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

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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 aryabhattai* 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 significantly higher accumulation of salicylic acid (SA), with a concomitant reduction in jasmonic acid (JA). These results indicate that *B. aryabhattai* strain SRB02 reduces the effects of *Fusarium* wilt disease in tomato by modulating endogenous phytohormones and amino acid levels.

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

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

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1. Introduction

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

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

Biological control of plant pathogens has been of great interest to researchers. Apart from pathogenic microbes, plants also have symbiotic or mutualistic interactions with a wide range of soilborne microbes, which protect plants from pathogens either directly or by inducing resistant mechanisms (Pieterse et al., 2014). These microbes associate with the plant roots and help enhance growth-related attributes by improving the uptake of essential ions and minerals, atmospheric nitrogen fixation, and protection from pathogens (Lugtenberg & Kamilova, 2009). These growth-promoting bacteria are mainly isolated from the rhizosphere of the plants. These microbes are commonly known as plant growth-promoting rhizobacteria (PGPR) (Kloepper, Lifshitz & Zablotovicz, 1989; Backer et al., 2018) and include organisms such as *Pseudomonas* spp. Other microbes are known as plant growth-promoting endophytic bacteria, plant growth-promoting fungi, or biocontrol fungi (BCF), including *Trichoderma* spp. and *Sebacinales* spp. These can play a role in plant growth and can stimulate plant immune systems (Shoresh, Harman & Mastouri, 2010; Singh et al., 2019). Endophytes are widely dispersed and can be found in diverse environments including the tropics, temperate zone, aquatics, xerophytics and deserts, tundra, geothermal soils, rainforests, mangroves, and coastal forests. They inhabit plant tissues such as endosperms, roots, leaves, stems, flowers, and fruits (Singh et al., 2017).

73   Generally, plant growth promotion may occur owing to the regulation of the plant hormonal system, modifications in
74   root architecture, production of siderophores, solubilization of soil minerals, activation of secondary mechanisms of
75   plant defense, and production of biochemicals (Pupin & Nahas, 2014; Backer et al., 2018).

76   PGPRs and endophytes have a non-pathogenic symbiotic life cycle associated with their host plant tissues; these
77   endophytes can be easily isolated from plant tissues (Arnold & & Lutzoni, 2007; Costa et al., 2012). Seeds are the
78   source of vertical dispersal of numerous seed-borne endophytes, or PGPRs (Ernst et al., 2003). Along with the
79   alleviation of biotic stress in plants, these PGPRs have been reported to help mitigate a wide range of abiotic stresses
80   as well (Shahzad et al., 2017a). Independent studies have reported the ameliorating effects of PGPRs on plant growth
81   and fungal diseases in tomato and sunflower (Shittu et al., 2009; Waqas et al., 2015). In addition, studies have revealed
82   the remediation abilities of PGPRs in soil contaminated with heavy metals (Jing, He & Yang, 2007; Bilal et al., 2018).
83   All of these impacts of PGPRs make them widely attractive as biofertilizers and soil microbe mediators (Backer et al.,
84   2018; Rosier, Medeiros & Bais, 2018). The positive effects of PGPRs on plant growth attributes are well known, but
85   the exact molecular mechanism(s) behind them have not yet been clearly demonstrated.

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

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

110   al., 2014), and degradation of pesticides (Pailan et al., 2015). In additions, various species of *Bacillus* have been
111   identified as plant growth promoting bacteria as well as biocontrol agents against various pathogenic fungi (Compant
112   et al., 2005; Shahzad et al., 2017a). Plant growth promoting rhizosphere bacteria employ a variety of strategies to
113   facilitate plant growth and survival under pathogenic attack by both direct and indirect mechanisms. The most common
114   direct mechanisms are phytohormone production, the acquisition of nutrients, and the control of pathogens through
115   various means, for example, through the synthesis of hydrolytic enzymes, antifungal compounds, lipopeptides, or
116   antibiotics. The indirect mechanisms include protection by triggering of specific defense-related pathways,
117   particularly the induction of systemic resistance (ISR) against pathogens and pests (Khan et al., 2012; Martínez-
118   Hidalgo et al., 2015) and the release of bacterial volatile compounds (Bernier et al., 2011). However, many
119   environmental factors influence the biological control potential of PGPR by either predisposing pathogens to microbial
120   antagonism, regulating the growth or production of metabolites by specific antagonists, or modulating disease
121   development and consequently the level of disease suppression achieved.

122   From our literature survey, it is evident that except for some reports in crops (*Xanthium italicum*, soybean, rice,
123   tomato, and wheat) there is a lack of information about the growth-promoting activity of *B. aryabhattai* and its role in
124   tolerance to biotic and abiotic stress in other plant species (Viljoen et al., 2019; Yoo et al., 2019). In this study, we
125   evaluated the plant growth-promoting abilities of *B. aryabhattai* SRB02 in tomato cultivars inoculated with
126   phytopathogenic fungus *FOL*.

127   **2. Materials and Methods**

128   *2.1. Growth of PGPR and FOL*

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

135   Briefly, a 0.5 cm^2 disc of active fungal mycelia of *FOL* was placed at the center of a 90 mm disposable plastic Petri
136   dish (SPL, Korea) containing LB agar (Becton, Dickinson and Company, France). The overnight bacterial culture of *B.*
137   *aryabhatttai* SRB02 was aseptically streaked around the fungal disc at equal distances in a square pattern. For the
138   untreated control, a fungal disc was placed on LB agar, as mentioned earlier, but instead of *B. aryabhatttai* SRB02, only
139   sterile water was streaked. For comparison, the effects of fungal growth inhibition of organic acids against the pathogen
140   were also evaluated. All of the plates were incubated at 28 °C for 7 d. After the incubation period, the inhibition zone
141   was measured and the percent inhibition was calculated according to the following formula.

142   Inhibition % = (diameter of fungus on control plate – diameter of fungus on SRB02 co-cultured plate) ×100
143   diameter of fungus on control plate

144   *2.2. Screening of tomato varieties for resistance to FOL*

145   In the current study, tomato seeds of four Korean cultivars (IT 252842-13 (Cultivar-1), IT 252869-14 (Cultivar-
146   2), IT 260627-16 (Cultivar-3), IT 259462-15 (Cultivar-4)) were selected for their response to the pathogen. Seeds
147   were sterilized with 2.5% sodium hypochlorite for 10 min and kept on wet paper towels inside Petri plates in an
148   incubator at 25 °C for 5 d. Horticultural soil, distilled water, and pots were autoclaved at 121 °C for 20 min. Uniformly
149   germinated seeds were transferred to separate trays filled with sterilized horticultural soil (Soil and Fertilizer
150   Technology, Korea). After one week, uniformly grown seedlings were transplanted to big pots with the dimensions
151   (LxWxH)-3.5 x 3 x 3 inches and volume 85-90gm. Plants were allowed to acclimatize for a few days, and the
152   experimental treatments were set up in triplicates, with each replicate containing at least six plants. The fungal spore
153   suspension of *FOL* strain KACC 40032 was prepared according to the protocol described by (Lichtenzveig et al.,
154   2006). Control plants were treated with distilled water, and plants were allowed to grow for 5 d. Plants to be treated
155   with the pathogen were inoculated by applying a spore suspension (10^6 conidia/mL) to the exposed roots of tomato
156   plants. The roots were then covered with soil. Plants were allowed to grow at relatively high humidity of $80 \pm 2\%$.
157   After 14 d of growth under the conditions mentioned above, the inoculated plants were assessed on the basis of
158   symptomatology (severity of plant wilting) and growth.

159   ### 2.3. In planta biocontrol assessment

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

173   ### 2.4. Extraction and quantification of amino acid content

174   The plant amino acids were extracted according to the protocol described by Khan et al. (2017)(Khan et al.,
175   2017), with some modifications. Briefly, the freeze-dried whole plant samples were ground to homogenate, and 100
176   mg powdered samples were hydrolyzed under a vacuum in 6N HCl at 110 °C followed by 80 °C for 24 h. The dried
177   residue was suspended in 0.02N HCl and filtered through a 0.45 μm filter. The amino acids were then quantified using
178   an automatic amino acid analyzer (Hitachi, Japan; L-8900). The experiments were conducted in triplicate, and each
179   replicate was comprised of six plants. The amino acid concentration was determined using relevant standards. These

180   standard known as amino acid standard mixture solution (type H) used for automatic amino acid investigation was

181   procured through Wako Pure Chemical Industries Ltd (Japan), and used for endogenous amino acids assessment.

182   *2.5. Jasmonic acid quantification*

183   For the quantification of endogenous jasmonic acid (JA) content, the optimized protocol described by McCloud

184   and Baldwin (1997) was used. Briefly, homogenized powder (0.3 g) from the immediately freeze-dried whole plant

185   samples was suspended in extraction buffer (70:30 v/v acetone and 50 mm citric acid), and 25 ng JA internal standard

186   ([9, 10-2H^2]-9, 10-dihydro-JA) was also added to the suspension. The extract suspension was kept overnight at room

187   temperature for evaporation of highly volatile organic solvents and to retain the less-volatile fatty acids. The

188   subsequent aqueous phase was filtered and then extracted with 30 mL diethyl ether three times. The collective extracts

189   were subsequently loaded onto a solid-phase extraction cartridge (500 mg of sorbent, aminopropyl). In addition, 7.0

190   mL of trichloromethane and 2-propanol (2:1 v/v) were used to wash the loaded cartridges. Then, the exogenous JA

191   and relevant standard were eluted with 1 mL of diethyl ether and acetic acid (98:2 v/v). Following evaporation, the

192   samples were esterified and analyzed by GCMS (6890N network GC system) and a 5973 network mass selective

193   detector (Agilent Technologies, Palo Alto, CA, USA) in the relevant ion mode. The relevant ion mode was selected

194   for JA determination. The ion fragment was examined at *m/z* = 83 AMU, corresponding to the base peaks of JA and

195   [9, 10-2H^2]-9, 10-dihydro-JA. The endogenous JA values were determined from the peak areas with respect to relevant

196   standards.

197   *2.6. Salicylic acid (SA) quantification*

198   The SA of SRB02-treated tomato plants was extracted and quantified according to the protocol described by

199   (Enyedi et al., 1992; Seskar, Shulaev & Raskin, 1998). Immediately freeze-dried whole plant tissues were

200   homogenized, and 0.2 g of homogenate powder was used for the extraction using 90% and 100% methanol. The pellets

201   were dried and re-suspended in 2.5 mL 5% trichloroacetic acid (TCA) and further partitioned with ethyl acetate,

202   cyclopentane, and isopropanol (ratio of 100:99:1, v/v). The upper organic layer containing free SA was used for air-

203   drying with nitrogen gas. The dry SA was again suspended in 1 mL 70% methanol and subjected to high-performance

204   liquid chromatography (HPLC) using a Shimadzu device outfitted with a fluorescence indicator (Shimadzu RF-

205   10AxL) with excitation at 305 nm and emission at 365 nm filled with a C18 reverse-phase HPLC column (HP Hypersil

206   ODS, particle size 5 μm, pore size 120 Å, Waters). The flow rate was maintained at 1.0 mL/min.

207   *2.7. Statistical analysis*

208   All experiments were replicated three times, and each replicate was comprised of six plants. Data were

209   statistically evaluated with Duncan multiple range tests and *t*-tests where appropriate, using SAS version 9.2 software

210   (Cary, NC, USA).

211   **3. Results**

212   *3.1. In vitro antifungal assay*

213   The *in vitro* antifungal activity of PGPR *B. aryabhattai* SRB02 was assessed against pathogenic *Fusarium*
214 *oxysporum* in dual culture. The results revealed that the PGPR *B. aryabhattai* SRB02 significantly inhibited the growth
215 of pathogenic *F. oxysporum,* as shown in Figure S1.

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

217   To determine the response of four tomato cultivars, the plants were challenged with a spore suspension of the
218 pathogen. The pathogen was applied to the exposed roots of tomato plants and incubated under higher relative
219 humidity to create a conducive environment for successful infection. After 14 d of inoculation, the cultivars revealed
220 a differential level of tolerance to the pathogen (Figure 1). The plant tolerance level was determined based on the
221 symptomatology (severity of plant wilting). In the susceptible plants, clear symptoms of wilting were evident.
222 Susceptible plants were also observed with retarded growth as compared to the tolerant plants. Based on the plant
223 growth attributes and resistance level, as shown in Figure 1, the most tolerant and susceptible tomato cultivars were
224 selected for further experiments.

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

226   Based on screening, the plant-growth-promoting and biocontrol efficiency of PGPR *B. aryabhattai* SRB02
227 against a virulent strain of *F. oxysporum* was investigated in both the selected tolerant and susceptible varieties (Figure
228 2). *B. aryabhattai* SRB02 significantly promoted plant growth, and interestingly reduced the disease in both tolerant
229 and susceptible tomato cultivars (Figure 2).
230   The growth-related traits of the disease-tolerant plants were significantly improved when applied with SRB02
231 alone. The plant height (PH) was improved by 37.4%, while RL was improved by 26.8% as compared to the water-
232 treated control plants. Other traits including seedling FW, seedling DW, and chlorophyll content were also improved
233 by 15.3%, 23.3%, and 5.8%, respectively. A similar trend was also observed when the tolerant plants were treated
234 with the pathogen and SRB02 together, compared to the pathogen-treated plants. The PH, RL, seedling FW, DW, and
235 chlorophyll content were improved by 124%, 6.4%, 15.8%, 42.3%, and 39.7%, respectively, compared to the plants
236 inoculated with the pathogen alone. SRB02 also improved the growth attributes of the disease-susceptible plants with
237 or without co-treatment by the pathogen. The PH of the disease-susceptible plants was improved by 14.1% with the
238 application of SRB02 in comparison with the water-treated control plants; however, the increase in PH was
239 significantly greater (105.7%) in plants treated with SRB02 and *F. oxysporum* combined as compared to the pathogen-
240 treated plants. Likewise, other traits were also improved in plants treated with PGPR alone as compared to the water-
241 treated plants and also in the PGPR and pathogen co-treated susceptible plants in comparison with the plants treated
242 with the pathogen alone. The RL, seedling FW, seedling DW, and chlorophyll content were improved by 9% and
243 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-
244 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

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

253   Furthermore, SRB02 treatment with *F. oxysporum*-inoculated plants of the tolerant cultivar accumulated 48.48%
254   more SA compared to plants not treated with the PGPR. More interestingly, the response of the *F. oxysporum*-
255   inoculated plants of the susceptible cultivar was more robust in the presence of SRB02, as these plants produced
256   74.60% more SA as compared to plants not treated with PGPR (Figure 4). However, no significant differences in SA
257   accumulation were observed in SRB02-treated plants of either tolerant and susceptible cultivars in the absence of *F.*
258   *oxysporum*.

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

260   The current study showed that *B. aryabhattai* SRB02 regulates amino acids in both disease-tolerant and
261   susceptible tomato plants in the presence or absence of *F. oxysporum* (Table 2). Under pathogenic infection by *F.*
262   *oxysporum*, *B. aryabhattai* SRB02 inoculation significantly enhanced aspartic acid (115.57% and 147.48%), threonine
263   (123.18% and 118.56%), serine (123.13% and 158.91%), glutamic acid (4.86% and 157.89%), glycine (131.82% and
264   143.58%), alanine (99.61% and 109.67%), valine (98.13% and 74.62%), methionine (239.06% and 172.93%),
265   isoleucine (42.60% and 97.82%), leucine (103.21% and 58.03%), tyrosine (138.45% and 65.45%), phenylalanine
266   (39.86% and 34.16%), lysine (113.15% and 98.03%), histidine (98.42% and 111.74%), arginine (108.69% and
267   157.18%), and proline (90.09% and 115.25%) in disease-susceptible and tolerant tomato plants, respectively (Table
268   2). Only cysteine was decreased by 9.65% and 21.82% in *B. aryabhattai* SRB02 applied to susceptible and tolerant
269   plants, respectively (Table 2).

270   Likewise, in the absence of the pathogen, *B. aryabhattai* SRB02 significantly enhanced aspartic acid (3.35% and
271   24.98%), threonine (32.99% and 118.56%), glutamic acid (4.86% and 157.89%), glycine (30.78% and 143.58%),
272   alanine (29.70% and 4.65%), cysteine (44.22% and 60.20%), valine (21.91% and 31.81%), methionine (132.35% and
273   31.17%), isoleucine (97.76% and 29.48%), leucine (36.52% and 32.40%), phenylalanine (114.92% and 77.20%),
274   histidine (22.81% and 41.48%), and arginine (35.98% and 23.95%) in the susceptible and tolerant plants, respectively
275   (Table 2). However, *B. aryabhattai* SRB02 showed an increase in serine-intolerant (9.73%) plants and a decrease of
276   13.22% in susceptible plants. Tyrosine was increased by 45.03% only in the tolerant plants when applied with PGPR,
277   while it was decreased by 51.52% in susceptible plants. Similarly, lysine was increased (35.12%) in the PGPR-applied
278   tolerant plants, while no significant difference was observed in the susceptible plants. In contrast, proline was
279   increased by 35.77% only in susceptible plants, while no significant difference was recorded in intolerant plants when
280   challenged with PGPR (Table 2).

5. Discussion

282   The use of microbial-based techniques in the management of plant diseases has gained significant attention in
283   recent years. In particular, PGPRs and their interactions with the plants under biotic or abiotic stress are gaining
284   importance, with the ultimate aim of improvement in protection of crops and increases in agricultural production.
285   These biocontrol approaches are eco-friendly and are becoming very popular, reliable, and long-lasting. Plant growth
286   improvement by PGPR is one of the outstanding characteristics of these naturally occurring microbes. The
287   improvements in plant growth and its ameliorating abilities with regard to plant diseases are determined by the
288   interactions between the host plant and PGPR (Vejan et al., 2016). PGPR improves plant growth and health by direct
289   or indirect mechanisms that can overcome diseases. The plant growth-promoting activity of PGPR bacteria has been
290   reviewed in detail by d Santoyo et al. (2016) (Xia et al., 2015; Santoyo et al., 2016). *Bacillus* and *Pseudomonas* species
291   are widely known as invaluable resources for plant growth promotion and the suppression of disease symptoms
292   (Sundaramoorthy & Balabaskar, 2013; Chaves-López et al., 2015). Over the last few decades, several studies have
293   reported on the beneficial aspects of *Bacillus* spp. as biocontrol and biofertilizer agents; e.g., *Bacillus licheniformis*,
294   *Bacillus subtilis*, *Bacillus cereus*, *Bacillus pumilus*, and *Bacillus amyloliquefaciens* (Pane & Zaccardelli, 2015; Han
295   et al., 2016). The plant growth promotion and other beneficial aspects of *Bacillus* strains can be attributed to their
296   ability to enhance the production of phytohormones such as auxin (IAA), and gibberellic acid (Gamalero & Glick,
297   2011).

298

299   A   wide   range   of   plant   species   are   infected   by   pathogens,   including   the   diverse   genera   of
300   *Alternaria*, *Botrytis*, *Fusarium*,   and *Rhizoctonia*. These pathogens result in severe losses to crop yield and
301   productivity, thereby posing a threat to food security. *F. oxysporum* is a devastating fungal pathogen that attacks the
302   vascular system and causes severe damages to tomato crops across the globe. Conversely, microbes, or PGPRs, found
303   in the rhizosphere of plants are directly associated with roots and are a vital source for plant growth promotion and
304   suppression of soilborne plant pathogens such as *F. oxysporum*. To isolate and evaluate the beneficial role of PGPR,
305   an appropriate *in vitro* experimental setup is required. Shahzad et al. (2017) (Shahzad et al., 2017a) reported plant
306   growth promotion by endophytic bacteria RWL-1 against the pathogenic infection by *FOL* in tomato. In addition, it
307   was recently reported that *B. aryabhattai SRB02* plays a role in oxidative and nitrosative stress tolerance and promotes
308   the growth of soybean and rice plants by modulating the production of phytohormones (Park et al., 2017a). However,
309   it was not clear whether *B. aryabhattai SRB02* could be used to rescue the plants from biotic stress. Hence, in the
310   present study, we subjected disease-tolerant and susceptible tomato plants to the PGPR *B. aryabhattai* SRB02 in the
311   presence and absence of a virulent strain of *FOL,* hypothesizing that SRB02 would rescue the plants from the disease
312   and improve their growth under stress conditions. Prior to inoculation by the pathogen, tomato plants were treated
313   with a cell suspension of *B. aryabhattai* SRB02. The SRB02 application improved the disease tolerance level of the
314   infected plants. In a previous study by Shahzad et al. (2017) (Shahzad et al., 2017a), PGPRs were shown to enhance
315   plant growth, reduce infection by the pathogen, and result in improved disease tolerance.
316   The present study showed that under pathogenic infection, the PGPR association rescued the plants from disease
317   and enhanced plant growth and biomass. This result might occur by restricting the pathogenic fungus, enhancing
318   nutrient uptake, and producing phosphate solubilization substances, or by induction of phytohormonal biosynthesis.

319   The present findings further strengthen the role of *Bacillus* species as a PGPR and biocontrol agent, as reported by
320   numerous researchers, against diverse diseases in various plant species, such as root wilting, damping off, fusarium
321   wilt, ring rot, and charcoal rot in tomato, soybean, banana, apple, and common bean, respectively (Yu et al., 2002;
322   Vitullo et al., 2012; Wang & Fobert, 2013; Chen et al., 2016; Torres et al., 2016). The current findings also indicate
323   that PGPR strains producing bioactive components may suppress the negative effects of pathogenesis and biotic stress
324   in infected plants. In addition, our study also confirmed and exhibited similar results to previous reports that organic
325   acids, as one of the many components produced by *Bacillus* species, can help rescue the plant from the disease.
326   Moreover, PGPR produces siderophores and organic acids, which mitigate the negative effects of pathogen-infected
327   sunflower plants (Waqas et al., 2015). From these studies, it is evident that biotic stress-related ameliorative effects
328   are commonly regulated by endogenous phytohormones such as SA and JA. Under normal and stress conditions,
329   phytohormone signaling and crosstalk play a vital role in plant growth and development. Accordingly, in the present
330   study, we found that inoculation with PGPR *B. aryabhattai SRB02* extensively modulated the endogenous levels of
331   JA and SA. Our findings are in conformity with previously elucidated phytohormonal regulation; i.e., increased SA
332   (Figure 4) and reduced JA (Figure 3) with PGPR, as revealed by independent studies (Khan et al., 2015; Waqas et al.,
333   2015; Shahzad et al., 2016, 2017b; Ali et al., 2017) comparing plants in the presence or absence of biotic stress.

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

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339   **Conflicts of Interest: The authors declare no conflict of interest**

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Figure 1

Figure 1. Response of four tomato cultivars against F. oxysporum f. sp. Lycopersici. Cultivar 1 showed tolerance, cultivar 2 was moderately tolerant, and cultivars 3 and 4 were susceptible based upon the symptomatology.
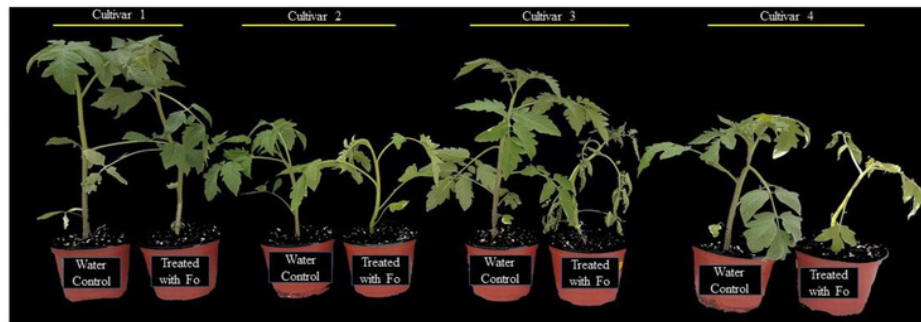
Figure 2

Figure 2. Effect of SRB02 on susceptible and tolerant tomato plants under inoculation with pathogenic F. oxysporum f. sp. lycopersici. The effect of SRB02 on tomato disease-tolerant plants

(A) and disease-susceptible plants (B) to ameliorate disease symptoms and promote growth parameter SRB02 improved the roots of both tolerant and susceptible plants in the presence and absence of the pathogen (C).
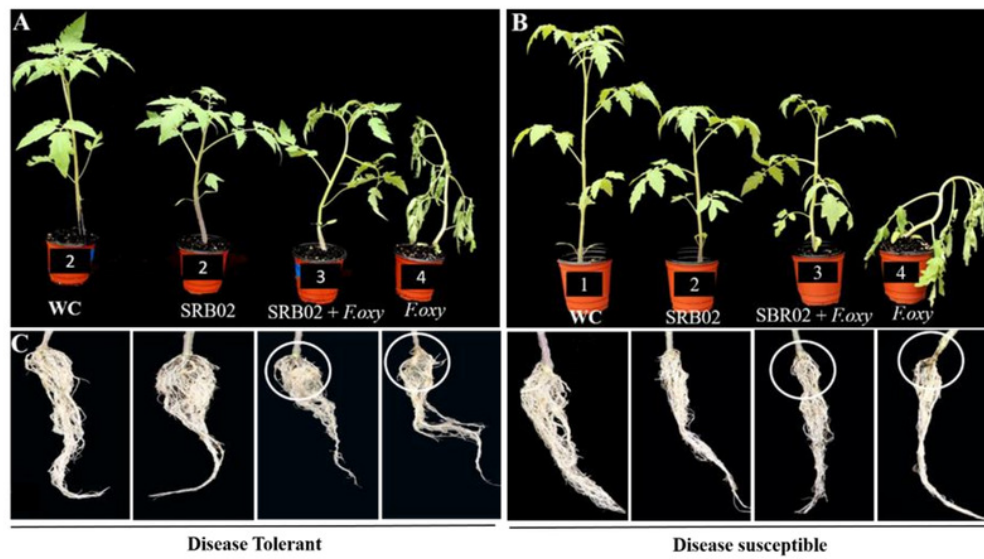
Figure 3

Figure 3. Regulation of jasmonic acid accumulation in plants.

A. Under pathogenic stress by Fusarium oxysporum, B. aryabhattai SRB02 significantly reduced the endogenous JA accumulation level in both susceptible and tolerant plants. Conversely, a reduction i
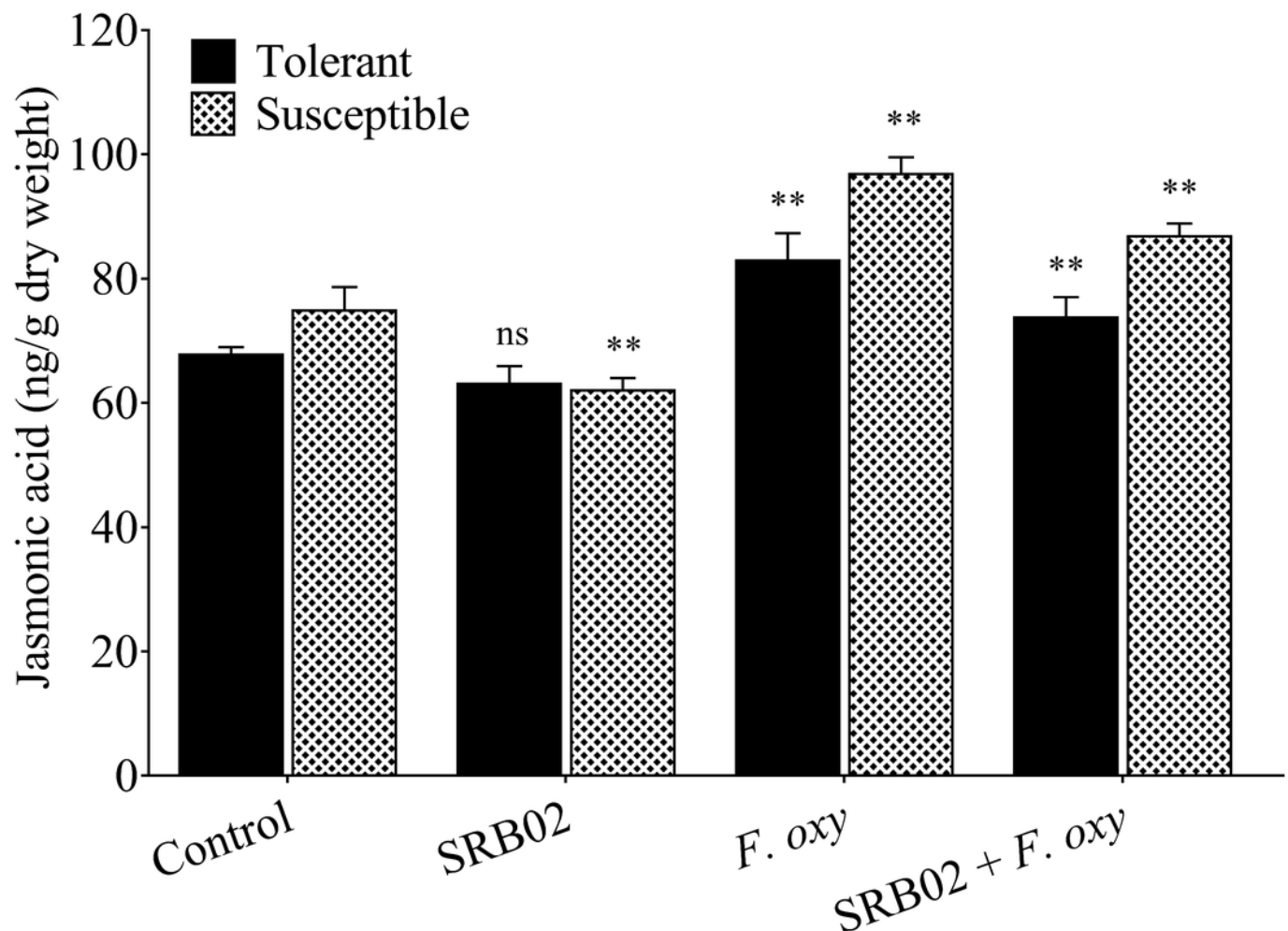
Figure 4. Regulation of salicylic acid accumulation in plants. In the presence of the pathogen, B. aryabhattai SRB02 significantly enhanced SA accumulation in both tolerant and susceptible plants, while in the absence of the pathogen, the plants displayed
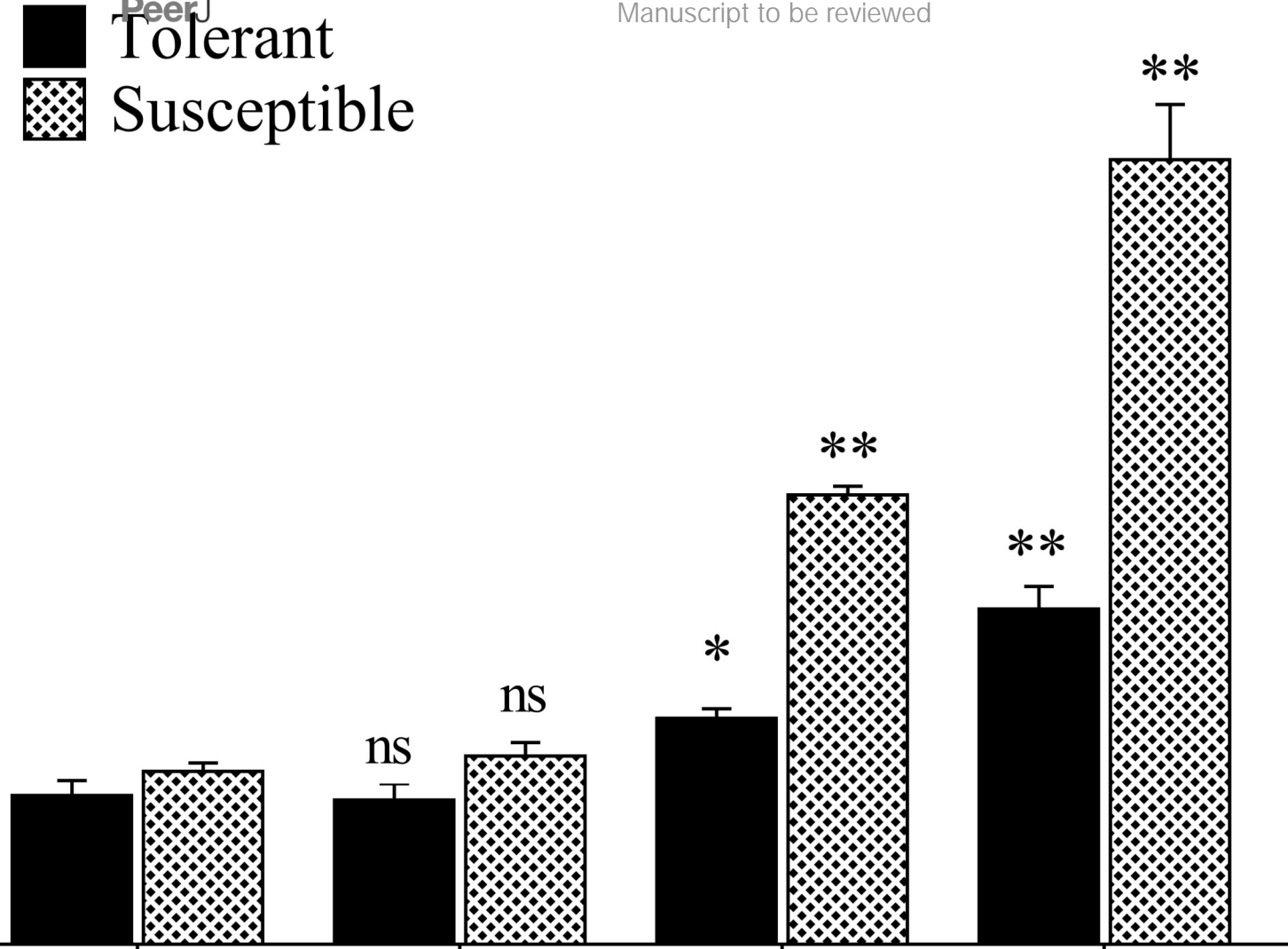
Table 1. Effects of SRB02 on growth parameters of tomato plants under control and biotic stress conditions

1   **Table 1. Effects of SRB02 on growth parameters of tomato plants under control and biotic stress**

2   **conditions**

Variety	Treatment	PH (cm)	RL (cm)	FW (g)	DW (g)	Chl.Cont. (SPAD)
Tolerant	Control	28.73 ± 0.40b	15.16 ± 0.34b	14.38 ± 0.05b	6.09 ± 0.25b	28.38 ± 0.37b
	SRB02	39.48 ± 0.96a	19.23 ± 0.64a	16.58 ± 1.00a	7.51 ± 0.60a	30.03 ± 0.40a
	F. oxysporum	17.50 ± 0.51b	14.25 ± 0.48b	13.59 ± 0.01b	5.18 ± 0.10b	23.38 ± 0.38b
	SRB02 + F. oxy	39.20 ± 0.88a	15.17 ± 0.34a	15.74 ± 0.42a	7.37 ± 0.27a	32.67 ± 0.48a
Susceptible	Control	29.88 ± 0.45b	18.75 ± 0.43b	16.11 ± 0.18b	6.89 ± 0.49b	27.11 ± 0.34b
	SRB02	34.10 ± 0.71a	20.43 ± 0.58a	14.42 ± 0.04a	7.10 ± 0.08a	28.17 ± 1.42a
	F. oxysporum	14.63 ± 0.62b	13.41 ± 0.76b	10.83 ± 0.57b	4.88 ± 0.18b	25.11 ± 1.07b
	SRB02 + *F. oxy*	30.10 ± 0.53a	19.38 ± 0.37a	14.36 ± 0.27a	6.08 ± 0.08a	40.51 ± 0.98a

3   Notes: PH, plant height; RL, root length; FW, fresh weight; DW, dry weight; Chl. Cont., chlorophyll content. Data

4   represent means ± SD of six replicates from three independent experiments. Each values in columns followed by

5   different letters are significantly different at $P \geq 0.05$.

Table 2. Regulation of Amino acids (mg/g DW) in tomato plants applied withSBR02 in the presence and absence of*F. oxysporum*f. sp.*lycopersici*.

Table 2. Regulation of Amino acids (mg/g DW) in tomato plants applied with SBR02 in the presence and absence of *F. oxysporum* f. sp. *lycopersici*.

Cultivar	Treatment	Asp	Thr	Met	ILE	Ser	Glu	Leu	Tyr	Gly	Phe	Lys	Cys	Val	His	Arg	Ala	Pro
Tolerant	Control	4.93 ± 0.12b	4.84 ± 0.27b	0.69 ± 0.17b	4.95 ± 0.22b	4.83 ± 0.11b	12.5 ± 0.32b	9.91 ± 0.11b	3.30 ± 0.60a	5.73 ± 0.22b	3.38 ± 0.32b	6.63 ± 2.69b	0.36 ± 0.02a	6.10 ± 0.21b	2.67 ± 0.32b	4.49 ± 0.45b	6.31 ± 0.21b	9.87 ± 1.15b
	SRB02	6.16 ± 0.07a	4.71 ± 0.27a	0.91 ± 0.08a	6.41 ± 0.24a	5.30 ± 0.14a	16.1 ± 0.33a	13.13 ± 0.4a	1.60 ± 0.15b	5.80 ± 0.16a	5.99 ± 0.14a	7.73 ± 1.52a	0.57 ± 0.03a	8.05 ± 0.14a	3.78 ± 0.31a	5.56 ± 0.47a	6.60 ± 0.18a	13.4 ± 0.74a
	F. oxy	2.96 ± 0.12b	3.05 ± 0.27b	0.32 ± 0.06b	2.51 ± 0.44b	2.70 ± 0.14b	7.36 ± 0.35b	6.21 ± 0.20b	2.29 ± 0.2b	3.24 ± 0.14b	5.92 ± 0.18b	5.29 ± 0.62b	0.53 ± 0.05a	3.54 ± 0.42b	1.25 ± 0.13b	2.82 ± 0.3b	4.06 ± 0.06b	4.67 ± 0.56b
	SRB02 + *F. oxy*	7.33 ± 0.46a	6.66 ± 0.22a	0.88 ± 0.02a	4.98 ± 0.09a	7.01 ± 0.22a	18.99 ± 0.2a	9.82 ± 0.26a	3.79 ± 0.25a	7.90 ± 0.15a	7.94 ± 0.19a	10.49 ± 2.26a	0.41 ± 0.01b	6.18 ± 0.19a	2.66 ± 0.33a	7.26 ± 0.6a	8.52 ± 0.34a	10.0 ± 0.7a
Susceptible	Control	6.14 ± 0.07b	4.41 ± 0.38b	0.73 ± 0.1b	3.02 ± 0.14b	5.90 ± 0.09b	7.77 ± 0.8b	9.28 ± 0.24b	2.50 ± 0.30a	5.31 ± 0.26b	3.57 ± 0.24b	8.44 ± 0.38b	0.39 ± 0.04a	6.30 ± 0.36b	2.96 ± 0.09b	4.67 ± 0.32b	6.36 ± 0.43b	11.5 ± 1.09b
	SRB02	6.34 ± 0.13a	5.87 ± 0.12a	1.71 ± 0.38a	5.99 ± 0.14a	5.12 ± 0.11a	14.1 ± 0.69a	12.67 ± 0.54a	3.60 ± 0.20b	6.95 ± 0.1a	7.69 ± 0.36a	11.4 ± 1.08a	0.55 ± 0.01a	7.68 ± 0.29a	3.63 ± 0.44a	6.35 ± 0.17a	8.25 ± 0.22a	12.5 ± 0.56a
	F. oxy	3.45 ± 0.47b	2.94 ± 0.13b	0.43 ± 0.05b	4.58 ± 0.35b	2.90 ± 0.13b	16.87 ± 0.15b	6.19 ± 0.30b	1.60 ± 0.30a	3.28 ± 0.2b	5.60 ± 0.29b	5.33 ± 0.36b	0.69 ± 0.07a	3.95 ± 0.16b	1.22 ± 0.28b	3.15 ± 0.23b	4.20 ± 0.21b	5.55 ± 0.38b
	SRB02 + *F. oxy*	7.45 ± 0.23a	6.57 ± 0.47a	1.47 ± 0.20a	6.54 ± 0.44a	6.48 ± 0.38a	17.69 ± 0.22a	12.59 ± 0.38a	3.90 ± 0.5b	7.60 ± 0.37a	7.83 ± 0.27a	11.37 ± 0.64a	0.62 ± 0.04b	7.83 ± 0.19a	2.43 ± 0.17a	6.57 ± 0.39a	8.38 ± 0.25a	10.55 ± 0.38a

Notes: Asp, Aspartic acid; Thr, Threonine; Met, Methionine; ILE, Isoleucine; Ser, Serine; Glu, Glutamic acid; Leu, Leucine; Tyr, Tyrosine; Gly, Glycine; Phe, Phenylalanine; Lys, Lysine; Cys, Cysteine; Val, Valine; His, Histidine; Arg, Arginine; Ala, Alanine; Pro, Proline. Each value represents means ± SD of three independent experiments. Each values in columns followed by different letters are significantly different at $P \geq 0.05$.