Effects of Marquandomyces Marquandii SGSF043 on Maize Growth Promotion and Soil Enzyme Activity

Xu Zheng , Bo Zhang , Feng Shi , Yuanlong Chen , Xiumei Zhao *

Posted Date: 31 January 2024

doi: 10.20944/preprints202401.2147.v1

Keywords: Metarhizium; Marquandomyces marquandii; Maize; Seed germination; Seedling growth

Article

Effects of *Marquandomyces marquandii* SGSF043 on Maize Growth Promotion and Soil Enzyme Activity

Xu Zheng [1,2,3,4,6], Bo Zhang [2,3,5,6,7], Feng Shi [2,3,5,6], Yuanlong Chen [2,3,4,6] and Xiumei Zhao [1,*]

1 Qiqihar Sub-academy of Heilongjiang Academy of Agricultural Sciences, Qiqihar, 161006, China
2 Engineering Research Center of Agricultural Microbiology Technology, Ministry of Education, Harbin, 150080, China
3 Heilongjiang Provincial Key Laboratory of Ecological Restoration and Resource Utilization for Cold Region, Harbin, 150080, China
4 Key Laboratory of Molecular Biology, College of Heilongjiang Province, Harbin, 150080, China
5 Key Laboratory of Molecular, College of Heilongjiang Province, Harbin, 150080, China
6 School of Life Sciences, Heilongjiang University, Harbin, 150080, China
7 Institute of Black Soil Conservation, Heilongjiang Academy of Agricultural Sciences, Harbin, 150086, China
* Correspondence: zxm0452@126.com

Abstract: In order to further clarify the growth-promoting effect of non-core *Metarhizium* spp. M. *marquandii* on plants, M. *marquandii* SGSF043, which was obtained from the pre-screening in the laboratory, was selected as a test strain, and the effects of the fungus fermentation broth during the stabilization period of the growth of this strain on the growth and growth of maize seeds were investigated by using fermentation broth as a submerged seed. germination, seedling growth, and inter-root soil enzyme activity of seedlings. The results are as follows: In the seed germination test, M. *marquandii* SGSF043 fermentation liquid had a certain inhibitory effect on the germination of maize seeds, and the 10-times dilution of the fermentation liquid significantly promoted the seed germination, and the effect was the best; After the germination test, the growth of maize germ was promoted by the treatment groups of 10 times and 1,000 times diluent; In the field plot experiment, the 100-fold dilution had the best growth promotion effect, which could promote the growth of cabbage seedlings and increase the seedling biomass. The results show that M. *marquandii* SGSF043 has the potential to promote the growth of maize and improve the soil environment, which provides a theoretical basis for the research and application of M. *marquandii* in farmland.

Keywords: *Metarhizium*; *Marquandomyces marquandii*; Corn; Seed germination; Seedling growth

1. Introduction

In recent years, due to the increase in maize yields and planting area, the use of chemical fertilizers has risen year by year, exacerbating soil stagnation, acidification, salinization, and reduced use of organic fertilizers, leading to crop yield reductions, increased resistance of phytophagous pests, increased production costs and environmental pollution, and a series of problems [1,2].To implement actions and strategies for sustainable food production systems, such as integrated pest management (IPM) and the development of organic agriculture, one of which is based on microorganisms and their metabolites for controlling pest and disease damage to crops, increasing plant "immunity" to abiotic stresses and promoting crop growth [3–6]. *Metarhizum* spp. is widely known as an entomopathogenic fungus, it is widely distributed throughout the world and usually survives in nature as a plant inter-root fungus [7], a plant endophyte [8], an insect pathogen [9], and a soil fungus. In 1883, Sorokin officially named the first species of *Metarhizium* as *Metarhizium* [10], and since then the research on *Metarhizium* as insect pathogens and biopesticides has begun. Based on previous studies, in addition to increasing plant resistance to pathogenic strains [11] and having strong insecticidal activity itself, it has been reported in recent years that S. aeruginosa can colonize different plant tissues to promote the growth of the host plant and has a positive impact on the growth indicators of the plant. For example, Enrique González Pérez et al [12] have shown that M. brunneum can colonize the roots of tomato, maize, Arabidopsis thaliana, peanut, soybean, cotton, coffee, and other plants, and it has a certain promotional effect on the biological indexes such as plant height of the seedlings, root length, and the above-ground and below-ground parts of the plants [13,14].

Xinggang Liao et al. showed a significant increase in leaf crown formation, stem length, mean stem leaf, and mean cob biomass in seeds treated with *Metarhizium* fungicide as compared to the control, and the main effect on maize yield was during early nutritive growth [15]. Meanwhile, plant photosynthesis is the basic unit of material accumulation and physiological metabolism in the process of plant growth, and it is also an important means of analyzing the influence of environmental factors on plant growth. It has been shown that endophytic fungi have an extremely strong ability to promote photosynthesis in plants by increasing the supply of carbon dioxide needed for photosynthesis, thus improving plant photosynthesis [16].

Soil enzymes are mainly derived from the cellular secretions of animals, plants, and microorganisms in the soil and the decomposition of their residues, which are important participants in the soil material cycle and energy transformation, and have a close relationship with the fertility level of the soil, which can characterize the dynamic equilibrium of the soil-microorganism-plant system to a certain extent [17]. There is an intrinsic link between soil enzymes and plant growth. The application of biotrophic agents can significantly improve the inter-root soil conditions of crops and create a suitable environment for soil enzyme production, and the increase in soil enzyme activity can also promote crop growth and development [18]. Some studies have shown that fungi fertilizer can regulate the micro-ecological environment of the plant root system and the activity of soil alkaline phosphatase and soil urease, enhance crop resilience, improve the ability of crops to absorb nutrients, and realize the effect of increasing yield and efficiency. However, it has also been shown that there was no significant increase in soil enzyme activities as influenced by environmental factors [19–21].

In this project, a strain of M. *marquandii* was isolated from the understory apomictic material in the Great Xing 'an Range in the preliminary study and was identified morphologically and by 16sRNA and named as M. *marquandii* SGSF043. Few studies have been reported on the growth promotion of field crops by M. *marquandii*. In this study, the seeds of "Meiyu" were soaked with the fermentation of M. *marquandii*. SGSF043.Respectively, to study the effect of this strain on the germination of corn seeds and the growth of seedlings, and at the same time, the inter-root soil enzyme activity of corn seedlings was measured, to clarify the role of M. *marquandii*. as well as M. *marquandii* on the promotion of maize and the role of the soil environment of the inter-root zone of seedlings, and the effect of M. *marquandii*. on the growth and development of maize seedlings was clarified. The results of the study are intended to provide a theoretical basis for further exploring the potential advantages of *Metarhizium* as a microbial fertilizer for crops.2. Materials and Methods

2.1. Experimental Strain

M. marquandii SGSF043. Isolated from litterfall in Nanweng River National Nature Reserve in the Great Xing ‘an Range of Heilongjiang province, China. The main plant species are *Tilia amurensis* Rupr. *Quercus mongolica* Fisch. *exTurcz* and *Fraxinus mandshurica* Rupr. The litter was divided into undecomposed, semi-decomposed, and decomposed layers from top to bottom and the samples were collected in layers. It was brought back to the laboratory and placed in a cool place then dried naturally for isolation and purification. The above strain is kept in the Mycological Collection Center of Wuhan University. (CCTCC No.M2020555).

Source of Experimental Maize: The “Meiyu” sweet and glutinous corn variety, with a harvest period of 75-80 days, was purchased from the seed market and is a common corn variety in Harbin.

2.2. Media Formulation and Fermentation Cultivation

Potato Dextrose Broth Medium (PDB): Weigh 25.0 g of this product in 1L of distilled water, heat, and boil until completely dissolved, pH 8.0, divided, autoclaved at 121℃ for 20 min, ready for use.

Culture conditions for the fermentation broth of *M. marquandii* SGSF043: The activated *M. marquandii* SGSF043 was transferred to PDB liquid medium (150 mL/bottle) and placed in a constant temperature shaker at 25 °C, 180 r·min^{-1} dark condition for 14d. The cultured fungal fermentation broth was filtered through sterile filter paper to obtain the spore-containing fermentation broth, in which the effective amount of viable fungal was measured to be 4.12×10^{9} CFU/mL.

2.3. Experimental Design

 doi:10.20944/preprints202401.2147.v1

The experiment was conducted in August 2022 in the greenhouse of Heilongjiang University. Maize seeds with full were selected to be sterilized by 70% alcohol for 30s and rinsed with sterile water for 5 times. For each sample of 30 maize seeds, before germination, the seeds were soaked in different treatments of fungal solution for 24h, and the soaked seeds were put into 28℃ incubators for germination. A total of five treatments were set up in the experiment: fungus filtrate was diluted with sterile water at 0 (Fermentation Solution); 10; 100; 1,000 times; PDB medium blank control, and three replications of each treatment.

2.4. Effect of Seed Soaking in Fermentation Solution on Germination of Maize Seeds

For each sample of 30 maize seeds, the seeds were immersed in different treatments of Fungal solution for 24 h before germination and subsequently placed in a glass petridish with sterilized filter paper for germination.

The germination was recorded as germination starting from the time when the radicle and endosperm leaked out, and the germination of the maize seeds was observed and recorded daily to calculate the germination potential (%), germination rate (%), and germination index. At the end of the seed germination test the radicle length, radicle thickness, radicle length, radicle thickness, and radicle length of young maize shoots were determined using a straightedge [22].

Germination potential (%) GP/%=$\frac{A_{2d}}{A_t} \times \mathbf{100}$ (1)

In the formula, "GP "is for germination potential（%）; "A_{2d}"is the amount of germination seeds in the first two days; "A_t "is the total amount of test seeds.

Germination rate (%) GE/%=$\frac{A_c}{A_t} \times \mathbf{100}$ (2)

In the formula, "GE" is germination rate（%）; "A_c" is the amount of germinated seeds when the seed reaches full germination; "A_t" is the total amount of test seeds.

Germination Index GI=$\sum(\boldsymbol{G_t/D_t})$ (3)

GI is the germination index; "G_t" is the amount of germination on day t; "D_t "is the corresponding amount of days to germination.

2.5. Determination of Biomass in Maize Seedlings

Seeds were treated with fungus solution before sowing in field plot trials, and samples were taken 20 d after corn sowing, five seedlings were selected for each treatment, and the roots of the plants were cleaned with tap water and the surface water was blotted out with filter paper to measure the plant height, root length, ground stem thickness, aboveground fresh weight, and belowground fresh weight.

2.6. Determination of Photosynthetic Indexes and Chlorophyll SPAD content in Maize Seedlings

Photosynthetic gas exchange parameters were determined using a LI-6400 photosynthesizer (USA). On September 2, 2022, from 9 a.m. to 11 a.m., plants with consistent growth were selected in each treatment to determine the relevant indexes, and each treatment was measured three times. The measured indices included net photosynthetic rate (*A*), transpiration rate (*E*), intercellular CO_2 concentration (*Ci*), and stomatal conductance (GH_2O).

The SPAD values of maize leaves were measured using a chlorophyll meter (FK-YL01) in the middle of the maize functional leaves, and each treatment was measured six times.

2.7. Determination of Inter-root Soil Enzyme Activities in Maize Seedlings

Soil Alkaline Phosphatase Kit (Solarbio LIFE SCIENCES, BC0285), Soil Neutral Protease Kit (Solarbio LIFE SCIENCES, BC0275), and Soil Uidase kit (Solarbio LIFE SCIENCES, BC0125) were

 doi:10.20944/preprints202401.2147.v1

used to determine the Enzyme activity in rhizosphere soil of maize seedlings in according to the manufacturer's instructions.

2.8. Statistical Analysis

Microsoft Excel and SPSS 27 were used for data processing and statistical analysis and GraphPad Prism 9 software package was used for plotting. The significance of differences between groups was tested at the 0.05 level by the LSD test in one-way ANOVA (one-way analysis of variance).

3. Results

3.1. Effect of Fermentation Solution Soaking on Germination of Maize Seeds

The data in Tables 1 and 2 show that the fermentation broth (Filtrate Stock,0) treatment group had some inhibitory effect on seed germination. Table 1 Among all the dilution treatments, the highest seed germination rate of 97% was recorded in the group treated with 10 times dilution of solution and was significantly higher than the other treatment groups with a germination time of 1 d ($P<0.05$). The differences in germination potential, germination index, and Control (PDB liquid medium) between the 10 times dilution and 1000 times dilution treatment groups were not significant ($P>0.05$) and were higher than the 100 times dilution treatment group.

As can be seen from the group diagram of some samples 2d after soaking in Figure 1, among all the dilution multiples, the embryo buds and embryonic axes of seeds in the treatment groups of 100 times fermentation solution and 1,000 times fermentation solution had the best growth, and with the increase of the dilution multiples of the fungal solution, the number of white lateral roots around the main roots of the seeds increased gradually in the form of a regular radial shape. Combined with the data in Table 2, it can be seen that the radicle thickness, radicle length, endosperm thickness, and embryonic axis length of maize seeds in the treatment group of 1,000 times dilution increased compared to the other treatment groups. They increased by 13.8%, 32.5%, 10.43%, and 27.1%, compared with the control group respectively. Among them, the greatest effect was on the germ length and embryonic axis length of maize seeds.

Table 1. Effect of fermentation solution on germination rate of corn seeds.

Treatment	Dilution times	Germination days/d	Germinating energy/%	Germination percentage/%	Germination index
	0	2	37±0.10c	15±1.90c	5.50±0.10c
M. Marquandii	10	1	97±0.10a	97±0.69a	14.53±0.06a
SGSF043	100	2	94±0.24b	94±1.39b	14.00±0.00b
	1000	2	97±0.10a	94±0.84b	14.50±0.10a
Control （PDB）	-	1	97±0.15a	94±2.12b	14.47±0.06a

The results are presented as the mean±standard deviation of three replicates. Different letters indicate the difference between different treatment groups ($P<0.05$)

Table 2. Effect of fermentation broth on the growth of corn seed embryos.

Treatment	Dilution times	Radicle length （cm）	Radicle coarse （cm）	Plumule length （cm）	Plumule coarse （cm）	Hypocotyl length （cm）
M. Marquandii	0	0.46±1.41c	0.19±0.56c	0.98±1.46d	0.54±0.76c	0.42±1.10c
SGSF043	10	12.16±3.48a	1.68±0.64ab	5.17±1.81ab	1.83±0.64ab	3.10±1.24b

 doi:10.20944/preprints202401.2147.v1

	100	8.17±4.36b	1.37±0.78b	4.58±2.76bc	1.59±0.77b	3.13±1.71b
	1000	11.38±4.51a	1.74±0.66a	5.56±1.73a	2.02±0.48a	4.07±1.24a
Control （PDB）	—	11.37±1.11a	1.52±0.41ab	4.20±1.39c	1.83±0.43ab	3.2±0.75b

The results are presented as the mean±standard deviation of three replicates. Different letters indicate the difference between different treatment groups (P<0.05)

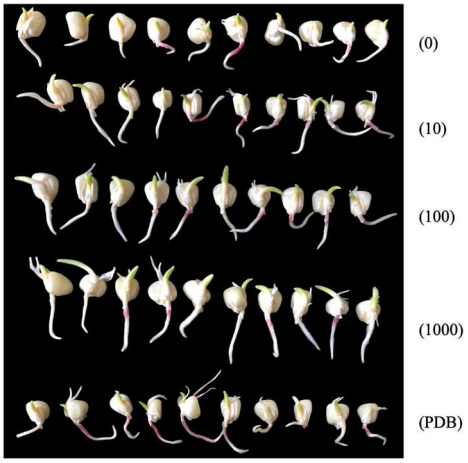

Figure 1. Effect of *M. marquandii* SGSF043 on germination of maize (Two days after germination). 0, *M. marquandii* SGSF043 fermentation Liquid; 10 ,10 times dilution of *M. marquandii* SGSF043; 100, 100 times dilution of *M. marquandii* SGSF043; 1000, 1,000 times dilution of *M. marquandii* SGSF043; PDB, Blank control.

3.2. Effect of Maize Seedling Biomass

A box-and-line plot was chosen to statistically graph the degree of data dispersion for each indicator of maize seedling biomass. Figure 2 shows the effect of *M. marquandii* on the biomass of maize seedlings, indicating that different dilutions of the fermentation filtrate of the test strain can promote the growth of maize seedlings, and the indicators of plant height, root length, diameter and thickness, aboveground fresh weight and underground fresh weight are higher than those of the control treatment. Different dilution concentrations had different effects on the seedling morphology of maize plants.

In Figure 2 (a), it can be seen that the difference in plant height between the 10 times dilution treatment group and the control group was significant (P<0.05) and also slightly higher than the 100 times and 1,000 times treatment groups. The relatively large dispersion of the 1,000 times plant height data indicates that the data fluctuated within the group.

The median of 10 times dilution was 58.5 cm, 100 times dilution was 52.9 cm, 1,000 times dilution was 53.4 cm, and the median of the control group was 48.4 cm. The height of maize seedlings in each fungal dilution treatment group was higher than that of the control treatment group, which was increased by 20.90%, 9.30%, and 10.33%, compared with the control group, respectively.

In Figure 2 (b), it can be seen that the root length of plants in all dilution treatment groups differed significantly (P<0.05) from that of the control group. 100 times solution root length data were more discrete, suggesting that the data fluctuated within the group. The median of 10 times dilution was 17.3 cm, the median of 100 times dilution was 14.7 cm, the median of 1,000 times dilution was 16.6 cm, and the median of the control group was 10.2 cm. The height of maize seedlings in each bacterial dilution group was higher than that of the control group, which was increased by 69.61%, 30.61%, and 62.75% compared with that of the control group, respectively.

In Figure 2(c), it can be seen that the difference between the above-ground fresh weight of the 100 times dilution treatment group and the other treatment groups was significant (P<0.05). The

 doi:10.20944/preprints202401.2147.v1

median of 10 times dilution was 8.425 g, 100 times dilution was 12.04 g, 1000 times dilution was 8.791 g, and the median of control treatment was 5.356 g. The above-ground fresh weight of maize in each dilution treatment group was increased by 57.3%, 125.8%, and 64.13%, compared with the control group, respectively.

In Figure 2 (d), the difference between the underground fresh weight of 100 times dilution treatment group and 1,000 times dilution treatment group was not significant (P>0.05). The median of 10 times dilution was 0.742 g, 100 times dilution was 1.038 g, 1000 times dilution was 0.943 g, and the median of control treatment was 0.457 g. The above-ground fresh weight of maize in the treatment groups of each bacterial dilution was increased by 62.36%, 127.13%, and 106.35%, compared with the control group, respectively.

From Figure 2(e), it can be seen that the maize seedling ground diameter thickness of each dilution treatment group was higher than that of the control treatment group, and the data were stable within the group. The median of 10 times dilution was 8.76 mm, 100 times dilution was 7.89 mm, 1,000 times dilution was 8.2 mm, and the median of the control treatment group was 6.6mm, and the treatments of each dilution increased the diameter of the ground roughness by 32.73%, 19.55%, and 24.24%, compared with the control group, respectively.

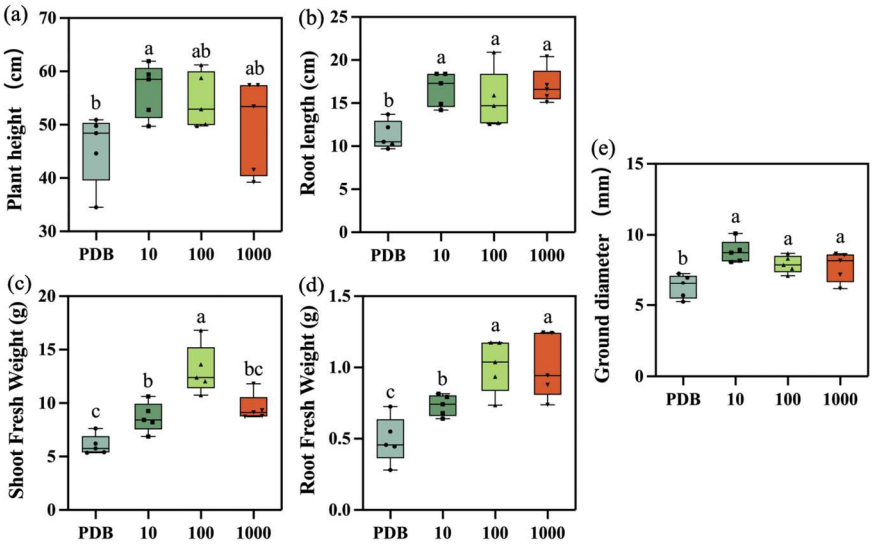

Figure 2. Effect of Maize Seedling Biomass. (a) Plant height; (b) Root length; (c) Shoot Fresh Weight; (d) Root Fresh Weight; (e) Ground diameter. The results are presented as the mean±standard deviation of three replicates. Different letters indicate the difference between different treatment groups (P<0.05).

3.3. Effect of Photosynthetic Indexes and Chlorophyll SPAD in Maize Seedlings

Different dilution treatments of the fermentation filtrate of *M. marquandii* had an effect on the photosynthetic properties of maize seedling leaves. Among them, different dilutions of fermentation solution increased the net photosynthetic rate (A) of maize seedlings (Figure a), which differed significantly (P<0.05) compared with the treatment of soaking in PDB sterilized liquid medium, and the data within the group of 10 times dilution of the fermentation solution were stable compared with those of the 100 times dilution treatment group and the 1,000 times dilution treatment group.

The transpiration rate (*E*) (Figure b) and stomatal conductance (GH_2O) (Figure d) of the plants in the 100 times dilution treatment group differed significantly (P<0.05) from those of the control treatment group. The transpiration rate of plants corresponding to 10 times dilution, 100 times dilution, and 1,000 times dilution was the most significant at 3.04 mmol/(m^2·s), 3.48 mmol/(m^2·s), and 2.5 mmol/(m^2·s), compared to 1.66 mmol/(m^2·s) in the control treatment group, respectively; The highest leaf stomatal conductance of 132.6 mmol/(m^2·s) was recorded for the 100 times dilution.

 doi:10.20944/preprints202401.2147.v1

The control treatment group plants had the highest interstitial carbon dioxide concentration (*Ci*) of 121 μmol/(m^2·s), and the 1,000 times dilution corresponded to the lowest interstitial carbon dioxide concentration (*Ci*) of 57.4 μmol/(m^2·s). The interstitial carbon dioxide concentrations (*Ci*) of the plants in the 10 times dilution and the 100 times dilution were 73.2 μmol/(m^2·s), and 85.4 μmol/(m^2·s), respectively.

The highest chlorophyll SPAD value of 41.12 was recorded for maize seedlings in the 100 times treatment group; 40.94 for the 10 times treatment group; 38.62 for the 1,000 times treatment group; and 38.92 for the control treatment group.

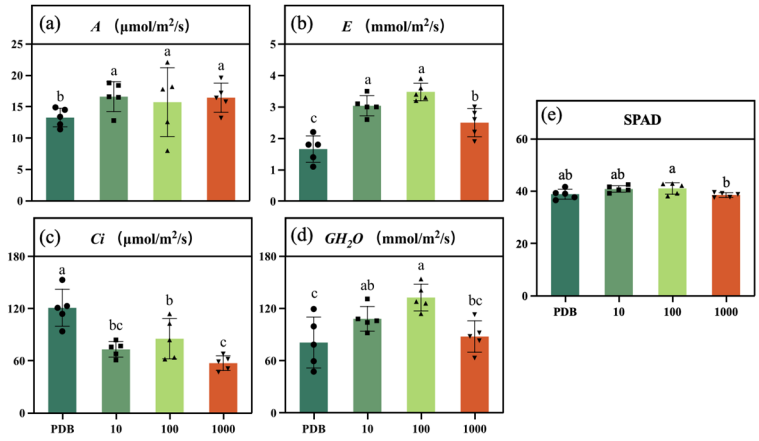

Figure 3. Effect of Maize Seedling Biomass. (a) *A*;(b) *E*;(c) *Ci*;(d) GH_2O;(e) SPAD. The results are presented as the mean±standard deviation of three replicates. Different letters indicate the difference between different treatment groups (P<0.05).

3.4. Inter-root Soil Enzyme Activities in Maize Seedlings

Soil alkaline phosphatase (Figure 4a), soil neutral protease (Figure 4b), and soil urease (Figure 4c) were measured in the inter-root of maize seedlings in different treatment groups. Differences between treatments in soil alkaline phosphatase and soil urease were not significant (P>0.05). Soil neutral protease (Figure 4b) was significantly (P<0.05) higher in the 100 times dilution neutral protease than in the other three treatment groups. The difference between 10 times dilution and 1,000 times dilution was not significant (P>0.05) and the values were lower than the control treatment group.

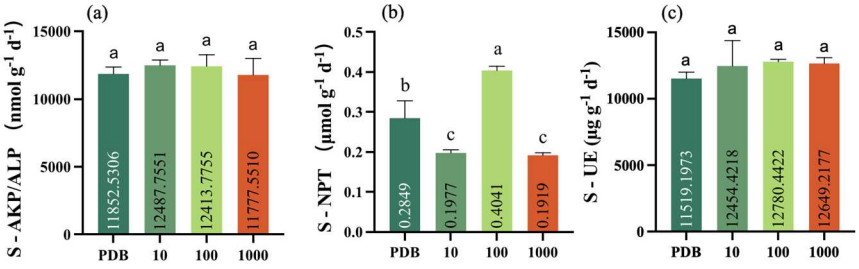

Figure 4. Inter-Root Soil Enzyme Activities in Maize Seedlings. (a) S-AKP/ALP;(b) S-NPT; (c) S-UE. The results are presented as the mean±standard deviation of three replicates. Different letters indicate the difference between different treatment groups (P<0.05).

4. Discussion

It has been shown that the association between *Metarhizium* spp. and plants and insects is mainly based on the nutritional basis [23]. *Metarhizium* spp. can transfer nitrogen produced by insects to plants for plant growth by parasitizing and killing the insects [8]. Plants provide carbon for

Metarhizium spp. Through photosynthesis [24], and vigorously growing plants can provide a more stable habitat for *Metarhizium* spp. , while the parasitic nature of *Metarhizium* spp. can further protect the healthy growth of plants. *Marquandomyces Marquandii* belongs to the genus of non-core *Metarhizium* spp. which are widely present in soil. *Marquandomyces Marquandii* belongs to the genus of non-core *Metarhizium* spp. which are widely present in soil. Some studies have shown that the secondary metabolites of *M. marquandii* not only have antimicrobial and antitumor activities [25], but also have the potential to be used in the removal of environmental pollutants (heavy metal pollution and pesticide residues, etc.), the control of crop pests and diseases, and the promotion of plant growth [26,27]. However, only a few studies have been reported on the plant growth promotion of this strain.

The experimental study was undertaken using the pre-existing Isolation and identification of *M. marquandii* SGSF043 from the Litter under the forest. Diluting the fungal fermentation broth at different multiples in the growth stabilization period and soaking the seeds, it was found that the seeds of undiluted fungal fermentation broth did not germinate, which indicated that the fermentation broth of *M.marquandii* SGSF043 had an inhibitory effect on the germination of maize seeds. The fermentation solution diluted by sterile water began to show the promotion effect on seed germination, among which, the highest seed germination rate was found in the group treated with 10 times dilution of bacterial solution, and the germination time was 1 d. After the seed germination test was completed, we continued to observe the growth of the seed germ and found that the germ and embryonic axis of seeds in the groups treated with 100 times fermentation solution and 1,000 times fermentation solution were the best in growth, and the seeds with 1,000 times dilution of seed had the most root hairs on the lateral root, which The seeds of 1,000 times dilution had the most lateral root hairs, which were in regular radial shape. Moreover, many studies have demonstrated that exogenous microorganisms can influence the root system configuration through colonization of the inter-root zone, mainly by affecting the growth of primary roots, lateral roots, and root hairs [28]. This is basically consistent with the results of the present study.

During the seedling growth period, different dilutions of the fermentation broth of *Marquandomyces marquandii* can promote the increase of various indicators of maize seedling height, root length, ground diameter, aboveground and underground fresh weight, and have different effects on the root morphology of maize seedlings. The growth indicators of the bacterial fermentation broth treatment group were better than those of the control group. In general, when the seeds soaked in the fermentation broth were planted into the soil, the 100 times dilution was the best for plant growth and development. The growth promotion of plants is first manifested in the changes of roots. Roots are not only an absorption organ, but also an organ that senses environmental changes. The growth and development of plant roots are closely related to the environment [29]. After the ermentation broth treatment, the root length of the seedlings is longer than that of the control group. The fresh weight of corn stems and roots in 100 times dilution solution is significantly higher than that of other treatment groups. With the change of environment, the seeds after the same treatment are cultured in Petri dishes and soil respectively, As the environment becomes more complex, the impact of *Marquandomyces marquandii* on plants has also changed accordingly. For example, the 1,000 times dilution in the Petri dish promotes the growth of seed germ and radicle. The best growth and development index of seedlings in the soil is 100 times dilution, and the required concentration of *Marquandomyces marquandii* increases. Therefore, it is presumed that *Marquandomyces marquandii* SGSF-043 may achieve the growth-promoting effect on maize by regulating the expression of genes related to lateral root formation, auxin synthesis, and transportation in maize, which needs further research through molecular research methods.

In this experiment, we found that the leaf *A* (Net Photosynthetic Rate), *E* (Transpiration Rate), and GH_2O (Stomatal Conductance) of each treatment group of the fermentation solution were higher than those of the treatment group, and the maize soaked by *M. marquandii* SGSF-043 could improve the photosynthetic capacity by increasing the photosynthetic gas exchange capacity and water uptake, and this is also consistent with the findings of the previous study mentioned that when the host endophytic fungi infested the plant roots, the fungus could consume the photosynthesized products in the host body to make the photosynthesis increase [30]. The interesting finding is that the *Ci*

(Intercellular carbon Dioxide Concentration) of each treatment group of the fermentation solution was lower than that of the blank control treatment group, which is hypothesized in conjunction with the results of the experiments corresponding to the other photosynthetic indexes, probably when photosynthesis is accelerated, the plant absorbs more carbon dioxide for photosynthesis from the environment and from other cells, which makes the intracellular inter-cellular carbon dioxide concentration lower, and the process of the plant absorbing carbon dioxide and water and converting them into chemical energy (glucose and oxygen) under the action of light. On the other hand, the cause of the decrease in intercellular CO_2 concentration could also be the accelerated rate of CO_2 consumption by the plant, resulting in a slower rate of CO_2 production. If the respiration of plants, plant chlorophyll content is one of the main indicators of the photosynthetic capacity of plants [31]. In the results of this study, the chlorophyll content of the plants in the treatment groups of fermentation solution did not differ much from the chlorophyll content of the control group, which is slightly different from the results of previous studies.

The addition of beneficial microorganisms can directly or indirectly with the microorganisms in the soil around the roots to varying degrees to increase soil enzyme activity, while closely related to the organic matter content and so on. Soil enzyme activities can reflect the type and strength of biochemical reactions in soil [32]. In this experiment, the inter-root soil alkaline phosphatase and soil urease contents of the fermentation solution treatment group were slightly higher than those of the control group. Soil-neutral protease content was significantly higher in the fermentation solution diluted 100 times in the treatment than in the other treatment groups. Inorganic nitrogen is released through the mineralization of organic nitrogen, which is an important source of nitrogen for plant growth. Soil inorganic nitrogen content characterizes the soil's ability to supply nitrogen. It has been shown that the highest neutral protease activity was found in soil with ammonium nitrogen alone, while the neutral protease activity was enhanced with the increase of ammonium nitrogen percentage [33]. It can be hypothesized that *M. marquandii* may produce inorganic ammonium nitrogen or recruit beneficial microorganisms that can produce ammonium nitrogen, thus directly or indirectly promoting the uptake and utilization of nutrients in the soil by plants, and the mechanisms related to the plant-promoting potential of *M.marquandii* will be investigated in more detail in the future.

5. Conclusions

The fermentation broth of *Marquandomyces marquandii* SGSF-043 can promote the germination of maize seeds and increase the biomass of corn seedlings at a suitable concentration. In the maize seed germination tests, the seed germination effect treated with 10 times dilution of bacteria fermentation broth was the best one. After the seed germination test, as the seed germs continued to grow, the radicle length, radicle diameter, germ length, germ diameter, and hypocotyl length of the seed were measured. It was found that the 100 times dilution and 1000 times dilution treatment groups had the best effect. Among them, the 1,000 times dilution had the most obvious effect on the elongation and growth of lateral roots around the seed main root, which increased by 13.8%, 32.5%, 10.43%, and 27.1% respectively compared with the control group. According to the field plot test, there were no significant differences in chlorophyll content among these treatments. According to the measurement data of maize growth and development, photosynthesis, and rhizosphere soil-related enzyme activities, it can be determined that the best effect of promoting corn growth and development is when the fermentation broth of *Marquandomyces marquandii* is diluted 100 times. It shows that *Marquandomyces marquandii* has the potential for development and utilization, which can be further studied and applied.

Author Contributions: Conceptualization, Bo Zhang and Feng Shi; data curation, Yuanlong Chen; formal analysis, Yuanlong Chen; methodology, Feng Shi and Yuanlong Chen; data curation, Bo Zhang, Xiumei Zhao; writing—original draft preparation, Xiumei Zhao; writing—review and editing, Xiumei Zhao; supervision. All authors have read and agreed to the published version of the manuscript.

Funding: This research has been carried out with the financial assistance of a grant from Heilongjiang Province for the funding of the scientific research project (CZKYF2021-2-C016); Xu Zheng was supported by an

experimental condition from Qiqihar Branch of Heilongjiang Academy of Agricultural Sciences &Engineering Research Center of Agricultural Microbiology Technology, Ministry of Education &Heilongjiang Provincial Key Laboratory of Ecological Restoration and Resource Utilization for Cold Region &Key Laboratory of Microbiology, College of Heilongjiang Province &Key Laboratory of Molecular Biology, College of Heilongjiang Province & School of Life Sciences, Heilongjiang University.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are contained within this article.

Acknowledgments: Bo Zhang and Feng Shi: Technical Support. Yuanlong Chen: Providing language help. Xiumei Zhao: Proofreading the article.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Zhao L, Xu Y, Chang J, Li M, Zhang Y, Dang Y, Wang M, Cheng Y, Zhang B. Screening, resistance and growth-promoting effect of endophytic bacteria with ACC deaminase activity isolated from soybean nodules. *Acta microbiologica Sinica.* 2016,56,6,1009-1021.
2. Xu J, Sun T, Li S. Application of microbial fertilizers in agricultural production of China. *Crops.* 2016,1,1-6.
3. Grasswitz TR. Integrated pest management (IPM) for small-scale farms in developed economies: Challenges and opportunities. *Insects.* 2019,10,6,179.
4. Ab Rahman SFS, Singh E, Pieterse CM, Schenk PM. Emerging microbial biocontrol strategies for plant pathogens. *Plant Science.* 2018,267,102-111.
5. Mansour, Ghorbanpour, Mahtab, Omidvari, Payman, Abbaszadeh-Dahaji, Reza, Omidvar, Khalil, Kariman. Mechanisms underlying the protective effects of beneficial fungi against plant diseases. *Biological Control Theory & Application in Pest Management.* 2018.
6. Maya, Raad, Travis, Glare, Helena, Brochero, Caroline, Müller. Transcriptional Reprogramming of Arabidopsis thaliana Defence Pathways by the Entomopathogen Beauveria bassiana Correlates With Resistance Against a Fungal Pathogen but Not Against Insects. *Frontiers in Microbiology.* 2019.
7. Index. In: Abd-Elsalam KA, Murugan K, eds. *Bio-Based Nanoemulsions for Agri-Food Applications.* Elsevier,2022,451-458.
8. Behie SW, Zelisko PM, Bidochka MJ. Endophytic Insect-Parasitic Fungi Translocate Nitrogen Directly from Insects to Plants. *Science.* 2012,336,1576-1577.
9. Vega FE, Goettel MS, Blackwell M, Chandler D, Jackson MA, Keller S, Koike M, Maniania NK, Monzon A, Ownley BH, Pell JK, Rangel DEN, Roy HE. Fungal entomopathogens: new insights on their ecology. *Fungal Ecology.* 2009,2,149-159.
10. Sorokin N. Plant parasites of man and animals as causes of infectious diseases. *J Military Med.* 1883,2, 268-291.
11. K SR, J BM. The insect-pathogenic fungus *Metarhizium* robertsii (Clavicipitaceae) is also an endophyte that stimulates plant root development. *American journal of botany.* 2012,99,1,101-107.
12. Pérez EG, Amaro MAO, Elihú B, Pablo DS, Francisco JBJ. The entomopathogenic fungus *Metarhizium* anisopliae enhances Arabidopsis, tomato, and maize plant growth. *Plant Physiology and Biochemistry.* 2022,176, 34-43.
13. Baron NC, Pollo AdS, Rigobelo EC. *Purpureocillium lilacinum* and *Metarhizium marquandii* as plant growth-promoting fungi. *Peerj.* 2020,8.
14. Elena GJ, Beatriz PJ, Alejandro P, Lecuona R. *Metarhizium* anisopliae (Metschnikoff) Sorokin promotes growth and has endophytic activity in tomato plants. *Adv Biol Res.* 2011,5,1,22-27.
15. Liao, XG, O'Brien, TR, Fang, WG, St, Leger, RJ. The plant beneficial effects of *Metarhizium* species correlate with their association with roots. *APPL MICROBIOL BIOT.* 2014,98,16,7089-7096.
16. Bangari MPS, Nataraja KN. Can endophytes minimize photosynthetic limitation? *Trends in Plant Science.* 2023.
17. Bais HP, Vepachedu R, Gilroy S, Callaway RM, Vivanco JM. Allelopathy and exotic plant invasion: From molecules and genes to species interactions. *Science.* 2003,5638,301.
18. Dali, Song, Xiangyin, Xi, Shaomin, Huang, Guoqing, Liang, Jingwen, Sun. Short-Term Responses of Soil Respiration and C-Cycle Enzyme Activities to Additions of Biochar and Urea in a Calcareous Soil. *PLOS ONE.* 2016,11,9.
19. Li D, Hockaday WC, Masiello CA, Alvarez PJJ. Earthworm avoidance of biochar can be mitigated by wetting. *Soil Biology & Biochemistry.* 2011,43,8,1732-1737.

20. Oleszczuk PJ, I.Futa, B.Pasieczna-Patkowska, S.Palys, E.Kraska, P. Effect of pesticides on microorganisms, enzymatic activity and plant in biochar-amended soil. *Geoderma: An International Journal of Soil Science.* 2014,214-215.
21. Xiao-Guang J, Chong-Sheng G, Guo-Hong L. Effect of Long-Term Fertilization on Soil Enzyme Activities Under Different Hydrothermal Conditions in Northeast China. *Agricultural sciences in China.* 2011,10,3,11.
22. Shagufta, Noor-un-Nisa, Jamali FA, Ahmad W, Ul-Allah S, Wahocho NA, Jamali MF, Shah SA. Comparative Effect of Varieties and Types of Containers on Seed Germination and Seedling Growth of Geranium (Palergonium graveolens). *Seeds.* 2023,2,1,165-176.
23. Barelli L, Moonjely S, Behie SW, Bidochka MJ. Fungi with multifunctional lifestyles: endophytic insect pathogenic fungi. *Plant Molecular Biology.* 2016,90,6,657-664.
24. Behie SW, Moreira CC, Sementchoukova I, Barelli L, Zelisko PM, Bidochka MJ. Carbon translocation from a plant to an insect-pathogenic endophytic fungus. *Nature Communications.* 2017,8.
25. Filipello Marchisio V, Curetti D, Cassinelli C, Bordese C. Keratinolytic and keratinophilic fungi in the soils of Papua New Guinea. *Mycopathologia.* 1991,115,2,113-119.
26. Mongkolsamrit S, Khonsanit A, Thanakitpipattana D, Tasanathai K, Luangsa-Ard J. Revisiting *Metarhizium* and the description of new species from Thailand. *Studies in Mycology.* 2020,95.
27. Szewczyk R, Sobon A, Slaba M, Dlugonski J. Mechanism study of alachlor biodegradation by Paecilomyces *marquandii* with proteomic and metabolomic methods. *Journal of Hazardous Materials.* 2015,291,52-64.
28. Tatematsu K. MASSUGU2 Encodes Aux/IAA19, an Auxin-Regulated Protein That Functions Together with the Transcriptional Activator NPH4/ARF7 to Regulate Differential Growth Responses of Hypocotyl and Formation of Lateral Roots in Arabidopsis thaliana. *THE PLANT CELL ONLINE.* 2004.
29. Oldroyd GED. Speak, friend, and enter: signalling systems that promote beneficial symbiotic associations in plants. *Nature Reviews Microbiology.* 2013,11,4,252-263.
30. Eisenhardt KM, Reuscher CM, Klug G. PcrX, an sRNA derived from the 3′-UTR of the Rhodobacter sphaeroides puf operon modulates expression of puf genes encoding proteins of the bacterial photosynthetic apparatus. *Molecular Microbiology.* 2018,110,3,325-334.
31. Pitman MG, Läuchli A. Global impact of salinity and agricultural ecosystems. *Salinity: environment-plants-molecules.* 2002,3,20.
32. Tu, C. M. Effect Of Four Experimental Insecticides On Enzyme Activities And Levels Of Adenosine Triphosphate In Mineral And Organic Soils. *Journal of Environmental Science & Health Part B.* 1990,25,6,787-800.
33. Christine, Wachendorf, Carola, Lampe, Friedhelm, Taube, Klaus, Dittert. Nitrous oxide emissions and dynamics of soil nitrogen under 15N-labeled cow urine and dung patches on a sandy grassland soil. *Journal of Plant Nutrition & Soil Science.* 2008.