

Evaluation potential of PGPR to protect tomato against *Fusarium* wilt and promote plant growth

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Abstract: Soilborne fungal diseases are most common among vegetable crops and have major implications for crop yield and productivity. Eco-friendly sustainable agriculture practices that can overcome biotic and abiotic stresses are of prime importance. In this study, we evaluated the ability of plant growth-promoting rhizobacterium (PGPR) *Bacillus aryabhatai* strain SRB02 to control the effects of tomato wilt disease caused by *Fusarium oxysporum* f. sp. *lycopersici* (strain KACC40032) and promote plant growth. *In vitro* bioassays showed significant inhibition of fungal growth by SRB02. Inoculation of susceptible and tolerant tomato cultivars in the presence of SRB02 showed significant protection of the cultivar that was susceptible to infection and promotion of plant growth and biomass production in both of the cultivars. Further analysis of SRB02-treated plants revealed a significantly higher production of amino acids following infection by *F. oxysporum*. Analysis of plant defense hormones after inoculation by the pathogen revealed a significantly higher accumulation of salicylic acid (SA), with a concomitant reduction in jasmonic acid (JA). These results indicate that *B. aryabhatai* strain SRB02 reduces the effects of *Fusarium* wilt disease in tomato by modulating endogenous phytohormones and amino acid levels.

Keywords: PGPR; *Bacillus aryabhatai*; SRB02; *Fusarium oxysporum*; Tomato wilt; Tomato; Plant growth

41 1. Introduction

42 Tomato (*Solanum lycopersicum* L.) is the second most economically important edible vegetable after
43 potato from the *Solanaceae* family and is widely cultivated and consumed around the world (Hanson & Yang,
44 2016). Tomato is used as a model plant for investigating the genetics and molecular aspects of disease resistance
45 mechanisms. The tomato crop is under threat worldwide owing to biotic and abiotic stresses that have caused
46 significant reductions in yield and productivity. One reason is that tomato is a host for nearly 200 species of
47 plant pathogens, including fungi, bacteria, nematodes, viruses, and others that infect plants at all developmental
48 stages (Stout, Kurabchew & Leite, 2017), reducing both yield and quality.

49 Vascular wilt is one of the most important fungal diseases of tomato and occurs wherever these crops are
50 grown. This disease is caused by the soilborne fungus *Fusarium oxysporum* f. sp. *lycopersici* (FOL). Three
51 different pathotypes have been identified so far, which can be further classified into three races, 1, 2, and 3,
52 based on various pathogenicity features during infection in tomato. Being soilborne, it is omnipresent and is
53 very hard to get rid of once introduced into the cropping system. If infection occurs at the nursery or seedling
54 stage, plants simply die back, whereas severe losses can occur if the disease appears in the field after
55 transplantation. The fungus can spread in different ways, such as through the transport of infested soil, irrigation
56 water, infected plants and transplants, and seeds (Jones et al., 2014). Infection occurs via the roots, causing
57 serious vascular damage and wilting of the plant that subsequently leads to cell death. In severe infections, more
58 than 80% of crop loss has been reported ([Worku & Sahe, 2018](#)). Some studies have reported the applicability
59 of protective fungicides as a possible remedy against the different strains of the pathogen. However, the use of
60 chemicals in agriculture has not only raised serious concerns regarding human health and environmental hazards
61 but is also considered responsible for the development of strains that are resistant to these widely used
62 agrochemicals (Zouari et al., 2016). Hence, eco-friendly alternates to chemical measures are needed.

63 Biological control of plant pathogens has been of great interest to researchers. Apart from pathogenic
64 microbes, plants also have symbiotic or mutualistic interactions with a wide range of soilborne microbes, which
65 protect plants from pathogens either directly or by inducing resistant mechanisms (Pieterse et al., 2014). These
66 microbes associate with the plant roots and help enhance growth-related attributes by improving the uptake of
67 essential ions and minerals, atmospheric nitrogen fixation, and protection from pathogens (Lugtenberg &
68 Kamilova, 2009). These growth-promoting bacteria are mainly isolated from the rhizosphere of the plants.
69 These microbes are commonly known as plant growth-promoting rhizobacteria (PGPR) ([Kloepper, Lifshitz &
70 Zablotoxicz, 1989; Backer et al., 2018](#)) and include organisms such as *Pseudomonas* spp. Other microbes are
71 known as plant growth-promoting endophytic bacteria, plant growth-promoting fungi, or biocontrol fungi
72 (BCF), including *Trichoderma* spp. and *Sebacinales* spp. These can play a role in plant growth and can stimulate
73 plant immune systems ([Shoresh, Harman & Mastouri, 2010; Singh et al., 2019](#)). Endophytes are widely
74 dispersed and can be found in diverse environments including the tropics, temperate zone, aquatics, xerophytics
75 and deserts, tundra, geothermal soils, rainforests, mangroves, and coastal forests. They inhabit plant tissues such
76 as endosperms, roots, leaves, stems, flowers, and fruits (Singh et al., 2017). Generally, plant growth promotion
77 may occur owing to the regulation of the plant hormonal system, modifications in root architecture, production
78 of siderophores, solubilization of soil minerals, activation of secondary mechanisms of plant defense, and
79 production of biochemicals (Pupin & Nahas, 2014; Backer et al., 2018).

80 PGPRs and endophytes have a non-pathogenic symbiotic life cycle associated with their host plant tissues;
81 these endophytes can be easily isolated from plant tissues (Arnold & Lutzoni, 2007; Costa et al., 2012). Seeds

82 are the source of vertical dispersal of numerous seed-borne endophytes, or PGPRs (Ernst et al., 2003). Along
83 with the alleviation of biotic stress in plants, these PGPRs have been reported to help mitigate a wide range of
84 abiotic stresses as well (Shahzad et al., 2017a). Independent studies have reported the ameliorating effects of
85 PGPRs on plant growth and fungal diseases in tomato and sunflower (Shittu et al., 2009; Waqas et al., 2015).
86 In addition, studies have revealed the remediation abilities of PGPRs in soil contaminated with heavy metals
87 (Jing, He & Yang, 2007; Bilal et al., 2018). All of these impacts of PGPRs make them widely attractive as
88 biofertilizers and soil microbe mediators (Backer et al., 2018; Rosier, Medeiros & Bais, 2018). The positive
89 effects of PGPRs on plant growth attributes are well known, but the exact molecular mechanism(s) behind them
90 have not yet been ~~clearly~~ demonstrated.

91 PGPRs affect plant growth by either direct or indirect means. The direct promotion of plant growth occurs
92 by a synthesis of complex compounds by the microbes—for instance, phytohormones such as indole-3-acidic
93 acid (IAA), gibberellic acid (GA3), zeatin, and abscisic corrosive (ABA)—or by incremental nutrient
94 accessibility by nitrogen fixation from the surrounding climate, thereby providing supplements for mineral
95 solubilization (Glick, 1995; Bhardwaj et al., 2014). The indirect method of plant growth promotion takes place
96 when PGPRs get involved in reducing the negative effects of one or more phytopathogenic microbes or fungi.
97 This occurs by the production of substantial antagonistic substances or by inducing resistance in plants against
98 the pathogens; for instance, the production of siderophores, hydrogen cyanide (HCN), hydrolytic proteins, etc.
99 (Glick, 1995; Mahmood, Gupta & Kaiser, 2009).

100 The role of antifungal PGPRs as biological control agents to control plant diseases has been widely
101 examined. PGPRs are considered either extracellular, including the genera *Agrobacterium*, *Arthrobacter*,
102 *Azotobacter*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Chromobacterium*, *Erwinia*, *Flavobacterium*,
103 *Micrococcous*, *Pseudomonas*, and *Serratia*, or intracellular, including the genera *Allorhizobium*,
104 *Bradyrhizobium*, *Mesorhizobium*, and *Rhizobium* (Martínez-Viveros et al., 2010; Gouda et al., 2018). The fact
105 that rhizospheric bacteria *Bacillus aryabhatai* strain B8W22 was previously identified and isolated from
106 cryotubes used for collecting air samples from the earth stratosphere (Shivaji et al., 2009) indicates that these
107 bacteria have cosmic ancestry. Moreover, different strains of the bacterium were isolated from the rhizosphere
108 in South Korea, India, and Tibet (Pailan et al., 2015; Lee et al., 2015; Yun et al., 2016). The plant growth-
109 promoting ability of *B. aryabhatai* was initially reported by (Lee et al., 2012), who demonstrated growth
110 promotion in *Xanthium italicum* plants. Similarly, Ramesh et al. (2014) (Ramesh et al., 2014) reported on *B.*
111 *aryabhatai* contributions to plant growth by enhancing the mobilization and bio-fortification of zinc in soybean
112 and wheat. More recently, *B. aryabhatai* strain SRB02 has been found to play a role in oxidative and nitrosative
113 stress tolerance and promotion of growth in soybean plants by modulating the production of phytohormones
114 (Park et al., 2017a). In addition, *B. aryabhatai* strains also show the ability for the biosynthesis of thermostable
115 alkaline phosphatase, anti-leukemic tumor-inhibiting L-asparaginase enzyme (Gill et al., 2013; Singh et al.,
116 2014), and degradation of pesticides (Pailan et al., 2015).

117 In additions, various species of *Bacillus* have been identified as plant growth-promoting bacteria as well
118 as biocontrol agents against various pathogenic fungi (Compant et al., 2005; Shahzad et al., 2017a). Plant
119 growth-promoting rhizosphere bacteria employ a variety of strategies to facilitate plant growth and survival
120 under pathogenic attack by both direct and indirect mechanisms. The most common direct mechanisms are
121 phytohormone production, the acquisition of nutrients, and the control of pathogens through various means, for
122 example, through the synthesis of hydrolytic enzymes, antifungal compounds, lipopeptides, or antibiotics. The

indirect mechanisms include protection by triggering of specific defense-related pathways, particularly the induction of systemic resistance (ISR) against pathogens and pests (Khan et al., 2012; Martínez-Hidalgo et al., 2015) and the release of bacterial volatile compounds (Bernier et al., 2011). However, many environmental factors influence the biological control potential of PGPR by either predisposing pathogens to microbial antagonism, regulating the growth or production of metabolites by specific antagonists, or modulating disease development and consequently the level of disease suppression achieved.

From our literature survey, it is evident that except for some reports in crops (*Xanthium italicum*, soybean, rice, tomato, and wheat) there is a lack of information about the growth-promoting activity of *B. aryabhatai* and its role in tolerance to biotic and abiotic stress in other plant species (Viljoen et al., 2019; Yoo et al., 2019).

In this study, we evaluated the plant growth-promoting abilities of *B. aryabhatai* SRB02 in tomato cultivars inoculated with phytopathogenic fungus *FOL*.

2. Materials and Methods

2.1. Growth of PGPR and *FOL*

B. aryabhatai SRB02 was isolated previously from the rhizosphere of a soybean field in the Chungcheong buk-do region of South Korea (Park et al., 2017b). Bacteria were cultured on LB agar or in broth (AppliChem, Darmstadt, Germany) media at 28 °C for 24 h. *F. oxysporum* f. sp. *lycopersici* strain KACC 40032 was obtained from the Korean Agricultural Culture Collection (KACC, <http://genebank.rda.go.kr>) and grown on potato dextrose agar plates at 28 °C for 7 d. The antifungal activity of *B. aryabhatai* SRB02 against *FOL* was evaluated following the protocol of (Shahzad et al., 2017a).

Briefly, a 0.5 cm² disc of active fungal mycelia of *FOL* was placed at the center of a 90 mm disposable plastic Petri dish (SPL, Korea) containing LB agar (Becton, Dickinson and Company, France). The overnight bacterial culture of *B. aryabhatai* SRB02 was aseptically streaked around the fungal disc at equal distances in a square pattern. For the untreated control, a fungal disc was placed on LB agar, as mentioned earlier, but instead of *B. aryabhatai* SRB02, only sterile water was streaked. For comparison, the effects of fungal growth inhibition of organic acids against the pathogen were also evaluated. All of the plates were incubated at 28 °C for 7 d. After the incubation period, the inhibition zone was measured and the percent inhibition was calculated according to the following formula.

$$\text{Inhibition \%} = \frac{(\text{diameter of fungus on control plate} - \text{diameter of fungus on SRB02 co-cultured plate}) \times 100}{\text{diameter of fungus on control plate}}$$

2.2. Screening of tomato varieties for resistance to *FOL*

In the current study, tomato seeds of four Korean cultivars ([IT 252842-13 \(Cultivar-1\)](#), [IT 252869-14 \(Cultivar-2\)](#), [IT 260627-16 \(Cultivar-3\)](#), [IT 259462-15 \(Cultivar-4\)](#)) were selected for their response to the pathogen. Seeds were sterilized with 2.5% sodium hypochlorite for 10 min and kept on wet paper towels inside Petri plates in an incubator at 25 °C for 5 d. Horticultural soil, distilled water, and pots were autoclaved at 121 °C for 20 min. Uniformly germinated seeds were transferred to separate trays filled with sterilized horticultural soil (Soil and Fertilizer Technology, Korea). After one week, uniformly grown seedlings were transplanted to big pots with the dimensions (LxWxH)-3.5 x 3 x 3 inches and volume 85-90gm. Plants were allowed to acclimatize for a few days, and the experimental treatments were set up in triplicates, with each replicate

161 containing at least six plants. The fungal spore suspension of *FOL* strain KACC 40032 was prepared according
162 to the protocol described by (Lichtenzveig et al., 2006). Control plants were treated with distilled water, and
163 plants were allowed to grow for 5 d. Plants to be treated with the pathogen were inoculated by applying a spore
164 suspension (10^6 conidia/mL) to the exposed roots of tomato plants. The roots were then covered with soil. Plants
165 were allowed to grow at relatively high humidity of $80 \pm 2\%$. After 14 d of growth under the conditions
166 mentioned above, the inoculated plants were assessed ~~on the basis of~~ based on symptomatology (severity of
167 plant wilting) and growth.

168 **2.3. *In planta* biocontrol assessment**

169 After the screening test, two cultivars (resistant and susceptible, one each) were selected based on disease
170 symptoms and growth under biotic stress. Seeds of the selected cultivars were surface-sterilized, germinated,
171 and grown before being transplanted to pots as mentioned previously. The plants were allowed to acclimatize
172 for a few days, and the experimental treatments were set up in triplicate, with each replicate containing at least
173 six plants. SRB02 was applied to plants by soil drenching with 10 mL SRB02 broth culture (4×10^8 cfu/mL) in
174 the root zone. The fungal spore suspension *FOL* strain KACC 40032 was prepared as mentioned previously.
175 Control plants were treated with distilled water, and plants were allowed to grow for 5 d. Plants to be treated
176 with the pathogen were inoculated by applying spore suspension (10^6 conidia/mL) to the exposed roots of
177 tomato plants. The roots were then covered with soil. The plants were allowed to grow at relatively high
178 humidity of $80 \pm 2\%$ because to further exploit the pathogenic impact of fungus. Data were recorded on growth
179 parameters such as plant height (PH), root length (RL), fresh weight (FW), dry weight (DW), and chlorophyll
180 content (Chl. Cont.) to determine the response of plants to infection in the presence or absence of SRB02. For
181 fresh plant biomasses, the plants were uprooted, carefully washed, and frozen in liquid nitrogen, and then
182 transferred to storage at -80°C until further analysis.

183 **2.4. *Extraction and quantification of amino acid content***

184 The plant amino acids were extracted according to the protocol described by Khan et al. (2017)(Khan et
185 al., 2017), with some modifications. Briefly, the freeze-dried whole plant samples were ground to homogenate,
186 and 100 mg powdered samples were hydrolyzed under a vacuum in 6N HCl at 110°C followed by 80°C for
187 24 h. The dried residue was suspended in 0.02N HCl and filtered through a $0.45\ \mu\text{m}$ filter. The amino acids
188 were then quantified using an automatic amino acid analyzer (Hitachi, Japan; L-8900). The experiments were
189 conducted in triplicate, and each replicate was comprised of six plants. The amino acid concentration was
190 determined using relevant standards. These is standard known as amino acid standard mixture solution (type H)
191 used for the automatic amino acid investigation was procured through Wako Pure Chemical Industries Ltd
192 (Japan), and used for endogenous amino acids assessment.

193 **2.5. *Jasmonic acid quantification***

194 For the quantification of endogenous jasmonic acid (JA) content, the optimized protocol described by
195 McCloud and Baldwin (1997) was used. Briefly, homogenized powder (0.3 g) from the immediately freeze-
196 dried whole plant samples was suspended in extraction buffer (70:30 v/v acetone and 50 mM citric acid), and
197 25 ng JA internal standard ([9, 10- 2H^2]-9, 10-dihydro-JA) was also added to the suspension. The extract
198 suspension was kept overnight at room temperature for evaporation of highly volatile organic solvents and to

199 retain the less-volatile fatty acids. The subsequent aqueous phase was filtered and then extracted with 30 mL
200 diethyl ether three times. The collective extracts were subsequently loaded onto a solid-phase extraction
201 cartridge (500 mg of sorbent, aminopropyl). In addition, 7.0 mL of trichloromethane and 2-propanol (2:1 v/v)
202 were used to wash the loaded cartridges. Then, the exogenous JA and relevant standard were eluted with 1 mL
203 of diethyl ether and acetic acid (98:2 v/v). Following evaporation, the samples were esterified and analyzed by
204 GCMS (6890N network GC system) and a 5973 network mass selective detector (Agilent Technologies, Palo
205 Alto, CA, USA) in the relevant ion mode. The relevant ion mode was selected for JA determination. The ion
206 fragment was examined at $m/z = 83$ AMU, corresponding to the base peaks of JA and [9, 10-2H²]-9, 10-dihydro-
207 JA. The endogenous JA values were determined from the peak areas ~~with respect to~~for relevant standards.

208 2.6. Salicylic acid (SA) quantification

209 The SA of SRB02-treated tomato plants was extracted and quantified according to the protocol described
210 by (Enyedi et al., 1992; Seskar, Shulaev & Raskin, 1998). Immediately freeze-dried whole plant tissues were
211 homogenized, and 0.2 g of homogenate powder was used for the extraction using 90% and 100% methanol.
212 The pellets were dried and re-suspended in 2.5 mL 5% trichloroacetic acid (TCA) and further partitioned with
213 ethyl acetate, cyclopentane, and isopropanol (ratio of 100:99:1, v/v). The upper organic layer containing free
214 SA was used for air-drying with nitrogen gas. The dry SA ~~was~~has again suspended in 1 mL 70% methanol and
215 subjected to high-performance liquid chromatography (HPLC) using a Shimadzu device outfitted with a
216 fluorescence indicator (Shimadzu RF-10AxL) with excitation at 305 nm and emission at 365 nm filled with a
217 C18 reverse-phase HPLC column (HP Hypersil ODS, particle size 5 μ m, pore size 120 Å, Waters). The flow
218 rate was maintained at 1.0 mL/min.

219 2.7. Statistical analysis

220 All experiments were replicated three times, and each replicate was comprised of six plants. Data were
221 statistically evaluated with Duncan multiple range tests and *t*-tests where appropriate, using SAS version 9.2
222 software (Cary, NC, USA).

223 3. Results

224 3.1. *In vitro* antifungal assay

225 The *in vitro* antifungal activity of PGPR *B. aryabhatai* SRB02 was assessed against pathogenic *Fusarium*
226 *oxysporum* in dual culture. The results revealed that the PGPR *B. aryabhatai* SRB02 significantly inhibited the
227 growth of pathogenic *F. oxysporum*, as shown in [Figure S1](#).

228 3.2. Response of tomato cultivars under pathogenic infection by *F. oxysporum*

229 To determine the response of four tomato cultivars, the plants were challenged with a spore suspension of
230 the pathogen. The pathogen was applied to the exposed roots of tomato plants and incubated under higher
231 relative humidity to create a conducive environment for successful infection. After 14 d of inoculation, the
232 cultivars revealed a differential level of tolerance to the pathogen ([Figure 1](#)). [The plant tolerance level was](#)
233 [determined based on the symptomatology \(severity of plant wilting\)](#). In the susceptible plants, clear symptoms
234 of wilting were evident. Susceptible plants were also observed with retarded growth as compared to the tolerant

plants. Based on the plant growth attributes and resistance level, as shown in [Figure 1](#), the most tolerant and susceptible tomato cultivars were selected for further experiments.

3.3. *Plant growth-promoting and ameliorative effects of B. aryabhattai SRB02 against FOL*

Based on screening, the plant-growth-promoting and biocontrol efficiency of PGPR *B. aryabhattai* SRB02 against a virulent strain of *F. oxysporum* was investigated in both the selected tolerant and susceptible varieties ([Figure 2](#)). *B. aryabhattai* SRB02 significantly promoted plant growth, and interestingly reduced the disease in both tolerant and susceptible tomato cultivars ([Figure 2](#)).

The growth-related traits of the disease-tolerant plants were significantly improved when applied with SRB02 alone. The plant height (PH) was improved by 37.4%, while RL was improved by 26.8% as compared to the water-treated control plants. Other traits including seedling FW, seedling DW, and chlorophyll content were also improved by 15.3%, 23.3%, and 5.8%, respectively. A similar trend was also observed when the tolerant plants were treated with the pathogen and SRB02 together, compared to the pathogen-treated plants. The PH, RL, seedling FW, DW, and chlorophyll content were improved by 124%, 6.4%, 15.8%, 42.3%, and 39.7%, respectively, compared to the plants inoculated with the pathogen alone. SRB02 also improved the growth attributes of the disease-susceptible plants with or without co-treatment by the pathogen. The PH of the disease-susceptible plants was improved by 14.1% with the application of SRB02 in comparison with the water-treated control plants; however, the increase in PH was significantly greater (105.7%) in plants treated with SRB02 and *F. oxysporum* combined as compared to the pathogen-treated plants. Likewise, other traits were also improved in plants treated with PGPR alone as compared to the water-treated plants and also in the PGPR and pathogen co-treated susceptible plants in comparison with the plants treated with the pathogen alone. The RL, seedling FW, seedling DW, and chlorophyll content were improved by 9% and 44.5%, 10.4% and 32.6%, 3%, and 24.6%, and 4% and 61.3% in plants treated with SRB02 alone and in plants co-treated with PGPR and the pathogen, respectively ([Figure 2](#), Table 1).

3.4. *B. aryabhattai regulates defense against F. oxysporum by modulating defense-related hormones in tomato*

Measurement of basal and induced levels of the plant defense-related hormones SA and JA following inoculation with *FOL* in the absence or presence of SRB02 revealed strict regulation of plant defense responses in SRB02-treated plants due to the regulation of the synthesis of both of these hormones ([Figures 3 and 4](#)). Interestingly, these results were observed in both the resistant and susceptible cultivars, indicating the high utility of SRB02 for field use even in susceptible crops. More specifically, SRB02-treated infected plants (tolerant and susceptible) produced significantly lower JA (11.10 % and 10.30 %, respectively) compared to control plants ([Figure 3](#)). Even the SRB02-treated plants in the absence of *FOL* accumulated lower JA (6.92% and 17.91%).

Furthermore, SRB02 treatment with *F. oxysporum*-inoculated plants of the tolerant cultivar accumulated 48.48% more SA compared to plants not treated with the PGPR. More interestingly, the response of the *F. oxysporum*-inoculated plants of the susceptible cultivar was more robust in the presence of SRB02, as these plants produced 74.60% more SA as compared to plants not treated with PGPR ([Figure 4](#)). However, no significant differences in SA accumulation were observed in SRB02-treated plants of either tolerant and susceptible cultivars in the absence of *F. oxysporum*.

274 3.5. *B. aryabhatai* SRB02 regulates amino acids in plants with or without biotic stress

275 The current study showed that *B. aryabhatai* SRB02 regulates amino acids in both disease-tolerant and
 276 susceptible tomato plants in the presence or absence of *F. oxysporum* (Table 2). Under pathogenic infection by
 277 *F. oxysporum*, *B. aryabhatai* SRB02 inoculation significantly enhanced aspartic acid (115.57% and 147.48%),
 278 threonine (123.18% and 118.56%), serine (123.13% and 158.91%), glutamic acid (4.86% and 157.89%),
 279 glycine (131.82% and 143.58%), alanine (99.61% and 109.67%), valine (98.13% and 74.62%), methionine
 280 (239.06% and 172.93%), isoleucine (42.60% and 97.82%), leucine (103.21% and 58.03%), tyrosine (138.45%
 281 and 65.45%), phenylalanine (39.86% and 34.16%), lysine (113.15% and 98.03%), histidine (98.42% and
 282 111.74%), arginine (108.69% and 157.18%), and proline (90.09% and 115.25%) in disease-susceptible and
 283 tolerant tomato plants, respectively (Table 2). Only cysteine was decreased by 9.65% and 21.82% in *B.*
 284 *aryabhatai* SRB02 applied to susceptible and tolerant plants, respectively (Table 2).

285 Likewise, in the absence of the pathogen, *B. aryabhatai* SRB02 significantly enhanced aspartic acid
 286 (3.35% and 24.98%), threonine (32.99% and 118.56%), glutamic acid (4.86% and 157.89%), glycine (30.78%
 287 and 143.58%), alanine (29.70% and 4.65%), cysteine (44.22% and 60.20%), valine (21.91% and 31.81%),
 288 methionine (132.35% and 31.17%), isoleucine (97.76% and 29.48%), leucine (36.52% and 32.40%),
 289 phenylalanine (114.92% and 77.20%), histidine (22.81% and 41.48%), and arginine (35.98% and 23.95%) in
 290 the susceptible and tolerant plants, respectively (Table 2). However, *B. aryabhatai* SRB02 showed an increase
 291 in serine-intolerant (9.73%) plants and a decrease of 13.22% in susceptible plants. Tyrosine was increased by
 292 45.03% only in the tolerant plants when applied with PGPR, while it was decreased by 51.52% in susceptible
 293 plants. Similarly, lysine was increased (35.12%) in the PGPR-applied tolerant plants, while no significant
 294 difference was observed in the susceptible plants. In contrast, proline was increased by 35.77% only in
 295 susceptible plants, while no significant difference was recorded in intolerant plants when challenged with PGPR
 296 (Table 2).

297 5. Discussion

298 The use of microbial-based techniques in the management of plant diseases has gained significant attention
 299 in recent years. In particular, PGPRs and their interactions with the plants under biotic or abiotic stress are
 300 gaining importance, with the ultimate aim of improvement in the protection of crops and increases in
 301 agricultural production. These biocontrol approaches are eco-friendly and are becoming very popular, reliable,
 302 and long-lasting. Plant growth improvement by PGPR is one of the outstanding characteristics of these naturally
 303 occurring microbes. The improvements in plant growth and its ameliorating abilities with regard to about plant
 304 diseases are determined by the interactions between the host plant and PGPR (Vejan et al., 2016). PGPR
 305 improves plant growth and health by direct or indirect mechanisms that can overcome diseases. The plant
 306 growth-promoting activity of PGPR bacteria has been reviewed in detail by d Santoyo et al. (2016) (Xia et al.,
 307 2015; Santoyo et al., 2016). *Bacillus* and *Pseudomonas* species are widely known as invaluable resources for
 308 plant growth promotion and the suppression of disease symptoms (Sundaramoorthy & Balabaskar, 2013;
 309 Chaves-López et al., 2015). Over the last few decades, several studies have reported on the beneficial aspects
 310 of *Bacillus* spp. as biocontrol and biofertilizer agents; e.g., *Bacillus licheniformis*, *Bacillus subtilis*, *Bacillus*
 311 *cereus*, *Bacillus pumilus*, and *Bacillus amyloliquefaciens* (Pane & Zaccardelli, 2015; Han et al., 2016). The
 312 plant growth promotion and other beneficial aspects of *Bacillus* strains can be attributed to their ability to

313 enhance the production of phytohormones such as auxin (IAA), ~~ethylene~~, and gibberellic acid (Gamalero &
314 Glick, 2011).

315
316 A wide range of plant species ~~are~~is infected by pathogens, including the diverse genera of
317 *Alternaria*, *Botrytis*, *Fusarium*, and *Rhizoctonia*. These pathogens result in severe losses to crop yield and
318 productivity, thereby posing a threat to food security. *F. oxysporum* is a devastating fungal pathogen that attacks
319 the vascular system and causes severe damages to tomato crops across the globe. Conversely, microbes, or
320 PGPRs, found in the rhizosphere of plants are directly associated with roots and are a vital source for plant
321 growth promotion and suppression of soilborne plant pathogens such as *F. oxysporum*. To isolate and evaluate
322 the beneficial role of PGPR, an appropriate *in vitro* experimental setup is required. Shahzad et al. (2017)
323 (Shahzad et al., 2017a) reported plant growth promotion by endophytic bacteria RWL-1 against the pathogenic
324 infection by *FOL* in tomato. ~~In addition~~Also, it was recently reported that *B. aryabhatai* SRB02 plays a role in
325 oxidative and nitrosative stress tolerance and promotes the growth of soybean and rice plants by modulating the
326 production of phytohormones (Park et al., 2017a). However, it was not clear whether *B. aryabhatai* SRB02
327 could be used to rescue the plants from biotic stress. Hence, in the present study, we subjected disease-tolerant
328 and susceptible tomato plants to the PGPR *B. aryabhatai* SRB02 in the presence and absence of a virulent
329 strain of *FOL*, hypothesizing that SRB02 would rescue the plants from the disease and improve their growth
330 under stress conditions. ~~Prior to~~Before inoculation by the pathogen, tomato plants were treated with a cell
331 suspension of *B. aryabhatai* SRB02. The SRB02 application improved the disease tolerance level of the
332 infected plants. In a previous study by Shahzad et al. (2017) (Shahzad et al., 2017a), PGPRs were shown to
333 enhance plant growth, reduce infection by the pathogen, and result in improved disease tolerance.

334 The present study showed that under pathogenic infection, the PGPR association rescued the plants from
335 disease and enhanced plant growth and biomass. This result might occur by restricting the pathogenic fungus,
336 enhancing nutrient uptake, and producing phosphate solubilization substances, or by induction of
337 phytohormonal biosynthesis. The present findings further strengthen the role of *Bacillus* species as a PGPR and
338 biocontrol agent, as reported by numerous researchers, against diverse diseases in various plant species, such
339 as root wilting, damping off, fusarium wilt, ring rot, and charcoal rot in tomato, soybean, banana, apple, and
340 common bean, respectively (Yu et al., 2002; Vitullo et al., 2012; Wang & Fobert, 2013; Chen et al., 2016;
341 Torres et al., 2016). The current findings also indicate that PGPR strains producing bioactive components may
342 suppress the negative effects of pathogenesis and biotic stress in infected plants. In addition, our study also
343 confirmed and exhibited similar results to previous reports that organic acids, as one of the many components
344 produced by *Bacillus* species, can help rescue the plant from the disease. Moreover, PGPR produces
345 siderophores and organic acids, which mitigate the negative effects of pathogen-infected sunflower plants
346 (Waqas et al., 2015). From these studies, it is evident that biotic stress-related ameliorative effects are commonly
347 regulated by endogenous phytohormones such as SA and JA. Under normal and stress conditions,
348 phytohormone signaling and crosstalk play a vital role in plant growth and development. Accordingly, in the
349 present study, we found that inoculation with PGPR *B. aryabhatai* SRB02 extensively modulated the
350 endogenous levels of JA and SA. Our findings ~~are in conformity~~conform with previously elucidated
351 phytohormonal regulation; i.e., increased SA ([Figure 4](#)) and reduced JA ([Figure 3](#)) with PGPR, as revealed by
352 independent studies (Khan et al., 2015; Waqas et al., 2015; Shahzad et al., 2016, 2017b; Ali et al., 2017)
353 comparing plants in the presence or absence of biotic stress.

354 **Author Contributions:** RBSN performed the experiments and analyzed the data. RBSN, RT, and RS drafted
355 the manuscript. MS, WA, and RBSN prepared illustrations, figures, tables, and references. B-WY, AH edited
356 the manuscript. B-WY contributed critical comments to the draft and approved the manuscript. All authors
357 contributed to the article and approved the submitted version.

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