Postprint: Mitigation Mechanism of Intercropping on Faba Bean Autotoxicity Under Benzoic Acid Stress

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Date: 2017-11-07T00:00:00+00:00

Abstract

Benzoic acid is one of the primary autotoxic substances responsible for continuous cropping obstacles in faba bean. This study employed a hydroponic experiment to investigate the effects of different benzoic acid concentrations [C0(0 mg L^{-1}), C1(50 mg L^{-1}), C2(100 mg L^{-1}), and C3(200 mg L^{-1})] on the growth of faba bean seedlings intercropped with wheat and the incidence of Fusarium wilt, and to explore the mechanism by which wheat-faba bean intercropping alleviates benzoic acid autotoxicity from a physiological resistance perspective, thereby providing a scientific basis for the rational utilization of intercropping to mitigate continuous cropping obstacles and achieve sustainable agricultural development. The results demonstrated that, compared with the C0 treatment, all benzoic acid treatments significantly inhibited faba bean seedling growth, with the inhibitory effect intensifying as treatment concentration increased; simultaneously, they significantly increased both the disease incidence and disease index of faba bean Fusarium wilt. The MDA content in faba bean roots and leaves was significantly elevated, whereas the activities of antioxidant enzymes (POD and CAT) and pathogenesis-related proteins (-1,3-glucanase and chitinase) decreased with increasing benzoic acid concentration. These findings indicate that benzoic acid treatments significantly suppressed faba bean growth, reduced physiological resistance, and promoted Fusarium wilt development. Compared with monocropped faba bean, wheat-faba bean intercropping significantly increased aboveground dry weight under benzoic acid stress (by 17.0%~47.1%), reduced disease incidence (by 11.1%~25.0%) and disease index (by 20.0%~42.1%); POD activity in roots and leaves increased by 12.9%~16.9% and 9.3%~24.9%, respectively, CAT activity increased by 10.3%~54.0% and 6.6%~20.5%, respectively, -1,3-glucanase and chitinase activities in roots increased by 4.7%~13.1% and 6.7%~15.8%, respectively, while MDA content decreased by 19.5%~25.4% and 20.5%~29.9%, respectively. Intercropping under the C2 treatment exhibited the optimal effect in enhancing antioxidant enzyme and pathogenesis-related protein activities, with the best disease resistance efficacy. These results demonstrate that wheat-faba bean intercropping effectively mitigates benzoic acid-induced Fusarium wilt damage and promotes faba bean growth by enhancing physiological resistance, representing an effective strategy for alleviating benzoic acid autotoxicity.

Full Text

Alleviation Mechanism of Intercropping with Wheat for Faba Bean Autotoxicity Under Benzoic Acid Stress

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Abstract: Benzoic acid is one of the main autotoxic substances causing continuous cropping obstacles in faba bean. Using a hydroponic experiment, this study investigated the effects of different benzoic acid concentrations [C0 (0 mg · L 1), C1 (50 mg · L 1), C2 (100 mg · L 1), and C3 (200 mg · L 1)] on the growth and Fusarium wilt development of faba bean seedlings intercropped with wheat. The objective was to explore the mechanism by which wheat-faba bean intercropping alleviates benzoic acid autotoxicity from the perspective of physiological resistance, providing a scientific basis for rationally utilizing intercropping to mitigate continuous cropping obstacles and achieve sustainable agricultural development. The results showed that, compared with the C0 treatment, all benzoic acid treatments significantly inhibited faba bean seedling growth, with the inhibitory effect intensifying as concentration increased. Simultaneously, benzoic acid significantly increased the incidence and disease index of Fusarium wilt, while markedly elevating malondialdehyde (MDA) content in both roots and leaves. In contrast, the activities of antioxidant enzymes (peroxidase [POD] and catalase [CAT]) and pathogenesis-related proteins (-1,3-glucanase and chitinase) decreased with increasing benzoic acid concentration. These findings indicate that benzoic acid significantly suppressed faba bean growth, reduced physiological resistance, and promoted Fusarium wilt development.

Compared with monocropped faba bean, wheat-faba bean intercropping significantly increased shoot dry weight by 17.0%–47.1% under benzoic acid stress, while reducing disease incidence by 11.1%–25.0% and disease index by 20.0%–42.1%. Intercropping enhanced POD activity by 12.9%–16.9% in roots and 9.3%–24.9% in leaves, and increased CAT activity by 10.3%–54.0% in roots and 6.6%–20.5% in leaves. Additionally, intercropping increased -1,3-glucanase and chitinase activities in faba bean roots by 4.7%–13.1% and 6.7%–15.8%, respectively, while decreasing MDA content by 19.5%–25.4% in roots and 20.5%–29.9% in leaves. The C2 treatment (100 mg · L ^{1}) showed the greatest effectiveness in enhancing antioxidant enzyme and pathogenesis-related protein activities, yielding the best disease control. These results demonstrate that wheat-faba bean intercropping alleviates benzoic acid-induced Fusarium wilt damage and promotes faba bean growth by enhancing physiological resistance, representing an effective measure to mitigate benzoic acid autotoxicity.

Keywords: Benzoic acid; Faba bean; Continuous cropping obstacle; Intercropping; Antioxidative enzyme; Pathogenesis-related protein; Fusarium wilt

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Introduction

Continuous cultivation of the same crop or crop family on the same land is widespread in China due to constraints including land scarcity, established farming practices, environmental conditions, and economic incentives [1]. Long-term monoculture leads to growth and developmental inhibition, severe soil-borne diseases, and substantial yield reductions—phenomena collectively known as continuous cropping obstacles [1]. For decades, research has shown that soil nutrient depletion, reduced soil enzyme activity, deterioration of rhizosphere microbial communities, and high incidence of soil-borne diseases all contribute to these obstacles. Recent studies have demonstrated that accumulation of phenolic acids in continuously cropped soils represents a crucial factor underlying continuous cropping obstacles in many crops [2-3]. Researchers have identified numerous phenolic compounds—including cinnamic acid, p-hydroxybenzoic acid, benzoic acid, vanillic acid, syringic acid, coumaric acid, and ferulic acid—in root exudates and soils under continuous cropping of various crops [3-4]. These phenolic acids have been proven to act as autotoxins causing continuous cropping obstacles in crops such as Chinese fir (*Cunninghamia lanceolata*), cucumber (*Cucumis sativus*), eggplant (*Solanum melongena*), and tomato (*Solanum lycopersicum*). Among these, benzoic acid is the predominant phenolic acid in continuously cropped soils and root exudates of watermelon (*Citrullus lanatus*), taro (*Colocasia esculenta*), and ginseng (*Panax ginseng*) [1,5]. In watermelon and ginseng, benzoic acid has been shown to significantly promote the occurrence of cucumber Fusarium wilt and ginseng rust disease [6-7]. Our previous field observations revealed poor faba bean (*Vicia faba*) emergence, weak growth, and severe Fusarium wilt under continuous cropping conditions. Using high-performance liquid chromatography, we detected seven phenolic acids (p-hydroxybenzoic acid, vanillic acid, syringic acid, ferulic acid, salicylic acid, cinnamic acid, and benzoic acid) in continuously cropped faba bean soils, with benzoic acid present at relatively high concentrations [8]. However, whether benzoic acid acts as an autotoxin causing faba bean continuous cropping obstacles remains unclear.

Intercropping systems that leverage allelopathic interactions represent a plantbased alternative to chemical fungicides, offering an effective strategy to eliminate disadvantages while preserving benefits for plant protection and relieving continuous cropping obstacles [5]. Yunnan Province possesses rich biological resources, and wheat (*Triticum aestivum*)-faba bean intercropping has long been a common planting pattern in Yunnan and southwestern China, playing an important role in agricultural production with extensive cultivation areas and significant yield, income, and disease control benefits [9]. Continuous faba bean cropping leads to severe soil-borne Fusarium wilt and substantial yield losses, representing a major constraint on faba bean production in China, particularly in Yunnan where damage is severe. Our previous research demonstrated that wheat-faba bean intercropping increased the quantity and diversity of faba bean rhizosphere microorganisms, thereby suppressing Fusarium wilt development [10-11]. However, to date, no studies have reported on the alleviating effects and mechanisms of wheat-faba bean intercropping on faba bean autotoxicity under autotoxin stress. This experiment investigated the effects of exogenous benzoic acid application combined with *Fusarium oxysporum* inoculation on monocropped faba bean growth, Fusarium wilt development, and faba bean antioxidant enzyme and pathogenesis-related protein activities to clarify benzoic acid' s role in faba bean continuous cropping obstacles. By comparing differences in growth, disease occurrence, and activities of pathogenesis-related proteins and antioxidant enzymes between monocropped and intercropped faba bean, we aimed to elucidate the alleviating effects of intercropping on benzoic acid autotoxicity and the underlying physiological mechanisms.

Materials and Methods

1.1 Experimental Materials The experiment was conducted in a greenhouse at the College of Resources and Environment, Yunnan Agricultural University, from October to December 2015. The wheat variety 'Yunmai 53' and faba bean variety '89-147' were purchased from the Yunnan Academy of Agricultural Sciences. Analytical-grade benzoic acid was obtained from Sinopharm Group Shanghai Co., Ltd. The pathogen *Fusarium oxysporum* f. *fabae* (FOF) was isolated from continuously cropped faba bean soil and maintained in our laboratory. The fungus was cultured on potato dextrose agar (PDA) plates at 28°C for 7 days. Mycelia were then scraped into sterile water, shaken to uniformity, filtered through two layers of gauze, and diluted to produce a spore suspension for inoculation.

1.2 Experimental Design The experiment employed a two-factor design. Factor A consisted of four benzoic acid concentrations: C0 (0 mg · L ^{1}, control), C1 (50 mg · L ^{1}), C2 (100 mg · L ^{1}), and C3 (200 mg · L ^{1}). Factor B comprised two planting patterns: faba bean monocropping (MF) and wheat-faba bean intercropping (IF). The eight treatment combinations were replicated three times, totaling 24 experimental units. Monocropped pots contained three faba bean plants, while intercropped pots contained three faba bean plants and nine wheat plants [Figure 1: see original paper]. An air pump provided continuous aeration.

1.3 Experimental Implementation Wheat and faba bean seeds were soaked at room temperature for 24 hours, germinated at 25°C, and simultaneously sown in sterile quartz sand saturated with Hoagland nutrient solution. When faba bean seedlings developed 4-6 true leaves, uniform seedlings were transplanted into 2 L containers filled with nutrient solution at different benzoic acid concentrations. For intercropping treatments, wheat seedlings (three-leaf stage) were transplanted simultaneously. Two days after benzoic acid treatment, *Fusarium oxysporum* spore suspension (1×10 $cfu \cdot mL^{1}$) was added to the nutrient solution.

1.4 Faba Bean Fusarium Wilt Investigation Fusarium wilt was surveyed 45 days after faba bean transplanting, with three faba bean plants examined per replicate in both monocropping and intercropping treatments. Disease severity was assessed using a five-grade classification system [11], after which incidence, disease index, and control efficacy were calculated using the following formulas:

Incidence (%) = (Number of diseased plants / Total number of plants surveyed) × 100 (1)
Disease index = Σ(Number of plants at each grade × Grade value) / (Highest grade value × Total number of plants surveyed) × 100 (2)
Relative control effect (%) = (Control disease index -Treatment disease index) / Control disease index × 100 (3)

1.5 Measurement Indicators 1.5.1 Growth Parameter Determination

At 45 days after transplanting (faba bean branching stage), three faba bean plants were sampled from each replicate to measure leaf number per plant, maximum leaf length, maximum leaf width, plant height, main root length, shoot dry weight, root dry weight, and root-shoot ratio.

1.5.2 Antioxidant Enzyme Activity and Membrane Lipid Peroxidation Determination

At 45 days after transplanting (branching stage), three faba bean plants were sampled per replicate. Fresh leaf and root samples were used to determine peroxidase (POD) and catalase (CAT) activities and malondialdehyde (MDA) content using the methods of Li Hesheng [12].

1.5.3 Pathogenesis-Related Protein Determination

At 45 days after transplanting (branching stage), three faba bean plants were sampled per replicate. Fresh root samples were used to determine pathogenesis-related protein activities. Chitinase activity was measured using a chitinase assay kit (Nanjing Jiancheng Bioengineering Institute), with one unit defined as the amount producing 1 mg of N-acetylglucosamine per gram of tissue per hour. -1,3-glucanase activity was determined using a -1,3-glucanase assay kit (Beijing Solarbio Science & Technology Co., Ltd.), with one unit defined as the amount producing 1 mg of reducing sugar per gram of tissue per hour.

1.6 Data Analysis Data were processed using Microsoft Excel 2007. Statistical analysis was performed using SPSS 20.0 software, with treatment differences tested for significance using the least significant difference (LSD) method at $P < 0.05$.

Results

2.1 Effects of Intercropping on Faba Bean Seedling Growth Under Benzoic Acid Stress The effects of benzoic acid treatments on faba bean seedling growth are presented in Table 1. Under monocropping conditions, all growth parameters—including leaf number, maximum leaf length, maximum leaf width, plant height, main root length, shoot dry weight, root dry weight, and root-shoot ratio—decreased significantly with increasing benzoic acid concentration. Compared with C0, the C1, C2, and C3 treatments reduced leaf number by 32.3%, 35.4%, and 41.5%; maximum leaf length by 15.6%, 32.4%, and 44.5%; maximum leaf width by 25.0%, 31.7%, and 50.3%; plant height by 18.0%, 34.2%, and 46.2%; shoot dry weight by 31.8%, 61.0%, and 76.2%; and root dry weight by 33.3%, 64.8%, and 83.3%, respectively. The C2 and C3 treatments also significantly reduced main root length by 8.18% and 28.4% compared with C0. These results demonstrate that benzoic acid treatment significantly inhibited faba bean growth.

Under all benzoic acid concentrations, wheat-faba bean intercropping promoted faba bean seedling growth. Without benzoic acid (C0), intercropping significantly increased leaf number, maximum leaf length, main root length, shoot dry weight, and root dry weight by 10.8%, 12.2%, 8.2%, 47.1%, and 59.3%, respectively. Under C1, intercropping significantly increased leaf number, shoot dry weight, and root dry weight by 10.6%, 40.8%, and 55.6%, respectively. Under C2, intercropping significantly increased maximum leaf length, maximum leaf width, shoot dry weight, and root dry weight by 6.1%, 7.0%, 31.0%, and 68.4%, respectively. Under C3, intercropping significantly increased maximum leaf length, shoot dry weight, and root dry weight by 3.6%, 17.0%, and 77.8%, respectively. These findings indicate that wheat-faba bean intercropping promoted faba bean seedling growth and alleviated the inhibitory effects of benzoic acid (Table 1).

2.2 Effects of Intercropping on Faba Bean Fusarium Wilt Under Benzoic Acid Stress As shown in Table 2 , under monocropping conditions, benzoic acid treatments C1, C2, and C3 significantly increased Fusarium wilt incidence by 33.3%, 50.0%, and 50.0%, and increased disease index by 25.0%, 137.5%, and 362.4%, respectively, compared with C0.

Under benzoic acid concentrations C1 and C2, intercropping significantly reduced Fusarium wilt incidence by 25.0% and 11.1% compared with monocropping, whereas no significant effect was observed under C0 and C3. Intercropping reduced disease index by 25.0%, 20.0%, 42.1%, and 21.6% under C0, C1, C2, and C3, respectively, with the greatest reduction occurring under C2. The relative control efficacy of intercropping on faba bean Fusarium wilt ranged from 20.0% to 42.1% across benzoic acid concentrations, with the highest efficacy (42.1%) achieved under C2.

2.3 Effects of Intercropping on POD and CAT Activities in Faba Bean Under Benzoic Acid Stress The effects of benzoic acid treatment on POD activity in faba bean roots and leaves are illustrated in Figure 2. Under monocropping conditions, POD activity in both roots and leaves initially increased then decreased with rising benzoic acid concentration. Compared with C0, the C1 treatment showed slightly higher POD activity in roots and leaves, though not significantly. The C2 and C3 treatments significantly reduced root POD activity by 15.7% and 31.4%, and leaf POD activity by 21.3% and 38.7%, respectively, indicating that POD activity decreased more substantially at higher benzoic acid concentrations.

Intercropping significantly increased root POD activity by 12.9%, 15.1%, 16.9%, and 14.6% under C0, C1, C2, and C3, respectively. Intercropping also enhanced leaf POD activity at all benzoic acid concentrations, with a significant increase of 24.9% observed under C2 (Figure 2). These results suggest that intercropping was most effective in increasing POD enzyme activity under the C2 benzoic acid concentration.

The effects of benzoic acid treatment on CAT activity are also shown in Figure 2. Under monocropping conditions, CAT activity in roots and leaves was lower under C1 than C0, though not significantly. The C2 and C3 treatments significantly reduced root CAT activity by 37.7% and 42.8%, and leaf CAT activity by 28.4% and 44.8%, respectively.

Intercropping increased CAT activity in both roots and leaves at all benzoic acid concentrations. Specifically, intercropping enhanced root CAT activity by 10.3%, 22.6%, 54.0%, and 21.7%, and leaf CAT activity by 6.6%, 9.2%, 11.5%, and 20.5% under C0, C1, C2, and C3, respectively (Figure 2).

2.4 Effects of Intercropping on MDA Content in Faba Bean Under Benzoic Acid Stress The effects of benzoic acid and intercropping on MDA content are presented in Figure 3 [Figure 3: see original paper]. Under monocropping conditions, MDA content in roots and leaves was slightly lower under C1 than C0, though not significantly. The C2 and C3 treatments significantly increased root MDA content by 28.9% and 42.6%, and leaf MDA content by 16.4% and 45.0%, respectively.

Intercropping significantly reduced MDA content in both roots and leaves at all benzoic acid concentrations. Specifically, intercropping decreased root MDA content by 25.4%, 22.6%, 20.0%, and 19.5%, and leaf MDA content by 20.5%, 29.9%, 24.5%, and 26.5% under C0, C1, C2, and C3, respectively.

2.5 Effects of Intercropping on Pathogenesis-Related Proteins in Faba Bean Seedlings Under Benzoic Acid Stress The effects of benzoic acid treatment on -1,3-glucanase and chitinase activities are shown in Figure 4 [Figure 4: see original paper]. Under monocropping conditions, -1,3-glucanase activity in faba bean roots was slightly higher under C1 than C0, though not significantly. The C2 and C3 treatments significantly reduced root -1,3-glucanase activity by 17.4% and 38.7%, respectively.

Intercropping increased root -1,3-glucanase activity by 4.7%, 7.9%, 13.1%, and 10.3% under C0, C1, C2, and C3, respectively, with the greatest enhancement observed under C2 (Figure 4).

Under monocropping conditions, chitinase activity in faba bean roots was slightly higher under C1 than C0, though not significantly. The C2 and C3 treatments significantly reduced chitinase activity by 23.6% and 39.4% ($P < 0.05$), demonstrating that chitinase activity decreased significantly with increasing benzoic acid concentration. Intercropping increased root chitinase activity by 6.7%, 7.8%, 15.8%, and 10.5% under C0, C1, C2, and C3, respectively, with the greatest enhancement occurring under C2.

Discussion

3.1 Effects of Intercropping on Faba Bean Growth and Fusarium Wilt Development Under Benzoic Acid Stress Allelopathic autotoxicity is widespread in agricultural production and contributes to continuous cropping obstacles in many crops, causing billions of dollars in annual losses worldwide. The autotoxicity responsible for continuous cropping obstacles in cucumber (*Cucumis sativus*), watermelon (*Citrullus lanatus*), and eggplant (*Solanum melongena*) is primarily caused by phenolic compounds in root exudates, including sinapic acid, syringic acid, vanillic acid (aldehyde), coumaric acid, gallic acid, p-hydroxybenzoic acid, phthalic acid, caffeic acid, ferulic acid, benzoic acid, salicylic acid, and cinnamic acid [13]. Autotoxins such as cinnamic acid and vanillin exhibit hormetic effects on eggplant root growth (root dry weight, fresh weight, and root activity), characterized by low-concentration stimulation and high-concentration inhibition [14]. Our results showed that under monocropping conditions, benzoic acid treatment inhibited faba bean seedling growth, with biomass reduction (shoot and root dry weight) being most pronounced. The inhibitory effect intensified with increasing benzoic acid concentration. Similar phenomena have been reported in cucumber continuous cropping studies, where cinnamic acid treatment followed by *Fusarium* inoculation significantly inhibited cucumber growth. This occurs because cinnamic acid exerts clear allelopathic inhibitory effects on cucumber roots, while *Fusarium* further compromises growth by destroying the vascular system [15].

Inoculation with *Fusarium oxysporum* under p-hydroxybenzoic acid stress exacerbated root tissue damage and promoted Fusarium wilt development in strawberry (*Fragaria ananassa*) [16]. Cinnamic acid, an important allelochemical in cucumber root exudates, can induce cucumber Fusarium wilt [17], while benzoic acid and cinnamic acid treatments significantly increase Fusarium wilt incidence in watermelon seedlings [18]. In our study, benzoic acid treatment significantly increased both incidence and disease index of Fusarium wilt in monocropped faba bean, indicating that benzoic acid aggravated disease development, with severity increasing as benzoic acid concentration rose. These findings align with reports that cinnamic acid, ferulic acid, and benzoic acid in melon root exudates significantly increase Fusarium wilt disease index in melon (*Cucumis melo*) [13].

As a cornerstone of traditional Chinese agriculture, intercropping offers advantages in yield improvement and nutrient resource utilization efficiency [19-20]. Employing intercropping to reduce soil-borne disease damage and alleviate continuous cropping obstacles has become a research focus in recent years [21]. Intercropping upland rice with watermelon significantly increased watermelon fresh weight and plant height, promoting watermelon growth [22]. In our study, wheat-faba bean intercropping enhanced leaf growth and increased faba bean shoot dry weight across all benzoic acid concentrations. Intercropping the medicinal plant *Atractylodes lancea* with peanut significantly reduced peanut soil-borne disease incidence and alleviated peanut continuous cropping obstacles [23], while upland rice-watermelon intercropping decreased watermelon Fusarium wilt disease index [5]. Our results showed that intercropping provided relative control efficacy of 20.0%–42.1% against faba bean Fusarium wilt across benzoic acid concentrations, with the best efficacy (42.1%) achieved under C2. These findings demonstrate that wheat-faba bean intercropping effectively alleviates benzoic acid-induced growth inhibition and mitigates the promotive effects of benzoic acid on Fusarium wilt development.

3.2 Physiological Mechanisms of Intercropping in Alleviating Benzoic Acid Autotoxicity Under normal conditions, reactive oxygen species production and scavenging maintain dynamic equilibrium in plants. CAT is a crucial enzyme system for scavenging reactive oxygen species and protecting plant cells, and its activity can serve as a physiological and biochemical indicator of disease resistance [22]. After pathogen infection, protective enzyme activity correlates positively with plant disease resistance [24-25]. Autotoxin stress reduces antioxidant enzyme activities (including POD and CAT) in crops such as eggplant and cucumber [14,26]. Under cinnamic acid treatment combined with *Fusarium* inoculation, grafted cucumber seedlings exhibited higher CAT activity than self-rooted seedlings, indicating that grafted cucumber roots possess a more robust antioxidant system [15]. Our results showed that under benzoic acid treatment combined with *Fusarium* inoculation, CAT activity in both roots and leaves of intercropped faba bean was higher than that of monocropped faba bean. This suggests that under benzoic acid stress, intercropped faba bean roots and leaves can rapidly enhance antioxidant system activity (CAT), effectively and timely scavenging free radicals, thereby improving faba bean resistance and mitigating benzoic acid' s promotive effects on Fusarium wilt. POD is a key enzyme in phenolic metabolism, participating in proline conversion and lignin synthesis in cell walls to control pathogen spread. In our study, intercropping significantly increased POD activity in faba bean roots and leaves under benzoic acid treatment and *Fusarium* inoculation, thereby inhibiting pathogen damage and protecting faba bean growth and metabolism. This indicates that wheat-faba bean intercropping enhances faba bean' s ability to resist *Fusarium* infection.

MDA content directly reflects the degree of membrane lipid peroxidation, with higher levels indicating greater cellular oxidative damage. Autotoxins accelerate membrane lipid peroxidation, causing nutrient leakage that stimulates pathogen growth and facilitates host invasion, ultimately resulting in high disease incidence and severe soil-borne diseases [27]. Treatment with cinnamic acid, a primary autotoxin in cucumber root exudates, significantly increased cucumber root MDA content, destroyed root cell membranes, and markedly increased ion leakage, thereby promoting Fusarium wilt development [15]. Our results showed that benzoic acid treatment combined with *Fusarium* inoculation significantly increased MDA content in faba bean seedling roots and leaves, while simultaneously increasing disease incidence and disease index. This indicates that under continuous faba bean cropping, benzoic acid promotes *Fusarium* infection and exacerbates Fusarium wilt by increasing membrane lipid peroxidation and nutrient leakage in faba bean seedlings. Intercropping significantly reduced MDA content in both roots and leaves across all benzoic acid concentrations, indicating that intercropping decreased MDA accumulation, reduced lipid peroxidation damage, lowered membrane permeability, and maintained cell membrane stability in faba bean seedlings under benzoic acid stress. This reduced nutrient supply to *Fusarium* and alleviated disease development. These findings are consistent with reports that MDA content in watermelon roots increased after inoculation with watermelon *Fusarium oxysporum* f. *sp. niveum* at 5, 10, and 15 days, but was significantly lower in watermelon roots grown with wheat than in monocropped watermelon [28].

Under benzoic acid stress, decreased POD and CAT activities in faba bean seedling roots and leaves, combined with increased MDA content and severe membrane damage, represent the primary reasons for reduced Fusarium wilt resistance. Intercropping enhanced antioxidant enzyme activity, reduced membrane lipid peroxidation, and significantly improved faba bean resistance to Fusarium wilt.

3.3 Effects of Intercropping on Pathogenesis-Related Proteins in Faba Bean Under Benzoic Acid Stress When host plants are infected by pathogens, defense responses are activated, pathogenesis-related proteins are expressed, and important hydrolases that degrade fungal cell walls are produced, thereby enhancing host resistance to pathogens [28]. Chitinase is a crucial hydrolase that directly degrades chitin—the main component of fungal cell walls—causing hyphal growth cessation, thickening and malformation, and even complete dissolution, thus resisting pathogen invasion. -1,3-glucanase is an important pathogenesis-related protein that plays a significant role in plant defense against fungal diseases [28]. Application of ferulic acid combined with *Fusarium* inoculation inhibited chitinase activity in watermelon roots and leaves and decreased -1,3-glucanase activity in watermelon leaves, thereby promoting Fusarium wilt development [29]. In our study, under *Fusarium* inoculation, the C1 benzoic acid treatment increased -1,3-glucanase and chitinase activities in faba bean roots, indicating activation of the root defense system. However, when benzoic acid concentration exceeded C1, both -1,3-glucanase and chitinase activities were significantly lower than the control, indicating gradual decline in the plant' s protective system function, inability to resist pathogen invasion, and consequently aggravated Fusarium wilt. This demonstrates that combined stress from benzoic acid and *Fusarium* exacerbated damage to faba bean' s defense system, leading to severe disease development.

Companion cropping with wheat enhanced watermelon resistance to Fusarium wilt by increasing -1,3-glucanase and chitinase activities in watermelon roots [28]. Watermelon-rice intercropping significantly increased chitinase and -1,3-glucanase activities in watermelon roots, thereby improving resistance to Fusarium wilt [29]. Our study showed that under benzoic acid application combined with *Fusarium* inoculation, wheat-faba bean intercropping increased chitinase and -1,3-glucanase activities in faba bean roots. Moreover, the enhancement of these pathogenesis-related proteins by intercropping increased with benzoic acid concentration, indicating that intercropping can significantly increase pathogenesis-related protein expression even under high benzoic acid accumulation, thereby improving faba bean resistance.

Our results demonstrate that the autotoxin benzoic acid inhibits faba bean seedling growth and reduces faba bean resistance by decreasing antioxidant enzyme and defense enzyme activities, thereby promoting Fusarium wilt development. The synergistic interaction between benzoic acid and *Fusarium* represents an important mechanism underlying faba bean continuous cropping obstacles. Wheat-faba bean intercropping alleviated benzoic acid-induced growth inhibition, enhanced defense enzyme activity, reduced membrane damage, and physiologically improved faba bean resistance to Fusarium wilt, thereby mitigating benzoic acid autotoxicity. This study confirms that rational intercropping is an effective measure for alleviating continuous cropping obstacles.

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Note: Figure translations are in progress. See original paper for figures.