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Article

Faba Bean−Wheat Intercropping Can Control the Occurrence of Faba Bean Fusarium Wilt by Alleviating the Inhibitory Effect of Benzoic Acid on Disease Resistance Metabolism and the Expression of Resistance Genes

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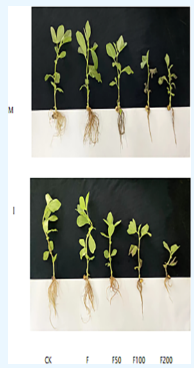
Cite This: *ACS Omega* 2023, 8, 2897−2906

ABSTRACT: *Background:* Continuous cropping leads to the accumulation of autotoxic substances in faba beans, which limits their global production. Intercropping is widely used to alleviate these problems. *Aim:* This study aims to explore the important role of *Fusarium oxysporum* f. sp. *fabae* (FOF) and benzoic acid stress in enhancing the occurrence of faba bean Fusarium wilt and the potential mechanism of faba bean−wheat intercropping to control the occurrence of this disease. *Methods:* We analyzed the pathogenic mechanism of FOF and benzoic acid and the defense response of faba bean−wheat intercropping against the autotoxicity of benzoic acid under hydroponic conditions that included the pathogen alone and in combination with different concentrations of benzoic acid. *Results:* The dual stress of FOF and benzoic acid inhibited the activity of defensive enzymes, the synthesis of defensive substances, and the expression of defensive genes in faba bean roots and reduced the disease resistance of faba bean. This shows that benzoic acid plays an important role in helping FOF cause disease. Faba bean−wheat intercropping improves plant resistance by alleviating benzoic acid stress and reducing the incidence and disease index of Fusarium wilt. *Conclusion:* The dual stress of FOF and benzoic acid promotes the occurrence of faba bean Fusarium wilt by destroying the root defense system of faba bean. Faba bean−wheat intercropping can effectively alleviate the autotoxicity of benzoic acid and control the occurrence of Fusarium wilt by improving the physiological and biochemical resistance of faba beans and the expression of defense genes.



■ INTRODUCTION

With the development of modern agriculture, large-scale, single, and intensive planting models have become common, and continuous cropping obstacles have become increasingly serious.[1] The hazards of continuous cropping obstacles primarily include soil compaction, the frequent occurrence of soilborne diseases, reduced crop yields, and even inability to germinate seeds. The frequent occurrence of soilborne diseases has always been an important factor that restricts actual production.[2] The accumulation of pathogens is an important reason for the frequent occurrence of soilborne diseases, and the exploration of the pathogenic mechanism of pathogens has always been an intensive area of research.[3] Research by Quadros et al. showed that *Fusarium oxysporum* f. sp. *phaseoli* (FOP) can cause peroxidative damage to crops by increasing the content of H_2O_2 and O_2^- in plant root cells, thereby promoting the occurrence of kidney bean Fusarium wilt.[4] Ren et al.'s research showed that *F. oxysporum* f. sp. *lycopersici* can inhibit the activity of polyphenol oxidase (PPO) in tomato roots to cause tomato Fusarium wilt.[5] The growth and physiological activities of pathogens will be regulated by the allelopathy of neighboring plants. Allelopathy is a natural process in which plants have positive or negative effects on the surrounding environment by releasing allelopathic substances.[6] Autotoxicity is special allelopathy. Autotoxicity will cause plants to inhibit their growth and the occurrence disease frequently.[7,8] Many studies have proven that autotoxicity increases the susceptibility of plants to pathogens and diseases.[9] Phenolic acid is an aromatic secondary metabolite and a typical autotoxic compound in plant root exudates. Research by Yang et al. showed that the autotoxic substances secreted by Chinese ginseng (*Panax notoginseng*) can cause oxidative damage to this species of plant by increasing the contents of H_2O_2 and O_2^- in its roots, thereby promoting the occurrence of continuous cropping obstacles.[10] Wang et al. showed that the four phenolic acids PA, HA, SA, and VA secreted by strawberries increase the risk of strawberry disease by inhibiting the activities of peroxidase (POD), catalase (CAT), and superoxide dismutase (SOD) in strawberry roots.[7] Studies by Ren et al. have shown that ferulic acid secreted by watermelon roots can inhibit the expression of defense genes in watermelon roots, reduce the defense ability of watermelon, and promote the occurrence of watermelon wilt.[11] However, in

Received: July 20, 2022
Accepted: November 28, 2022
Published: January 10, 2023

actual production, plants are not faced with the individual stress of pathogens or autotoxic substances but the dual stress produced by the synergistic effect of autotoxic substances and pathogens. These studies cited did not explore the pathogenic mechanism from the dual stress of autotoxic substances and pathogens.

Several chemical and biological methods have been developed to control the occurrence of plant diseases.[12] However, chemical agents have obvious side effects. For example, moderate administration of urea can control the occurrence of soilborne diseases. However, in actual production, to pursue better disease control effects, people tend to administer excess urea. This in turn leads to an increase in the number of pathogens in the soil and a high incidence of diseases.[13] In addition, a biological control agent is a green and good method of disease control. However, the effectiveness of prevention and control in actual production is reduced and it is highly susceptible to environmental factors.[14] For example, *Pythium oligandrum* has received widespread attention as a biocontrol agent, but under field conditions, this biocontrol agent cannot survive for a long time in plants, and its effect is greatly reduced.[15] Therefore, species diversity is a better way to control the disease, for example, crop rotation and intercropping. Some studies have shown that the rotation of legumes and cereal crops effectively controls the occurrence of soilborne diseases of legumes.[16] Similar findings were found in intercropping systems. The intercropping of marigold and tomato inhibited the damage caused by soilborne tomato early blight.[17] Also, the intercropping of garlic and cruciferous vegetables reduced the occurrence of soilborne white rot of garlic.[18] It shows that intercropping can effectively control the occurrence of diseases.[19] Qi et al. found that cotton and melon intercropping can effectively reduce the content of O_2^- in melon compared with melon monocropping, alleviate the peroxidation damage of melon, and improve its disease resistance.[20] Research by Cheng et al. has shown that garlic−tomato intercropping improves the ability of tomatoes to scavenge oxidative free radicals and reduces the risk of disease by increasing the activities of tomato SOD, POD, and phenylalanine ammonia lyase (PAL).[21] Gao et al. showed that soybean−corn intercropping can increase the resistance of soybean to crown rot by increasing the expression of soybean pathogenic response genes (PRs) and effectively control the occurrence of soybean crown rot.[22] However, these studies did not explore the mechanism of disease control from intercropping under the dual stress of autotoxic substances and pathogens. In addition, they did not examine the effect of intermediate crops on the expression of plant defense genes in the faba bean−wheat intercropping system.

Faba beans are grown as food and animal feed all over the world.[23] However, owing to years of continuous cropping, the frequent occurrence of Fusarium wilt restricts the production of faba beans worldwide.[24] In southwest China, the planting pattern of faba bean−wheat intercropping is often used to control the occurrence of faba bean Fusarium wilt. Research efforts by our group have explored the pathogenic mechanism of faba bean Fusarium wilt factoring in faba bean autotoxicity.[3] Among them, benzoic acid has been focused on as an important autotoxic substance of faba bean.[25] Previous research has shown that benzoic acid can reduce the resistance of faba bean root tissue, thereby increasing the incidence of faba bean Fusarium wilt.[25] However, it did not explore pathogenic mechanisms of benzoic acid that could involve changes in physiological and biochemical resistance and the expression of defense genes nor did it explore the mitigation effect of faba bean−wheat intercropping on the autotoxicity of benzoic acid. This study aims to (1) reveal the effects of benzoic acid and stress by pathogens on the occurrence of faba bean Fusarium wilt and its mechanism of action and (2) examine the potential mechanism of faba bean−wheat intercropping to control faba bean Fusarium wilt by studying its effects on plant physiological and biochemical resistance and the expression of defense genes.

■ MATERIALS AND METHODS

Test Materials. Varieties 89-147 of faba bean (Vicia faba L.) and Yunmai53 of wheat (Triticum aestivum L.) were obtained from Yunnan Academy of Agricultural Sciences (Yunnan Sheng, China). Benzoic acid was obtained from China Pharmaceutical Group Shanghai Medical Instrument Co. Ltd. (Shanghai, China). FOF was isolated from a faba bean monoculture at the Plant−Microbe Laboratory at Yunnan Agricultural University (Kunming, China) and cultured on potato dextrose agar (PDA) for 7 days at 28 °C. The mycelia were stored at 4 °C.

Greenhouse Cultivation. We established a hydroponic experiment in the greenhouse of Yunnan Agricultural University (Kunming, China). Faba beans and wheat were planted in pots and grown from October 2021 to May 2022 in M (six faba beans per pot) or I (three faba beans and nine wheat per pot) mode until May 2020 in no stress (CK), pathogen alone stress (F), pathogen and 50 mg·L^{-1} benzoic acid double stress (F50), pathogen and 100 mg·L^{-1} benzoic acid double stress (F100), and pathogen and 200 mg·L^{-1} benzoic acid (F200). Faba beans and wheat were grown under 26/22 °C light for 14 h every day. Each treatment was repeated three times.Faba bean seeds were soaked at room temperature for 24 h and germinated in sterile quartz sand at 25 °C. After the seedlings grew 4−6 leaves and were approximately 10 cm high, they were transplanted into a 3 L plastic pot (upper diameter 25 cm, lower diameter 13 cm, height 16 cm), which contained different concentrations of benzoic acid and 2 L of 1 × 10^6 mL^{-1} Hoagland nutrient solution of an FOF spore suspension. The faba bean plants were grown with or without the wheat seedlings. Next, we changed the Hoagland nutrient solution of the same concentration of benzoic acid and 1 × 10^6 mL^{-1} FOF spore suspension every 3 days, and the position of the pot was changed randomly. The nutrient solution was stored in the pot, and air was pumped through the solution 24 h a day.

Evaluation of Fusarium Wilt. Fusarium wilt was studied 45 days after the transplantation of faba beans. Single-cropped faba beans were studied in three pots per treatment and six plants in each pot for a total of 18 plants. Intercropped faba beans were investigated in three pots per treatment and three plants in each pot for a total of nine plants.

$$\text{incidence} = \frac{\text{number of diseased plants}}{\text{total number of plants investigated}} \times 100\%$$

$$\text{disease index} = \frac{\Sigma(\text{number of diseased plants at each level} \times \text{level})}{\text{the highest level} \times \text{total number of plants investigated}} \times 100$$

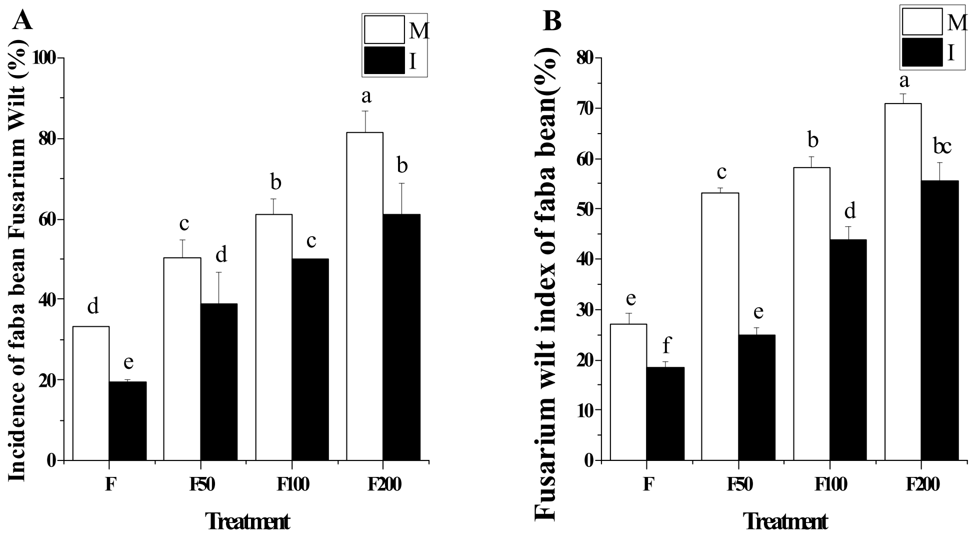


Figure 1. Effects of different concentrations of benzoic acid (0, 50, 100, 200 mg·L^{-1}) on (A) incidence and (B) disease index of faba bean (*Vicia faba*) Fusarium wilt inoculated with FOF under monocropping and intercropping modes. F, *F. oxysporum* f. sp. *fabae*; M, monocropping; I, intercropping. The data represent the mean ± standard error of three biological replicates. Different letters for each index indicate significant differences at $p < 0.05$ (Tukey's test).

Determination of the Activity of Defensive Enzymes in Faba Bean Roots. Samples were collected after the faba bean plants had grown for 45 days (faba bean branching stage). Three faba bean plants were harvested each time. Fresh root samples were used to determine the activities of PAL, SOD, and PPO and the contents of hydrogen peroxide (H_2O_2) and superoxide anions (O_2^-). All of the activities and contents were measured using kits from Sino Best Biological Technology Co., Ltd. (Shanghai, China) following the manufacturer's instructions: O_2^- (Art. No., YX-C-A407; specification, 50T/48S), H_2O_2 (Art. No., YX-C-A400; specification, 50T/48S), SOD (Art. No., YX-C-A500; specification, 50T/48S), PAL (Art. No., YX-C-A604; specification, 50T/48S), and PPO (Art. No., YX-C-A404; specification, 50T/24S).

Determination of Total Phenolics and Total Flavonoids in Faba Bean Roots. Samples were collected after the faba bean plants had grown for 45 days (the faba bean branching stage). Three faba bean plants were harvested each time. Fresh root samples were used to determine the content of total phenolics and total flavonoids. All of the activities and contents were measured using kits from Sino Best Biological Technology Co., Ltd. (Shanghai, China) following the manufacturer's instructions as described: total phenolics (Art. No., YX-C-A507; specification, 50T/24S) and total flavonoids (Art. No., YX-C-A506; specification, 50T/48S).

Determination of Defensive Gene Expression in Faba Bean Root Systems. Samples were collected from faba beans as described above. For every three repetitions of faba beans, the harvested faba bean roots were quickly stored in liquid nitrogen for subsequent determination. Fluorescence quantitative PCR was used to detect the expression of the four defense genes *Vf*PR1, *Vf*PR2, *Vf*PR5, and *Vf*PR10 in faba bean roots.

Specific primer sequences and putative functions of the plant PR genes examined are listed in Tables S1−S5 in the supporting file.

Statistical Analysis. All of the data were analyzed using Origin 2018 (OriginLab, Northampton, MA) and SPSS 20.0 (IBM, Inc., Armonk, NY). Significant differences between treatments were evaluated using a two-factor analysis of variance (ANOVA) followed by a least significant difference (LSD) test at the 5% probability level.

■ RESULTS

Effect of FOF and Benzoic Acid Stress on the Occurrence of Faba Bean Fusarium Wilt and the Mitigation Effect of Intercropping. As shown in Figure 1A, the F50, F100, and F200 treatments significantly increased the incidence of faba bean Fusarium wilt compared with F in the single cropping mode. With an increase in the concentration of benzoic acid, the incidence of faba bean Fusarium wilt significantly increased and reached its highest value in the F200 treatment. Compared with monocropping, faba bean−wheat intercropping significantly reduced the incidence of faba bean fusarium wilt under both pathogen stress and the combination of benzoic acid and pathogen stress by 41.65, 23.14, 18.18, and 25.00%, respectively.

As shown in Figure 1B, the F50, F100, and F200 treatments significantly increased the disease index of faba bean Fusarium wilt compared with F in the monocrop mode. With an increase in benzoic acid concentration, the incidence of faba bean Fusarium wilt significantly increased and reached its highest value in the F200 treatment. Compared with monocropping, faba bean−wheat intercropping significantly reduced the disease index of faba bean Fusarium wilt under both pathogen stress and benzoic acid and pathogen stress by 32.11, 52.90, 24.93, and 21.56%, respectively.

Effects of FOF and Benzoic Acid Stress on the Contents of H_2O_2 and O_2^- in Faba Bean Roots and the Mitigation Effect of Intercropping. As shown in Figure 2A, F has no significant effect on the content of H_2O_2 in the faba bean roots compared with CK in the single cropping mode. Compared with CK and F, the F50, F100, and F200 treatments significantly increased the content of H_2O_2 in the faba bean roots, and with an increase in the concentration of benzoic acid, the content of H_2O_2 in faba bean roots increased significantly, reaching its highest value in the F200 treatment.

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ACS Omega 2023, 8, 2897−2906

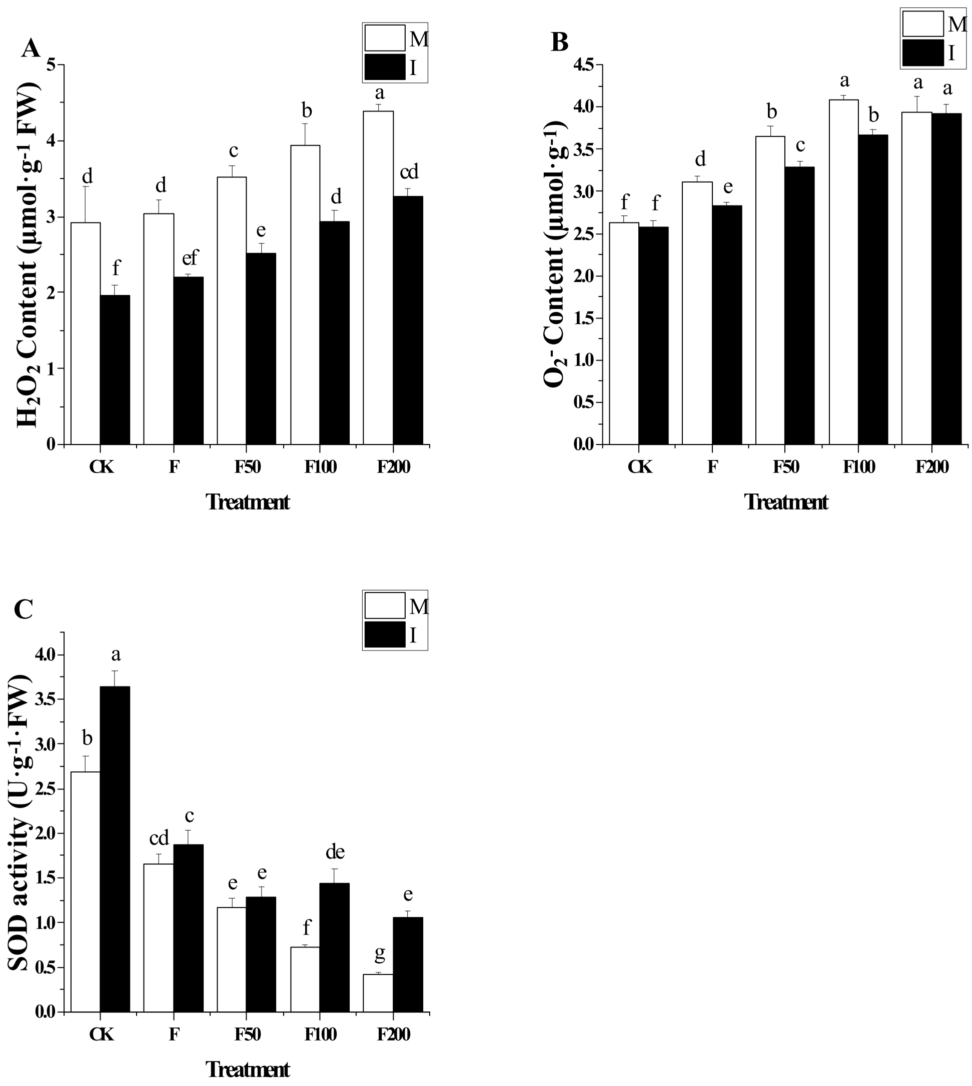

Figure 2. Effects of different concentrations of benzoic acid (0, 50, 100, 200 mg·L^{-1}) on (A) H_2O_2 and (B) O_2^- contents. (C) SOD activity in faba bean (*Vicia faba*) roots inoculated with FOF under monocropping and intercropping modes. F, *F oxysporum* f. sp. *Fabae*; M, monocropping; I, intercropping; H_2O_2, hydrogen peroxide; SOD, superoxide dismutase. Data represent the mean ± standard error of three biological replicates. Different letters for each index indicate significant differences at $p < 0.05$.

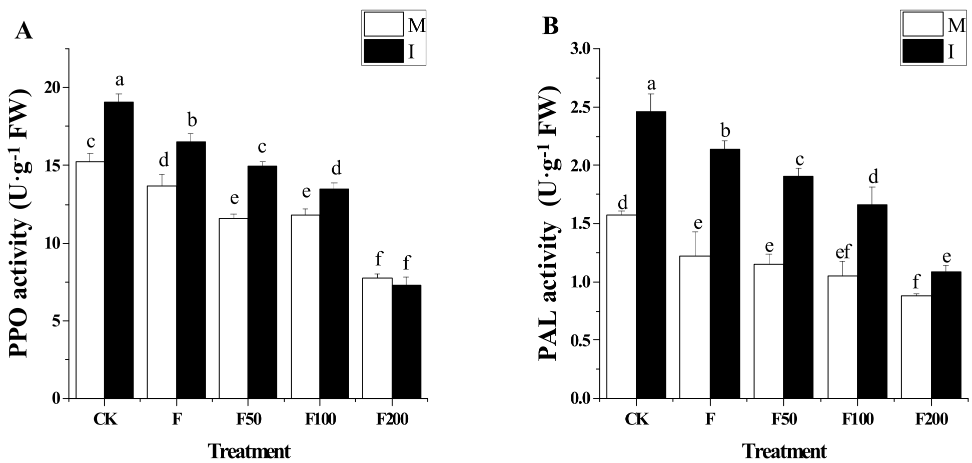

Figure 3. Effects of different concentrations of benzoic acid (0, 50, 100, 200 mg·L^{-1}) on (A) PPO activity and (B) PAL activity in faba bean (*Vicia faba*) roots inoculated with FOF under monocropping and intercropping modes. F, *F. oxysporum* f. sp. *Fabae*; M, monocropping; I, intercropping; PAL, phenylalanine ammonia lyase; PPO, polyphenol oxidase. Data represent the mean ± standard error of three biological replicates. Different letters for each index indicate significant differences at $p < 0.05$.

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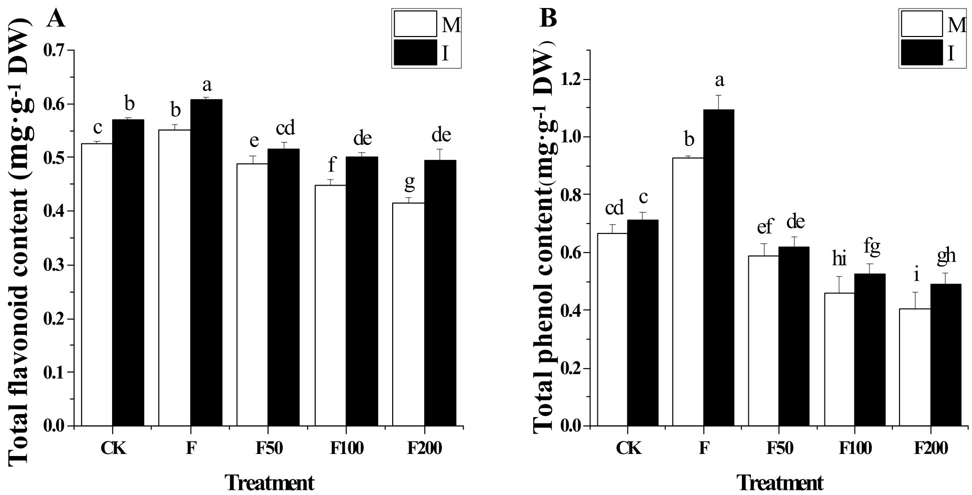

Figure 4. Effects of different concentrations of benzoic acid (0, 50, 100, 200 mg·L^{-1}) on (A) total flavonoids and (B) total phenolics in faba bean (*Vicia faba*) roots inoculated with FOF under monocropping and intercropping modes. F, *F. oxysporum* f. sp. *Fabae*; M, monocropping; I, intercropping. Data represent the mean ± standard error of three biological replicates. Different letters for each index indicate significant differences at $p < 0.05$.

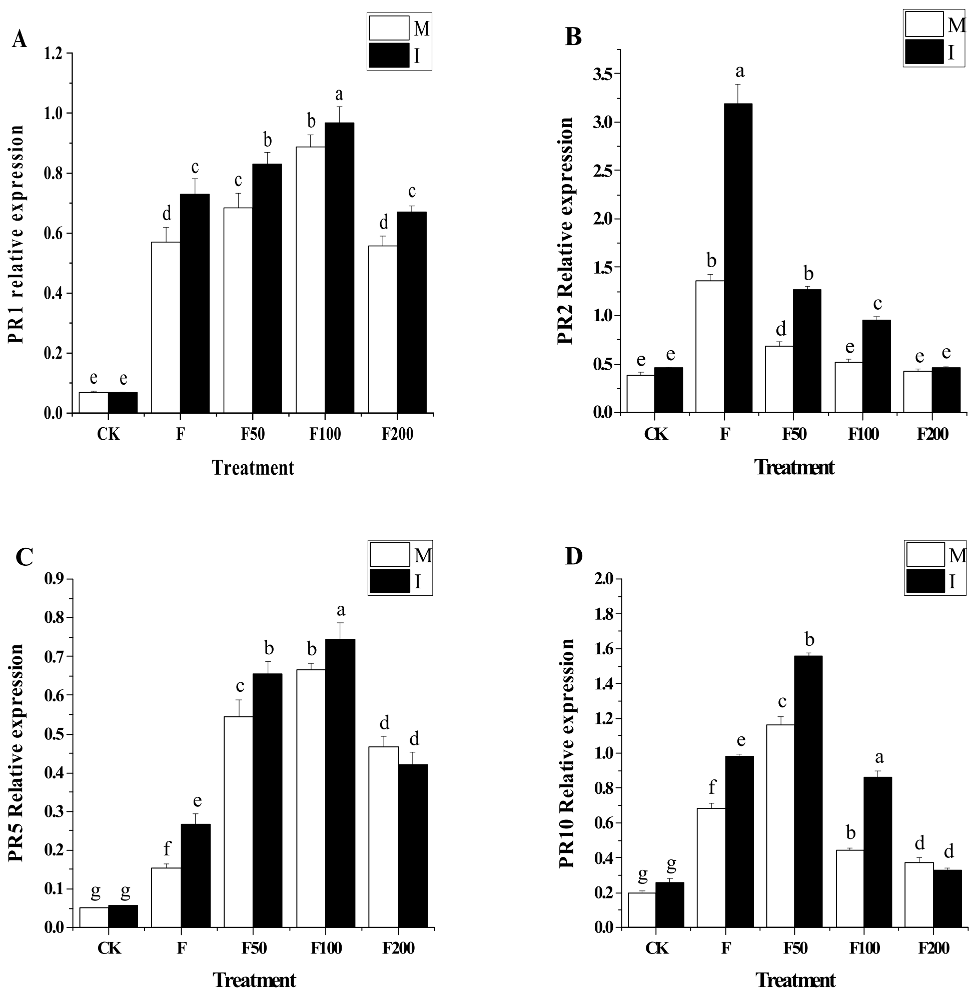

Figure 5. Effects of different (0, 50, 100, 200 mg·L^{-1}) concentrations of benzoic acid on gene expression of (A) PR1, (B) PR2, (C) PR5, and (D) PR10 in faba bean (*Vicia faba*) roots inoculated with FOF under monocropping and intercropping modes. F, *F. oxysporum* f. sp. *Fabae*; M, monocropping; I, intercropping. Data represent the mean ± standard error of three biological replicates. Different letters for each index indicate significant differences at $p < 0.05$.

Compared with monocropping, faba bean−wheat intercropping significantly reduced the content of H_2O_2 in faba bean roots in all treatments by 32.47, 27.30, 28.44, 25.41, and 25.49%, respectively.

https://doi.org/10.1021/acsomega.2c04569
ACS Omega 2023, 8, 2897−2906

As shown in Figure 2B, compared with CK in the single cropping mode, F significantly increased the content of O_2^- in faba bean roots. Compared with CK and F, the F50, F100, and F200 treatments significantly increased the content of H_2O_2 in faba bean roots, and with an increase in the concentration of benzoic acid, the content of O_2^- in faba bean roots gradually increased, but the difference between the F100 and F200 treatments was not significant. Compared with monocropping, faba bean−wheat intercropping significantly reduced the content of O_2^- in faba bean roots in the F, F50, and F100 treatments.

As shown in Figure 2C, compared with CK in the single cropping mode, F significantly reduced the SOD activity in faba bean roots. Compared with CK and F, the F50, F100, and F200 treatments significantly reduced the activity of SOD in the faba bean roots, and the activity of SOD gradually decreased with an increase in the concentration of benzoic acid. Compared with monocropping, faba bean−wheat intercropping significantly increased the activity of SOD in faba bean roots in the CK, F100, and F200 treatments by 35.53, 99.63, and 153.75%, respectively.

Effects of FOF and Benzoic Acid Stress on Defensive Enzyme Activities in Faba Bean Roots and the Mitigation Effect of Intercropping. As shown in Figure 3A, compared with CK in the single cropping mode, F significantly reduced the PPO activity in faba bean roots. Compared with CK and F, the F50, F100, and F200 treatments significantly reduced the activity of PPO in faba bean roots, and the inhibitory effect gradually increased as the concentration of benzoic acid increased. Compared with monocropping, faba bean−wheat intercropping in the CK, F, F50, and F100 treatments significantly increased the activity of PPO in the faba bean root system, but there was no significant difference between monocropping and intercropping in the F200 treatment.

As shown in Figure 3B, compared with CK in the single cropping mode, F significantly reduced the activity of PAL in the faba bean roots. Compared with CK and F, the F200 treatment significantly reduced the activity of PAL in faba bean roots, and the inhibitory effect gradually increased as the concentration of benzoic acid increased. Compared with monocropping, faba bean−wheat intercropping significantly increased the activity of PAL in in all of the treatments in faba bean roots by 56.62, 74.53, 65, 58.24, and 23.68%, respectively.

Effects of FOF and Benzoic Acid Stress on the Contents of Total Phenolics and Total Flavonoids in Faba Bean Roots and the Mitigation Effect of Intercropping. As shown in Figure 4A, compared with CK, F significantly increased the contents of total flavonoid in faba bean roots in the single cropping mode. Compared with CK and F, the F50, F100, and F200 treatments significantly reduced the content of total flavonoids in the faba bean roots, and the content of total flavonoids gradually decreased with an increase in the concentration of benzoic acid and reached its lowest value in the F200 treatment. Compared with monocropping, faba bean−wheat intercropping significantly increased the content of total flavonoids in the faba bean roots in all treatments.

As shown in Figure 4B, compared with CK in the single cropping mode, F significantly increased the total phenolic contents in faba bean roots. Compared with CK and F, the F50, F100, and F200 treatments significantly reduced the content of total phenols in the faba bean roots, and the content of total phenolics in faba bean roots gradually decreased with an increase in the concentration of benzoic acid. Compared with monocropping, faba bean−wheat intercropping significantly increased the content of total phenolics in faba bean roots in the F, F100, and F200 treatments.

Effects of FOF and Benzoic Acid Stress on the Expression of PR Defense Genes in Faba Bean Roots and the Mitigation Effect of Intercropping. As shown in Figure 5A, compared with CK in the single cropping mode, F significantly increased the expression of the PR1 gene in faba bean roots. Compared with F, the F50 and F100 treatments significantly increased the expression of the PR1 gene in faba bean root system, and F200 had no significant difference compared with F. Compared with monocropping, faba bean−wheat intercropping significantly increased the expression of the PR1 gene in faba bean roots in F, F50, F100, and F200 treatments.

As shown in Figure 5B, compared with CK, F significantly increased the expression of the PR2 gene in faba bean roots in the single cropping mode. Compared with F, the F50, F100, and F200 treatments significantly reduced the expression of the PR2 gene in faba bean roots, and the expression of the PR2 gene gradually decreased with an increase in benzoic acid concentration. However, there was no significant difference between the F100 and F200 treatments. Compared with monocropping, the F, F50, and F100 treatments of faba bean−wheat intercropping significantly increased the expression of the PR2 gene in faba bean roots by 134.11, 85.19, and 83.51%, respectively.

As shown in Figure 5C, F significantly increased the expression of the PR5 gene in the faba bean root system compared with CK in the single cropping mode. Compared with F, the F50, F100, and F200 treatments significantly increased the expression of the PR5 gene in faba bean roots. However, the relative expression of the PR5 gene showed an obvious downward trend in the F200 treatment. Compared with monocropping, faba bean−wheat intercropping significantly increased the expression of the PR5 gene in faba bean roots in the F, F50, and F100 treatments.

As shown in Figure 5D, F significantly increased the expression of the PR10 gene in the faba bean root system compared with CK in the single cropping mode. Compared with F, the F50 treatment significantly increased the expression of the PR10 gene in the faba bean root system, but the F100 and F200 treatments significantly reduced the expression of the PR10 gene in the faba bean root system. Compared with monocropping, the F, F50, and F100 treatments of faba bean−wheat intercropping significantly increased the expression of the PR10 gene in the faba bean roots by 43.23, 34.08, and 95.74%, respectively.

■ DISCUSSION

Autotoxicity refers to the process by which plants or their residues release toxic chemicals into the environment during decomposition, thereby inhibiting the germination and growth of the same plant and serving as a common cause of plant continuous cropping obstacles.[26] Benzoic acid is one of the most important autotoxic substances in crops and can promote the occurrence of faba bean Fusarium wilt.[3] In this study, we found that the double stress of pathogen and benzoic acid (F50, F100, F200) significantly increased the incidence rate and disease index of faba bean Fusarium wilt and reached the maximum incidence rate and disease index when the

https://doi.org/10.1021/acsomega.2c04569
ACS Omega 2023, 8, 2897−2906

concentration of benzoic acid reached 200 mg·L^{-1}, which was the same as the research on cinnamic acid on the occurrence of faba bean Fusarium wilt.[3] The results showed that benzoic acid was highly effective at promoting the occurrence of faba bean Fusarium wilt. Species diversity planting is a green planting mode, which has proven its strong ability to control disease and increase yields.[16] Diversity in agroecosystems leads to a reduction in the incidence of soilborne diseases of crops. For example, the rotation of legumes and nonlegumes can effectively control the occurrence of legumes fusarium wilt.[16] Similar findings have been found in intercropping systems. For example, the intercropping of *Atractylodes macrocephala* and peanut (*Arachis hypogaea*) effectively controlled the occurrence of peanut Fusarium wilt.[27] This is the same as the results obtained in this study. Compared with faba bean monoculture, faba bean and wheat intercropping significantly reduced the incidence rate and disease index of faba bean Fusarium wilt under FOF alone or FOF combined with benzoic acid stress. This shows that compared with monoculture, faba bean−wheat intercropping can effectively alleviate the stress of FOF and benzoic acid on faba bean, which is reflected in improved physiological and biochemical resistance and defense gene expression.

Stress-resistant plants usually have a rapid increase in intracellular reactive oxygen species (ROS) after stress.[28] These ROS are strong oxidants and can destroy the structure of cell membranes, reduce the resistance of crops, and increase the risk of disease.[29] Compared with F, F50, F100, and F200 significantly increased the contents of the H_2O_2 and O_2^- ROS in faba bean roots. With an increase in the concentration of benzoic acid, the contents of H_2O_2 and O_2^- in faba bean roots increased significantly, reaching their highest value in the F200 treatment, which is consistent with the results of Yang et al.[10] Yang et al. showed that ginsenosides secreted by *P. notoginseng* could cause oxidative damage to itself by increasing the contents of H_2O_2 and O_2^- in its root system, thus promoting the occurrence of continuous cropping obstacles of *P. notoginseng*.[10] In this study, on the basis of F treatment, the double stress of benzoic acid and FOF further increased the degree of peroxidation of faba bean root cells and caused peroxidative damage to the faba bean root cells. Under all of the stress treatments, compared with monoculture, faba bean and wheat intercropping significantly reduced the contents of H_2O_2 and O_2^- in the root cells of faba bean. Qi et al. found that compared with melon (*Cucumis* sp.) monoculture, cotton (*Gossypium hirsutum*) and melon intercropping effectively reduced the content of O_2^- in melon cells, which is consistent with our results.[20] Both studies showed that compared with monoculture, intercropping could effectively remove the peroxidative damage to cells and enable them to reach a healthy state. To maintain normal physiological metabolism, crops will enhance the protective enzyme system in their tissues, maintain the dynamic balance of production and clearance of ROS in crop cells, and alleviate the peroxidative damage of crop cells.[30] SOD is an enzyme synthesized by plants that effectively scavenges free oxygen and can oxidize H_2O_2 and O_2^- to H_2O and O_2, respectively. Therefore, the activity and synthesis of SOD can reflect the antioxidant capacity of crops. The results showed that compared with CK and F, F50, F100, and F200 significantly decreased the activity of SOD in the faba bean root system. Wang et al. showed that the exogenous addition of autotoxic substances secreted by strawberry (*Fragaria* x *ananassa*) roots significantly reduced the activities of SOD, POD, and CAT in strawberry roots, which was similar to our results.[7] These results show that compared with F, benzoic acid can further inhibit the activity of SOD in the faba bean root system and aggravate the damage to the faba bean root system. Therefore, the synergistic effect of benzoic acid and FOF is an important reason for the high incidence of faba bean Fusarium wilt. Under the treatments of F100 and F200, compared with monoculture, faba bean−wheat intercropping significantly increased the activity of SOD in faba bean roots and the antioxidant capacity of faba bean roots. This result shows that when the concentration of benzoic acid is below 100 mg·L^{-1}, the defense system of faba bean can resist the damage of benzoic acid. When the concentration is higher than 100 mg·L^{-1}, faba bean cannot resist this damage. Ren et al. showed that the intercropping of rice (*Oryza sativa*) and watermelon (*Citrullus lanatus*) can significantly improve the antioxidant enzyme activity of watermelon, improve the resistance of watermelon, and effectively alleviate the occurrence of watermelon Fusarium wilt, which is consistent with our results.[31] These results showed that the faba bean−wheat intercropping may improve the antioxidant capacity of faba bean roots by increasing the SOD activity of faba bean roots, which may be the mechanism of intercropping to control the occurrence of faba bean Fusarium wilt.

After plants are stressed, they will improve their resistance through a series of reactions to resist the damage of stress. The phenylpropanoid metabolic pathway is the main disease-resistant reaction in plants. L-phenylalanine catalyzes the reaction in plants through phenylalanine ammonia lyase (PAL) and polyphenol oxidase (PPO) to produce flavonoids and phenolics.[32] Many studies have shown that the production of phenolics and flavonoids is one of the important signs of resistant responses to plant disease and important secondary metabolites in plants and changes in their content are closely related to plant disease resistance.[33] We found that F50, F100, and F200 significantly inhibited the contents of phenolics and flavonoids in faba bean root cells compared with the CK and F, and this inhibition reached its maximum at a concentration of 200 mg·L^{-1}. We also found that F50, F100, and F200 significantly reduced the activity of PPO in the faba bean root system compared with CK and F; F50, F100, and F200 significantly reduced the activity of PAL in the faba bean root system compared with CK; and only F200 significantly inhibited the activity of PAL compared with F. This is consistent with the results of Wang et al., who showed that the exogenous addition of autotoxic substances in the strawberry (*Fragaria* x *ananassa*) planting system reduces the activity of strawberry defense enzymes.[7] These results show that benzoic acid may reduce the production of flavonoids and phenolic disease resistance compounds by inhibiting the activities of two key enzymes (PPO and PAL) in the phenylpropanoid metabolism of faba bean to reduce the disease resistance of faba bean roots and promote the occurrence of faba bean Fusarium wilt. In this study, we also found that, compared with monoculture faba bean, faba bean−wheat intercropping significantly increased the contents of phenolics and flavonoids in the faba bean root system in all stress treatments. This result was similarly confirmed in the wheat−watermelon intercropping system. Research by Xu et al. showed that wheat−watermelon intercropping significantly increased the contents of total phenolics and flavonoids in the watermelon root system and effectively controlled the occurrence of watermelon

https://doi.org/10.1021/acsomega.2c04569
ACS Omega 2023, 8, 2897−2906

Fusarium wilt.[34] In all of the treatments, compared with faba bean monoculture, faba bean−wheat intercropping significantly increased the activity of PAL in faba bean roots. PPO also has the same trend among monoculture treatments, but the difference is that when the concentration of benzoic acid reaches 200 mg·L^{-1}, compared with monoculture, faba bean−wheat intercropping has no significant effect on the activity of PPO, which could be because the high concentration of benzoic acid has substantially damaged the biosynthetic pathway of PPO and intercropping has been unable to alleviate this damage. This is similar to the results found by Wang et al. in the eggplant (*Solanum melongena*)/garlic (*Allium sativum*) intercropping model.[35] Eggplant and garlic intercropping significantly increased the activities of PAL and other antioxidant enzymes in eggplant roots, thereby improving the disease resistance of eggplant.[35] These results showed that faba bean−wheat intercropping could improve the activities of PPO and PAL in faba bean roots, promote the metabolism of phenylpropanoids, and then increase the contents of phenolics and flavonoids in faba bean roots to improve the disease resistance of faba bean. Therefore, faba bean−wheat intercropping may help to control the occurrence of Fusarium wilt by promoting phenylpropanoid metabolism in faba bean roots.

Disease resistance and pathogenicity between plants and pathogens are very complex interactive processes. Pathogen infection, abiotic stress and related signal molecules, immune reactions, and systemic acquired resistance (SAR) can stimulate the accumulation of pathogenesis-related proteins (PRs). The production and accumulation of such proteins play an important role in the resistance of plants to biological and abiotic stresses.[36] Research by Zhang et al. showed that increasing the expression of PRs in *P. notoginseng* plants can effectively improve their resistance to *F. solani* and thus control the occurrence of root rot in *P. notoginseng*.[37] PR1 has been proven to help resist fungal invasion and the spread of bacteria and alleviate stress. The β-PR2 protein with 1,3-glucanase activity can degrade fungal cell walls. The specific permeability of the plasma membrane to PR5 contributes to the degradation and destruction of fungal and bacterial pathogens and inhibits their growth and development. PR10 participates in the responses of plant defense and has antibacterial activity and *in vitro* ribonuclease activity. PR1, PR2, PR5, and PR10 can all act effectively to inhibit the growth and activities of pathogenic bacteria, thus helping to protect plants against infection by these pathogens. Therefore, the expression of PR genes can indicate the disease resistance of crops.[38−40] We found that the relative expression of PR1, 2, 5, and 10 genes responded similarly to stress. Compared with CK, F, F50, F100, and F200 all first increased and then decreased. The difference is that PR1 and PR5 began to decrease rapidly when the concentration of benzoic acid reached 200 mg·L^{-1}, while PR2 and PR10 decreased at 50 and 100 mg·L^{-1}, respectively. This could be because the synthetic genes of proteins related to different disease courses respond differently to benzoic acid. Based on these results, we believe that benzoic acid could reduce the disease resistance of faba bean roots by inhibiting the synthesis of PRs in faba bean roots, thus promoting the occurrence of faba bean Fusarium wilt. In this study, we found that, compared with monoculture faba bean, faba bean−wheat intercropping significantly increased the relative expression of PR1, PR2, PR5, and PR10 genes in faba bean roots in all of the stress treatments, which could be because some allelochemicals secreted by wheat in the faba bean−wheat intercropping system can be used as signal factors to stimulate the expression of faba bean PR genes.[11] Gao et al. showed that soybean (*Glycine max*)/maize (*Zea mays*) intercropping can improve the resistance of soybean to crown rot by increasing the expression of soybean PR genes and effectively control the occurrence of soybean crown rot, which is consistent with our results.[22] These results indicated that wheat and faba bean intercropping could improve the disease resistance of faba bean by promoting the synthesis of PRs in the faba bean root system and then help faba bean control the occurrence of Fusarium wilt.

In conclusion, the results of this study show that benzoic acid can promote the occurrence of faba bean Fusarium wilt by aggravating the oxidative stress of faba bean roots, destroying the phenylpropanoid metabolism of faba bean roots, reducing the production of resistant substances, and inhibiting the synthesis of PRs. Faba bean−wheat intercropping can alleviate the oxidative stress of faba bean roots, promote the phenylpropanoid metabolism of faba bean roots, improve the synthesis of resistant compounds and PRs, and then control the occurrence of faba bean Fusarium wilt.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.2c04569.

RNA extraction, cDNA preparation, preparation of genomic DNA removal mixture, reverse transcription reaction system, reverse transcription reaction PCR program, qPCR reaction conditions, and defense gene primer sequence (PDF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by the Natural Science Foundation of China (31860596). The authors thank all reviewers who participated in the review and MJEditor (www.mjeditor.com) for its linguistic assistance during the preparation of this manuscript.

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https://doi.org/10.1021/acsomega.2c04569
ACS Omega 2023, 8, 2897−2906