Effects of Bt Gene Transformation on Growth, Physiology, and Phosphorus Transporter Gene Expression in Maize Inoculated with Arbuscular Mycorrhizal Fungi (Postprint)

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Abstract

To analyze the similarities and differences in the response of Bt corn and conventional corn to inoculation with arbuscular mycorrhizal fungi, this study comparatively analyzed the arbuscular mycorrhizal fungal colonization rate, expression levels of phosphorus transporter genes, growth, and nutrient utilization status in the roots of two Bt corn varieties '5422Bt1' (Bt11) and '5422CBCL' (Mon810) and their isogenic conventional corn variety '5422' under both inoculated and non-inoculated conditions with Funneliformis mosseae. The results showed that: at 50 d and 80 d of growth, the arbuscular mycorrhizal fungal colonization rates in the roots of Bt corn '5422Bt1' and '5422CBCL' were significantly higher than those of conventional corn '5422' , being 13.54% and 11.24% higher at 50 d, and 9.83% and 6.70% higher at 80 d, respectively; at the 50 d sampling, there were no significant differences in the expression levels of phosphorus transporter genes in the roots of corn '5422Bt1' , '5422CBCL' , and '5422' between inoculated and non-inoculated treatments; under both inoculated and non-inoculated conditions, the dry weight of corn '5422Bt1' was significantly higher than that of the corresponding treatments of '5422CBCL' , but showed no significant difference compared to '5422' ; at the 80 d sampling, the root length, root surface area, and root volume of corn '5422Bt1' under non-inoculated conditions were significantly higher than those of '5422' and '5422CBCL' under non-inoculated conditions. The introduction of the Bt gene primarily affected the nitrogen absorption and utilization of the two Bt corn varieties ('5422Bt1' and '5422CBCL') at the seedling stage (50 d) and maturity stage (80 d); compared with the conventional corn variety '5422' , the synthesis of Bt protein consumed some nitrogen and phosphorus. The three corn varieties responded differently to AMF inoculation; the inoculation treatment enhanced the nitrogen absorption and utilization of '5422Bt1' and '5422CBCL' at both the seedling stage

(50 d) and maturity stage (80 d). Under phosphorus nutrient conditions that met the growth requirements of corn, inoculation with arbuscular mycorrhizal fungi had no significant effect on the expression levels of phosphorus transporter genes or phosphorus absorption and utilization in Bt corn. The effects of Bt gene introduction and F. mosseae inoculation on the growth and nutrient utilization of Bt corn were related to the variety characteristics formed by different transformation events.

Full Text

Effect of Bt Gene Insertion on Growth, Physiology, and Phosphorus Transporter Gene Expression in Corn Colonized by Arbuscular Mycorrhizal Fungi

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Abstract

To analyze the similarities and differences in responses between Bt corn and conventional corn to arbuscular mycorrhizal fungi (AMF) inoculation, this study compared AMF colonization rates, phosphorus transporter gene expression levels, growth characteristics, and nutrient utilization in two Bt corn varieties ('5422Bt1' [Bt11] and '5422CBCL' [Mon810]) and their isogenic conventional counterpart '5422' , with and without inoculation of *Funneliformis mosseae*. At 50 and 80 days after planting, AMF colonization rates in the roots of Bt corn varieties '5422Bt1' and '5422CBCL' were significantly higher than in conventional corn '5422' , with increases of 13.54% and 11.24% at 50 days, and 9.83% and 6.70% at 80 days, respectively. At the 50-day sampling, no significant differences in phosphorus transporter gene expression were observed among '5422Bt1' , '5422CBCL' , and '5422' under either inoculated or non-inoculated conditions. The dry weight of '5422Bt1' was significantly higher than that of '5422CBCL' under both treatments, but did not differ significantly from '5422' . At 80 days, non-inoculated '5422Bt1' exhibited significantly greater root length, surface area, and volume compared to non-inoculated '5422' and '5422CBCL' . Bt gene insertion primarily affected nitrogen absorption and utilization in the two Bt corn varieties during both seedling (50 d) and maturity (80 d) stages, with Bt protein synthesis consuming some nitrogen and phosphorus compared to the conventional variety. The three corn varieties responded differently to AMF inoculation, which enhanced nitrogen absorption and utilization in '5422Bt1' and '5422CBCL' at both growth stages. Under phosphorus-sufficient conditions, AMF inoculation did not significantly affect phosphorus transporter gene

expression or phosphorus absorption and utilization in Bt corn. The effects of Bt gene insertion and *F. mosseae* inoculation on Bt corn growth and nutrient utilization were associated with variety characteristics determined by different transformation events.

Keywords: Bt corn; Arbuscular mycorrhizal fungi; Growth characteristics; Nutrient utilization; Phosphorus transporter gene

Introduction

Arbuscular mycorrhizal fungi (AMF) are beneficial eukaryotic microorganisms in soil that form mutualistic symbioses with over 80% of terrestrial plants, enhancing water and mineral absorption, promoting host plant growth, and improving resistance to salinity, drought, heavy metals, and disease. However, these beneficial fungi are readily influenced by host plant characteristics.

Bt (*Bacillus thuringiensis*) corn (*Zea mays*) represents one of the most rapidly commercialized insect-resistant genetically modified crops, with global cultivation reaching 5.391 million hectares in 2015. Bt corn roots secrete insecticidal Bt proteins that maintain activity in soil for over 180 days, potentially affecting the symbiotic relationship between corn and AMF. Previous studies on Bt corn impacts on AMF colonization have yielded inconsistent results. Cheeke et al. [**?**, **?**] reported significantly lower AMF colonization in Bt corn compared to conventional corn without effects on growth, while Seres et al. [**?**] observed similar reductions in field experiments. Conversely, Ren et al. [**?**] found significantly higher colonization rates in Bt corn inoculated with *Funneliformis mosseae* (formerly *Glomus mosseae*), with greater growth promotion than in conventional corn. Zeng et al. [**?**] also reported higher AMF colonization after five consecutive seasons of Bt corn cultivation. Other studies found no significant effects of Bt gene insertion on colonization rates or growth, suggesting that nutrient content changes relate to transformation events rather than the Bt trait itself.

These studies focused primarily on colonization rates and growth impacts, without investigating underlying molecular mechanisms. AMF enhances mineral element acquisition, particularly phosphorus. The phosphorus transporter gene *ZEAma:Pt1;6* is a key regulator of phosphorus uptake and utilization, often induced by AMF colonization. Whether AMF inoculation induces *ZEAma:Pt1;6* expression in Bt corn and whether Bt gene insertion affects this expression remain unknown.

The Bt corn varieties '5422Bt1' (Bt11) and '5422CBCL' (Mon810), produced by Beck' s Superior Hybrids, are widely used in ecological risk assessment studies. *Funneliformis mosseae* is the predominant species colonizing corn roots in field conditions. Sampling at 50 days captures peak vegetative growth, while 80 days reflects complete nutritional development. This study examined these Bt varieties and their conventional counterpart '5422' to investigate AMF col-

onization, *ZEAma:Pt1;6* expression, and growth/nutrient utilization differences at both stages, providing scientific guidance for Bt corn biosafety management.

Materials and Methods

1.1 Experimental Materials The Bt corn varieties '5422Bt1' (Bt11) and '5422CBCL' (MON810) and their isogenic conventional variety '5422' were obtained from Beck' s Superior Hybrids (USA) through Dr. Cindy Nakatus at Purdue University. Both Bt varieties express the Cry1Ab insecticidal protein. The AMF inoculum consisted of *Funneliformis mosseae* from the Chinese Academy of Sciences' AMF Germplasm Resource Bank, provided by Dr. Yao Qing at South China Agricultural University as spores, mycorrhizal root fragments, and hyphae propagated on corn. The substrate was river sand sieved through 2 mm mesh and sterilized by dry heat at 180°C for 2 hours.

1.2 Experimental Design The inoculation experiment was conducted on June 8, 2014, in a greenhouse at South China Agricultural University. Each corn variety received two treatments: inoculation with *F. mosseae* and non-inoculation control. Plastic pots (15 cm height, 16.5 cm diameter) were sterilized with 0.1% potassium permanganate for 24 hours. Each pot contained 1.5 kg sterilized river sand covered with 100 g AMF inoculum. Uniform seeds (treated with 10% H_2O_2 for 5 minutes) were sown and covered with 0.5 kg sand, then watered thoroughly. Hoagland nutrient solution was applied every 3 days with supplemental watering as needed. One plant was grown per pot, with 20 replicates per treatment. At 50 and 80 days after planting, three plants per replicate were sampled for mycorrhizal colonization and physiological measurements.

1.3 Measurement Methods 1.3.1 AMF Colonization Rate Determination

Thirty root fragments (1 mm diameter) per replicate were washed, stained with trypan blue, and examined microscopically. Colonization was scored using standard categories (0, <1%, <10%, <50%, >50%, >90%) and calculated using "Mycocalc" software [**?**].

1.3.2 Phosphorus Transporter Gene Expression Analysis

Total RNA was extracted from 100 mg root samples using the RNAprep Pure Plant Kit (Tiangen, Beijing). cDNA synthesis was performed with PrimeScript™ RT Master Mix (Takara, Dalian), followed by qRT-PCR using SuperReal PreMix Plus (SYBR Green) (Tiangen, Beijing). The 20 L reaction contained 1 L cDNA, 10 L 2× SuperReal PreMix Plus, 0.6 L each of forward (5 -CCGTCAACAACATGGTGACAGG-3) and reverse (5 -AGAAGCGGAAGAAGCAGAGCGT-3) primers, and RNase-free water. Cycling conditions were: initial denaturation at 95°C for 15 min, followed by

40 cycles of 95°C for 30 s, 60°C for 30 s, and 72°C for 30 s. Relative expression of *ZEAma:Pt1;6* was calculated using the $2^{-}\Delta\Delta$Ct method [**?**].

1.3.3 Growth Parameter Measurement

Plant height was measured from stem base to tip. Root characteristics were analyzed by scanning with an EPSON V700 dual-light scanner and quantifying length, surface area, and volume using WinRHIZO REG 2009 software [**?**]. Shoots and roots were oven-dried at 105°C for 30 minutes, then at 75°C to constant weight for biomass determination.

1.3.4 Nutrient Content Analysis

Total organic carbon, nitrogen, phosphorus, and potassium in leaves and roots were determined according to Bao [**?**].

1.4 Statistical Analysis Data were analyzed using SPSS 13.0. One-way ANOVA with Duncan' s multiple comparison tested differences among varieties, while independent t-tests compared inoculated vs. non-inoculated treatments. Significance was set at $P < 0.05$. Values are presented as means $\pm$ standard error.

Results

2.1 AMF Colonization and Phosphorus Transporter Gene Expression At both sampling times, AMF colonization rates in Bt varieties '5422Bt1' and '5422CBCL' were significantly higher than in conventional '5422' , showing increases of 13.54% and 11.24% at 50 days, and 9.83% and 6.70% at 80 days, respectively [Figure 1: see original paper]. These results align with Zeng et al. [**?**] and Ren et al. [**?**] but contrast with Cheeke et al. [**?**], likely due to differences in tested varieties. The *ZEAma:Pt1;6* gene encodes a high-affinity phosphorus transporter that enhances phosphorus acquisition and is often induced by AMF [**?**, **?**]. However, at 50 days, no significant differences in *ZEAma:Pt1;6* expression were detected among '5422' , '5422Bt1' , and '5422CBCL' under either treatment, nor between inoculated and non-inoculated plants within each variety [Figure 1: see original paper]. This agrees with Saia et al. [**?**], suggesting that phosphorus availability was sufficient for growth without requiring AMF-induced transporter expression.

2.2 Growth and Nutrient Responses **2.2.1 Plant Height and Biomass**

At 50 days, non-inoculated varieties showed no significant differences in height, total dry weight, or root dry weight, though '5422Bt1'shoot dry weight exceeded '5422CBCL' . Inoculation significantly increased '5422' height above its control and above both Bt varieties. '5422Bt1' total, shoot, and root dry weights under inoculation were significantly higher than '5422CBCL' . At 80 days, no height or total/aboveground biomass differences existed among varieties or treatments,

though root dry weight differed: non-inoculated '5422Bt1' exceeded '5422' and '5422CBCL' , while inoculated '5422Bt1' and '5422CBCL' had lower root weights than their controls, with '5422Bt1' remaining higher than '5422CBCL' . These findings indicate Bt gene insertion influenced growth characteristics, particularly shoot biomass at 50 days and root biomass at 80 days, and modified variety-specific responses to AMF inoculation.

2.2.2 Root Growth Characteristics

Roots are primary organs for water and nutrient acquisition, with length and density critically affecting plant performance. At 50 days, no significant differences in root length, surface area, or volume were observed among varieties or treatments , indicating neither Bt insertion nor AMF affected early root development. By 80 days, non-inoculated '5422Bt1' showed significantly greater root length, surface area, and volume than '5422' and '5422CBCL' , demonstrating that the Bt11 event enhanced mature root system vigor. However, AMF inoculation did not further promote root growth in '5422Bt1' .

2.2.3 Nutrient Content

Nitrogen, phosphorus, potassium, and organic carbon contents reflect plant nutrient utilization efficiency. At 50 days without inoculation, '5422Bt1' exhibited higher total and root nitrogen content than '5422CBCL' and higher root nitrogen than both other varieties, while phosphorus, potassium, and organic carbon did not differ among varieties . Under inoculation at 50 days, only '5422Bt1' root nitrogen was lower than '5422CBCL' , and its phosphorus content was lower than both other varieties; '5422CBCL' shoot potassium exceeded '5422' . Inoculation reduced '5422Bt1' total and shoot nitrogen compared to its control, while increasing '5422CBCL' total and root nitrogen.

At 80 days without inoculation, '5422' showed higher total and shoot nitrogen, total phosphorus, root phosphorus, total potassium, and root potassium than '5422CBCL' , and higher total potassium than both Bt varieties. Under inoculation at 80 days, only '5422' total organic carbon was lower than the Bt varieties. Inoculation increased '5422CBCL' total nitrogen while decreasing its organic carbon. These results demonstrate that Bt gene insertion primarily affected nitrogen absorption and utilization in both Bt varieties at both growth stages, with Bt protein synthesis consuming nitrogen and phosphorus relative to conventional corn. The three varieties responded differently to AMF inoculation, which enhanced nitrogen uptake in '5422Bt1' and '5422CBCL' at both stages.

Discussion and Conclusion

This study demonstrates that Bt gene insertion affected growth characteristics and AMF responses in two transgenic corn varieties, with effects dependent on

transformation events, consistent with Cheeke et al. [**?**]. Mycorrhizal colonization reflects symbiotic affinity between plants and fungi [**?**]. The significantly higher AMF colonization in '5422Bt1' (Bt11) and '5422CBCL' (Mon810) compared to conventional '5422' indicates enhanced fungal establishment in Bt varieties. Inoculation more strongly promoted '5422' height at the seedling stage (50 d) than the Bt varieties, while more strongly increasing '5422Bt1' total and shoot dry weight than '5422CBCL' . The Bt11 event improved mature root vigor (80 d) in '5422Bt1' , though AMF inoculation did not further enhance root growth.

Bt gene insertion primarily influenced nitrogen absorption and utilization in both Bt varieties at seedling and maturity stages, with Bt protein synthesis consuming nitrogen and phosphorus, consistent with Feng et al. [**?**]. The three varieties exhibited distinct AMF responses, with inoculation increasing nitrogen uptake in '5422Bt1' and '5422CBCL' at both stages but not affecting *ZEAma:Pt1;6* expression or phosphorus utilization. This contrasts with Ren et al. [**?**], Jia et al. [**?**], and Fu et al. [**?**], likely because Hoagland solution applied every 3 days provided sufficient phosphorus, eliminating the need for AMF-mediated enhancement [**?**].

In conclusion, Bt gene insertion promoted AMF colonization without affecting phosphorus transporter gene expression. The impacts on growth and nutrient utilization were transformation event-dependent: '5422Bt1' dry weight exceeded '5422CBCL' and did not differ from '5422' ; '5422Bt1' root length, surface area, and volume surpassed both other varieties; '5422' nitrogen, phosphorus, and potassium contents exceeded '5422CBCL' but not '5422Bt1' . The three varieties responded differently to AMF inoculation, which enhanced nitrogen absorption in '5422Bt1' and '5422CBCL' .

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