Evaluation of wheat genotype responses to *Trichoderma afroharzianum* strain T22

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Independent project in Biology, A2E - Agriculture • 30 credits
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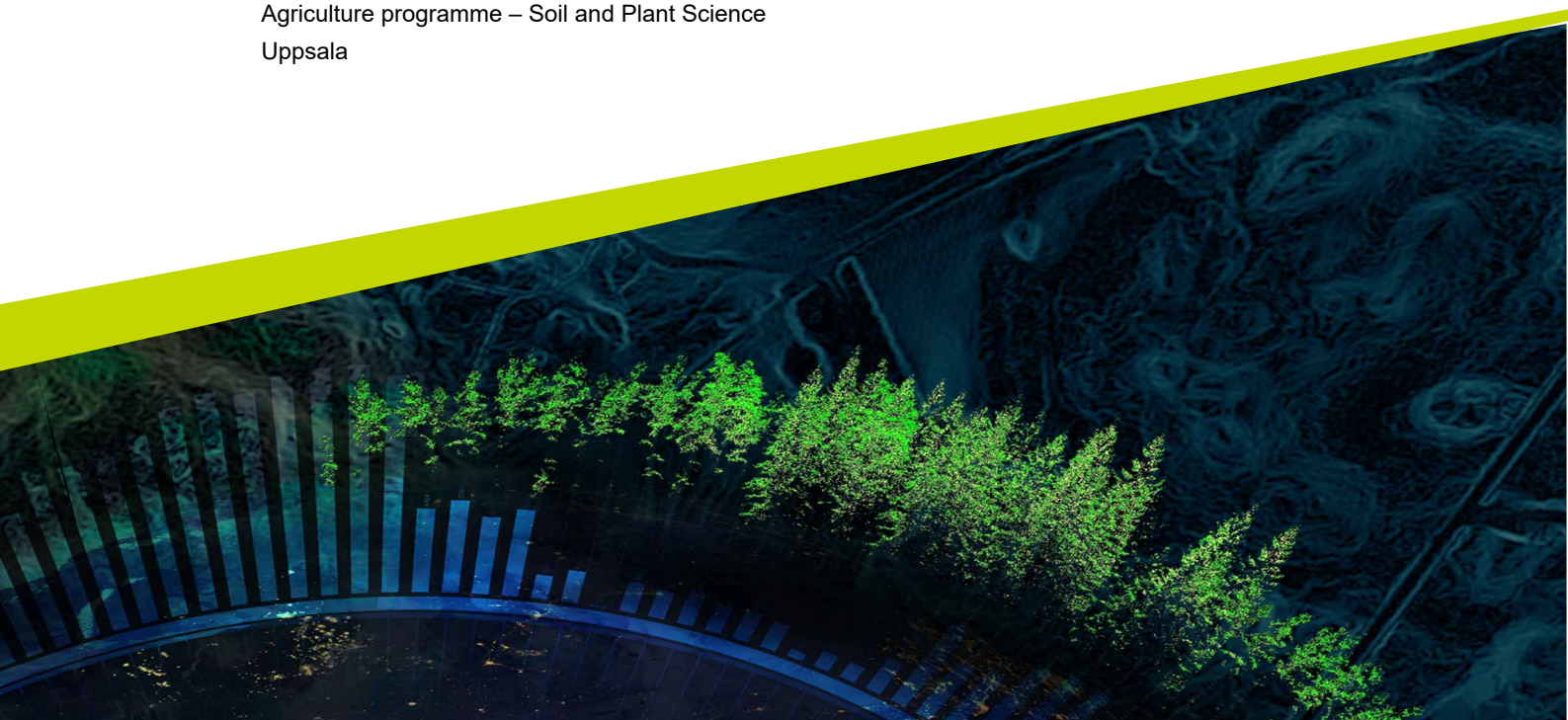

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Abstract

Trichoderma afroharzianum strain T22 is used in agriculture as a biocontrol agent and plant growth promoter. However, its effects can vary depending on the plant species and genotype. In this study, 190 winter wheat genotypes were evaluated for their differential responses to *T. afroharzianum* T22 inoculation and mock-treated controls. Growth traits (shoot length, root length, and dry weight) and disease incidence (root browning) were assessed under controlled conditions. The results showed that most wheat genotypes had a neutral response to inoculation, with no significant reduction in shoot and root length. Some genotypes had a negative response, whereas only a small number exhibited increased growth. Browning of roots and stems occurred more frequently in *Trichoderma*-treated plants, while similar but less frequent discoloration appeared in the mock treatment. Genotypes with more than 50% of plants showing browning frequently exhibited reduced growth, suggesting a potential link between browning of the roots and stem base and growth suppression. While this browning is not interpreted as a direct disease symptom, its increased occurrence in treated plants may be related to additional stress experienced by the roots during exposure to *Trichoderma*. Overall, these findings highlight that the effect of *T. afroharzianum* T22 on wheat is strongly genotype-dependent, with growth promotion being rare and growth inhibition being more prevalent. This underscores the need for careful genotype-specific evaluations before agricultural application.

Keywords: wheat, *Trichoderma afroharzianum*, T22, plant growth-promoting fungi, genotype variation, biocontrol, pathogenicity

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Abbreviations

ANOVA	Analysis of Variance
BCA	Biological Control Agent
BLUE	Best Linear Unbiased Estimate

1. Introduction

Global food demand is projected to increase by 35 –56% by 2050 compared to 2010. Wheat, one of the most important crops, accounts for approximately 20% of the global energy and protein intake (Kettlewell et al. 2023), and is cultivated on 200 million hectares worldwide (Ortiz et al. 2008). Key factors influencing wheat growth and grain development include temperature, water, and fertilizers (Filip et al. 2023).

Wheat cultivation faces significant challenges due to the decline in global arable land and climate change. Climate change increases many stressors, including heat stress, drought, flooding, and pests (Grote et al. 2021). At the same time, pesticide use must be reduced by 50% by 2030 (McGinley et al. 2023). Achieving this goal requires that we replace pesticides with alternative methods and products (Chaudhary et al. 2025).

Various approaches have been employed to address these challenges, including the application of biological control agents and plant growth-promoting microorganisms (Goh et al. 2013), as well as breeding disease-resistant crop genotypes (Grote et al. 2021). Due the global importance of wheat, extensive research has been conducted on wheat species to understand their specific genotypic traits (Filip et al. 2023).

1.1 Growth promoting microbes

Plant roots release a variety of chemical signals that attract beneficial microbes, initiating symbiotic relationships in which these microorganisms attach to, penetrate, and colonize the root system (Sood et al. 2020). Such microbe-mediated associations are important for improving the plant health, as plant growth–promoting microbes collectively enhance soil quality and increase plant tolerance to both biotic and abiotic stressors (Lugtenberg & Kamilova 2009). By improving nutrient availability and plant growth, agricultural practices can be made more resilient and resource-efficient by making them less reliant on synthetic fertilizers and pesticides (Lugtenberg & Kamilova 2009).

1.2 *Trichoderma* spp.

Trichoderma spp. are a group of free-living filamentous ascomycetous fungi belonging to the class Deuteromycetes (Contreras-Cornejo et al. 2024). This genus, typically known for producing asexual spores, is commonly found in soil (Daulatbhai Vasait et al. 2023), on plant roots, and on decaying plant debris (Pfordt et al. 2023) in almost all terrestrial ecosystems (Contreras-Cornejo et al.

2024). Initially, *Trichoderma* was primarily regarded as a soil saprotroph (Del Carmen H. Rodríguez et al. 2021) due to its ability to decompose organic matter, particularly dead fungi and oomycetes. However, with advancing knowledge, the genus is now also recognized for its mycoparasitic nature and its capacity to interact with plants, including rhizosphere colonization and endophytism, depending on the species (Woo et al. 2023).

Trichoderma-based products are increasingly valued in agriculture for their ability to enhance soil health, promote plant growth (Saadaoui et al. 2023), and increase yields (Stewart & Hill 2014). These products can be applied in various forms, such as soil inoculants, seed treatments, biopesticides, and biofertilizers. *Trichoderma* spp. are commonly found in the rhizosphere, and certain species can colonize plant roots and exist as endophytes (Saadaoui et al. 2023). While Trichoderma-based products are widely used in agriculture for their ability to enhance plant growth and increase yields (Saadaoui et al. 2023), the potential risks to non-target organisms, such as pollinators, remain uncertain. Laboratory studies on *Bombus terrestris* exposed to commercial *Trichoderma* formulations found no lethal or sublethal effects on adult worker bees or larvae, and no fungal growth on bee bodies (Mommaerts et al. 2008). However, the diversity of *Trichoderma* species and formulations, as well as differences in field exposure conditions, mean that possible negative effects on pollinators cannot be entirely ruled out, and further research under realistic field conditions is needed.

Many species within the genus *Trichoderma* are known to produce up to 1,000 secondary metabolites, many of which possess antibacterial, antifungal, and growth-promoting properties (Mommaerts et al. 2008). The genus suppresses pathogen growth both directly through hyperparasitism, antibiosis, and competition for space and nutrients, and indirectly by enhancing plant resilience to biotic and abiotic stresses, as well as by improving nutrient uptake and growth (Sood et al. 2020). Some species of *Trichoderma* are among the most commonly used fungal biocontrol agents (BCAs), accounting for approximately 90% of commercially available fungal BCAs (Modrzewska et al. 2022). *T. atroviride*, *T. virens*, *T. afroharzianum*, *T. longibrachiatum*, *T. reesei*, and *T. gamsii* are among the species most commonly used as biological control agents and for other practical applications (Modrzewska et al. 2022). Studies across 26 *Trichoderma* species have shown that *T. afroharzianum* has the highest number of unique metabolites, most of which have antifungal properties and many of which promote plant growth (Rush et al. 2021).

Trichoderma species and even individual isolates exhibit significant variation in their phytohormone-related biosynthetic genes. These genes, and the production of phytohormone-like compounds, have been linked to root colonization, hyphal growth promotion of plant performance under abiotic stress, and activation of the antioxidant machinery in plants (Woo et al. 2023). Among

the phytohormones influenced by *Trichoderma*, gibberellins play a role in regulating plant growth and development, including seed germination, flowering, stem extension, and aging. During cereal germination, they also stimulate the production of hydrolytic enzymes, improving nutrient mobilization. The fungus has also shown the ability to solubilize insoluble phosphate, increasing nutrient availability and further promoting plant growth (Abdenaceur et al. 2022).

Trichoderma has also been reported to modulate auxin transport within plants, thereby promoting plant growth. However, excessive auxin accumulation, caused by Trichoderma-mediated rhizosphere acidification, can lead to inhibited root growth (Woo et al. 2023).

Many *Trichoderma* strains that influence plant growth and development through phytohormone modulation and nutrient solubilization are now commercially registered for their growth-promoting and plant-protective effects (Woo et al. 2023). For example, *T. atroviride* and *T. gamsii* are used as fungicides (EFSA Journal 2013; 2015) while *T. afroharzianum* T22 functions both as a fungicide and a growth-promoter (Lewis et al. 2016). Despite these benefits, Trichoderma-based products cannot currently be marketed as biostimulants in Europe. Regulations differ by country; in some places, *Trichoderma* can be sold as an inoculant or biostimulant without verified efficacy. There is an ongoing debate on how to regulate their use as both BCAs and biostimulants because their effects depend on the plant host and environmental conditions (Woo et al. 2023).

1.2.1 *Trichoderma harzianum* complex

The *Trichoderma harzianum* complex includes at least 14 species that can be found worldwide in many different environments growing on various substrates. It is commonly found in soil, on decomposing plants, on other fungi, and as an endophyte within plants (Chaverri et al. 2015). Species within the complex differ in traits, such as metabolite production and host range (Zhang et al. 2015), and is commonly used in biotechnology due to its effectiveness on controlling soilborne diseases and its growth promoting effect (Chaverri et al. 2015), and it is also applied as a BCA and growth promoter in agriculture (Lewis et al. 2016). The species complex was revised in 2015 to include at least 14 species, with *Trichoderma afroharzianum* (Figure 1.) described as a new species (Chaverri et al. 2015).

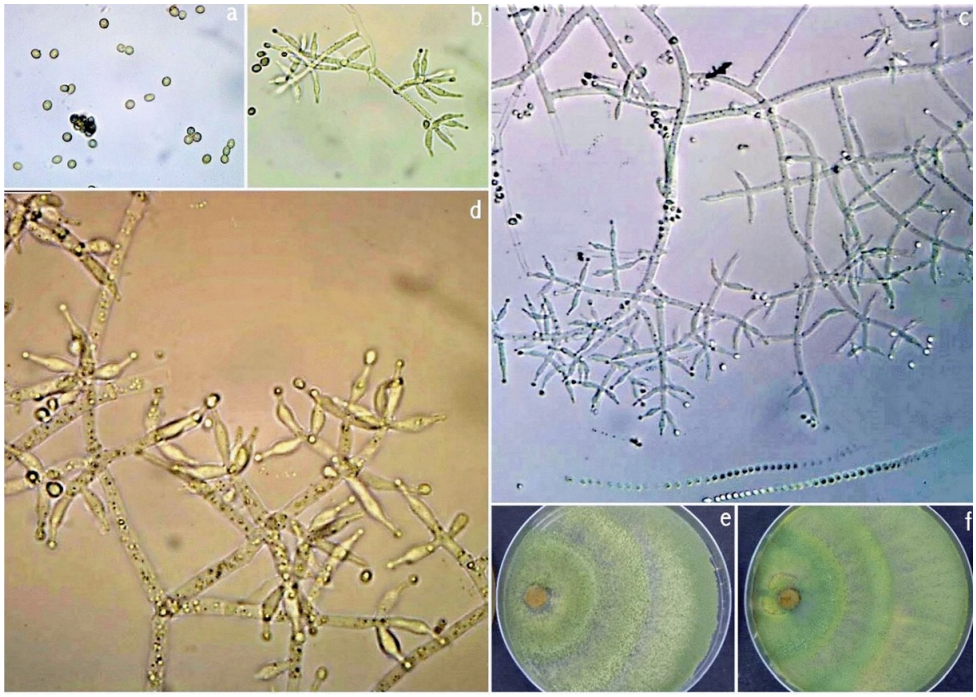


Figure 1. *Trichoderma afroharzianum. (a) Conidia, (b–d) Conidiophores and phialides, (e–f) (Jambhulkar et al. 2024)*

1.2.2 *Trichoderma afroharzianum* T22

Inoculation with *Trichoderma afroharzianum* T22 has been shown to increase growth and nutrient uptake in sorghum, specifically increasing Zn, Mn, Mg, and Ca in leaves and Fe in roots (Kabir et al. 2024), as well as in other crops such as sugar beet, maize and tomato. However, the effect has been shown to vary depending on the plant genotype. In sugar beet, maize and tomato, responses range from growth stimulation to growth inhibition, indicating genetic differences among the genotypes (Schmidt et al. 2020).

Sorghum inoculated with *T. afroharzianum* T22 showed increased expression of genes associated with increased auxin signalling, or accumulation. Auxin response factors regulate key developmental processes such as cell division, differentiation, and organ formation (Kabir et al. 2024). Inoculated sorghum roots also exhibit increased expression of genes involved in water and nutrient transport (Kabir et al. 2024). Additionally, genes involved in chloroplast function, CO_2 assimilation, and photosystem II activity, showed increased expression in sorghum inoculated with *T. afroharzianum* T22. However, the change in expressed genes in sorghum still require further studies to confirm their specific contributions to growth promotion (Kabir et al. 2024). In another study on cherry rootstocks, the strain was shown to significantly increase gibberellin (GA3) and indole-3-acetic acid (IAA) levels by 71% and 49% in leaves and by 143% and 40% in roots, respectively, 10 days after treatment (Contreras-Cornejo et al. 2024).

1.2.3 Pathogenicity of *T. afroharzianum* and its implications for plant and pollinator safety in the field

Despite the many beneficial effects observed in *Trichoderma,* there have been indications that some species in this genus can be pathogenic to maize, with the earliest report made in 1910. Since 2020, observations have been made in Europe, with *T. afroharzianum* causing ear rot disease in maize in Germany, Italy, and France (Pfordt et al. 2024). The disease in maize is marked by extensive mycelial growth and the production of green conidia between the kernels and on the outer husk surface. Infected cobs are typically smaller and show soft rot symptoms, along with premature germination of kernels within the husk leaves (Figure 2). Infection also reduced the germination rate, and resulted in stunted and deformed seedling growth, compared with the control. In addition to these visible symptoms, infections also reduce both the dry and fresh matter content (Pfordt et al. 2024).

Figure 2. *Disease symptoms (100% disease severity) of* Trichoderma *ear rot infection after artificial inoculation under field conditions on maize the cultivar Mallory. (A, B) Massive production of greenish spores on husk leaves and around the kernels after artificial inoculation; (C) early germination of infected kernels (Pfordt et al. 2024)*

The pathogenicity of *T. afroharzianum* in maize has raised concerns if the species can cause disease symptoms in other cereal crops (Pfordt et al. 2023), especially since the fungus thrives in dry weather and high temperatures, which is expected to occur more frequently with climate change (Pfordt et al. 2024). In a study on wheat, barley, and sorghum, *T. afroharzianum* was point-inoculated into the center of two florets. Distinct symptoms were observed two weeks after inoculation, such as tan or brown discoloration of the base of a floret within the spikelets. Later on, the infected spikelets developed a dark brown color, and the infection spread up the ear until all spikelets were discolored (Figure 3) (Pfordt et al. 2023).

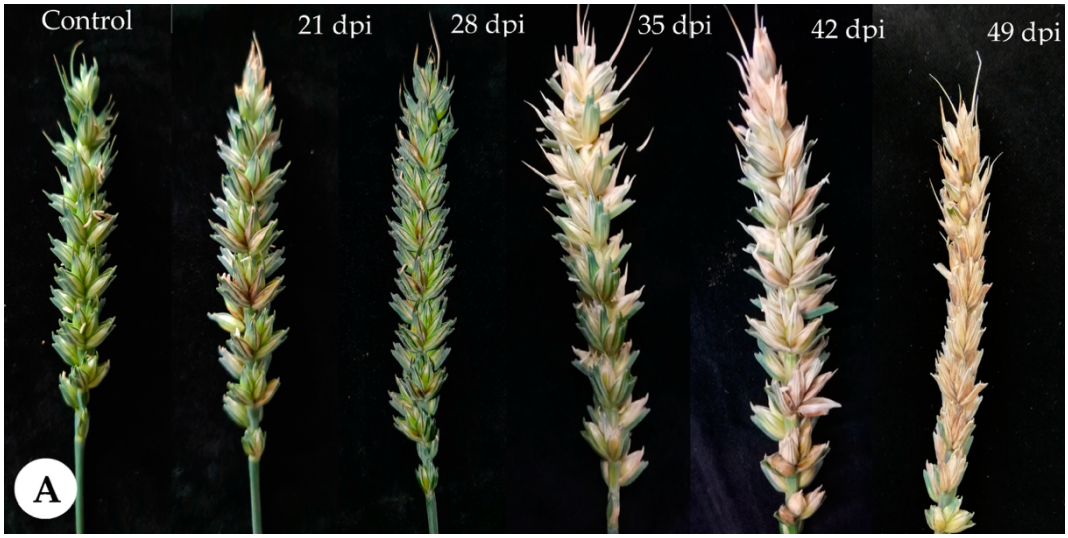


Figure 3. *Disease symptoms in spring wheat at 21, 28, 35, 42 and 49 days post inoculation with pathogenic T. afroharzianum isolates (Pfordt et al. 2023).*

In addition, some concerns have been raised regarding the potential risks of certain *Trichoderma* species to non-target organisms; however, studies on commercial Trichoderma-based biofungicides indicate minimal risk to pollinators. Laboratory evaluations of formulations containing *T. harzianum* showed no lethal or sublethal effects on *Bombus terrestris* worker bees (Mommaerts et al. 2008). While these results suggest that commercially used strains pose low risk to pollinators, other *Trichoderma* strains may have pathogenic potential, so their safety under field conditions should be carefully evaluate.

1.2.4 Phenotypic variation in wheat genotypes: Insights from previous research on the same panel used in this study

Several studies have used the same large panel of winter wheat genotypes to explore genetic variation in response to several different biotic and abiotic factors (Chaudhary et al. 2024). One of these studies observed significant variation among 190 genotypes for the biocontrol efficacy of *Clonostachys rosea* in controlling Fusarium foot rot caused by *Fusarium graminearum*. A positive correlation was also observed between disease susceptibility and plant genotype-dependent *C. rosea* biocontrol, indicating that more susceptible genotypes had a better effect against *F. graminearum* (Chaudhary et al. 2024).

A similar study of 183 genotypes in the same large genotype panel explored the efficiency of *C. rosea* as a BCA against septoria tritici blotch (STB) caused by *Zymoeptoria tritici.* A similar pattern was observed, but with a weak correlation, where more susceptible genotypes showed a better effect of *C. rosea* against STB. Furthermore, SNP markers linked to disease resistance against STB and SNP markers linked to biocontrol efficiency have been shown to be located on different chromosomes. This makes it possible to breed for more resistant

genotypes without decreasing the efficiency of *C. rosea.* (Chaudhary et al. 2025). A comparison between the two studies showed that genes linked to biocontrol efficiency against *F. graminearum* and *C. rosea* are located on different chromosomes, indicating that plant genotype-mediated biocontrol efficacy can be specific to different pathogens and/or different plant organs (Chaudhary et al. 2024).

2. Aim and objectives

The aim of this study was to investigate how winter wheat genotypes vary in their responses to the fungal biostimulant *Trichoderma afroharzianum* T22, focusing on its capacity to stimulate growth and any potential pathogenic effects.

Therefore, we hypothesized that (1) the growth-promoting effect of *T. afroharzianum* T22 will vary among wheat genotypes, and (2) If pathogenicity occurs, the severity and incidence of disease symptoms will be genotype-dependent, reflecting variation in host defense and susceptibility.

Accordingly, this study has two main objectives: (1) to evaluate the growth responses of 190 winter wheat genotypes following inoculation with *T. afroharzianum* T22, and (2) to examine potential pathogenicity and any associated disease manifestations following inoculation with *T. afroharzianum* T22.

3. Methods

3.1 Plant material

This study evaluated 190 winter wheat genotypes, comprising landraces and cultivars sourced from the Nordic Genetic Resources Center (Alnarp, Sweden) (Appendix 1). The material represents diverse germplasms collected from: Sweden, Afghanistan, Germany, Finland, Norway, and the Netherlands (Appendix 1). To reduce damage to the roots for further examination, wheat seeds were cultivated in pots filled with sand.

3.2 Fungal cultivation and inoculum preparation

Spores of *T. afroharzianum* T22 stored at minus 80 degrees were revived on PDA petri dishes and stored at 25 °C until growth had covered the plate and the mycelia turned green. Spores were collected by adding sterilized water to the colonies, scraping off the spores, and filtering them through a Mira cloth. The spore concentration was determined using a light microscope and hemocytometer using the Bürker chamber cell count method. The suspension was diluted to a concentration of $1*10^6$ colony-forming units per ml (cfu/ml).

3.3 Bioassay setup

Forty seeds from each genotype, stored at 4 °C, were distributed into Falcon tubes, with 20 seeds designated for the mock treatment and 20 seeds designated for T22 inoculation. All seeds were surface sterilized by immersion in 6% sodium hypochlorite (NaClO) for 5 minutes, followed by three rinses with sterile water. The experimental setup involved the following treatments:

I) seeds coated with *Trichoderma* at $1*10^6$ cfu/ml
II) seeds treated with sterile water (mock)

For the *Trichoderma* treatment, 10 ml of spore suspension was added to the inoculation tubes, and 10 ml of sterile water was added to the mock. To ensure uniform spore contact, all the tubes were placed on a rotary shaker at 110 rpm for 30 minutes. After the incubation, the suspensions were decanted.

To assess phenotypic variation among 190 winter wheat genotypes in response to *Trichoderma* treatment, an *in vivo* bioassay was performed across six batches. Each batch included a subset of genotypes. A randomized complete block design was implemented within each batch, with five trays per treatment assigned randomly, resulting in five biological replicates per genotype.

To control for potential variations between batches, three reference genotypes (Kranich, Stava, and Festival) were included in each tray. Each tray contained 40 plastic pots (5 × 5 × 5 cm) filled with moistened sand (Rådasand, 0.5–1 mm grain size). Two seeds were sown per pot and placed evenly in holes 2 cm deep and 1.5 cm wide (Figure 4 (A)). Trays were maintained in a growth chamber under controlled conditions: a 16-hour light period (200 µmol m^{-2} s^{-1}) and 8 hours of darkness at 20 °C (Figure 4 (B)). After 14 days, seedlings were harvested and measured for shoot and root length, then scored for disease symptoms using a 0–1 scale, where 0 indicated healthy plants without visible symptoms and 1 indicated the presence of root and stem browning. The harvested plants were dried in an oven for 3 days, and their dry weight was measured using a scale with a precision of 0.01 g.

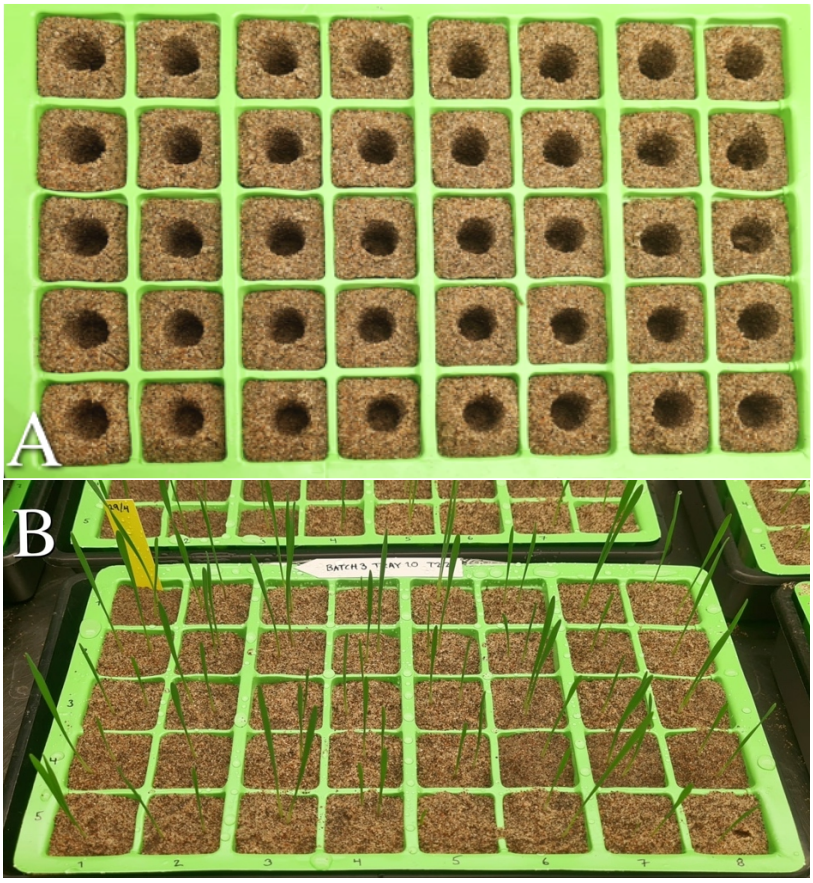


Figure 4 *(A) Tray contained 40 plastic pots (5 × 5 × 5 cm) filled with moistened sand (Rådasand, 0.5–1 mm grain size). Two seeds were sown per pot and placed evenly in holes 2 cm deep and 1.5 cm wide. (B) Trays were maintained in a growth chamber under controlled conditions: a 16-hour light period (200 µmol m^{-2} s^{-1}) and 8 hours of darkness at 20 °C.*

3.4 Statistical analysis

Statistical analyses were performed within a linear modelling framework to assess the effects of experimental design factors and biological variables on the measured traits. This approach accounted for sources of variation inherent to the study design and explicitly modelled the interaction between treatment and genotype.
The model was specified as follows:

$$\mathbf{Y = \mu + Ba + Ba/Tr + T + G + T \times G + \varepsilon}$$

Where:

Y = The response variables (disease score, shoot length, and root length),
μ = The overall mean,
Ba = Effect of different batches
Ba/Tr = Tray-level effects within batches
T = Treatment effect (two levels: T22 and mock),
G = indicates the plant genotype (identified by nordID),
T × G = The interaction between treatment and plant type
ε = Residual error.

Analysis of variance (ANOVA) was used to evaluate the significance of the model terms at $\alpha = 0.05$. Model assumptions were verified through graphical assessment of residuals for normality and homogeneity of variance.

Post-hoc analyses were conducted to estimate the genotype marginal means, yielding the best linear unbiased estimators (BLUEs) within each treatment group. Subsequent pairwise comparisons across treatments were performed with Tukey's Honestly Significant Difference adjustment, to control for the family-wise error rate. Pearson correlation coefficients between traits and treatments were calculated based on BLUE estimates.

All analyses were conducted using R version 2024.12.1+563 (R Core Team, 2024). Linear models were fitted using the lm() function (Wilkinson & Rogers 1973), and ANOVA was performed using the anova() function (Everitt 1992). Marginal means and pairwise contrasts were obtained using emmeans () and pairs () functions (Searle et al. 1980). Correlation analyses utilized the stat_cor () function, whereas data processing and visualization were performed using the tidyverse suite of packages (Wickham et al. 2019).

Disease incidence was compared between the mock and *Trichoderma* treatment groups. Data were reformatted so that each row represented the disease status of one plant. Counts were calculated for (1) diseased plants, and (2)

diseased genotypes, where a genotype was considered diseased if any plant had a disease index of 1.

To evaluate disease consistency, the proportion of diseased plants was calculated (disease index = 1) for each genotype under the mock and *Trichoderma* treatments. Plant-level data were summarized by genotype and treatment, and genotypes were ordered by disease proportion under *Trichoderma* to facilitate comparisons. Proportions were visualized with grouped bar charts, including a 0.5 threshold line, and split into three parts for clarity.

4. Results

A total of 190 winter wheat genotypes were evaluated for their responses to the fungal biostimulant *T. afroharzianum* T22, focusing both on its capacity to stimulate growth and any potential pathogenic effects.

4.1 Disease index

Disease incidence, assessed at both plant and genotype levels, differed between the mock and *Trichoderma* treatments. The number of plants and genotypes with a disease index of 1 was higher in the *Trichoderma* treatment than that in the mock treatment (Figure 5 A and B).

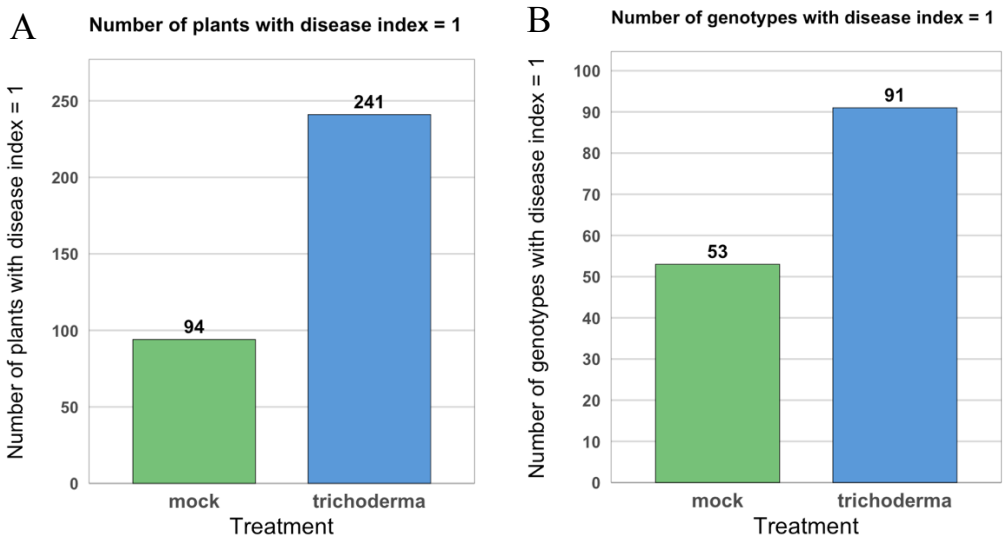
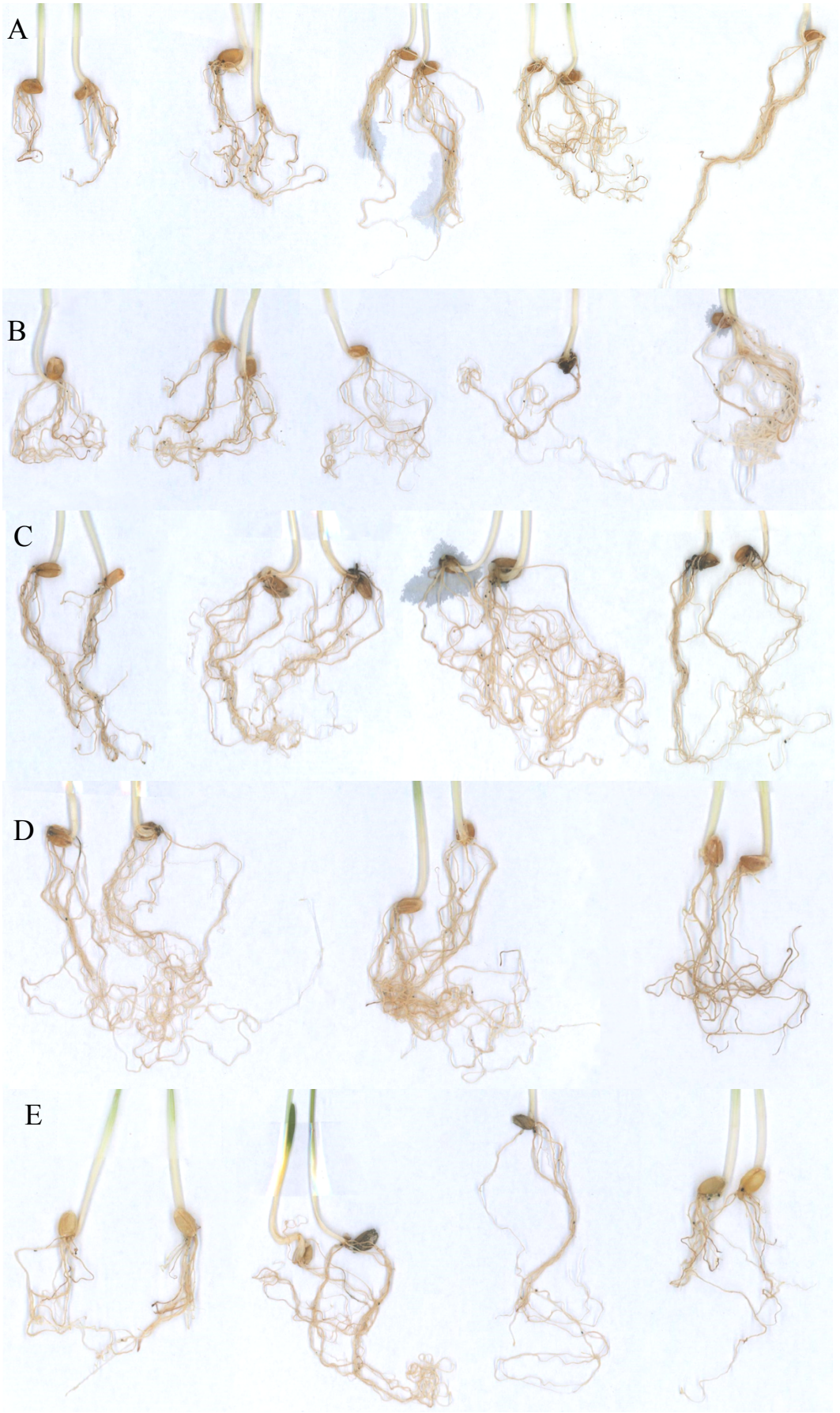


Figure 5. *Number of (A) plants and (B) genotypes with disease index = 1 in the mock and Trichoderma treatments.*

The symptoms observed were primarily browning of the root tips, with occasional browning at the stem base (Figure 6A-I). Browning was frequently observed in mock-treated plants, making it difficult to specifically attribute root browning to *Trichoderma* treatment. Nonetheless, a higher incidence of browning was recorded in the *Trichoderma* treatment than that in the mock treatment. Six genotypes (593179, 593127, 593162, 593169, 593172, and 593192) showed a root browning proportion greater than 50%, while three genotypes (593137, 593138, and 593168) exhibited a root browning proportion of 50% (Figure 7).


A
B
C
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E

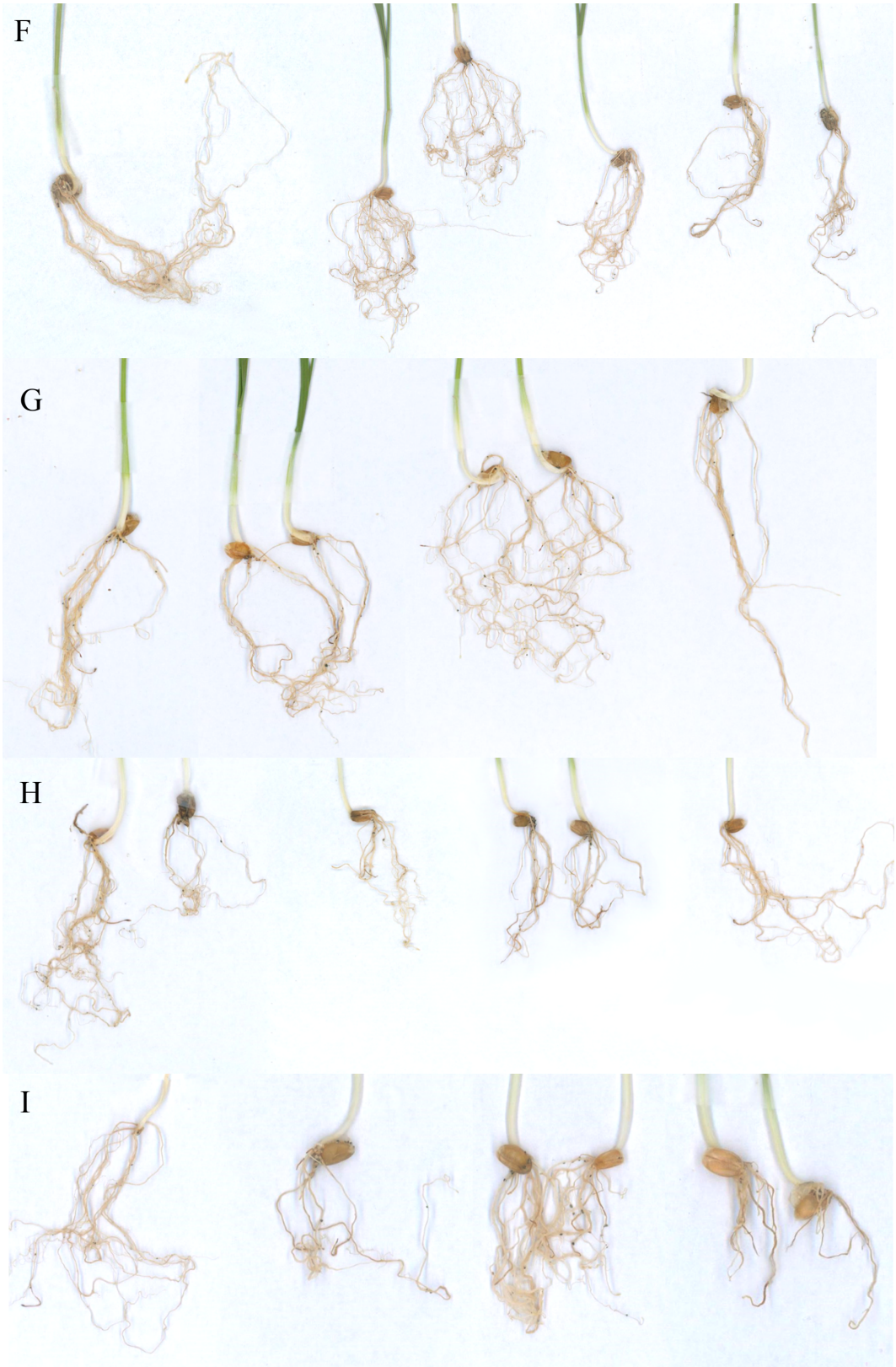

Figure 6. *Genotypes showing root and stem browning symptoms with a proportion of browning equal to 50% or greater than 50%. Symptoms are visible as root browning and stem base browning. Proportion > 50%: (A) 593162, (B) 593169, (C) 593172, (D) 593179, (E) 593192, (F) 593127, Proportion = 50%: (G) 593168, (H) 593137, (I) 593138*

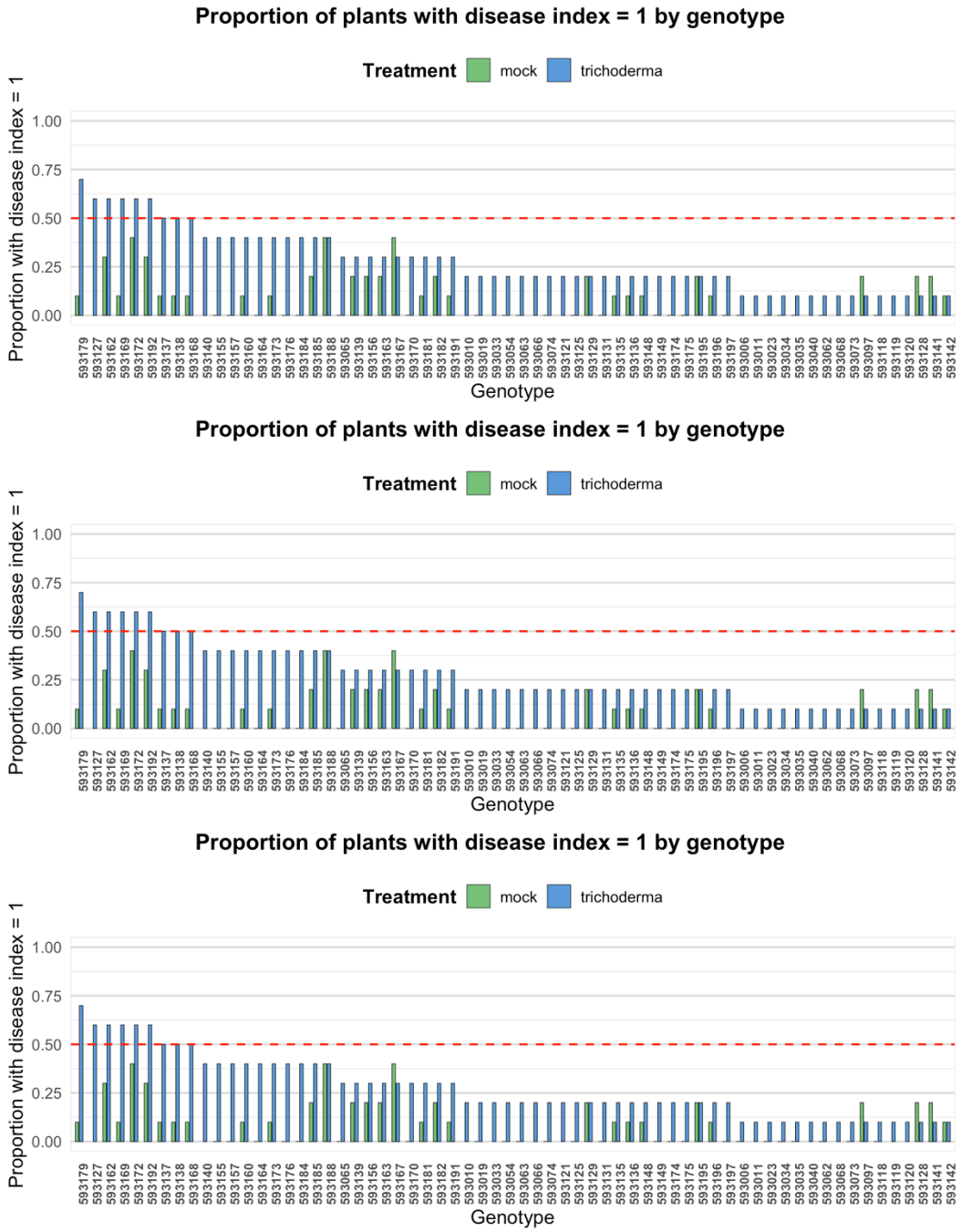

Figure 7. *Proportion of plants with root and stem browning that were assigned a disease (browning) index = 1 according to genotype and treatment. Genotypes were ordered based on the proportion of plants showing disease (browning) under the Trichoderma treatment. Bars represent the proportion of plants with root and stem browning (disease) for the mock (green) and Trichoderma (blue) treatments. The dashed red line indicates the 50% disease threshold. Data were split into three plots for clarity.*

4.2 Effects on wheat growth variables

To evaluate the impact on various traits (shoot length, root length, and dry weight), analysis of variance (ANOVA) was conducted (Tables 1, 2, and 3). Diagnostic plots were visually inspected to verify the model assumptions. Fitted versus residual plots displayed randomly dispersed points, supporting homoscedasticity, whereas the QQ plots indicated normality, as the points closely followed the diagonal reference line (Appendix 2). Significant treatment effects ($p < 0.05$) were identified for all measured traits (Tables 1, 2, and 3). The analysis showed significant variation among the genotypes in shoot and root lengths. Additionally, the genotype-by-treatment interaction (Genotype × Treatment) was significant for all traits, indicating genotypic differences in the response to the treatments (Tables 1, 2, and 3).

Table 1. Results of two-way ANOVA from linear mixed model analysis of shoot length.

Term	Df	Sum Sq	Mean Sq	F value	Pr(>F)	Significance
batch	5	120810,4	24162,07	34,20922	2,38E-33	***
Genotype	189	583866,2	3089,239	4,373816	8,17E-61	***
Treatment	1	44794,84	44794,84	63,42156	3,08E-15	***
batch:rep	24	80289,14	3345,381	4,736467	4,32E-13	***
Genotype:Treatment	188	291103,9	1548,425	2,192295	5,24E-16	***
Residuals	1651	1166106	706,303			

*** = p < 0.001, ** = p < 0.01, * = p < 0.05.

Table 2. Results of of two-way ANOVA from linear mixed model analysis of root length.

Term	Df	Sum Sq	Mean Sq	F value	Pr(>F)	Significance
batch	5	208661,7	41732,35	62,09141	1,91E-59	***
Genotype	189	236090,4	1249,156	1,858554	2,49E-10	***
Treatment	1	120432,4	120432,4	179,1852	7,17E-39	***
batch:rep	24	89992,19	3749,675	5,578947	1,84E-16	***
Genotype:Treatment	188	398482,4	2119,587	3,153624	6,51E-35	***
Residuals	1651	1109656	672,1115			

*** = p < 0.001, ** = p < 0.01, * = p < 0.05.

Table 3. Results of of two-way ANOVA from linear mixed model analysis of dry weight.

Term	Df	Sum Sq	Mean Sq	F value	Pr(>F)	Significance
batch	5	0,097113	0,019423	38,91803	7,07E-38	***
Genotype	189	0,239715	0,001268	2,541416	1,15E-22	***
Treatment	1	1,56E-06	1,56E-06	0,003126	0,955421	
batch:rep	24	0,044605	0,001859	3,724048	3,56E-09	***
Genotype:Treatment	188	0,131985	0,000702	1,406729	0,000471	***
Residuals	1651	0,823956	0,000499			

*** = p < 0.001, ** = p < 0.01, * = p < 0.05.

4.3 Comparison between phenotypic responses

Correlations between treatments showed a positive correlation with shoot length ($R = 0.23$, $p = 0.001$) and dry weight ($R=0.32$, $p < 0.001$) (Figure 8A and C), and a negative correlation with root length ($R = -0.24$, $p = 0.001$) (Figure 8B).

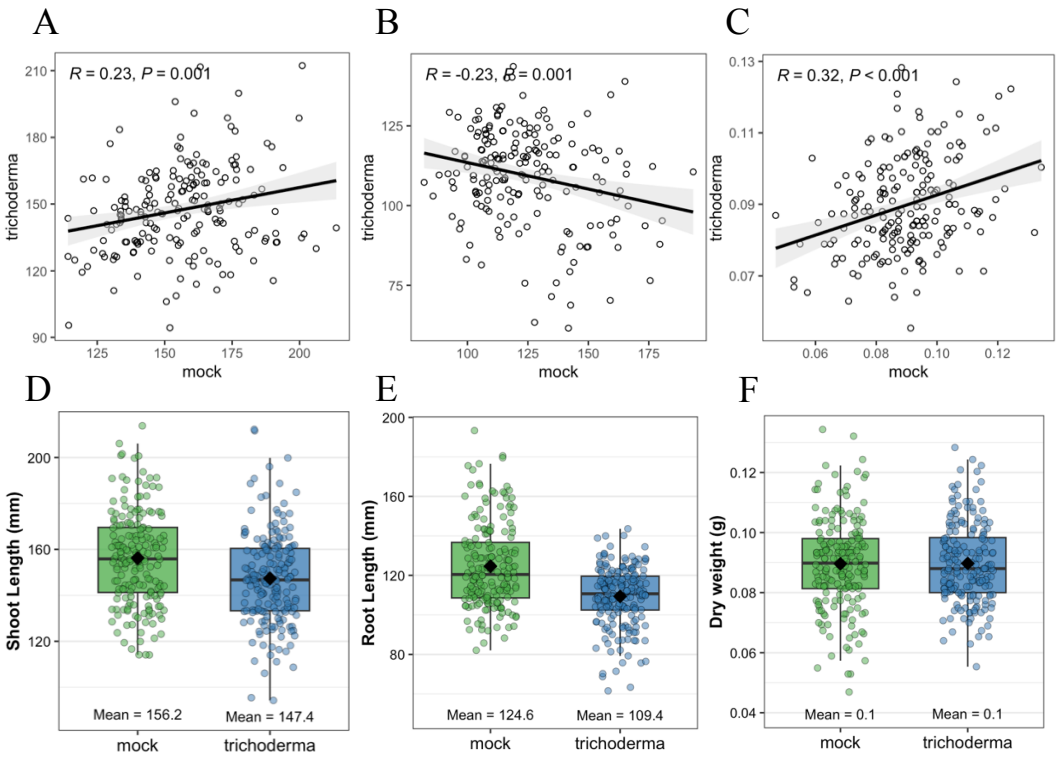

Figure 6. *Pearson correlation analysis between treatments T22 and Mock in 190 winter wheat genotypes for the traits: (A) shoot length, (B) root length, and (C) dry weight. Each data point represents the BLUE (Best Linear Unbiased Estimate) of a genotype across the two treatments. Box plots comparing the BLUEs of genotypes under Trichoderma and Mock treatments for shoot length (D), root length (E), and dry weight (F). The thick horizontal line within each box represents the median, whereas the black diamonds indicate the mean estimate for each treatment.*

Five genotypes exhibited increased shoot length, while 34 exhibited reduced growth compared to the mock treatment (Figure 9A). For root length, one genotype demonstrated increased growth, whereas 44 showed reduced growth (Figure 9B).

Nine genotypes exhibited increased dry weight, whereas nine exhibited reduced dry weight (Figure 9C). Because of the very low germination rate, the Nelson genotype was excluded from the comparison of significant genotypes in Appendix 3.

Among the genotypes that showed a significant response to *Trichoderma*, none showed a simultaneous increase in shoot and root length, nor an increase in one trait and a decrease in the other (Appendix 4). However, the majority of the genotypes showed simultaneous decreases in root and shoot length (Appendix 4).

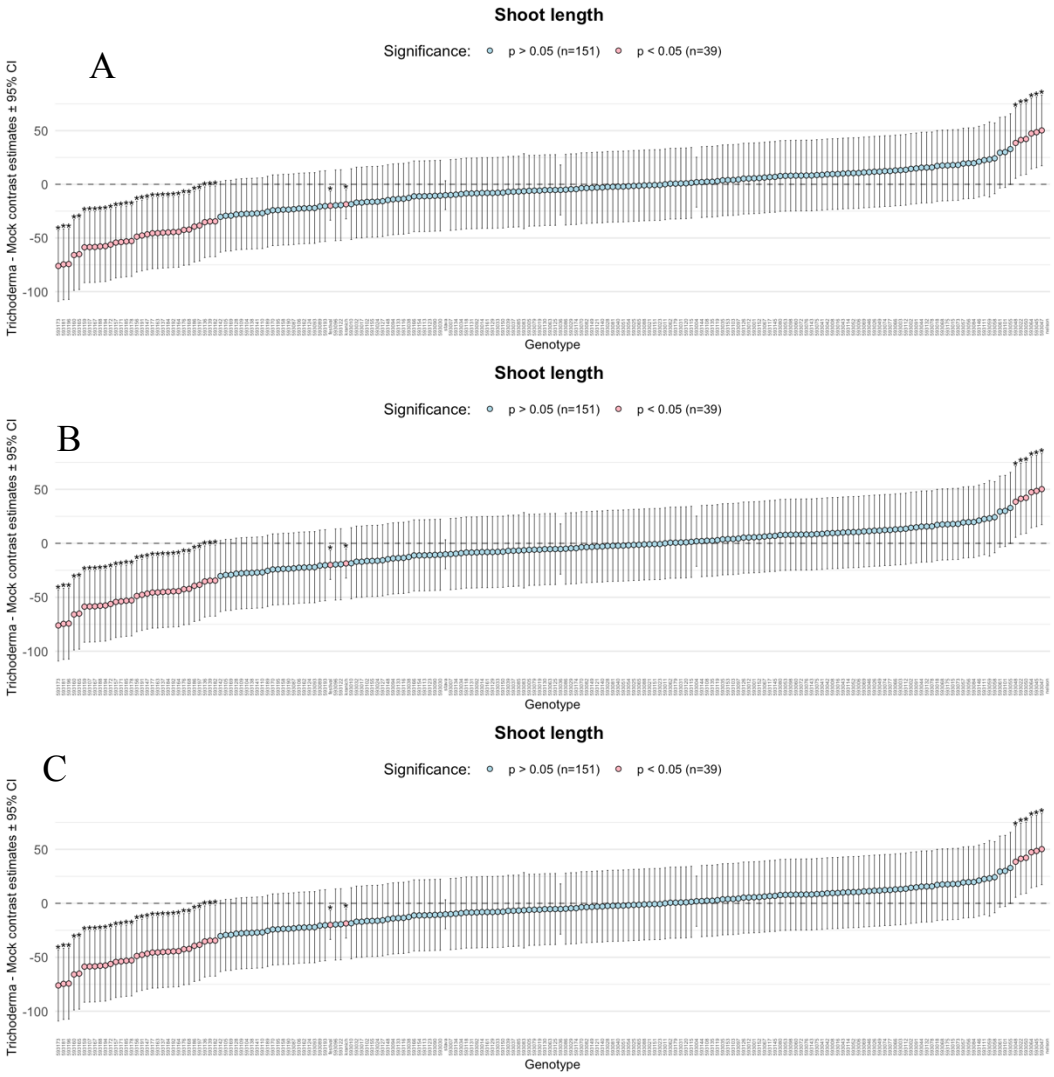

Figure 7. *Inter-treatment pairwise contrast estimates between treatments T22 and Mock for 190 winter wheat genotypes assessing shoot length (A), root length (B), and dry weight (C). Pairwise contrasts were estimated for each genotype using the post-hoc Tukey test. Each point represents the estimated mean difference between treatments for a given genotype, with vertical error bars showing 95% confidence intervals. Points whose confidence intervals overlap with the horizontal line at zero indicate non-significant contrast. Data points marked in pink denote statistically significant differences ($p < 0.05$). Note: Genotype ordering varies across panels.*

Three genotypes (593179, 593162, and 593169) that had a proportion of diseased plants (browning of roots and stem) greater than 50%, simultaneously showed a decrease in root length (Appendix 3 and Figure 6). Two genotypes (593172 and 593192) that showed a proportion of diseased plants greater than 50%, and two genotypes (593137 and 593168) that showed a proportion of diseased plants equal to 50% simultaneously showed a decrease in root and shoot length (Appendix 3 and Figure 6).

5. Discussion

Trichoderma afroharzianum T22 is often recognized as a beneficial fungus, with numerous studies reporting improvements in nutrient uptake, and overall growth across diverse plant species (Woo et al. 2023; Kabir et al. 2024). However, the effects of *T. afroharzianum* have shown varying results depending on plant genotype (Schmidt et al. 2020) and certain strains have also shown to be pathogenic or stress-inducing effects under certain conditions (Pfordt et al. 2024). Beyond plant responses, the use of biocontrol agents under field conditions also raises questions regarding potential impacts on non-target organisms, including pollinators. While *Trichoderma* spp. is generally regarded as safe, changes in plant physiology, or secondary metabolites induced by fungal symbionts could indirectly influence pollinator behavior or performance. Although such effects remain largely unexplored, they highlight the importance of evaluating biocontrol agents, not only for plant compatibility, but also for broader agroecosystem interactions (Mommaerts et al. 2008).

Similarly, in our study we observed reduced shoot and root length in plants treated with *T. afroharzianum* T22, indicating that the strain can induce growth inhibition rather than stimulation in certain genotypes according to plant genetic background. The majority of genotypes in this study responded neutrally, with no significant effect from inoculation (Figure 9).

Correlation analysis of shoot length showed that wheat genotypes with long shoots in the mock treatment also tended to have long shoots when inoculated with *T. afroharzianum* T22.

Root length showed a negative correlation, showing that genotypes with long roots in the mock treatment tended to have shorter roots when inoculated with *Trichoderma* (Figure 8B). Although modest, the negative correlation was statistically significant. This response may be due to the ability of *Trichoderma* to increase acidification in the rhizosphere, which can lead to the accumulation of auxin in the plant, thereby inhibiting root growth (Woo et al. 2023).

Dry weight showed a positive correlation indicating that genotypes with high biomass under Mock also had to have high biomass when inoculated with *Trichoderma* (Figure 8C). However, the accuracy of the dry weight measurements may have been affected by the scale's limitation in measuring weights below 0.01 g.

Genotype-level analyses showed that phenotypic responses to *Trichoderma* inoculation varied among the genotypes, ranging from positive to negative effects on plant growth. Previous studies using the same large panel of winter wheat genotypes have reported similar genotype-dependent variations in response to

inoculation with biological control agents, such as *Clonostachys rosea* (Chaudhary et al. 2024; 2025).

Together, these findings suggest that compatibility between wheat and beneficial microbes is influenced by genotype-dependent factors, potentially governed by distinct genetic loci that determine the outcome of plant–microbe interactions.

None of the genotypes that responded significantly showed a combination of increased shoot and root length, nor a mixed pattern of one trait increased while the other decreased (Appendix 3). Instead, the pattern was simultaneous suppression of both shoot and root growth, indicating systemic stress or pathogenic effects. Similar observations have been observed in maize, where infections with *T. afroharzianum* have led to stunted growth even at 0% visible disease (Pfordt et al. 2024).

The results of this study showed that *T. afroharzianum* T22 inoculation more often led to growth suppression than stimulation in the tested wheat genotypes, which is consistent with previous reports showing strong genotype-specific responses. For example, Schmidt et al. (2020) observed that among three sugar beet genotypes, one exhibited increased shoot and root biomass, whereas two showed growth reductions of up to 30 %, depending on the substrate. Similarly, Kabir et al. (2024) reported that only one of two sorghum genotypes responded positively to T22 inoculation, while the other showed little or no growth promotion, highlighting that the fungus can either stimulate or suppress growth depending on the host genotype. These findings highlight that plant genotypes should be considered when applying biological control agents, as responses to *T. afroharzianum* T22 varied widely among wheat genotypes.

Several genotypes with high root browning incidence also showed reduced shoot and root growth (Appendix 3; Figure 6), indicating that browning is associated with systemic response rather than localized growth suppression. The correlation between root browning and reduced growth in certain wheat genotypes may reflect a shift in resource allocation from growth to defence in response to *T. afroharzianum* T22. Similar defense-associated trade-offs have been reported for other systems. In sugar beets, *T. afroharzianum* T22 induced genotype-dependent changes in biomass and activation of defence-related genes (*WRKY70* and *PR-1*) (Schmidt et al. 2020). Similarly, in quinoa, inoculation with *T. harzianum* T22 resulted in browning and stunting of lateral roots under axenic conditions, indicating a strong physiological response to the fungus (Rollano-Peñaloza et al. 2018). Such responses suggest that root browning can be linked either to direct fungal effects or to defense-related phenolic oxidation and lignification, and that activation of these processes may result in growth costs. The balance between growth promotion and defence activation likely depends on host genotype and environmental context (Harman 2006)

Given the frequent occurrence of browning in mock-treated plants, it is not possible to draw any conclusions that *Trichoderma* T22 causes root browning. However, the higher incidence observed in the T22 treatment group is noteworthy. This browning is not interpreted as a direct disease symptom; however, its increased occurrence in treated plants compared with mocks could reflect additional stress experienced by the roots during exposure to *Trichoderma.* Additional studies using genotypes showing a high incidence of browning are required to clarify whether T22 contributes to root browning and, if so, whether this response is systemic or reflects a modest degree of pathogenicity.

6. Conclusion

This study demonstrates that wheat genotypes vary substantially in their responses to *Trichoderma afroharzianum* strain T22, revealing a clearly genotype-dependent interaction. Among the 190 genotypes evaluated most exhibited neutral growth responses, while smaller subsets showed either growth promotion or growth inhibition. These results indicate that T22 is not a universally beneficial biostimulant in wheat; rather, its effects range from positive to negative depending on the genetic background of the host.

Genotypes that developed root browning also displayed reduced shoot and root growth, suggesting that T22 induces a stress response in certain genotypes. This provides evidence that *T. afroharzianum* T22 does not function as a general biostimulant or root-associated symbiont across wheat as a whole. Instead, the fungus interacts through distinct physiological pathways that differ among genotypes, producing positive, neutral, or adverse outcomes. The occurrence of T22-induced growth inhibition in several genotypes further underscores the need for caution when considering the agricultural use of this strain.

Overall, the findings highlight the importance of evaluating biocontrol organisms across broad and diverse genetic panels prior to their implementation in crop production. The substantial variation observed here indicates that T22 should only be applied to wheat genotypes for which compatibility has been confirmed. Continued research into the mechanisms underlying these contrasting responses will be essential for the safe, predictable, and effective integration of biological solutions into wheat cultivation.

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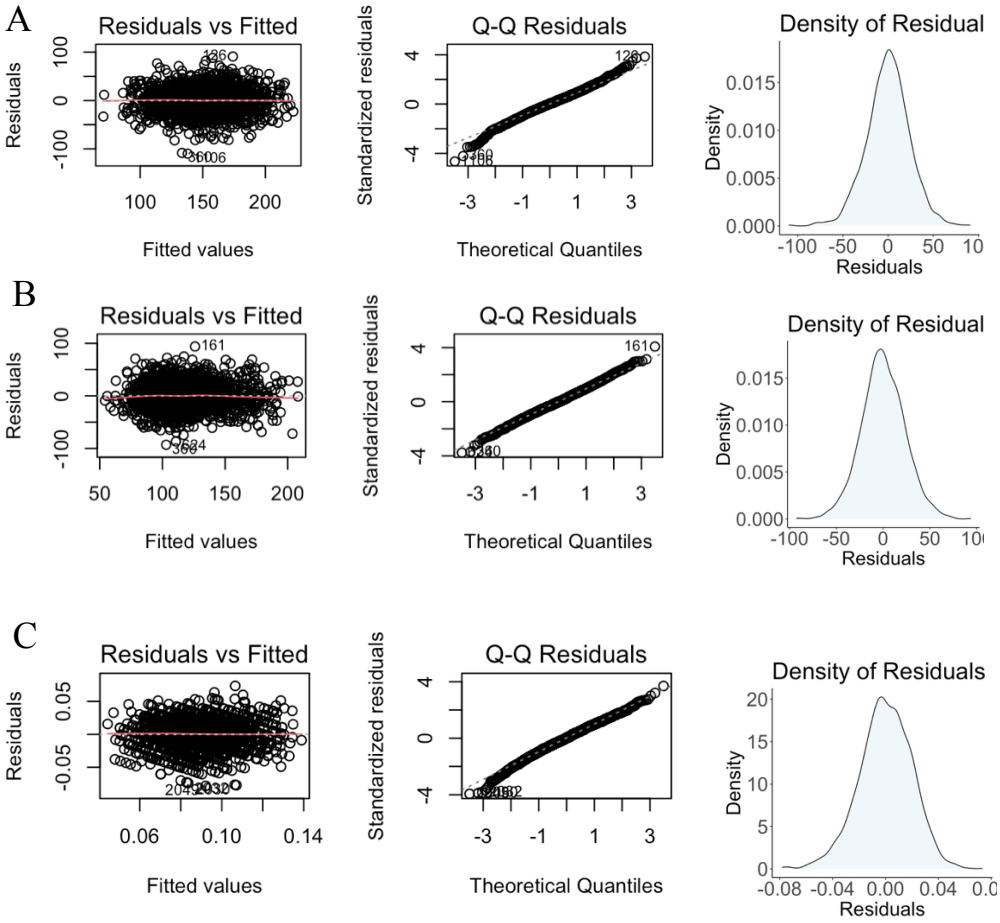
7. Appendix

Appendix 1 List of the 190 winter wheat genotypes included in the T. afroharzianum T22 bioassay.

nordID	genID	cultivar	release year	country	Accession type
NGB16916	593001	Galicia	2010	Denmark	Cultivar
NGB1	593002	Iduna	1911	Sweden	Cultivar
NGB10	593003	Äring II	1936	Sweden	Cultivar
NGB11	593004	Äring III	1940	Sweden	Cultivar
NGB11316	593005	Kalle	1990	Norway	Cultivar
NGB11317	593006	Rida	1976	Norway	Cultivar
NGB11425	593007	Starke II - LR	1968	Denmark	Unknown
NGB12	593008	Eroica	1943	Sweden	Cultivar
NGB12242	593010	Pansar I	1915	Sweden	Cultivar
NGB12243	593011	Ergo II	1949	Sweden	Cultivar
NGB12244	593012	Konge III	1939	Denmark	Cultivar
NGB13023	593014	Ritmo		Netherlands	Unknown
NGB13430	593015	Finnish Winter Wheat (Pi181455)		Finland	Unknown
NGB13442	593016	Winter Wheat From Bohuslän			Unknown
NGB13444	593017	-		Sweden	Landrace
NGB13445	593018	Ångermanland		Sweden	Landrace
NGB13446	593019	Tystofte smaahvede	1909	Denmark	Cultivar
NGB13576	593021	Urban	1981	Germany	Cultivar
NGB13659	593022	Bjørke	1997	Norway	
NGB14	593023	Aros	1947	Sweden	Cultivar
NGB14114	593024	Gunbo	1997	Sweden	Cultivar
NGB14115	593025	Mjölner	1996	Sweden	
NGB14116	593026	Rental	1993	Sweden	Cultivar
NGB14118	593028	Rudolf rubin	1921	Sweden	Cultivar
NGB14286	593029	S-5		Sweden	Landrace
NGB15	593030	Eroica II	1951	Sweden	Cultivar
NGB15070	593031	Kirsten	1997	Denmark	Cultivar
NGB15071	593032	Lone	1994	Denmark	Cultivar
NGB15072	593033	Brandt	1999	Denmark	Cultivar
NGB15075	593034	Karat	2000	Denmark	Cultivar
NGB15076	593035	Arlo		Denmark	Cultivar
NGB16	593036	Banco	1953	Sweden	Cultivar
NGB16675	593037	Saxild	2002	Denmark	Cultivar
NGB16679	593038	Abba	2002	Denmark	Cultivar
NGB16852	593039	Konsul	1994	Sweden	Cultivar
NGB16853	593040	Rektor	1981	Denmark	Cultivar
NGB16909	593041	Probat	2000	Denmark	Cultivar
NGB16910	593042	Stakado	1995	Denmark	Cultivar
NGB17	593043	Ertus	1953	Sweden	Cultivar
NGB17135	593044	Sampo	1933	Finland	Cultivar
NGB17137	593045	Väinö		Finland	Cultivar
NGB17141	593046	Pitkävihneinen maatiainen		Finland	Landrace
NGB17142	593047	Kökar		Finland	Landrace
NGB18	593048	Starke	1959	Sweden	Cultivar
NGB18629	593049	Olympia	1941	Finland	Cultivar
NGB19	593050	Trond	1960	Sweden	Cultivar
NGB2	593051	Standard	1921	Sweden	Cultivar
NGB20	593052	Thor	1961	Sweden	Cultivar
NGB21	593053	Norre	1962	Sweden	Cultivar
NGB21864	593054	Otso	1989	Finland	Cultivar
NGB22	593055	Starke II	1968	Sweden	Cultivar
NGB23	593056	Holme	1972	Sweden	Cultivar
NGB23170	593057	Kuikka		Finland	Landrace
NGB23171	593058	Istäsuomalainen		Finland	Landrace
NGB23345	593059	Alrø	1999	Denmark	Cultivar
NGB23346	593060	Dirigent	1999	Denmark	Cultivar
NGB23347	593061	Facet	1995	Sweden	Cultivar
NGB23348	593062	Frimegu	1995	Denmark	Cultivar
NGB23349	593063	Hybris	1998	Denmark	Cultivar

NGB23350	593064	Junker	1988	Sweden	Cultivar
NGB23351	593065	Miller	2000	Denmark	Cultivar
NGB23352	593066	Pentium	1996	Denmark	Cultivar
NGB23353	593067	Revelj	2000	Sweden	Cultivar
NGB23356	593068	Skjaldar	1976	Norway	Cultivar
NGB23357	593069	Solist	1999	Denmark	Cultivar
NGB23358	593070	Terra	1994	Denmark	Cultivar
NGB23360	593072	Wasmo	1999	Denmark	Cultivar
NGB23363	593073	Cardos	1998	Germany	Cultivar
NGB23364	593074	Gefion	1998	Denmark	Cultivar
NGB23678	593075	Loyal		Sweden	Cultivar
NGB23679	593076	Ambition		Denmark	Cultivar
NGB23681	593077	Mariboss		Denmark	Cultivar
NGB23682	593078	Hereford		Denmark	Cultivar
NGB23780	593079	Agrestis	2001	Denmark	Cultivar
NGB24	593080	Walde	1945	Sweden	Cultivar
NGB2434	593081	Folke	1981	Sweden	Cultivar
NGB2435	593082	Holger	1981	Sweden	Cultivar
NGB25	593083	Sture	1975	Sweden	Cultivar
NGB26	593084	Helge	1980	Sweden	Cultivar
NGB3	593085	Jarl	1925	Sweden	Cultivar
NGB31181	593086	Cymbal	2012	Sweden	Cultivar
NGB31730	593087	Penta Sejet	2001	Denmark	Cultivar
NGB334	593088	Linna	1965	Finland	Cultivar
NGB343	593089	Nisu	1966	Finland	Cultivar
NGB344	593090	Vakka	1959	Finland	Cultivar
NGB347	593091	Aura	1976	Finland	Cultivar
NGB348	593092	Jyvä	1965	Finland	Cultivar
NGB4	593093	Ankar	1928	Sweden	Cultivar
NGB4494	593094	Borstvete från Gotland		Sweden	Landrace
NGB4770	593096	Als	1923	Denmark	Cultivar
NGB4783	593097	Storvik sjundeå		Finland	Landrace
NGB4799	593098	Atchena K.62		Afghanistan	Landrace
NGB5	593101	Saxo	1929	Sweden	Cultivar
NGB5147	593102	Squarehead II	1909	Sweden	Cultivar
NGB5151	593103	Deh Kundi K.244		Afghanistan	Landrace
NGB5152	593104	Gusalek K.17		Afghanistan	Landrace
NGB5153	593105	Hunsballe R	1955	Denmark	Cultivar
NGB6	593106	Ankar II	1928	Sweden	Cultivar
NGB6383	593107	Skandia	1935	Sweden	Cultivar
NGB6388	593108	Lading skæghvede		Denmark	Landrace
NGB6392	593109	Kabel K.238		Afghanistan	Landrace
NGB6691	593110	Lantvete från Halland		Sweden	Landrace
NGB6692	593111	Lantvete från Uppsala		Sweden	Landrace
NGB6693	593112	Kotte	1950	Sweden	Cultivar
NGB6694	593113	Extra squarehead	1900	Sweden	Cultivar
NGB6695	593114	Bore	1902	Sweden	Cultivar
NGB6696	593115	Grenadier II	1907	Sweden	Cultivar
NGB6697	593116	Extra squarehead II	1909	Sweden	Cultivar
NGB6698	593117	Pudel	1910	Sweden	Cultivar
NGB6699	593118	Renodlat sammetsvete	1910	Sweden	Cultivar
NGB6700	593119	Sol	1911	Sweden	Cultivar
NGB6701	593120	Sol II	1916	Sweden	Cultivar
NGB6702	593121	Thule II	1917	Sweden	Cultivar
NGB6703	593122	Pansar III	1919	Sweden	Cultivar
NGB6704	593123	Svea I	1924	Sweden	Cultivar
NGB6705	593124	Riddar	1922	Sweden	Cultivar
NGB6706	593125	Birgitta	1922	Sweden	Cultivar
NGB6707	593126	Pansar III	1923	Sweden	Cultivar
NGB6708	593127	Kron	1925	Sweden	Cultivar
NGB6709	593128	Stål	1927	Sweden	Cultivar
NGB6710	593129	Sol III	1929	Sweden	Cultivar
NGB6712	593130	Bore II	1931	Sweden	Cultivar
NGB6713	593131	Gyllen II	1935	Sweden	Cultivar
NGB6714	593132	Thule III	1936	Sweden	Cultivar
NGB6715	593133	Sol IV	1937	Sweden	Cultivar
NGB6716	593134	Gyllen II	1938	Sweden	Cultivar
NGB6717	593135	Skandia II	1939	Sweden	Cultivar
NGB6718	593136	Gluten	1939	Sweden	Cultivar
NGB6719	593137	Borg	1943	Sweden	Cultivar
NGB6720	593138	Skandia III B	1955	Sweden	Cultivar
NGB6721	593139	Hansa Svalöf	1945	Sweden	Cultivar

NGB6722	593140	Pärl II	1946	Sweden	Cultivar
NGB6723	593141	Odin	1949	Sweden	Cultivar
NGB6724	593142	Robur	1949	Sweden	Cultivar
NGB6725	593143	Svale	1955	Sweden	Cultivar
NGB6726	593144	Diana	1957	Sweden	Cultivar
NGB6727	593145	Ölve	1959	Sweden	Cultivar
NGB6728	593146	Seba	1969	Sweden	Cultivar
NGB6729	593147	Virgo	1968	Sweden	Cultivar
NGB6730	593148	Solid	1973	Sweden	Cultivar
NGB6731	593149	Hildur	1976	Sweden	Cultivar
NGB6773	593150	Hankkijan ilves	1984	Finland	Cultivar
NGB7	593151	Äring	1932	Sweden	Cultivar
NGB7027	593152	Dania	1926	Denmark	Cultivar
NGB7034	593153	Mendel	1950	Sweden	Cultivar
NGB7043	593154	Bagelgrom K.87		Afghanistan	Landrace
NGB7044	593155	Kabel K.162		Afghanistan	Landrace
NGB7045	593156	Kabel K.165		Afghanistan	Landrace
NGB7183	593157	Små II, Tystofte	1915	Denmark	Cultivar
NGB7184	593158	Storaks Abed	1967	Denmark	Cultivar
NGB7193	593159	Gusalek K.10 A		Afghanistan	Landrace
NGB7194	593160	Vama K.40 A		Afghanistan	Landrace
NGB7195	593161	Øtofte I.56	1956	Denmark	Cultivar
NGB7482	593162	Kosack	1984	Sweden	Cultivar
NGB7483	593163	Sleipner	1988	Sweden	Cultivar
NGB7484	593164	Rurik	1986	Sweden	Cultivar
NGB8	593165	Ergo	1934	Sweden	Cultivar
NGB8189	593166	Dronning	1940	Sweden	Cultivar
NGB8194	593167	Konge II	1939	Denmark	Cultivar
NGB8197	593168	Stand tystofte	1907	Denmark	Cultivar
NGB8198	593169	Lantvete från Värmland		Sweden	Landrace
NGB8199	593170	Gammalt Svenskt lantvete		Sweden	Landrace
NGB8672	593171	Salut	1982	Sweden	Cultivar
NGB8933	593172	Borg Abed	1966	Denmark	Cultivar
NGB8937	593173	Bankuta		Sweden	Cultivar
NGB8946	593174	Brødtorp Pajo		Denmark	Landrace
NGB8957	593175	Enger		Norway	Landrace
NGB8968	593176	Haukiala Pirola		Finland	Landrace
NGB8973	593177	Ideal	1929	Denmark	Cultivar
NGB8999	593178	Sammets	1910	Sweden	Cultivar
NGB9	593179	Standard II	1936	Sweden	Cultivar
NGB9016	593181	Trifolium 14	1925	Denmark	Cultivar
NGB9017	593182	Tystofte Stakket	1967	Denmark	Cultivar
NGB9020	593184	Varma Tammisto	1933	Finland	Cultivar
NGB9057	593185	Hallandsvete		Sweden	Landrace
NGB9062	593186	Mendel II	1952	Sweden	Cultivar
NGB9078	593188	Kabel K.161		Afghanistan	Landrace
NGB9079	593189	Pandshir K.156 A		Afghanistan	Landrace
NGB9080	593190	Pandshir K.157		Afghanistan	Landrace
NGB9118	593191	Nana	1975	Denmark	Cultivar
NGB9119	593192	Sarah	1976	Denmark	Cultivar
NGB9122	593193	Anja	1980	Denmark	Cultivar
NGB9123	593194	Kraka	1980	Denmark	Cultivar
NGB9925	593195	Portal	1990	Germany	Cultivar
NGB9952	593196	Tjelvar	1984	Sweden	Cultivar
NGB9953	593197	Tryggve	1990	Sweden	Cultivar
Nelson		Nelson	2011	Germany	
Kranich	C1	Kranich	2007	Germany	
NGB13479	C2	Stava	1995	Sweden	Cultivar
Festival	C3	Festival			



Appendix 2. *Residuals vs fitted, Normal Q-Q and Density plots for Model of bioassay with treatment. (a) shoot length, (b) root length, (c) dry weight.*

Appendix 3 Genotypes showing significant response from Trichoderma. Columns marked pink indicates decreased length or weight and columns marked green indicates increase in length or weight.

Root length	festival	kranich	593059	593061	593133	593137	593139	593142	593144	593147
	593155	593156	593157	593158	593159	593160	593162	593163	593164	593166
	593167	593168	593169	593170	593171	593172	593173	593174	593175	593176
	593177	593178	593179	593181	593182	593184	593185	593186	593188	593190
	593191	593192	593193	593194	593195	593196				
Shoot length	festival	kranich	593022	593045	593047	593048	593050	593064	593107	593136
	593137	593139	593147	593156	593157	593159	593160	593163	593164	593165
	593167	593168	593171	593172	593173	593176	593177	593178	593181	593182
	593184	593185	593186	593188	593191	593192	593194	593196	593197	
Dry weight	593015	593022	593023	593035	593046	593047	593048	593053	593070	593074
	593081	593086	593087	593102	593104	593109	593121	593175	593196	

Appendix 4 Genotypes showing significant responses to Trichoderma, with multiple traits significantly affected (x indicates significant change).

Genotype	Root length increase	Shoot length increase	Dry weight increase	Root length decrease	Shoot length decrease	Dry weight decrease
593022		x				x
593047		x				x
593048					x	x
festival				x	x	
kranich				x	x	
593137				x	x	
593139				x	x	
593147				x	x	
593156				x	x	
593157				x	x	
593159				x	x	
593160				x	x	
593163				x	x	
593164				x	x	
593167				x	x	
593168				x	x	
593171				x	x	
593172				x	x	
593173				x	x	
593175				x		x
593176				x	x	
593177				x	x	
593178				x	x	
593181				x	x	
593182				x	x	
593184				x	x	
593185				x	x	
593186				x	x	
593188				x	x	
593191				x	x	
593192				x	x	
593194				x	x	
593196			x	x	x	

Popular science summary

Wheat is one of the most important crops in the world, providing approximately 20% of the global energy and protein intake. However, wheat cultivation is facing increasing challenges due to climate change, pests, and the need to reduce the use of chemical pesticides.

To make agriculture more sustainable, scientists are exploring alternatives, such as beneficial microbes that can support plant growth and protect crops from disease. One such microbe is *Trichoderma afroharzianum* strain T22, a fungus that has been widely tested in crops, such as maize, tomato, and sorghum. It is known for its ability to promote growth and for its potential to protect plants against pathogens. However, its effects are not always positive and may depend on the plant genotype and growing conditions.

In this study, 190 different winter wheat genotypes were tested to determine their response to inoculation with *T. afroharzianum* T22. The results showed that most genotypes did not benefit from the fungus. Instead, many plants had shorter roots and shoots compared to untreated controls. Some genotypes also showed browning of roots and stems, symptoms that might be linked to mild pathogenic effects but needs further studies to distinguing the cause. Only a few wheat genotypes showed increased growth when treated with the fungus.

These findings suggest that while *T. afroharzianum* T22 can be beneficial in some crops, its use in wheat may be risky without testing specific varieties first. The results highlight the importance of matching biological control agents to the right crop genotypes to ensure safe and effective agricultural applications.

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