Effect of *Trichoderma* on the Growth, Maturity, and Yield of Corn in the Heilongjiang Province

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Abstract. Heilongjiang is the main corn producing province in China. However, the unique geographic and climatic characteristics severely reduced corn growth and yields in this area. The aims of this study were to identify the effects of *Trichoderma* agent on the growth, quality, yield and maturity of corn in Heilongjiang Province. The *Trichoderma* agent were obtained by thick-walled spores of *Trichoderma viride* strain Tv-1511 using deep-liquid fermentation. We showed that Tv-1511 played pivotal roles in promoting corn growth (plant height, stem thickness and leaf development) and corn kernels development (kernels length, width, thickness, and 100-grain weight). In relation to corn maturity and quality, we observed an increase in dry matter content by 11.24%, nitrogen content by 31.69%, and amino acid content by 31.77% to 75.65% in inoculated Tv-1511 as compared to controls at early maturity stage. Moreover, Tv-1511 can significantly enhance the corn yield, which increased by 11.13% and 9.06% at early and late maturity stages, respectively. Further, the total economic income were increased over 1700 CNY per hectare in inoculated Tv-1511 as compared to controls. This study provided a theoretical basis for the application of *Trichoderma* agent in corn production in Northeast China.

Keywords: *Trichoderma*; corn; maturity; growth promotion; increase production

1 Introduction

Corn, one of the world's major food crops, has a broad distribution. China is the world's second largest producer of corn, and it has the largest sown area and total output [1]. As the "king of feed", corn is also significant for China's livestock industry. China's main corn-producing areas are concentrated in the northeast, north, and southwest regions, and of these areas, Heilongjiang is the largest corn-growing province [2]. In 2023, Heilongjiang's corn planting area reached 6,452,000 hectares, producing a total of 43.79 million tons of corn, accounting for more than 30% of the country's total output. Hei-

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longjiang Province has a diverse climate with four distinct seasons, and climatic conditions have a significant impact on corn growth and maturity, often lowering the yield. As the demand for corn increases, there is a need for improved planting techniques, especially in Heilongjiang, to rationally arrange sowing and fertilizing according to climatic conditions and promote the early maturity of corn using microbial inoculum and other means.

At present, the use of environmentally friendly microbial fungi for the green production of corn has received much attention. *Trichoderma* spp., a widely distributed genus of beneficial fungi that produce a rich variety of secondary metabolites having a broad range of biological activities, have been widely used in agriculture [3-4]. For example, *Trichoderma* spp. significantly promote plant seed germination, seedling growth, and yield increase [5-7]. Additionally, they provide considerable protection against many important plant pests and diseases [8-10]. However, there are few studies on *Trichoderma* spp promoting early maturity in plants. Previously, we screened and obtained a strain of *Trichoderma viride*, Tv-1511 (conservation number is CGMCC No. 16800), with good growth-promoting, enzyme-producing, anti-stress, and spore-producing characteristics.

Herein, we tested the growth-promoting effect of Tv-1511 on corn in Heilongjiang. We also determined the effect of Tv-1511 on corn quality, yield, and maturity. Our study will provide a theoretical basis for the application of *Trichoderma* in Heilongjiang province.

2 Materials and Methods

2.1 Materials

T. viride strain Tv-1511, was provided by the Institute of Biology, Qilu University of Technology (Shandong Academy of Sciences). Corn (LH 869), was purchased from Henderson Limagrain Seeds Ltd. *Trichoderma* agent was provided by Jinxing (Rizhao) Agricultural Science and Technology Co. Ltd, and the concentration of the *Trichoderma* spp. in the fermentation broth was approximately 10^9 CFU·mL^{-1}.

2.2 Field Application of *Trichoderma* Agent

Field experiment was conducted from May 20 to October 8, 2023 in an experimental field of the Hulan District, Harbin City, Heilongjiang Province. The annual temperature changes in Hulan District are shown in Fig.1. Conventional fertilization was used as the control (CK), while the application of *Trichoderma* agent was the treatment group (T). Corn was sown on May 20 and sprayed with *Trichoderma* agent four times between June 6 and July 20. Sampling and measurement were conducted on the early stage (September 25) and late stage of maturity (October 8), respectively. Six sampling points each were set up in the control and treatment fields, and four adjacent plants were counted at each sampling point. All corn plants were uniformly fertilized with the required nutrients according to local fertilization practices.

2.3 Measurement Items and Methods

2.3.1 Corn Growth Indicators

Plant height was determined by measuring the distance from the ground to the top of the plant. Stem thickness was determined by measuring the diameter of a corn plant at 5 cm from the ground with a Vernier caliper. Leaf length was quantified by measuring the length from the petiole to the leaf tip of the first leaf blade under the first spike. Leaf width was measured as the widest point in the direction perpendicular to the main vein on the first leaf blade under the first spike.

2.3.2 Corn Kernel Indicators

Corn were collected and threshed. These corn kernels underwent drying in the oven at 105°C for 20 min and then drying at 50°C for 48 h until the kernels were completely dry. Afterward, the dried corn kernels were mixed well, and 10 kernels of uniform sizes were selected from each group to determine the length, width, thickness, and weight of each kernel.

2.3.3 Corn Quality

The corn kernel was crushed, and three replicates were set up for each group to determine the changes in dry matter, protein, starch, fat, and amino acid content in the corn kernels by using the drying, Kjeldahl, enzymatic, and Soxhlet extraction methods, respectively.

2.3.4 Corn Yield

Corn was harvested at maturity stages, and the corn spike weight recorded. The corn yield per unit area was converted with a standard moisture content of 14%. Then, the number of plants in an hectare of corn based on the spacing of the plants and rows was calculated and converted to yield in kg per hectare.

2.4 Statistical Analyses

All experimental data were statistically analyzed using Excel 2016 software. Additionally, the data were statistically analyzed using IBM SPSS 25; a p-value of ≤ 0.05 indicated a significant difference in the analysis of variance method. Plots were made with Origin 2021. All box plots, with the actual data points on the left and the box plots on the right, had horizontal lines from top to bottom for the upper edge, upper quartile Q3, median, lower quartile Q1, and lower edge. The data points within the rectangle in the box plots were the mean, and the data points outside the rectangle were outliers. In all plots, * denotes $0.01 < p \leq 0.05$, **denotes $0.001 < p \leq 0.01$, and *** demotes $p \leq 0.001$.

3 Results

3.1 Effect of *Trichoderma* Agent on Corn Growth

The effect of the *T. viride* strain Tv-1511 on the growth of corn in Heilongjiang was analyzed. The overall plant height, stem thickness, leaf length, and leaf width of the corn treated with Tv-1511 increased by 9.87%, 6.18%, 3.75%, and 5.99%, respectively, compared with those corns in the control group (Fig. 2). These data indicated that Tv-1511 had a significant effect on the changes of growth indexes in corn, such as plant height, stem thickness and leaf development.

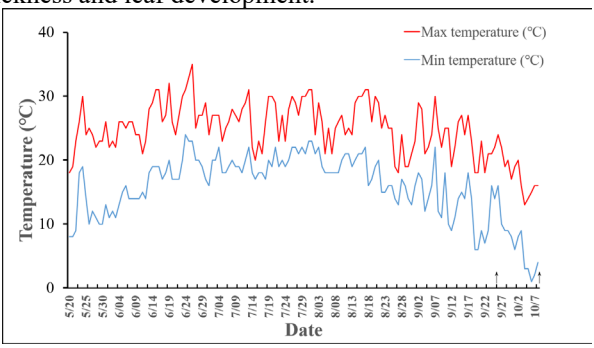

Fig. 1. Temperature change in Heilongjiang province from 20 May to 8 October. The arrows in the figure represent sampling points.

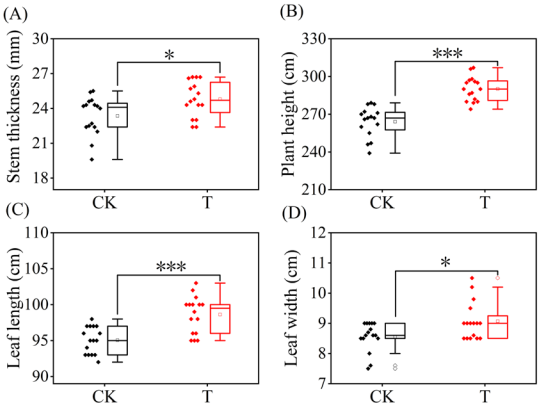

Fig. 2. Effect of *Trichoderma* agent on corn growth. (A) Stem thickness. (B) Plant height. (C) Leaf length. (D) Leaf width. CK: conventional fertilization. T: *Trichoderma* agent Tv-1511 treatment.

3.2 Effect of *Trichoderma* Agent on Corn Kernels

The size and weight of kernels are one of the main indicators of their fullness. The effect of *Trichoderma* agent on corn kernels was analyzed by measuring the kernel indicators on September 25 and October 8.

The application of the *Trichoderma* agent Tv-1511 significantly increased the fullness of the corn kernels. Specifically, on September 25, the corn kernels length, width, thickness, and 100-grain weight of the treatment group increased by 9.28%, 20.9%, 11.04%, and 14.56%, respectively, compared with those of the control group. On October 8, the corn kernels length, width, thickness, and 100-grain weight of the treatment group increased by 7.75%, 16.8%, 9.22%, and 12.31% (Fig. 3), respectively, compared with those of the control group. These results indicated that *Trichoderma* agent Tv-1511 significantly improved the fullness of the corn kernels, and the increase was most significant on September 25.

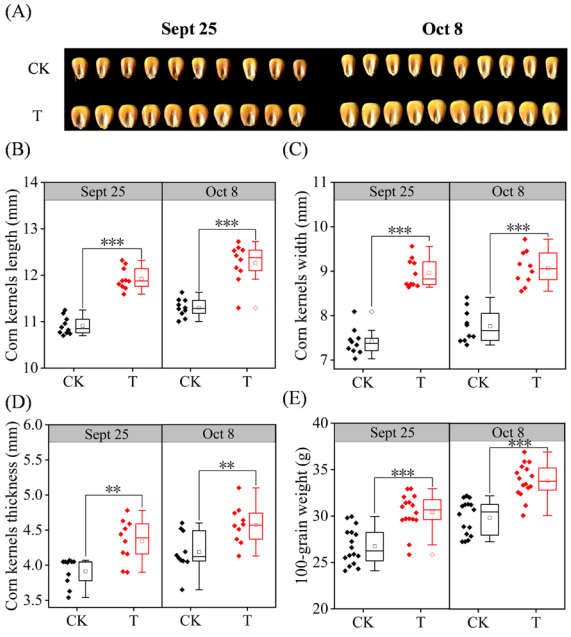

Fig. 3. Effect of *Trichoderma* agent on corn kernels. (A) Phenotype of corn kernels. Statistics of corn kernels length (B), width (C), thickness (D) and 100-grain weight (E). CK: conventional fertilization. T: *Trichoderma* agent Tv-1511 treatment.

3.3 Effect of *Trichoderma* Agent on Corn Maturity and Quality

The dry matter content of corn kernel generally increases with a decrease in moisture content. Thus, the differences in corn maturity and quality can be compared on the basis of the dry matter content as well as the nutrient (e.g., proteins, fats, starch) contents in the corn kernel. In this study, Tv-1511 significantly increased the dry matter content as well as the nutrient content in the corn kernels. Specifically, the dry matter content of

corn in the treatment group increased by 11.24% (September 25), and 12.39% (October 8) (Fig. 4A), respectively, compared with those of the control group. In addition, the nitrogen content of the corn in the treatment group increased by 31.69% (September 25) and 22.51% (October 8) (Fig. 4B), compared with that in the control group. Although the effect of the Tv-1511 on the starch content and fat content was insignificant (Fig. 4C, D); the slightly upward trend still indicated a greater energy reserve in the kernels, which contributed to the overall energy density and quality of corn. Therefore, the use of *Trichoderma* agent Tv-1511 could significantly improve the maturity and quality of corn.

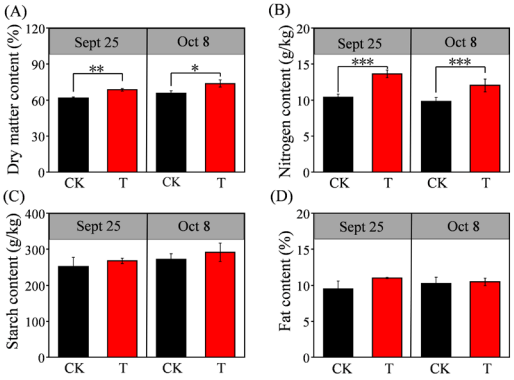

Fig. 4. Effect of *Trichoderma* agent on corn maturity and quality. Statistics of dry matter content (A), nitrogen content (B), starch content (C) and fat content in corn. CK: conventional fertilization. T: *Trichoderma* agent Tv-1511 treatment.

3.4 Effect of *Trichoderma* Agent on the Amino Acid Content of Corn

Amino acids are basic components of the proteins in corn kernels. They participate in the construction of plant cells and act as important metabolic regulators, affecting the growth and resistance of plants. An increase in the amino acid content of corn kernels can help improve the adaptability of corn to adversity and indirectly increase the yield. In this study, amino acid contents were increased in all treatment group as compared to that in the control group. Among these amino acid, glutamic acid (Glu), aspartic acid (Asp), and leucine (Leu) contents increased most significantly, by 67.69%, 26.85%, and 56.02%, respectively, in the early stages of maturity and by 42.51%, 21.30%, and 30.63%, respectively, in the late stages of maturity (Fig.5).

The effect of Tv-1511 on amino acid content was more significant in the early maturity stage than in the later maturity stage. Maybe, in the early maturity stage, the metabolism of kernel activity is more active, and the rate of amino acid accumulation and conversion is higher. *Trichoderma* agent Tv-1511 could effectively promote amino acid synthesis and transport, thereby significantly enhancing the amino acid content. At the later maturity stage, the seed metabolism tends to stabilize, and amino acid synthesis decreases; as a result, the effect of *Trichoderma* agent Tv-1511 is weaker.

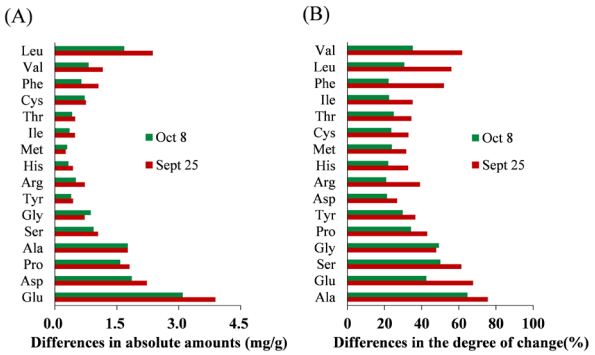

Fig. 5. Effect of *Trichoderma* agent on amino acid content of corn. (A) Differences in the absolute amount of hydrolysed amino acids increased in the T group compared to the CK. (B) Differences in the degree of hydrolysed amino acids increased in the T group compared to the CK. CK: conventional fertilization. T: *Trichoderma* agent Tv-1511 treatment.

3.5 Effect of *Trichoderma* AGEnt on CORN YIeld

Trichoderma agent also had some effects on corn yield. Tv-1511 treatment significantly increased the single-plant ear weight, single-plant grain weight, and yield of corn. At the early maturity stage of corn, the single-plant ear weight, single-plant grain weight, yield of the treatment group increased by 16.31%, 15.90%, and 11.13%, respectively, compared with those of the control group. At the later maturity stage of corn, the single-plant ear weight, single-plant grain weight, and yield of the treatment group increased by 10.73%, 9.20%, and 9.06%, respectively, compared with those of the control group (Fig.6).

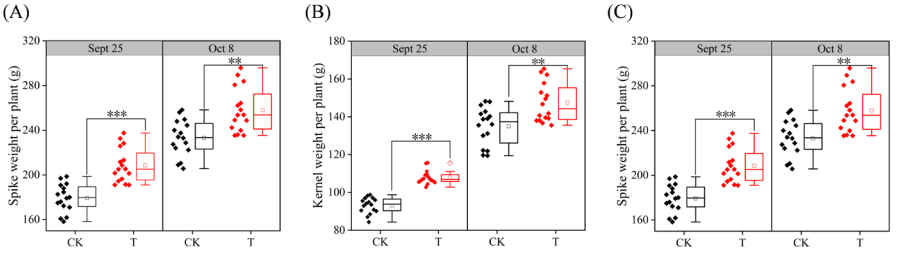

Fig. 6. Effect of *Trichoderma* agent on corn yield at different times. (A) Spike weight per plant. (B) Kernel weight per plant. (C) Yield. CK: conventional fertilization. T: *Trichoderma* agent Tv-1511 treatment.

Table 1. Economic analysis results

	Unit	Sep 25	Otc 8
Corn yield of CK	kg/hm^2	8671.91	10788.52
Corn yield of T	kg/hm^2	9637.25	11765.47

Increased corn yield of Tv-1511 agent	kg/hm^2	965.34	976.95
Increased income of selling corn	CNY/hm^2	2606.42	2637.77
Cost of Tv-1511 agent	CNY/hm^2	900.00	900.00
Increased revenue per hectare	CNY/hm^2	1706.42	1737.77

Note: CK: conventional fertilization. T: *Trichoderma* agent Tv-1511 treatment.

3.6 Economic Benefit Analysis

At the late maturity stage, the yield of the *Trichoderma* agent Tv-1511 treatment group was 9.06% higher than that of the control, with an increase of 976.95 kg per hm^2. With the purchase price of corn at CNY 2.7/kg, the treatment increased the income by CNY 2637.77/hm^2. *Trichoderma* agent and application costs totaling 900 CNY per hm^2, and the increase in revenue was 1737.77 CNY per hm^2. If the *Trichoderma* agent treatment promoted corn planting of 100 thousand hm^2 per year, the corn yield will increase by 97695 tons per year, and the revenue will increase by 174 million CNY per year. Similarly, at the early maturity stage, the corn yield of *Trichoderma* agent treatment will increase by 96534 tons per year, and the revenue will increase by 171 million CNY per year (Table 1). The above calculation has not taken into account the benefits brought about by the improvement of corn quality, the future cost reduction and efficiency. Therefore, the application of *Trichoderma* agent in corn could achieve good economic benefits.

4 Discussion and Conclusions

Trichoderma, a well-known filamentous fungi genus in agroecology, is ubiquitously present in soil and root ecosystems. It has been widely recognised for its ability to promote plant growth and antagonize pathogenic bacteria. *Trichoderma* has a significant promoting effect on seed germination, seedling growth, crop yield, and plant resistance [11-13]. Consistent with this observation, we observed that corn treated with *Trichoderma* agent Tv-1511 showed better growth advantages. Part of the reason for this may be that *Trichoderma* produce diverse secondary metabolites with various biological activities.

The maturity of corn is critical to yield and quality; if corn is not fully developed at optimal maturity, it may be damaged by early frosts [14]. Located in the northeast of China, Heilongjiang Province has a cold climate and a short frost-free period, causing crops susceptible to disasters, such as spring cold, spring drought, and autumn frosts, which ultimately weakens nutrient uptake, and leading to a decrease in yield. Therefore, it is crucial to promote the early maturity of corn [15]. In this study, we found that *Trichoderma* agent Tv-1511 significantly increased the dry matter content and maturity of corn kernels and increased nutrient accumulation. Therefore, when dealing with adverse weather conditions such as premature cooling, the application of *Trichoderma* agent in corn could achieve higher production and economic benefits.

Currently, the solid fermentation process is commonly used to produce *Trichoderma* agent [16]. However, this process required high level of experimental conditions and

sophisticated laboratory equipment. Moreover, the shelf-life of *Trichoderma* agents produced by solid fermentation is short, only about 10 months. *Trichoderma* agents can be obtained by liquid (LF) and solid-state fermentation (SSF). *Trichoderma* conidia were produced in a SSF system, while *Trichoderma* thick-walled spores were produced by LF. Thick-walled, resistant spores formed by the direct differentiation of hyphae, can survive under unfavorable conditions. Compared with *Trichoderma* conidia, Thick-walled spores can significantly increase shelf life and overall reliability of *Trichoderma* agents. Therefore, obtaining thick walled spores of *Trichoderma* through deep-liquid fermentation is an effective method for its large-scale application. [17]. In this study, we used liquid submerged fermentation technology to obtain *Trichoderma* agent. By optimizing the medium and fermentation process, we achieved a stable *Trichoderma* agent with fermentation cycle ≤ 7 days, viable bacteria count≥1 billion CFU/mL, and a spore formation rate ≥85%. Thus, the liquid fermentation technology we used could significantly reduce costs, enhance the performance of *Trichoderma* agent, and facilitate the wider application in agriculture.

Acknowledgments

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