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RODRIGO MATTOS SILVA GALEANO

Isolamento, caracterização bioquímica e potencial de *Trichoderma* spp. para promoção de crescimento da soja

CAMPO GRANDE – MS
MARÇO – 2024

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Tese de doutorado apresentada ao Programa Multicêntrico de Pós-Graduação em Bioquímica e Biologia Molecular – PMBqBM – SBBq, da Universidade Federal do Mato Grosso do Sul, como requisito para a obtenção do grau de Doutor.

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TERMO DE APROVAÇÃO

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Á minha avó Ilca de Mattos,
sempre em minha memória,

dedico.

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RESUMO

Nas regiões agrícolas do Cerrado brasileiro, o teor de nutrientes é significativamente baixo, levando muitos agricultores a aplicar frequentemente fertilizantes. Entretanto, a crise recente de fertilizantes tem impulsionado a busca por alternativas mais sustentáveis e econômicas para o manejo agrícola. Os bioinsumos à base de *Trichoderma* são capazes de aumentar a disponibilidade de nutrientes para as plantase protegê-las contra o ataque de fitopatógenos. Além disso, esses fungos podem melhorar a qualidade do solo ao colonizar a rizosfera, aumentando a atividade de enzimas envolvidas em processos biogeoquímicos. Por isso, o objetivo deste estudo foi selecionar, caracterizar, e avaliar o efeito da inoculação de novos isolados de *Trichoderma* spp. no crescimento e na produtividade da soja. Para isso, realizou-se um isolamento a partir de solo rizosférico de plantas soja cultivadas em São Gabriel do Oeste, MS, Brasil. Os fungos foram confrontados a fitopatógenos da cultura da soja, e os três isolados escolhidos foram caracterizados quanto à presença de mecanismos que promovemo crescimento de plantas e tolerância a diferentes condições e agroquímicos. Esses isolados foram posteriormente inoculados em sementes de soja (cv. Nidera NS6601), e o efeito na germinação e desenvolvimento inicial foi determinado. O efeito da inoculação dos isolados na produtividade foi avaliado em experimentos de campo utilizando duas cultivares de soja (Nidera NS6601 e DM 69IX60RSF 12X RR2 PRO) e duas doses de fertilização fosfatada (400 e 200 kg ha^{-1}) de superfosfato simples (SSP). Após 48 dias da semeadura (DAS), foram realizadas coletas para análises foliares e avaliação das atividades enzimáticas do solo rizosférico das plantas. Dos 66 isolados de *Trichoderma* spp. obtidos, os isolados GT-8 (*T. viride*), GT-31 (*T. reesei*), e GT-32 (*T. longibrachiatum*) foram selecionados. Eles mostraram diversas habilidades promotoras de crescimento *in vitro*, incluindo produção de ácido indol-3-acético, solubilização de fosfato, síntese de sideróforos, além de tolerância a condições adversas e crescimento em meios com diferentes agroquímicos. Os isolados não inibiram a germinação das sementes e promoveram significativamente o crescimento das plântulas, com aumento notável na parte aérea e raízes, especialmente quando co-inoculados, aumentando em 50,3% e 48,8%, respectivamente. No campo, a inoculação de GT-32 (200 kg ha^{-1}) na soja (cv. Nidera NS6601) resultou em aumento de 4,3% na produtividade de grãos. Enquanto que para a cultivar DM 69IX60RSF 12X RR2 PRO, o uso de GT-31 e GT-32, na meia dose de fertilizante, proporcionou aumentos de 22,7% e 18,6% na produtividade de grãos, comparado à testemunha. Plantas inoculadas apresentaram maior teor de clorofilas, compostos fenólicos, flavonóides e respostas antioxidantes em ambas as condições de fertilização, com maiores atividades das enzimas catalase, peroxidase e ascorbato peroxidase. Além disso, as atividades enzimáticas foram mais elevadas no solo rizosférico das plantas de soja inoculadas. As descobertas deste estudo demonstraram o potencial de novas cepas de *Trichoderma* para aumentar o crescimento e produtividade da soja, e minimizar o uso de fertilizantes no campo. Por fim, o uso destas novas cepas também pode ser uma estratégia sustentável para melhorar a saúde e a fertilidade do solo.

Palavras-chave: bioinsumo, solubilização de fosfato, ácido indol-3-acético, agroquímicos, fertilizante, FPCV.

ABSTRACT

In the agricultural soils of the Brazilian Cerrado, the nutrient content is significantly low, leading many farmers to frequently apply fertilizers. However, the recent fertilizer crisis has driven the search for more sustainable and economical alternatives for agricultural management. Bio-inputs based on *Trichoderma* are able to increase the availability of nutrients for plants and protect them from attack by phytopathogens. In addition, these fungi can improve soil quality by colonizing the rhizosphere, increasing the activity of enzymes involved in biogeochemical processes. Therefore, the aim of this study was to select, characterize and evaluate the effect of inoculation of new *Trichoderma* spp. isolates on soybean growth and productivity. The fungi were isolated from the rhizospheric soil of soybean plants grown in São Gabriel do Oeste, MS, Brazil. The isolates were tested against soybean crop pathogens, and the three isolates chosen were characterized for their plant growth-promoting mechanisms, tolerance to different conditions and agrochemicals. Theisolates were then inoculated into soybean seeds (cv. Nidera NS6601), and their effect on germination and initial development was determined. The effect of inoculation of the isolates on productivity was assessed in field experiments using two cultivars of soybean (Nidera NS6601 and DM 69IX60RSF 12X RR2 PRO), and two doses of phosphate fertilization (400 and 200 kg ha^{-1}) of simple superphosphate (SSP). After 48 days of planting, samples were collected for leaf analysis and evaluation of the enzymatic activities of the rhizospheric soil of the plants .Of the 66 *Trichoderma* spp. isolates obtained, GT-8 (*T. viride*), GT-31 (*T. reesei*), and GT-32 (*T. longibrachiatum*) were selected. They showed various plant growth-promoting abilities *in vitro*, including indole-3-acetic acid production, phosphate solubilization, siderophore synthesis, as well as tolerance to adverse conditions and growth in media with different agrochemicals. They did not inhibit seed germination and significantly promoted seedling growth, with a notable increase in the shoot and root of the plants, especially when co-inoculated, increasing by 50.3% and 48.8% respectively. In the field, GT-32 inoculation (200 kg ha^{-1}) on soybeans (cv. Nidera NS6601) resulted in a 4.3% increase in grain yield. While for the cv. DM 69IX60RSF 12X RR2 PRO, the use of GT-31 and GT-32 at half the fertilizer dose resulted in yield increases of 22.7% and 18.6%, respectively, compared to the control. Inoculated plants showed a higher content of chlorophylls, phenolic compounds, flavonoids and antioxidant responses in both fertilization conditions, with higher activities of the enzymes catalase, peroxidase and ascorbate peroxidase. Moreover, the enzyme activities were higher in the rhizospheric soil of inoculated soybean plants. The findings of this study demonstrated the potential of new *Trichoderma* strains to increase soybean growth and productivity, and minimize the use of fertilizers in the field. Finally, the use of these new strains could also be a sustainable strategy to improve soil health and fertility.

Keywords: bio-input, phosphate solubilization, indole-3-acetic acid, agrochemicals, fertilizer, PGPF.

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LISTA DE SÍMBOLOS, ABREVIATURAS E UNIDADES

x g	Força centrífuga relativa
µg/mL	Micrograma por mililitro
µg P/mL	Micrograma de fósforo por mililitro
ºC	Grau Celsius
6-PP	6-pentil-α-pirona
ACC	1-aminociclopropano-1-carboxilato
AIA	Ácido indol-3-acético
Al	Alumínio
ANOVA	Análise de variância
APX	Ascorbato peroxidase
BCAs	*Biological control agents*
Ca	Cálcio
CAT	Catalase
CWDEs	*Cell Wall Degrading Enzymes*
CMC	Carboximetilcelulose
CO_2	Dióxido de carbono
CONAB	Companhia Nacional do Abastecimento
CYA	*Czapek yeast extract agar*
DAS	Dias após a semeadura
DNA	Ácido Desoxirribonucleico
DNS	Ácido 3,5-dinitrosalicílico
EDPC	Enzimas de degradação da parede celular
EDTA	Ácido etilenodiamino tetra-acético
FAS	Serviço Agrícola Estrangeiro
FDA	Diacetato de fluoresceína
Fe	Ferro
GNP	Número de grãos por vagem
g/kg	Grama por quilograma
GY	Produtividade de grãos
GYB	Produtividade de grãos em sacas
h	Hora

ha	Hectare
HFP	Altura da inserção da primeira vagem
ITS	*Internal transcribed spacer*
K	Potássio
M1000	Massa de mil grãos
MEA	*Malt extract agar*
mg	miligrama
min	Minuto
mL	Mililitro
mm	Milímetro
mM	Milimolar
mPa	Milipascal
MPCP	Microrganismos Promotores do Crescimento de Plantas
Mg	Magnésio
mg g^{-1} FW	Miligrama por grama de peso fresco
MGI	Inibição do crescimento micelial
N	Nitrogênio
NaCl	Cloreto de Sódio
NAGase	*N*-acetil-*β*-D-glucosaminidase
NBP	Número de ramos por planta
NGP	Número de grãos por planta
nm	Nanômetro
NRP	Pequenos peptídeos não ribossômicos
NPP	Número de vagens por planta
P	Fósforo
PCA	*Principal component analysis*
PCR	*Polymerase chain reaction*
PDA	*Potato-dextrose-agar*
PDB	*Potato-dextrose broth*
PEG	Polietilenoglicol
PH	Altura da planta
pH	Potencial hidrogeniônico
POD	Peroxidase

p-NPP	*para*-nitrofenilfosfato
p-NPNAG	*para*-nitrofenil-β-*N*-acetilglucosamina
®	Registrado
ROS	*Reactive oxygen species*
rpm	Rotação por minuto
s	segundos
RBA	*Agar Rose Bengal*
RSI	Resistência Sistêmica Induzida
SISGEN	Sistema Nacional de Gestão do Patrimônio Genético
spp.	Espécies
SSP	Superfosfato Simples
TSM	*Trichoderma* selective medium
TLE	*Trichoderma liquid enzyme medium*
U/mg	Unidades por miligrama
U/mL	Unidades por mililitro
v/v	Volume por volume
VOCs	Compostos orgânicos voláteis
w/v	Peso por volume
Zn	Zinco

SUMÁRIO

1. INTRODUÇÃO

No cenário global,o Brasil se destaca como o maior produtor e exportador de soja. Segundo a Companhia Nacional do Abastecimento (CONAB, 2023), a safra 2022/2023 superou em 23,1% o recorde de produção alcançado na safra anterior, com 154.609,5 mil de toneladas. A cultura é de grande importância em vários setores, como agroindústria, indústria química, fabricação de rações para animais, alimentação humana, além dos grãos serem fonte alternativa para produção de biocombustível (FREITAS, 2004; LIU *et al*., 2020; QIN *et al*., 2022).

Apesar das tecnologias e estratégias de manejo atuais serem avançadas, há fatores que limitam a obtenção de altos rendimentos de grãos no campo. Por exemplo, as perdas que ocorrem anualmente devido às doenças causadas por uma variedade de fungos, nematóides, bactérias e vírus (BANDARA *et al*., 2020). As perdas por doenças fúngicas e a severidade destas dependem de diversos fatores, como o ano, região de plantio, cultivar de soja usada, práticas de manejo para o controle de doenças, e virulência da cepa (LIN *et al*., 2022).

No Brasil, as doenças economicamente importantes que mais acometem a cultura da soja são a ferrugem-asiática, causada por *Phakopsora pachyrhizi* (WRATHER *et al*., 2010), a podridão de carvão, causada por *Macrophomina phaseolina* (LODHA; MAWAR, 2020), o mofo branco, causado por *Sclerotinia sclerotiorum* (SMOLIŃSKA; KOWALSKA, 2018), e o tombamento de plântulas causado por certas espécies de *Fusarium* (LIN *et al*., 2022). Muitas práticas são recomendadas para o manejo destas doenças, incluindo o controle químico com fungicidas. Entretanto, a aplicação excessiva e a longo prazo destes insumos vem causando diversos impactos ambientais nos últimos anos, como contaminação de ecossistemas, impactos na saúde humana e dos animais, além do desenvolvimento de resistência dos patógenos aos fungicidas (PRASHAR; SHAH, 2016; ALBUQUERQUE *et al*., 2016;

CAPELLA *et al*., 2023). Isso implicana necessidade urgente por alternativas mais sustentáveis visando a substrituição completa ou parcial desses insumos químicos.

Um outro problema enfrentado pela agricultura brasileira é a aquisição de fertilizantes no mercado externo. O país é altamente dependente da importação de fertilizantes, correspondendo a 85% dos aplicados nos solos agrícolas brasileiros (MAPA, 2022). Desde 2020, o mercado global de fertilizantes tem sido afetado por diversos problemas, incluindo as limitações impostas pela pandemia da COVID-19, além da crise energética na China e em países europeus, e a guerra entre Rússia e Ucrânia (ARNDT *et al*., 2023; BROWNLIE *et al*., 2023). Esses fatores afetaram a oferta destes insumos, disparando os preços no mercado internacional. Fertilizantes fosfatados, por exemplo, aumentaram em cerca de 100% o preço de mercado (BROWNLIE *et al*., 2023).

Logo, alternativas sustentáveis e economicamente viáveis estão sendo cada vez mais priorizadas. Entre essas, está o uso de microrganismos benéficos para biocontrole de patógenos e promoção de crescimento vegetal, sendo uma das alternativas mais sustentáveis e seguras para o meio ambiente (SOUMARE *et al*., 2019; MACIK *et al*., 2020).

Fungos do gênero *Trichoderma* apresentam mecanismos eficientes para o controle de fitopatógenos no campo. Entre os mecanismos utilizados por esses fungos para a supressão de patógenos, estão a antibiose, competição por espaço e/ou nutrientes, e micoparasitismo pela produção de enzimas capazes de degradar estruturas de resistência (escleródios, microescleródios) e parede celular dos fitopatógenos, e indução de resistência sistêmica (POVEDA, 2021). Esses fungos também possuem propriedades que atuam diretamente no metabolismo vegetal, aumentando o crescimento e produtividade, como a produção de fitormônios reguladores de crescimento (TYŚKIEWICZ *et al*., 2022). Além disso, *Trichoderma* é capaz de aumentar a disponibilidade de nutrientes para as plantas, como o

fósforo, característica de grande interesse para a formulação de inoculantes (ALORI *et al*., 2017; SOUMARE *et al*., 2019).

Os biofungicidas à base de *Trichoderma* são utilizados no Brasil em diversas culturas, incluindo a da soja. Esses insumos estão bem consolidados no manejo de doenças causadas por fungos habitantes do solo. Entretanto, o papel duplo como biocontrolador e promotor de crescimento vegetal ainda é pouco explorado e as formulações para esta finalidade ainda são limitadas no mercado. Alguns produtos apresentam em seus rótulos a informação de que as cepas utilizadas podem promover o crescimento direto de culturas. No entanto, a principal finalidade dessas cepas é o biocontrole de patógenos. Portanto, com o aumento do interesse nessa alternativa pela agricultura brasileira, torna-se necessário a busca por novas cepas de *Trichoderma* com propriedades para promoção de crescimento direta para o sistema agrícola. Pesquisas com o objetivo de selecionar e caracterizar novas cepas contribuirão para a promoção de uma agricultura mais sustentável e menos onerosa, contribuindo para aumentar a competitividade do país no cenário global.

2. REVISÃO DE LITERATURA

2.1 Histórico da cultura da soja no Brasil

A soja (*Glycine max* [L.] Merril) é uma das culturas de maior relevância no mercado internacional atual em termos de produção. Historicamente, a soja possui sua primeira evidência histórica no nordeste da China, entre 1700 e 1100 a.C, embora existam relatos do seu cultivo já em 2300 a.C. (HARTMAN *et al*., 2011). A Figura 1 ilustra a disseminação dessa cultura no mundo a partir do centro de origem, na China. No continente americano, o cultivo dessa oleaginosa se iniciou em 1804 no estado da Pensilvânia, Estados Unidos (HYMOWITZ *et al*., 1987).

Figura 1 - Mapa da origem e a difusão geográfica da cultura soja no mundo. (Fonte: Extraído de BONETTI, 1970)

No Brasil, o primeiro registro indica que Gustavo D'Utra tentou introduzir a soja na Bahia em 1882, sem êxito no cultivo (D'UTRA, 1882). As primeiras cultivares não tiveram êxito pois não se adaptaram às condições edafoclimáticas do país. Em 1908, esse cenário mudou a partir da introdução de novas variedades de soja trazidas por imigrantes japoneses (LÖBBE, 1945). Após novos estudos e experimentos, a cultura foi oficialmente incorporadaao Brasil em 1914, no estado do Rio Grande do Sul, com os primeiros relatos de plantio comercial datando de 1923 (MAGALHÃES, 1981).

A partir dos anos 40, houve uma notável expansão da cultura da soja no mercado brasileiro, tornando-se produtor pela primeira vez nas estatísticas internacionais em 1949, com aumento de mais de 50 vezesna produção (DALL'AGNOL, 2016). O sucesso no cultivo de trigo durante o inverno (maio a novembro) e de soja no verão (novembro a abril) na região Sul do país contribuiu para essa expansão (BRUM *et al*., 2004). A partir de 1950, políticas específicas de incentivo ao cultivo do trigo também estimularam o rápido crescimento da produção da leguminosa.

O destaque da soja no agronegócio brasileiro na década de 70 consolidou essa cultura como uma das principais do Brasil, colocando o país em destaque no contexto mundial (HIRAKURI; LAZZAROTTO, 2014; DALL'AGNOL, 2016). Ao longo dos anos, a produtividade e a área de plantio da cultura atingiram níveis surpreendentes. Isso foi alcançado devido a ínumeros avanços científicos e tecnológicos, como melhoria de cultivares adaptadas ao clima tropical, manejo de solos, adubação balanceada, melhorias no sistema de plantio direto, fixação biológica do nitrogênio e outras práticas agrícolas (NETO *et al*., 2010; ROCHA *et al*., 2018; de SOUZA *et al*., 2019; UMBURANAS *et al*., 2022). Recentemente, por exemplo, ROTUNDO *et al*. (2022) desenvolveram um aplicativo que auxilia na otimização das taxas de fertilização, incluindo o preço de fertilizantes e grãos, no estado do Mato Grosso.

A expansão do plantio da soja do sul para o cerrado brasileiro ocorreu a partir de 1980, incluindo o sul de Goiás, o sul de Mato Grosso do Sul e o Triângulo Mineiro (DOMINGUES; BERMANN, 2012). Com o passar dos anos, as áreas de cultivo avançaram no país, principalmente, devido à implementação de centros de pesquisas nacionais focados no melhoramento genético e estudos sobre os aspectos fisiológicos, bioquímicos e agronômicos da cultura (CAMPOS, 2010; DALL'AGNOL, 2016).

Atualmente, a soja ocupa uma posição de destaque no mercado brasileiro agrícola, sendo a principal *commodity* do país devido aos seus mais variados usos como matéria-prima, e cerca de 240 mil fazendas produzem soja no país (BICUDO DA SILVA *et al*., 2020). A soja representa uma significativa fonte de proteínas e outros nutrientes essenciais para diversos países, principalmente os asiáticos (LIU *et al*., 2020). Os grãos são compostos de aproximadamente 35–40% de proteínas, 20% de lipídios, 9% de fibra alimentar (QIN *et al*., 2022). Devido ao seu altoteor de proteínas, a soja é utilizada na produção de rações de alta qualidade para animais (REZENDE *et al*., 2023).

Ademais, observou-se nos últimos anos um aumento significativo no seu consumo na alimentação humana (SINGH, 2010; QIN *et al*., 2022). Dentre as diversasregiões do mundo que consomem alimentos à base de soja, os países asiáticos se destacam pelo alto consumo diário desses alimentos (RIZZO; BARONI, 2016). Na indústria, essa oleaginosa é usada para fabricação de óleo de soja, biodiesel, biocompósitos, produtos de limpeza e beleza (PLOSCHUK *et al*., 2022). Em 2020, o Brasil alcançou o 1° lugar no *ranking* como o maior produtor de soja do mundo, superando os Estados Unidos. Atualmente, o Brasil, Estados Unidos e Argentina representam aproximadamente 80% da produção mundial desta oleaginosa (Figura 2).

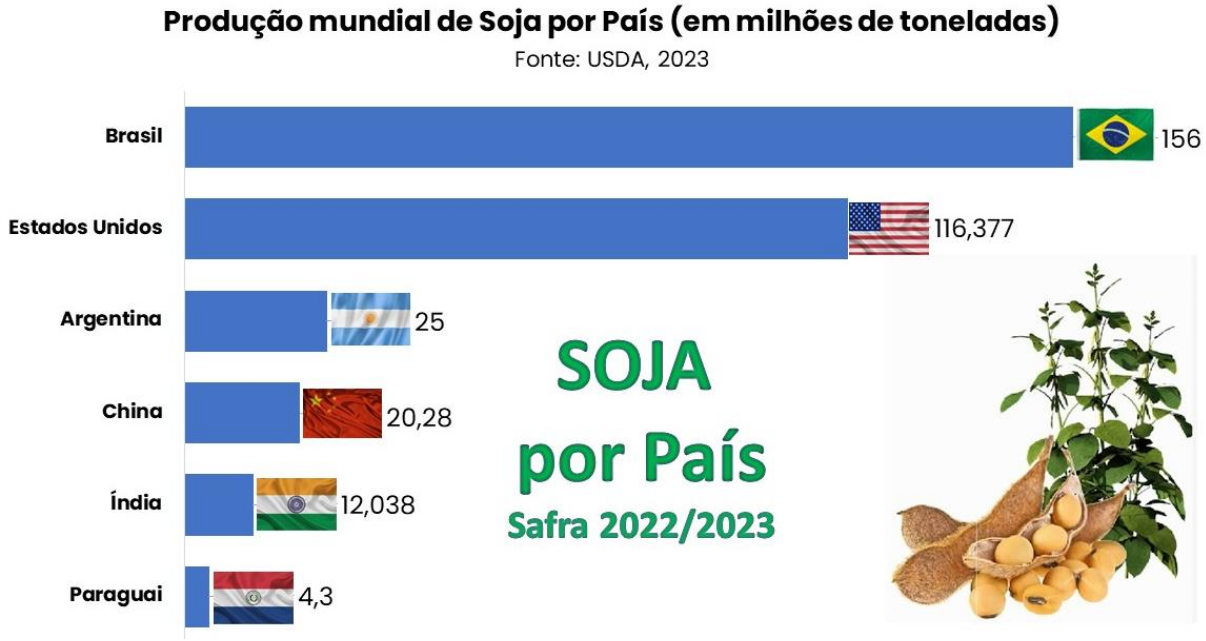

Figura 2 - Ranking dos maiores produtores de soja do mundo. (Fonte: Extraído de BOSCHIERO, 2023)

De acordo com a CONAB (2023), a produtividade obtida foi superior em 23,2% em relação à safra 2022/23, com recordes de produção observados em vários estados. O Brasil produziu cerca de 154.605,9 mil toneladas em uma área de 44.079,8 mil hectares, representando um aumento de 4,4% em comparação coma safra anterior. No ranking nacional, o estado de Mato Grosso do Sul (MS) ocupa o 5° lugar na produção da oleaginosa. Na última safra 2022/2023, o estado de MS encerrou o ciclo com 15,006 milhões de toneladas, cobrindo 4,005 milhões de hectares (SIGA/MS, 2023). No entanto, apesar do aumento observado nas áreas de cultivo, produtividade e exportação do grão nos últimos anos, existem fatores limitantes que afetam a produtividade da soja, tornando o processo dispendioso e levando ao aumento das perdas no campo.

Na última safra, por exemplo, algumas áreas de cultivo de MS enfrentaram um regime irregular de chuvas, caracterizado por períodos de estiagem e baixa umidade no solo, resultando em uma redução da produtividade (SIGA/MS, 2023). De acordo com SILVA *et al*.

(2023), o aumento das temperaturas e os períodos de seca prolongada no Cerrado brasileiro têm impacto negativo na produtividade da soja. Todavia, os produtores mostram maior preocupação com as mudanças climáticas de curto prazo, pois acreditam que investimentos no setor agrícola poderão contornar esses problemas, o que é discordado por diversos pesquisadores (RODRIGUES *et al*., 2022; SILVA *et al*., 2023).

O aumento da incidência de doenças, como a ferrugem asiática (agente causal: *Phakopsora pachyrhizi*), e o mofo-branco (agente causal: *Sclerotinia sclerotiorum*), também está preocupando os produtores rurais. O clima tem sido apontado como uma das principais causas dessas doenças, e o aumento da aplicação de fungicidas está entre as estratégias principais de manejo fitossanitário (FAVERIN, 2023). No entanto, essas medidas impactam negativamente os ecossistemas agrícolas e naturais, aumentando a resistência de patógenos e afetando a fertilidade dos solos (HARTMANN; SIX, 2023).

Na região do Cerrado brasileiro, estudos destacam que o desmatamento destinado à expansão agrícola, juntamente com práticas convencionais de manejo de doenças e nutrição de plantastêm desencadeado impactos negativos na qualidade do solo desse bioma, e ameaça à biodiversidade (RAMPELOTTO *et al*. 2013; dos SANTOS *et al*., 2022; PROCÓPIO; BARRETO, 2021). A expansão desenfreada da agricultura nas regiões de Cerrado exacerbou os problemas fitossanitários, exigindo a implementação urgente de medidas mais sustentáveis para garantir a manutenção da produção agrícola nessas terras aráveis (PROCÓPIO; BARRETO, 2021). Além disso, a crise dos fertilizantes resultou no aumento dos preços no mercado global, dificultando o acesso aos fertilizantes fosfatados e potássicos (BROWNLIE *et al*., 2023). Isso, por sua vez, deve ter consequências já na próxima safra 2023/24 (CAMARGO, 2023).

2.2. O uso de insumos químicos na cultura da soja

Nas últimas décadas, a difusão de inovações tecnológicas na cultura da soja tem redefinido o agronegócio brasileiro, consolidando o país como uma das maiores potências globais desse setor. O aumento notável na produtividade da cultura no país é diretamente atribuído a uma série de tecnologias, como sementes, inoculantes para fixação biológica do nitrogênio, fertilizantes, agrotóxicos, e maquinaria avançada (ARTUZO *et al*., 2018; BOLFE *et al*., 2020; UMBURANAS *et al*., 2022). Em virtude disso, os produtores dependem, consideravelmente, do fornecimento destes insumos, que estão relacionados diretamente aos custos de produção.

Entre eles, os fertilizantes estão entre os mais dependentes de importação. Em 2021, a importação desses insumos atingiu um recorde de 41,6 milhões de toneladas, representando aumento de 21% em relação a 2020, quando foram importados 34,25 milhões de toneladas (CONAB, 2022). Esse volume corresponde a 85% dos fertilizantes utilizados nos solos agrícolas do Brasil (MAPA, 2022). Segundo o Instituto de Pesquisa Econômica Aplicada (IPEA, 2022), os fertilizantes de potássio (K) (38%), fósforo (P) (33%) e nitrogênio (N) (29%) são os mais comumente empregados na agricultura brasileira. A cultura da soja é dependente, principalmente, de importações de fertilizantes fosfatados e potássicos.

A soja não necessita de adubação nitrogenada, uma vez que sua associação simbiótica com bactérias do gênero *Bradyrhizobium* pode suprir as necessidades de N (SANTOS *et al*., 2019). Para isso, as sementes de soja são tratadas com inoculantes contendo cepas recomendadas de *Bradyrhizobium*. Entretanto, outros nutrientes são limitantes para a produtividade desta oleaginosa, como P e K.

Depois do N, o P é o segundo nutriente mais limitante para o crescimento de plantas. Nos solos agrícolas, a concentração desse elemento é relativamente baixa, sendo apenas 0,1% desse P disponível para absorção pelas raízes das plantas (ALORI *et al*., 2017). Esse cenário

se deve à baixa mobilidade no solo e a sua alta reatividade com cálcio (Ca), alumínio (Al) e ferro (Fe), resultando em sua precipitação (RAWAT *et al*., 2021). Além disso, o P pode ser imobilizado ou adsorvido à matéria orgânica presente no solo (KISHORE *et al*., 2015). Esses eventos ocorrem nos solos do Cerrado brasileiro, uma vez que eles são pobres em nutrientes e ácidos, com elevadas concentrações de alumínio (BUSTAMANTE *et al*. 2012; PROCÓPIO; BARRETO, 2021). A prática comum de aplicação defertilizantes fosfatados busca garantir o suprimento de P para as plantas. No entanto, a maior parte desse nutriente é rapidamente imobilizada pelos cátions no solo, tornando-se indisponível para absorção pelas raízes (RAWAT *et al*., 2021). Em doses maiores para superar esses efeitos, surgem problemas ambientais, como a contaminação de águas subterrâneas e superficiais (ALORI *et al*., 2017; MOREIRA *et al*., 2018).

Uma notável elevação dos preços destes fertilizantes foi observada em 2022. Os fosfatados, por exemplo, aumentaram cerca de 100%, de US$ 400 para US$ 800 por tonelada, enquanto o potássio aumentou em 170%, aumentando de US$ 290 para US$ 780 por tonelada (PEDROZO, 2022).

A crescente dependência do comércio exterior de insumos, expõe o agronegócio brasileiro vulnerável às flutuações do mercado internacional. Apesar do recorde de importações, o Brasil enfrentou dificuldades para adquirir fertilizantes no mercado global devido às restrições causadas pela pandemia de COVID-19 e pela crise energética que afetou países europeus e a China (ARNDT *et al*., 2023; BROWNLIE *et al*., 2023). Além disso, a guerra entre Rússia e Ucrânia limitou ainda mais as importações desses insumos, impactando diretamente a agricultura brasileira. A Rússia é o principal fornecedor de adubos e fertilizantes, responsável por cerca de 22% das importações totais, conforme dados do COMEXSTAT (2021). Com menor oferta no mercado, é previsível que os custos dos insumos

para a produção de soja aumentem nas próximas safras, afetando, consequentemente, a produção agrícola como um todo.

Por essa razão, a crescente preocupação no mercado nacional tem sido acompanhada pela busca por alternativas que possam reduziressa dependência do mercado externo. Paralelamente, outra grande preocupação da agricultura moderna é o uso excessivo de fertilizantes inorgânicos e agrotóxicos prejudiciais ao meio ambiente (PRASHAR; SHAH, 2016). O Brasil está entre os quatro maiores consumidores de agrotóxicos do mundo (juntamentecom Estados Unidos, União Europeia e China), representando mais de 20% do uso global desses insumos (ALBUQUERQUE *et al*., 2016; PAUMGARTTEN, 2020). Isso se deve ao fato de vários incentivos públicos, incluindo isenções totais ou parciais de impostos aplicados no comércio desses produtos químicos, o que facilita a sua aquisição e aplicação em larga escala no país (CAPELLA *et al*., 2023).

O papel dos fertilizantes químicos em fornecer nutrientes para as plantas (BHANDARI, 2014), e dos agrotóxicos (que incluem pesticidas, fungicidas, bactericidas e nematicidas) na erradicação e/ou controle de pragas e fitopatógenos (SOARES; PORTO, 2007) é bem compreendido. Entretato, o uso destes insumos ao longo das últimas décadas tem causando poluição ambiental e apresentado riscos para a saúde animal e humana.

A aplicação excessiva de fertilizantes inorgânicos provoca alterações na estrutura físico-química do solo, prejudicando suas funções, o equilíbrio ecológico, a conservação da biodiversidade do solo, além de aumentar a emissão de gases de efeito estufa e a eutrofização de corpos d’água, resultando na contaminação de lençóis freáticos (PRASHAR e SHAH, 2016; JACOBY *et al*., 2017). Os fertilizantes de fosfato, devido à sua composição,são uma das principais fontes de entrada de metais pesados no solo, acarretando efeitos prejudiciais para as plantas, águas e microbiota (FREITAS *et al*., 2009).

2.3. Doenças fúngicas na cultura da soja

A soja pode ser afetada por diversas doenças causadas por fungos patogênicos que habitam o solo, com perdas nas lavouras que podem chegar até 100% na produtividade (GRIGOLLI, 2015). Entre as principais doenças, estão o mofo branco (agente causal: *S. sclerotiorum*), a podridão de carvão da raiz (agente causal: *Macrophomina phaseolina*) e o tombamento de plântulas por *Fusarium* (LIN *et al*., 2022).

S. sclerotiorum desenvolve-se, principalmente, em períodos de alta umidade relativa do ar e temperaturas amenas, sendo presentes principalmente nos estados do Sul e Centro-Oeste do Brasil (SEVERO, 2021). Plantas acometidas pela doença apresentam murchas e necroses que podem levar até a morte. Na soja, a fase mais crítica está entre a floração plena e o início da formação de vagens e enchimento de grãos (SMOLIŃSKA; KOWALSKA, 2018).

Por outro lado, *M. phaseolina* é favorecida em temperaturas altas e baixa disponibilidade hídrica, em que os micro-escleródios podem germinar e produzir massa de hifas (REYES-FRANCO *et al*., 2006). Os sintomas são mais visíveis nos estágios finais da planta, em que o patógeno entra em sua fase necrotrófica, tornando a aparência da planta escura e empoeirada pelo bloqueio de feixes vasculares através de suas estruturas, secreção de enzimas e toxinas patogênicas (LODHA; MAWAR, 2020).

Para o manejo destas doenças, diversos métodos de controle são utilizados, incluindo o uso do controle químico por agrotóxicos. O uso intensivo de agrotóxicos nas últimas décadas tem impulsionado estudos abrangendo seus impactos em âmbitos sociais, econômicos e ambientais (BROVINI *et al*., 2021; DREONI *et al*., 2002). Esses insumos afetam espécies não-alvo e a qualidade do solo, pois suas moléculas ao penetrarem nas camadas do horizonte do solo, prejudicam microrganismos envolvidos na ciclagem de nutrientes, além de águas superficiais e subterrâneas (BHANDARI, 2014; HOSSAIN, 2017). Eles também afetam negativamente trabalhadores agrícolas, comunidades vizinhas e a população distante pelo

contato direto e indireto, desencadeando diversos distúrbios e até câncer (MARTIN *et al*., 2018; RANI *et al*., 2021).

Neste contexto, uma alternativa mais sustentável e segura para as culturas é o uso do controle biológico, minimizando os impactos causados por estes agroquímicos. Neste método, utilizam-se microrganismos, como bactérias e fungos, que apresentem mecanismos de ação para o controle de doenças de plantas. Controlando essas doenças, esses microrganismos podem indiretamente promover o crescimento e desenvolvimento vegetal, por isso, também são nomeados como microrganismos promotores de crescimento de plantas (GLICK, 2014; TYŚKIEWICZ *et al*., 2022).

2.4. Microrganismos Promotores do Crescimento de Plantas (MPCP)

MPCP são aqueles capazes de aumentar o crescimento e melhorar o desenvolvimento vegetal. Esses microrganismos podem ser encontrados no solo, na região rizosférica ou colonizando raízes e outras partes das plantas (GLICK, 2014; ADEDAYO; BABALOLA, 2023). Eles são responsáveis por diversas atividades no solo, entre elas a ciclagem de nutrientes, que podem melhorar o fornecimento de nutrientes para as raízes das plantas, auxiliando no crescimento e desenvolvimento vegetal (ALORI *et al*., 2017; VERMA *et al*., 2021). O uso de MPCP como, por exemplo,inoculantes e biofungicidas, tem sido proposto como uma das alternativas mais promissoras e sustentáveis para o crescimento e desenvolvimento de culturas (ALORI *et al*., 2017; SOUMARE *et al*., 2020; JIAO *et al*., 2021; LOPES *et al*., 2021; TYŚKIEWICZ *et al*., 2022).

Os MPCP apresentam diversos mecanismos para promover o crescimento vegetal, classificados em diretos e indiretos (Figura 3).

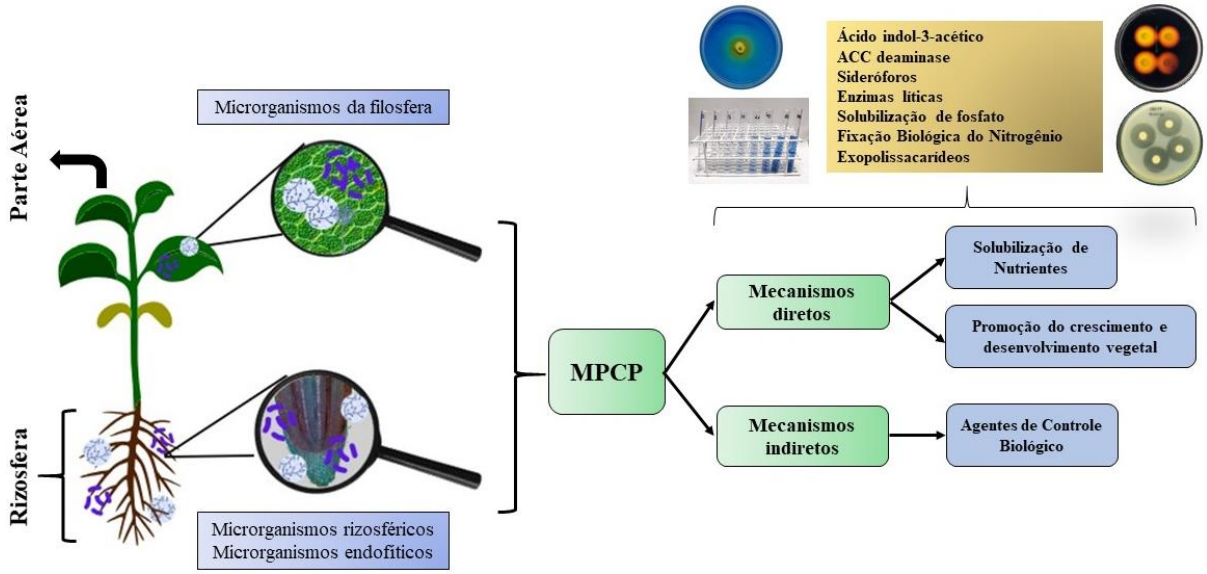

Figura 3 - Mecanismos diretos e indiretos usados por Microrganismos Promotores de Crescimento de Plantas (MPCP). (Fonte: Autoria Própria)

Entre os mecanismos diretos, está a bioestimulação através da produção de fitormônios, como o ácido indol-3-acético (AIA) (BORAH *et al*., 2023). A biofertilização também exerce influência direta no metabolismo vegetal, aumentando a disponibilidade de nutrientes minerais, como o P e o K, e a fixação biológica do nitrogênio pelas bactérias diazotróficas (KOUR *et al*., 2020; BORAH *et al*., 2023; RAWAT *et al*., 2021). Os mecanismos indiretos abrangem todas as propriedades responsáveis para o biocontrole de fitopatógenos, incluindo a produção de enzimas de degradação da parede celular (EDPC) e resistência sistêmica induzida (RSI) (ZEHRA *et al*., 2021). Assim, a busca por estratégias mais sustentáveis, impulsionada pela crise de fertilizantes que elevou os preços no mercado global, tem aumentado o interesse por inoculantes contendo MPCP, bactérias e/ou fungos, capazes de solubilizar e mineralizar nutrientes no solo (MACIK *et al*., 2020; FATIMA *et al*., 2021).

No Brasil, a Lei n° 6.934 de 1981 classifica como inoculante a substância que contenha microrganismos com a atuação favorável ao desenvolvimento vegetal.

Microrganismos formulados em produtos biológicos para uso no controle de microrganismos patogênicos e pragas agrícolas são chamados de agentes biológicos de controle. Apesar de possuírem baixa toxicidade e periculosidade (Decreto n° 4.074 de 2002), esses agentes foram categorizados como agrotóxicos segundo a Lei n° 7.802 de 1989, a Lei de agrotóxicos e afins, recentemente revogada pela Lei nº 14.785, de 2023, mas com as mesmas especificações. Para fungos do gênero *Trichoderma*, por exemplo, o termo mais adequado seria biofungicida.

2.5. *Trichoderma*: Uso no controle biológico

Fungos do gênero *Trichoderma* pertencem ao filo Ascomycota, classe Sordariomycetes, ordem Hypocreales e família Hipocreaceae. São filamentosos, encontrados nos mais diversos ambientes, algumas espécies com distribuição cosmopolita, enquanto outras possuem uma distribuição mais restrita (DRUZHININA *et al*., 2011). Os conidióforos de *Trichoderma* são organizados de maneira próxima à piramidal e padrões de ramificações desiguais. A coloração dos conídios facilita o reconhecimento deste gênero, com diferentes tons de verde (ABREU; PFENNING, 2019).

As espécies de *Trichoderma* podem ser de vida livre, sobre madeira em decomposição ou estar colonizando a rizosfera e tecidos vegetais quando habitantes do solo (ZIN; BADALUDDIN, 2020). Quando endófitos, curiosamente as espécies de *Trichoderma* estabelecem-se principalmente nos caules das plantas (EVANS *et al*., 2003). No mercado mundial, diversas espécies de *Trichoderma* são usadas em bioformulações para o biocontrole de doenças e pragas no mundo, como *T. viride*, *T. harzianum*, *T. asperelloides*, *T. asperellum*, *T. hamatum*, *T. longibrachiatum*, *T. koningii*, *T. koningiopsis*, *T. virens*, *T. afroharzianum* (YAO *et al*., 2021).

Na agricultura, é bem entendido o papel deste gênero no controle biológico de doenças, uma vez que possuem diversos mecanismos para proteção de plantas contra doenças

e pragas (SCUDELETTI *et al.*, 2021; SENGER *et al.*, 2023). De forma simplificada, o controle biológico é o uso de microrganismos (ou organismos) que ocorrem naturalmente para controlar doenças ou pragas de plantas (RAYMAEKERS *et al.*, 2020). Um dos conceitos mais amplamente aceitos pelos fitopatologistas para o controle biológico é o proposto por COOK e BAKER (1983), que define o controle biológico como "a redução da soma de inóculo ou das atividades determinantes da doença, provocada por um patógeno, realizada por um ou mais organismos que não o homem".

Entre os organismos, estão os antagonistas dos fitopatógenos, entre eles, os fungos filamentosos do gênero *Trichoderma*. Esses fungos atuam no controle de doenças usando diversos mecanismos ancestrais compartilhados por várias espécies do gênero, como micoparasitismo, competição, antibiose e resistência sistêmica induzida (RSI) (Figura 4).

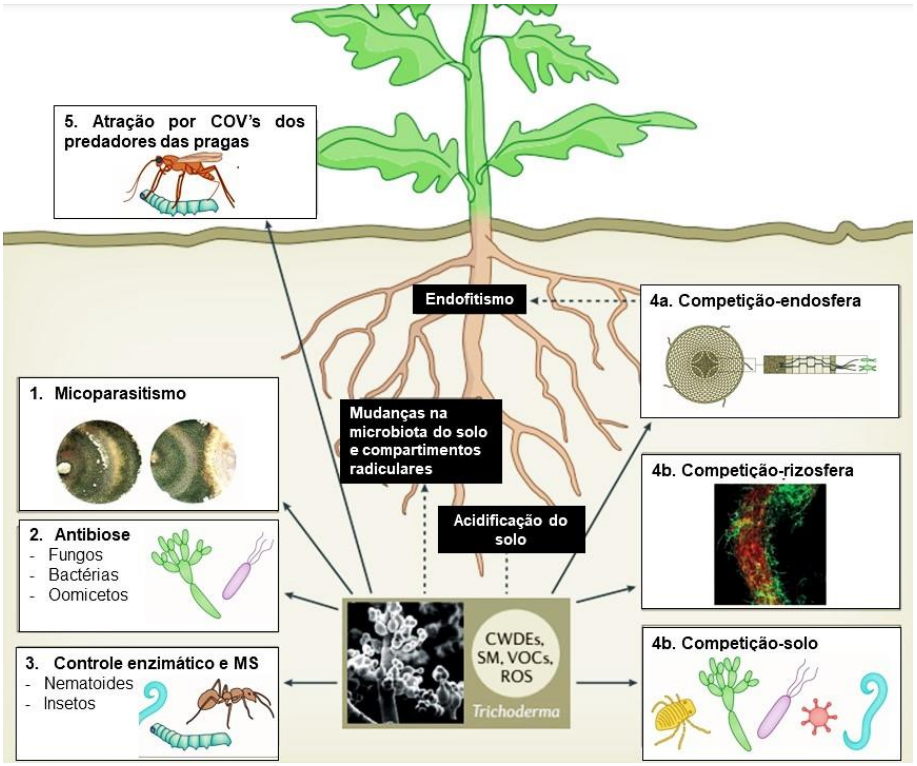

Figura 4 - Mecanismos usados por *Trichoderma* para o controle biológico em plantas. CWDEs, enzimas de degradação da parede celular de fitopatógenos; metabólitos secundários,

MS; compostos orgânicos voláteis, VOCs; espécies reativas de oxigênio, ROS. (Fonte: Extraído e adaptado de WOO et al., 2023).

O uso de biofungicidas contendo *Trichoderma* já é uma realidade no Brasil, com diversos produtos disponíveis no mercado (MACENA *et al*., 2020; ZIN; BADALUDDIN, 2020). No micoparasitismo, o *Trichoderma* adere à superfície da parede celular de fungos fitopatogênicos, secretam EDPC, e se alimenta ao absorver nutrientes por meio de suas hifas. Enzimas como *N*-acetil-β-D-glucosaminidases (NAGases), endoquitinases, β-1,3-gluanases e proteases, desempenham um papel crucial na degradação da parede celular dos patógenos (POVEDA, 2021). Algumas espécies de *Trichoderma* são conhecidas por serem micoparasitas agressivos, como *T. viride, T. harzianum, T. asperellum* e *T. asperelloides*, sendo utilizados como biofungicidas no Brasil para o controle de doenças (WOO *et al*., 2014).

Na antibiose, isolados de *Trichoderma* secretam metabólitos secundários que atuam em vias de sinalização e interação com outros microrganismos (RAJANI *et al*., 2021). Os metabólitos secundários pertencem a diversas classes, como pequenos peptídeos não ribossômicos (NRP), policetídeos, terpenóides, butenolídeos e pironas, como 6-pentil-α-pirona (6-PP) (RAMADA *et al*., 2019). Muitos desses compostos possuem efeito antagônico aos patógenos por serem tóxicos, ou induzirem a resistência sistêmica em plantas (RAMADA *et al*., 2019; WOO *et al*., 2023).

Na literatura, muitos estudos mostraram a atividade antifúngica de diversos metabólitos produzidos por espécies de *Trichoderma*, incluindo fitopatógenos que atacam plantas de soja, como *Sclerotinia*, *Sclerotium*, *Rhizoctonia*, *Corynespora*, *Macrophomina*, *Fusarium* e *Colletrotrichum* (KHAN *et al*., 2020). Compostos como 6-PP produzido por *T. viride, T.atroviride* e *T. harzianum*, também atua como regulador do crescimento das plantas, semelhante aos efeitos observados pelo AIA (VINALE *et al*., 2008).

Enquanto na competição, algumas espécies de *Trichoderma* disputam por nutrientes, espaço, oxigênio, luze sítios de infecção na planta com os patógenos, reduzindo as chances do desenvolvimento de doenças (RAYMAEKERS *et al*., 2020). A habilidade de ser um bom competidor por recursos ambientais parece ser mais restrita a espécies de *Trichoderma* com distribuição cosmopolita (DRUZHININA *et al*., 2011). O êxito de *Trichoderma* na colonização da rizosfera é atribuído à sua notável tolerância ao estresse oxidativo, uma vez que a rizosfera é um ambiente com um alto teor desse estresse. Isso ocorre devido às respostas das plantas ao ataque de patógenos, que inclui a produção de espécies reativas de oxigênio (ROS) (MORÁN-DIEZ *et al*., 2010).

A RSI também deve ser mencionada como um mecanismo de biocontrole para a proteção de plantas, mesmo na ausência de contato direto com o patógeno, *Trichoderma* ativa respostas moleculares e bioquímicas na planta que se associa (Figura 5).

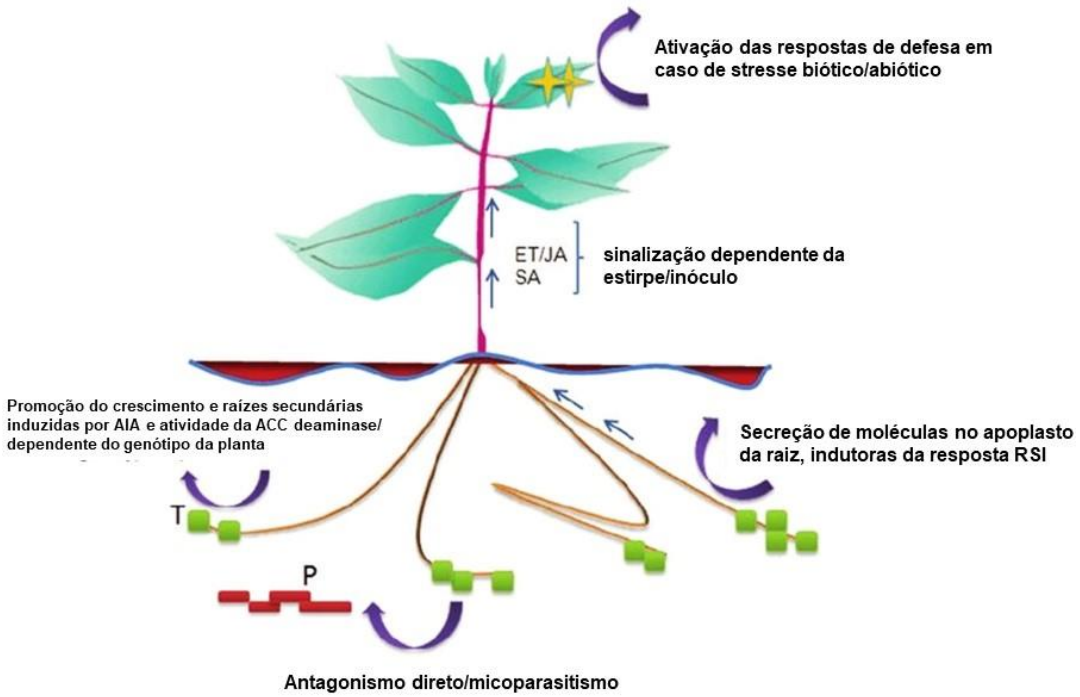

Figura 5 - Sinalização molecular de *Trichoderma*-planta e os efeitos induzido na planta por *Trichoderma*. AIA (ácido indol-3-acético); T (*Trichoderma)*; P (patógeno); RSI (resistência

sistêmica induzida); ET (etileno) ; JA (ácido jasmônico); SA (ácido salicílico). (Fonte: HERMOSA et al., 2012).

A indução de resistência de plantas é uma condição fisiológica de reforço da capacidade de defesa a patógenos (CHOUDHARY *et al*., 2007). Na planta, a indução por *Trichoderma* está diretamente ligada às cascatas de sinalização envolvendo os hormônios etileno/ácido jasmônico e ácido salicílico (MACÍAS-RODRÍGUEZ *et al*., 2020). Algumas espécies de *Trichoderma* podem ativar a RSI por diferentes vias, usando proteínas, enzimas e metabólitos secundários. Essa ativação é um pré-condicionamento ("*priming*"), que potencializa as respostas moleculares e bioquímicas das plantas para possíveis ataques de patógenos e a estresses abióticos (PASCHOLATI; DALIO, 2018).

Muitas estratégias são utilizadas na agricultura para o manejo destas doenças, como escolhas de sementes, cultivares, rotação de culturas, plantio direto, aplicação de agrotóxicos, e uso de biofungicidas (SMOLIŃSKA; KOWALSKA, 2018; SEVERO, 2021). O uso de *Trichoderma* para diminuir a densidade do inóculo dos patógenos pode contribuir para minimização dos impactos causados por fungicidas (ASAD, 2022). Portanto, o primeiro critério de seleção de cepas potenciais para o biocontrole é a realização de ensaios de antagonismo contra fitopatógenos de interesse econômico.

As perspectivas futuras indicam um crescimento na utilização desses insumos biológicos, o que impulsionará a vantagem competitiva do país, uma vez que esses produtos são menos onerosos em comparação aos agrotóxicos (RODIGUES *et al*., 2023). Em 2019, 21 produtos à base de *Trichoderma* estavam disponíveis no mercado (BETTIOL *et al*., 2019). Por isso, a busca de novos produtos com formulações contendo novas cepas é necessária para aumentar a oferta do mercado nacional.

2.6. *Trichoderma*: Promotor de crescimento vegetal

A interação planta-*Trichoderma* pode promover o crescimento, pois o fungo é capaz de solubilizar nutrientes no solo, tais como P, K, Zn e Fe, aumentando assim a abosorção pelas raízes. Estes microrganismos apresentam mecanismos que tornam esses elementos mais biodisponíveis para as plantas (Figura 6).

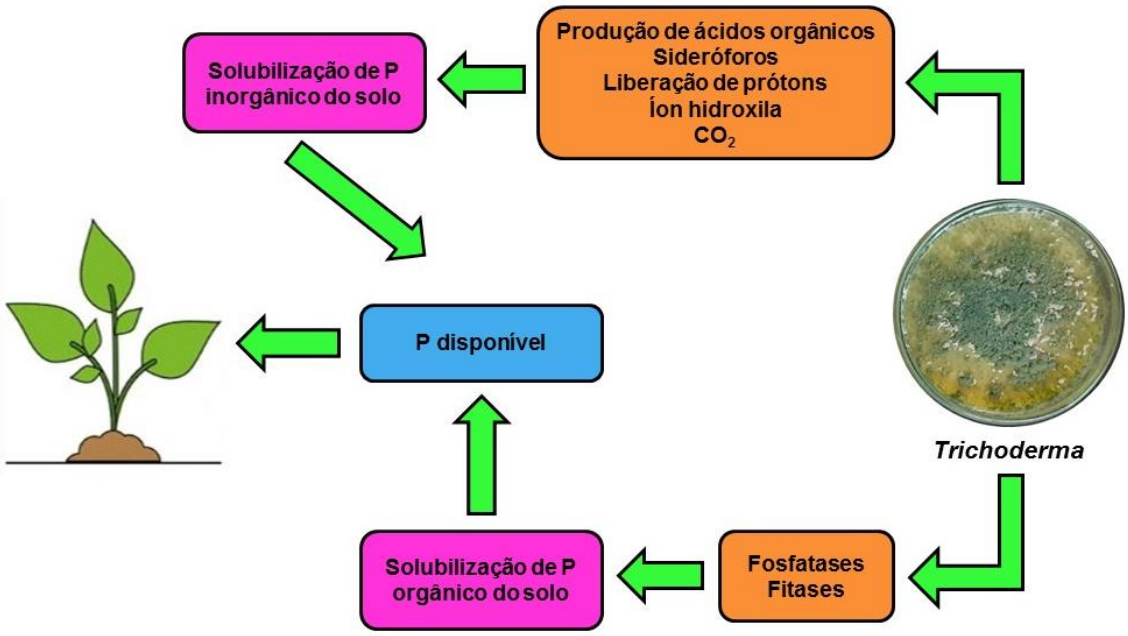

Figura 6 - Apresentação esquemática da solubilização de fosfato por *Trichoderma* solubilizadores de fosfato. P, fósforo; CO_2, gás carbônico. (Fonte: Adaptado de RAWAT *et al*., 2021).

Entre esses mecanismos, destaca-se a produção de ácidos orgânicos (Ex.: ácido ascórbico, ácido cítrico, glucônico e fumárico), que atuam como quelantes dos cátions ligados ao fósforo, além de síntese de moléculas de baixo peso molecular, como sideróforos, e liberação de prótons (ALORI *et al*., 2017; BONONI *et al*., 2020). Em solos do Cerrado, por exemplo, onde são registradas elevadas concentrações de Al (PROCÓPIO; BARRETO, 2021), o P do solo em complexos de Al-P, pode ser solubilizado por espécies de *Trichoderma*

que apresentam tolerância a pH mais ácido. A mineralização do fósforo de origem orgânica no solo é realizada pela ação de enzimas fosfatases e fitases (KHARE; YADAV, 2017).Os fungos têm sido reconhecidos por sua notável habilidade em disponibilizar P para as plantas, em parte devido à sua maior capacidade de colonizar o solo e produzir uma quantidade maior de ácidos orgânicos em comparação com as bactérias (SHARMA *et al*., 2013; ALORI *et al*., 2017).

Fungos do gênero *Trichoderma* também são reconhecidos por apresentarem outros mecanismos que atuam diretamente no metabolismo vegetal, promovendo o crescimento e a saúde de plantas. Um exemplo é a capacidade de estimular o crescimento e desenvolvimento das plantas por meio da produção de auxinas, como o AIA (CONTRERAS-CORNEJO *et al*., 2009; ZHANG *et al*., 2019; ZIN; BADALUDDIN, 2020). O AIA é a auxina mais presente no metabolismo de plantas e regula inúmeros processos do desenvolvimento e crescimento vegetal, como a divisão celular, diferenciação e formação de órgãos e raízes secundárias (SPAEPEN; VANDERLEYDEN, 2011). Cepas de *Trichoderma* produtores de AIA promovem maior arquitetura de raízes, que consequentemente aumenta a absorção de água e nutrientes (CONTRERAS-CORNEJO *et al*., 2009; YADAV *et al*., 2011).

Assim como outros MPCP, diversas cepas de *Trichoderma* foram identificadas como produtoras de sideróforos, metabólitos que se ligam com alta afinidade ao Fe férrico no solo (SYED *et al*., 2023). Por meio desse complexo, as plantas podem aumentar a captação do Fe, especialmente em condições de baixa concentração desse elemento (KHAN *et al*., 2018). Quando o Fe forma complexos com o P, a sua ligação com os sideróforos resulta na liberação do P no solo (RAWAT *et al*., 2021). Além de desempenharem esse papel nutricional, os sideróforos podem exercer um efeito direto no controle de patógenos do solo, inibindo seu crescimento por meio da privação de ferro (SAHA *et al*., 2016).

Algumas espécies de *Trichoderma* podem atenuar os impactos do estresse abiótico em plantas. Em condições de estresse, como salinidade e seca, as plantas modulam os níveis de determinados hormônios, incluindo o etileno (GLICK, 2014). Quando o estresse perdura, os níveis de etileno aumentam, causando efeitos deletérios, como senescência prematura, abscisão de órgãos, e menor crescimento, prejudicando a produtividade (SINGH *et al*., 2015). Alguns isolados de *Trichoderma* têm a capacidade de sintetizar a enzima 1-aminociclopropano-1-carboxilato (ACC) deaminase, que cliva o ACC, precursor do etileno, reduzindo as concentrações do hormônio e mitigando os efeitos prejudiciaisnas plantas (SYED *et al*., 2020; RAUF *et al*., 2021). Além disso, linhagens de *Trichoderma* produzem uma variedade de compostos orgânicos voláteis (COVs) que possuem a capacidade de inibir fitopatógenos e estimular o crescimento das plantas (SILVA *et al*., 2021).

Em relação à promoção de crescimento da soja, existem relatos sobre o aumento da produtividade da soja com aplicação de *Trichoderma* (MACENA *et al*., 2020; BUSO *et al*., 2021; SENGER *et al*., 2023). O uso combinado de *Trichoderma* e fertilizantes pode ser uma estratégia para aumentar o crescimento de plantas de soja (BONONI *et al*., 2020). No entanto, experimentos em campo para avaliar o efeito da inoculação de *Trichoderma* na produtividade da soja com menor aplicação de fertilizantes ainda são limitados.

A interação de espécies de *Trichoderma* com a soja pode auxliar na germinação e desenvolvimento inicial de plântulas de soja (CARDORE *et al*., 2020). Recentemente, SENGER *et al*. (2023) relataram aumentos médio acima de 8,9% na produtividade da soja usando inoculante contendo uma cepa de *T. asperelloides*. Também tem sido relatado que plantas de soja inoculadas com *Trichoderma* apresentaram maior teor de açúcares, metabólitos secundários e responstas antioxidantes (RODRÍGUEZ-HERNÁNDEZ *et al*.; 2023). Nos últimos anos, vários relatórios de pesquisa também destacaram os benefícios da colonização de *Trichoderma* na rizosfera de plantas cultiváveis, evidenciando um aumento

nas atividades enzimáticas do solo, especialmente de enzimas envolvidas nos ciclos biogeoquímicos, como fosfatase, fitase, protease, amilase e β-glucosidase (TENG *et al*., 2015; MAO; JIANG, 2021). Essa atuação deste fungo melhora a fertilidade e saúde dos solos agrícolas.

Contudo, há escassez de pesquisas direcionadas ao *Trichoderma* como agente promotor de crescimento vegetal para a formulação de inoculantes no Brasil. Isso pode ser atribuído ao fato de que, ao longo de décadas, cepas de *Trichoderma* têm sido predominantemente empregadas como agentes de controle biológico, enquanto seu papel como promotor de crescimento tem sido menos explorado. Dessa forma, quando formulações são desenvolvidas para o mercado, essas são registradas como agrotóxicos pela legislação brasileira (Lei nº 14.785, 2023). Entre os produtos que contém como ingrediente ativo *Trichoderma* disponíveis no mercado, existe uma formulação registrada como inoculante à base de diferentes cepas de *T. koningiopsis*, *T. harzianum* e *T. asperellum*, o ICB Nutrisolo®.

Nos últimos anos, houve um notável aumento no isolamento e seleção denovas cepas de *Trichoderma* promissoras para controle biológico e promoção de crescimento vegetal. As pesquisas dedicadas ao isolamento e caraterização de espécies de *Trichoderma* nativas do Cerrado brasileiro,visando incrementar o rendimento da soja nessa região, ainda são limitadas. No entanto, essas pesquisas são essenciais para impulsionar a prática da agricultura sustentável nesses sistemas de produção. Assim como ocorre com produtos biológicos contendo outros microrganismos, é crucial contar com relatórios que abordem o impacto da inoculação de *Trichoderma* no crescimento, produtividade da soja e na qualidade do solo. Tais informações são indispensáveis para orientar a seleção de cepas em estudos subsequentes, voltados à formulação de inoculantes e/ou biofungicidas.

3. OBJETIVOS

3.1 Objetivo geral

Isolar, caracterizar bioquimicamente e avaliar o potencial de *Trichoderma* spp. na promoção de crescimento de plantas de soja.

3.2. Objetivos específicos

a) Isolar e selecionar fungos rizosféricos do gênero *Trichoderma*em relação ao antagonismo a fungos fitopatogênicos que acometem a cultura da soja;

b) Determinar mecanismos promotores de crescimento vegetal pelos isolados;

c) Identificar morfológica e molecularmente os isolados mais promissores;

d) Caracterizar fisiologicamente os isolados em relação a tolerância a diferentes condiçõesin *vitro*;

e) Avaliar o efeito da inoculação de cepas na germinação de sementes e em plântulas de soja por parâmetros agronômicos e bioquímicos;

f) Avaliar o efeito da inoculação de cepas no crescimento e produtividade de duas cultivares de soja no campo sobduas condições de fertilização;

g) Investigar as repostas bioquímicas nas plantas inoculadas com *Trichoderma*;

h) Verificar as atividades enzimáticas do solo rizosférico de plantas de soja.

4. CAPÍTULO 1

Artigo aceito na Revista *Journal of Plant Growth Regulation*

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New strains of *Trichoderma* with potential for biocontrol and plant growth promotion improve early soybean growth and development

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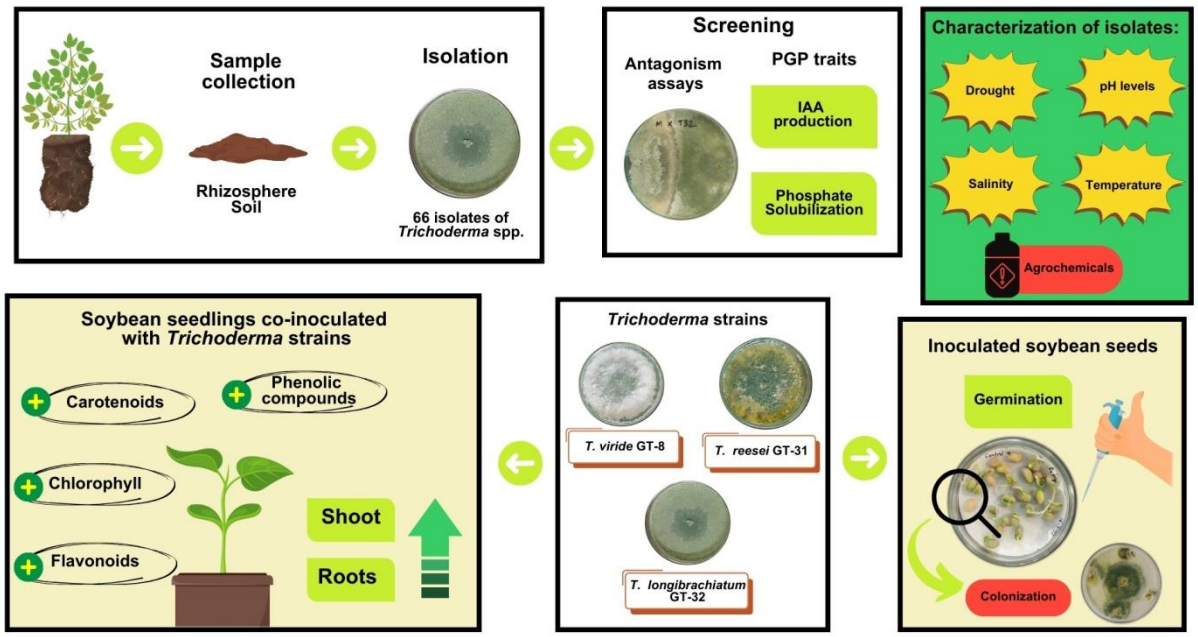
Graphical Abstract



Abstract

Bioformulations with *Trichoderma* strains offer cost-effective and sustainable options for integrated disease management and plant nutrition. Therefore, this study aimed to selected isolates of *Trichoderma* with antagonistic and plant growth-promoting potential, specifically in the early development stage of soybean seedlings. *Trichoderma* isolated from the soybean rhizosphere were screened to asses their antagonistic activity against three phytopathogens; as well as their capability for indole-3-acetic acid (IAA) synthesis and phosphate solubilization. Three promising were further examined for their tolerance to various in vitro conditions and agrochemicals. Experiments were performed to assess the effect of single inoculation and co-inoculation of strains on the growth and bichemical responses during early soybean development. Nine isolates showed effective antagonism against phytopathogens. Among them, *T. reesei* GT-31 and *T. longibrachiatum* GT-32 showed the highest IAA production, with 175.8 and 119.6 µg/mL, respectively, while the *T. viride* GT-8 showed the highest phosphate solubilization activity after 10 days of growth (285.6 µg P/mL). These strains

displayed robust growth under various conditions and agrochemical treatments. The co-inoculation of three strains resulted in even higher dry shoot and root weights, increasing by 50.3% and 48.8%, respectively, compared to non-inoculated seedlings. Co-inoculated plants also exhibited elevated chlorophyll (31.9%), carotenoids (24.9%), flavonoids (13.2%), and phenolic compounds (42.3%). The results suggest that employing the three strains with beneficial mechanisms for plants could significantly enhance the growth and early development of soybean seedlings. Overall, these findings highlight the potential of these novel *Trichoderma* strains to enhance plant growth and offer benefits in soybean crops, providing a sustainable strategy for agriculture.

Keywords: agrochemical, biocontrol, indole-3-acetic acid, PGP, phosphate solubilization, *Trichoderma*

Introduction

On a global scale, soybean [*Glycine max* (L.) Merr.] is one of the prominent commodities in the world. Currently, Brazil and the United States are the largest producers of this crop, accounting for approximately 69% of the global production (USDA 2022). Recent data have shown that soybean production reached 388.01 million tons in the 2022/2023 season, representing an increase of approximately 7.7% compared to the previous harvest (Statista 2023). The strong demand from the global market is one of the factors driving large-scale production (CONAB 2022), and farmers meet this need through expanding cultivation areas, increased use of fertilizers, advanced machinery and precision agriculture technologies, as well as an increased application of pesticides for crop disease management (Cherubin et al. 2022).

Among the diseases that limit crop yield are those caused by soil-borne fungi, including *Macrophomina phaseolina*, responsible for charcoal rot (Lodha and Mawar 2020), certain *Fusarium* species that cause seedling damping-off (Lin et al. 2022), and *Sclerotinia sclerotiorum*, the causative agent of white mold (Smolińska and Kowalska 2018). These diseases are intensified by the presence of high levels of soil inoculum, particularly in the form of sclerotia for *S. sclerotiorum* and microsclerotia for *M. phaseolina* (Smolińska and Kowalska 2018; Lin et al. 2022). Several agricultural practices are employed to reduce the inoculum density of these pathogens in the soil. This includes techniques such as biosolarization, use of resistant cultivars, crop rotation using non-host crops, direct seeding, chemical control, and biological control (Willbur et al. 2019; Marquez et al. 2021). Since there are numerous other diseases affecting this crop, phytosanitary management heavily relies on broad-spectrum fungicides. However, global concerns regarding the widespread use of these fungicides and other pesticides have sparked debates in agricultural countries due to their negative impacts on the environment, chronic effects on human health, and the

emergence of pesticide-resistant species. This necessitates urgent and sustainable alternatives for integrated disease management (Albuquerque et al. 2016; Wani et al. 2022; Agyekum et al. 2023; Capella et al. 2023).

Additionally, various efforts are being promoted in collaboration with the private sector for the more rational use of chemical fertilizers in intensive agriculture (Villoria et al. 2022). Over the past decades, the intensive management of fertilizers to increase agricultural productivity has led to several environmental disorders. Many ecological problems have already been reported, such as soil degradation due to nutrient imbalance (Prashar and Shah 2016), modifications in the physicochemical composition of the soil affecting its microbiota and microbiome functions (Hartmann and Six 2023), and water pollution resulting in the eutrophication of aquatic environments (de Azevedo Morgado et al. 2023).

Bioinputs containing microorganisms as biological control agents (BCAs) and biofertilizers have established themselves as a sustainable agricultural practice, aiming to mitigate the adverse environmental impacts caused by agrochemicals (Woo et al. 2023). *Trichoderma* spp. is a genus of fungi with species that have been used for many decades as BCAs in the management of plant diseases (Bettiol 2019; Poveda 2021). These fungi are found in a wide range of habitats around the world and can colonize the rhizosphere and/or become endophytes after penetrating plant tissues, assisting in the suppression of soil pathogens (Poveda 2021). The success as BCAs is because this genus possesses valuable attributes for biocontrol, such as mycoparasitism, antibiosis, competition for space and nutrients, and the induction of systemic resistance in plants (Asad 2022; Guzmán-Guzmán et al. 2023; Rodrigues et al. 2023). Numerous species of *Trichoderma* are capable of mycoparasitizing phytopathogenic fungi in crops, such as *T. harzianum* (Giordano et al. 2023), *T. viride* (Abdelrhim et al. 2023), *T. atroviride* (El-Benawy et al. 2020), and *T. longibrachiatum* (Sridharan et al. 2021).

Some strains of *Trichoderma* can tolerate stressful environments, such as high and low temperatures, saline soils, and drought (Zhang et al. 2019; Scudeletti et al. 2021). Additionally, there are reports of isolates tolerant to various agrochemicals, such as certain herbicides and fungicides (Escudero-Leyva et al. 2022). Therefore, there are several formulations of biofungicides containing *Trichoderma* that can be used for seed treatment, applied directly to the soil, or via foliar application (Rodrigues et al. 2023). It is important to remember that *Trichoderma* can also promote plant growth and enhance the productivity of various crops such as soybean (Senger et al. 2022), cotton (Silva et al. 2022), sugarcane (Scudeletti et al. 2021), rice (Cortés-Rojas et al. 2021), and coriander (Abdelrhim et al. 2023). These fungi can improve root architecture and other plant organs through the production of phytohormones, such as indole-3-acetic acid (IAA) (Machado-Rosa et al. 2023). *Trichoderma* can also assist in meeting nutritional needs of plants by solubilizing minerals from chemical fertilizers or the soil itself, such as phosphorus (Paul and Rakshit 2021). Previous studies have also shown that *Trichoderma* can accelerate seed germination and seedling survival through the production of various secondary metabolites, including gibberellins and IAA (You et al. 2016; Campos et al. 2020; Senger et al. 2022).

The antagonistic role of *Trichoderma* is a common topic in the literature; however, the dual role of biocontrol and plant growth promotion needs more investigation. Therefore, selecting competent strains of *Trichoderma* as BCAs and for plant growth promotion is desirable for the development of a more sustainable and cost-effective integrated disease management approach compared to agrochemicals. In recent years, various formulations containing more than one strain of *Trichoderma* have become available in the market (Bettiol 2019). The inoculation of two or more strains of *Trichoderma,* or other microorganisms, aims to enhance the beneficial effects promoted by a single strain, therefore, the technique aims to

exploit the most outstanding capabilities of each strain (da Costa et al. 2022; Stummer et al. 2022; Syed et al. 2022).

The Brazilian Cerrado, one of the most important biomes in South America, covers a portion of several states in Brazil with an area of approximately 2 million km^2, including the state of Mato Grosso do Sul (Bonanomi et al. 2019). However, deforestation for land conversion to agriculture is one of the main causes of degradation of this ecosystem (Procópio and Barreto 2021). In order to promote sustainable agriculture in the Brazilian Cerrado regions, research focusing on the isolation and characterization of native *Trichoderma* species aimed at promoting the growth and yield of soybeans could boost the increased use of this alternative in agriculture. In the literature, there are more reports on the impacts of rhizobia inoculation and co-inoculation with *Azospirillum brasilense* on biological nitrogen fixation in soybean (Barbosa et al. 2021). In this context, the aim of this study was to isolate and select new *Trichoderma* strains from agricultural field of Mato Grosso do Sul with antagonistic and plant growth-promoting potential. Furthermore, a comprehensive characterization was conducted on the most promising isolates in relation to their tolerance to a variety of *in vitro* environmental conditions and to the agrochemicals commonly used in soybean cultivation. Finally, the effect of inoculating three isolates was evaluated on the germination, root colonization, and early development of soybean seedlings.

Materials and Methods

Collection Area and Isolation of *Trichoderma* spp.

The collection of 15 soil samples from the soybean rhizosphere was conducted at an Experimental Farm in São Gabriel do Oeste city, MS, Brazil (19°46’07”S 54°61’60”W) (Fig. S1). The region is situated at an elevation of 646 m and has a red and dystrophic Latosol soil with clayey texture of approximately 65%. Five subsamples of soil near the roots were

collected at a depth of 0-15 cm in the soil profile. The subsamples were mixed, stored in polypropylene bags, and kept refrigerated.

The rhizosphere soil was sieved, and 10 g of soil from each sample was suspended in 90 mL of sterile saline solution (NaCl 0.9%). The suspensions were homogenized under agitation at 150 rpm for 30 min at room temperature. Serial dilutions were performed until a concentration of 1 x 10^{-4} was reached. In triplicate, aliquots of 1.0 mL from the 1 x 10^{-3} and 1 x 10^{-4} dilutions were inoculated in Petri dishes (90 mm x 15 mm) containing culture medium. Four media were used for isolation: potato dextrose agar (PDA), *Trichoderma* selective medium (TSM) (Elad et al. 1981), DOC2 medium (Shimazu and Sato 1996) and Rose Bengal Agar (RBA) medium (King et al. 1979) with chitin as source carbon (0.2% w/v), and all media were supplemented with chloramphenicol (50 µg/mL). The plates were incubated at 28 °C (12 h photoperiod) for 5 days. Starting from the 3rd day of incubation, the plates were observed daily. Fungal colonies that exhibited similarity to *Trichoderma* spp., as per the classification by Gams and Bissett (1998), were transferred to plates containing sterile PDA media. Subsequent pick-ups were performed to determine the purity of the colonies, and the isolates were preserved using silica gel and the Castellani method (1939).

Antagonism of *Trichoderma* spp. against Phytopathogens

The antagonistic potential of the isolates against three phytopathogenic fungi was assessed using the dual culture method on PDA as described by Dennis and Webster (1971). The phytopathogens *Sclerotinia sclerotiorum*, *Macrophomina phaseolina* and *Fusariumsolani* were generously provided by the Phytopathology Laboratory of Anhanguera-Uniderp University, Campo Grande/MS-Brazil. Initially, the biocontrol activity of *Trichoderma* spp. isolates against *M. phaseolina* was assessed using 7-day-old colonies. The plates were incubated at 28 °C with a 12-hour photoperiod, and the assay was terminated once complete

colonization of the pathogens occurred on the control plates (without *Trichoderma*). The percentage of mycelial growth inhibition (MGI) by the isolates was calculated using the equation proposed by Rahman et al. (2009). Isolates exhibiting MGI greater than 50% were subsequently evaluated against *F.solani,* and later against *S. sclerotiorum*.

Plant Growth-Promoting (PGP) Traits

IAA Synthesis

In triplicate, three mycelium discs (5 mm in diameter) were inoculated into 50 mL of Czapek broth medium supplemented with 5 mM L-tryptophan. The cultures were incubated at 28 °C with agitation at 110 rpm for 72 h. Subsequently, the cultures were centrifuged at 10,000 x g for 10 min, and the supernatant was utilized for analysis following Salkowski's method (Gordon and Weber 1951). The absorbance of the samples was measured at 530 nm, and the values were determined by constructing a standard calibration curve using IAA (Sigma-Aldrich, USA).

Phosphate Solubilization Ability

The ability of *Trichoderma* spp. to solubilize phosphate was investigated using Pikovskaya broth medium (1948)containing $CaHPO_4$ (0.5 w/v).. In triplicate, three mycelium discs (5 mm in diameter) were inoculated into 50 mL of the medium, and the cultures were incubated at 28 °C with constant agitation at 110 rpm for a duration of 10 days. Evaluations were conducted on days 3, 5, 7 and 10. Following incubation, the cultures were centrifuged at 8,000 x g for 20 min, and the concentration of available phosphorus was determined using the ascorbic acid-molybdate method on the supernatant (Murphy and Riley 1962). Additionally, the final pH of the culture media was measured using a digital pH meter.

Morphological and Molecular Identification

Three isolates (GT-8, GT-31, and GT-32) were selected to continue this study. They were chosen because of their promising results in the IAA production test (GT-31 and GT-32) and the higher phosphate solubilization value for GT-8.The morphology of the isolates was evaluated using colony characteristics and microscopy (Gams and Bissett 1998). The fungi were cultured on CYA (Czapek yeast extract agar), MEA (Malt extract agar) and PDA media for 5 days at 28 ℃. For molecular identification, total genomic DNA was extracted, and internal transcribed spacer (ITS) regions were amplified for molecular identification of the isolates. The universal primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') were used for ITS region amplification following the method described by White et al. (1990). PCR was conducted using a Veriti TM 96-Well Thermal Cycler (Applied Biosystems) with the following amplification program: initial denaturation at 95 ℃ for 2 min, followed by 38 denaturation cycles at 95 °C for 15 s, annealing at 56 ℃ for 15 s, extension at 72 °C for 30 s, and a final extension cycle at 72 ℃ for 7 min. The amplified samples were sequenced, and the sequences were manually aligned and adjusted using BioEdit 7.0.5.4 software (Hall 1999). Species identification was performed using the BlastN algorithm of the National Center for Biotechnology Information (NCBI). The obtained sequences were submitted to GenBank, and a phylogenetic tree was constructed using MEGA 6.0 software based on the Neighbor-Joining method with 1000 bootstrap replications.

Production of Cell Wall-Degrading Enzymes

The isolates were inoculated (three 5 mm diameter mycelium plugs) into flasks containing 50 mL of *Trichoderma* liquid enzyme (TLE) medium (Ramada et al. 2016). The culture medium was supplemented with 0.5% (w/v) of the dry cell wall of *M. phaseolina* or *S.*

sclerotiorum as enzyme inducers, and the pH was adjusted to 5.0. The dry cell walls of each pathogen were prepared according to Pasquoto-Stigliani et al. (2023).The flasks were incubated for 96 h under agitation (110 rpm) at 28 °C. The cultures were then filtered, centrifuged and the supernatant was recovered to measure the enzymatic activities. Enzymatic assays for phosphatase (Leitão et al. 2010), β-1,3-glucanase (Ramada et al. 2010), N-acetyl-β-D-glucosaminidase (NAGase) (da Costa et al. 2021), chitinase (Miller 1959), and protease (da Costa et al. 2021), were performed using *p*-nitrophenyl phosphate (*p*-NPP), laminarin, *p*-nitrophenyl-β-*N*-acetylglucosamine (*p*-NPNAG), colloidal chitin, and azocasein as substrates, respectively. Enzyme activities were expressed as U/mL.

Tolerance of *Trichoderma* Strains to Different Conditions *in vitro*

The isolates were subjected to various stress conditions to assess their tolerance, including salinity (0, 50, 150, 300, 500, and 1000 mM NaCl), different pH levels (ranging from pH 4.5 to 7.0), temperature (22, 28, 32, and 38 °C), and drought induced by polyethylene glycol (PEG) (8% w/v, equivalent to approximately -0.1 mPa of water potential). For each experiment, mycelium discs (5 mm) of *Trichoderma* spp. were placed at the center of Petri plates containing modified PDA medium according to each test. The colony diameter was measured after 7 days of growth at 28°C. Additionally, the isolates growth was evaluated in potato-dextrose broth (PDB) medium. For PEG-induced drought, concentrations of -0.1, -0.2, and -0.3 mPa of water potential were employed. Mycelium plugs (5 mm) were inoculated into 125 mL flasks containing 25 mL of the medium and incubated for 7 days at 28 °C with agitation at 110 rpm. The dry weight of the mycelia was determined (in mg) and the values were converted into percentages, with the mean dry weight of the control cultures considered as 100%. All experiments were performed in triplicate.

Tolerance of *Trichoderma* Strains to Agrochemicals

Mycelial Growth Analysis

The tolerance of the isolates to atrazine, haloxyfop-p-methyl (Glint), glyphosate (ZAPP), glufosinate-ammonium (G-ammonium), and ethiprole (Curbix) was assessed. The concentration of each chemical in the PDA medium corresponded to the field dose indicated on the package leaflet (Table S1). The plates were incubated at 28 °C for 7 days, and the colony diameter (in mm) was measured. The assays were also conducted in PDB medium, with incubation at 28 °C and stirring at 110 rpm for 7 days. The dry weight of the mycelia was determined, and the values were expressed as a percentage (%), considering the cultures without agrochemicals as 100%. All assays were performed in triplicate.

Cell Viability Analysis

In each microplate well, 150 µL of PDB medium supplemented with the herbicides atrazine, haloxyfop-p-methyl, glyphosate, glufosinate-ammonium, the insecticide ethiprole, and the fungicide mancozeb were incubated at two concentrations: the recommended field dose and half the dose (Table S1). Additionally, 50 µL of a spore solution in PDB (1 x 10^3 spores/mL) was added to each well. The microplates were then incubated at 28 °C for 24, 48, and 72 h, with eight independent replicates for each time point. At every 24-hour interval, 10 µL of Alamar Blue dye (0.1 mg/mL of resazurin in PBS) was added to each well, and the absorbances were measured at 570 nm and 600 nm using a microplate spectrophotometer (SpectraMax Plus 384®). The results were expressed as the percentage of resazurin reduction, calculated using the equation described by Larson et al. (1997).

Effect of *Trichoderma* Strains Inoculation in Seed Germination

A suspension of conidia in sterile distilled water was diluted at a ratio of 1:9 in a carboxymethyl cellulose solution (0.5% w/v), resulting in a final conidia concentration of 1 x 10^8 per mL. The seeds (Nidera NS 6601 cultivar) were immersed in 70% ethanol for 1 min, followed by a 4-min treatment with 3% sodium hypochlorite. Subsequently, they were rinsed with sterile distilled water five times. The seeds were treated with a commercial inoculant containing *Bradyrhizobium japonicum*. The experiment was designed in a randomized manner with four treatments (T) and five replicates, with each replicate consisting of 25 seeds: T1 - control without *Trichoderma*; T2 - seeds inoculated with *T. viride* GT-8; T3 - inoculation with *T. reesei*GT-31; T4 - inoculation with *T. longibrachiatum* GT-32. The seeds were inoculated at a rate of 2.0 mL of the fungal suspension per 100 g of seeds. After a 30-min resting period, the seeds were sown on germitest paper in plates and incubated at 25 °C for 7 days in a growth chamber. The percentage (%) of germination was obtained after 7 days, and the mean germination time was calculated following the method described by Al-Mudaris (1998).

Seedlings Colonization Test

Fifteen seedlings from each treatment of the germination assay were transferred to plates containing sterile filter paper. After 12 days of sowing, the seedlings were subjected to the same disinfection procedure as described in the previous assay. The radicles were then cut into approximately 1 cm fragments and placed in Petri plates containing PDA medium. Three plates were used per treatment, with each plate containing five radicle fragments. Following an incubation period of 5 days at 28 °C, the growth of *Trichoderma* isolates was observed, and the radicle colonization was calculated as a percentage (%) using the formula described by Cortés-Rojas et al. (2021).

Promotion of Soybean Seedling Growth by *Trichoderma* Strains

The experimental design employed a completely randomized design with five replicates for each treatment (T): T1 - control without *Trichoderma*; T2 - seeds inoculated with *T. viride*GT-8; T3 - inoculation with *T. reesei* GT-31; T4 - inoculation with *T. longibrachiatum* GT-32; T5 - co-inoculation of the three isolates. The seeds (Nidera NS 6601 cultivar) were prepared as previously described for the germination test. For co-inoculation, a spore suspension was prepared using a 1:1:1 ratio of the three isolates. Two seeds were sown in nursery bags (8 cm x 15 cm) filled with 200 g of soil (pH 6.7; 26.1 g/kg of organic matter). The bags were maintained at an average temperature of 24-27 °C under a 14-h light and 10-h dark regime. Manual irrigation was performed daily to maintain 80% of field capacity. After 15 days from the start of emergence, the seedlings were collected for the following analyses: fresh weight of the shoot and root, dry weight of the shoot and root. Dry weight was obtained by drying the samples in an oven at 65 °C for three days. The first trifoliate leaf of the seedlings was collected for biochemical analysis.

Biochemical Analyses

Total chlorophyll and carotenoids were obtained by macerating 100 mg of leaves (n = 3) with liquid nitrogen. A volume of 1.5 mL of acetone (85% v/v) was added to extract the pigments. The absorbances of the extracts were measured at 663 nm (chlorophyll a), 647 nm (chlorophyll b), and 470 nm (carotenoids). Concentration values were calculated using the equations described by Lichtenthaler and Buschmann (2001). Flavonoids and phenolic compounds were quantified (n = 3) following the methods outlined by Assis et al. (2020) and Arnaldos et al. (2001), respectively, with some modifications. Briefly, 100 mg of leaves were macerated with liquid nitrogen and resuspended in 5.0 mL of methanol (80% v/v). The samples were then homogenized and centrifuged at 3,000 rpm for 20 min.

For the quantification of flavonoids, 0.5 mL of the supernatant was mixed with 0.5 mL of $AlCl_3$ (2% w/v) and 2.5 mL of ethanol. After incubation in the dark for 50 minutes, absorbance readings were taken at 420 nm, and the concentration values were obtained from a standard curve constructed using different concentrations of rutin (ranging from 5 to 100 µg/mL). To determine the concentration of phenolic compounds, 500 µL of the extract was mixed with 2.0 mL of Na_2CO_3 solution (2% w/v) and 150 µL of Folin-Ciocalteau reagent. The mixture was incubated in the dark for 50 minutes, and the absorbance was measured at 750 nm. The concentration values were calculated using a calibration curve generated with different concentrations of gallic acid (ranging from 10 to 80 µg/mL). Protein content was determined using the Bradford method (1976) with a 100 mM potassium phosphate buffer (pH 7.8) containing 1% (w/v) polyvinylpyrrolidone. A standard solution of bovine serum albumin (1 mg/mL) was employed.

Data Analysis

The *in vitro* experiments were conducted three times, and the results were expressed as means ± standard deviation (SD). Analysis of variance (ANOVA) was used to compare the data from the antagonism experiment and PGP characteristics. The Tukey's test was performed for statistical analysis using the SISVAR software (Ferreira 2011). Characterization data related to tolerance to different environmental conditions and agrochemicals were analyzed using one-way ANOVA with the Tukey's test in GraphPad Prism software version 8.0. To assess the relationships between the biochemical analysis of the leaves and the treatments, principal component analysis (PCA) was conducted using R version 4.2.1 software with the Factoextra package.

Results

Isolation of *Trichoderma* spp.

A total of 66 isolates, exhibiting morphological similarities to the genus *Trichoderma*, were obtained from the rhizospheric soil of soybean using four different culture media. These isolates were cultivated using four distinct culture media, and subsequently deposited in the Culture Collection of the Universidade Federal de Mato Grosso do Sul (UFMS) in Campo Grande/MS, Brazil. Among the employed culture media , PDA demonstrated the highest efficacy for isolation, yielding 40 strains. TSM resulted in the isolation of 14 fungi, followed by RBA with 7 fungi, and DOC2 with 5 fungi.

Antagonism of *Trichoderma* spp. against Phytopathogens

The isolates were initially tested against a strain of *M. phaseolina*, and the results indicated that 58 strains exhibited more than 50% inhibition of its mycelial growth. The inhibition values, expressed as MGI, ranged from 52.9% to 72.6% (Table S2). In this study, the focus was on presenting the results obtained for the nine selected isolates (Table 1). Among these isolates, GT-12 exhibited the highest inhibition of *M. phaseolina* growth (72.59%), followed by GT-38 (71.38%) and GT-7 (70%).

Table 1 Antagonism activity of *Trichoderma* spp. against the phytopathogens

Isolates	MGI (% ± SD)		
	M. phaseolina[*]	*F. solani*[**]	*S. sclerotiorum*[**]
GT-4	68.5 ± 1.7 [abc]	77.0 ± 0.6	76.1 ± 3.4
GT-7	70.0 ± 1.1 [abc]	84.4 ± 3.9	75.6 ± 1.1
GT-8	52.9 ± 1.2 [d]	77.0 ± 1.3	80.9 ± 2.6
GT-12	72.6 ± 0.6 [a]	80.7 ± 2.8	77.4 ± 0.9
GT-19	68.2 ± 1.3 [abc]	84.8 ± 3.9	74.8 ± 1.2

GT-28	66.5 ± 2.1 [bc]	81.1 ± 7.3	74.9 ± 0.9
GT-31	66.3 ± 2.6 [bc]	83.7 ± 5.0	77.2 ± 1.9
GT-32	65.6 ± 1.1 [c]	79.5 ± 1.5	75.7 ± 4.2
GT-38	71.5 ± 3.6 [ab]	80.4 ± 2.3	75.2 ± 3.1

The values presented in the table represent means with ± standard deviation (SD) obtained from a triplicate experiment

*Means that share the same letter within a column indicate no statistically significant difference between treatments, as determined by the Tukey test

**No statistically significant differences were observed between means based on Tukey's test

It is worth noting that although isolates GT-31 and GT-32 showed lower suppression values, both exhibited growth inhibition over the pathogen (Fig. 1a).

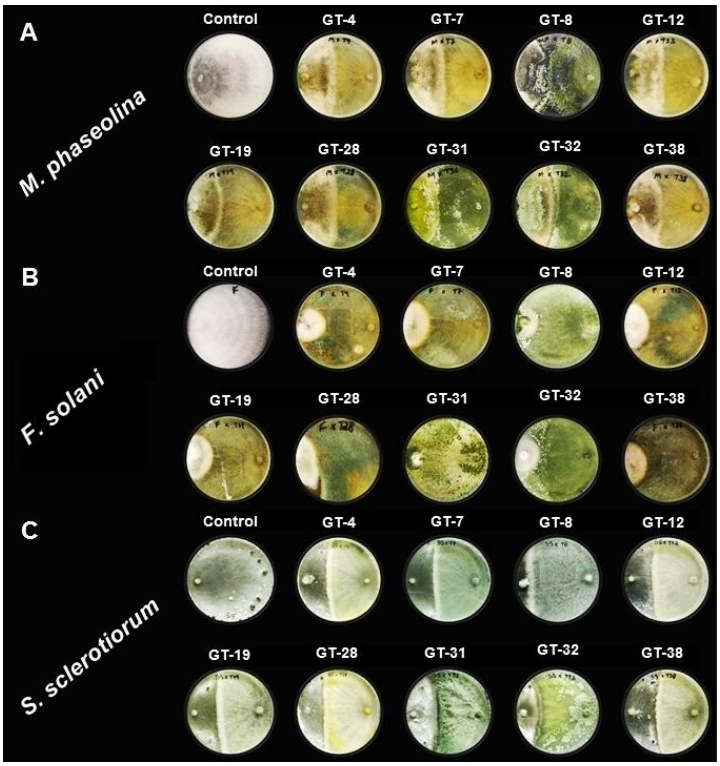


Fig. 1 Antagonistic activity of *Trichoderma* isolates against (**a**) *M. phaseolina*, (**b**) *F.solani*, and (**c**) *S. sclerotiorum* by dual culture method (Mycelial discs of the pathogens were inoculated on the left side, while mycelial discs of *Trichoderma* were inoculated on the right side)

Regarding *F.solani* and *S. sclerotiorum*, the antagonists exhibited higher MGI values, ranging from 77% to 84.8% and 74.8% to 80.9%, respectively. Statistical analysis indicated no significant difference ($p > 0.05$) among the isolates. However, variations in the interaction between the isolates and the two pathogens were observed, including differences in sporulation and color change of the mycelium on the pathogen (Fig. 1b, c).

PGP traits

Throughout the three-day evaluation period, all nine *Trichoderma* isolates were found to synthesize IAA at varying concentrations, ranging from 1.13 to 175.8 µg/mL (Table 2). Notably, isolate GT-31 exhibited the highest IAA concentration in the supernatant, measuring 175.8 ± 2.4 µg/mL, which was significantly different from the other fungi. GT-32 closely followed with a concentration of 119.6 ± 11.2 µg/mL.

Table 2 Indole-3-acetic acid (IAA) production and phosphate solubilization by *Trichoderma* spp.

Isolates	IAA (µg/mL)*	Phosphate solubilization (µg P/mL)*				pH
		3 days	5 days	7 days	10 days	
GT-4	1.13 ± 0.2 ^{f}	256.6 ± 34.9 ^{b}	264.2 ± 12.3 ^{ab}	133.1 ± 18.9 ^{b}	113.8 ± 4.7 ^{c}	5.18
GT-7	29.53 ± 0.7 ^{c}	83.8 ± 17.6 ^{ef}	69.6 ± 2.3 ^{d}	71.1 ± 3.8 ^{c}	70.6 ± 6.9 ^{d}	5.2
GT-8	23.06 ± 0.7 ^{d}	234.2 ± 28.6 ^{b}	272.9 ± 39.7 ^{a}	263.7 ± 10.6 ^{a}	285.6 ± 4.4 ^{a}	4.83
GT-12	7.01 ± 0.3 ^{e}	197.6 ± 13.6 ^{bc}	226.1 ± 6.9 ^{b}	276.9 ± 10.2 ^{a}	238.3 ± 16.3 ^{b}	4.74
GT-19	12.09 ± 0.5 ^{e}	167.7 ± 21.6 ^{cd}	65.6 ± 1.5 ^{d}	60.0 ± 6.9 ^{c}	18.3 ± 4.0 ^{f}	5.25
GT-28	18.56 ± 0.8 ^{d}	148.3 ± 4.9 ^{cde}	133.1 ± 22.1 ^{c}	79.3 ± 9.1 ^{c}	45.2 ± 5.7 ^{e}	5.09
GT-31	175.8 ± 2.4 ^{a}	81.3 ± 6.9 ^{f}	276.4 ± 7.7 ^{a}	272.4 ± 8.7 ^{a}	266.8 ± 1.5 ^{a}	4.34
GT-32	119.6 ± 11.2 ^{b}	633.3 ± 55.9 ^{a}	272.9 ± 1.5 ^{a}	251.5 ± 2.6 ^{a}	73.2 ± 4.0 ^{d}	4.96
GT-38	17.66 ± 0.6 ^{d}	112.3 ± 20.4 ^{def}	52.3 ± 6.8 ^{d}	76.7 ± 22.9 ^{c}	46.8 ± 8.6 ^{e}	5.31

The values presented in the table are means with ± standard deviation (SD) obtained from an experiment performed in triplicate

Means that are followed by the same letter in the column indicate no statistically significant difference between treatments, as determined by the Tukey's test

Besides IAA synthesis, the isolates were also assessed for their ability to solubilize inorganic phosphate *in vitro*. The results revealed that all isolates demonstrated the capability to solubilize calcium phosphate in Pikovskaya's broth medium throughout the evaluation period (Table 2). Among them, strains GT-32, GT-8, and GT-4 displayed the highest values of soluble phosphorus after 3 days of incubation, with concentrations of 633.3 ± 55.9, 234.2 ± 28.6, and 256.6 ± 34.9 µg P/mL, respectively. However, after 10 days of growth, isolates GT-8 (285.6 ± 4.4 µg P/mL) and GT-31 (266.8 ± 1.5 µg P/mL) exhibited significantly higher levels of available phosphorus in their filtrates compared to the other isolates. Additionally, a decrease in pH was observed in all fungal culture filtrates after 10 days of incubation, ranging from 7.0 (initial pH) to values between 4.34 and 5.31 (Table 2). Isolates GT-31 and GT-32, which showed the highest IAA production values, together with isolate GT-8, which demonstrated the highest phosphate solubilization capacity, were selected for further investigations.

Morphological and Molecular Identification

The *Trichoderma* isolates GT-8, GT-31 and GT-32 were chosen for further investigation, focusing on their morphology. The conidia of strains GT-8, GT-31 and GT-32 were germinated on CYA, MEA and PDA media for five days of incubation. Differences in colony coloration were observed among the three media, with the presence of green-colored colonies on CYA and BDA media (Fig. 2a).

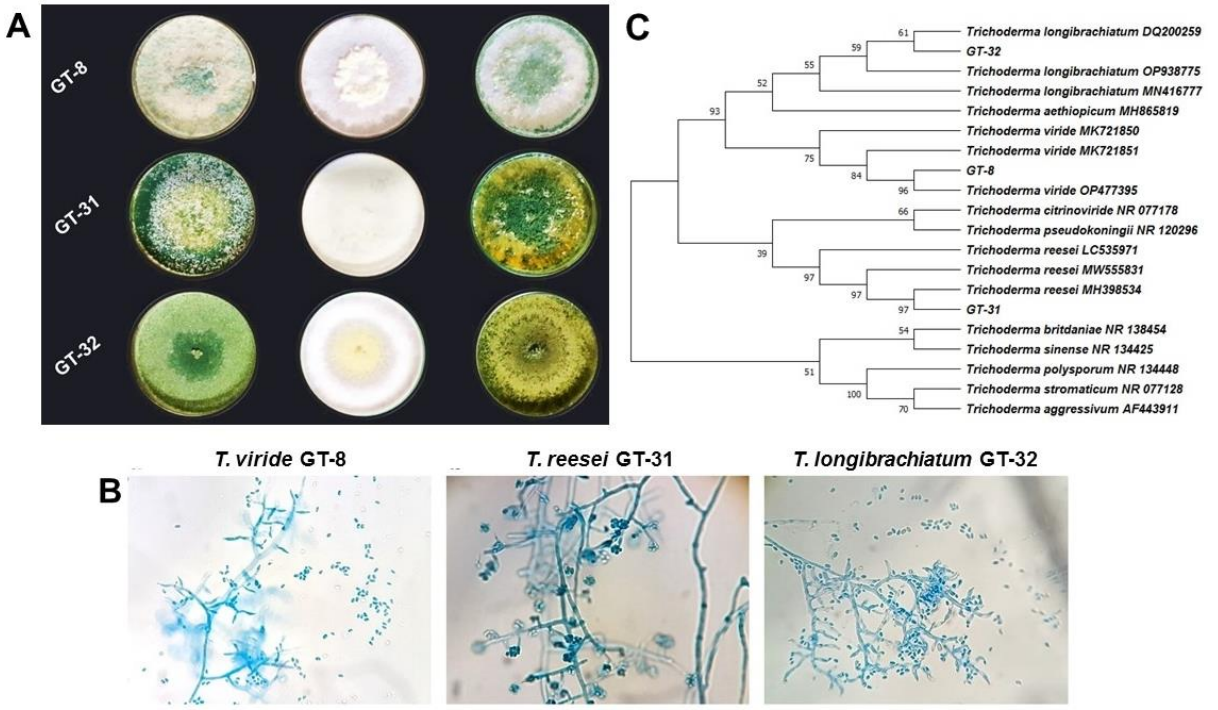


Fig. 2 (**a**) The *Trichoderma*strains GT-8 (*T. viride*), GT-31 (*T. reesei*), and GT-32 (*T. longibrachiatum*) were cultured for 5 days on Petri plates containing CYA, MEA, and PDA medium, arranged from left to right (**b**) Under a light microscope, conidiophores and conidia of *T. viride* GT-8, *T. reesei* GT-31, *T. longibrachiatum* GT-32 were examined (**c**) Phylogenetic tree was constructed using the Neighbor-Joining method based on the sequences of the ITS region from *T. viride* GT-8, *T. reesei* GT-31 and *T. longibrachiatum* GT-32. The values at each point in the tree branches represent bootstrap support calculated from 1.000 replicates, indicating the reliability of the branching pattern in the tree

Under microscopic observation, GT-8 isolate slides exhibited spherical conidia and conidiophores arranged in a pyramidal pattern (Fig. 2b), while GT-31 and GT-32 showed conidiophores with sparser branching and more irregularly arranged phialides. However, molecular identification is essential to confirm the species. Based on sequencing of the ITS region, GT-8 was identified as *T. viride*, GT-31 as *T. reesei*, and GT-32 as *T. longibrachiatum*. The obtained accession numbers in GenBank were OQ826673, OQ826674, and OQ826675, for *T. viride* GT-8, *T. reesei* GT-31, and *T. longibrachiatum* GT-32, respectively. By aligning the sequences in MEGA, the evolutionary history was determined using the Neighbor-joining method, which clustered isolates GT-8, GT-31, and GT-32 with

the species *T. viride* MK721850 (100%), *T. reesei* MH398534 (99.67%), and *T. longibrachiatum* DQ2000259 (100%), respectively (Fig. 2c).

Production of Cell Wall-Degrading Enzymes

The presence of the cell wall of the phytopathogens *M. phaseolina* and *S. sclerotiorum* induced the *Trichoderma* strains to produce enzymes that act in the degradation of the cell wall (Table 3).

Table 3 Enzymatic activity (U/mL) of cell wall-degrading enzymes by *Trichoderma* strains using the dry cell wall of *M. phaseolina* (Mp) and *S. sclerotiorum* (Ss) as an inducer substrate

Enzymes	*T. viride* GT-8		*T. reesei* GT-31		*T. longibrachiatum* GT-32	
	Mp	Ss	Mp	Ss	Mp	Ss
Phosphatase	3.11± 0.46 ^{cd}	5.29 ± 0.25 ^{bc}	6.72± 0.85 ^{b}	17.84 ± 1.42 ^{a}	2.57± 0.16 ^{d}	4.09 ± 0.9 ^{cd}
β-1,3-glucanase	13.2 ± 1.04 ^{bc}	12.07 ± 0.84 ^{c}	13.98 ± 0.94 ^{bc}	16.58 ± 1.87 ^{ab}	19.28 ± 2.03 ^{a}	15.76 ± 0.46 ^{bc}
NAGase	6.71± 0.59 ^{bc}	8.21 ± 0.43 ^{ab}	5.28 ± 0.22 ^{cd}	9.44 ± 1.24 ^{a}	1.33 ± 0.16 ^{e}	4.09 ± 0.96 ^{d}
Chitinase	0.019 ± 0.01 ^{b}	0.022 ± 0.02 ^{b}	0.024 ± 0.00 ^{b}	0.049 ± 0.07 ^{a}	0.013 ± 0.00 ^{b}	0.032 ± 0.04 ^{ab}
Protease	3.6 ± 0.4 ^{b}	4.7 ± 0.46 ^{ab}	4.13 ± 0.68 ^{b}	4.83 ± 0.21 ^{ab}	3.8 ± 0.1 ^{b}	5.43 ± 0.59 ^{a}

Values are means with ± standard deviation (SD)

Means followed by the same letter in the row indicate no statistically significant difference between treatments based on the Tukey's test

TLE culture medium with glucose as a carbon source did not induce the activity of these enzymes (data not shown). Briefly, the highest enzyme activities were observed in the presence of the *S. sclerotiorum* cell wall. Except for protease and β-1,3-glucanase activities, the *T. reesei* GT-31 strain showed significantly higher production values for the enzymes phosphatase (17.84 ± 1.42 U/mL), NAGase (9.44 ± 1.24 U/mL), and chitinase (0.049 ± 0.07 U/mL).

Tolerance of *Trichoderma* Strains to Different Conditions *in vitro*

Variations in temperature had no impact on the mycelial growth of *T. viride* GT-8, *T. reesei* GT-31, and *T. longibrachiatum* GT-32 isolates (Fig. S2a). Additionally, these strains demonstrated robust growth in the presence of NaCl concentrations ranging from 50 to 500 mM (Fig. S2b). However, when exposed to 1000 mM NaCl, the mycelial growth diameter significantly decreased by 3.6%, 14.28%, and 19.61% for *T. viride* GT-8 ($P = 0.0022$), *T. reesei* GT-31 ($P = 0.0031$), and *T. longibrachiatum* GT-32 ($P = 0.0012$), respectively. The presence of PEG at -0.1 MPa allowed the fungi to grow (Fig. S2c). Nevertheless, the colony characteristics were altered; for instance, *T. viride* GT-8 did not exhibit sporulation along the mycelium, while *T. reesei* GT-31 and *T. longibrachiatum* GT-32 showed colonies with a more yellowish color (Fig. S3). Interestingly, the isolates exhibited no significant difference in growth across all tested pH values, even when compared to the control cultures at pH 5.5 (Fig. S2d).

During the temperature test in PDB, 28 °C served as the control treatment, and the growth rate remained unaffected for the three isolates (Fig. 3a). However, when subjected to 1000 mM NaCl concentration, *T. viride* GT-8 ($P = 0.0223$) and *T. reesei* GT-31 ($P = 0.0133$) exhibited a significant reduction in growth, with a decrease of 49.7% and 39.1% in dry mycelium weight, respectively (Fig. 3b). *T. longibrachiatum* GT-32 demonstrated enhanced growth in the presence of NaCl within the range of 50-300 mM, with a notable increase of 43.3% in growth at 150 mM NaCl ($P = 0.0064$). In the PEG assay, the growth of the isolates among the treatments did not exhibit any statistical difference based on Tukey's test ($P < 0.05$) (Fig. 3c). Moreover, the results showed that there was also no significant difference in the dry mycelium weight values at all tested pH values (Fig. 3d).

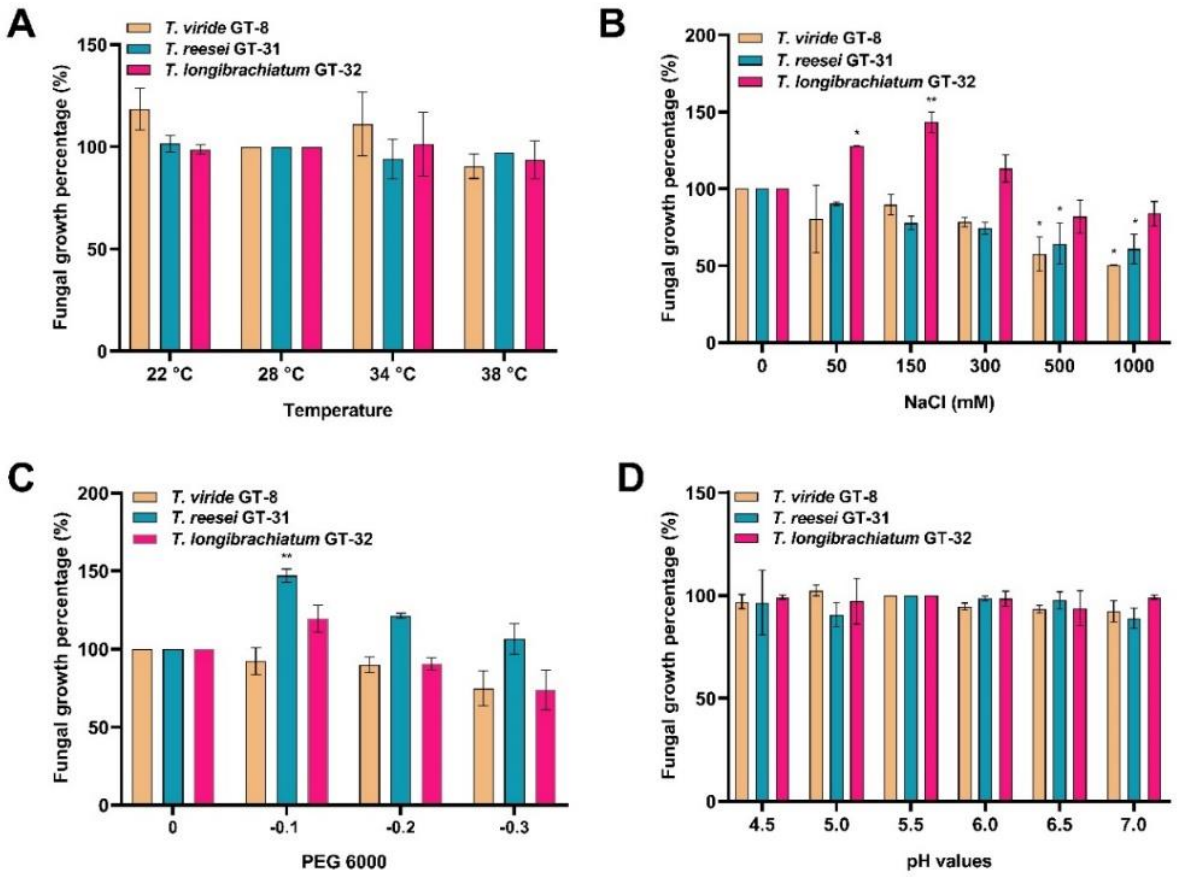


Fig. 3 Growth of *Trichoderma*strains in PD broth medium after 7 days under different conditions of (**a**) temperature (**b**) salinity, (**c**) drought by PEG 6000, and (**d**) pH. Asterisks represent significant differences from the control treatment (*, p-value < 0.05; **, p-value < 0.01) as determined by Tukey's test

Tolerance of *Trichoderma* Strains to Agrochemicals

The agrochemical tolerance test conducted for soybean cultivation revealed distinct growth responses among the three isolates. Surprisingly, exposure to the insecticide Curbix had no significant effect on the complete colonization of the culture medium by the fungi (Fig. 4a).

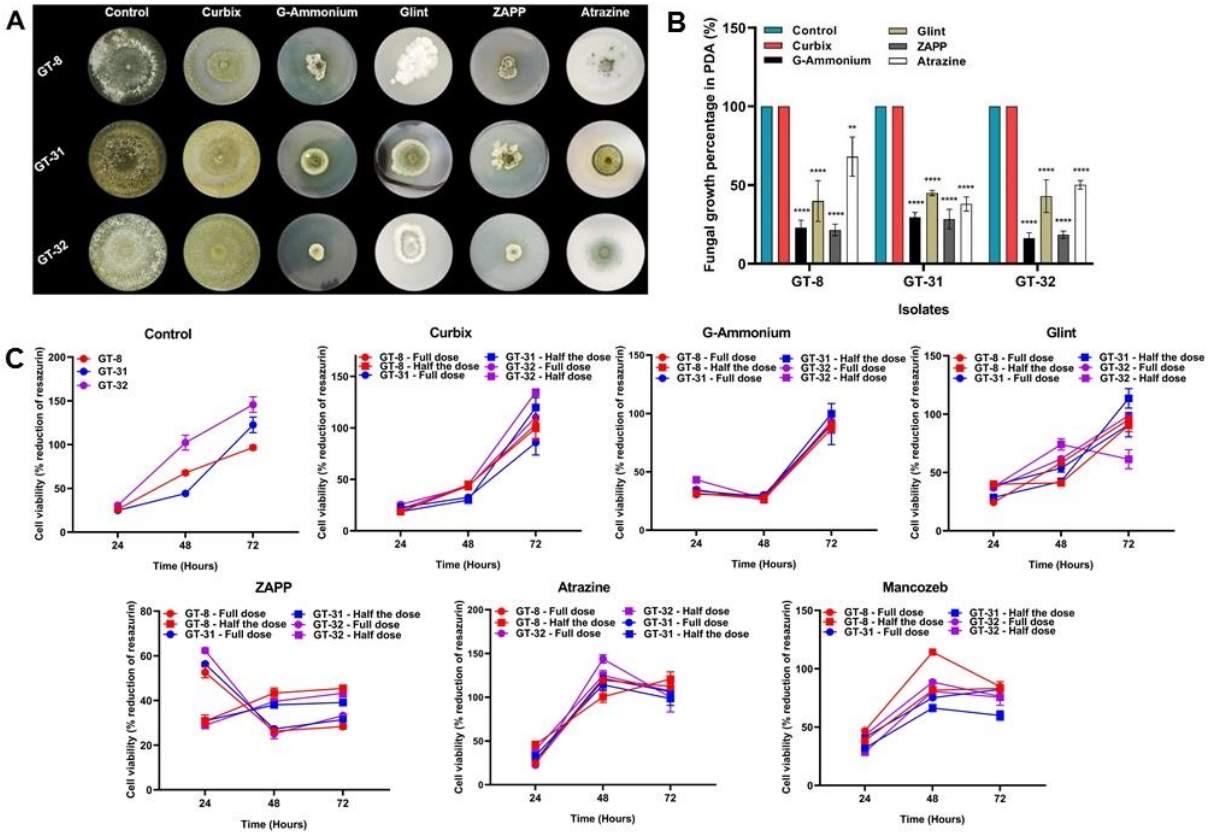


Fig. 4 (**a**) *Trichoderma* strains growth in Petri plates containing PDA medium supplemented with agrochemicals at the recommended field dose (**b**) Growth (%) of *Trichoderma* strains in the presence of agrochemicals in PDA medium. Asterisks represent significant differences from the control treatment (**, p-value < 0.01; ****, p-value < 0.0001; Tukey's test) (**c**) Analysis of cell viability by measuring of resazurin reduction (%) by *Trichoderma* strains exposed to agrochemicals at the recommended field dose and half the dose after 24, 48 and 72 h

Effect of *Trichoderma* Strains Inoculation in Seed Germination and Seedlings Colonization Test

After 7 days of the assay, the presence of isolate growth on the seeds was observed (Fig. 5a). However, the results indicated that the percentage of germination did not show a significant difference compared to the control (Table 4).

Table 4 Effect of *Trichoderma* strains in germination, average germination time, and colonization (%) of soybean rootlets of cultivar (Nidera NS 6601)

Treatments	Germination*	Germination time*	Colonization**
Control	82.0 ± 2.3	4.6 ± 0.04	0
T. viride GT-8	80.0 ± 10.3	4.56 ± 0.11	30.0 ± 14.1 [b]
T. reesei GT-31	89.0 ± 3.8	4.51 ± 0.01	100.0 [a]
T. longibrachiatum GT-32	81.0 ± 6.8	4.61 ± 0.03	100.0 [a]

Values are means with ± standard deviation (SD)

*No statistically significant differences were observed

**Means followed by the same letter in the column indicate no statistically significant difference between treatments based on the Tukey's test

This suggests that the fungi do not inhibit seed germination. Moreover, the average germination time did not exhibit a significant difference among the evaluated treatments ($p > 0.05$). Colonies of the *T. viride* GT-8 isolate were observed only on 30% of the radicles (Table 4). In contrast, treatments with *T. reesei* GT-31 and *T. longibrachiatum* GT-32 showed colony growth on PDA in 100% of the radicles (Fig. 5b).

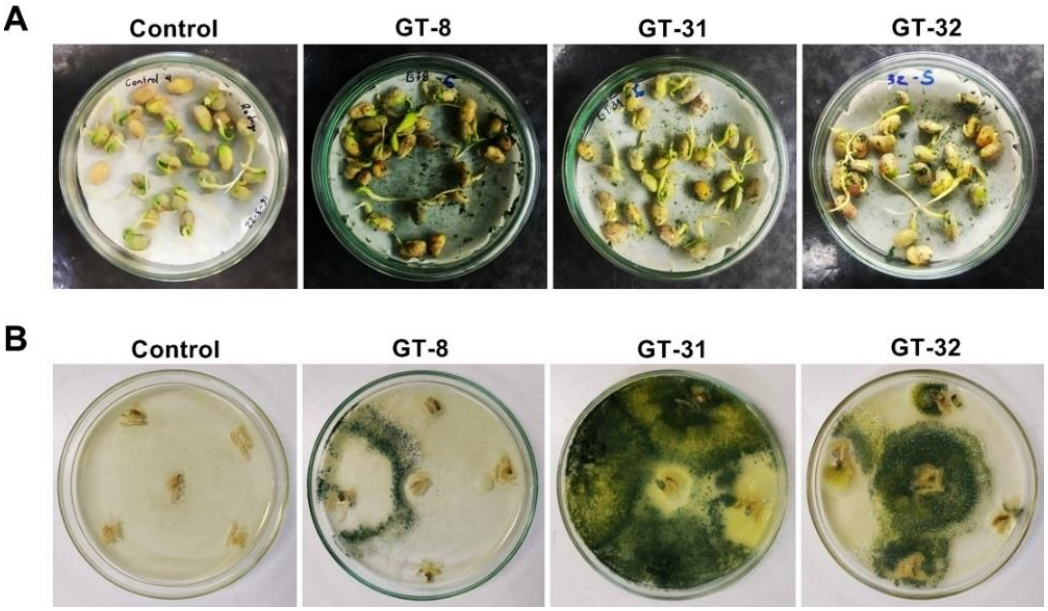


Fig. 5 (**a**) Soybean seeds (Nidera NS 6601) inoculated with *Trichoderma* strains after 7 days of sowing on germitest paper (**b**) Colonization test of the inner tissue of radicles by *Trichoderma* strains

Promotion of Soybean Seedling Growth by *Trichoderma* Strains

The effect of seed inoculation with *T. viride* GT-8, *T. reesei* GT-31, and *T. longibrachiatum* GT-32 on the early development of soybean seedlings was investigated (Fig. 6).

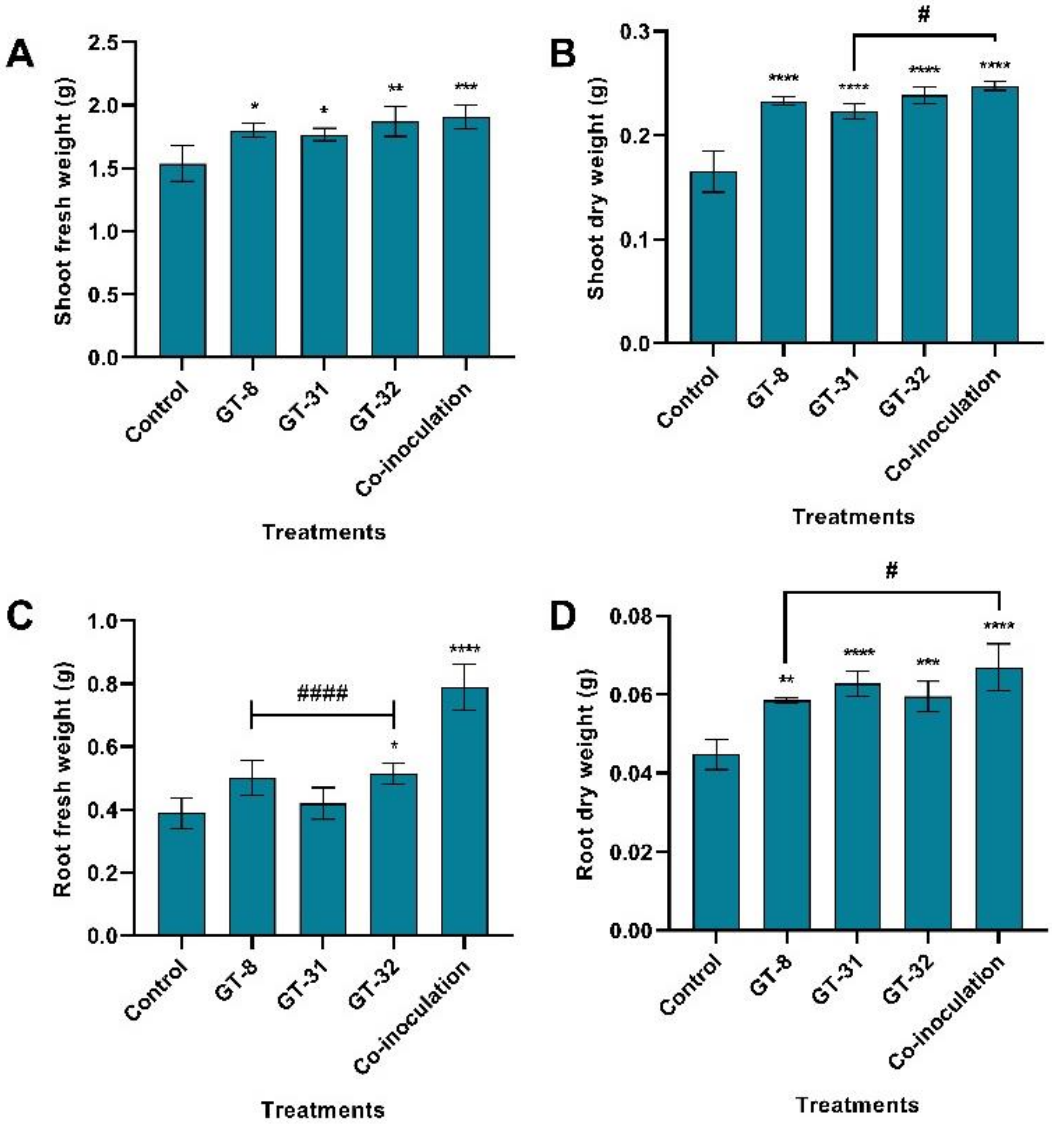


Fig. 6 Effect of inoculation with *T. viride* GT-8, *T. reesei* GT-31, *T. longibrachiatum* GT-32 and co-inoculation of the isolates on the initial development of soybean (Nidera NS 6601 cultivar). (**a**) Shoot fresh weight of seedlings (**b**) Shoot dry weight of seedlings (**c**) Root fresh weight of seedlings (**d**) Root dry weight of seedlings. Values are means with ± standard deviation (SD), obtained from an experiment repeated three times with five replicates. Asterisks represent significant differences from the control treatment (*, p-value < 0.05; **, p-value < 0.01; ***, p-value < 0.001; ****, p-value < 0.0001; as determined by Tukey's test). Hash (#) symbols represent

significant differences from the co-inoculation treatment (#, p-value < 0.05; ####, p-value < 0.0001; as determined Tukey's test)

The results revealed that the inoculated seedlings exhibited a significant increase in shoot fresh weight ($p < 0.05$). Co-inoculation of the three isolates resulted in a remarkable 23.4% induction ($P = 0.0007$) compared to the control, surpassing the values obtained for single-isolate treatments (Fig. 6a). The influence on shoot dry weight was even more pronounced ($P < 0.0001$), with increases of 41.25%, 34.55%, 44.85%, and 50.3% for *T. viride* GT-8, *T. reesei* GT-31, *T. longibrachiatum* GT-32, and co-inoculation, respectively (Fig. 6b). Notably, co-inoculation showed significant differences compared to the *T. reesei* GT-31 treatment ($P < 0.0001$).

Moreover, co-inoculation led to a significant increase in root fresh weight ($P < 0.0001$), exhibiting a remarkable 102.83% increase compared to the uninoculated control (Fig. 6c). When comparing treatments involving the single inoculation of *Trichoderma* strains, the combined use of isolates also significantly induced root growth ($P < 0.0001$). For root dry weight, the application with *T. reesei* GT-31 and co-inoculation significantly outperformed the other treatments, resulting in increases of 40% and 48.8%, respectively, compared to the uninoculated control ($P < 0.0001$) (Fig. 6d). These findings indicate that the inoculation of soybean seeds with the selected *Trichoderma* strains, particularly through co-inoculation, has a substantial positive impact on shoot and root growth, which may lead to improved early seedling development and potential benefits for soybean cultivation.

Biochemical Analyses

The total chlorophyll content in the seedlings was significantly higher in the inoculated treatments (Fig. 7a). The treatment with *T. reesei* GT-31 showed the highest increase, with a remarkable 56.9% rise ($P < 0.0001$), followed by the co-inoculation

treatment, which exhibited a 31.9% increase compared to the control (P = 0.0002). Co-inoculation of isolates also significantly contributed to the carotenoid content compared to the other treatments, resulting in a 24.9% increase compared to the control, reaching a concentration of 0.216 mg g^{-1} FW (Fig. 7b).

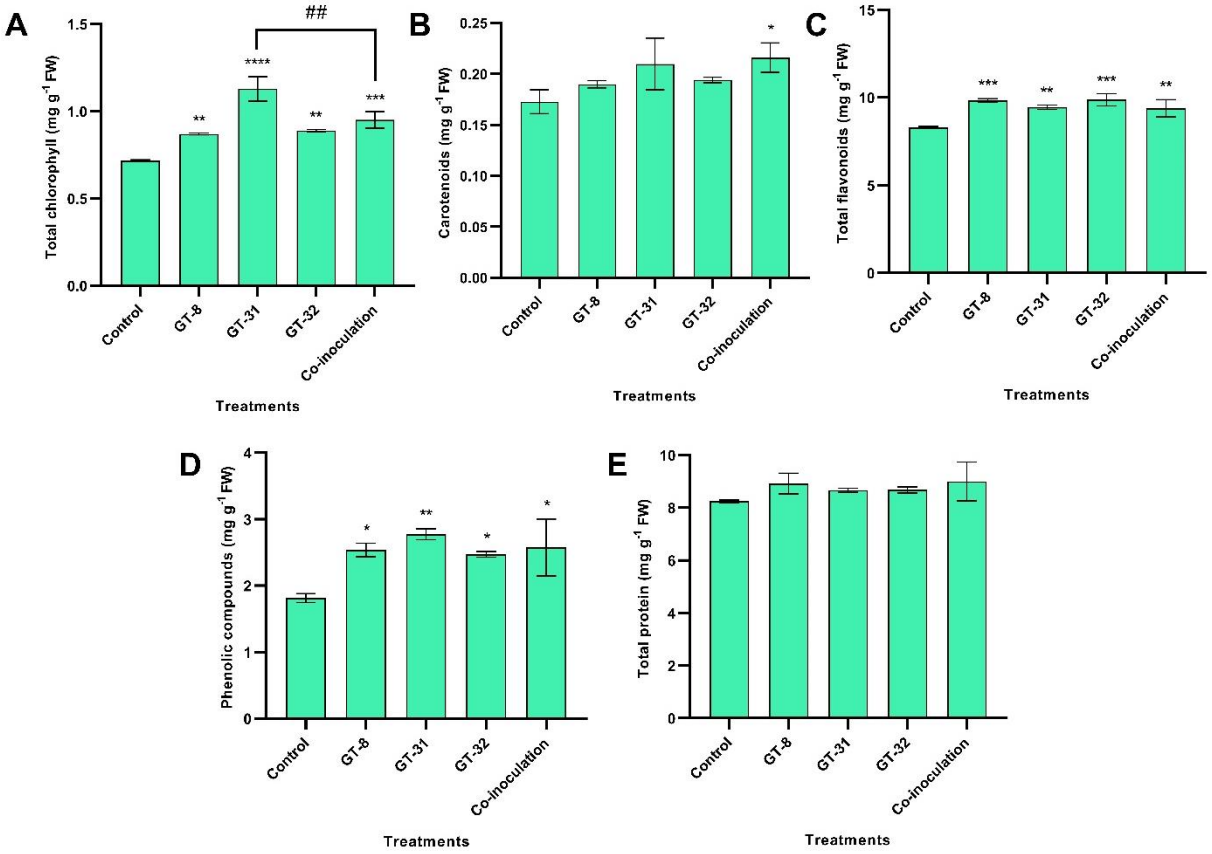


Fig. 7 Effect of inoculation of soybean seedlings with *T. viride* GT-8, *T. reesei* GT-31, *T. longibrachiatum* GT-32 and co-inoculation of isolates on various biochemical parameters (**a**) total chlorophyll, (**b**) carotenoids, (**c**) total flavonoids, (**d**) phenolic compounds, and (**e**) total proteins. Values are means with ± standard deviation (SD) of an experiment repeated three times with five replicates. Asterisks represent significant differences from the control treatment (*, p-value < 0.05; **, p-value < 0.01; ***, p-value < 0.001; ****, p-value < 0.0001; Tukey's test). Hash represent significant differences from the co-inoculation treatment (#, p-value < 0.05; Tukey's test)

Moreover, seedlings inoculated with *Trichoderma* strains displayed significantly higher concentrations of flavonoids in the methanolic extract (Fig. 7c). In terms of total phenolic compounds, the presence of *Trichoderma* led to significant increase of 40% (P = 0.0151), 53.1% (P = 0.002), 36.4% (P = 0.0274), and 42.1% (P = 0.0108) for the treatments with *T. viride* GT-8, *T. reesei* GT-31, *T. longibrachiatum* GT-32, and co-inoculation, respectively (Fig. 7d). However, no statistical difference was observed among the treatments for total protein analysis (Fig. 7e). These results demonstrate that seedling inoculation with *Trichoderma* spp., particularly *T. reesei* GT-31 and co-inoculation, significantly enhances the content of important plant metabolites, such as chlorophyll, carotenoids, flavonoids and phenolic compounds, which may contribute to the overall growth and health of the soybean seedlings.

PCA

The correlation between seedling growth analyses (fresh and dry weight of shoot and roots) and biochemical parameters (total chlorophyll, carotenoids, flavonoids, phenolic compounds, and total proteins) with different treatments, both without and with *Trichoderma*, was evaluated using principal component analysis (PCA) (Fig. 8). In the PCA plot, PC1 - the first principal component (DM1) explains 54.8% of the variance in the data, and PC2 - the second principal component (DM2) accounts for 15.4% of the variation (Fig. 8a). The Bi-plot ordination diagram clearly shows a distinct separation between the non-inoculated treatment and the *Trichoderma* treatments, indicating that the presence of *Trichoderma* strains significantly influenced the growth and biochemical responses of the seedlings (Fig. 8b). Notably, the variables of fresh shoot weight, fresh root weight, dry shoot weight, and dry root weight were highly correlated and positively associated with the co-inoculation of the three isolates.

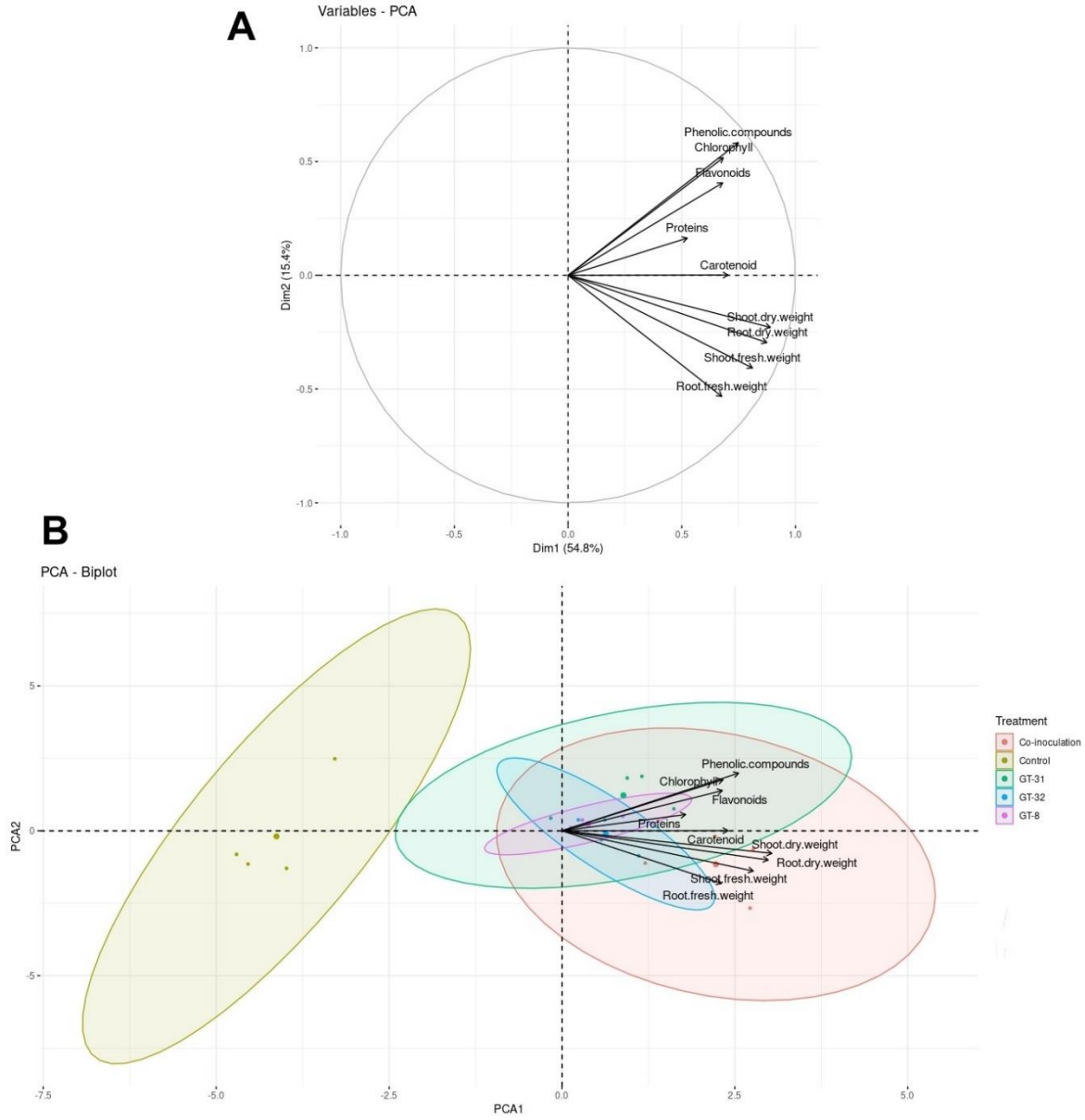


Fig. 8 Principal component analysis (PCA) on the evaluated biochemical parameters in soybean leaves inoculated with four *Trichoderma* treatments, showing the correlations between variables and the application of the isolates. (**a**) Correlation circle (**b**) Bi-plot ordination diagram

Discussion

Trichoderma-based bioinputs have long been used for agricultural diseases control and have proven to be beneficial for plant growth and development (Cortés-Rojas et al. 2021; Senger et al. 2022; Silva et al. 2022). This study obtained 66 native *Trichoderma* isolates

from the soybean rhizosphere. The use of four culture media for *Trichoderma* isolation was essential for successfully obtaining fungi from this genus, with PDA as a general medium for soil fungi, TSM as selective for *Trichoderma* (Elad et al., 1981), and RBA also mentioned for *Trichoderma* isolation (Siddiquee, 2017). Interestingly, five isolates were obtained using DOC2 medium, characterized by a high pH (10.0) and high copper concentration (Shimazu and Sato, 1996), suggesting a possible ability of these isolates to tolerate more adverse environments. Similar previous research has successfully identified and isolated rhizospheric strains of *Trichoderma* in soybean and other agricultural crops, demonstrating their potential for both biocontrol and promoting plant growth (Zhang et al. 2017; Liu et al. 2020; Yu et al. 2021). These microorganisms, along with others in the rhizosphere, are attracted by root exudates and can promote plant growth by controlling pathogens. (Lombardi et al. 2018; Dutta et al. 2023). Therefore, the antagonism assay against phytopathogens was crucial in selecting strains with agricultural potential.

The first pathogen selected for screening was *M. phaseolina*, primarily due to the complexity of managing charcoal rot disease (Lodha and Mawar 2020). Due to the climatic conditions of the Brazilian Cerrado region, this pathogen can thrive in cultivable areas, because it is favored by higher temperatures and minimal water stress (Pandey and Basandrai 2021). This disease presents challenges as there are no known systemic fungicides specifically designed for its control, and identifying resistant cultivars, especially those with R genes that confer resistance against one or more strains of one pathogen, remains elusive (Li et al. 2020; Marquez et al. 2021). Rajani et al. (2021) also observed the inhibition of mycelial growth in *S. sclerotiorum* and *M. phaseolina* by *Trichoderma* strains in dual culture method. Yu et al. (2021) showed that some *Trichoderma* strains inhibited the mycelial growth of *F. oxysporum* and *F. camptocerus* by more than 80%.

Trichoderma employs mechanisms such as mycoparasitism, competition for nutrients and space, and antimicrobial production to control phytopathogens (Woo et al. 2023). In dual culture assays, the hyphae of the *Trichoderma* adhere to the pathogen's mycelium, leading to mycoparasitism (Poveda 2021). *Trichoderma* secretes enzymes that break down the cell wall of pathogens, which contains chitin, membrane proteins, and β-glucans (da Costa et al. 2021). The cell wall degrading enzymes produced by *Trichoderma* strains (chitinases, NAGase, phosphatase, protease, and β-1,3-glucanase) cleave the cell wall polymers and release glucose, amino acids, and N-acetyl-β-D-glucosamine monomers, which are used as nutrients by the fungi (Erazo et al. 2021; Poveda 2021). In this study, the induction of the production of these enzymes was observed in contact with the cell wall of *M. phaseolina* and *S. sclerotiorum*. These enzymes were present in high concentrations in the presence of the pathogens' cell walls compared to the medium containing only glucose as a carbon source (data not shown). These findings were similar to those observed by Javeria et al. (2020). Gajera et al. (2021) also reported increased activity of chitinases and β-1,3-glucanases in the presence of *M. phaseolina* cell wall by *Trichoderma* isolates. These enzymes represent important markers in the selection of *Trichoderma* strains for disease control in agriculture. This is attributed to the enhanced production of these enzymes, as revealed by proteomic analyses conducted during the interaction between *Trichoderma* and the cell wall of pathogenic fungi (Ferreira e Musumeci 2021). For further studies, the identification and isolation of these biomolecules could improve plant immunity through molecular tools.

In this study, all the tested *Trichoderma* strains demonstrated the ability to produce IAA *in vitro*. The inoculation of this strains can significantly improve root architecture, leading to enhanced water and nutrient uptake, even under stressful conditions (Tyśkiewicz et al. 2022). Bader et al. (2020) reported that IAA-producing *T. harzianum* strains increased the growth of tomato plants. Zhang et al. (2019) showed that *T. longibrachiatum* T6 increased the

expression levels of endogenous IAA in rice seedling roots and improved tolerance to saline stress. In soils from the Brazilian Cerrado, low phosphate availability was observed, mainly due to P immobilization processes (Procópio and Barreto 2021). A considerable portion of soil phosphate is not readily available to plants, and the ability of microorganisms like *Trichoderma* to solubilize phosphate has gained significant interest, specially in the context of the global increase in the price of phosphate fertilizers and the pursuit of environmentally friendly agriculture (Ibrahim et al. 2022; Brownlie et al. 2023). The findings of this study revealed that all *Trichoderma* isolates tested were capable of solubilizing phosphate. *Trichoderma* achieves this by making phosphorus bioavailable through the excretion of organic acids that reduce the pH values of the medium *in vitro* (Fatima et al. 2022). Bononi et al. (2020) prospected for *Trichoderma* strains capable of solubilizing phosphate and observed an increase in growth and phosphorus absorption by soybeans.

The isolates, namely GT-8, GT-31, and GT-32, displayed high phylogenetic similarity to *T. viride* (99.9%), *T. reesei* (99.83%), and *T. longibrachiatum* (99.84%), respectively. *T. viride* is widely utilized in product formulations for integrated disease management, while there are fewer products and studies reported for *T. longibrachiatum* (Bettiol et al. 2019; Ferreira and Musumeci 2021). On the other hand, *T. reesei* fungi are extensively employed in the industry as a source of enzymes in the cellulolytic complex (Fang et al. 2021), but their role as plant growth promoters and biocontrollers remains underexplored in the scientific literature.

It is crucial to acknowledge that plants face various abiotic stresses in the field (dos Santos et al. 2022). Interestingly, in this study, varying concentrations of NaCl, temperature, pH values, and the presence of PEG did not disrupt the growth of the isolates. The tolerance of the isolates to a pH of 4.5 to 5.5 suggests that these strains can thrive in Brazilian Cerrado soils, which are notoriously characterized by low pH levels (Procópio and Barreto 2021).

Remarkably, *T. longibrachiatum* GT-32 exhibited significant growth enhancement within NaCl concentration range of 50-300 mM, indicating its potential for use in saline environments. This finding aligns with recent research by Liu et al. (2023), who selected a salt-tolerant strain of *T. longibrachiatum* using PDA modified with NaCl and reported its growth-promoting effects on cowpea under salt stress. Salinity-tolerant *T. longibrachiatum* T6 promoted growth and improved tolerance of wheat under salt stress (Zhang et al. 2019). Additionally, Rawal et al. (2022) employed PEG to select drought-tolerant *Trichoderma* strains, which showed promising results in alleviating water deficit stress in tomato.

T. viride GT-8, *T. reesei* GT-31, and *T. longibrachiatum* GT-32 displayed the ability to grow in the presence of agrochemicals in PDA and PD broth. Similar observations were made by Widmer (2019) and Escudero-Leyva et al. (2022), who demonstrated the tolerance and conidial viability of different *Trichoderma* strains to various agrochemicals. These encouraging findings suggest that these *Trichoderma* isolates can thrive in environments with residual pesticides or could potentially be applied in conjunction with agrochemicals, either during seed treatment, in the furrows, or through foliar application. Although direct contact between *Trichoderma* and these agrochemicals is reduced in agricultural soils, in vitro assays are needed as a guide on the tolerance of these strains (Celar and Kos 2022).

In agricultural management, one of the methods commonly employed for *Trichoderma* application is seed treatment with spores (Ferreira and Musumeci 2021). In this study, the inoculation of spores from the isolates suspended in CMC did not hinder or reduce seed germination rates, nor did impact the average germination time. This finding indicates that the inoculum concentration used in the assay was suitable and well-tolerated by the seeds. CMC, a cellulose-derived polymer, acts as an adhesive, enhancing the dispersion of conidia on the seed surface (Giordano et al. 2023). Interestingly, *T. viride* GT-8 and *T. reesei* GT-31 exhibited high efficiency in colonizing the inner tissue of the rootlets, suggesting that these

fungi can establish mutualistic relationships with this specific cultivar and reside as endophytes. *Trichoderma* can also establish themselves in the rhizosphere, on the surface of plant tissues (epiphytes), or even penetrate the internal tissues of plants and live as endophytes, a lifestyle observed in several *Trichoderma* isolates (Tseng et al. 2020; Giordano et al. 2023).

Notably, the combined use of the three isolates showed even more promising results, with greater gains observed in both fresh and dry root weights. Similar findings were reported by Cardore et al. (2020), who observed enhanced soybean seedling development upon inoculation with *Trichoderma* in conjunction with *B. japonicum*. The seedling growth stage is crucial for establishing plants in the soil (Dehnavi et al., 2020), making it essential to investigate the effects of inoculation with these isolates during this critical phase. The greater impact of the co-inoculation of isolates on root growth may corroborate the hypotheses of Eslahi et al. (2020) and Salem et al. (2024), who observed that the production of IAA by *Trichoderma* can promote better root architecture. This can lead to a significant increase in the absorption, translocation and accumulation of nutrients (e.g. phosphorus) and water in plant leaves. This can contribute substantially to subsequent improved shoot development, driven by a significant increase in photosynthesis and photosynthate production.

The inoculated seedlings displayed significantly higher chlorophyll and carotenoid contents, indicating an increased photosynthetic rate and, consequently, higher sugar synthesis, in line with previous studies by do Rêgo Meneses et al. (2022). Sofy et al (2021) demonstrated that inoculation of *T. harzianum* increased the concentration of chlorophylls and carotenoids in spinach plants subjected to normal conditions and salinity. The observed increase in total chlorophyll concentration can be attributed to the greater root growth observed in the fresh and dry weight results, which, in turn, provides enhanced water and nutrient absorption from the soil (Zhang et al. 2020). The production of IAA by the strains

could increase the surface area of the roots by elongating and improving the development of lateral and adventitious roots, thereby enabling plants to more efficiently exploit nutrients and water in the soil, including magnesium (Tyśkiewicz et al. 2022). Magnesium is an important macronutrient used by plants and plays a crucial role in chlorophyll formation (Peng et al. 2019).

The rise in flavonoids and other phenolic compounds due to *Trichoderma* inoculation indicates an improvement in the plant's antioxidant system. This enhancement helps to prevent damage to plant cells caused by reactive oxygen species (Elkelish et al. 2020; Paul and Rakshit 2023). Recently, Younes et al. (2023) observed an increase in the concentration of flavonoids and phenolic compounds in onions inoculated with *T. viride*. Also, another study that demonstrated a similar increase in these compounds in soybean when treated with *Trichoderma* (Yasnawan et al. 2019).

The pursuit of maximizing crop productivity, especially in soybean, has led to negative consequences due to the improper use of fertilizers and pesticides, as well as deforestation to expand cultivable areas, as observed in the Brazilian Cerrado region (Procópio and Barreto 2021; Song et al. 2021; Agyekum et al. 2023). As a result, there is a growing demand for more economical and sustainable alternatives, such as *Trichoderma*-based bioinputs (Woo et al. 2023). This study demonstrated that inoculation with the *Trichoderma* strains significantly increased the growth of seedlings soybean ($P < 0.05$). According to PCA, co-inoculation emerged as a standout approach in terms of the fresh and dry weight of the shoot and root, indicating that the combined use of the three isolates holds promise for maximizing plant growth. In co-inoculation techniques, microorganisms are simultaneously introduced to act synergistically, leveraging the combined effects of individual strains (da Costa et al. 2022; Stummer et al. 2022; Syed et al. 2022). It is hypothesized that plants inoculated with *Trichoderma* benefited from growth-promoting characteristics, such as

auxin synthesis, which increased root growth and enhanced water and nutrient absorption, including phosphate solubilization in the soil.

However, further investigations are required to confirm the underlying mechanisms of interaction for growth promotion and biological control of pathogens. More studies on the mechanisms involved in pathogen suppression and direct growth promotion are needed to obtain more comprehensive information on the benefits of the interaction between these strains and different soybean cultivars, as well as other crops. Furthermore, it is important to conduct experiments under field conditions to evaluate the performance of these isolates in biological control and plant growth promotion. These studies will provide valuable insights into the practical application and effectiveness of *Trichoderma*-based bioinputs as a sustainable and eco-friendly approach for agricultural management.

Conclusion

This study highlights the potential of three *Trichoderma* strains (*T. viride* GT-8, *T. reesei* GT-31, and *T. longibrachiatum* GT-32) as biocontrol agents against and exhibited plant growth-promotion traits. Their ability to tolerate various conditions and thrive in the presence of agrochemicals further enhances their potential as versatile bioinputs. Both the individual and combined use of these isolates showed promising results in enhancing growth and biochemical responses during the early development of soybean seedlings. The study suggests that these strains show promise for use in bioformulations for sustainable agriculture, supporting eco-friendly practices in the Brazilian Cerrado. In the future, experiments under field conditions involving these three *Trichoderma* strains will be considered to understand the effect of inoculation of these isolates on soybean yield.

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Author contributions

RMSG and FFZ conceived and designed this study. RMSG conducted all the experiments. SMS contributed to the isolation and antagonism assays. JVSR, ALOS, and NCAG contributed to the characterization of isolates. BOC, SFL, MSB contributed to the promotion of soybean seedling growth assay. GCG, DCM and FFZ contributed with biochemical analysis. RMSG and FFZ wrote the paper. RMSG, NCAG, and FFZ reviewed and edited the manuscript. All authors have read and approved the final manuscript.

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Data Availability

All the data used in this study are presented in the form of a table or figure.

Declarations

Ethics approval

Not applicable.

Competing interest

The authors declare that they have no conflict of interest.

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New strains of *Trichoderma* with potential for biocontrol and plant growth promotion improve early soybean growth and development

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Supplementary Information

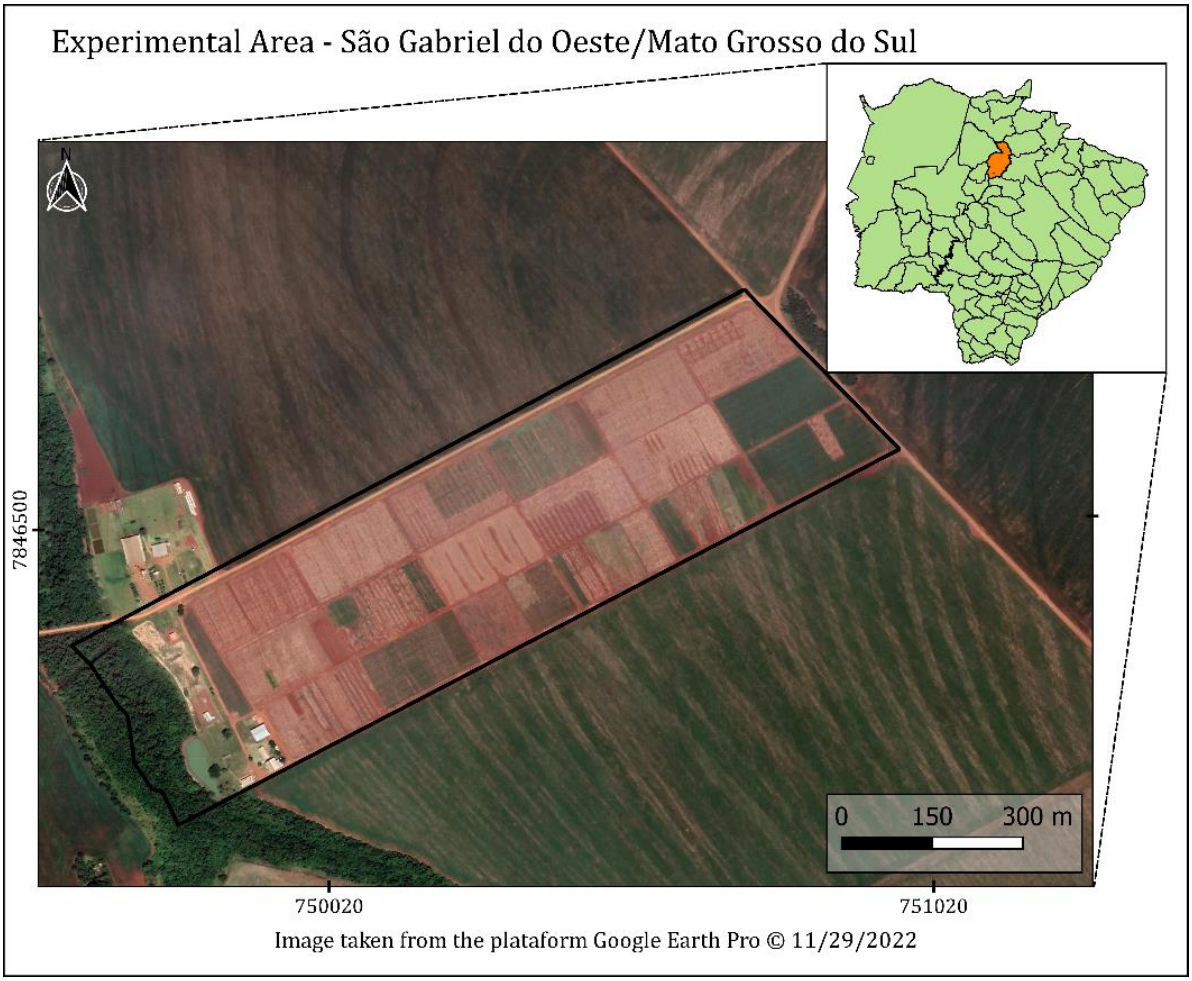

Fig. S1 Collection area of 15 soybean rhizospheric soil samples in an experimental farm in São Gabriel do Oeste, MS, Brazil (19°46'07"S 54°61'60"W)

Table S1 Pesticides used in this study. The concentration of agrochemicals added to the culture medium are presented following the recommended dose by the manufacturers and considering a spray tank of 200 L.

Commercial name	Pesticide class	Active ingredient	Concentration	Recommended dose	Concentration used in the culture medium
Atrazine	Herbicide	Atrazine	500 g/L	4.5 L/ha	22.5 mL/L
Glint	Herbicide	Haloxyfop-P-methyl	125 g/L	0.45 L/ha	2.25 mL/L
ZAPP QI 620	Herbicide	Glyphosate (N-[phosphonomethyl]glycine)	620 g/L	2.0 L/ha	10 mL/L

G-ammonium	Herbicide	Glufosinate ammonium salt	200 g/L	2.0 L/ha	10 mL/L
Curbix	Insecticide	Etiprole	200 g/L	0.25 L/ha	1.25 mL/L
Unizebe	Fungicide	Mancozebe	750 g/kg	2,0 kg/ha	10 g/L

Table S2 Antagonism activity of *Trichoderma* spp. against the phytopathogens *M. phaseolina*, *Fusarium* sp., and *S. sclerotiorum*

Isolates	Culture medium	MGI (% ± SD)		
		M. phaseolina	*F.solani*	*S. sclerotiorum*
GT-1	DOC2	59.3 ± 0.6	76.1 ± 1.7	63.0 ± 1.7
GT-2	PDA	55.2 ± 4.6	71.5 ± 6.4	n.d.
GT-3	PDA	60.4 ± 1.3	75.9 ± 1.7	n.d.
GT-4	PDA	68.5 ± 1.7	77.0 ± 0.6	76.1 ± 3.4
GT-5	TSM	54.3 ± 3.1	77.0 ± 0.3	n.d.
GT-6	PDA	53.2 ± 2.3	74.8 ± 2.6	n.d.
GT-7	PDA	70.0 ± 1.1	84.4 ± 3.9	75.6 ± 1.1
GT-8	PDA	52.9 ± 1.2	77.0 ± 1.3	80.9 ± 2.6
GT-9	PDA	55.7 ± 2.6	82.2 ± 5.9	58.9 ± 2.2
GT-10	PDA	57.0 ± 1.3	81.5 ± 6.4	60.0 ± 1.1
GT-11	PDA	48.6 ± 0.6	n.d.	n.d.
GT-12	PDA	72.6 ± 0.6	80.7 ± 2.8	77.4 ± 0.9
GT-13	PDA	54.3 ± 2.5	73.7 ± 3.6	n.d.
GT-14	PDA	50.7 ± 2.8	74.8 ± 2,8	n.d.
GT-15	PDA	45.6 ± 4.8	73.7 ± 3.7	n.d.
GT-16	PDA	66.3 ± 0.6	84.1 ± 4.5	n.d.
GT-17	PDA	55.0 ± 1.5	75.6 ± 2.2	n.d.
GT-18	PDA	46.5 ± 1.3	n.d.	n.d.
GT-19	PDA	68.2 ± 1.3	84.8 ± 3.9	75.8 ± 1.2
GT-20	PDA	55.2 ± 2.8	73.0 ± 4.5	n.d.
GT-21	DOC2	55.7 ± 2.6	86.3 ± 2.3	65.2 ± 1.8

GT-22	PDA	65.4 ± 1.4	76.7 ± 1.9	66.3 ± 1.2
GT-23	PDA	45.9 ± 0.6	n.d.	n.d.
GT-24	PDA	50.0 ± 2.2	73.7 ± 3.9	n.d.
GT-25	PDA	51.9 ± 0.7	74.8 ± 2.8	n.d.
GT-26	PDA	58.2 ± 2.6	73.7 ± 4.2	70.2 ± 0.3
GT-27	PDA	58.7 ± 1.4	78.1 ± 1.7	65.0 ± 0.6
GT-28	PDA	66.5 ± 2.1	81.1 ± 7.3	74.9 ± 0.9
GT-29	PDA	68.0 ± 0.9	77.4 ± 0.6	64.6 ± 2.7
GT-30	PDA	49.3 ± 4.0	n.d.	n.d.
GT-31	PDA	66.3 ± 2.6	83.7 ± 5.0	74.1 ± 4.8
GT-32	PDA	65.6 ± 1.1	79.5 ± 1.5	75.7 ± 4.2
GT-33	DOC2	54.4 ± 1.1	76.3 ± 1.7	70.0 ± 1.9
GT-34	PDA	61.9 ± 0.6	76.5 ± 1.4	n.d.
GT-35	PDA	53.3 ± 1.1	75.6 ± 1.9	n.d.
GT-36	PDA	56.7 ± 1,1	72.2 ± 5.1	60.4 ± 2.6
GT-37	PDA	58.9 ± 1.1	75.9 ± 2.3	n.d.
GT-38	PDA	71.5 ± 3.6	80.4 ± 2.3	75.2 ± 3.1
GT-39	PDA	47.2 ± 2.5	n.d.	n.d.
GT-40	PDA	58.2 ± 1.3	75.2 ± 2.8	n.d.
GT-41	PDA	58.1 ± 0.8	81.9 ± 6.1	63.1 ± 0.6
GT-42	PDA	54.8 ± 1.3	77.4 ± 0.6	64.9 ± 4.2
GT-43	PDA	46.3 ± 0.6	n.d.	n.d.
GT-44	PDA	64.8 ± 0.6	71.1 ± 5.9	n.d.
GT-45	DOC2	59.1 ± 1.2	82.0 ± 5.9	56.5 ± 0.3
GT-46	DOC2	55.5 ± 2.1	77.0 ± 1.3	n.d.
GT-47	TSM	55.0 ± 0.6	75.6 ± 1.9	n.d.
GT-48	TSM	54.3 ± 3.1	77.0 ± 0.6	n.d.
GT-49	TSM	54.4 ± 1.1	76.3 ± 1.7	70.0 ± 1.9
GT-50	TSM	55.3 ± 0.3	87.4 ± 1.3	n.d.
GT-51	TSM	49.7 ± 0.9	61.5 ± 5.6	n.d.

GT-52	TSM	55.2 ± 0.6	74.8 ± 1.3	n.d.
GT-53	TSM	59.1 ± 0.9	74.4 ± 3.3.	n.d.
GT-54	TSM	59.6 ± 1.3	73.0 ± 0.6	57.2 ± 1.5
GT-55	TSM	54.1 ± 2.4	74.6 ± 2.9	n.d.
GT-56	TSM	56.7 ± 1.1	78.2 ± 0.6	n.d.
GT-57	TSM	57.4 ± 0.6	84.8 ± 3.9	n.d.
GT-58	TSM	63.9 ± 1.5	76.3 ± 1.7	n.d.
GT-59	TSM	57.4 ± 0.6	73.7 ± 3.9	63.0 ± 2.8
GT-60	RBA	55.9 ± 0.6	70.0 ± 2.2	59.8 ± 1.4
GT-61	RBA	57.4 ± 2.3	79.6 ± 8.2	n.d.
GT-62	RBA	60.0 ± 2.2	88.5 ± 0.6	71.4 ± 2.3
GT-63	RBA	52.3 ± 2.9	86.3 ± 2.3	n.d.
GT-64	RBA	53.3 ± 1.1	77.0 ± 0.6	n.d.
GT-65	RBA	53.0 ± 1.3	73.9 ± 2.6	n.d.
GT-66	RBA	57.6 ± 0.9	80.4 ± 7.4	n.d.

The values presented in the table represent means with ± standard deviation (SD) obtained from a triplicate experiment

n.d.: not determined

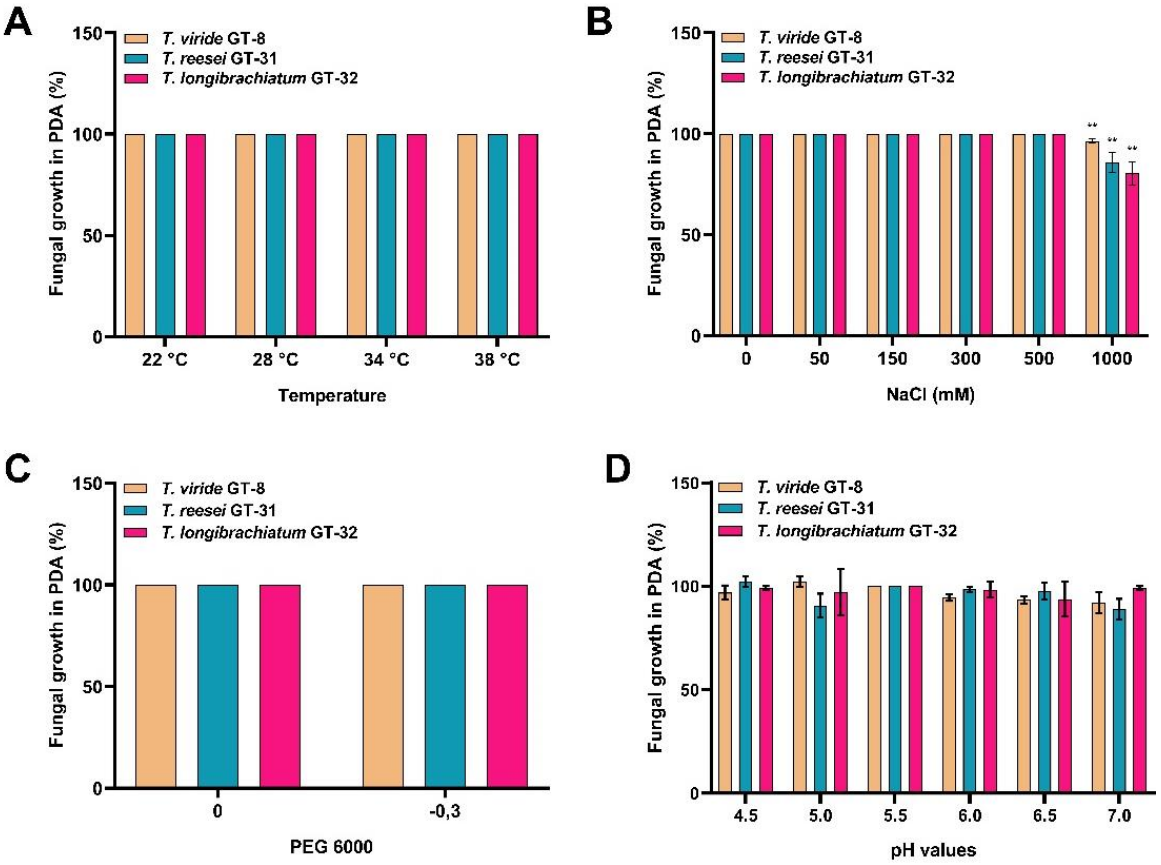

Fig. S2 Growth of *Trichoderma* spp. in PDA medium after 7 days under different conditions of (**A**) temperature (**B**) salinity (**C**) drought by PEG 6000 and (**D**) pH. Asterisks represent significant differences from the control treatment (**, p-value < 0.01) as determined by Tukey's test

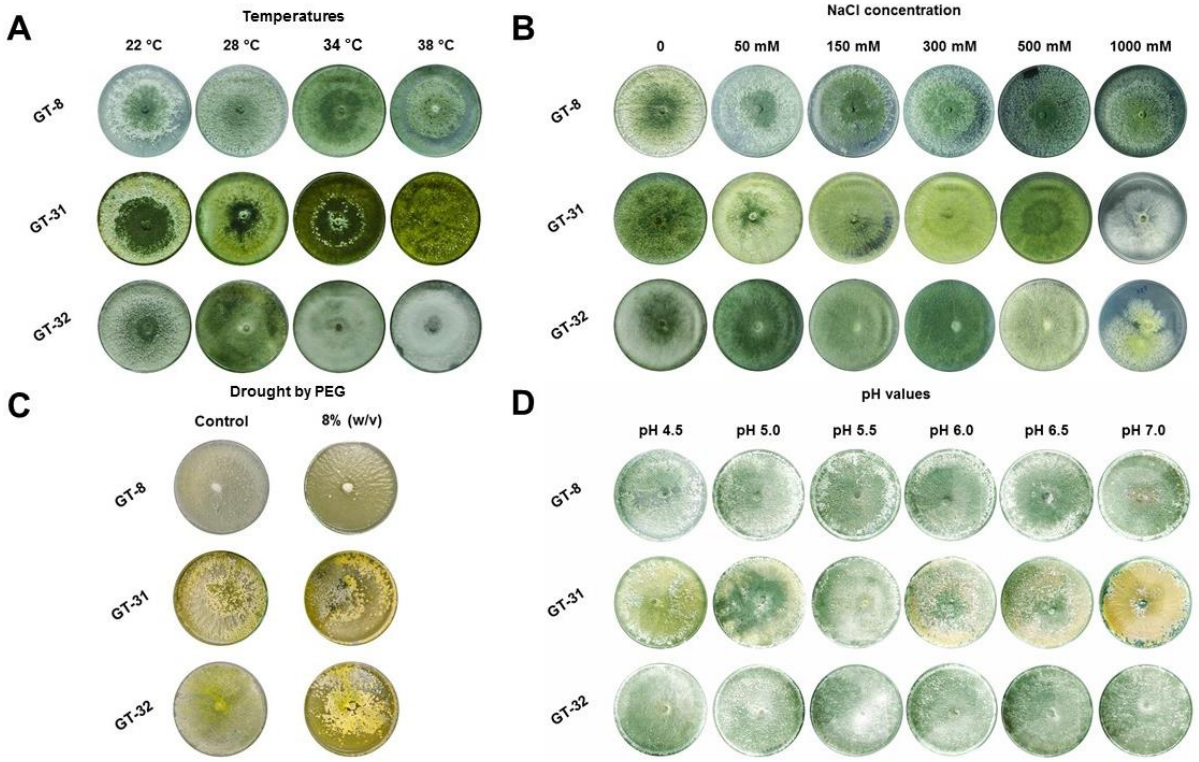

Fig. S3 Growth of *Trichoderma* spp. in Petri plates containing PDA medium after 7 days under different conditions of (A) temperature (B) salinity, (C) drought by PEG 6000, and (D) pH

5. CAPÍTULO 2

Artigo nas normas da Revista *Environmental Research*

(fator de impacto 8.3, qualis A1)

New phosphorus-solubilizing *Trichoderma* strains: Mechanisms to promote the growth of soybean plants and agroecosystem sustainability strategies

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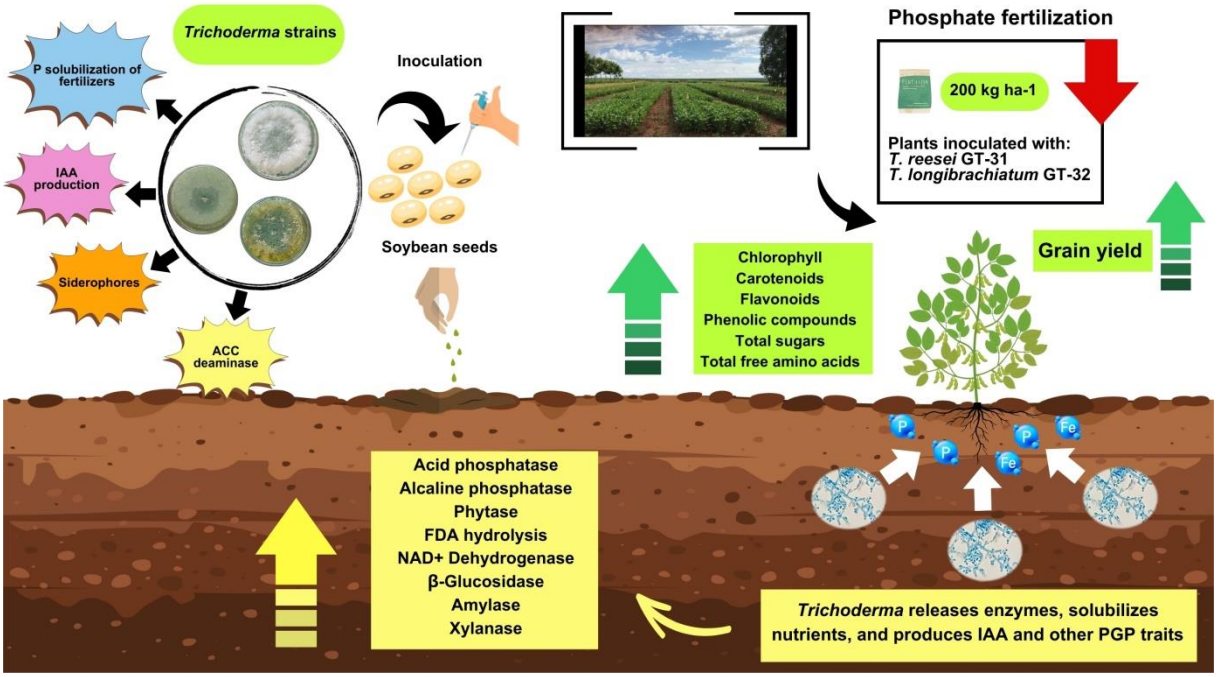
Graphical Abstract



Abstract

In Brazilian Cerrado soils, the phosphorus content is significantly low, leading manyfarmers to frequently apply phosphate fertilizers. However, these inputs are derived from non-renewable sources, and Brazil is dependent on the foreign market to acquire them.*Trichoderma* has traits that can improve the fertility and health of the soil and promote plant growth. In this study, native *Trichoderma* strains (*T. viride* GT-8, *T. reesei* GT-31, and *T. longibrachiatum* GT-32) were characterized for plant growth-promoting traits, including phosphate solubilization from fertilizers. The study assessed the impact of their inoculation on soybean growth and grain yield under two phosphate fertilization conditions: 400 and 200 kg ha^{-1} of simple superphosphate. Two field experiments were conducted with two cultivars (Nidera NS6601 IPRO and DM 69IX60RSF 12X RR2 PRO). Leaf and rhizospheric soil samples were collected for biochemical analyses. The strains showed phosphate solubilization from fertilizers and exhibited other plant growth-promoting traits. Inoculation of GT-32 (200 kg ha^{-1}) on cv. Nidera NS6601 resulted in a 4.3% increase in grain yield. For the second

cultivar, the use of GT-31 and GT-32 (200 kg ha^{-1}) resulted in grain yield increases of 22.7% and 18.6%, respectively. Inoculated plants showed higher shoot dry weight, chlorophyll content, phenolic compounds, flavonoids and antioxidant responses under both fertilization conditions. Furthermore, enzymatic activities were higher in the rhizospheric soil of plants inoculated with GT-31 and GT-32 strains. These findings demonstrated the potential of the GT-31 and GT-32 strains to improve the growth and soybean yield with less fertilizer use. Also, the use of these new strains could be a sustainable strategy for improving soil health and fertility.

Keywords: Biofertilization, *Trichoderma*, soil enzymes, siderophore, phosphate solubilization, sustainable agriculture.

1. Introduction

Soybean (*Glycine max* (L.) Merr.) is a crop of great global relevance, with the United States and Brazil accounting for 69% of global production (Statista, 2023). However, its cultivation is linked to various environmental problems, including those arising from the excessive use of chemical fertilizers. Undoubtedly, these inputs help with plant nutrition, providing nutrients that are often not readily available in the soil, especially phosphorus (Bindraban et al., 2020). In agricultural soils, only 0.1% of the total phosphorus stock is available for plant uptake due to its low mobility and high affinity with cations in the soil, resulting in its precipitation (Alori et al., 2017; Rawat et al., 2021). It is also immobilized or adsorbed in the soil's organic matter (Rawat et al., 2021).

The regular application of fertilizers guarantees the supply of nutrients to the plants (Dincă et al., 2022), but the use of phosphate fertilizers is challenging because phosphorus precipitates rapidly with soil cations, making it unavailable to plants (Bindraban et al., 2020). Furthermore, improper management using higher doses of fertilizers can alter the structure of the soil, harming health and fertility (Dincă et al., 2022; Penuelas et al., 2023). These agrochemicals can leach into surface and groundwater and cause eutrophication (de Azevedo Morgado et al., 2023). Fertilizers are also among the main ways in which heavy metals enter the soil (Zwolak et al., 2019). Therefore, other management strategies are needed to mitigate these problems.

Another challenge faced by agricultural countries is the acquisition of these agrochemicals in the global market. Recently, the prices of these products have skyrocketed and imports have suffered (Arndt et al., 2023). This increase was due to the restrictions imposed by COVID-19, climatic events in China, the energy crisis in European countries, and the conflicts between Russia and Ukraine, because Russia is the largest fertilizer exporter in the world (Arndt et al., 2023; Brownlie et al., 2023). Limited access to these inputs can result

in lower food productivity, as well as contributing to higher grain prices (Abay et al., 2023). Urgent measures are needed to overcome these problems, which is why more sustainable and economical alternatives are gaining more attention.

For decades, fungi from the genus *Trichoderma* have been employed to manage phytopathogens in crops, including soybean, through mechanisms such as mycoparasitism, antibiosis, induction of systemic resistance and competition for nutrients and infection sites (Bader et al., 2020; Poveda, 2021; Rodrigues et al., 2023). In addition, *Trichoderma* can stimulate the growth and development of plants in mutualistic relationships by colonizing the rhizosphere (Sood et al., 2020). These fungi can be used as powerful biofertilizers because they have the ability to increase the availability of nutrients for plants by solubilizing minerals in the soil (Rawat et al., 2021; Bedine et al., 2022). As a result, plants can effectively acquire nutrients from fertilizer and soil, optimizing fertilization and reducing the excessive use of fertilizers in agricultural fields (Bononi et al., 2020; Woo et al., 2023).

Additionally, *Trichoderma* can produce auxins, such as indole-3-acetic acid (IAA), increasing the rooting and vigor of roots, consequently improving the efficiency of water and nutrient absorption (Bader et al., 2020). *Trichoderma* can synthesize siderophores, which chelate iron ions, helping plants absorb iron (Sood et al., 2020). These fungi can also help plants tolerate abiotic stresses by producing the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which cleaves the ACC molecule, the precursor of ethylene in plants. Under abiotic stress, high concentrations of this hormone can cause harmful effects on plants (Singh et al., 2023). *Trichoderma* can also improve soil fertility by increasing the activity of enzymes that act in various biogeochemical processes and, by colonizing the rhizosphere(Paul and Rakshit, 2021; Anshu et al., 2022). This fungus can recruit and increase the presence of other beneficial microorganisms near the roots (Hang et al., 2022; Wang et al., 2023).

Because of all these characteristics, *Trichoderma* is attractive for the formulation of biofertilizers. This alternative for agricultural management can help agroecosystems, as it is ecologically sustainable besides helping to minimize the impact of fertilizers (Sood et al., 2020). Several studies have shown the beneficial effects of *Trichoderma* inoculation in increasing soybean growth (Bononi et al., 2020; Paul e Rakshit, 2021; Senger et al., 2022). However, field-scale studies in the Brazilian Cerrado using native Brazilian *Trichoderma* strains and their effect on growth, productivity, and the enzymatic activity of rhizospheric soybean soil are scarce. Therefore, the aim of this study was to characterize three *Trichoderma* strains in terms of their ability to solubilize phosphate from different fertilizers in vitro and the presence of other properties that promote plant growth. Also, to evaluate the effect of *Trichoderma* inoculation on the growth and productivity of two soybean cultivars under two fertilization conditions, their biochemical responses and the enzymatic activities of the soil after the introduction of *Trichoderma*.

2. Materials and methods

2.1. Origin of *Trichoderma* strains

Three *Trichoderma* strains were used in this study: *Trichoderma viride* GT-8, *T. reesei* GT-31, and *T. longibrachiatum* GT-32, and the GenBank access numbers obtained were OQ826673, OQ826674, and OQ826675, respectively. These strains are deposited in the Culture Collection of the Federal University of Mato Grosso do Sul (UFMS), Campo Grande/MS, Brazil. The fungi were cultivated periodically in test tubes and Petri plates containing potato-dextrose-agar (PDA) medium.

2.2. Phosphate solubilization from phosphate fertilizers

Four phosphate fertilizers were tested: simplex strong thermo phosphate (11% P_2O_5), thin thermo phosphate (14% P_2O_5), granulated thermo phosphate (17% P_2O_5), and MV27 (27% P_2O_5). Three plugs of mycelium (5.0 mm) were inoculated into flasks containing 50 mL of modified PVK medium (Pikovskaya, 1948), substituting $Ca_3(PO_4)_2$ for the fertilizers (0.5% w/v). The cultures were incubated at 28 °C under agitation (110 rpm). The concentration of soluble P was determined after 3, 5, 7 and 10 days using the method of Murphy and Riley (1962). The pH variation in the medium was monitored using a digital pH meter.

2.3. Determination of plant growth-promoting traits

IAA synthesis was investigated at different time intervals using Czapek medium with 5 mM tryptophan. In flasks containing 50 mL of the medium, three plugs of mycelium (5 mm) were added and the cultures incubated at 28 °C (110 rpm) for 7 days. Aliquots were collected every 24 h, and the concentration of IAA was determined using the method of Gordon and Weber (1951). Siderophore synthesis was detected in a modified Czapek solid medium supplemented with 20% (v/v) Chrome Azurol S (CAS) reagent (Schwyn and Neilands, 1987). Also, conidia (1 x 10^8 conidia/mL) were inoculated (1% v/v) into Czapek broth and cultivated at 28 °C (110 rpm) for 21 days. Aliquots collected every 3 days and the supernatants were recovered for analysis according to Machuca and Milagres (2003). ACC deaminase activity was determined in synthetic medium (SM) and the enzymatic assay was performed according to Viterbo et al. (2010).

Ammonia production was assessed in peptone medium inoculated with mycelium disks and incubated at 28 °C (110 rpm) for 72 h. Detection was carried out using Nessler's reagent (Cappuccino and Sherman, 1992). The following enzymes were evaluated: amylase, protease, xylanase, phosphatase and phytase. In 50 mL of TLE culture medium (Ramada et al., 2016) with wheat bran (1% w/v), three plugs of mycelium were added, and the cultures

were incubated at 28 °C (110 rpm) for 7 days. Aliquots were collected every 24 h, centrifuged (8,000 x g for 5 min) and the supernatant used as an enzyme source. Amylase and xylanase activities were determined by quantifying reducing sugars according to Miller (1959). The protease, phytase and phosphatase assays were carried out according to Sarath et al. (1996), Heinonen and Lahti (1981) and Leitão et al. (2010), respectively, with some modifications. The specific information for each test is shown in Table S1.

2.4. Preparing the inoculum for the field experiments

The strains were cultivated in tilted PDA medium at 28 °C for 7 days, with 12 h of photophase per day. Suspensions of conidia were obtained using sterilized distilled water and scraping the surface of the medium. The concentration of conidia was calibrated to 1 x 10^8 spores/mL^{-1} by diluting the suspensions in carboxymethylcellulose (CMC) solution (0.5% w/v).

2.5. Experiment I

The first experiment was implemented during the 2021/2022 harvest in the experimental area of the Chapadão do Sul Campus of the UFMS, a region with a humid tropical climate (Aw) according to Köppen, and an altitude of 810 m (18º 48'S and 52°36'O). Annual temperatures range from 13 to 28 °C (Cunha et al., 2013). Rainfall data and temperatures during the experiment are shown in Fig. S1A. The soil, classified as dystrophic Red Latosol (Santos et al., 2018), was previously sampled (0.00-0.20 m) for physical-chemical characterization (Table S2). The Nidera NS6601 IPRO cultivar was used in this experiment. The design used was randomized blocks in a factorial scheme, with four replications. The factors were made up of *Trichoderma* inoculums and two phosphate

fertilization conditions, 400 and 200 kg ha^{-1} with simple superphosphate (SSP), totaling 32 plots (Table 1).

Table 1. Treatments carried out on soybean seeds of the two cultivars Nidera NS6601 IPRO and DM 69IX60RSF 12X RR2 PRO in the 2021/2022 and 2022/2023 harvests, respectively, using inoculation with *Trichoderma* strains and two conditions of phosphate fertilization with simple superphosphate (SSP) (18% P_2O_5).

Treatments
T1 – Control without *Trichoderma* inoculation+ complete fertilization of 400 kg ha^{-1} of SSP
T2 – Inoculation with *T. viride* GT-8 + complete fertilization of 400 kg ha^{-1} of SSP
T3 – Inoculation with *T. reesei* GT-31 + complete fertilization of 400 kg ha^{-1} of SSP
T4 – Inoculation with *T. longibrachiatum* GT-32 + complete fertilization of 400 kg ha^{-1} of SSP
T5 – Control without Trichoderma inoculation + half fertilization of 200 kg ha^{-1} of SSP
T6 – Inoculation with *T. viride* GT-8 + half fertilization of 200 kg ha^{-1} of SSP
T7 – Inoculation with *T. reesei* GT-31 + half fertilization of 200 kg ha^{-1} of SSP
T8 – Inoculation with *T. longibrachiatum* GT-32 + half fertilization of 200 kg ha^{-1} of SSP

The seeds were treated with Masterfix® inoculant containing *Bradyrhizobium japonicum* strains SEMIA 5079 and SEMIA 5019. To inoculate the *Trichoderma* strains, a ratio of 2.0 mL of conidia suspension per 100 g of seeds was used. Sowing took place manually on Nov 25, 2021, in experimental plots with five 5.0 m long rows, spaced 0.45 m apart, with 18 seeds per meter. The three central rows were defined as the useful area for the evaluations. Crop management, including weeds, pests and diseases control, followed the standards used in the region. Before harvesting, with the plants at growth stage R8, five plants from each plot were collected and the following parameters were assessed: Plant height (PH),

height of first pod insertion (HFP), number of branches per plant (NBP), number of pods per plant (NPP), grains number per pods (GNP), number of grains per plant (NGP), mass of grains per plant (MG), and mass of one thousand grains (M1000). To determine grain yield (GY), the entire plot was harvested and threshed and the mass was adjusted to 13% moisture. Harvesting took place 125 days after planting.

2.6. Experiment II

A 2nd experiment was carried out during the 2022/2023 harvest in the same experimental area. The rainfall data and temperatures during the experiment are shown in Fig. S1B. The soil was also characterized (Table S2). In this experiment, the DM 69IX60RSF 12X RR2 PRO cultivar was used, maintaining the same seed treatment conditions and experimental design adopted in Experiment I (Table 1). Manual sowing was carried out on Oct. 20, 2022. At 48 DAS (days after sowing), rhizospheric soil, leaf samples and aerial parts of soybean plants were collected for analysis. Shoot dry weight was obtained by drying the samples in an oven at 65 °C for 72 h. At the end of the cycle, the same analyses were carried out as in Experiment I.

2.6.1. Analysis of the biochemical parameters of growth promotion

Leaves were collected by walking around the plot in irregular directions and choosing the second or third trefoils of five random plants in each plot, starting from the apex of the central stem. The leaves were collected in centrifuge tubes (50 mL) and immediately stored in liquid nitrogen for biochemical analysis.

2.6.1.1 Total chlorophyll and carotenoid content

The extraction process was carried out according to Ross (1974). The absorbances of the samples was measured at 663 (chlorophyll *a*), 647 (chlorophyll *b*) and 470 nm (carotenoids). The concentration of the pigments was obtained using the formula from Lichtenthaler and Buschmann (2001).

2.6.1.2. Flavonoids, phenolic compounds, sugars and total free amino acids

The methanolic extract was prepared from 100 mg of fresh leaves, macerated in liquid nitrogen, and 5.0 mL of methanol (80% v/v) was added. After centrifugation at 3,000 x g for 20 min, the supernatants were stored at -20 °C. The flavonoid content was determined as described by Assis et al. (2020). Phenolic compounds were quantified according to Arnaldos et al. (2001). The total sugar content was determined using the phenol-sulfuric acid method (Dubois et al., 1951). The content of reducing sugars was assessed using the dinitrosalicylic acid (DNS) reagent (Miller, 1959). Free amino acid content was investigated using the protocol of Sandhya et al. (2010).

2.6.1.3. Antioxidant enzymes

Fresh leaves (100 mg) were macerated in liquid nitrogen and resuspended in 5.0 mL of 100 mM potassium phosphate buffer (pH 7.8), containing 10 mM EDTA, 200 mM ascorbate and 1% (w/v) polyvinylpyrrolidone. The samples were transferred to tubes protected from light and centrifuged at 3,000 x *g* for 20 min. The supernatant was used for analysis of antioxidant enzyme activity and total protein concentration (Bradford, 1976). The catalase assay was carried out according to the method of Havir and McHale (1987). The activity of ascorbate peroxidase was assessed following the method described by Nakano and Asada (1981). Peroxidase activity was measured according to Campos and Silveira (2003).

2.6.2. Soil enzymatic activities

Using an auger, three composite soil samples were collected at random per plot, from five sub-samples equidistant at 12 cm and 12 cm deep, one collected in the crop row and two in each inter-row. The samples were homogenized, packed in polypropylene bags and refrigerated until analysis. In the laboratory, the soil was sieved for enzymatic analysis. The hydrolysis of fluorescein diacetate (FDA) was estimated according to Ghini et al. (1998). Acid and alkaline phosphatase activities were estimated according to the method of Tabatabai and Bremner (1969), using sodium acetate buffer (pH 5.0) and borate buffer (pH 11.0), respectively. β-glucosidase was determined using the method proposed by Tabatabai (1982). For the phytase assay, 0.1 g of soil was added to test tubes containing 5 mL of sodium acetate buffer (pH 5.0) with 10 mM phytic acid. The assay was carried out using the method described by Heinonen and Lahti (1981). NADH dehydrogenase activity was determined using triphenyltetrazolium chloride (TTC) according to Casida et al. (1964). Soil amylase and xylanase activities were investigated according to the method of Guan et al. (1986).

2.7. Statistical analysis

The biochemical characterization tests of the strains were carried out in triplicate and the data were presented as mean ± standard deviation. The data obtained from the leaf, shoot dry weight and soil analyses were subjected to One-Way ANOVA using the Tukey test ($P < 0.05$) in GraphPad Prism software version 8.0. Statistical differences between the treatments in the field experiments were analyzed using the Scott-Knott test with significance levels of $P < 0.05$ and $P < 0.01$ using SISVAR software (Ferreira, 2019).

3. Results

3.1. Phosphate solubilization from phosphate fertilizers

The *Trichoderma* strains were able to phosphate solubilization from the four fertilizers evaluated (Fig. 1).

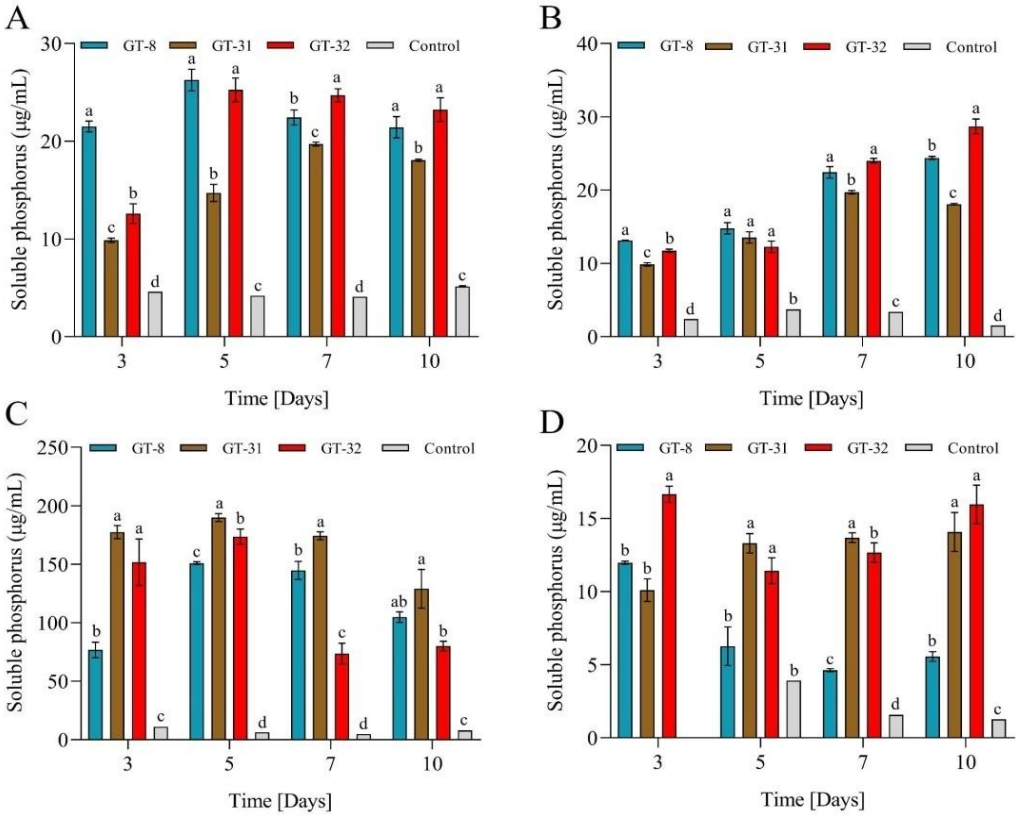


Fig. 1. Phosphate solubilization by *Trichoderma* strains. The fungi were grown in PVK medium with different phosphate fertilizers (µg/mL) after 3, 5, 7, and 10 days of incubation (a) Thin thermo phosphate (b) Granulated thermo phosphate (c) MV27 (d) Simplex strong thermo phosphate. Equal letters indicate that there is no statistical difference between treatments on each day of analysis according to the Tukey test ($P < 0.05$)

After 10 days of incubation, GT-8 and GT-32 showed similar levels of solubilization for thin thermo phosphate, with 21.4 ± 1.1 and 23.2 ± 1.2 µg/mL, respectively (Fig. 1A). For granulated thermo phosphate, GT-32 stood out after 10 days of growth, with 28.7 ± 1.0

µg/mL (Fig. 1B). In the MV27 assay, GT-31 showed the highest solubilizing activity throughout the period evaluated, differing significantly from the other strains (Fig. 1C). For simplex strong thermo phosphate, GT-31 (14.1 ± 1.3 µg/mL) and GT-32 (15.9 ± 1.3 µg/mL) exhibited the highest solubilization activity after 10 days of incubation (Fig. 1D). The pH values of the *Trichoderma* culture filtrates were lower than those of the uninoculated controls, except for the cultures of GT-8 and GT-31 grown with MV27 (Fig. S2).

3.2. Determination of plant growth-promoting traits

All three strains synthesized IAA throughout the incubation periods (Fig. 2a).

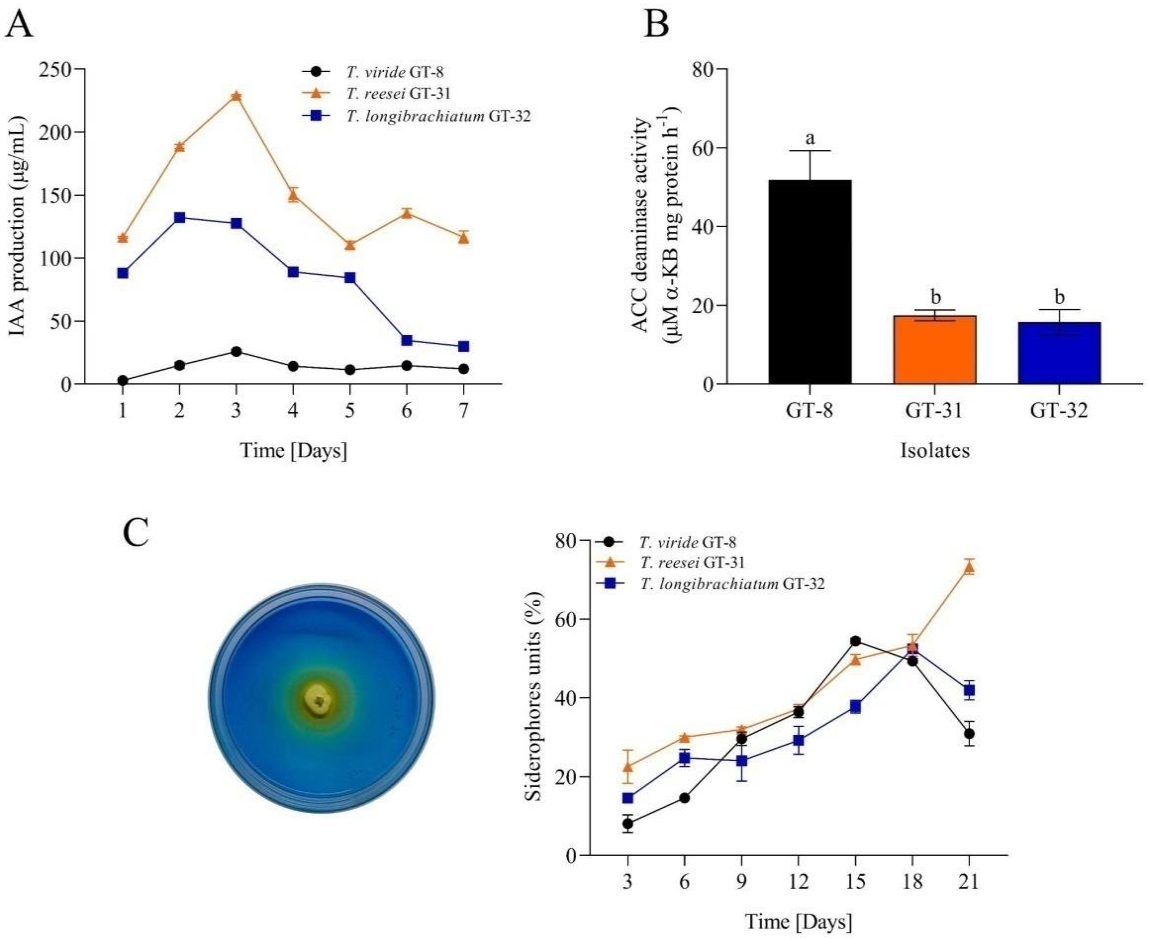


Fig. 2. (a) IAA production (µg/mL) by *Trichoderma* strains. The line bars represent the standard errors of the means of an experiment carried out in triplicate (b)ACC deaminase activity of *Trichoderma* strains. Different

letters indicate statistical difference using the Tukey test ($P < 0.05$) (c) Synthesis of siderophores by *Trichoderma* strains: left – Assay in Czapek-CAS solid medium, right – Analysis in Czapek broth medium during 21 days of incubation

GT-31 showed the highest IAA production, reaching a maximum peak after three days of incubation (229.4 ± 0.6 µg/mL). Following GT-32 showed the second-highest production, with its peak occurring on the second day of growth (132.1 ± 1.5 µg/mL). GT-8, on the other hand, exhibited the highest ACC deaminase activity among the strains, with 51.9 ± 7.4 µM α-CB mg protein h^{-1} (Fig. 2b). Also, the strains showed positive reactions for ammonia production after the addition of Nessler's reagent (Fig. S3). GT-31 showed the ability to synthesize siderophores in solid medium (Fig. 2c). Interestingly, the strains synthesized siderophores in Czapek broth medium (Fig. 2c). GT-31 exhibited the highest siderophore production, reaching 73.3% in siderophore units after 21 days of incubation.

The strains produced the enzymes over the periods evaluated in TLE medium (Fig. 3).

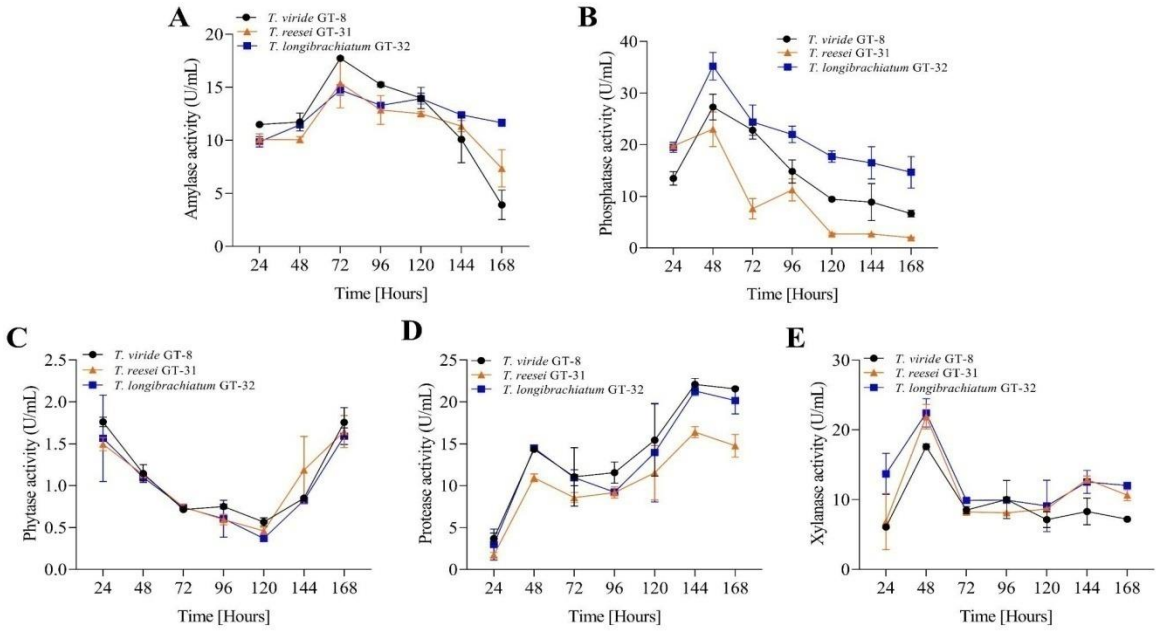


Fig. 3. Enzymatic activities of *Trichoderma* strains grown in TLE medium at 28 °C (24–168 h) (a) Amylase (b) Phosphatase (c) Phytase (d) Protease (e) Xylanase

Amylase production peaked at 72 hours, with the highest activity observed for GT-8, with 17.7 ± 0.1 U/mL (Fig. 3a). Acid phosphatase activity increased after 48 hours (Fig. 3b), with GT-32 exhibited the highest activity (35.2 ± 2.7 U/mL). Phytase production displayed two peaks at 24 and 168 hours (Fig. 3c). The highest protease activity occurred after 144 h (Fig. 3d), with GT-8 (22.1 ± 0.7 U/mL) and GT-32 (21.3 ± 0.5 U/mL) having the highest activities. Xylanase production peaked after 48 h, with the highest production values obtained for GT-31 and GT-32, with 22.4 ± 2.0 and 21.9 ± 1.8 U/mL, respectively (Fig. 3e).

3.3. Experiment I

The inoculation of *Trichoderma* strains significantly affected the following variables in the cv. NS6601 IPRO: HFP, NPP, GNP, NGP, MGP and GY (Table S3). The recommended HFP for soybean plants is above 10 cm, but excessively high values can reduce the number of pods per plant. In accordance with these recommendations, the results indicated that the treatments GT-31 and GT-32, at the full dose of fertilizer, had the highest averages (Table 2).

Table 2. Height of first pod insertion (HFP), number of pods per plant (NPP), mass of grains per plant (MGP), and grain yield (GY) of soybean plants (Nidera NS6601 IPRO) inoculated with *Trichoderma* strains under two phosphate fertilization conditions.

Treatments	HFP		NPP		MGP		GY	
	400 kg	200 kg	400 kg	200 kg	400 kg	200 kg	400 kg	200 kg
Control	16,10 bB	17,35 aA	42,25 aA	37,10 bD	18,14 aB	15,87 bC	4046 aA	4043 aB
GT-8	18,15 aA	16,20 bB	43,45 aA	41,75 aB	20,84 aA	18,72 bB	4187 aA	3970 bB
GT-31	16,30 aB	16,00 aB	42,65 aA	45,05 aA	19,41 aB	20,36 aA	4175 aA	4157 aA
GT-32	15,70 aB	14,70 aC	42,35 aA	40,20 aC	18,12 aB	17,89 aB	4050 bA	4225 aA

Equal lowercase letters in the rows do not differ statistically from each other at 5% significance between fertilization conditions for each treatment.

Equal capital letters do not differ statistically at 5% significance between treatments.

When was used half the fertilizer dose, the inoculated treatments showed values closer to 10 cm. GT-31 provided the highest NPP (45.05) at a dose of 200 kg ha^{-1}. Among the treatments, the use of the strains resulted in a higher number of GNP (Fig. S4b). For MGP, treatments with GT-8 (full dose) and GT-31 (half dose) promoted greater grain mass, with an increase of 14.9% and 12.2%, respectively, compared to the full dose control. Plants inoculated with GT-32 at a dose of 200 kg ha^{-1} resulted in higher GY, surpassing the non-inoculated control at the full dose by 4.3%.

3.4. Experiment II

For the cv. DM 69IX60RSF 12X RR2 PRO, *Trichoderma* application significantly increased the shoot dry weight of the soybean plants at both fertilizer doses (Fig. S5). At 400 kg ha^{-1}, inoculation of GT-32 led to a significantly increased in dry weight by 143.1% and 118.5% compared to the controls at the full and half doses of fertilization, respectively. At half the dose, the greatest gains were observed for treatments with GT-8 and GT-32, surpassing the values obtained for non-inoculated plants by 46.1% and 55.9%, respectively.

Inoculation with the strains impacted all evaluated variables, with the exception of GNP (Table S4). Inoculated plants exhibited the recommended height for HFP (Table 3).

Table 3. Height of first pod insertion (HFP), number of pods per plant (NPP), mass of grains per plant (MGP), grain yield (GY), and grain yield in bags ha^{-1} (GYB) of soybean plants (DM 69IX60RSF 12X RR2 PRO) inoculated with *Trichoderma* strains under two phosphate fertilization conditions.

Treatments	HFP		NPP		MGP		GY	
	400 kg	200 kg	400 kg	200 kg	400 kg	200 kg	400 kg	200 kg
Control	9,62 Ac	9,90 aC	51,93 aB	42,88 bB	20,05 aD	20,49 aD	4702 aA	4250 bB
GT-8	12,70 aA	10,85 bBC	51,00 aB	53,50 aA	21,53 bC	25,68 aC	4396 aB	4460 aB
GT-31	10,32 bBC	11,35 aAB	53,67 aAB	54,38 aA	24,67 aB	23,95 aB	4530bAB	5214 aA
GT-32	11,13 bB	12,30 aA	57,83 aA	52,67 bA	25,99 aA	22,02 bA	4782 bA	5040 aA

Equal lowercase letters in the rows do not differ statistically from each other at 5% significance between fertilization conditions for each treatment.

Equal capital letters do not differ statistically at 5% significance between treatments.

The combination of GT-32 and the full dose resulted in the highest NPP (57.83), followed by GT-31 (54.38) at half the dose, with an increase of 7.75% and 4.7%, respectively. Notably, among the treatments, the inoculation of GT-32 with 400 kg ha^{-1} of SSP significantly increased the MGP compared to the other treatments. The M1000 values were higher with the *Trichoderma* treatments (Fig. S6). When using half the fertilizer dose, it was observed that the application of GT-31 and GT-32 provided a significant increase in GY by 22.7% (or 16.1 bags) and 18.6% (or 13.2 bags), respectively, compared to the control. In comparison to both the control and the full dose treatment, the increases obtained were 10.9% (or 8.5 bags) and 7.2% (or 5.6 bags) for GT-31 and GT-32, respectively.

3.4.1. Analysis of the biochemical parameters of growth promotion

Inoculation of GT-31 significantly increased total chlorophyll content at 400 kg ha^{-1} of SSP (Fig. 4a). At half the dose, plants inoculated with GT-8 and GT-31 showed a significant increase of 38.9% and 39.5%, respectively, in chlorophyll content compared to the control. Regarding total carotenoids, greater increases were observed in the inoculated plants at the 200 kg ha^{-1} fertilization compared to the non-inoculated control (Fig. 4b).

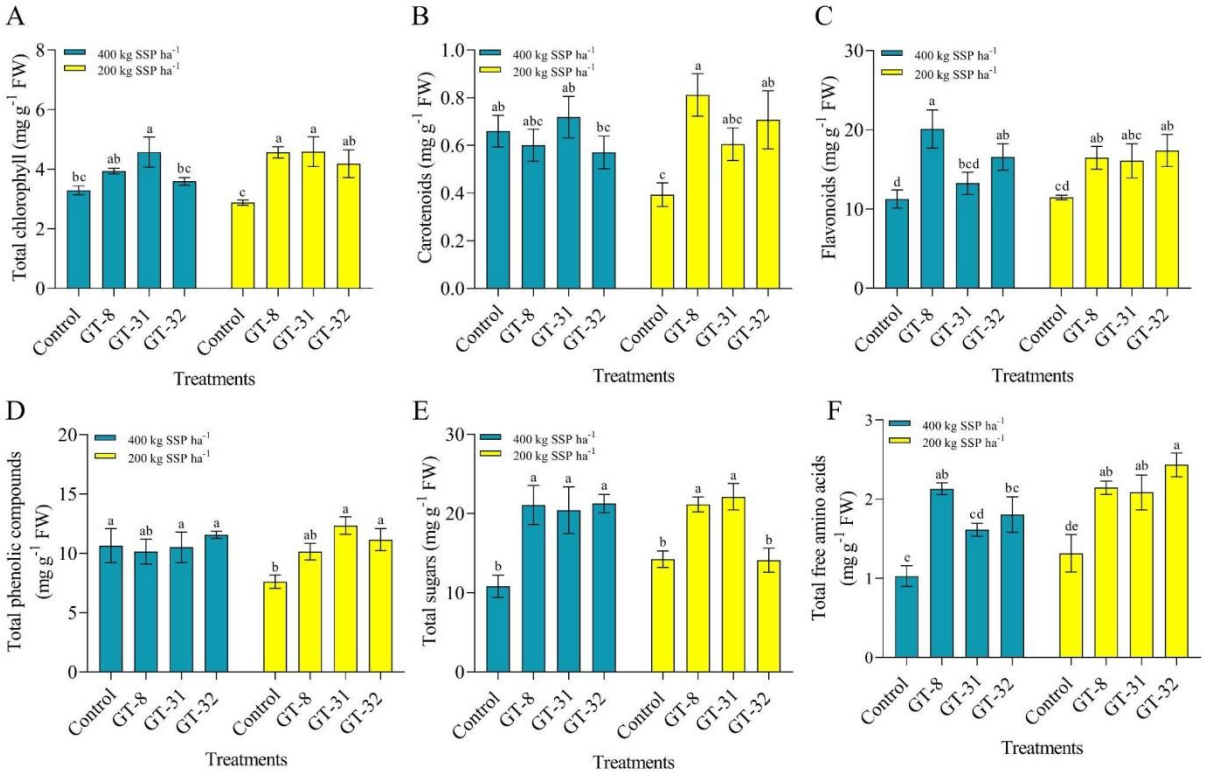


Fig. 4. Effect of *Trichoderma* strains inoculation on the content of (A) total chlorophyll (B) carotenoids (C) flavonoids (D) total phenolic compounds (E) total sugars (F) total free amino acids in soybean leaves harvested 48 DAS subjected to two phosphate fertilization conditions. Equal letters indicate no statistical difference between treatments according to Tukey's test ($P < 0.05$).

Inoculation with GT-8 resulted in the greatest increase, of 106.6%. At 400 kg ha^{-1} of SSP, a significant increase in flavonoid content was also observed with GT-8, with 20.1 mg g^{-1}FW (Fig. 4c). Moreover, at half the dose, the use of *Trichoderma* resulted in a significant increase in flavonoid content compared to non-inoculated controls.

Plants inoculated with GT-31 and GT-32 showed significant increases in total phenolic compounds with 200 kg ha^{-1} of SSP, rising by 61.8% and 46.8%, respectively (Fig. 4d). There was no significant difference between these results and those obtained in the treatments with the full dose. Similarly, the levels of total reducing sugars were higher in the *Trichoderma* treatments (Fig. S7). Total sugar contents were significantly higher in plants inoculated with GT-8 and GT-31 under both fertilization conditions (Fig. 4e). On the other

hand, inoculation with GT-32 (200 kg ha^{-1} of SSP) resulted in a higher concentration of total free amino acids, with 2.4 ± 0.1 mg g^{-1} FW (Fig. 4f).

While there was no significant difference in total protein content (Fig. S7b), differences in antioxidant enzyme activity were observed among treatments (Fig. 5).

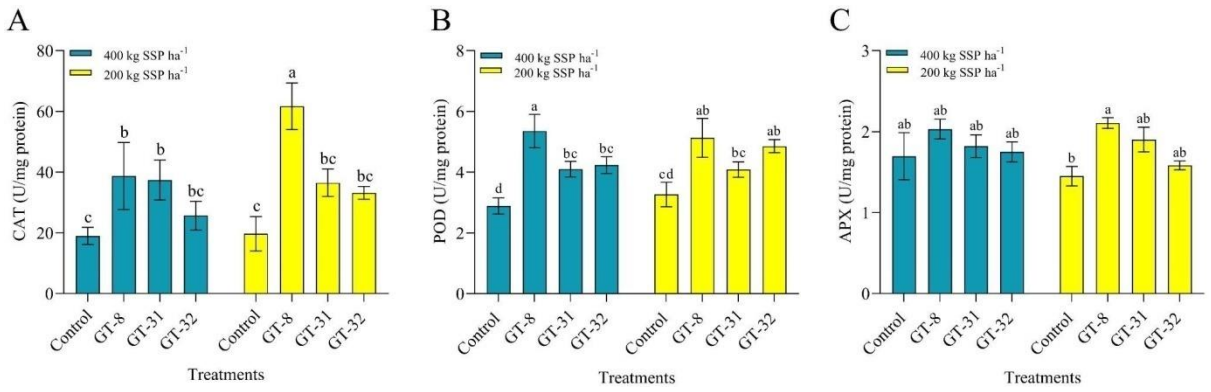


Fig. 5. Effect of *Trichoderma* strains inoculation on the activity of (A) catalase (CAT) (B) peroxidase (POD) (C) and ascorbate peroxidase (APX) in soybean leaves harvested 48 DAS subjected to two phosphate fertilization conditions. Equal letters indicate no statistical difference between treatments according to Tukey's test ($P < 0.05$).

CAT activity was higher in plants received *Trichoderma* inoculation, with the highest activity observed with GT-8 (61.7 U/mg) at 200 kg ha^{-1} of SSP (Fig. 5a). Plants inoculated with GT-8 also showed higher activity of POD (Fig. 5b) and APX (Fig. 5c). For POD, increases of 85.5% and 56.9% were observed compared to the controls at doses of 400 and 200 kg ha^{-1} of SSP, respectively. APX activity was significantly higher at half the fertilizer dose, with 2.1 U/mg protein.

3.4.2. Soil enzymatic activities

The activity of the acid phosphatase showed a significant increase in soils where plants were inoculated with *Trichoderma* (Fig. 6a). For example, GT-32 increased activity by 105.2% compared to the control with 400 kg ha^{-1} of SSP. Similarly, an enhancement in

alkaline phosphatase activity was observed in soils with inoculated plants (Figure 6b). The highest activity was observed for GT-8 (52.4%) at 400 kg ha^{-1} of SSP, with 101.9 µg of *p*-nitrophenol g^{-1} of dry soil h^{-1}. For phytase activity, treatments with GT-31 showed the highest activity values, increasing by 141.7% and 137.5% for 400 and 200 kg ha^{-1} of SSP, respectively (Fig. 6c). About NAD^+ dehydrogenase activity, a significant increase was observed for treatments with GT-31, increasing by 44.74% and 96.5% for 400 and 200 kg ha^{-1} of SSP, respectively (Fig. 6d).

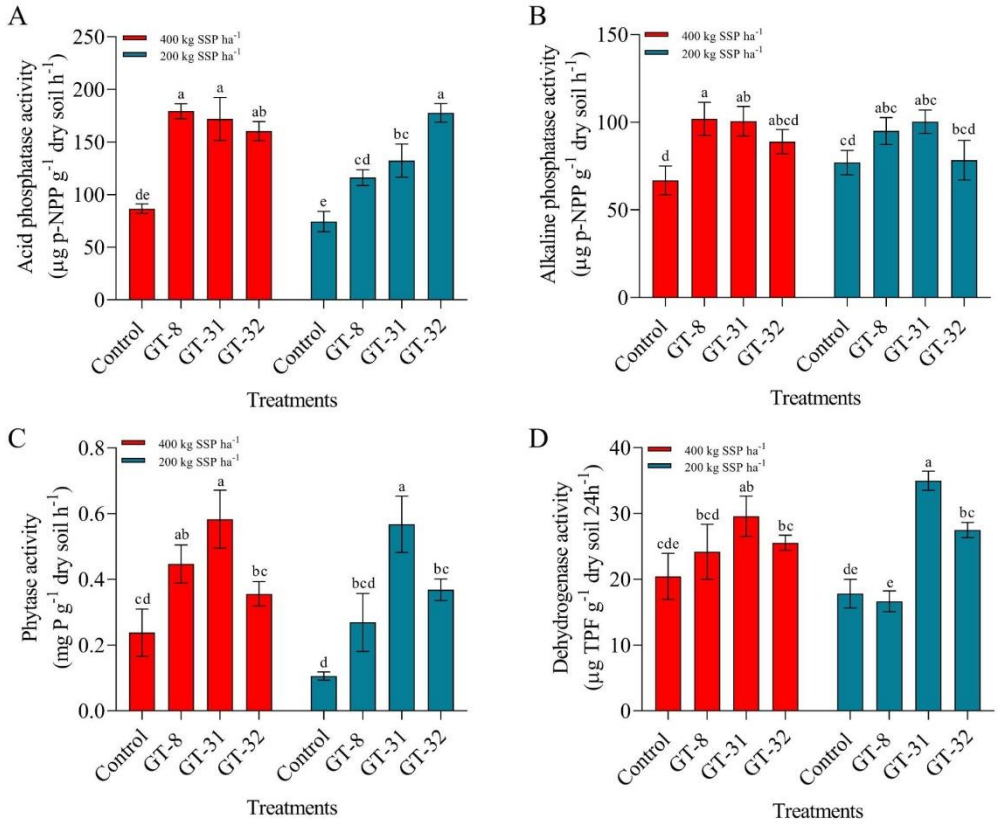


Fig. 6. Activity of (A) acid phosphatase (B) alkaline phosphatase (C) phytase and (D) NAD^+ dehydrogenase in the rhizospheric soil of soybeans inoculated with *Trichoderma* strains and collected 48 DAS and subjected to two phosphate fertilization conditions. Equal letters indicate no statistical difference between treatments according to Tukey's test ($P < 0.05$).

The β-glucosidase assay indicated high activity for the GT-32 treatment at 400 kg ha^{-1} of fertilization, with 273.4 µg of p-nitrophenol g^{-1} dry soil h^{-1} (Fig. 7a). At half the dose, more

significant increases were observed with GT-8 (120.5%) and GT-32 (108.2%) treatments compared to the non-inoculated treatment. Hydrolysis of FDA exhibited a percentage increase in *Trichoderma* treatments (Fig. 7b). GT-31 treatment showed the greatest increase in hydrolysis, with 24.4% at 400 kg ha^{-1}, and 27.3% at 200 kg ha^{-1}, compared to the control at full dose. For amylase, greater significant differences were observed between the treatments using half the dose of SSP (Fig. 7c). The highest activity was observed for soil with GT-8-treated plants, with 23.03 mg glucose g^{-1} dry soil 24 h^{-1}, a 54.5% increase compared to the control. Finally, soil xylanase activity revealed that the presence of GT-31 also led to higher enzyme production at 200 kg ha^{-1} of SSP, with 25.1 mg glucose g^{-1} dry soil 24 h^{-1} (Fig. 7d). At 400 kg ha^{-1} of SSP, xylanase activity for GT-32 was significantly higher at 67.9% compared to the control soil.

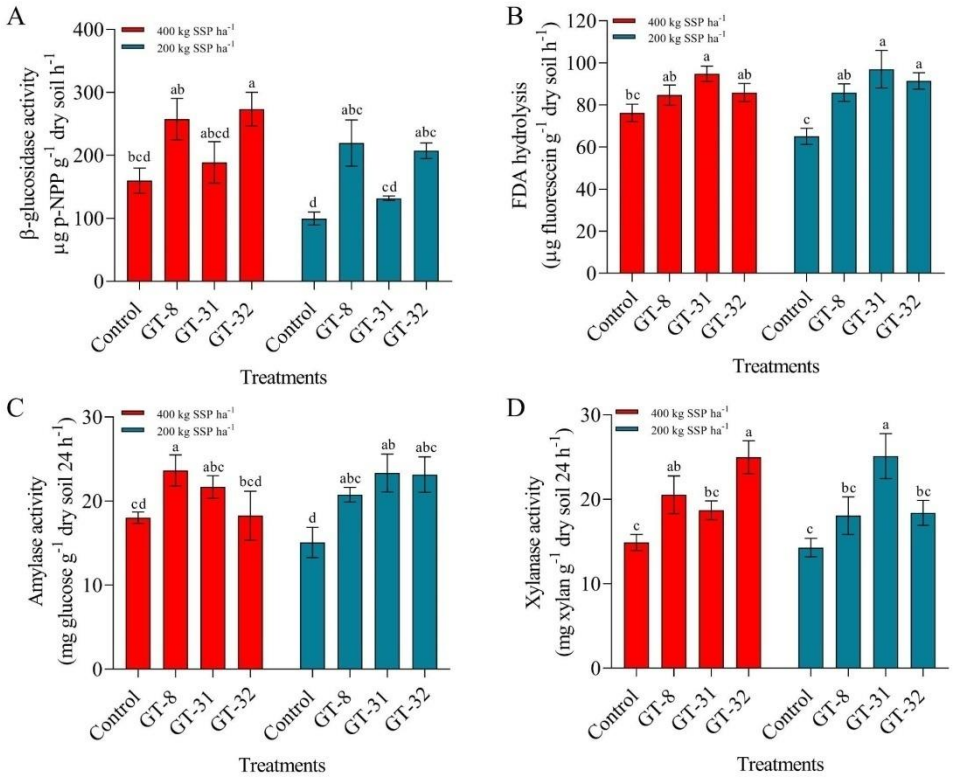


Fig. 7. Activity of (A) β-glucosidase (B) FDA hydrolysis (C) amylase activity and (D) xylanase in the rhizospheric soil of soybeans inoculated with *Trichoderma* strains and collected 48 DAS and subjected to two

phosphate fertilization conditions. Equal letters indicate no statistical difference between the treatments according to Tukey's test ($P < 0.05$).

4. Discussion

Modern agriculture has been seeking alternatives to mitigate the economic and ecological impacts caused by chemical fertilizers, especially phosphates (Abay et al., 2023; Brownlie et al., 2023). Within this context, *Trichoderma* has multiple biochemical properties that play roles in the ecosystem promoting plant growth and making them desirable for formulating biofertilizers (Saldaña-Mendoza et al., 2023). In this study, the *Trichoderma* strains showed mechanisms that trigger beneficial effects on plants. Galeano et al. (2023) obtained these strains from the rhizosphere of soybean plants grown in the state of Mato Grosso do Sul, Brazil, they were selected based on the results of antagonism to phytopathogens, phosphate solubilizing activity, IAA production, and tolerance to different *in vitro* conditions and agrochemicals.

In this study, the *Trichoderma* strains were able to increase the concentration of soluble phosphorus from four phosphate fertilizers tested. The ability to solubilize phosphate has been reported for different *Trichoderma* species (Ikram et al., 2019; Bader et al., 2020; Nykiel-Szymańska et al., 2020; de Andrade Reis et al., 2021). However, these studies investigated this ability using tricalcium phosphate as an insoluble source of phosphorus. The solubilization of phosphate from fertilizers is poorly investigated. In this process, *Trichoderma* secretes organic acids that reduce the pH around the cells and act as chelators of phosphorus-bound cations by their hydroxyl and carboxyl groups, forming complexes with metals (Bononi et al., 2020; Rawat et al., 2021). The reduction in pH observed is also the result of gas exchange (O_2/CO_2) during respiration and proton release (Alori et al., 2017; Rawat et al., 2021). Previously, Galeano et al. (2023) reported the biosynthesis of IAA by *Trichoderma* strains after three days of incubation. In this study, IAA synthesis was

investigated at different incubation times, and the values obtained were higher than those observed by Wang and Zhuang (2019), whose values ranged from 0.8 to 23.4 μg/mL^{-1}, and Bader et al. (2020), with 7.8 to 21.14 μg/mL^{-1}. IAA-producing *Trichoderma* can increase the accumulation of this auxin in roots, helping to improve the development of lateral roots (Woo et al., 2023). Therefore, plants can absorb nutrients and water available in the soil more efficiently by improving root architecture (Saldaña-Mendoza et al., 2023).

Trichoderma also secrete siderophores, low molecular weight molecules that have a high affinity for iron, chelating it from insoluble sources and increasing its availability to plants (Saha et al., 2016; Sood et al., 2020). In addition, siderophores have also been reported to act in the solubilization of phosphate in the soil by chelating iron from Fe-P (Rawat et al., 2021). In this study, the *Trichoderma* strains demonstrated the ability to synthesize siderophores. Several other *Trichoderma* strains have been reported as siderophore producers (Nykiel-Szymańska et al., 2019; Wang e Zhuang, 2019; Syed et al., 2023).

Some *Trichoderma* strains synthesize ACC deaminase, an enzyme that cleaves ACC, the precursor of ethylene in plants, alleviating the deleterious effects of high concentrations of this hormone in stressed plants (Rauf et al., 2021). In this study, all three strains produced the enzyme, with the highest activity observed for GT-8. Other studies have shown that ACC deaminase-producing *Trichoderma* improved the growth of corn (Zhang et al., 2020), soybeans (Zhang et al., 2017), and wheat (Rauf et al., 2021) under environmental stress. Ammonia excretion observed by the strains can be an alternative source of nitrogen and act indirectly on plant growth by suppressing pathogens (Naziya et al., 2020).

In this study, the three strains produced amylase, phosphatase, phytase, protease and xylanase on all the days evaluated. Phosphatases and phytases are directly involved in the P cycle in the soil, acting in the mineralization processes (Rawat et al., 2021). In carbon, sulfur or nitrogen limited conditions, proteases act to recycle proteins in the soil (Tandon et al.,

2018). Amylase and xylanase act in the carbon cycle; xylanases also act as microorganism-associated molecular patterns (MAMPs), activating induced systemic resistance (ISR) against phytopathogens (Guo et al., 2021).

Overall, the three strains have different abilities to promote plant growth and are crucial for the formulation of biofertilizers. Previously, it was reported that these strains were able to promote growth and biochemical responses during the early soybean growth and development (Galeano et al., 2023). In this study, the efficiency of *Trichoderma* strains was evaluated on two soybean cultivars under field conditions (in vivo) and with two doses of phosphate fertilization: 400 kg ha^{-1} (full dose) and 200 kg ha^{-1} (half dose) of SSP. Inoculation of soybean cv. NS6601 IPRO with *Trichoderma* strains affected some variables, such as HFP, GNP and GY. In particular, plants inoculated with GT-32 + 200 kg ha^{-1} showed an increase in GY of 4.3%.

Interestingly, the cv. DM 69IX60RSF 12X RR2 PRO showed the best response to inoculation, affecting all the variables analyzed, with the exception of GNP. Soybean plants inoculated with GT-31 and GT-32 showed significantly higher GY, with increases of 22.7% and 18.6%, respectively, at 200 kg ha^{-1} of SSP. Notably, when compared to the control treatment + 400 kg ha^{-1} of SSP, the inoculation of these strains also provided greater GY. These findings are in line with the study of Senger et al. (2023), who observed increases between 8.9% and 15.1% in GY with *T. asperelloides* inoculation. Chagas Júnior et al. (2021) reported GY gains in soybeans inoculated with *T. asperellum*, with variations of 3.97% and 13.02% between harvests. Previous studies have also reported that the application of *Trichoderma* resulted in increases in plant biomass, such as the shoot dry weight (Yusnawan et al., 2019; Bader et al., 2020; Swain et al., 2021; Chagas Junior et al., 2022).

The use of *Trichoderma* strains also affected the metabolism of soybean plants, increasing the content of chlorophylls, carotenoids, sugars, phenolic compounds, flavonoids

and free amino acids. The plants increased their photosynthetic capacity showing an increase in the content of photosynthetic pigments and, therefore, a greater accumulation of their products, such as carbohydrates (Elkelish et al., 2020). This was evidenced by the higher concentration of sugars in the plants inoculated with *Trichoderma*. Similar results were observed by do Rêgo Meneses et al. (2022) in plants inoculated with *T. asperelloides*. The increase in the content of total free amino acids in plants inoculated with *Trichoderma* was also reported by Rodríguez-Hernández et al. (2023). In the literature, there are reports that flavonoids and other phenolic compounds can help antioxidant responses in plants, avoiding the deleterious effects of ROS (Chen et al., 2019; Elkelish et al. 2020). In response to abiotic or biotic stresses, phenolic compounds absorb and neutralize free radicals, eliminating species such as singlet oxygen and peroxides (Shah and Smith, 2020; Yusnawan et al., 2021).

In agricultural ecosystems, plants are exposed to various environmental adversities and respond to these stresses by producing secondary metabolites and oxidative scavenging enzymes (Seleiman et al., 2021). In this study, we observed higher percentage enzyme activity of catalase, peroxidase and ascorbate peroxidase in plants inoculated with *Trichoderma* strains. It is important to mention that the soybean plants faced a period of drought and, probably, the inoculation with *Trichoderma* increased the antioxidant responses to overcome oxidative damage. Scudeletti et al. (2021) observed an increase in the activity of antioxidant enzymes in sugarcane plants under drought stress and inoculated with *T. asperellum*. Khomari et al. (2017) reported an increase in catalase and peroxidase activity in soybean inoculated with *T. harzianum* under salt stress.

When applied to seeds, *Trichoderma* propagules, germinate and colonize the plant roots and/or rhizosphere (Rodrigues et al., 2023). In the rhizospheric soil, *Trichoderma* secretes various enzymes that act on the soil's biogeochemical processes, increasing the nutrients available to plants and improving soil fertility (Teng et al., 2015; Mao e Jiang, 2021;

Rawat et al., 2021). Moreover, this enzyme pool also acts to suppress phytopathogens around the roots (Woo et al., 2023). In this study, it was observed that rhizospheric soils with plants inoculated with *Trichoderma* showed an increase, at different levels, in the activity of phosphatase, phytase, dehydrogenase, amylase, xylanase, FDA hydrolysis and β-glucosidase. These findings indicate that inoculation with *Trichoderma* not only improves the physiological and biochemical responses of soybeans, but also the microbial activity of the soil by increasing the solubilization and transfer of nutrients. In the soil, phosphatases act to mineralize phosphorus from organic matter (Rawat et al., 2021). Bononi et al. (2020) also reported an increase in the activity of these enzymes in soybean soils inoculated with *Trichoderma*.

Phytic acid of plant origin is the most abundant form of organic P in the soil, and phytases cleave this compound releasing inositol and inorganic phosphorus (Rawat et al., 2021). The findings of this study suggest that the strains can increase the mineralization of P in the soil (Bononi et al., 2020; Rizwanuddin et al., 2023). The activity of dehydrogenases can be used as an indicator of soil quality, whose activity occurs only in living cells, making it possible to measure total microbial activity (Chaudhary et al., 2021). In this study, the increase in the activity of this enzyme may suggest that the presence of strains increased the metabolic activities of the soil microbial community (Chaudhary et al., 2022). Recently, Mao and Jiang (2021) reported that the use of *T. hamatum* in soil cultivated with pepper also led to an increase in dehydrogenase activity.

The activities of proteases, lipases and esterases can be measured by the hydrolysis of FDA. In this study, soils with GT-31 and GT-32 showed higher activity values, which indicated greater microbial activity (Chaudhary et al., 2021). Similarly, Teng et al. (2015) reported increased hydrolysis of FDA in soil inoculated with *T. reesei*. β-glucosidase, amylase, and xylanase are involved in the C cycle, aiding in the biogeochemical cycle of the

soil. In this study, the use of *Trichoderma* stimulated the activity of these enzymes in both fertilization conditions. These findings suggest a higher rate of decomposition of, for example, cellulose and hemicellulose in the soil by β-glucosidases and xylanases, as well as indicates greater microbial activity in the soil (McBride et al., 2020; Chaudhary et al., 2022).

This study showed that the *Trichoderma* strains have multiple ecological functions that directly and indirectly promote plant growth. Notably, using half of the dose of SPP with GT-31 and GT-32 proved to be the most promising and sustainable strategy for obtaining higher grain yields. Inoculation also affected the biochemical responses of the plants in both fertilization conditions. These results indicate the high potential of these strains for promoting soybean growth. In addition, the use of these strains seems to be a good strategy for improving the fertility and health of agricultural soils. The hypothesis of this study is that the traits for plant growth promotion observed by the strains in the laboratory were expressed in the field. For example, when considering treatments with half the dose of fertilizer (200 kg ha^{-1}), plants inoculated with strains GT-31 and GT-32 may have had increased access to phosphorus through the solubilization activity of both the phosphate present in the soil and the fertilizer. During the association with these strains, it is likely that IAA accumulated in plant tissues, forming more robust roots and, consequently, increasing the absorption not only of phosphorus but also of other nutrients and water.

In this field-scale study, conidial suspensions in CMC were inoculated directly onto soybean seeds. In the future, other formulations for delivering the propagules and different application methods will be evaluated to determine the performance of these strains. Furthermore, studies in different regions should be carried out to verify the efficiency of these strains in other soil and climatic conditions. This study has provided valuable information on the practical application and efficiency of the GT-31 and GT-32 strains as a possible

sustainable and environmentally friendly approach to agricultural management aimed at less fertilizer application.

5. Conclusion

This study demonstrated the potential of new *Trichoderma* strains for recommendation as biofertilizers. The strains showed multiple plant growth-promoting abilities, such as phosphate solubilization from fertilizers, synthesis of IAA, siderophores, ACC deaminase, and ammonia. Inoculation of the strains affected many variables in the field experiment using two soybean cultivars and two fertilization conditions. Treatments with the *T. reesei* GT-31 and *T. longibrachiatum* GT-32 provided higher yields in cv. DM 69IX60RSF 12X RR2 PRO at 200 kg ha^{-1} of SSP. There was also greater microbial activity in the soil, demonstrating the potential of these strains to minimize the use of fertilizers in the field as well as improving soil health and fertility.

Credit author statement

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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New phosphorus-solubilizing *Trichoderma* strains: Mechanisms to promote the growth of soybean plants and agroecosystem sustainability strategies

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Supplementary Information

Table S1 Information on the assays conducted with enzymes from three strains of *Trichoderma*.

Enzyme	Substrate	Enzyme assay conditions
Amylase	Sodium acetate buffer 50 mM (pH 5.0) with starch (1% w/v)	Incubation time: 10 min at 55 °C Reaction stopped with DNS reagent
Protease	Sodium phosphate buffer 50 mM (pH 6.5) with casein (1% w/v)	Incubation time: 10 min at 50 °C Reaction stopped with trichloroacetic acid (10% w/v)
Xylanase	Sodium acetate buffer 50 mM (pH 5.0) with xylan (1% w/v)	Incubation time: 10 min at 55 °C Reaction stopped with DNS reagent
Phosphatase	Sodium acetate buffer 50 mM (pH 4.8) with *p*-nitrophenyl phosphate 5 mM	Incubation time: 10 min at 37 °C Reaction stopped with NaOH 0.1 M
Phytase	Sodium acetate buffer 100 mM (pH 5.0) with phytic acid 10 mM	Incubation time: 30 min at 50 °C Reaction stopped with a solution of ammonium molybdate, sulfuric acid, acetone (1:1:2), and citric acid

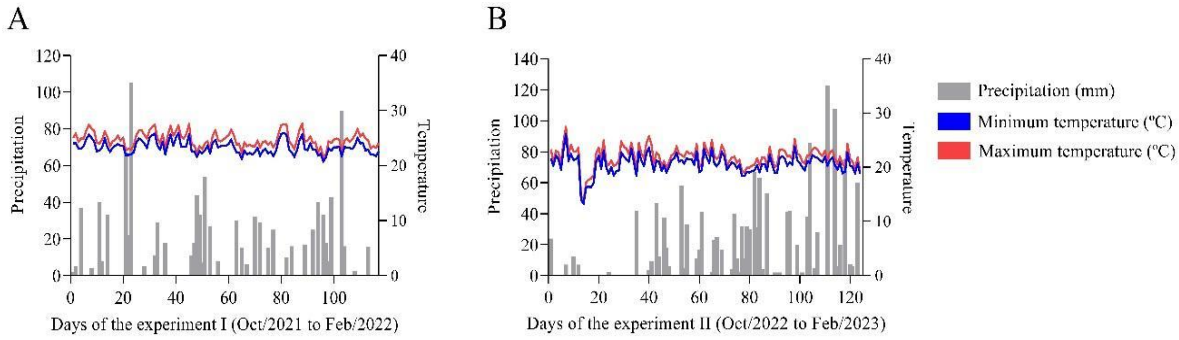

Fig. S1 Precipitation (mm) and temperatures (°C) in the experimental area during the experimental periods (a) Experiment I (b) Experiment II.

Table S2 Chemical and physical characteristics of the soil used in Experiment I and II of the first (2021/2022) and second harvest (2022/2023) in the experimental area of the Chapadão do Sul Campus, UFMS, MS, Brazil.

Parameter	Mean value	
	Experiment I	Experiment II
pH in $CaCl_2$	5.0	5.0
Organic matter (g dm^{-3})	24.5	26.6

Clay (%)	49.5	49.5
Silt (%)	5	5
Sand (%)	45.5	45.5
P available (mg dm^{-3})	13.0	15.9
K (mg dm^{-3})	101	74
Ca ($cmol_c$ dm^{-3})	3.4	3.4
Mg ($cmol_c$ dm^{-3})	1.3	1.3
H^+ + Al ($cmol_c$ dm^{-3})	4.5	4.3
Fe (mg dm^{-3})	59	54
Mn (mg dm^{-3})	15.2	14.4
Zn (mg dm^{-3})	5.1	4.1

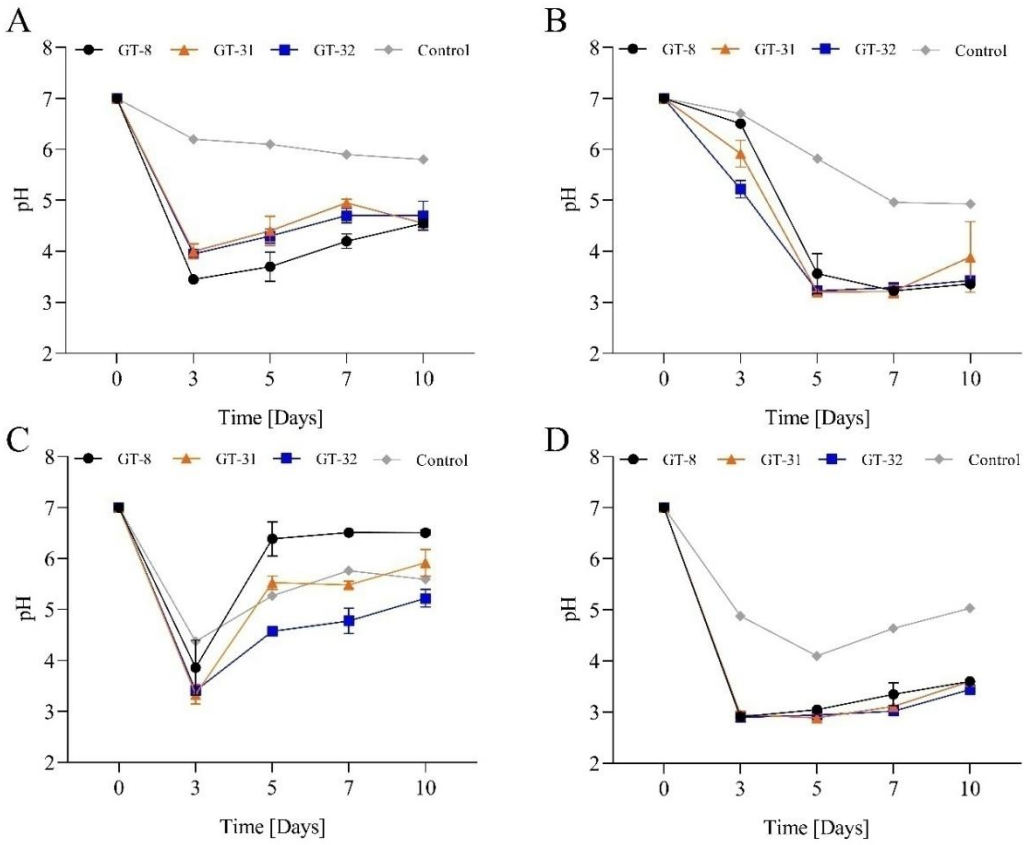

Fig. S2 pH values of culture filtrates of *Trichoderma* strains grown in PVK medium with different phosphate fertilizers after 3, 5, 7, and 10 days of incubation (a) Thin thermo phosphate (b) Granulated thermo phosphate (c) MV27 (d) Simplex strong thermo phosphate. Line bars represent the standard errors of means.

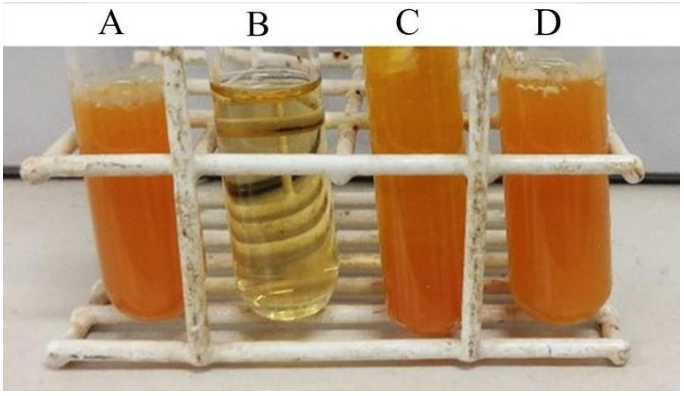

Fig. S3 Ammonia production by *Trichoderma* strains. The formation of a brownish color after the addition of Nessler's reagent indicated positive. (a) *T. viride* GT-8 (b) Control without inoculation (c) *T. reesei* GT-31 (d) *T. longibrachiatum* GT-32

Table S3. ANOVA of growth parameters and productivity of soybean (Nidera NS6601 IPRO) inoculated with *Trichoderma* strains under two phosphate fertilization conditions (400 and 200 kg ha^{-1}).

SV	DF	PH	HFP	NBP	NPP	GNP
Block	3	1.9617	2.1083	0.3458	0.7267	0.0095
Fertilizer (F)	1	85.8050**	2.0000^{ns}	0.0012^{ns}	21.7800*	0.0078^{ns}
Isolate (I)	3	1.9650^{ns}	5.8083**	0.1142^{ns}	25.6633**	0.0661*
F x I	3	5.7250^{ns}	3.6367**	0.1087^{ns}	19.2700**	0.0128^{ns}
Error	21	2.4569	0.7036	0.0594	3.7467	0.0147
CV (%)	-	1.94	5.14	5.56	4.63	4.06
Mean	-	80.74	16.31	4.39	41.85	2.98
SV	DF	NGP	MG	M1000	GY	
Block	3	1.9567	0.1172	11.1907	34968.3695	
Fertilizer (F)	1	298.2903*	9.1220*	12.0295^{ns}	1963.5556^{ns}	
Isolate (I)	3	613.1289**	14.7240**	1.9149^{ns}	24261.7033^{ns}	
F x I	3	179.9392^{ns}	4.4405*	6.1743^{ns}	51287.26018*	
Error	21	58.9275	1.3177	3.8439	13342.7618	
CV (%)	-	6.14	6.12	1.31	2.81	
Mean	-	125.01	18.77	150.10	4106.58	

SV – Source of variation, DF – Degrees of freedom, CV – Coefficient of variation

ns = not significant; * = significant at 5%; ** = significant at 1% by Scott Knott test

Plant height (PH), height of first pod insertion (HFP), number of branches per plant (NBP), number of pods per plant (NPP), grains number per pods (GNP), number of grains per plant (NGP), mass of grains per plant (MG), mass of one thousand grains (M1000), grain yield (GY)

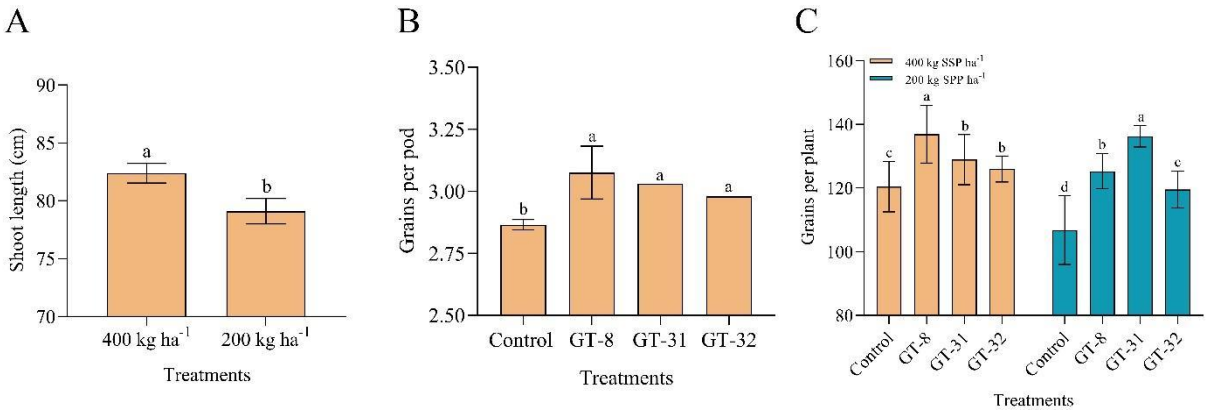

Fig. S4 Effect of inoculation of *Trichoderma* strains on soybean plants (Nidera NS6601 IPRO) under two phosphate fertilization conditions (a) Shoot length (b) Grains per pod (c) Grains per plant. Line bars represent standard errors of the means obtained (n = 5 per block). Equal letters indicate that there is no statistical difference between treatments according to the Scott-Knott test (P < 0.05)

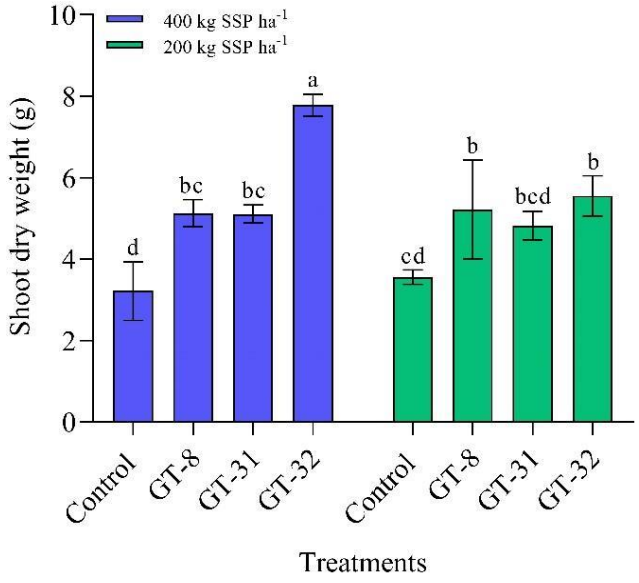

Fig. S5 Effect of inoculation of *Trichoderma* strains on the shoot dry weight of soybean plants harvested at 48 DAS under two phosphate fertilization conditions. Equal letters indicate that there is no statistical difference between treatments according to the Tukey test (P < 0.05)

Table S4 ANOVA of growth parameters and productivity of soybean (DM 69IX60RSF 12X RR2 PRO) inoculated with *Trichoderma* strains under two phosphate fertilization conditions (400 and 200 kg ha^{-1}).

SV	DF	PH	HFP	NBP	NPP	GNP
Block	3	4.9683	0.2803	0.0900	0.2852	0.0128
Fertilizer (F)	1	162.0000**	0.1953^{ns}	0.6050*	60.5000**	0.0020^{ns}
Isolate (I)	3	66.8483**	7.1045**	0.3900*	94.9100**	0.0123^{ns}
F x I	3	38.8567**	3.8878**	1.7217**	56.7403**	0.0046^{ns}
Error	21	4.4217	0.4667	0.0824	6.9708	0.0094
CV (%)	-	2.34	6.20	3.97	5.05	3.21
Mean	-	89.69	11.02	7.23	52.23	3.02
SV	DF	MG	M1000	GY	GYB	
Block	3	1.6982	7.4852	8903.3333	2.4732	
Fertilizer (F)	1	0.0045^{ns}	0.3584^{ns}	153458.0000*	42.6272*	
Isolate (I)	3	28.1223**	55.6350**	520194.0000**	144.4984**	
F x I	3	22.4373**	11.0758^{ns}	444060.6667**	123.3502**	
Error	21	0.4101	4.8048	23784.8571	6.6069	
CV (%)	-	2.78	1.64	3.30	3.30	
Mean	-	23.05	133.65	4671.75	77.86	

SV – Source of variation, DF – Degrees of freedom, CV – Coefficient of variation

ns = not significant; * = significant at 5%; ** = significant at 1% by Scott Knott test

Plant height (PH), height of first pod insertion (HFP), number of branches per plant (NBP), number of pods per plant (NPP), grains number per pods (GNP), mass of grains per plant (MG), mass of one thousand grains (M1000), grain yield (GY), and grain yield in bags ha^{-1} (GYB)

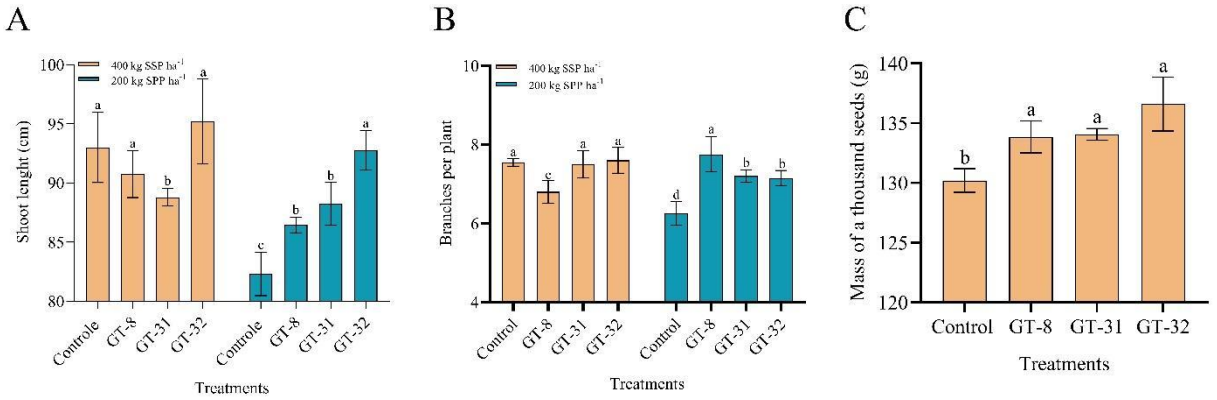

Fig. S6 Effect of inoculation of *Trichoderma* strains on soybean plants (DM 69IX60RSF 12X RR2 PRO) under two phosphate fertilization conditions (a) Shoot length (b) Branches per plant (c) Mass of a thousand seeds. Line bars represent standard errors of the means obtained (n = 5 per block). Equal letters indicate that there is no statistical difference between treatments according to the Scott-Knott test ($P < 0.05$)

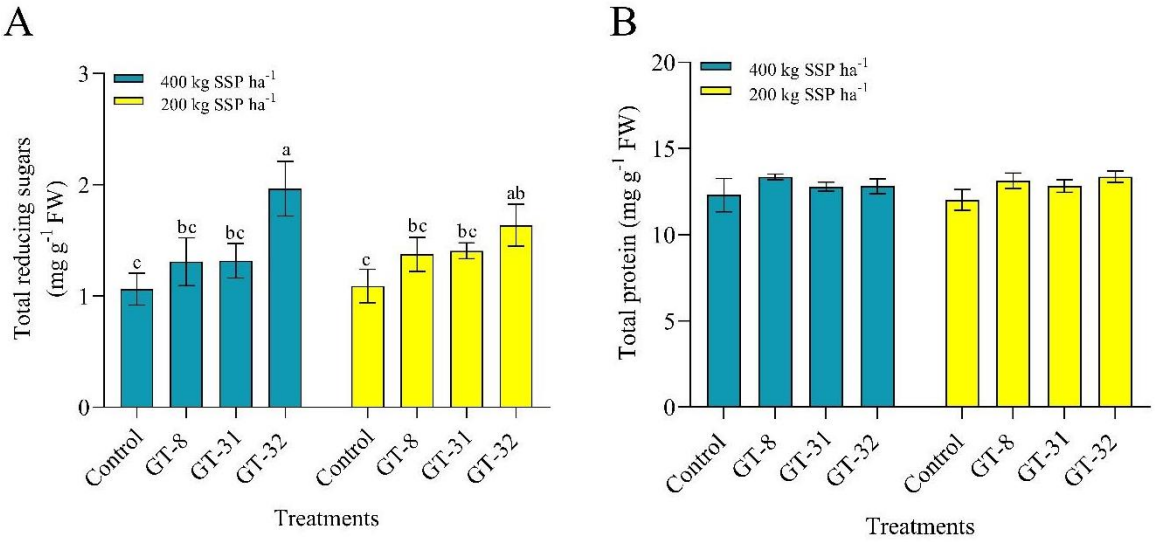

Fig. S7 Effect of inoculation of *Trichoderma* strains on (a) Total reducing sugars (b) and Total proteins in soybean leaves harvested 48 DAS under two phosphate fertilization conditions. Equal letters indicate that there is no statistical difference between treatments according to the Tukey test ($P < 0.05$)

6. CONCLUSÃO

- Este estudo destaca o potencial de três cepas de *Trichoderma* (*T. viride* GT-8, *T. reesei* GT-31 e *T. longibrachiatum* GT-32) como agentes de biocontrole e para a promoção do crescimento da soja;
- As cepas apresentaram múltiplas capacidades de promoção do crescimento das plantas, como solubilização de fosfato de fertilizantes, síntese de IAA, sideróforos, ACC desaminase e amônia;
- A capacidade dessas cepas de tolerar diversas condições e crescer na presença de agroquímicos mostou o potencial para bioformulações;
- Tanto o uso individual quanto combinado dessas cepasapresentaram resultados promissores no aumento do crescimento e nas respostas bioquímicas durante o desenvolvimento inicial de mudas de soja;
- A inoculação das cepas de *Trichoderma* afetou diversas variáveis no experimento de campo utilizando duas cultivares de soja e duas condições de fertilização fosfatada;
- Os tratamentos com GT-31 e GT-32 proporcionaram maiores valores de produtividade de grãos na cv. DM 69IX60RSF 12X RR2 PRO em 200 kg ha^{-1} de SSP;
- Houve também maior atividade microbiana no solo, demonstrando o potencial destas cepas para minimizar o uso de fertilizantes no campo, bem como melhorar a saúde e a fertilidade do solo.

O estudo sugere que essas cepas são promissoras para uso em bioformulações para agricultura sustentável, apoiando práticas ecologicamente corretas no Cerrado brasileiro.

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ANEXO 1

Ministério do Meio Ambiente
CONSELHO DE GESTÃO DO PATRIMÔNIO GENÉTICO
SISTEMA NACIONAL DE GESTÃO DO PATRIMÔNIO GENÉTICO E DO CONHECIMENTO TRADICIONAL ASSOCIADO

Comprovante de Cadastro de Acesso

Cadastro nº AE5129A

A atividade de acesso ao Patrimônio Genético, nos termos abaixo resumida, foi cadastrada no SisGen, em atendimento ao previsto na Lei nº 13.123/2015 e seus regulamentos.

Número do cadastro:	**AE5129A**
Usuário:	**UFMS**
CPF/CNPJ:	**15.461.510/0001-33**
Objeto do Acesso:	**Patrimônio Genético**
Finalidade do Acesso:	**Pesquisa**

Espécie

Trichoderma longibrachiatum
Trichoderma viride
Trichoderma reesei
Trichoderma sp

Título da Atividade:	**Fungos filamentosos do gênero Trichoderma isolados do solo e região rizosférica da soja com potencial para biocontrole e promoção de crescimento vegetal**

Equipe

FABIANA FONSECA ZANOELO	**UFMS**
Rodrigo Mattos Silva Galeano	**UFMS**
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Nathalia Nunes Glienke	UFMS
João Victor Souza Ribeiro	UFMS
Isabela Pavão Vargas	UFMS
Izabella Marques de Assis	UFMS
Hellen Natália Cardoso Malheiro	UFMS

Data do Cadastro: **02/02/2024 12:41:03**

Situação do Cadastro: **Concluído**

Conselho de Gestão do Patrimônio Genético

Situação cadastral conforme consulta ao SisGen em **12:41** de **02/02/2024**.

SISTEMA NACIONAL DE GESTÃO DO PATRIMÔNIO GENÉTICO E DO CONHECIMENTO TRADICIONAL ASSOCIADO - **SISGEN**