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Mitigating drought effects in maize with *Trichoderma harzianum* (strain – ESALQ 1306): a bioinoculant for sustainable agriculture

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Abstract: Agriculture faces increasing challenges due to climate change, underscoring the importance of beneficial microorganisms for enhancing crop resilience and improving soil health. However, the performance of microbial inoculant strains can vary widely depending on the cultivated species and environmental conditions. This study evaluated the ESALQ 1306 strain of *Trichoderma harzianum*, a soil fungus recognised as a biological control agent for crops such as soybean and strawberry, investigating its potential as a growth promoter in maize (*Zea mays* L.). Field experiments were conducted with three commercial cultivars (DKB255, DKB360, and 2B810) over two growing seasons, one under irrigation and the other under severe natural drought. The results revealed that *Trichoderma* (ESALQ 1306) significantly increased plant height, biomass, and grain yield, particularly under drought stress, despite lacking a formal recommendation for maize. The cv. DKB360 showed the greatest response, with yield increases of up to 60% compared to untreated controls. Inoculation also improved nutrient uptake, especially nitrogen, highlighting its potential to maintain soil health and fertility. These findings demonstrate that the ESALQ 1306 strain of *Trichoderma* is a promising soil bioinoculant for agriculture, capable of improving maize performance under both optimal and stressful conditions. However, it is important to emphasise that genotype-specific responses highlight the need to align bioinoculant application with selecting specific cultivars to ensure inoculation success. This insight is crucial for guiding future breeding programs and establishing clear regulatory guidelines for commercialising biological products, fostering sustainable and resilient agricultural systems.

Keywords: endophytic microorganism; drought tolerance; maize yield; biological inoculants; innovation; plant-microbe interaction

As the world's population continues to grow, ensuring global food security will depend on technological advances that increase agricultural yields, particularly in maize cultivation, which has recently surpassed wheat to become the most widely planted crop globally (Santos Júnior et al. 2019).

In this worrying scenario, adopting new and more productive sustainable agricultural practices is necessary. This new agricultural model is expected to rely heavily on artificial intelligence to optimise the use of resources and production factors (Mana et al. 2024). This new agricultural model is also expected to encourage greater adoption of safer and more natural biotechnological agricultural products, such as endophytic microorganisms, to produce more nutritious and healthier foods. Thus, the introduction of endophytic microorganisms is a significantly promising avenue for sustainable innovation in agriculture (Anand et al. 2023). Endophytes can be a useful tool in the biological control of plant diseases, reducing the need for agrochemicals such as fungicides, chemical nematicides, and fertilisers, which are notorious for causing greenhouse gas emissions during production and application (Anand et al. 2023). At the same time, reducing the use of agrochemicals reduces the risk of soil and water resource contamination, thereby improving environmental quality and minimising adverse impacts on ecosystems. Endophytes can also promote plant growth and increase agricultural yields, thus limiting the need to expand cultivation areas (Alsherif et al. 2022, Anand et al. 2023).

Among the various endophytes known to humans, *Trichoderma harzianum* spp. has attracted increasing attention from both scientific and agricultural communities due to its versatile adaptability to diverse environments and substrates (Stewart and Hill 2014, Anand et al. 2023, Awad-Allah et al. 2023). Initially recognised as a biological control agent, *Trichoderma* has demonstrated the ability to solubilise soil nutrients, enhance plant defences against biotic and abiotic stresses, synthesise plant growth-promoting agents, and offer potential for food biofortification (Ali et al. 2022, Woo et al. 2022, Anand et al. 2023). Therefore, the application of *Trichoderma harzianum* in agriculture can play a pivotal role in reducing deforestation and in the mitigation of the effects of global warming by increasing yield in areas already planted, thereby reducing the need to open new cultivation areas (Anand et al. 2023, Araújo et al. 2023, Awad-Allah et al. 2023).

However, a deeper investigation into the interactions between plants and endophytes, including *Trichoderma*, is necessary because the efficacy of endophyte inoculation may vary according to environmental conditions and inoculation methods. It is a common occurrence that endophytic strains showing positive results in controlled laboratory settings do not replicate the same effectiveness in field conditions, potentially leading to colonisation failures or non-expression of previously observed traits in laboratory or greenhouse environments (Smyth et

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al. 2011, Fadiji and Babalola 2020, Mengistu 2020). There are also studies reporting that the inoculation of maize plants with endophytic microorganisms, such as endophytic fungi, such as *Trichoderma*, or even endophytic bacteria, such as *Azospirillum*, did not influence the yield characteristics of the maize crop (Dartora et al. 2016, Mahato and Neupane 2017, Santos Júnior et al. 2019), or that the results may vary depending on the dose used (Araújo et al. 2023).

Based on this background, our research group conducted preliminary trials indicating that the endophytic fungus *Trichoderma harzianum* (strain ESALQ 1306; MAPA 22318), currently registered only as a soil-applied fungicide and nematicide, may also act as a maize growth promoter, with the magnitude of the response depending on the cultivar genotype. The insights gained from this hypothesis will provide novel information and refined direction for plant breeding programs, aiding in the identification and development of cultivars that, upon inoculation, exhibit not only enhanced yield but also improved nutritional values, even under adverse climatic conditions. Such discoveries will also aid in the development of new laws and guidelines for the commercialisation of seeds, enabling producers to be informed about the potential yield response of cultivars and their food biofortification capabilities, thereby promoting greater economic sustainability and productive control in maize cultivation.

MATERIAL AND METHODS

Site description and overview of study design. This study was conducted in the experimental fields of UNESP – Campus Ilha Solteira (51°22'W, 20°22'S, 335 m a.s.l.), in Brazil. The local climate, categorised as Aw-type by the Köppen classification, is predominantly humid and tropical, characterised by a wet season during summer and a dry season in winter. The region's average yearly temperature is 23.5 °C, with an annual rainfall of 1 370 mm and a relative humidity typically between 70% and 80%. The soil at the site is described as Rhodic Hapludox (Santos et al. 2018), having been used for annual crop cultivation for over 29 years, including 13 years under a conservation tillage system. The most recent crop grown before this study was soybeans. For the experiment, we selected the maize cvs. DKB360, DKB255, and 2B810PW, which are commonly used and recommended across Brazil's primary maize-producing regions (south, southeast, and central west). These three commercial cultivars are early-maturing with high yield potential. All carry *Bt* trait packages for lepidopteran control, along with herbicide tolerance, and are recommended for summer and/or second-crop (safrinha) planting. Their primary differences involve plant height, grain type, and disease tolerance profiles. DKB 255 averages 250 cm in height and produces semi-dent grain. It shows moderate tolerance to white leaf spot, *Cercospora* leaf spot, northern corn leaf blight (*Exserohilum turcicum*), but is moderately susceptible to the corn stunt complex. DKB 360 is 230 cm tall with dent grain; it is broadly adapted and shows good tolerance to grain rot in tropical second-crop conditions. It is tolerant to white leaf spot and moderately tolerant to *Cercospora* leaf spot, southern rust, and the corn stunt complex, with moderate tolerance to northern corn leaf blight. In contrast, 2B810 PW averages 219 cm in height with an ear insertion of 106 cm; this stature supports improved standability and allows for higher plant populations. The selection of these cultivars reflects typical maize production conditions in Brazil, which has recently become the world's largest maize exporter (Food and Agriculture Organisation of the United Nations 2023a).

Figure 1 summarises the entire workflow of our study, from preliminary tests for cultivar and endophyte selection, through inoculation with *Trichoderma harzianum* (ESALQ 1306), to field evaluation and statistical analysis of growth and yield responses.

Environmental conditions during the experiment. During the 2020/2021 growing season, irrigation was applied as needed with a centre-pivot system, delivering an average depth of 14 mm every ~72 h. By contrast, no irrigation was possible in the 2021/2022 crop season, as evidenced by the local weather station data shown in Figure 2. Because a severe regional drought left local tributaries of the Paraná River, the main water source for the experiment, completely dry. The Paraná, South America's second-largest river, fell to its lowest level in 77 years, disrupting irrigation and river transport in Brazil and Argentina.

To inoculate the maize cultivars, the endophytic fungus *Trichoderma harzianum* (STRAIN ESALQ 1306) was applied through the commercial product TRICHODERMIL®, which is registered as a biological fungicide and nematicide for crops such as beans, strawberries, sugarcane, soybeans, and pineapple. Preliminary greenhouse trials using sterilised soil showed enhanced leaf elongation rates in seed-

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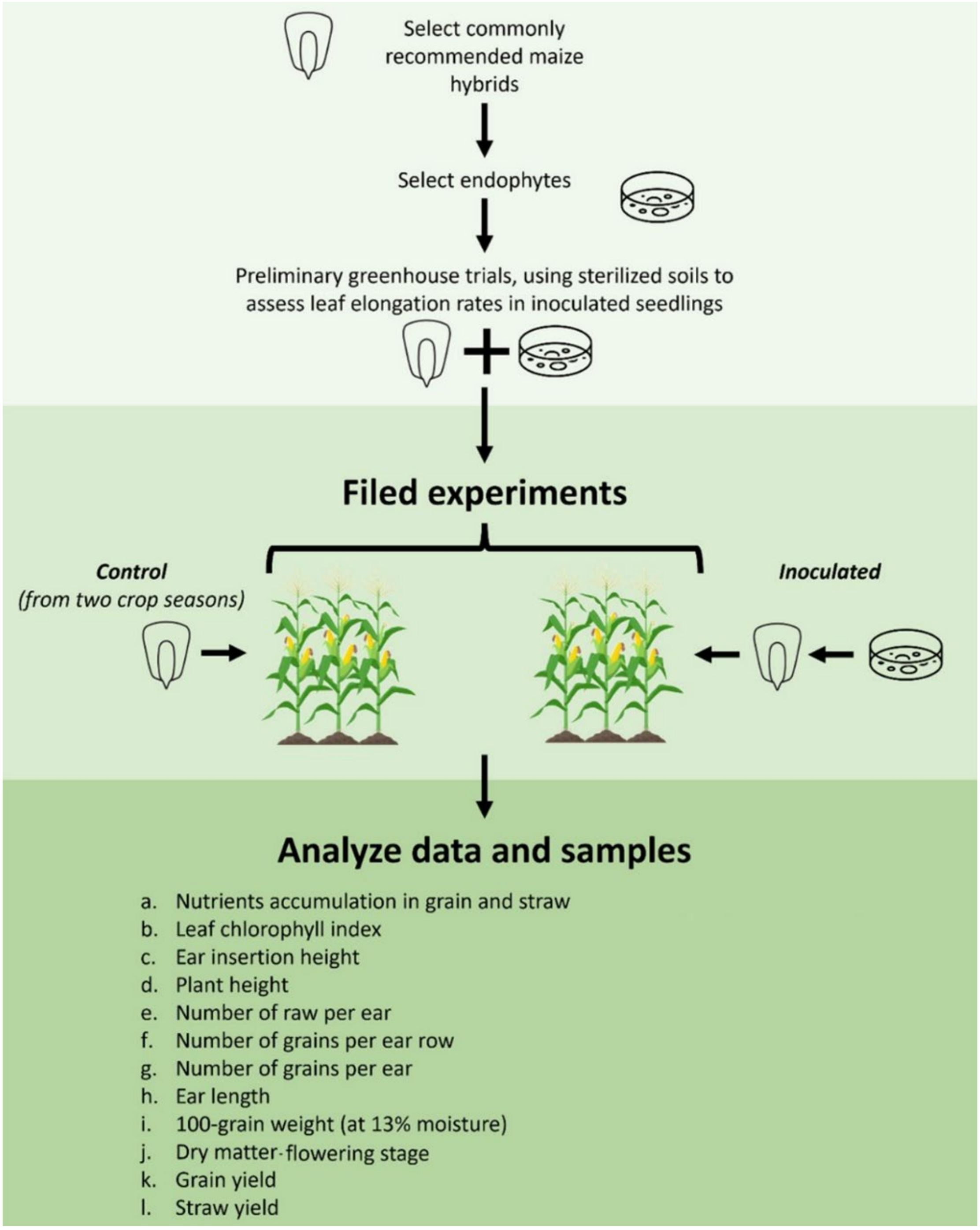

Figure 1. Flowchart of the study with *Trichoderma harzianum* (ESALQ 1306) in corn

lings inoculated with the fungus. These trials led to determining an optimal dosage of 2 mL/kg of seeds at planting. All seeds were pre-treated with agrochemicals (Ciantraniliprole, Tiametoxam, and a fungicide combination of METALAXIL-M and FLUDIOXONIL), which were deemed biologically compatible with *Trichoderma* (Loureiro et al. 2020). The experimental setup included units of six 5-m rows, and the evaluation was conducted on the four central rows, excluding 0.5 m at both ends. Row spacing was maintained at 0.45 cm, with a density of 3.5 plants per meter, targeting a plant population of approximately 78 000 per hectare, in line with the chosen cultivars and available technology level. Initial site preparation was achieved by applying glyphosate (1 800 g/ha) and 2,4-D (670 g/ha), followed by land preparation with a shredder.

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An herbicide application with paraquat (Gramoxone 200®) was performed 3 days before sowing, following the crop's standard recommendations. Soil chemical properties were assessed before the experiment, following the methods outlined by Malavolta et al. (1997) and Raij et al. (2001). To achieve a base saturation of 70% recommended by Raij et al. (2001), 2.5 t/ha of dolomitic limestone (ECCE = 88%) was applied 65 days before sowing. At planting, fertiliser was applied to supply 32 kg N/ha, 48.9 kg P/ha, and 53.1 kg K/ha, based on soil analysis and maize nutritional requirements. Post-emergence weed control consisted of glyphosate at 1.56 kg/ha at the V3 growth stage.

Pest control was performed using a mixture of thiamethoxam, lambda-cyhalothrin, and methomyl. Harvest occurred at R8 when the plants were dry, with manual collection of the maize plots. The experimental design was a randomised block in a 3 × 2 factorial arrangement, involving three maize cultivars (DKB360, DBK255, and 2B810PW) and two treatments (inoculated with *Trichoderma harzianum* and control – not inoculated), with four replications.

The experimental design was a randomised block design in a 3 × 2 factorial scheme, with four replications. When the interaction between cultivar and inoculation was significant, the means were compared using two distinct tests. The *F*-test was used to compare the effect of inoculation (control *vs*. inoculated) within each cultivar. In contrast, the Tukey's test was used to compare the cultivar means within each treatment. All analyses were performed using Sisvar software (Ferreira 2019) at a 5% significance level.

Growth and yield analysis. Ear insertion height, measured from the ground level to the first ear; plant height, measured from ground level to panicle apex at harvest, number of rows per ear, number of grains per ear row, number of grains per ear, ear length, 100-grain weight, determined at 13% moisture content, grain yield (kg/ha) at 13% moisture content, harvest index calculated using (Bremner 2016) formula:

$$\text{harvest index (\%)} = (\text{grain yield/total biomass}) \times 100$$

Leaf chlorophyll index measurements at the R1 stage using a portable chlorophyll meter (CCM-200, Opti-Sciences, Hudson, USA).

Nutrient analysis. Nutrient concentration and accumulation in grains and straw at the flowering stage, including N, P, K, Ca, Mg, S, B, Cu, Fe, Mn, and Zn. The determination followed the methodology adapted from Malavolta et al. (1997), which involved drying, weighing, and grinding plant materials. The accumulations of nutrients were calculated based on their respective dry matter and the concentrations of nutrients, using the equation:

$$\text{dry matter weight (kg/ha)} \times \text{nutrient concentration (g/kg or mg/kg)}$$

where: macronutrients in g/kg, and micronutrients in mg/kg.

RESULTS

According to Figure 3A, inoculation resulted in higher leaf chlorophyll levels in all cultivars, indicating a possible increase in the photosynthetic capacity of the treated plants.

According to Figure 3B, in the 2020/2021 crop season, inoculation resulted in greater dry mass at flowering for all inoculated cultivars. However, DKB

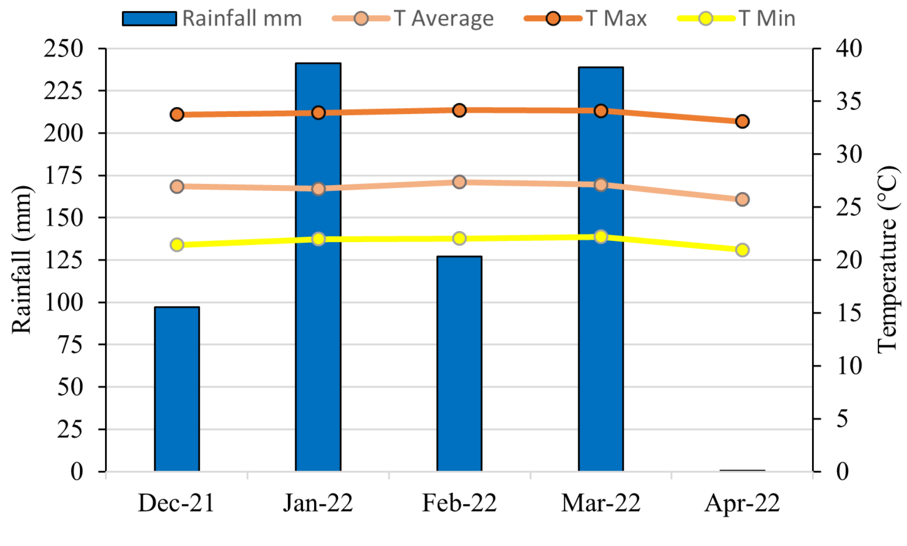

Figure 2. Rainfall, maximum and minimum temperatures (T). Weather station of the Extension and Research Farm of the School of Engineering – UNESP, December to April 2021–2022

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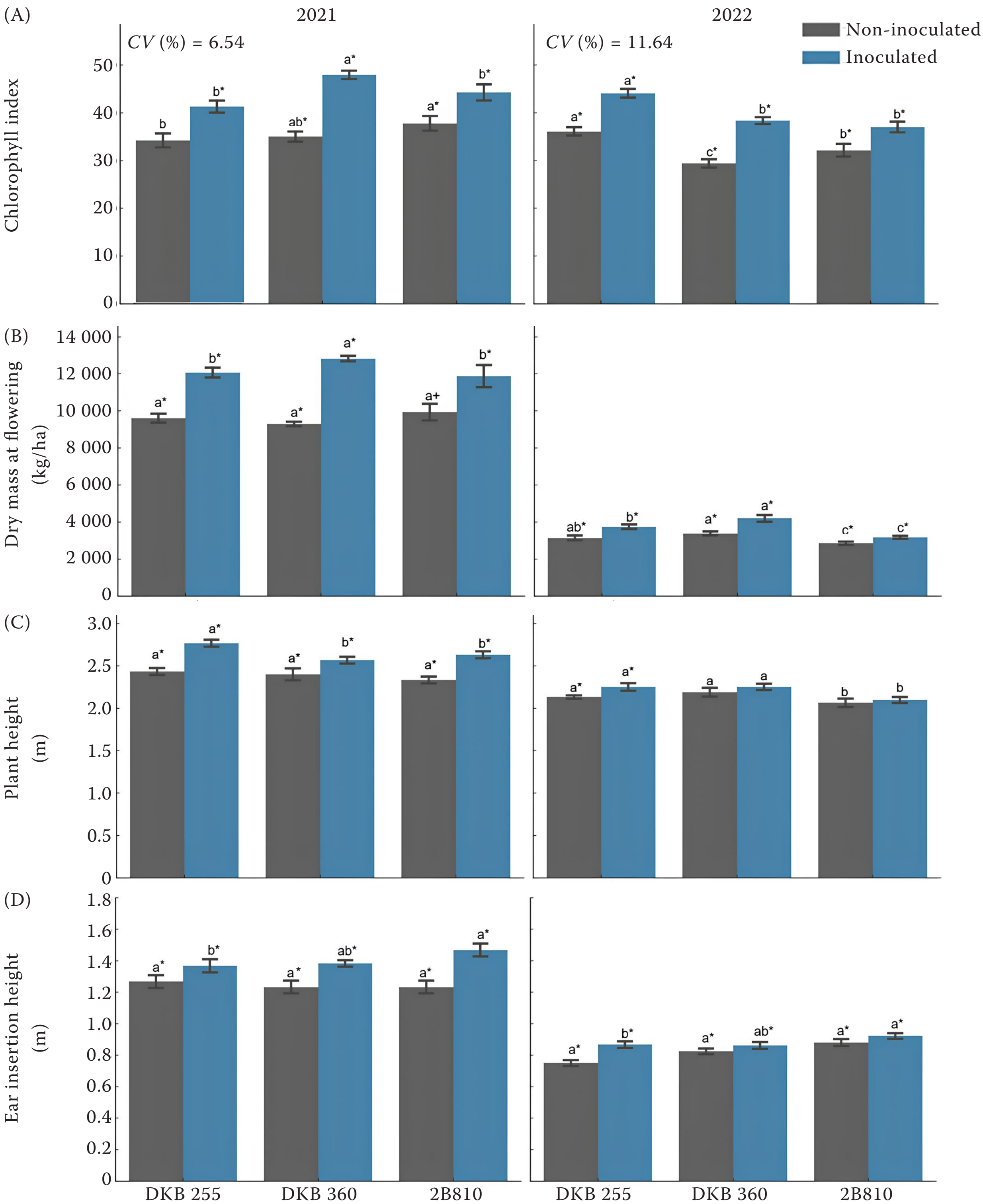

Figure 3. (A) Chlorophyll index (SPAD); (B) dry mass at flowering; (C) plant height and (D) ear insertion height of three corn cultivars in the 2020/2021 and 2021/2022 crop seasons, with and without *Trichoderma harzianum* inoculation. Distinct letters on the bars indicate significant differences among the cultivars within the treatments, both inoculated and non-inoculated (control), as determined by the Tukey's test ($P \leq 0.05$). *Indicate the difference between inoculated and non-inoculated (control) plants within each corn cultivar by the test-F ($P \leq 0.05$). Bars indicate the standard deviation (SD)

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360 showed the highest biomass accumulation, followed by DKB 255. In the 2B810 cultivar, the response to inoculation for this variable was less pronounced. In the 2021/2022 crop season, drought reduced the total accumulated biomass for all cultivars; however, inoculated plants showed a smaller relative reduction in biomass. DKB 360 maintained higher dry matter with inoculation, while 2B810 continued to show a lower response to inoculation.

According to Figure 3C, regarding the plant height variable in the 2020/2021 crop season, inoculation resulted in greater plant height for all cultivars. During the 2021/2022 crop season, the height of plants was reduced due to drought.

According to Figure 3D, in the 2020/2021 crop season, the ear insertion height was greater for all inoculated plants. In the 2021/2022 crop season, drought reduced the height of the first ear; however, the inoculated treatment still showed higher values than the non-inoculated one.

According to Figure 4A, in the 2020/2021 crop season, under irrigated conditions in the 2020/2021 crop season, inoculation did not result in a significant difference in the number of rows per ear between the inoculated treatment and the control. In the 2021/2022 crop season, marked by drought, inoculation resulted in a higher number of rows per ear for all cultivars, demonstrating its positive effect under adverse conditions.

According to Figure 4B, in the 2020/2021 crop season, inoculation resulted in more kernels per row in the inoculated plants, except for DKB 360, which showed no significant difference between treatments. During the drought period in the 2021/2022 crop season, all inoculated cultivars demonstrated a higher number of kernels per row compared to the control, reinforcing the benefits of inoculation even under adverse conditions.

In the 2020/2021 crop season, inoculation resulted in longer ears in the inoculated plants, except for

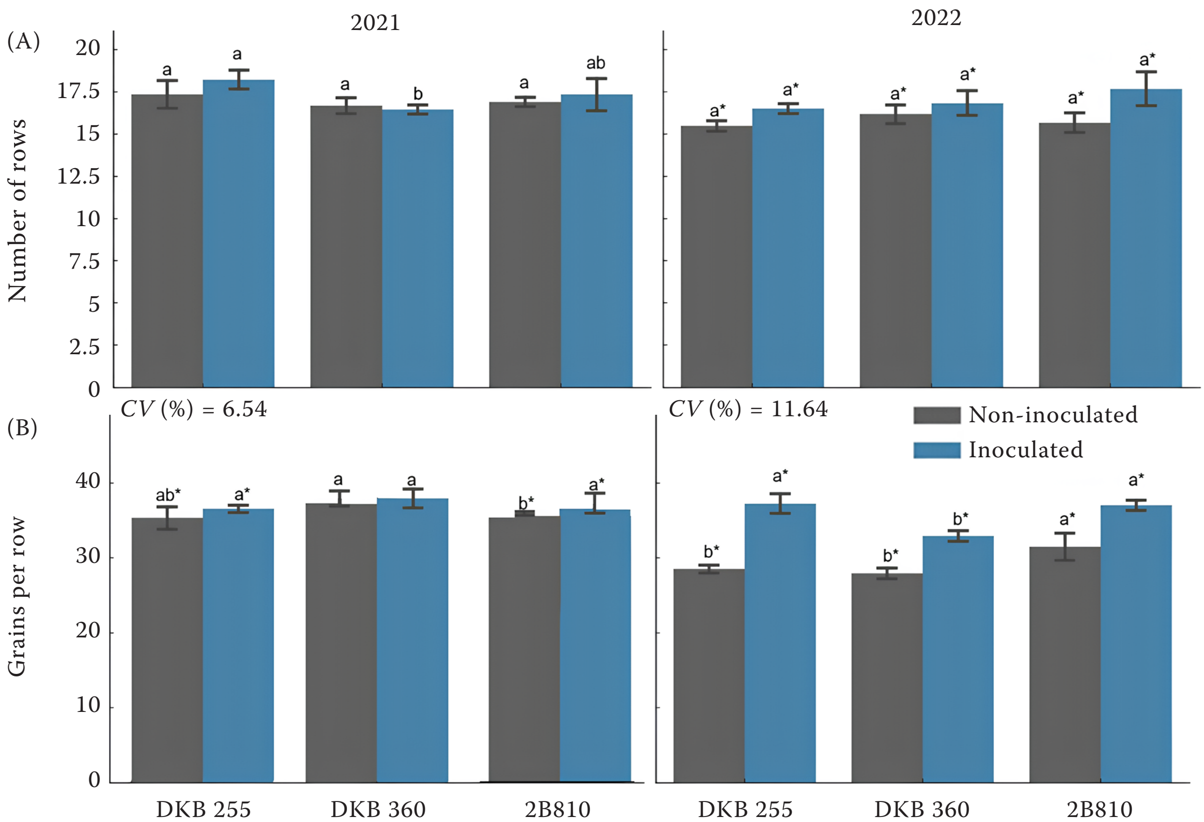

Figure 4. (A) Number of rows and (B) grains per row of three corn cultivars in the 2020/2021 and 2021/2022 crop seasons, with and without *Trichoderma harzianum* inoculation. Distinct letters on the bars indicate significant differences among the cultivars within the treatments, both inoculated and non-inoculated (control), as determined by the Tukey's test ($P \leq 0.05$). *Indicate the difference between inoculated and non-inoculated (control) plants within each corn cultivar by the test-F ($P \leq 0.05$). Bars indicate the standard deviation (SD)

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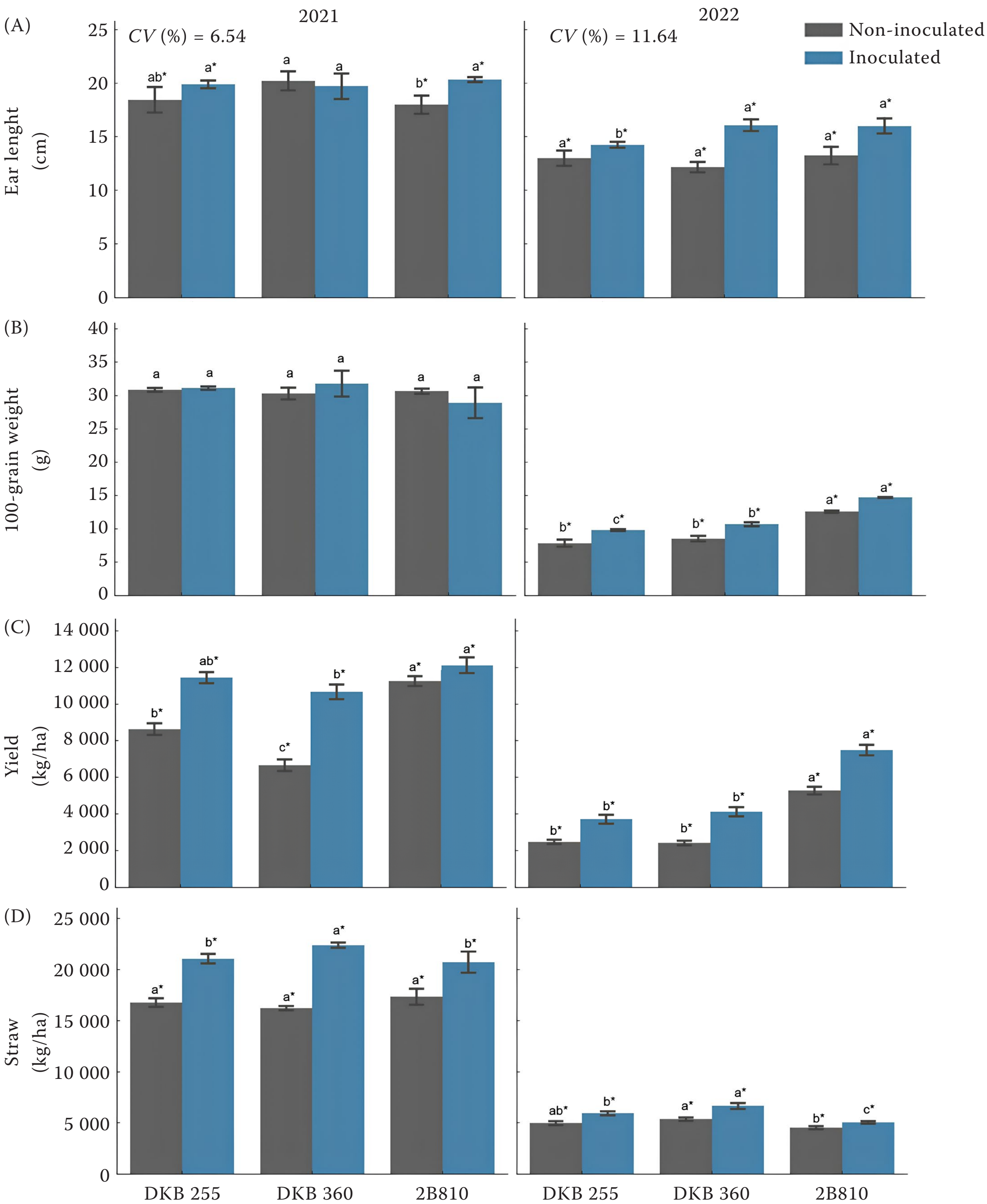

Figure 5. (A) Ear length; (B) 100-grain weight; (C) yield of three and (D) straw of three corn cultivars in the 2020/2021 and 2021/2022 crop seasons, with and without *Trichoderma harzianum* inoculation. Distinct letters on the bars indicate significant differences among the cultivars within the treatments, both inoculated and non-inoculated (control), as determined by the Tukey's test ($P \leq 0.05$). *Indicate the difference between inoculated and non-inoculated (control) plants within each corn cultivar by the test-F ($P \leq 0.05$). Bars indicate the standard deviation (SD)

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DKB 360, which showed no significant difference compared to the control. During the drought period in the 2021/2022 crop season, all inoculated cultivars exhibited larger ears than the control (Figure 5A).

According to Figure 5B, under irrigated conditions in the 2020/2021 crop season, inoculation did not result in a significant difference in 100-grain weight for any of the cultivars evaluated. In the drought-affected 2022 growing season, inoculation significantly increased 100-grain weight relative to the uninoculated control across all evaluated cultivars. Despite the overall reduction in grain weight caused by the drought, inoculated plants outperformed the control.

In the 2020/2021 crop season, all inoculated cultivars showed higher yields compared to the control, with DKB 360 exhibiting the most positive response, followed by DKB 255. The difference between treatments was less pronounced in 2B810 (Figure 5C). In the 2021/2022 crop season, drought significantly reduced the yield of all cultivars. However, even under adverse conditions, inoculation provided greater yield gains than the control. DKB 360 maintained notable yield stability, with inoculated plants producing 60% more than the control. On the other hand, 2B810 showed a less pronounced response to inoculation, with only a 7% increase compared to the control (Figure 5C).

According to Figure 5D, in the 2020/2021 crop season, straw production was higher for all inoculated plants, particularly for DKB 360, which showed the most pronounced response to inoculation. On the other hand, 2B810 had a less significant response compared to DKB 360. In the 2021/2022 crop season, drought reduced the final biomass of all plants. However, inoculated plants still achieved better results than the control, especially DKB 360, which produced a significantly larger amount of straw than the other cultivars.

The data from Figures 5C and 5D show a significant increase in yield and straw yield for all three cultivars following inoculation with *Trichoderma harzianum*. It is particularly noteworthy that the response to inoculation varied among the cultivars. DKB360 exhibited the greatest increase in yield as a result of inoculation.

Nutritional data.

Nutrients in dry matter during the flowering period in 2020/2021. According to Table 1 data, for the three maize cultivars studied (DKB255, DKB360, and 2B810), inoculation with *Trichoderma harzianum* generally resulted in increased levels of calcium, iron, potassium, magnesium, nitrogen, phosphorus, and sulfur. However, a trend toward decreased copper and zinc levels was observed in response to inocula-

Table 1. Nutrients in dry matter during the flowering period in the 2020/2021 crop season for inoculated and non-inoculated cultivars

Nutrient	*CV* (%)	Cultivar					
		DKB255		DBK360		2B810	
		control	inoculated	control	inoculated	control	inoculated
Macroelement (kg/ha)							
N	6.0	210^{A*}	282^{b*}	214^{A*}	332^{a*}	215^{A*}	301^{b*}
P	6.6	29^{A*}	32.5^{a*}	26.2^{A*}	34.9^{a*}	26.9^{A*}	35.2^{a*}
K	5.52	182^{A*}	275^{b*}	174^{A*}	311^{a*}	161^{A*}	237^{c*}
Ca	7.16	32.4^{A*}	44.2^{ab*}	30.9^{A*}	49.0^{a*}	29.5^{A*}	46.9^{ab*}
Mg	6.33	24.3^{A*}	31.5^{ab*}	22.3^{AB*}	33.3^{a*}	19.8^{B*}	28.7^{b*}
S	4.21	19.3^{B*}	28.5^{b*}	20.5^{B*}	36.9^{a*}	23.0^{A*}	27.1^{b*}
Microelement (g/ha)							
Fe	5.65	$11\,271^{B*}$	$14\,634^{b*}$	$15\,032^{A*}$	$16\,915^{a*}$	$5\,526^{C*}$	$9\,477^{c*}$
Mn	9.87	$1\,222^{B}$	$1\,369^{b}$	$1\,116^{C*}$	$1\,573^{ab*}$	$1\,481^{A*}$	$1\,775^{a*}$
Zn	10.37	$1\,491^{A*}$	759^{ab*}	$1\,060^{B*}$	683^{b*}	$1\,098^{B*}$	931^{a*}
Cu	6.03	$2\,739^{B*}$	$4\,449^{a*}$	$2\,068^{C*}$	$1\,102^{c*}$	$3\,564^{A*}$	$3\,104^{b*}$

Uppercase letters in the row indicate the differences between the non-inoculated cultivars. Lowercase letters in the row indicate the differences between the inoculated cultivars. The asterisk indicates a significant difference in relation to the inoculation of the cultivar compared with the same uninoculated cultivar. Tukey (5%) = *F*-value

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Table 2. Nutrients in dry matter during the flowering period in the 2021/2022 crop season for inoculated and non-inoculated cultivars

Nutrient	*CV* (%)	Cultivar					
		DKB255		DBK360		2B810	
		control	inoculated	control	inoculated	control	inoculated
Macroelement (kg/ha)							
N	4.77	56.0^{B*}	74.9^{b*}	74.7^{A*}	104.5^{a*}	59.1^{B*}	71.1^{b*}
P	7.53	6.56^{B*}	8.07^{b*}	8.77^{A*}	10.53^{a*}	7.93^{A}	7.86^{b}
K	3.74	53.1^{B}	53.4^{c}	61.3^{A*}	98.8^{a*}	52.5^{B*}	70.1^{b*}
Ca	7.13	10.90^{A}	11.37^{b}	10.12^{A*}	14.42^{a*}	8.66^{B*}	10.46^{b*}
Mg	8.01	5.63^{B}	5.78^{c}	7.78^{A*}	10.50^{a*}	6.93^{A*}	7.96^{b*}
S	5.67	5.99^{B}	5.71^{c}	7.24^{A*}	11.71^{a*}	5.56^{B*}	7.28^{b*}
Microelement (g/ha)							
Fe	7.22	7 063^{A*}	4 942^{a*}	5 354^{B}	5 435^{a}	3 284^{C}	3 772^{b}
Mn	11.19	404^{A*}	709^{a*}	389^{A*}	495^{b*}	349^{A}	345^{c}
Zn	9.28	582^{A*}	234^{a*}	370^{B*}	215^{a*}	426^{B*}	192^{a*}
Cu	6.17	584^{B*}	492^{b*}	691^{A*}	333^{c*}	749^{A*}	1 077^{a*}

Uppercase letters in the row indicate the differences between the non-inoculated cultivars. Lowercase letters in the row indicate the differences between the inoculated cultivars. The asterisk indicates a significant difference in relation to the inoculation of the cultivar compared with the same uninoculated cultivar. Tukey (5%) = *F*-value

tion for some of the evaluated parameters, except for cv. DKB255, which almost doubled the copper levels in the dry matter during the flowering period.

Nutrients in dry matter during the flowering period in 2021/2022. As per Table 2 data, the inoculation in crop season 2021/2022 provided different results for the three maize cultivars evaluated (DKB255, DKB360, and 2B810), with nutrients that were elevated or reduced as a function of the cultivar analysed; however, the inoculation provided a consistent increase in nitrogen levels for all cultivars

Table 3. Nutrients in maize grains in the 2020/2021 crop season for inoculated and non-inoculated cultivars

Nutrient	*CV* (%)	Cultivar					
		DKB255		DBK360		2B810	
		control	inoculated	control	inoculated	control	inoculated
Macroelement (kg/ha)							
N	6.36	142^{AB*}	219^{a*}	115^{B*}	156^{b*}	173^{A*}	199^{a*}
P	4.08	49.0^{B*}	73.5^{a*}	37.0^{C*}	64.3^{b*}	60.9^{A*}	68.8^{ab*}
K	4.06	79.4^{B*}	118.5^{b*}	56.9^{C*}	106.2^{c*}	100.6^{A*}	128.8^{a*}
Ca	8.16	2.011^{A*}	2.951^{a*}	0.892^{C*}	2.760^{a*}	1.407^{B*}	1.720^{b*}
Mg	8.49	18.7^{A*}	29.3^{a*}	10.1^{B*}	25.6^{b*}	18.6^{A*}	22.7^{b*}
S	7.00	12.1^{B*}	20.6^{a*}	8.4^{C*}	14.3^{b*}	14.6^{A*}	16.2^{b*}
Microelement (g/ha)							
Fe	17.44	344^{b}	407^{b}	272^{b}	424^{b}	1 404^{A*}	796^{a*}
Mn	14.18	130^{B}	149^{a}	103^{C*}	153^{a*}	157^{A}	162^{a}
Zn	8.13	732^{A*}	1 740^{a*}	502^{B}	582^{b}	428^{B}	485^{b}
Cu	8.68	2 519^{A*}	5 119^{a*}	2 084^{B*}	1 337^{b*}	289^{C}	347^{c}

Uppercase letters in the row indicate the differences between the non-inoculated cultivars. Lowercase letters in the row indicate the differences between the inoculated cultivars. The asterisk indicates a significant difference in relation to the inoculation of the cultivar compared with the same uninoculated cultivar. Tukey (5%) = *F*-value

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Table 4. Nutrients in maize grains in the 2021/2022 crop season for inoculated and non-inoculated cultivars

Nutrient	*CV* (%)	Cultivar					
		DKB255		DBK360		2B810	
		control	inoculated	control	inoculated	control	inoculated
Macroelement (kg/ha)							
N	9.86	42.5^B*	53.1^b*	40.2^B*	57.7^b*	82.2^A*	137.9^a*
P	6.54	14.6^B*	20.0^b*	12.4^B*	22.8^b*	27.6^A*	44.3^a*
K	6.90	27.3^B*	37.5^b*	20.1^C*	39.8^b*	47.1^A*	75.3^a*
Ca	9.03	0.519^B*	0.444^c*	0.291^C*	0.960^b*	1.107^A	1.743^a
Mg	8.44	3.97^B*	6.30^c*	3.53^B*	9.49^b*	10.98^A*	18.46^a*
S	9.17	3.63^B*	4.97^b*	2.96^B*	5.35^b*	7.19^A*	13.10^a*
Microelement (g/ha)							
Fe	13.65	165.2^A*	565.3^a*	96.8^B*	161.0^c*	205.8^A*	262.1^b*
Mn	15.00	33.1^B*	51.2^b*	35.8^B*	56.4^b*	76.2^A*	92.8^a*
Zn	11.57	91.7^C	138.0^c	175.3^B	214.7^b	430.0^A*	1096.4^A*
Cu	8.77	91.8^C	109.8^c	697.3^B*	473.1^b*	1416.3^A*	3083.9^a*

Uppercase letters in the row indicate the differences between the non-inoculated cultivars. Lowercase letters in the row indicate the differences between the inoculated cultivars. The asterisk indicates a significant difference in relation to the inoculation of the cultivar compared with the same uninoculated cultivar. Tukey (5%) = *F*-value

evaluated. A decrease in zinc levels was also observed in response to inoculation in all cultivars evaluated.

Nutrients in maize grains – crop season 2020/2021. As per Table 3 data, inoculation generally resulted in a differential increase in nutrients in maize grains from different cultivars. However, the cv. DKB255 was the only one that showed a significant positive change in zinc levels.

Nutrients in maize grains – crop season 2021/2022. All three cultivars exhibited increased

Table 5. Nutrients in straw in the 2020/2021 crop season for inoculated and non-inoculated cultivars. Uppercase letters in the row indicate the differences between the non-inoculated cultivars

Nutrient	*CV* (%)	Cultivar					
		DKB255		DBK360		2B810	
		control	inoculated	control	inoculated	control	inoculated
Macroelement (kg/ha)							
N	18.01	81.0^B	112.3^b	79.1^B*	126.6^ab*	144.7^A*	163.8^a*
P	9.62	17.8^B*	28.0^a*	11.4^C*	29.9^a*	22.1^A*	31.3^a*
K	5.95	237^A	235^ab	151^B*	242^a*	149^B*	213^b*
Ca	8.57	44.0^B*	71.1^b*	45.8^B*	82.1^b*	61.0^A*	99.6^a*
Mg	7.85	31.0^A*	49.9^b*	36.4^A*	62.8^a*	36.9^A*	52.1^b*
S	6.81	22.6^A*	37.0^b*	25.5^A*	45.2^a*	22.5^A*	33.1^b*
Microelement (g/ha)							
Fe	11.60	4 869^A*	2 307^c*	5 235^A	5 274^a	2 378^B*	4 184^b*
Mn	12.00	1 445^C*	2 796^b*	1 764^B*	2 808^b*	2 519^A*	3 971^a*
Zn	17.28	963^A	698^c	620^A*	1 847^a*	910^A*	1 378^b*
Cu	7.03	5 227^A	5 220^c	3 371^B*	9 504^a*	4 615^A*	6 661^b*

Lowercase letters in the row indicate the differences between the inoculated cultivars. The asterisk indicates a significant difference in relation to the inoculation of the cultivar compared with the same uninoculated cultivar. Tukey (5%) = *F*-value

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Table 6. Nutrients in straw in the 2021/2022 crop season for inoculated and non-inoculated cultivars

Nutrient	*CV* (%)	Cultivar					
		DKB255		DBK360		2B810	
		control	inoculated	control	inoculated	control	inoculated
Macroelement (kg/ha)							
N	14.63	26.8^{A*}	51.2^{a*}	25.2^{A*}	36.2^{b*}	20.8^{C}	25.8^{c}
P	10.53	3.50^{A*}	5.70^{b*}	3.46^{A*}	8.21^{a*}	4.41^{A*}	6.15^{b*}
K	5.77	45.0^{B*}	75.4^{a*}	48.5^{B*}	69.8^{a*}	62.2^{A*}	54.5^{b*}
Ca	6.95	20.2^{A*}	17.4^{b*}	13.7^{B*}	22.0^{a*}	10.7^{C*}	15.3^{b*}
Mg	9.29	13.08^{A}	12.74^{b}	11.54^{A*}	18.01^{a*}	8.03^{B*}	11.45^{b*}
S	6.95	6.94^{B*}	9.87^{b*}	8.20^{A*}	13.07^{a*}	5.93^{B*}	8.57^{c*}
Microelement (g/ha)							
Fe	15.14	849^{C}	$1\,039^{b}$	$1\,697^{A}$	$1\,543^{a}$	$1\,290^{B*}$	542^{c*}
Mn	9.06	750^{A}	820^{a}	561^{B*}	802^{a*}	375^{C*}	642^{c*}
Zn	16.46	195^{A*}	673^{a*}	197^{A*}	527^{b*}	250^{A*}	160^{c*}
Cu	8.90	$1\,672^{A}$	$1\,539^{b}$	$1\,026^{C*}$	$2\,609^{a*}$	$1\,298^{B}$	$1\,148^{c}$

Uppercase letters in the row indicate the differences between the non-inoculated cultivars. Lowercase letters in the row indicate the differences between the inoculated cultivars. The asterisk indicates a significant difference in relation to the inoculation of the cultivar compared with the same uninoculated cultivar. Tukey (5%) = *F*-value

nutrient levels in the straw due to inoculation, with a few exceptions, as observed in Table 4. The cv. DKB360 showed increases in almost all nutrients, except for a decrease in copper content. The cv. 2B810 had substantial increases in zinc, almost doubling its concentration in the grains.

Nutrients in straw – crop season 2020/2021. For straw nutrients in the crop season 2020/2021, each cultivar exhibited distinct changes in nutrient levels, either increasing or decreasing, depending on the specific cultivar. Notably, there was a uniform enhancement in nitrogen levels across all cultivars following inoculation Table 5.

Nutrients in straw – crop season 2021/2022. The data presented in Table 6, regarding nutrients in the straw resulting from inoculation in the 2021/2022 crop season, also varied according to the genotype of the cultivar, with varying results in nutrient levels in the straw of the three cultivars. However, for all three cultivars, inoculation increased nitrogen levels.

Notably, the cv. DKB360 exhibited a superior response to inoculation, showing significant increases in nutrient concentrations in both straw and grains. Although producing more grains, the cv. 2810 showed a lower straw yield in relation to inoculation. The cvs. DKB255 and DKB360 are potential candidates for future food biofortification research.

DISCUSSION

The *Trichoderma harzianum* (ESALQ 1306 strain) differentially improved the growth and yield of the three maize cultivars, exhibiting a notable capability in promoting the growth of maize plants (Tables 1 and 2). These results are similar to those found by Ali et al. (2022), Araújo et al. (2023), and Fadiji and Babalola (2020), who also reported positive effects of *Trichoderma* inoculation on plant growth. However, our findings differ from those of Mahato and Neupane (2017), who assessed the effects of inoculating maize plants with a *Trichoderma* strain that had a negative influence on crop growth. Although all cultivars showed improvements with inoculation, each cultivar responded differently to inoculation, which may be related to its unique genetic characteristics. The cv. DKB360 showed high responsiveness to inoculation with the *Trichoderma* strain, resulting in greater crop growth, yield, and nutrition in maize straw and grains. In turn, the cv. 2B810 showed the lowest percentage increase in yield. This could be explained by the high base yield of this cultivar, indicating that there may be a limit to how much inoculation can improve yield in cultivars that are already highly productive.

Despite the scarcity of studies assessing the performance of *Trichoderma* sp. with different plant

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genotypes, our results are similar to those found by Schmidt et al. (2020), who evaluated the variation in efficiency of Trichoderma inoculation on different beet genotypes in relation to plant growth. This study can offer valuable insights for future research into maize yield, especially considering the importance of choosing the right cultivar to maximise the benefits of inoculation with endophytes such as *Trichoderma*.

The *Trichoderma harzianum* improved the nutritive value of the three maize cultivars; these results reinforce the growth-promoting capacity of *Trichoderma harzianum* (Tables 1 and 2). All three cultivars exhibited a positive response to inoculation with *Trichoderma harzianum*, resulting in overall increases in nutrient levels. In this regard, the cv. DKB360 showed the highest increase in all nutrients among the inoculated maize cultivars. Knowing the nutrient levels in straw is important because straw can be an integral part of the animal diet (Zhang et al. 2020).

In both seasons, we can conclude that inoculating maize grains with Trichoderma harzianum tends to increase the most nutrients in the grains of the three cultivars. In this context, *Trichoderma harzianum* employs various mechanisms to enhance plant nutrient absorption (Azarmi et al. 2011, Santiago et al. 2011, Ali et al. 2022, Araújo et al. 2023). For instance, *Trichoderma* contributes to the protection and expansion of the root system, increasing the effective area for nutrient absorption; it induces root growth through the production of plant hormones, resulting in a more efficient root system for nutrient absorption (Araújo et al. 2023, Awad-Allah et al. 2023, Boorboori and Zhang 2023). *Trichoderma* can secrete various organic acids that play a crucial role in facilitating the acquisition of soil nutrients such as phosphorus and nitrogen, making them more available to plants (Viswanath et al. 2020, Paul and Rakshit 2021). Conversely, a decrease in the copper and zinc levels was observed in response to *Trichoderma* strain inoculation. These results may be related to *Trichoderma's* ability to regulate the availability of these elements in the soil (Viswanath et al. 2020, Paul and Rakshit 2021, Ali et al. 2022, Araújo et al. 2023). Some *Trichoderma* strains are known for their soil bioremediation capability, acting as a filter that can increase heavy metal content near roots but regulate and prevent their absorption by the plant, thus aiding in biomass increment even in relatively contaminated environments (Babu et al. 2014, Khalid et al. 2021, Karmaita et al. 2023). However, according to a study by de Santiago et al. (2011), which assessed zinc absorption in wheat plants, it was noted that in soils poorer in these nutrients, the reduction in absorption of these elements may be more pronounced.

Overall, *Trichoderma harzianum* (ESALQ 1306 strain) as an inoculant can benefit the nutritional quality of maize grains, although these benefits vary according to the plant genotype. These data also help us better understand how inoculation affects soil fertility and nutrient exportation; this information could guide more sustainable agricultural management practices (Chen et al. 2023).

The occurrence of an extreme drought event facilitated the observation of the effects of inoculation on cultivars under drought conditions. Drought stress altered and improved the impact of *Trichoderma* on maize yield and nutritional value; all cultivars benefited from inoculation under drought conditions. The *Trichoderma harzianum* mitigated part of the negative impact of drought on these cultivars, possibly by enhancing water use efficiency and aiding in the plants' nutrient acquisition (Fadiji and Babalola 2020, Mengistu 2020, Araújo et al. 2023, Boorboori and Zhang 2023).

In particular, the cv. DKB360 showed the most significant increases in nutrient content, as well as in straw and grain yield, after inoculation. These findings indicate that this cultivar is an excellent candidate for advanced genomic studies, which could further our understanding of the genes associated with improved responsiveness to the inoculation process. On the other hand, inoculation resulted in increased nitrogen levels for all three cultivars, which is a significant observation because nitrogen is a crucial macronutrient for plant growth. These results are consistent with those of Singh et al. (2019), who discussed how inoculation with *Trichoderma* can alter the regulation of transcription and activation of signal transduction in relation to N metabolism.

Therefore, in this scenario where global agriculture faces growing challenges due to climate change and rapid population growth, our study demonstrated how the introduction of endophytes, such as *Trichoderma harzianum*, is a promising tool for sustainable innovation with the ability to promote efficient reduction in the use of agrochemicals and increase agricultural yield even under conditions of extreme climatic events such as prolonged drought (Paul and Rakshit 2021, Anand et al. 2023, Araújo et al. 2023). These discoveries provide valuable insights and contribute to ensuring food security in the coming decades, thereby reducing deforestation by increasing crop yields and alleviating the pressure to open new agricultural areas.

Our work demonstrated that the endophytic fungus *Trichoderma harzianum* (ESALQ 1306 strain), registered exclusively as a fungicide and nematicide for the biological control of soil diseases, can act as a powerful plant growth promoter in maize cultivation. In addition, we demonstrated that the selection of maize cultivars significantly impacts the efficacy of inoculation with *Trichoderma harzianum*, influencing nutrient availability and the quantity and nutritional quality of the grains. This study is pioneering in its focus on the importance of considering the genotype of traditionally cultivated maize plants in commercial plantations, particularly in relation to the effectiveness of inoculation with *Trichoderma harzianum*. Such discoveries have significant implications for cost planning because of the price of inoculants in relation to their effectiveness for specific cultivars. The results of our study also provide valuable information for plant breeding programs, potentially contributing to the creation of new standards for seed sales, requiring companies to inform about the responsiveness of their cultivars to the main biological products on the market to ensure that producers are well-informed about the expected performance of the varieties in relation to inoculation.

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