens: An exclusive focus on tomato as a model plant. *Biocatal. Agric. Biotechnol.* **2021**, *33*, 101993. [\[CrossRef\]](#)
42. Bektas, H.; Hohn, C.E.; Waines, J.G. Root and shoot traits of bread wheat (*Triticum aestivum* L.) landraces and cultivars. *Euphytica* **2016**, *212*, 297–311. [\[CrossRef\]](#)
43. Baysal, Ö.; Soylu, E.M.; Soylu, S. Induction of defence-related enzymes and resistance by the plant activator acibenzolar-S-methyl in tomato seedlings against bacterial canker caused by *Clavibacter michiganensis* ssp. *michiganensis*. *Plant Pathol.* **2003**, *52*, 747–753. [\[CrossRef\]](#)
44. Livak, K.J.; Schmittgen, T.D. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods* **2001**, *25*, 402–408. [\[CrossRef\]](#)
45. Molinari, S.; Fanelli, E.; Leonetti, P. Expression of tomato salicylic acid (SA)-responsive pathogenesis-related genes in Mi-1-mediated and SA-induced resistance to root-knot nematodes. *Mol. Plant Pathol.* **2014**, *15*, 255–264. [\[CrossRef\]](#)
46. Hu, J.; Jiang, J.; Wang, N. Control of citrus Huanglongbing via trunk injection of plant defense activators and antibiotics. *Phytopathology* **2018**, *108*, 186–195. [\[CrossRef\]](#) [\[PubMed\]](#)
47. Atamian, H.S.; Eulgem, T.; Kaloshian, I. SIWRKY70 is required for Mi-1-mediated resistance to aphids and nematodes in tomato. *Planta* **2012**, *235*, 299–309. [\[CrossRef\]](#)
48. Chandrasekaran, M.; Chun, S.C. Expression of PR-protein genes and induction of defense-related enzymes by *Bacillus subtilis* CBR05 in tomato (*Solanum lycopersicum*) plants challenged with *Erwinia carotovora* subsp. *carotovora*. *Biosci. Biotechnol. Biochem.* **2016**, *80*, 2277–2283. [\[CrossRef\]](#)
49. Zhou, J.; Wang, J.; Zheng, Z.; Fan, B.; Yu, J.-Q.; Chen, Z. Characterization of the promoter and extended C-terminal domain of Arabidopsis WRKY33 and functional analysis of tomato WRKY33 homologues in plant stress responses. *J. Exp. Bot.* **2015**, *66*, 4567–4583. [\[CrossRef\]](#)
50. Ray, S.; Mondal, S.; Chowdhury, S.; Kundu, S. Differential responses of resistant and susceptible tomato varieties to inoculation with *Alternaria solani*. *Physiol. Mol. Plant Pathol.* **2015**, *90*, 78–88. [\[CrossRef\]](#)
51. Worrall, D.; Holroyd, G.H.; Moore, J.P.; Glowacz, M.; Croft, P.; Taylor, J.E.; Paul, N.D.; Roberts, M.R. Treating seeds with activators of plant defence generates long-lasting priming of resistance to pests and pathogens. *New Phytol.* **2012**, *193*, 770–778. [\[CrossRef\]](#)

52. Anup, C.P.; Melvin, P.; Shilpa, N.; Gandhi, M.N.; Jadhav, M.; Ali, H.; Kini, K.R. Proteomic analysis of elicitation of downy mildew disease resistance in pearl millet by seed priming with β -aminobutyric acid and *Pseudomonas fluorescens*. *J. Proteom.* **2015**, *120*, 58–74. [[CrossRef](#)] [[PubMed](#)]
53. Baysal, Ö.; Gürsoy, Y.Z.; Örneke, H.; Duru, A. Induction of oxidants in tomato leaves treated with DL- β -Amino butyric acid (BABA) and infected with *Clavibacter michiganensis* ssp. *michiganensis*. *Eur. J. Plant Pathol.* **2005**, *112*, 361–369. [[CrossRef](#)]
54. Dong, X. NPR1, all things considered. *Curr. Opin. Plant Biol.* **2004**, *7*, 547–552. [[CrossRef](#)] [[PubMed](#)]
55. Zimmerli, L.; Métraux, J.-P.; Mauch-Mani, B. β -Aminobutyric acid-induced protection of Arabidopsis against the necrotrophic fungus *Botrytis cinerea*. *Plant Physiol.* **2001**, *126*, 517–523. [[CrossRef](#)]
56. Uehara, T.; Sugiyama, S.; Matsuura, H.; Arie, T.; Masuta, C. Resistant and susceptible responses in tomato to cyst nematode are differentially regulated by salicylic acid. *Plant Cell Physiol.* **2010**, *51*, 1524–1536. [[CrossRef](#)]
57. Tsai, C.H.; Singh, P.; Chen, C.W.; Thomas, J.; Weber, J.; Mauch-Mani, B.; Zimmerli, L. Priming for enhanced defence responses by specific inhibition of the Arabidopsis response to coronatine. *Plant J.* **2011**, *65*, 469–479. [[CrossRef](#)]
58. Martínez-Aguilar, K.; Ramírez-Carrasco, G.; Hernández-Chávez, J.L.; Barraza, A.; Alvarez-Venegas, R. Use of BABA and INA as activators of a primed state in the common bean (*Phaseolus vulgaris* L.). *Front. Plant Sci.* **2016**, *7*, 653. [[CrossRef](#)]
59. Vogt, T. Phenylpropanoid biosynthesis. *Mol. Plant* **2010**, *3*, 2–20. [[CrossRef](#)]
60. Cumplido-Nájera, C.F.; González-Morales, S.; Ortega-Ortíz, H.; Cadenas-Pliego, G.; Benavides-Mendoza, A.; Juárez-Maldonado, A. The application of copper nanoparticles and potassium silicate stimulate the tolerance to *Clavibacter michiganensis* in tomato plants. *Sci. Hortic.* **2019**, *245*, 82–89. [[CrossRef](#)]
61. Eulgem, T.; Rushton, P.J.; Robatzek, S.; Somssich, I.E. The WRKY superfamily of plant transcription factors. *Trends Plant Sci.* **2000**, *5*, 199–206. [[CrossRef](#)]
62. Mao, G.; Meng, X.; Liu, Y.; Zheng, Z.; Chen, Z.; Zhang, S. Phosphorylation of a WRKY transcription factor by two pathogen-responsive MAPKs drives phytoalexin biosynthesis in Arabidopsis. *Plant Cell* **2011**, *23*, 1639–1653. [[CrossRef](#)]
63. Liu, S.; Ziegler, J.; Zeier, J.; Birkenbihl, R.P.; Somssich, I.E. Botrytis cinerea B05. 10 promotes disease development in Arabidopsis by suppressing WRKY33-mediated host immunity. *Plant Cell Environ.* **2017**, *40*, 2189–2206. [[CrossRef](#)] [[PubMed](#)]
64. Knoth, C.; Ringler, J.; Dangl, J.L.; Eulgem, T. Arabidopsis WRKY70 is required for full RPP4-mediated disease resistance and basal defense against *Hyaloperonospora parasitica*. *Mol. Plant-Microbe Interact.* **2007**, *20*, 120–128. [[CrossRef](#)] [[PubMed](#)]
65. Li, J.; Zhong, R.; Palva, E.T. WRKY70 and its homolog WRKY54 negatively modulate the cell wall-associated defenses to necrotrophic pathogens in Arabidopsis. *PLoS ONE* **2017**, *12*, e0183731. [[CrossRef](#)] [[PubMed](#)]
66. Smith, J.E.; Mengesha, B.; Tang, H.; Mengiste, T.; Bluhm, B.H. Resistance to *Botrytis cinerea* in *Solanum lycopersicoides* involves widespread transcriptional reprogramming. *BMC Genom.* **2014**, *15*, 334. [[CrossRef](#)]
67. AbuQamar, S.; Chai, M.-F.; Luo, H.; Song, F.; Mengiste, T. Tomato protein kinase 1b mediates signaling of plant responses to necrotrophic fungi and insect herbivory. *Plant Cell* **2008**, *20*, 1964–1983. [[CrossRef](#)]

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