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Induce resistance against bacterial speck disease of tomato *Pseudomonas syringae* pv. *tomato* by the chemical inducer ß-aminobutyric acid

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Abstract

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The ability of ß-aminobutyric acid to induce resistance tomato (*Lycopersicon esculentum* Mill. var. GS12) seedlings against bacterial speck disease was studied. Different concentrations were used as: 100, 200, 300, 400, 500, 600 mg/lafter tomato plants were sprayed with 10^8CFU/ml foliar *Pseudomonas syringae* pv. *tomato* suspension.

The results revealed that the concentration of 100 mg/l of ß-aminobutyric acid was the most efficient in reducing disease incidence and severity, furthermore it was found that all used concentrations improved tomato seedlings growth compared with control.

The response of the treated tomato seedlings to the different concentration of ß-aminobutyric acid to induce systemic acquired resistance against bacterial speck (*P. syringae* pv. *tomato*) was found to be correlated to fluctuations with the amount of some compounds known to interfere with the defense mechanism against pathogen invading including: free salicylic acid, ethylene, chitinase, peroxidase, ß-1,3- glucanase enzymes, protein content, and total phenolic compounds. The biochemical analysis indicated that tomato seedlings sprayed with 100 mg/l of ß-aminobutyric acid produced highest amounts of ethylene 9 and 15 days post inoculation which was significantly different from control, while highly recovered free salicylic acid was 12 days post inoculation. The recovered level of endogenous enzymes peroxidase and ß-1,3- glucanase was increased over time post inoculation, where the highest amount was 15 days post inoculation. The highest recovered chitinase was 9 days post inoculation and protein compounds contents were increased gradually over time and the highest amount were 12 days post inoculation, in which, it was found that was significantly different compared with control. These results were correlated with low level of disease incidence and severity compared with control and with the high recovered population of *P. syringae* pv. *tomato* of 5.38*10^8CFU /ml after 21 days of inoculation. The results revealed that there is no correlation between total phenolic contents in tomato seedlings treated with ß-aminobutyric acid on induces systemic resistance.

Keywords: Pseudomonas syringae pv. *Tomato;* induce resistance; ß-aminobutyric acid
Abbreviations: ßABA*:* ß-aminobutyric acid; dpi*:* days post inoculation; SA*:* salicylic acid

Introduction

Tomato *(Lycopersicon esculentum* Mill.) is one of the important economic crops grown in Jordan. The area planted to tomato in 2016 was 15643.5 hectares with total production of 726440 tons (Department of Statistics, 2016).

Bacterial speck of tomato caused by *Pseudomonas syringae* pv. *tomato* (Okabe) attacks tomato leaves, fruits and stems, making production of disease free seedlings very difficult and drastically affects the yield (Good & Sasser, 1980). The disease was first reported in Jordan during 1991 (Khlaif, 1991). Within the last decade, bacterial speck of tomato has become a serious problem in many tomato production areas. The disease was reported to reduce the fruit quality due to lesions that formed on the fruit surface with 75% of the fruit surface area in the early infection and with 5% of the fruit surface area due to late infection (Yunis et al., 1980).

Different methods were used to control tomato speck of these; fungicides, bactericides (Pernezy et al., 1995; Duffy & Defago, 1999) and cultural practices (Colin & Chafik, 1986). Unfortunately, these methods were found to be inefficient due to the ability of bacteria to build up resistance strains. Therefore, there is need for an effective way to protect plant from pathogens without endangering people or the environment (Colin & Chafik*,* 1986).

All plants possess active defense mechanisms against pathogens attack. These mechanisms fail when a plant is infected by a virulent pathogen because the pathogen avoids triggering or suppressing resistance reactions, or evades the effects of activated defenses. If defense mechanisms are triggered by a stimulus prior to infection by a plant pathogen, disease can be reduced. Induced resistance is not the reaction of resistance where there is none, but the activation of latent resistance mechanisms that are expressed upon subsequent events (Van Loon et al., 1998).

Induced resistance is a phenomenon by which a plant exhibits an increased level of resistance by utilizing the plant defense mechanism to restrict the pathogen progress without changing in its basic genetic constitutions. Resistance can be triggered by a predisposing infection with necrotic pathogen, by colonization of the rhizosphere with certain plant growth -promoting rhizobacteria or by treating with certain chemicals such as salicylic acid (SA) and ß-amino butyric acid (ßABA) (Siegrist et al., 1997; Jakab et al., 2001; Herman & Smart, 2008; Tamm et al., 2011; Kim et al., 2013; Baccelli & Mauch-Mani, 2016; Wilkinson et al., 2018; and Yoo et al., 2018). Resistance could be induced locally and called localized acquired resistance, or systemically through all plant tissue which is commonly referred as systemic acquired resistance (SAR) (Van loon et al., 1998).

Different chemicals have been used to induce resistance against different plant pathogens of these: benzothiadiazole (Bion) (Friedrich et al., 1996; Benhamou & Belangaer, 1998; Godard et al., 1999), salicylic acid (Ckmus & Sayar, 1991), 3- aminobutyric acid (Junjun et al., 1996; Jakab et al., 2001; Cohen, 2002; Tamm et al., 2011) and potassium phosphate (K_2HPO_4) (Orober et al., 1996; Becot et al., 2000) and acibenzolar-S-methyl (Herman & Smart, 2008).

Aminobutyric acid is secondary, non-protein amino acids plant metabolites which have the ability to protect different plant species against different pathogens. However, a high degree of specificity among aminobutyric acid isomers were detected; and only β-aminobutyric acid (ßABA) isomer (also known as 3- ABA according to IUPAC nomenclature) reported to activate the systemic acquired resistance pathway depending on SA accumulation, while no changes were detected in plants treated with â-aminobutyric acid before infection (Zimmerli et al., 2000)

The chemical 3-aminobutyric acid have been used to induce resistance against different plant pathogens of these; *Fusarium oxysporium* f. sp. *Lycopersici* (Junjun et al., 1996), *Peronospora parasitica* (Zimmerli et al., 2000), *Plazmopara viticola* and *Bremialactucae* (Tamm et al., 2011), *Alternaria brassicicola* and *Colletotrichum higginsianum* (Kim et al., 2013) and *Botrytis cinerea* (Wilkinson et al., 2018).

Moreover, several studies were performed about the effect of amino butyric acid isomers; gamma-aminobutyric acid (GABA) and ßABA; on plant growth. It was found that Synthetic GABA involved in the plant growth regulations and development (Ramesh et al., 2015; Li et al., 2016). Whereas, ßABA treatment of kimchi cabbage seedlings significantly reduced primary root elongation and cotyledon development in a dose-dependent manner (Kim et al., 2013). Also, Rajaei & Mohamadi (2013) reported that ßABA had no significant effect on seed germination of canola but in abscisic acid and $CoCl_2$ treated seeds; germination was reduced compared to the control. Abscisic acid inhibited root growth more than ßABA. Combined use of abscisic acid and ßABA treatment, had an additional inhibitory effect on shoot and root growth.

Therefore, this research was conducted in order to test the ability of the following concentrations of ßABA; 100, 200, 300, 400, 500, and 600 mg/l; of aqueous solution to induce systemic acquired resistance in tomato seedlings against tomato speck and its effect on plant growth.

Materials and Methods

Bacterial Isolate

Pseudomonas syringae pv. *tomato* was isolated from a diseased tomato plant collected from a nursery in the Jordan

Valley suspected to be infected with tomato speck. The obtained isolate was subjected to physiological, nutritional and pathological tests for characterization and identification as described by Schaad et al. (2001); In Kring & Holt (1984); and Brudbary (1986). The same tests were run against a reference culture of *Pseudomonas syringae* pv. *tomato* (NCP-PB1106).

ß-Aminobutyric acid

The different tested concentrations of ß-ßABA48% (University of Hoenheim, Institute of Phytomedicine, Germany) are in Table 1.

Greenhouse experiment

Greenhouse experiments were conducted at the University of Jordan campus to test the ability of the different concentrations of ßABAto induce systemic acquired resistance in tomato seedlings *Lycopersicon esculentum* Mill. cv. GS12 against tomato speck *P. syringae* pv. *tomato*.

Five to four weeks old tomato seedlings were transplanted into plastic pots (500g of sterilized soil mixture) Soil: Peat moss 2:1, watered with 25 ml of selected concentration suspension. Each treatment was replicated four times.

One week later, all treated tomato seedlings were artificially inoculated with 10^8 CFU/ml of *P. syringae* pv. *tomato* suspension prepared from 24 hours old culture to the lower surface of the true full leaves of the treated seedlings. The treated tomato seedlings were randomly and kept under mist chamber to maintain high relative humidity around 100% and temperature of 24 ± 3°C.

Other treatments were as follows:

The first set of tomato seedlings were inoculated only with *P. syringae* pv. *tomato* and kept in the greenhouse under mist chamber.

Second set of tomato seedlings were treated with the chemical inducers only to serve as control and kept on greenhouse bench.

Third set of tomato seedlings was left without any treatment and kept in the greenhouse bench.

The tomato seedlings were checked periodically for the development of speck symptoms. Then treatments were evaluated according to the response of the treated tomato seedlings to the challenged pathogen taking into consideration the following parameters: disease incidence, severity and vegetative growth.

The disease incidence was calculated as the percentage of infected leaves out of total leaves for tomato seedlings in the same treatment and the average of readings was calculated.

The number of specks per each plant in each treatment was counted and the severity of each infected tomato seedling was determined according to 0-6 modified scale of Pilowsky & Zutra (1982) as follow: 0, 1,2, 3,4, 5, and 6 equals to no lesion, 1-10 lesions per plant, 11-20 lesions per plant, 21-40 lesions per plant, 41-100 lesions per plant, 101-200 lesions per plant, and more than 200 lesions per plant, respectively. The average of severity of all tomato infected seedlings in each treatment was calculated and the overall average of disease severity for all tested tomato seedlings, in the different replicates of each treatment was calculated.

The leaf area, plant height, foliage fresh and dry weight and root of 8 tomato seedlings from the different replicates of each treatment were randomly chosen.

Population dynamic of P. syringae pv. tomato

Leaf samples consisted of 15-20 leaves taken from the tomato seedlings treated with chemical inducer were randomly taken from the treated tomato seedlings from all replicates of each treatment at 0, 3, 6, 9, 12, 15, 18, and 21 days after inoculation, brought to the laboratory mixed to make a composite sample, 0.5 g were randomly taken from each sample, and serial dilutions were prepared.

After that 0.1 ml of the 10^{-3}, $l0^{-4}$ was taken aseptically with sterile pipette, spreaded on the surface of KB dried plates supplemented with rifampicin (50 mg/liter) and cyclohexamide (100 mg/liter). Four plates were inoculated from each dilution for each sample, incubated at 25±2°C and checked for bacterial growth. Plates were subjected periodically to long wave ultra violet light (367 nm) to detect fluorescent colonies. Fluorescent colonies were counted in each plate then the CFU/ml were calculated for each plate and average CFU/ml for the four samples for each treatment was estimated. Representative fluorescent colonies were sub-cultured on new KB medium plates, subjected to biochemical tests as described by Schaad et al. (2001) to prove their identity to *P. syringae* pv. *tomato.* Log of bacterial population was computed and plotted against time (Saskia et al., 1997).

Table 1. The different tested concentrations of ß-aminobutyric acid 48%

Chemical Inducer	Concentrations	Source
ß-aminobutyric acid (48% active ingredient)	100, 200, 300, 400, 500 and 600 mg/l of aqueous solution	University of Hoenheim, Institute of Phytomedicine, Germany

Biochemical analysis

The most effective concentration of ßABA was chosen for biochemal analysis based on disease incidence and severity for tomato seedlings treated with ßABA.

The response of the treated tomato seedlings was correlated to fluctuation with the amount of some compounds: free SA, ethylene, chitinase, peroxidase, ß-1,3-glucanase enzymes, protein content, and total phenolic compound. Fifteen leaves from each treatment were randomly collected after 3, 6, 9, 12, and 15 days of inoculation. Mixed and subjected to the following experiments:

A – Extraction, purification and quantization of free salicylic acid

Free SA was evaluated according to Smith-Becker et al. (1998); and Enyedi et al. (1992) and identified by its characteristic UV absorption spectrum, its co elution with standard SA, and its visible fluorescence upon exposure to UV (214 nm) light (Leon et al., 1995; smith-bicker et al., 1998; Spletzer et al., 1999). Quantification of free SA in tomato leaves was performed by constructing a standard curve for the relationship between peak height and the concentration of the SA standards.

B – Ethylene estimation

Ethylene content was determined according to Hoffland et al. (1997); Knoester et al. (1999); Lund et al. (1998); and Penninckx et al. (1998). A standard curve was constructed for the relationship between peak area and different concentrations of standard ethylene. Standard concentration of 4, 2, 1, 0.1, and 0.001 ppm were prepared and injected in GC.

C – Enzymes extraction

A total of 0.5 g from frozen leaves of the composite sample were macerated with l0 uL of extraction buffer per 1 mg of fresh weight in 50 mM cold sodium phosphate buffer pH7.5, centrifuged at 17000 x g for 20 min at 4°C, then the different enzyme activities were immediately assayed:

1. Peroxidase activity

Peroxidase activity was determined according to Brisset (2000). Oxidation of guaiacol to tetraguaiacol amount was determined by measuring the absorbance at 470 nm for 2 min at 30°C spectrophtometrically (Model 6100 Spectrophotometer JENWAY).

2. ß -1,3-glucanase

ß -1,3-glucanase was determined according to Brisset (2000), carboxymethyl-curdlan- Remazol Brilliant Blue (CM-curdlan-RBB, Loewe Biochemica, Otterfing, Germany) was used as a substrate for ß-1,3-glucanase enzyme. Gluconase activity was calculated from the differences in the absorbance of the mixture at 600nm between duplicate extracts incubated 1 and 2 hrs, respectively.

3. Chitinase

The protocol of Loewe Biochemica Company was followed for the quantification of chitinase enzyme. Chitinase activity was expressed as 1 pmol of GlcNAc (N-acetyle glucosamine) ml^{-1} min^{-1} (Sathiyabama & Balasubramanian, 1999)

D – Total protein determination:

Lowy (1951) procedure was followed to determine the protein content in tomato tissue by measuring the intensity of the blue color developed after the reaction of the protein solution with alkaline copper and Folin's - Ciocaltues reagent.

For protein contents quantification, a standard curve was constructed for the relationship between the absorbance and the standard protein concentration. Standard protein concentrations were prepared from stock solution of Bovine Serum Albumin (BSA) 22% containing 220 ppm. Dilutions of 25, 50, 75, 100, 125 and 150 mg/l were prepared, then 0.5 ml from each dilution was added to 5 ml of protein reagent, after 5 min 0.5 ml of folin's-ciocalties reagent (1:1 folin's -ciocltuse to water) was added, then the absorbance was measured after 10 min at 750 nm using spectrophotometer (Model 6100 Spectrophotometer JENWAY). Sterile distilled water was used as blank.

E – Total phenolic compounds

Total phenols in the leaves and stems were determined according to Johnson & Schaall (1957). A standard curve was prepared from stock solution of 10000 ppm chlorogenic acid ($C_{l6}H_{l8}O_9$), and the absorbance of 10000, 7500, 5000, 2500, and 1250 ppm concentrations was measured at 725 nm using the spectrophotometer (Model 6100 Spectrophotometer JENWAY).

Statistical analysis

The layout of the greenhouse experiments was Split Plot Design-with Complete Randomized arrangement, and the laboratory experiment was arranged in Randomized Design. All data collected; disease incidence, severity, vegetative parameters, average amounts of the recovered enzymes (peroxidase, ß-1,3-glucanase and chitinase), protein and phenolic compounds were statistically analyzed using SAS program. The different significant means were separated by LSD ($P \leq 10.05$).

Results

Greenhouse Experiment

1. Disease incidence and severity

The disease incidence and severity increased during the first four weeks of inoculation, except for tomato seedlings treated with 100 mg/l, where it showed the lowest incidence and severity which significantly differed from other treatments (Table 2).

On the other hand, the different vegetative parameters were found to be affected with various degrees in the different treatments, but no significant differences occurred for the same vegetative parameter of tomato seedlings watered with different concentrations of ßABA except the leaf area. Significant differences occurred between the control values and the values of the same parameters for the different treatments (Table 3).

2. Population of *Pseudomonas syringae* pv. *tomato*

Pseudomonas syringae pv. *tomato* was recovered from leaves of the treated tomato seedlings with the ßABAartificially inoculated with a suspension of 108 CFU/ml prepared from 24 hours old culture *P. syringae* pv. *tomato* (Figure 1). In general, the recovered population of treated infected tomato was lower than infected control.

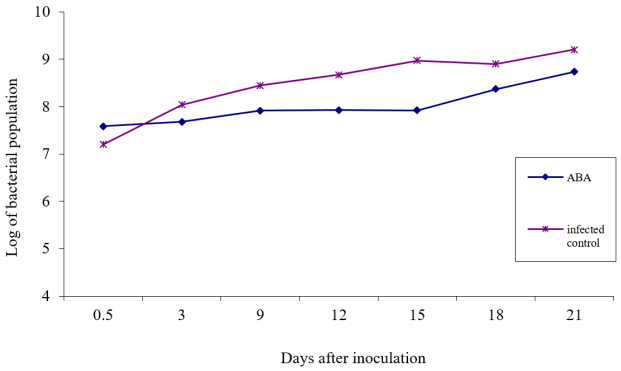

Fig. 1. Recovered population of *Pseudomonas Syringae* pv. *tomato* from tomato seedlings sprayed with aminobutyric acid

Table 2. Disease incidence and severity of tomato speck on tomato seedlings cv. GS12 sprayed with different concentrations of ß-aminobutyric acid and artificially inoculated with *Pseudomonas syringae* pv. *tomato* one week later

Amino Butyricacid Conc., mg/l	Time in week post inoculation							
	Wk1		Wk2		Wk3		Wk4	
	Incidence	Severity	Incidence	Severity	Incidence	Severity	Incidence	Severity
600	95a*	5.2a	97a	5.5a	100a	5.9a	100a	6.0a
500	94**	4.7ab	98a	5.0a	100a	5.6ab	100a	6.0a
400	88ab	4.2b	91a	5.1a	94ab	5.4b	96a	5.8a
300	76b	2.8c	64b	4.0b	66c	4.3c	67b	4.2c
200	84b	2.7c	51c	3.1c	54d	3.2d	52c	3.3d
100	41c	2.2c	39d	2.3d	36e	2.1e	35d	2.2e
Control	43c	2.4c	64b	3.7bc	891b	4.5c	68b	5.1b
L.S.D.	12.14	0.77	11.87	0.76	10.78	0.49	12.45	0.45

*Average of 4 replicates

**Means followed with the same letter are not significantly different at $p \leq 0.05$ within column

Table 3. Plant length, root weight, foliage weight, foliage dry weight and leaf area for tomato plants cv. GS12 sprayed with different concentrations of ß-aminobutyric acid and artificially inoculated with *Pseudomonas syringae* pv. *tomato* suspension one week later

Conc., mg/l	Plant height, cm	Root weight, g	Foliage weight, g	Foliage dry weight, g	Leaf area, cm^2
600	33.80a*	4.42ab**	11.74a	1.27a	249.88b
500	34.00a	3.42ab	12.00a	1.32a	251.04b
400	32.30a	3.32b	11.68a	1.41a	267.88b
300	33.00a	3.60ab	12.02a	1.44a	333.44a
200	37.20a	3.90ab	12.18a	1.60a	240.50b
100	37.30a	4.54a	12.26a	1.50a	269.92b
Control	22.29b	3.57ab	6.00b	0.90b	143.77c
L.S.D.	5.28	1.15	2.73	0.41	58.71

*Average of 4 replicates

**Means followed with the same letter are not significantly different at $p \leq 0.05$ within column

3. Ethylene content

Ethylene was recovered from all tomato seedlings treated with the selected concentration of aminobutyric acid; where the ethylene contents significantly differ after 9 and 15 days post inoculation (dpi) (Table 4).

Table 4. Recovered ethylene (nmol/g/hr) from tomato seedlings cv. GS12 sprayed with ß-aminobutyric acid (100mg/l) and artificially inoculated with *Pseudomonas syringae* pv. *tomato* one week later

Treatment	Days after inoculation				
	3	6	9	12	15
ßABA (100 mg/l)	3.48bc*	2.84b	5.74a	4.60b	7.78a
Infected control	2.15bc	3.07b	2.82b	4.23b	3.67b
Control	1.10c	1.25b	1.65b	2.87b	2.04b
L.S.D.	3.22	3.92	1.92	2.03	2.04

*Average of 4 replicates

**Means followed with the same letter are not significantly different at $p \leq 0.05$ within column

4 Free salicylic acid content

The recovered free SA followed the same pattern in all treatments and decreased from three up to six dpi, where the highest recovered free SA was recorded 12 dpi from the tomato seedlings treated with ßABA, and differ significantly with other treatments (Table 5).

Table 5. Recovered free salicylic acid (µg/g) from tomato seedlings cv. GS12 sprayed with ß-aminobutyric acid (100mg/l) and artificially inoculated with *Pseudomonas syringae* pv. *tomato* one week later

Treatment	Days after inoculation				
	3	6	9	12	15
ßABA (100mg/l)	12.00b*	9.40d**	8.76c	14.03a	3.70c
Infected control	13.29b	11.6b	10.71b	9.15c	4.91c
Control	5.07d	7.34e	7.00d	9.00c	3.32c
L.S.D.	3.29	1.00	1.49	2.03	1.65

*Average of 4 samples

**Means followed with the same letter are not significantly different at $p \leq 0.05$ within column

5 Enzyme extraction

A Peroxidase content

Peroxidase enzyme was detected in all tomato seedlings treated with the selected concentration of ßABAat the different sampling times, where the lowest level of peroxidase was recovered from tomato seedlings treated with ßABA (100 mg/l) after three days of inoculation (Table 6).

B ß-l,3-glucanase content

The recovered amount of ß-l,3-glucanase ranged from 0.11 up to 4.58 Δ absorbance/mg protein/min from tomato seedlings treated with ßABA (100 mg/l) (Table 7).

Table 7. Recovered ß-l,3-glucanase enzyme (Δ absorbance/mg protein/min) from tomato seedlings cv. GS12 sprayed with ß-aminobutyric acid (100 mg/l) and artificially inoculated with *Pseudomonas syringae* pv. *tomato* one week late

Treatment	Days after inoculation				
	3	6	9	12	15
ßABA (100 mg/l)	0.11c*	2.57c **	4.58ab	0.34c	2.93a
Infected control	0.22c	1.43de	2.53b	0.04c	1.20d
Control	0.15c	1.88d	3.2b	0.02c	1.13d
L.S.D.	0.13	0.90	4.58	1.75	0.33

*Average of 4 samples

**Means followed with the same letter are not significantly different at $p \leq 0.05$ within column

C Chitinase content

The highest amount of chitinase was recovered from tomato seedlings treated with ßABA (100 mg/l), infected control and control treatment did not differ significantly from each other (Table 8).

6. Protein content

The recovered amount of protein from tomato seedlings treated with ßABA (100 mg/l) increased up to 12 days after

Table 6. Recovered peroxidase enzyme (nmol tetraguaiacol/mg protein/min) from tomato seedlings cv. GS12 sprayed with ß-aminobutyric acid (l00 mg/l) and artificially inoculated with *Pseudomonas syringae* pv. *tomato* one week later

Treatment	Days after inoculation				
	3	6	9	12	15
ABA (100 mg/l)	281.87d*	388.13d**	335.77d	383.90d	503.17c
Infected control	350.21d	555.64b	220.07e	208.6f	139.53e
Control	189.54e	261.00f	129.43f	103.30e	171.67e
L.S.D.	33.10	11.12	12.57	58.64	42.67

*Average of 4 samples

**Means followed with the same letter are not significantly different at $p \leq 0.05$ within column

Table 8. Recovered chitinase enzyme (pmol of GlcNAc ml^{-1} mid^{-1}) from tomato seedlings cv. GS12 sprayed with ß-aminobutyric acid (100 mg/l) and artificially inoculated with *Pseudornonas syringae* pv. *tomato* one week later

Treatment	Days after inoculation				
	3	6	9	12	15
ABA (100 mg/l)	0.09b	0.20a	0.59b	0.16 d	0.42b
Infected control	0.02c	0.03b	0.48b	1.09b	0.37b
Control	0.02c	0.01b	0.43c	0.91bc	0.33b
L.S.D.	0.02	0.12	0.15	0.32	0.21

*Average of 4 samples

**Means followed with the same letter are not significantly different at $p \leq 0.05$ within column

Table 9. Recovered protein (ng/g) from tomato seedlings cv. GS12 sprayed with ß-aminobutyric acid (100 mg/l) and artificially inoculated with *Pseudomonas syringae* pv. *tomato* one week later

Treatment	Days after inoculation				
	3	6	9	12	15
ßABA (100 mg/l)	0.54b*	0.45b**	0.50d	0.88a	0.52bc
Infected control	0.51bc	0.52ab	0.61cd	0.45c	0.43c
Control	0.43d	0.52ab	0.75bcg	0.45c	0.44c
L.S.D.	0.063	0.33	0.16	0.09	0.22

*Average of 4 samples

**Means followed with the same letter are not significantly different at $p \leq 0.05$ within column

inoculation, where the highest amount was 0.88 ng/g recovered from tomato seedlings after 12 dpi (Table 9).

7. Total phenolic compounds content

Phenolic compounds were recovered from all tomato seedlings treated with 100 mg/l ßABA, where there was no significant difference between the infected control and treated plants (Table 10).

Discussion

Induced disease resistance is the phenomenon by which a plant exhibits an increased level of resistance without changing of its basic genetic constitute. Induction of resistance in dicots is correlated with the accumulation of SA or ethylene and or jasmonic acid (Friedrich et al., 1996) and activation of set of genes which include gene coding for pathogensis-related protein of these chitinase, peroxidase and β-1,3-glucanase (Siegrist et al., 1997), also phenolic compounds were found to increase in infected plants and correlated to the level of resistance (Agrios, 2005).

The results of this study indicated that watering tomato seedlings with the concentration of 100 mg/l of ßABA resulted in reduction of the incidence and severity of bacterial speck disease of tomato in comparison to the incidence and severity of the disease resulted from watering with the other concentrations. Wilkinsona et al. (2018) demonstrated that ßABA induces post-harvest resistance in tomato fruit against *Botrytis cinerea* with no penalties in yield. Also, Kim et al. (2013), found that pretreatment of kimchi cabbage with ßABA conferred induced resistance against challenges by the two different classes of fungal pathogens; *Alternaria brassicicola* and *Colletotrichum higginsianum*; in a dose-dependent manner. Jakab et al. (2001), presented evidence that the protective effect of ßABA is due to a potentiating of natural defense mechanisms against biotic and abiotic stresses. ßABA had a primary role in modulation hormonal defense signaling; especially the special role of abscisic acid against various attacks (Baccelli & Mauch-Mani., 2016). Also, artificial infection of *Arabidobsis thaliana* with *Pseudomonas syringae* pv. *tomato* led to an increase endogenous ßABA levels over time in leaf tissues (Thevenet et al., 2017).

Furthermore, the ßABA resulted in improving the growth of tomato seedlings more than control, in term of different measured growth parameters especially foliage weight and leaf area. These results partially agreed with a study done by Wilkinsona et al. (2018) in which they reported that treatment of tomato seedlings with ßABA induce resistance against *Botrytis cineria* with no yield reduction; where, there was a delay in fruit ripening (Wilkinsona et al., 2018). In the other hand, these findings were disagreed with Kim et al. (2013), who reported that treatment of kimchi cabbage

Table 10. Recovered phenolic compounds (ppm) from tomato seedlings cv. GS12 sprayed with ß-aminobutyric acid (100 mg/l) and artificially inoculated with *Pseudomonas syringae* pv. *tomato* one week

Treatment	Days after inoculation				
	3	6	9	12	15
ABA (100 mg/l)	2529.0b*	4099.33c**	5035.70c	6081.0b	5130.3b
Infected control	2621.7b	5630.00b	6142.70bc	5233.3b	3807.7c
Control	3282.3b	4613.33c	5476.70c	5115.0b	2895.0c
L.S.D.	946.94	571.72	1210.20	1053.50	1011.90

*Average of 4 samples

**Means followed with the same letter are not significantly different at $p \leq 0.05$ within column

seedlings with ßABA significantly reduced primary root elongation and cotyledon development in a dose-dependent manner, which adverse effects were similar to the plant response to exogenous abscisic acid application. While Rajaei & Mohamadi (2013) reported that ßABA had no significant effect on seed germination of canola but in abscisic acid and $CoCl_2$ treated seeds, germination was reduced compare to the control, abscisic acid inhibited root growth more than ßABA.

Also, the function specificity of amino butyric acid isomers was illustrated in different studies. It was reported that when wheat and barley plants under biotic and abiotic stresses, GABA rapidly accumulates in plant tissues in response, and regulates plant growth (Ramesh et al., 2015). Also, Li et al. (2016), reported that exogenous GABA application improved seedling growth in maize, GABA involved in various physio-metablical mechanisms which might lead to improvement in morphological growth of maize. Our findings about the effect of ßABA on improving tomato growth could be considered partially agreed with these studies.

Results of the greenhouse experiments and laboratory tests conducted on tomato seedlings watered with ßABA(100 mg/l), indicated that ßABA reduced the disease incidence by 13.05% after two week of inoculation compared with the incidence in the control treatment. Also, there was a significant increase in the studied growth parameters of the ßABA treated tomato seedlings with different concentrations compared with control.

The biochemical analysis indicated that tomato seedlings watered with ßABA (100 mg/l) produced 5.74 and 7.78 nmol/g/h ethylene, 9 and 15 dpi, respectively that were significantly different from control. This found to be agreed with Zimmerli et al. (2000), who reported that ßABA activates abscisic acid signaling intermediate and also alters the expression level of some genes implicated in signaling by ethylene.

Whereas, 14.03 ug/g of free SA was recovered from treated tomato seedlings 12 dpi, which was significantly different compared with control. Zimmerli et al. (2000) reported that free SA increased in tomato seedlings treated with aminobutyric acid. While Wilkinsona et al. (2018) reported that SA along with its glycosylated forms (salicylic acid glucoside/salicylic acid glucosyl ester) did not differ between treatments after ßABA application to tomato seedlings.

The recovered levels of peroxidase enzyme were increased gradually with time; in term of dpi; the highest recovery was 15 dpi, in which reached 503.17 nmol tetraguaiacol/mg protein/min, and was correlated with the lowest recovery from control treatments. While, in the case of β-1,3-glucanaseenzyme; the highest recovered levels were 9 and 15 dpi that were 4.58 and 2.93 absorbance/mg protein/min respectively, which were differed significantly compared with control, the same results were obtained by Brisset (2000) who reported that the peroxidase and β-1,3-glucanase induced SAR pathway in plant tissues, as a defensive mechanism.

Moreover, the highest recovered amount of chitinase was 0.59 pmol of GlcNAc $ml^{-1}min^{-1}$, but was not significantly different compared with infected control. However, chitinase enzyme levels increased by induced systemic acquired resistance (Grenier & Asselin., 1990; Orober et al., 1996 and Siegrist et al., 1997).

Fortunately, the highly recovered enzymes of treated tomato seedlings were correlated with low level of disease incidence and severity 1-2 weeks post inoculation and with the high recovered population of *Pseudomonas syringae* pv. *tomato* of $5.38x10^8$ CFU/ml after 21 days of inoculation. These findings could illustrate the effect of ßABA in inducing SAR against bacterial speck disease.

Protein and total phenolic compounds contents were increased gradually, where the highest amounts were recorded 12 dpi, their amounts were 0.88 ng/g and 6081 ppm, respectively. At this time, the protein content was significantly different compared with infected control, while the total phenolic compounds were not significantly different.

Thus, ßABA could be considered a weak chemical resistance inducer against tomato speck disease, the obtained results are in conflict with the findings of (Junjun et al., 1996; Zimmerli et al., 2000), in which, they reported high aminobutyric acid induce high level of systemic resistance in treated tomato seedlings, in the other hand, in agreement with their finding in which aminobutyric acid induced systemic resistance through SA-dependant pathway and correlated with accumulation of SA which serve as signal and in signal transduction.

Conclusion

The results revealed that the ßABA induce systemic resistance to certain extent against bacterial speck disease of tomato *Pseudomonas syringae* pv. *tomato* (Jordanian isolates) on tomato seedlings cv. GS12. It was found that watering tomato seedlings with ßABA (100 mg/l) had a positive effect on ethylene, SA, peroxidase, β-1,3-glucanase and protein contents of the artificially infected tomato seedlings. Whereas, it was found that this chemical inducer had no significant effect on chitinase enzyme and total phenolic compounds contents. Also, ßABA stimulates vegetative growth of tomato seedlings, this effect could illustrate their indirect role in induce systemic resistance through improving plant vigor. Moreover, resistance induction for crop plants is environ-

mentally sound management strategy, which can be applied in the field coincided with other management strategies.

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