

Cell wall modifications during the interaction of two forms of *Bacillus thuringiensis* with mycorrhizal fungi

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Abstract

The interactions between arbuscular mycorrhizal fungi (AMFs) and *Bacillus thuringiensis* can enhance plant growth, yet their combined effects under soluble and insoluble phosphorus conditions remain unclear. This study evaluated three AMF species, *Funneliformis mosseae* (AMF1), *Funneliformis caledonium* (AMF2), and *Acaulospora langula* (AMF3), in combination with vegetative and endospore forms of *B. thuringiensis* in strawberry grown under insoluble P, and P-available conditions. Under insoluble P, vegetative *B. thuringiensis* produced the greatest growth improvements, particularly with AMF3 (up to 89% above the uninoculated control) and AMF2 (up to 80%). Dual inoculation with vegetative cells increased root length, biomass. Under P-available soil, dual inoculation reduced AMF colonization (11%), root length and biomass, reflecting indicating downregulation of symbiosis when external P is sufficient. FTIR analyses showed that vegetative cells enhanced polysaccharide deposition, whereas endospores increased lignin-associated features. Overall, the benefits of AMF-*B. thuringiensis* were most evident under conditions of insoluble P, emphasizing their potential to enhance nutrient uptake in low P soil.

Keywords: Arbuscular mycorrhizal fungi; *Bacillus thuringiensis*; Cell wall composition; Phosphorus; Strawberry

Introduction

Arbuscular mycorrhizal fungi (AMF) form symbiotic associations with most terrestrial plants and enhance nutrient uptake, especially phosphorus, by extending the functional root surface through their hyphal networks (Shukla et al., 2025). However, little is known about how specific AMF species interact with vegetative and endospore forms of *Bacillus* under insoluble-P conditions. This study addresses these gaps by examining interactions between AMF species and *B. thuringiensis* (vegetative and endospore forms) under soluble and insoluble phosphorus environments.

Materials and methods

The study utilized day-neutral strawberry plants ('San Andreas'). After a month of chilling at 4 °C, the plants were transferred to a 25 °C greenhouse. Three types of arbuscular mycorrhizal fungi (*Funneliformis mosseae* (AM1), *F. caledonium* (AM2), and *Acaulospora langula* (AM3)), a bacterium (*Bacillus thuringiensis* subsp. kurstaki), and phosphorus levels (soluble P [KH₂PO₄] and insoluble P [Ca₅(PO₄)₃OH]) were chosen for the experiment. After two months, root samples were collected and frozen to measure root growth and physiological traits (Root colonization, Root length, total root fresh weight, and total root dry weight, FTIR spectroscopy analysis) (Fallah and afshar-Mohammadian, 2026).

Results and discussion

In this study, under insoluble P conditions, all combinations of *B. thuringiensis* and AMFs significantly enhanced root length and biomass, along with root colonization with the vegetative form of *B. thuringiensis* generally exhibiting stronger effects than the endospore form (Fig. 1).

The highest increases were observed in *B. thuringiensis* +AMF3, followed by *B. thuringiensis* +AMF2 and *B. thuringiensis* +AMF1 (Fig. 1), indicating that both the identity of the AMF species and the metabolic state of *B. thuringiensis* influence P mobilization and root proliferation.

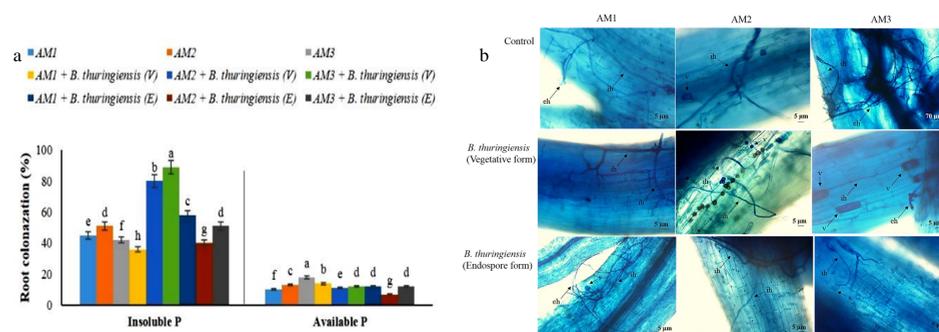


Fig. 1. Root colonization of strawberry plants by AMFs co-inoculated with *B. thuringiensis* under insoluble P (a,b) and available P (a) conditions.

Interestingly, under P-available conditions, dual inoculation often reduced root length, dry weight, and root colonization compared with the controls (Figure 1). This pattern suggests that plants may downregulate symbiotic associations when external P is sufficient, thereby reducing the carbon costs associated with maintaining microbial partners (Peng et al., 2025).

FTIR analysis (Figs. 2,3) provided further insights into the biochemical modifications induced by AMFs and *B. thuringiensis*. Peaks in the carbohydrate region (1000–1200 cm⁻¹) were more pronounced in treatments involving vegetative *B. thuringiensis*, suggesting enhanced deposition of cellulose and hemicellulose (Kang et al., 2015), a pattern consistent with studies showing microbe- or stress-induced reinforcement of polysaccharide matrices in roots (Oliveira et al., 2009; El-Mahdy et al., 2025). By contrast, the endospore form induced stronger absorbance in the lignin- and phenolic-associated region (1400–1600 cm⁻¹), along with more defined hydrogen-bond networks in the 3200–3300 cm⁻¹ region (Pan et al., 2019).

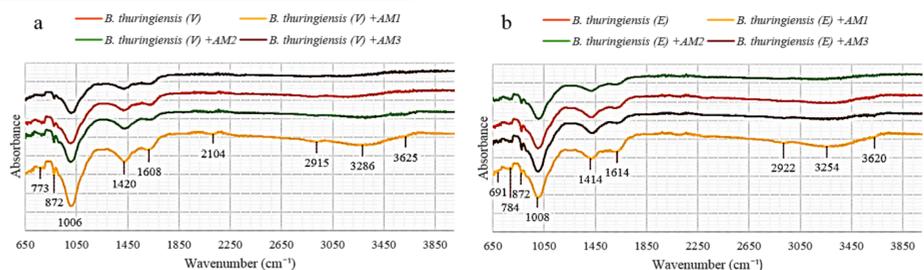


Fig. 2. FTIR spectra of strawberry root cell walls: Interaction with *B. thuringiensis* (V) (a); interaction with *B. thuringiensis* (E) (b) under insoluble P.

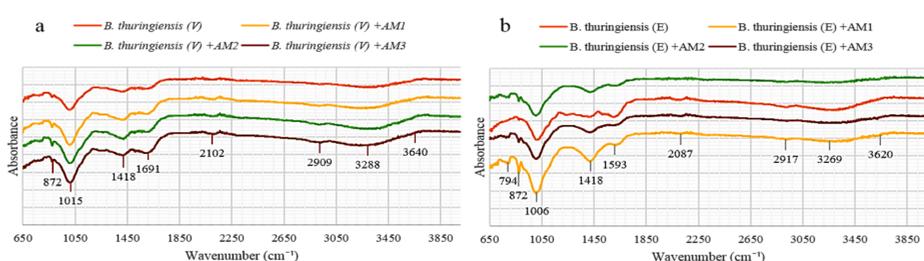


Fig. 3. FTIR spectra of strawberry root cell walls: Interaction with *B. thuringiensis* (V) (a); interaction with *B. thuringiensis* (E) (b) under available P.