HELBERT ROCHA REIS

INTERAÇÕES ECOLÓGICAS E NUTRICIONAIS ENTRE *Talaromyces aculeatus* E O EUCALIPTO

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Microbiologia Agrícola, para obtenção do título de *Doctor Scientiae*.

Orientador: Maurício Dutra Costa

Coorientadores: Marisa Vieira de Queiroz
Mateus Ferreira Santana

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Dedico à minha mãe, Norma, pelo suporte incondicional e constante torcida.

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RESUMO

REIS, Helbert Rocha, D.Sc., Universidade Federal de Viçosa, agosto de 2022. **Interações ecológicas e nutricionais entre *Talaromyces aculeatus* e o eucalipto**. Orientador: Maurício Dutra Costa. Coorientadores: Marisa Vieira de Queiroz e Mateus Ferreira Santana.

O uso intensivo de fertilizantes e pesticidas em plantios representa elevado custo para as atividades agrícolas e florestais, além de causar danos aos ecossistemas. Diante dessa realidade, alternativas capazes de amenizar problemas de fertilidade do solo e o impacto de fitopatógenos, ao mesmo tempo que aumentam a produtividade e diminuem os efeitos indesejáveis ao ambiente, representam mercado promissor e em constate crescimento. Os fungos promotores de crescimento de planta (PGPF – Plant Growth Promoting Fungi) são capazes de beneficiar os plantios ao estimular as repostas das plantas à estresses bióticos e abióticos, aumentar a eficiência de acesso e uso de nutrientes, estimular o crescimento e desenvolvimento vegetal, melhorar a qualidade do solo, dentre outros meios. O fungo *Talaromyces aculetaus* apresenta grande potencial para o desenvolvimento de tecnologias aplicáveis nos cultivos, considerando sua versatilidade metabólica, que o capacita a produzir grande variedade de compostos e enzimas, além de apresentar crescimento vigoroso e esporulação eficiente, características desejáveis para bioinoculantes. Apesar do fato de que inúmeras espécies de PGPF já tiveram os mecanismos de promoção de crescimento caracterizados, aspectos importantes das interações entre fungos e plantas ainda não foram devidamente elucidados. O presente trabalho tem o objetivo de entender a capacidade de *T. aculeatus* de promover crescimento de diferentes espécies de eucalipto, assim como investigar a influência da fertilidade do solo nos resultados dessas interações. O primeiro capítulo resume as informações disponíveis relacionadas com os aspectos do estabelecimento das interações e a influência da disponibilidade de nutrientes no solo sobre a promoção de crescimento. O segundo capítulo explora como a disponibilidade de fósforo pode afetar a interação entre *T. aculeatus* e *E. grandis*. As plantas foram submetidas a cinco diferentes dosagens de fósforo, entre 35 e 290 mg kg^{-1} de P no solo. Os resultados demonstraram que o efeito da inoculação do fungo foi concentrado nas doses mais baixas de P, especialmente 70 e 140 mg kg^{-1}, sugerindo que nesse intervalo a planta é mais suscetível à colonização do fungo. Houve aumentos de até 260 % na matéria seca e até 808 % no conteúdo de nutrientes das plantas de *E. grandis* inoculadas. O fungo *T. aculeatus* também teve comprovada sua

capacidade de produção de ácido indolacético, síntese de sideróforos e solubilização de diferentes fontes de fosfato. Os dados sugerem que a disponibilidade de fósforo possui grande influência no estabelecimento da interação entre *T. aculeatus* e *E. grandis*, e que essa interação é mais eficiente quando o P é limitante no solo. O terceiro capítulo demonstra os efeitos da inoculação de *Talaromyces aculeatus* em seis espécies diferentes de eucalipto: *Eucalyptus grandis, Eucalyptus urophylla, Eucalyptus urograndis, Eucalyptus saligna, Eucalyptus cloeziana* e *Corymbia torelliana.* As espécies *E. grandis* e *E. urograndis* foram as mais beneficiadas pela inoculação do fungo, principalmente pela maior aquisição de nutrientes por *E. grandis* e incremento de matéria seca de 32,21 % por *E. urograndis*. As espécies *E. urophylla* e *C. torelliana* manifestaram aumentos na matéria seca e aquisição de nutrientes especialmente nas raízes. Por fim, *E. saligna* e *E. cloeziana*, apesar de não apresentarem incrementos na matéria seca, exibiram aumentos na aquisição de nutrientes.

Palavras-chave: Fungos rizosféricos. Promoção de crescimento. Nutrição. Fósforo.

ABSTRACT

REIS, Helbert Rocha, D.Sc., Universidade Federal de Viçosa, August, 2022. **Ecological and nutritional interactions between *Talaromyces aculeatus* and eucalypt**. Adviser: Maurício Dutra Costa. Co-advisers: Marisa Vieira de Queiroz and Mateus Ferreira Santana.

The use of fertilizers and pesticides in plantations represents great cost to agricultural and forestry activities, in addition to causing damage to ecosystems. Given this reality, alternatives capable of alleviating soil fertility problems and the impact of phytopathogens, while increasing productivity and reducing undesirable effects on the environment, have represented a promising and constantly growing market. Plant growth promoting fungi (PGPF) are able to benefit crops by stimulating plant responses to biotic and abiotic stresses, increasing access and use of nutrients, stimulating plant growth, improving soil quality, among others. The fungus *Talaromyces aculetaus* offers great potential for the development of technologies applicable to crops due to its metabolical versatility, this fungus produces a wide variety of compounds and enzymes, in addition to presenting vigorous growth and efficient sporulation, desirable characteristics for bioinoculants. Despite the fact that numerous species of PGPF have already had their growth promotion mechanisms characterized, important aspects of the interactions between fungi and plants have not been properly elucidated. The present work aims at understanding the ability of *T. aculeatus* to promote growth of different eucalyptus species, as well as to investigate the influence of soil fertility on the results of the interaction. The first chapter summarizes the available information related to fungal plant interactions and the influence of soil nutrient availability on growth promotion. The second chapter explores how phosphorus availability can affect the interaction between *T. aculeatus* and *E. grandis*. The plants were submitted to five different levels of phosphorus, between 35 and 290 mg kg^{-1} of P in the soil. The results showed that the fungal inoculation effect was concentrated at the lowest P doses, especially 70 and 140 mg kg^{-1}, suggesting that in this interval the plant is more susceptible to fungus colonization. There were increases of up to 260% in dry matter and up to 808% in the nutrient content of *E. grandis* inoculated with *T. aculeatus*. The fungus *T. aculeatus* was also proven to produce indoleacetic acid, synthesize siderophores and solubilize different sources of phosphate. The data suggest that phosphorus availability has a great influence on the establishment of the interaction between *T. aculeatus* and *E. grandis*, and that this interaction is considerably more efficient when P is limiting in the soil. The third chapter demonstrates the effects of

inoculation of *Talaromyces aculeatus* on six different eucalypt species: *Eucalyptus grandis, Eucalyptus urophylla, Eucalyptus urograndis, Eucalyptus saligna, Eucalyptus cloeziana* and *Corymbia torelliana*. The species *E. grandis* and *E. urograndis* were the most benefited by the fungus inoculation, especially in the acquisition of nutrients by *E. grandis* and by increases in dry matter of 32.21 % to *E. urograndis*. The species *E. urophylla* and *C. torelliana* showed increases in dry matter and nutrient acquisition, especially on roots. Finally, *E. saligna* and *E. cloeziana*, despite not showing increases in dry matter, exhibited increases in the acquisition of nutrients.

Keywords: Rhizospheric fungi. Growth promotion. Nutrition. Phosphor.

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INTRODUÇÃO GERAL

O plantio de eucalipto representa a maior parcela de cultivo florestal em diversas partes do mundo, inclusive no Brasil. Devido à grande versatilidade de uso, variedade de espécies, alta adaptabilidade e produtividade, o eucalipto possui posição de destaque na economia mundial.

No Brasil, o cultivo de eucalipto concentra-se especialmente nos estados de Minas Gerais, São Paulo e Mato Grosso do Sul, alcançando a marca de 9,55 milhões de hectares plantados. A madeira proveniente desse cultivo é destinada à fabricação de diversos produtos com forte apelo mercadológico, como celulose, painéis de madeira, papel e carvão.

No ano de 2020, os produtos madeireiros, como pisos e painéis de madeira, celulose, papel, madeira serrada e carvão vegetal, renderam receita bruta para o país de 116,6 bilhões de reais, o que representa crescimento de 17,6 % em comparação com o ano de 2019. O setor destaca-se nos dados econômicos nacionais, representando média de 1,0 % do PIB e se coloca na posição 22, dentre 50 atividades mais importantes para a economia nacional. O segmento também é destaque no exterior, representando 4,8 % de participação de produtos da cadeia no total de exportações. O Brasil alcançou desempenho recorde, estabelecendo-se como o maior exportador de celulose no comércio mundial desde 2018.

Os plantios de eucalipto, assim como qualquer outro cultivo, sofrem perdas consideráveis decorrentes das limitações nutricionais dos solos e pela ação de patógenos. Esse panorama induz maior requerimento por substâncias químicas nos cultivos, como pesticidas e fertilizantes, que além de representarem investimentos consideráveis, também podem ameaçar o equilíbrio das regiões de plantio e ecossistemas adjacentes. Considerando o papel crucial que a microbiota desempenha nos processos do solo e desenvolvimento vegetal, o emprego de microrganismos apresenta-se como promissora ferramenta para a redução da aplicação de insumos agrícolas nos plantios.

Os microrganismos do solo atuam pelos mais diversos mecanismos de promoção de crescimento vegetal, que incluem produção de fitormônios, solubilização de nutrientes, indução da resistência sistêmica, síntese de sideróforos, dentre outros. Tais mecanismos beneficiam o desenvolvimento vegetal ao exercer funções importantes no estabelecimento e manutenção das interações, no crescimento vegetal, na descontaminação de solos, na supressão de doenças, nas respostas ao estresse e na aquisição de nutrientes.

Os fungos promotores de crescimento de planta (PGPF) ocupam posição de destaque dentre esses microrganismos por desempenharem papel central nos processos do solo, como a ciclagem de nutrientes, degradação da matéria orgânica e supressão de patógenos. A microbiota do solo sofre forte influência de fatores bióticos e abióticos, o que inclui a dinâmica da complexa rede de comunicação na rizosfera, tanto entre plantas e microrganismos, quanto entre os próprios microrganismos, assim como as características físico-químicas dos solos.

O fungo utilizado nesse estudo foi isolado em 2016 da rizosfera de *Eucalyptus grandis*, no setor de Silvicultura da Universidade Federal de Viçosa, Minas Gerais. O isolamento foi parte do projeto de mestrado intitulado "Isolamento e caracterização de fungos promotores de crescimento da rizosfera de eucalipto", defendido em 2018. O isolado teve suas capacidades de promoção de crescimento de *E. grandis* testadas e foi identificado como *Talaromyces aculeatus* por meio de técnicas moleculares, utilizando as regiões ITS, Beta-tubulina e RPB2.

O potencial metabólico de *Talaromyces aculeatus* é bastante vasto, sendo a espécie capaz de produzir inúmeras enzimas de interesse comercial, como dextranases, beta-glicosidades e quitinase. Além disso, estudos também exploraram sua capacidade de produzir diversos metabólitos, incluindo compostos antimicrobianos e até substâncias citotóxicas. Quanto a promoção de crescimento, o fungo já teve sua capacidade demonstrada para palmeira (oil palm) e sorgo. Os mecanismos empregados pelo fungo incluem produção de ácido indolacético, fitases, sideróforos e fosfatases.

Essa tese elucidou aspectos das interações estabelecidas pelo fungo promotor de crescimento *T. aculeatus,* avaliando os mecanismos empregados na promoção de crescimento vegetal, sua capacidade de promover crescimento de diferentes espécies de eucalipto e a influência das condições nutricionais do solo, particularmente diferentes dosagens de fósforo, no estabelecimento das interações com *Eucalyptus grandis.*

CAPÍTULO 1:
INFLUÊNCIAS ECOLÓGICAS E NUTRICIONAIS NA PROMOÇÃO DE CRESCIMENTO DE PLANTAS MEDIADA POR FUNGOS

Rizomicrobioma

A rizosfera é definida como a região que sofre influência direta das raízes das plantas e apresenta alta atividade microbiana graças à deposição de exsudados radiculares que são disponibilizados para microbiota do solo. A grande oferta de recursos na rizosfera faz com que ela seja um nicho extremamente cobiçado pelas comunidades microbianas, onde elas competem pelos nutrientes e algumas naturalmente irão se estabelecer com maior sucesso que outras (McNear Jr., 2013).

A rizosfera não é um espaço precisamente delimitado, manifestando-se realmente como um gradiente químico, físico e biológico próximo as raízes das plantas (McNear Jr., 2013). A região que compreende a rizosfera pode ainda ser dividida em três zonas: endorizosfera, rizoplano e ectorizosfera. A endorizosfera é formada por partes do córtex e endoderme, onde os microrganismos podem ocupar a região apoplástica. O rizoplano inclui a epiderme e mucilagem, enquanto a ectorizosfera se estende do rizoplano até o solo não rizosférico (McNear Jr., 2013).

Uma das estratégias de resposta ao estresse empregada pelas plantas é modificações da morfologia da raiz, alterando as características químicas da rizosfera e recrutando microrganismos que potencialmente concederão benefícios a elas naquelas condições (McNear Jr., 2013). Os microrganismos por sua vez liberam vitaminas, aminoácidos, fitormônios, ácidos orgânicos, compostos antimicrobianos, enzimas líticas, dentre outros, impactando de forma significativa populações de outros microrganismos, aspectos fisiológicos da planta e características químicas e físicas do solo (Naik et al., 2019).

Os efeitos de determinadas classes de metabólitos secundários podem ser críticos nas dinâmicas de respostas ao estresse mediada por microrganismos da rizosfera e sua produção pode ser amplificada durante esses eventos. Apigenina e flaxetina, por exemplo, atuam como metabólitos chave ao impactarem as comunidades microbianas da rizosfera, recrutando microrganismos que auxiliam as plantas na aquisição de nitrogênio e ferro, respectivamente (Hong et al., 2021).

Portanto, as flutuações na estrutura e composição das comunidades microbianas podem resultar em impactos significativos para a dinâmica do solo e para o desenvolvimento vegetal, como por exemplo, no estabelecimento de interações, respostas às condições de estresse e na competição de plantas (Sasse et al., 2018; Prabha et al., 2019; Jamil et al., 2022).

Fungos Promotores de Crescimento de Planta

Os fungos possuem grande importância na qualidade do solo e desenvolvimento vegetal, justamente por exercerem papéis cruciais nos processos vinculados a eles, tais como decomposição de material orgânico, ciclagem de nutrientes, estruturação das partículas e supressão de patógenos (Yedidia et al., 2001; Singh et al., 2006; Dini-Andreote and Raaijmakers, 2018; Kariman et al., 2018).

Os fungos promotores de crescimento de planta (PGPF – Plant Growth Promoting Fungi) estabelecem interações com as plantas e impactam seu desenvolvimento por meio de diferentes mecanismos de promoção, dos quais merecem destaque a produção de fitormônios, solubilização de minerais, síntese de sideróforos, emissão de compostos voláteis e a indução da resistência sistêmica.

Os fitormônios produzidos por PGPF possuem o potencial não apenas de estimular o crescimento radicular e caulinar das plantas, mas também desempenham importantes papéis na sinalização das interações entre plantas e microrganismos e entre os próprios microrganismos do solo. Os fitormônios mais frequentemente associados a importantes funções na promoção de crescimento, sistemas defensivos das plantas e comunicação da rizosfera são o ácido indolacético, ácido giberélico, citocininas, etileno, ácido abscísico, jasmonato e ácido salicílico.

As auxinas possuem grande influência no crescimento, desenvolvimento e regulação de processos fisiológicos em todas as partes da planta, além de serem essenciais como moléculas sinalizadoras nas interações entre microrganismos e plantas. Como exemplo, a promoção de crescimento de *Eucalyptus globulus* conferida pelo fungo *Chaetomium cupreum* é consequência da regulação da síntese e metabolismo de ácido indolacético. Além disso, o fungo ainda tem a capacidade de proteger a planta contra efeitos tóxicos advindos de altas concentrações de metais no solo, principalmente por meio da supressão de transportadores de metal da planta (Ortiz et al., 2019).

Por outro lado, as auxinas ainda podem ser importantes nos ciclos de infecção de patógenos, que ao produzirem e secretarem o fitormônio conseguem suprimir as defesas mediadas pelo ácido salicílico (Eichmann et al., 2021).

Os efeitos da regulação de auxinas podem ser ainda mais abrangentes, afetando a interação da planta com outros microrganismos do rizomicrobioma. O fungo *Phomopsis liquidambari* ao estabelecer interações com *Arachis hypogaea,* favorece a interação da planta com bactérias do gênero *Bradyrhizobium*. A interação do fungo com a planta é dependente de

sinalização de auxinas, e essa mesma sinalização é usada por *A. hypogaea,* para ativar as vias simbióticas responsáveis pela associação com as bactérias e aumentar o metabolismo de carbono no nódulo de fixação de nitrogênio (Zhang et al., 2018).

O fungo *Trichoderma longibrachiatum* promove crescimento do trigo e confere maior tolerância a salinidade por meio da produção de ácido indolacético e aumento na atividade de ACC desaminase na planta. Os benefícios da inoculação fúngica estão associados ao aumento nas concentrações de ácido indolácetico e diminuição na síntese do etileno pela ação da ACC desaminase, alterações que também reverberam no maior nível de expressão do gene *SOS1*, o que confere melhor regulação no transporte de sódio e consequentemente diminui os efeitos negativos da alta salinidade no crescimento dessa cultura (Zhang et al., 2019).

Quanto as giberelinas, apenas as bioativas possuem efeitos na promoção de crescimento de planta, incluindo alongamento da raiz, aumento no número de raízes laterais, germinação de sementes, crescimento do caule, desenvolvimento das flores e sementes.

As giberelinas não atuam no desenvolvimento e crescimento dos fungos, porém como são produzidas por eles principalmente em condições de estresse, provavelmente conferem vantagens para sobrevivência deles. Esses fitormônios também foram associadas a patogênese de fungos, ao interferir com a sinalização de jasmonato e assim prejudicar as defesas da planta contra a infeção (Salazar-Cerezo et al., 2018).

O antagonismo entre giberelinas e jasmonato também é importante para a colonização do fungo benéfico *P. liquidambari* durante o florescimento de *Arabidopsis thaliana.* A sinalização de jasmonato inibe a biossíntese de giberelinas e assim impede o acúmulo de açúcares solúveis na raiz, consequentemente reduz a colonização pelo fungo durante esse período de florescimento, dada a menor oferta de recursos para o microrganismo (Zhang et al., 2021).

A regulação da sinalização de giberelinas nas plantas envolve a repressão de genes relacionados a biossíntese, produção de receptores e enzimas que catabolizam giberelinas ativas. Um exemplo são as proteínas regulatórias nas plantas, da família DELLA, que reprimem o crescimento. As giberelinas bioativas causam a degradação dessas proteínas, resultando em alterações na expressão gênica e permitindo assim que a estimulação do crescimento ocorra (Salazar-Cerezo et al., 2018).

As citocininas possuem funções importantes para plantas, fungos e a interação entre os mesmos. Nas plantas estão envolvidas no desenvolvimento e crescimento da planta, germinação de sementes, senescência, desenvolvimento de raízes laterais, dentre outros

processos. Para os fungos, as citocinas desempenham papéis no desenvolvimento de hifas, aquisição de nutrientes e virulência, inclusive atuando no estabelecimento de associações micorrízicas e no processo de infecção por fitopatógenos hemibiotróficos (Jing and Starder, 2019; Anand et al., 2022).

A solubilização de minerais está associada principalmente à produção de ácidos orgânicos que quelatam os cátions, assim liberando os ânions na solução do solo. A solubilização de P é a mais explorada devido a importância do elemento para nutrição das plantas e por sua interação com as partículas do solo. Muitas espécies de fungos já foram caracterizadas como eficientes solubilizadoras de fosfato, como *Aspergillus niger*, que possui capacidade de produção de ácidos orgânicos eficientes no processo de liberação de P (Ikhajiagbe et al., 2020; Murali et al., 2021; Nascimento et al., 2021).

Além da capacidade de solubilização de fosfatos pela liberação de ácidos orgânicos, o fungo *A. niger* promove crescimento do trigo, ampliando o acesso da planta a nutrientes por meio da degradação de celulose, aumentando a disponibilidade de potássio. O isolado modifica a composição da comunidade microbiana do solo, incluindo redução de fungos patogênicos como consequência da sua inoculação (Wang et al., 2018).

Os sideróforos são compostos de baixo peso molecular que possuem a capacidade de capturar o ferro férrico e torná-lo disponível em sua forma assimilável para plantas e outros microrganismos, especialmente em ambientes onde o elemento é limitante (Schwyn and Neilands, 1987). As funções dos sideróforos não se limitam apenas à disponibilização de ferro, mas também estão associadas às interações entre plantas e microrganismos, solubilização de fosfato, efeitos antimicrobianos e na virulência de fungos que infectam plantas e animais (Chowdappa et al., 2020; Murali et al., 2021).

Sideróforos produzidos pelos fungos *Penicillium chrysogenum, Aspergillus terreus* e *Aspergillus sydowii* apresentam efeitos antibacterianos, sendo capazes de inibir os patógenos *Ralstonia solanacearum* e *Xanthomonas oryzae,* representando assim um meio de proteção dos cultivos de amendoim e arroz contra a infecção dos seus respectivos patógenos (Chowdappa et al., 2020). Em condições de limitação de ferro, o sequestro do elemento pelos sideróforos priva o acesso do patógeno a ele, resultando na eliminação do patógeno (Chowdappa et al., 2020).

Os PGPF também possuem a capacidade de produzir e emitir compostos orgânicos voláteis (VOCs – Volatile Organic Compounds), que apresentam grande diversidade química e possuem múltiplos efeitos, incluindo promoção e inibição de crescimento vegetal, efeitos

antimicrobianos, alterações na expressão gênica, dentre outros (Lemfack et al., 2017; Quintana-Rodriguez et al., 2018; Jamil et al., 2022).

Como exemplo, o fungo *Trichoderma asperellum* emite compostos voláteis com atividade antifúngica contra os patógenos *Corynespora cassiicola* and *Curvularia aeria*, que infectam a alface. Além disso, os VOCs aumentam a atividade de enzimas degradadoras de parede celular, aumentam o teor de clorofila e promovem crescimento das folhas e raízes da alface (Wonglom et al., 2020).

A resistência sistêmica induzida (ISR – Induced Systemic Resistance) permite que a planta responda prontamente e de forma robusta a condições estressantes, uma vez que o fungo promotor de crescimento ao interagir com a planta desencadeia a expressão de enzimas e compostos defensivos (Hammerschmidt et al., 1995; Choudhary et al., 2007). A indução dessas respostas é resultado da interferência do PGPF na complexa rede de sinalização de fitormônios, especialmente mediada por jasmonato e etileno na ISR (Tucci et al., 2011).

O fungo *T. harzianum* possui a capacidade de induzir ISR em *Brassica napus* e *Raphanus alboglabra,* além disso o filtrado da sua cultura induz resistência sistêmica adquirida (SAR – Systemic Acquired Resistance) contra o patógeno *Sclerotinia sclerotiorum*. As defesas dependentes da sinalização de ácido salicílico e jasmonato/etileno reduziram a manifestação de sintomas nas folhas infectadas por *S. sclerotiorum*, portanto estão envolvidas na regulação para as respostas dos dois tipos de resistência mencionados (Alkooranee et al., 2017).

O óxido nítrico, composto volátil emitido por *T. harzianum* e *T. asperellum*, é requerido nas raízes de *A. thaliana* para desencadear a ISR nas folhas contra o patógeno *Botrytis cinerea.* O acúmulo de óxido nítrico induz a expressão de *MYB72,* fator de transcrição essencial para desencadear ISR em *A. thaliana.* As respostas na parte aérea das plantas são desencadeadas pela expressão dos genes *PDF1.2* e *PR1*, dependentes da sinalização de jasmonato e acído salicílico, respectivamente (Pescador et al., 2022).

Apesar da quantidade considerável de estudos que exploram a importância dos fungos promotores e as características das interações com as plantas (Venturi and Keel, 2016; Li-Hua et al., 2016; Zhang et al., 2017; Oskiera et al., 2017; Naik et al., 2019), alguns aspectos permanecem pouco esclarecidos, tais como a influência de fatores nutricionais do solo no estabelecimento das interações e as capacidades de um mesmo isolado eficientemente promover crescimento de diferentes espécies de plantas.

Capacidade dos PGPF de promoverem crescimento de diferentes plantas

Atualmente não há informações concretas sobre como diferentes espécies de plantas respondem a um mesmo fungo rizosférico e quais mecanismos envolvem o estabelecimento dessas interações. Tratando-se especificamente de eucalipto, os estudos são ainda mais escassos, a maioria deles realizado com bactérias (Santigo et al., 2015; Souza et al., 2022), e não abordam aspectos relacionados à especificidade dos microrganismos rizosféricos, se restringindo aos benefícios conferidos pelos microrganismos quando inoculados em determinada espécie do gênero *Eucalyptus* (Zaldúa and Sanfuentes, 2010; Vitorino et al., 2016; Ortiz et al., 2019).

Os fungos promotores podem demonstrar desempenhos distintos quando inoculados em diferentes plantas. Essas diferenças se estendem tanto no grau de benefício conferido a planta, quanto em quais aspectos de crescimento e aquisição de nutrientes essa planta será beneficiada pela inoculação do fungo (Baron et al., 2020).

Além disso, foi demonstrada a indicação de que aspectos filogenéticos da planta são altamente influentes nas interações com a microbiota do solo, podendo superar até mesmo aspectos nutricionais em determinadas situações. A promoção de crescimento de diferentes subespécies de eucalipto foi associada a receptividade dessas plantas para as comunidades de fungos no solo, uma vez que as diferentes dosagens de nitrogênio impostas não influenciaram o estabelecimento das interações (Wooliver et al., 2018).

As plantas potencialmente dispõem de mecanismos sofisticados que permitem o reconhecimento e favorecimento de interações com microrganismos benéficos, uma vez que frequentemente patógenos e microrganismos benéficos estão filogeneticamente próximos. A interação é dependente desse reconhecimento, comunicação e supressão, ao menos parcial, das defesas das plantas. Os mecanismos pelos quais a planta realiza essa distinção e seleção dos microrganismos ainda não foram claramente elucidados (Rodriguez et al., 2019).

O reconhecimento dos microrganismos pode ocorrer por meio de receptores da planta chamados de receptores de reconhecimento de padrões (RRPs), que por sua vez atuam na detecção dos chamados padrões moleculares associados a microrganismos ou patógenos (PMAMs ou PMAPs). O mecanismo aciona a imunidade da planta e respostas são ativadas, como a produção e deposição de compostos, realocação de nutrientes, mudanças em sinalizações e alterações transcricionais (Macho and Zipfel, 2014; Rodriguez et al., 2019).

A interação do fungo *T. harzianum* com tomateiro foi investigada por meio de análises de transcriptoma em três momentos diferentes desde o contato inicial: 24, 48 e 72 horas. A

colonização de *T. harzianum* é regulada por vias de interação de fitormônios. No primeiro momento há indução da síntese de ácido salicílico e acúmulo de espécies reativas de oxigênio, resultando no controle do crescimento do fungo. Em seguida, o ácido salicílico causa inibição da síntese e sinalização do etileno e jasmonato, garantindo colonização controlada pelo fungo nas raízes da planta. Por fim, mudanças na morfologia da raiz são causadas pelo aumento na sinalização do etileno e auxina, e junto às alterações no transporte de nutrientes, o crescimento da planta é estimulado (De Palma et al., 2019).

Apesar de o mecanismo descrito anteriormente trazer informações sobre as respostas causadas por microrganismos benéficos, sobretudo sua capacidade de fomentar a indução da resistência sistêmica da planta, não explica como a planta realiza a distinção do microrganismo, considerando que os PMAMs podem ser muito parecidos com os PMAPs, ou até mesmo idênticos (Rodriguez et al., 2019).

Algumas possibilidades já foram consideradas por estudos, como a de que alguns mecanismos atuam como interruptores moleculares, os quais apesar de desempenhar funções relacionadas à simbiose, também possuem papel importante nas respostas de defesa da planta (Rodriguez et al., 2019).

Outra hipótese explorada está relacionada com a ação de efetores, moléculas capazes de modificar função de proteínas do hospedeiro, ocasionando mudanças nas respostas defensivas da planta. Tal funcionalidade não é restrita aos patógenos, mas aplica-se também a fungos micorrízicos, fungos endofíticos e bactérias fixadoras de nitrogênio (Miwa and Okazaki, 2017). Os genes que codificam essas proteínas estão em constante modificação devido à pressão do processo de coevolução bastante notável entre fitopatógenos e plantas (Uhse and Djamei et al., 2018).

Qualquer molécula secretada por microrganismos atuante no estabelecimento de interações com o hospedeiro pode ser reconhecida como efetor e funcionalmente categorizada como efetor específico do hospedeiro ou não específico do hospedeiro (Rangel and Bolton, 2022). Além disso, tais efetores podem ser proteicos, o que inclui efetores clássicos, sintetizados nos ribossomos e peptídeos modificados pós tradução, e os não proteicos, representados por RNAs pequenos e efetores de metabólitos secundários (Rangel and Bolton, 2022).

Diferentemente de patógenos e fungos micorrízicos, que reconhecidamente dispõem de diversos efetores para estabelecer interações com as plantas, o nível de abrangência do papel dessas moléculas para fungos endofíticos e rizosféricos ainda não foi devidamente

elucidado (Liao et al., 2019). Proteínas já detectadas em *T. harzianum*, como o efetor Epl1 e as celulases Thph1 e Thph2, foram relacionadas com a indução de genes defensivos nas plantas, assim como a proteína ThPG1 foi vinculada à colonização das raízes pelo fungo (Ramírez-Valdespino et al., 2019).

Estudos já revelaram que os genomas de fungos saprofíticos podem apresentar considerável homologia nos genes de efetores com os genomas de patógenos intimamente relacionados a eles. Assim como que o comportamento saprofítico pode estar associado às divergências entre os genes de efetores expressos por fungos saprofíticos e patógenos (Rangel and Bolton, 2022).

O transcriptoma da interação de *A. thaliana* com o fungo benéfico *Colletotrichum tofieldiae* e o patógeno *Colletotrichum incamum* foi analisado e comparado, assim como análises dos genomas dos fungos foram realizadas, revelando que a diferença entre eles se restringia a menor quantidade de genes de efetores, expansão das famílias de proteínas relacionadas a metabolismo secundário e de ligação a quitina, e menor ativação de genes relacionados a patogenicidade quando em interação com a planta (Hacquard et al., 2016). Essas divergências demonstram como alterações no repertório genômico dos fungos são influentes no resultado das interações com as plantas.

Os pequenos RNAs (sRNA – small RNA) também já foram caracterizados como moléculas influentes nas interações de fungos e seus hospedeiros. Por exemplo, pequenos RNAs produzidos por *B. cinerea* são capazes de suprimir as defesas de *A. thaliana* (Wang et al., 2017). O efetor *Bc-siR37* possui quinze potenciais genes alvo no hospedeiro, incluindo genes codificadores de quinases, fatores de transcrição e enzimas modificadoras de parede celular. Diante da indução na expressão do efetor durante o processo de infecção por *B. cinerea*, pelo menos oito desses genes são suprimidos. Assim como, superexpressão de *Bc-siR37* ou mutações em seus genes alvo, resultaram em maior susceptibilidade da planta à doença causada por *B. cinerea* (Wang et al., 2017).

Outro exemplo em que RNAs atuam decisivamente nas interações, é o do fungo ectomicorrízico *Pisolithus microcarpus,* ao codificar o microRNA *Pmic_miR-8* que é transferido para as raízes de *Eucalyptus grandis*, provocando alterações na expressão gênica do hospedeiro, favorecendo assim o estabelecimento da relação simbiótica entre eles (Wong-Bajracharya et al., 2022). A inibição do efetor resulta menor grau de colonização do fungo nas raízes, em contrapartida, o aumento dos níveis do efetor resulta em integração superior do fungo nas raízes de *E. grandis* (Wong-Bajracharya et al., 2022).

Portanto, apesar das informações disponíveis relacionadas à interação das plantas com os microrganismos, consequentes respostas e as moléculas envolvidas, determinados detalhes dos mecanismos pelos quais a planta diferencia e seleciona os microrganismos para estabelecer interações ainda permanecem desconhecidos.

Influência da fertilidade do solo na promoção de crescimento

Outro aspecto importante, ainda não devidamente explorado, é o grau de influência do nível de fertilidade do solo para o estabelecimento das interações entre fungos promotores de crescimento e plantas. A capacidade de promoção de crescimento de diversos isolados fúngicos, especialmente pertencentes ao gênero *Trichoderma*, já foi demonstrada em diferentes condições físico-químicas do solo (Harman et al., 2011; Saba et al., 2012; Chowdappa et al., 2013; Li-Hua et al., 2016; Oskiera et al., 2017; Umadevi et al., 2018; Ozimek et al., 2018). Inclusive, quando comparado com o uso de fertilizantes, a inoculação dos isolados fúngicos pode resultar em desenvolvimento vegetal mais eficiente (Doni et al., 2014).

Os mecanismos de promoção apresentados pelos isolados influenciam o desenvolvimento vegetal de acordo com as necessidades da planta em determinadas condições (Babu et al., 2014; Li-Hua et al., 2016; Prabha et al., 2019). As plantas inclusive quando em condições nutritivas adequadas podem selecionar microrganismos produtores de fitormônios, como auxinas, para investir em incrementos na biomassa. Em contrapartida, em solos pobres em nutrientes, as plantas podem selecionar microrganismos solubilizadores de nutrientes, para auxiliar na captação de recursos para suprir suas deficiências, e assim aumentar sua chance de sucesso naquelas condições (Costa et al., 2020).

As comunidades microbianas sofrem alterações em decorrência da disponibilidade de nutrientes no solo, além do fato de que as plantas, através dos exsudados radiculares disponibilizados na rizosfera, manipulam a estrutura e a composição das comunidades microbianas do solo (Sasse et al., 2018; Plassart et al., 2019). Adicionalmente, o genótipo da planta e as características físico-químicas do solo demonstram ampla influência nas redes de interações entre fungos e bactérias que compõem o rizobioma (Bonito et al., 2019).

A composição molecular de diferentes compostos adicionados ao solo influencia o desenvolvimento vegetal, aquisição de fósforo e nitrogênio, colonização e crescimento de fungos micorrízicos e impacta a estrutura das comunidades microbianas do solo (Cozzolino et al., 2016). Os estudos disponíveis demonstram que em condições específicas de cultivo e

fertilidade, o fungo promotor de crescimento pode influenciar, até mesmo negativamente, a aquisição de macro e micronutrientes pela planta (De Santiago et al., 2013; Li et al., 2015). Tais informações reafirmam a importância do controle da qualidade dos compostos adicionados ao solo, pois diversos efeitos indesejáveis podem resultar das condições impostas pela fertilidade do solo, como competição entre microrganismos e planta, diminuição na aquisição de macro e microelementos, redução de populações microbianas benéficas e prejuízos ao crescimento das plantas.

A natureza dos fertilizantes pode influenciar diretamente a atividade de fungos promotores de crescimento em interação com a planta. Compostos orgânicos e inorgânicos com a mesma quantidade de P foram adicionados à cultura do milho junto com inóculo de *T. harzianum* para avaliar a potencial influência desses materiais na promoção de crescimento desempenhada pelo fungo (Vinci et al., 2018). Nos tratamentos sem fertilizantes, a inoculação do fungo resultou em decréscimos na biomassa do caule e nos conteúdos de P e N na planta, caracterizando competição entre planta e fungo pelos nutrientes. Já nos tratamentos fertilizados, tanto com compostos orgânicos, quanto com inorgânicos, foi observado efeitos sinérgicos com o inóculo fúngico, caracterizado pela maior aquisição de nutrientes, maior crescimento das plantas e maior atividade fotossintética. Porém, a inclusão de compostos inorgânicos resultou em desempenho inferior quando comparado com os compostos orgânicos, e as plantas apresentaram menor biomassa do caule e níveis de nitrogênio inferiores (Vinci et al., 2018). A análise de metabólitos detectou compostos tipicamente produzidos pela planta durante episódios de estresse, sugerindo que os fertilizantes inorgânicos não foram capazes de disponibilizar a quantidade de fósforo ideal durante o crescimento da planta, uma vez que o P liberado na rizosfera é rapidamente convertido em formas insolúveis e pode desencadear competição entre plantas e microrganismos para sua solubilização (Vinci et al., 2018).

As plantas possuem mecanismos adaptativos para lidar com a baixa disponibilidade de fósforo, as chamadas phosphate starvation responses (PSRs). Testes com *A. thaliana* demonstraram que esses sistemas são coordenados com a imunidade da planta e em situação de privação de fosfato, o regulador *PHR1* e seu parálogo *PHL1* são importantes. Plantas com esses reguladores mutados, além de demonstrar níveis muito inferiores de PSRs, também apresentaram aumentos nas repostas defensivas contra colonização de suas raízes. A regulação negativa do sistema imune da planta por *PHR1* é direta e funcional, portanto, o regulador em questão ativa as repostas para lidar com limitação de fósforo, ao mesmo tempo

em que reprime o sistema imune da planta e a composição da comunidade microbiana é consequentemente alterada (Castrillo et al., 2017).

A privação de fósforo também provoca alterações nas repostas imunes de *A. thaliana* na interação com o fungo *C. tofieldiae.* Análises do transcriptoma revelaram que quando o fosfato era disponibilizado em condições suficiente, respostas defensivas da planta eram ativadas. Em contrapartida, quando o fosfato era limitante, a respostas defensivas eram suprimidas e assim a colonização pelo fungo era favorecida (Hacquard et al., 2016). Considerando a interação de *A. thaliana* com o fungo patogênico *C. incanum,* fortemente relacionado com *C. tofieldiae,* as respostas defensivas da planta eram mantidas independentemente da disponibilidade de P (Hacquard et al., 2016).

A influência do enriquecimento de nitrogênio na promoção de crescimento é distinta para as diferentes linhagens de eucalipto, mesmo aquelas com pequeno distanciamento filogenético (Wooliver et al., 2018). A fertilização não altera fortemente os níveis de respostas entre a planta e a microbiota, pois essa interação é filogeneticamente estruturada. Portanto, a promoção de crescimento é atribuída aos diferentes níveis de receptividade das plantas pelos fungos do solo que desempenham esse papel, nesse caso em particular, fungos não-filamentosos e Dark Septate Endophytes (DSE). As plantas que demonstraram menor crescimento mantiveram a associação com fungos ectomicorrízicos e reduziram a colonização pelos outros grupos de fungos citados (Wooliver et al., 2018). Adicionalmente, existem evidências que sugerem que o nível de alocação de carbono que a planta destina ao fungo ectomicorrízico pode influenciar o crescimento dela em situações de enriquecimento por nitrogênio no solo (Johnson et al., 2008).

Os fungos promotores de crescimento vegetal possuem influência no perfil metabólico da planta, impactando o crescimento, desenvolvimento, processos defensivos e biossíntese de moléculas (Brotman et al., 2012). Determinados fatores de transcrição relacionados com a promoção de crescimento e defesa vegetal podem ser estimulados, enquanto outros fatores podem ser inibidos (Brotman et al., 2012; Liao et al., 2019). As atuações de tais fatores traduzem-se em aumento da sinalização mediada por fitormônios importantes nas respostas de defesa e interações, diminuição da susceptibilidade a infecções por patógenos e aumento de moléculas, enzimas e intermediários de vias metabólicas (Brotman et al., 2012; Liao et al., 2019).

Estudos que abordam essa relação de nutrição e estabelecimento das interações podem revelar como a presença do fungo promotor de crescimento pode interferir no metabolismo e

nas defesas da planta concomitantemente (Liao et al., 2019). Por exemplo, o metabolismo da planta pode ser modulado para investir mais em metabolismo primário e genes de defesa, ao mesmo tempo que ocorre menor expressão de genes relacionados com transporte e metabolismo secundário (De Palma et al., 2016).

Conclusão

Apesar do considerável volume de informações já disponíveis sobre a promoção de crescimento vegetal mediada por fungos, muitos aspectos ainda precisam ser mais profundamente explorados para que a aplicação das tecnologias que envolvem esses organismos seja eficaz e vantajosa para os cultivos. Considerar aspectos relacionados com as capacidades dos fungos de promover crescimento de diferentes culturas e a avaliação de como aspectos da fertilidade do solo podem alterar a interação do fungo com a planta, é crucial para o desenvolvimento de bioinoculantes.

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CAPÍTULO 2:

INFLUENCE OF SOIL PHOSPHOROUS AVAILABILITY ON *Eucalyptus grandis* GROWTH PROMOTION BY *Talaromyces aculeatus*

Abstract

Eucalypt cultivation deals with challenges to its productivity, mostly represented by poor soils and pathogen occurrence. Currently, the concern about the indiscriminate use of chemical fertilizers and pesticides has gained attention and brought focus on new ways to achieve productivity with reduction of costs and lower levels of environmental damages. The use of soil microorganisms to benefit plant development is a powerful tool already successfully applied and researches are still bringing up useful information to amplify their potential. Plant growth promoting fungi (PGPF) have great potential as beneficial microorganisms applied to plant cultivation, especially because their important roles on soil processes. They can impact plants through different and already well characterized promoting mechanisms, such as phosphorous solubilization, phytohormones production and siderophores synthesis. Despite of the considerable amount of information about how PGPF can increase plant productivity, health and responses to stresses, some topics are still poorly explored and details of their interactions remains unclear. The aim of this study was to investigate how changes on soil nutrition, particularly P variation, can impact the interaction outcomes between *Talaromyces aculeatus* and *Eucalyptus grandis*. Firstly, *T. aculeatus* was transformed with a plasmid containing hygromycin resistance and red fluorescent protein genes, applying protoplast-PEG technique. Transformation was confirmed through fluorescent microscopy and the transformed isolates were purified and submitted to a growth promotion test (*in vitro*) on *E. grandis* seedlings to select one of them to greenhouse experiment. Wild-type and transformed fungi were also submitted to some promotion mechanisms experiments: indoleacetic acid production, phosphate solubilization and siderophores production. Seedlings of *E. grandis* were obtained through seed germination and posteriorly they were cultivated in vases with 3.0 dm^3 of red-yellow oxisol. Three treatments (wild-type fungus, transformed fungus and control), with three repetitions each, were kept for each one of the phosphorous doses (35, 70, 140, 215 and 290 mg kg^{-1}), during a total of 60 days in greenhouse. After experiment period, height and dry matter were measured, plant tissues were used to analyze micro and micronutrients and the obtained data were submitted to variance analysis, regression and plant nutrient use efficiency calculation. Wild-type *Talaromyces aculeatus* inoculation caused growth and nutritional gains to *E. grandis*, while its transformed version caused considerably losses on plant development at the same P interval. Moreover, regression analyses indicated interaction between factors to almost all responses. Therefore, *T. aculeatus* promoted growth to *E. grandis*, especially on 70 and 140

mg kg^{-1} doses of phosphorous. According to data, the amount of phosphorous available on soil had significant influence on interaction between *T. aculeatus* and *E. grandis,* thus interaction establishment are consistently stronger when phosphorus is limiting.

Introduction

Eucalypt industry is economically important around the world because it generates several products with high value and demand worldwide. In Brazil, the sector presents continuum growth and between 2010 and 2020 the value added to GDP (Gross Domestic Product) was 10.2 %, while Brazil's GDP only grew 2.7 % in the same period (IBA, 2021). As any cultivation, eucalypt plantations deal with several challenges especially imposed by pathogens and nutrients limitations (IBA, 2021). Therefore, wider understanding on eucalypt interaction with soil microbiome can offer new tools to achieve higher productivity with lower costs and less environmental negative impacts.

Microorganisms play very important roles in the soil quality, including organic matter decomposition, nutrients cycling, pathogens suppression and contribution to soil structure (Yedidia et al., 2001; Singh et al., 2006; Dini-Andreote and Raaijmakers, 2018). Fungi are protagonists of these crucial soil processes; thus, they have been explored as powerful tools to enhance plant health and development, reducing the use of chemicals on agricultural and forestry activities (Ahkami et al., 2017; Paliwoda & Mikiciuk, 2020). Consequently, they can provide financial economy, higher productivity and environmentally sustainable practices.

Plant growth promoting fungi (PGPF) can benefit plants in several ways through different promotion mechanisms. The main mechanisms are the production of phytohormones, phosphate solubilization, biosynthesis of siderophores, induction of systemic resistance, emission of volatile organic compounds, production of antibiotics and other metabolites (Gupta et al., 2015; Rodriguez et al., 2019).

Currently, several species of fungi already had characterized their mechanisms of plant growth promotion, especially the ones belonging to *Trichoderma* genus. There is a considerable number of studies covering the potential of these fungi in helping plants to develop vigorously and deal with biotic and abiotic stress (Paliwoda & Mikiciuk, 2020). However, fungi from other genera are also being studied and the results suggest great potential, such as *Aspergillus* and *Penicillium* (Paliwoda & Mikiciuk, 2020).

Additionally, there are few studies which approached nutritional aspects related to growth promotion and they indicated important contributions of these factors to the resulting

interaction between fungi and plants, which includes that even beneficial fungi can compete with plants in certain nutritional conditions and that other aspects might be more definitive for the interaction establishment (Wooliver et al., 2018; Vinci et al., 2019). However, there is no enough information to clearly establish if variation on soil nutrients would directly affect the relationship between PGPFs and plants or not.

The PGPF *Talaromyces aculeatus* is poorly explored for growth promotion. There are several studies focused on the production of metabolites, enzymes and antibiotics (Lee et al., 2013; Zhang et al., 2017; Krishnamurthy et al., 2018). Also, there are few studies related to P solubilization, production of phytohormones and siderophores, but there is no deeper investigation about its growth promotion potential, especially none of the studies tested its potential to eucalypt growth promotion (Babu et al., 2014; Istina et al., 2015; Efthymiou et al., 2018; Raymond et al., 2018).

The PGPF *Talaromyces aculeatus* on this work was isolated from the rhizosphere of *Eucalyptus grandis* from the silviculture sector of Universidade Federal de Viçosa, in 2016, as part of a study of *E. grandis* growth promotion for master dissertation (Reis 2018). Posteriorly, the isolate was identified using molecular tools and selected to this study due its capacity of *E. grandis* growth promotion and desirable features, such as rapid growth and efficient sporulation.

The aim of this study was to investigate the potential of *T. aculeatus* on *E. grandis* growth promotion and understand how P fertilization can affect the interaction outcomes. Particularly, how the influence of different P doses on soil can impact fungal interaction with the plants and the results from it. This study hypothesizes that variation on P availability on soil considerably affects the interaction between *T. aculeatus* and *E. grandis*.

Materials and Methods

Transformation

The transformation of *T. aculeatus* was made to obtain isolates expressing the red fluorescent protein (RFP) to investigate *T. aculeatus* colonization on *Eucalyptus grandis* rhizosphere. Firstly, the isolate was submitted to a test in which the concentration of hygromycin B capable to inhibit its growth was defined. Suspension of spores with concentration of 10^7 was applied to Petri dishes with PDA (Potato Dextrose Agar) containing

different concentrations of hygromycin B (Sigma-Aldrich), ranging from 0 to 400 µg mL^{-1}. The Petri dishes were incubated at 28 °C and the fungal growth was observed on the following days until it was possible to define 350 µg mL^{-1} as the minimum inhibitory concentration for *T. aculeatus* growth.

The following step was the generation of protoplasts. It was applied through spread plate 250 µL of spore solution with concentration of 10^8 in six Petri dishes with PDA covered with cellophane on its surface. The plates were incubated at 28 °C for 16 hours, then 800 mg of mycelium were revested and transferred to an Erlenmeyer flask containing 5 mL of KCL 0.8 M, phosphate buffer 10 mM and 30 mg of Lysing Enzymes (*Trichoderma harzianum* – Sigma-Aldrich). The mixture was incubated on shaker for 4 hours at 30 °C and 80 rpm. It was counted on microscope, using Neubawer chamber, the number of 10^7 protoplasts and after purification steps, the concentration of protoplasts was about 10^6.

The chosen plasmid included hygromycin B resistance gene (*hph* - hygromycin B phosphotransferase of *Escherichia coli*) regulated by PtrpC promoter of *Aspergillus nidulans* and PtDsRed cassette with PtoxA-5'UTR promoter controlling DsRed gene (reef coral *Discosoma* sp.) and the terminator Tnos.

The transformation methodology applied was using protoplasts and polyethylene glycol (PEG) (Pereira et al., 2004). Two tubes received 200 µL of protoplast suspension each: only one of them received 5 µg of plasmid because the other one was to originate the control group. The tubes then received 50 µL of PEG 6000 60% (Sigma-Aldrich) and were incubated on ice for 20 minutes. After incubation, 500 µL of PEG are added and the tubes are incubated at room temperature for another 20 minutes. Finally, three plates received 500 µL each of the mixture from the treatment tube and three plates received 500 µL of the mixture on control tube, applied through pour plate in PDA added with sucrose at 0.56 M to stabilize the protoplasts. The six plates were incubated at 28 °C and the hygromycin B at 350 µg mL^{-1} was added on the next day with 10 mL of PDA, covering the entire surface of the medium in the plates.

During the next following 10 days, the plates were constantly observed and twelve colonies appeared on medium surface of the three treatment plates. Three of them were selected through fluorescence microscopy, submitted to monosporic purification and kept on plates with PDA.

Growth promotion test

The transformed fungus was selected based on the growth promotion test *in vitro*, which was conducted during 45 days, at 30 ºC with 12 hours photoperiod. The seeds were superficially disinfected and inserted on sterile flasks containing washed and sterile sand. After germination, *E. grandis* seedlings were transplanted, one per flask, and each one received on roots three 6 mm discs with mycelium from each of the three tested transformed isolates or the wild-type fungus (TW). A control group, with no inoculation, was also kept. Each treatment was maintained with four repetitions. The field capacity was kept around 60% with distilled water addition and Clark solution was added regularly, twice a week.

Fungal impact on plant development was evaluated based on plant morphological features: number of leaves, height, root length and dry matter. The data was submitted to ANOVA and the means were compared by Tukey test ($p < 0.5$).

Inodole-3-acetic acid production

The evaluation of indole-3-acetic acid (IAA) production consisted in the extraction of three discs containing the fungal mycelium from colonies growing in PDA for 7 days at 30 ºC, and inoculation in flasks with 50 mL of Czapek-Dox enriched with L –tryptophan at 5 mmol L-1. The flasks were kept under agitation at 180 rpm and 28 ºC for 7 days. The supernatant was collected and filtered in filter paper with 8-µm pores. The detection of IAA followed the method of Salkowski reagent (Bric et al., 1991). The tubes received 1 mL of the supernatant and 1 mL of Salkowski reagent. After 30 minutes of reaction the samples were read in a spectrophotometer at 540 nm, based on a calibration curve of IAA with concentrations ranging from 0 to 100 µg mL-1.

Siderophore production

Siderophore production was also determined using three discs of 6 mm from the colonies and inoculated into 25 mL of CAA liquid medium enriched with succinic acid (Cabellero-Mellado et al., 2007). The flasks were incubated during 7 days at 28 ºC under agitation at 180 rpm. After this period, three portions of 3 mm were extracted from the mycelium and transferred to CAA-CAS solid medium. The plates were incubated for 7 days

at 28 ºC, and after this period, the appearance of yellow halos around the colonies was used as an indication of hydroxamate-type siderophore production. Three discs from the colonies were inoculated in 25 mL of Simoon and Tessman medium (Simon & Tessman, 1963) and incubated for 10 days at 28 ºC under agitation of 130 rpm. The samples were filtered and the supernatant collected to quantify siderophore production. The calibration curve used as reference was made with EDTA ranging from 0 to 100 mg L^{-1}. The reaction occurred combining 1 mL of the supernatant with 100 µL of CAS solution. After 15 minutes of reaction, the mixture was read in spectrophotometry at 630 nm.

Phosphate solubilization

The isolates also had their capabilities of phosphate solubilization tested for four different phosphorous (P) sources: tricalcium phosphate, iron phosphate, aluminum phosphate and Araxá phosphate. Three 6 mm discs from the colonies were inoculated in 50 mL of NBRIP medium (Nautiyal, 1999) with the respective phosphate at 3 g L-1. The flasks were incubated for 7 days at 28 ºC under agitation of 150 rpm. The supernatant was collected and filtered in filter paper with 8-µm pores. The determination of soluble phosphorus was made by colorimetric method (Braga & Defelipo, 1974). The fungal biomass was determined through mycelium drying at 105 ºC until constant weight, followed by incineration for 6 hours at 500 ºC in a muffle, so that phosphate particles that remained attached to the mycelium could be calculated and discounted.

Greenhouse experiment

On the main experiment, conducted on greenhouse, the plants were cultivated during 60 days in plastic vases with 3.0 dm^{-3} of Latossolo Vermelho-amarelo distrófico (Red-yellow oxisol - chemical and physical analysis on Table 1) collected from the Viçosa region (coordinates: 20° 46' 009'' S 42° 52' 43.4'' W). The soil was submitted to different nutritional levels, forming a gradient, where P was offered in five different doses: 35, 70, 140, 215 and 290 mg kg^{-1} de P ($Ca(H_2PO_4)_2$). The rest of the fertilization was made according to Novais et al. (1991) recommendations with few changes: 100 mg kg^{-1} of N (NH_4NO_3), 100 mg kg^{-1} of K (KCl), 190 mg kg^{-1} of Ca ($CaCl_2$), 4.00 mg kg^{-1} of Zn ($ZnSO_4 . 7 H_2O$), 1.33 mg kg^{-1} of Cu ($CuSO_4 . 5 H_2O$) and 30 mg kg^{-1} of Mg ($MgSO_4 . 7 H_2O$). The microelements B,

Table 1 - Physical and chemical soil composition from Viçosa region, Minas Gerais.

Mineral Fraction (%)										
Fine sand		**Coarse sand**		**Silt**		**Clay**		**Texture**		
9.2		14.1		4.2		72.4		Very Clayey		
Chemical Analysis										
pH	**P**	**K^+**	**H+Al**	**Al^{3+}**	**Ca^{2+}**	**Mg^{2+}**	**CEC**	**V**	**M**	**OM**
H_2O	mg dm^{-3}		$cmol_c$ dm^{-3}					%		dag Kg^{-1}
4.92	0.4	0	3.4	0.39	0.22	0.03	3.65	6.8	60.9	1.6

Mn, Mo and Fe were not added because the soil already contained appropriate quantities of them.

The treatments consisted to: I) Inoculation of *T. aculeatus* wild-type; II) Inoculation of the selected transformed *T. aculeatus*; III) Control treatment, without inoculation. The 5x3 factorial experiment (5 phosphate doses x three inoculation treatments) was built in completely randomized design with three repetitions.

The seedlings were obtained by seeds germination in sterile sand, after superficial disinfection. The seeds were immersed in 70% alcohol for 1 minute and washed in distilled and autoclaved water, followed by immersion in 20% H_2O_2, and after seven minutes, they were successively washed. The seeds were inserted in the washed and autoclaved sand contented in plastic recipient in the laboratory. After germination, the plants were transferred to greenhouse. Once a week the plants received Clark nutritive solution on full force (Clark, 1975) and the necessary amount of distilled water to maintain the sand field capacity in 60%. Water addition was controlled based on sand recipient's weight, then it was possible to measure water loss and replenish it. Four weeks later the plants were transplanted, one per vase, and each one received on roots three 6 mm discs of mycelium from the colony of *T. aculeatus* wild type or the selected transformed fungus.

The irrigation was executed twice per day and during the experiment, on regular time intervals, a portion of soil, kept in vases separately for each of the soil P doses without any plant, was collect to analyze the availability of P in the soil along the experiment time. The P concentrations were obtained through extraction with acidic solution (chloridric and sulfuric acids) and colorimetric assay for P quantification (Defelipo and Ribeiro, 1981).

After 60 days of experimentation, the plants were collected and the following variables were evaluated: shoot, root and total dry matter, plant height, nutrients content and concentration (Donagema et al., 2011).

The quantification of nutrients was performed on Soil Science Department (DPS) at Universidade Federal de Viçosa. Nitrogen quantification was made through sulfuric digestion and application of Kjeldahl method. All the other nutrients were submitted to nitropercloric digestion and analyzed on Plasma ICP-OES-Optima 8300 (Sarruge & Haag, 1974).

The nutrient utilization efficiency index (E) was calculated according to Siddiqi and Glass (1981), using the formula: $(E) = (\text{Total Dry Matter})^2/(\text{total nutrient content})$. The data was submitted to ANOVA and the means were compared by Tukey test ($p < 0.05$). Statistical analyses were executed on R 4.2.1 software.

Results

Growth promotion test

The initial objective was to obtain one transformed isolate of *T. aculeatus* with the gene RFP to allow the indirect quantification of the wild-type fungus on the rhizosphere. However, the transformed isolates obtained (T1, T3 and T4) did not behave exactly like the wild type on terms of plant growth promotion. During the test performed *in vitro*, T3 did not differentiate statistically from the wild type and also manifested difference from control group (Figure 1). On the other hand, the three transformed isolates did not present statistical difference from each other, although T1 and T4 did not even differentiate from control group (Figure 1). Considering the described outcomes, T3 was the best candidate available, so it was selected to being applied on the greenhouse study (Figure 2).

Indoleacetic acid production

The wild-type and transformed isolates produced the amount of 11.06 µg mL $^{-1}$ and 13.09 µg mL $^{-1}$ of IAA, respectively. The amount produced by T3 was significantly greater than TW, according to ANOVA F Test ($p < 0.05$).

Siderophore production

Both isolates presented the capacity of hydroxamate-type siderophore production revealed by the yellow halo formed around the colony (Figure 3). The amount produced by the wild-type fungus was 8.17 µg mL $^{-1}$, while the transformed fungus produced 8.86 µg mL $^{-1}$. The difference was not significant according to ANOVA F Test ($p < 0.05$).

P solubilization

Considering solubilized P and solubilization (%), fungus T3 presented higher performance than wild-type fungus to all four tested phosphates (Table 2). Moreover, there were differences on solubilization level between the four P sources (Table 2). Both wild-type and transformed *T. aculeatus* had their best performances with calcium phosphate, followed by Araxá natural phosphate, and then iron and aluminium phosphates (Table 2). Additionally,

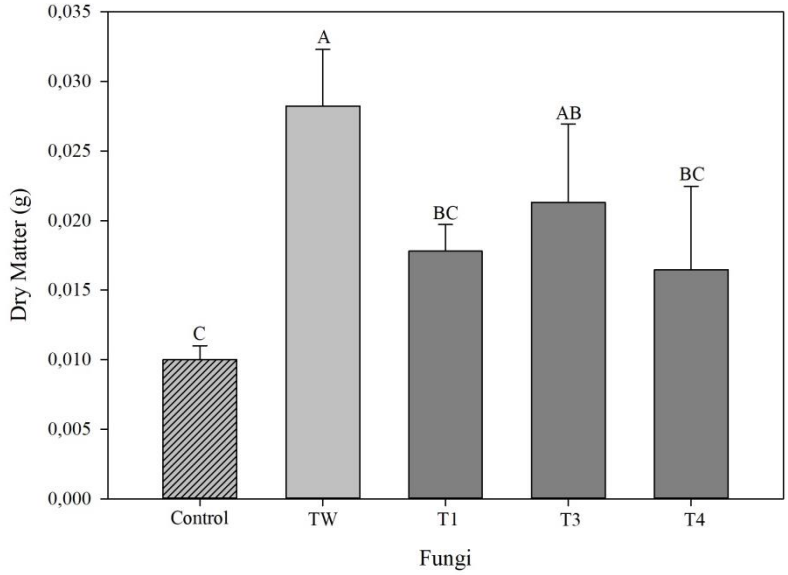

Figure 1 – Plant growth promotion test (*in vitro*) indicating the fungal inoculation impact on plant dry matter. TW represents the Wild type and T1, T2 and T3 represent the isolates transformed through protoplast-PEG method. Columns with the same letter are not significantly different by the Tukey test ($p < 0.05$). The bars represent the standard deviation.

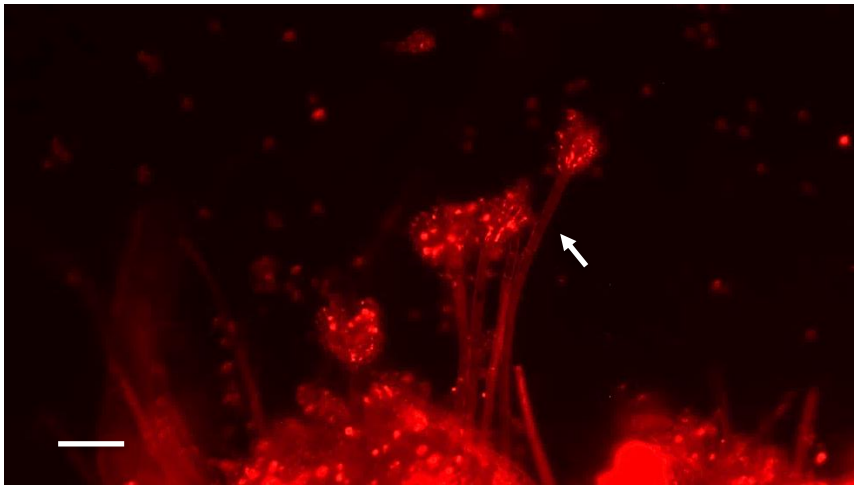

Figure 2 – Fluorescent conidiophores and spores from the transformed fungus T3. The transformed fungus was obtained applying protoplast-PEG technique. The plasmid used in the process contains Red Fluorescent Protein and Hyrgromycin resistance genes. Magnification: 100x, scale bar: 30 µm.

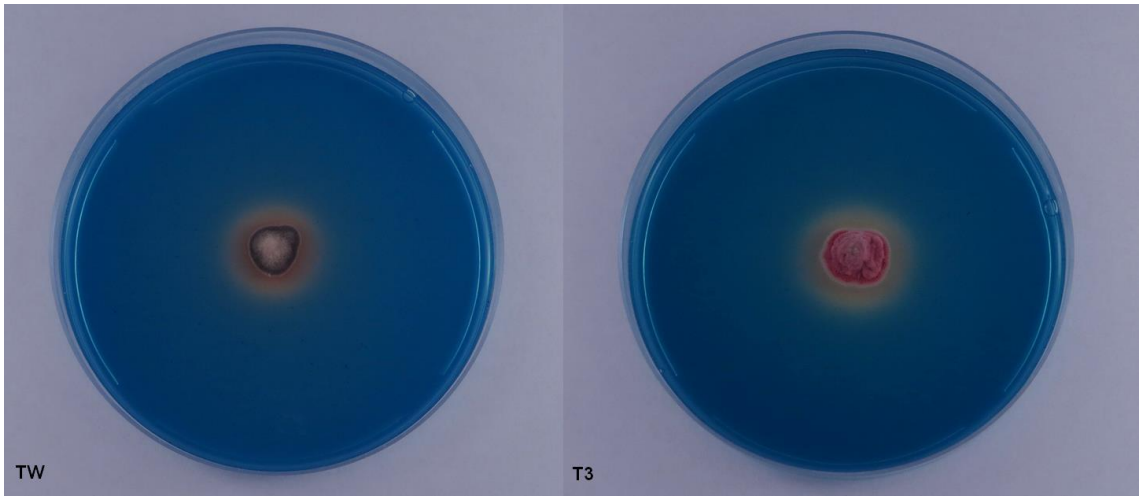

Figure 3 – Detection of hydroxamate-type siderophore production by Chome Azurol S assay (CAS). Yellow halos formed around the colonies indicate siderophore production by the fungi.

Table 2 – Phosphate solubilization of four different sources of P by wild-type and transformed T3 *T. aculeatus*. Experiment conducted during 7 days, at 28 ºC, 180 rpm, inoculation on NBRIP medium

Phosphate	**Fungal Isolate**	
	TW	**T3**
	Solubilized P (mg L^{-1})	
Calcium ($Ca_3(PO_4)_2$)	78.55 Ab	172.71 Aa
Iron ($FePO_4$)	-3.50 Cb	16.58 Ca
Aluminium ($AlPO_4$)	-5.77 Cb	17.64 Ca
Araxá Natural Phosphate	5.88 Bb	58.32 Ba
Mean	18.79	66.31
CV (%)	12.15	
	Solubilization (%)	
Calcium ($Ca_3(PO_4)_2$)	13.11 Ab	28.82 Aa
Iron ($FePO_4$)	-0.70 Cb	3.33 Ca
Aluminium ($AlPO_4$)	-0.76 Cb	2.31 Ca
Araxá Natural Phosphate	1.41 Bb	13.95 Ba
Mean	3.26	12.10
CV (%)	12.53	
	pH	
Calcium ($Ca_3(PO_4)_2$)	4.95 Aa	4.38 Ab
Iron ($FePO_4$)	2.20 Ca	1.96 Cb
Aluminium ($AlPO_4$)	1.97 Da	1.91 Ca
Araxá Natural Phosphate	3.52 Ba	3.08 Bb
Mean	3.16	2.83
CV (%)	1.61	
	Titratable Acidity (mmol H+ L^{-1})	
Calcium ($Ca_3(PO_4)_2$)	5.03 Bb	17.60 Aa
Iron ($FePO_4$)	7.54 Ab	15.09 Ba
Aluminium ($AlPO_4$)	5.03 Bb	12.57 Ca
Araxá Natural Phosphate	2.51 Cb	12.57 Ca
Mean	5.03	14.46
CV (%)	0	
	Biomass (mg)	
Calcium ($Ca_3(PO_4)_2$)	113.66 Ba	45.00 Ab
Iron ($FePO_4$)	70.99 Ca	52.00 Aa
Aluminium ($AlPO_4$)	101.33 Ba	34.33 Ab
Araxá Natural Phosphate	228.67 Aa	47.66 Ab
Mean	128.66	44.75
CV (%)	14.26	

Uppercase letters represent significant statistical difference between phosphates and lowercase letter between fungi, according to Tukey test ($p < 0.05$).

negative values to iron and aluminium phosphates solubilization suggests P immobilization by wild-type *T. aculeatus* (Table 2).

It was possible to notice that pH varied significantly in function to both fungi and phosphates treatments (Table 2). Wild-type fungus presented significant different pH values between all phosphates and theses values were higher when compared to transformed fungus (Table 2). Fungus T3 also presented statistical differences on pH values between the phosphates, except for iron and aluminium (Table 2).

Titratable acidity was also impacted by fungi and phosphate type (Table 2). The values were consistently higher for fungus T3 than the wild fungus and the highest value occurred with calcium phosphate, followed by iron and aluminium with the same value, and finally Araxá phospate (Table 2). Alternatively, to wild-type fungus, the highest value was with iron phosphate, followed by calcium and aluminium phosphates, and in the lower position, Araxá natural phosphate (Table 2).

Fungal biomass was affected by phosphate type on wild-type fungus treatment, but it did not vary significantly on T3 treatment (Table 2). The highest value for wild-type fungus biomass was with Araxá natural phosphate, followed by aluminium and calcium phosphates, and finally, iron phosphate (Table 2). Moreover, biomass of wild-type fungus was significantly higher than fungus T3, except in the iron phosphate treatment (Table 2).

Greenhouse experiment

Plant growth

The greenhouse experiment revealed that variation on P doses in the soil had significant influences on interactions between *T. aculeatus* and *E. grandis*. It was clear that in the low doses (70 and 140 mg kg^{-1}) the presence of the fungi resulted in effects on plant development, positively in the case of the wild type and negatively with the transformed fungus selected (Table 3). On higher doses (215 and 290 mg kg^{-1}), the differences generally were not significant, despite of few exceptions (Table 3).

On the first dose, 35 mg kg^{-1} of P, the plant development was considerably compromised due the limitation imposed by the lack of P, consequently the plants did not grow much. When the soil was submitted to 70 mg kg^{-1} of P, the plants presented the highest differences between inoculation with TW and control group, indicating increases of 58.62 %

Table 3 – Impacts of wild-type and transformed *Talaromyces aculeatus* T3 on *Eucalyptus grandis* submitted to different P doses. Experiment conducted in greenhouse for 60 days.

Treatments	P (mg kg $^{-1}$)					
	35	**70**	**140**	**215**	**290**	**Mean**
			Height (cm)			
TW	7.50 b	23.00 a	37.50 a	38.20 a	40.50 b	29.34
T3	9.25 ab	17.50 b	24.00 c	38.50 a	46.50 a	27.15
Control	10.50 a	14.50 c	32.00 b	33.33 b	40.50 b	26.17
Mean	9.08	18.33	31.17	36.68	42.50	
CV(%)	4.57					
			SDM (g)			
TW	0.07 ns	1.56 a	7.76 a	8.82 ns	8.60 ns	5.36
T3	0.08	0.24 b	1.56 c	7.55	8.73	3.63
Control	0.18	0.37 b	5.75 b	7.59	8.77	4.53
Mean	0.11	0.72	5.02	7.99	8.70	
CV(%)	6.16					
			RDM (g)			
TW	0.02 ns	0.17 a	1.19 a	1.86 a	1.87 a	1.02
T3	0.04	0.04 c	0.66 b	1.19 b	1.25 b	0.64
Control	0.02	0.11 b	1.36 a	1.12 b	1.79 a	0.88
Mean	0.03	0.11	1.07	1.39	1.64	
CV(%)	4.73					
			TDM (g)			
TW	0.09 ns	1.73 a	8.95 a	10.68 a	10.47 ns	6.38
T3	0.12	0.28 b	2.26 c	8.74 b	9.98	4.28
Control	0.20	0.48 b	7.11 b	8.71 b	10.56	5.41
Mean	0.14	0.83	6.11	9.38	10.34	
CV(%)	5.58					

The letters represent significant statistical difference and "ns" indicates no significant differences, according to Tukey test ($p < 0.05$). SDM – Shoot Dry Matter, RDM – Root Dry Matter and TDM – Total Dry Matter.

on plant height, 321.62 % on shoot dry matter, 54.55 % on root dry matter and 260.42 % considering plant total dry matter (Table 3). Additionally, *T. aculeatus* (T3) caused increase of 20.69 % on plant height and reduction of 175 % on root dry matter, (Table 3).

Considering the P dose of 140 mg kg^{-1}, the inoculation with TW presented significant increases of 17.19 % on plant height, 34.96 % on shoot dry matter and 25.88 % on total dry matter, however there was no significant difference on the root dry matter (Table 3). On the other hand, the negative effects of T3 were higher on this dose, causing decreases of 33.34 % on plant height, 268.59 % on shoot dry matter, 106.06 % on root dry matter and 214.60 % considering the total dry matter, when compared with the control group (Table 3).

On the second highest dose, 215 mg kg^{-1}, the significant effects caused by inoculation of TW were limited to increases of 14.61 % on plant height, 66.07 % on root dry matter and 22.62 % considering the total dry matter (Table 3). Additionally, the inoculation of T3 not only did not cause any negative effects on plant development, but also it caused an increase of 15.51 % on plant height, when compared to control group (Table 3).

On the final and higher P dose, 290 mg kg^{-1}, only T3 manifested significant differences from control treatment, presenting an increase of 14.81 % on plant height and a decrease of 43.20 % on root dry matter (Table 3).

Nutrient concentration

Considering nutrients concentration, most of the significant changes happened on micronutrients, particularly on the three first P doses (Tables 4 and 5). On the first dose, wild-type fungus caused reductions on shoot nutrients concentration, but on root nutrients concentration there were increments on K, S and Zn (Tables 4 and 5).

The transformed fungus also impacted nutrient acquisition on shoot on the first P dose, but it only caused reduction on Fe and increments on Zn and B. On root tissues, the fungus increased S and Zn (Tables 4 and 5).

On the second P dose, 70 mg kg^{-1}, the outcome was drastically different for the wild-type fungus, which caused increases on shoot nutrients concentration, especially P and Zn (Tables 4 and 5). Transformed fungus only caused a rise of 121.46 % of Zn on shoot nutrients concentration, while on roots the increments were about 37.91 % of Ca, 75.32 % of Cu, 12.15 % of Fe and 113.01 % of Zn (Tables 4 and 5).

Table 4 – Impacts of wild-type and transformed T3 *Talaromyces aculeatus* on shoot (right) and root (left) macronutrients concentration of *Eucalyptus grandis* submitted to different P doses. Experiment conducted in greenhouse for 60 days.

Treatments	P (mg kg $^{-1}$)						P (mg kg $^{-1}$)					
	35	70	140	215	290	Mean	35	70	140	215	290	Mean
	N (dag kg^{-1})						N (dag kg^{-1})					
TW	-	-	2.69	2.95	2.45	2.70 ns	-	-	1.57	1.53	1.37	1.49 ns
T3	-	-	3.11	2.90	2.42	2.81	-	-	-	1.55	1.38	1.47
Control	-	-	2.87	2.97	2.51	2.78	-	-	1.29	1.55	1.28	1.37
Mean	-	-	2.89 A	2.94 A	2.46 B		-	-	1.43	1.54	1.34 ns	
CV(%)	15.19						8.07					
	P (dag kg^{-1})						P (dag kg^{-1})					
TW	0.08 b	0.23 a	0.24 a	0.16 ns	0.14 ns	0.17	0.11	0.12	0.15	0.13	0.11	0.12 ns
T3	0.22 a	0.13 b	0.14 b	0.18	0.16	0.17	0.12	0.09	0.14	0.13	0.12	0.12
Control	0.15 a	0.12 b	0.11 b	0.18	0.15	0.14	0.08	0.09	0.08	0.16	0.12	0.11
Mean	0.15	0.16	0.16	0.17	0.15		0.10	0.10	0.12	0.14	0.12 ns	
CV(%)	12.52						12.12					
	K (dag kg^{-1})						K (dag kg^{-1})					
TW	1.22	1.53	1.56	1.46	1.39	1.43 b	0.90	0.81	1.40	1.86	1.49	1.29 ns
T3	1.74	1.53	1.62	1.86	1.57	1.66 a	0.61	0.85	1.13	1.54	1.56	1.14
Control	1.56	1.45	1.44	1.52	1.59	1.51 ab	0.53	0.90	1.21	1.69	1.56	1.18
Mean	1.51	1.50	1.54	1.61	1.52 ns		0.68 C	0.85 C	1.25 B	1.70 A	1.54 AB	
CV(%)	11.27						12.51					
	Ca (dag kg^{-1})						Ca (dag kg^{-1})					
TW	0.46	0.67	0.59	0.63	0.73	0.62 ns	0.32 ns	0.30 ab	0.38 a	0.42 a	0.52 ns	0.39
T3	0.53	0.55	0.54	0.66	0.74	0.60	0.26	0.34 a	0.35 a	0.35 b	0.45	0.35
Control	0.62	0.44	0.52	0.63	0.82	0.61	0.29	0.25 b	0.25 b	0.43 a	0.50	0.34
Mean	0.54 B	0.55 B	0.55 B	0.64 AB	0.76 A		0.29	0.30	0.33	0.40	0.49	
CV(%)	9.90						10.93					
	Mg (dag kg^{-1})						Mg (dag kg^{-1})					
TW	0.19 b	0.28 ns	0.18 ab	0.20 ns	0.19 ns	0.21	0.24	0.17	0.15	0.21	0.19	0.19 ns
T3	0.24 ab	0.23	0.22 a	0.20	0.18	0.21	0.22	0.18	0.14	0.15	0.19	0.18
Control	0.27 a	0.23	0.17 b	0.20	0.17	0.21	0.15	0.18	0.16	0.17	0.19	0.17
Mean	0.23	0.25	0.19	0.20	0.18		0.20	0.18	0.15	0.18	0.19 ns	
CV(%)	6.14						12.70					
	S (dag kg^{-1})						S (dag kg^{-1})					
TW	0.19 b	0.38 a	0.26 ns	0.26 ns	0.19 ns	0.26	0.17	0.17	0.13	0.13	0.12	0.14 ns
T3	0.32 a	0.25 b	0.27	0.26	0.20	0.26	0.18	0.15	0.13	0.11	0.12	0.14
Control	0.27 a	0.25 b	0.22	0.26	0.18	0.24	0.12	0.14	0.11	0.14	0.11	0.12
Mean	0.26	0.29	0.25	0.26	0.19		0.16	0.15	0.12	0.13	0.12 ns	
CV(%)	12.86						16.84					

The uppercase letters indicate statistical difference between phosphate doses, lowercase letters between inoculation treatments and "ns" represents no significant difference, according to Tukey test ($p < 0.05$). Table cells filled with "-" represent missing value due to plant sample limitation.

Table 5 – Impacts of wild-type and transformed *Talaromyces aculeatus* T3 on shoot (right) and root (left) micronutrients concentration of *Eucalyptus grandis* submitted to different P doses. Experiment conducted in greenhouse for 60 days.

Treatments	P (mg kg $^{-1}$)												P (mg kg $^{-1}$)											
	35		70		140		215		290		Mean		35		70		140		215		290		Mean	
	Cu (mg kg^{-1})												Cu (mg kg^{-1})											
TW	9.66	b	21.38	a	19.41	a	11.34	ns	9.22	ns	14.20		20.06	ns	17.26	b	16.59	a	12.12	ns	8.94	ab	14.99	
T3	18.41	a	15.01	b	15.55	ab	14.06		10.04		14.61		24.71		23.39	a	13.63	ab	11.85		11.87	a	17.09	
Control	17.92	a	13.34	b	12.79	b	13.56		8.89		13.30		13.75		13.34	c	10.98	b	14.27		6.91	b	11.85	
Mean	15.33		16.58		15.92		12.99		9.38				19.51		18.00		13.73		12.75		9.24			
CV(%)	9.06												14.61											
	Fe (mg kg^{-1})												Fe (mg kg^{-1})											
TW	41.35	c	125.18	a	66.82	ab	70.17	ns	60.06	ns	72.72		10721.09	ns	13696.86	b	6487.23	b	4646.30	a	4404.30	b	7991.16	
T3	74.96	b	90.37	b	79.89	a	62.10		64.45		74.35		16533.06		15576.21	a	7747.91	a	3757.83	b	5661.73	a	9855.35	
Control	111.69	a	83.31	b	45.98	b	67.34		64.80		74.62		10549.88		13888.34	b	4370.79	c	3424.95	b	3799.24	b	7206.64	
Mean	76.00		99.62		64.23		66.54		63.10				12601.34		14387.14		6201.98		3943.03		4621.76			
CV(%)	8.74												6.01											
	Zn (mg kg^{-1})												Zn (mg kg^{-1})											
TW	54.02	b	125.77	a	50.23	b	45.41	b	56.55	ns	66.40		433.16	a	279.06	b	169.46	b	173.08	ns	166.16	b	244.18	
T3	113.21	a	126.83	a	100.10	a	122.45	a	54.12		103.34		320.08	a	611.80	a	224.57	a	151.98		291.37	a	319.96	
Control	66.59	b	57.27	b	42.42	b	53.35	b	77.06		59.34		165.93	b	287.21	b	152.08	b	151.04		121.11	b	175.47	
Mean	77.94		103.29		64.25		73.74		62.58				306.39		392.69		182.04		158.70		192.88			
CV(%)	10.39												11.08											
	Mn (mg kg^{-1})												Mn (mg kg^{-1})											
TW	662.86	b	1187.74	ns	218.97	b	178.05	ns	172.56	ns	484.04		120.25	ns	78.95	b	35.71	b	24.26	ns	32.39	a	58.31	
T3	1164.40	a	1037.49		464.34	a	186.66		177.08		605.99		128.94		116.24	a	69.08	a	32.45		25.52	ab	74.45	
Control	1188.93	a	873.14		175.55	b	186.66		176.02		520.06		91.42		110.04	a	27.90	b	29.39		20.87	b	55.92	
Mean	1005.40		1032.79		286.29		183.79		175.22				113.54		101.74		44.23		28.70		26.26			
CV(%)	4.86												5.88											
	B (mg kg^{-1})												B (mg kg^{-1})											
TW	24.39	c	25.44	ns	12.16	b	11.98	ns	10.59	a	16.91		74.72	ns	58.98	ns	22.83	ns	17.14	b	17.78	ns	38.29	
T3	49.78	a	27.06		17.48	a	11.58		10.74	a	23.33		61.87		81.64		22.85		23.53	a	16.15		41.21	
Control	32.79	b	27.67		9.99	b	10.21		7.75	b	17.68		64.29		70.06		21.49		18.65	b	20.61		39.02	
Mean	35.65		26.72		13.21		11.26		9.69				66.96		70.23		22.39		19.77		18.18			
CV(%)	4.20												13.99											

The letters indicate statistical difference and "ns" represents no significant difference, according to Tukey test ($p < 0.05$). Table cells filled with "-" represent missing value due to plant sample limitation.

On the third P dose, higher nutrients concentrations on shoot caused by wild fungus inoculation were about 116.31 % of P and 51.76 % of Cu. On roots, the shifts were 53.31 % of Ca, 51.12 % of Cu and 48.42 % of Fe (Tables 4 and 5). On the other hand, the transformed fungus impacted even more the nutrients concentration, especially micronutrients. The increases on concentration on shoot were 27.73 % of Mg, 73.74 % of Fe, 135.98 % of Zn, 164.50 % of Mn and 74.88 % of B. On roots the increments were of 38.48 % of Ca, 77.27 % of Fe, 47.67 % of Zn and 147.63 % of Mn (Tables 4 and 5).

On two highest P doses, inoculation consequences on nutrients concentrations were considerable weaker than the previous doses (Tables 4 and 5).

Nutrient content

Considering nutrients content, inoculation effects on shoot were concentered mostly on second and third P doses. On the other hand, on root tissues, the effects were considerably present on all the doses, except for the first one (Tables 6 and 7).

On the first dose, inoculation caused few effects on plants nutrients content, which included only a reduction of Ca on shoot, considering wild-type fungus treatment. However, on the transformed fungus treatment, there was reduction of Ca on shoot and considerable increment of Fe on roots (Tables 6 and 7).

Nutrient content of plants from the second P dose, 70 mg kg^{-1}, revealed huge positive impacts caused by wild-type fungus inoculation, particularly on shoot tissues (Tables 6 and 7). All nutrients on shoot were impacted and the increments were about 478.59 % of N, 745.27 % of P, 345.98 % of K, 503.90 % of Ca, 395.05 % of Mg, 516.84 % of S, 582.87 % of Cu, 515.12 % of Fe, 808.79 % of Zn, 467.20 % of Mn and 286.95 % of B (Tables 6 and 7). Moreover, on the roots, the only significant alteration was a gain of 104.79 % of Cu. In contrast, the alterations on transformed fungus treatment were comparatively very subtle and in opposite direction, restricted to decreases of 130.24 % of Fe and 136.54 % of Mn (Tables 6 and 7).

When the plants were submitted to 140 mg kg^{-1} of P, inoculation outcomes where very important and it was clear the opposite effects that the two different fungi caused on plant nutrients contents (Tables 6 and 7). On shoot tissues, wild-type fungus enhanced 190.73 % of P, 45.29 % of K, 42.54 % of Mg, 61.29 % of S, 103.96 % of Cu, 94.71 % of Fe, 59.97 % of

Table 6 – Impacts of wild-type and transformed *Talaromyces aculeatus* on shoot (left) and root (right) macronutrients content of *Eucalyptus grandis* submitted to different P doses. Experiment conducted in greenhouse for 60 days.

Treatments	P (mg kg $^{-1}$)						P (mg kg $^{-1}$)					
	35	**70**	**140**	**215**	**290**	**Mean**	**35**	**70**	**140**	**215**	**290**	**Mean**
			N (mg)						**N (mg)**			
TW	-	-	209.33 a	259.87 ns	210.86 ns	226.69	-	-	18.47 ns	28.37 a	25.59 a	24.14
T3	-	-	49.33 b	219.05	208.45	158.94	-	-	-	18.51 b	17.87 b	18.19
Control	-	-	165.91 a	225.85	215.67	202.48	-	-	17.51	17.42 b	22.80 a	19.24
Mean	-	-	141.52	234.92	211.66		-	-	17.99	21.43	22.09	
CV(%)	7.46						8.99					
			P (mg)						**P (mg)**			
TW	0.06 ns	3.61 a	18.35 a	13.73 ns	11.92 ns	9.53	0.02	0.21	1.83	2.36	2.14	1.31 a
T3	0.17	0.31 b	2.06 c	13.60	14.08	6.04	0.04	0.04	0.09	1.57	1.60	0.67 b
Control	0.29	0.43 b	6.31 b	13.71	12.57	6.66	0.01	0.10	1.12	1.74	2.12	1.02 b
Mean	0.17	1.45	8.91	13.68	12.86		0.02 B	0.12 B	1.01 AB	1.89 A	1.95 A	
CV(%)	14.43						11.25					
			K (mg)						**K (mg)**			
TW	0.88 ns	24.11 a	120.84 a	128.85 ns	119.70 ns	78.88	0.21 ns	1.40 a	16.69 a	34.45 a	27.93 a	16.14
T3	1.33	3.61 b	25.59 c	140.41	138.03	61.79	0.22	0.36 b	7.45 b	18.41 b	20.18 b	9.32
Control	2.91	5.41 b	83.17 b	115.26	138.28	69.01	0.11	1.00 ab	16.39 a	19.03 b	28.04 a	12.91
Mean	1.71	11.04	76.53	128.17	132.00		0.18	0.92	13.51	23.96	25.38	
CV(%)	8.10						6.06					
			Ca (mg)						**Ca (mg)**			
TW	0.32	10.10	46.22	55.58	62.36	34.92	0.07 ns	0.51 ns	4.55 a	7.87 a	9.67 a	4.53
T3	0.40	1.29	8.52	49.76	64.33	24.86	0.10	0.14	2.29 c	4.13 c	5.80 c	2.49
Control	1.06	1.67	30.07	47.98	71.70	30.50	0.06	0.27	3.40 b	4.83 b	8.88 b	3.49
Mean	0.59	4.35	28.27	51.11	66.13		0.08	0.31	3.41	5.61	8.12	
CV(%)	10.80						7.77					
			Mg (mg)						**Mg (mg)**			
TW	0.13 ns	4.27 a	14.16 a	17.84 ns	16.67 ns	10.61	0.05 ns	0.29 a	1.75 a	3.93 a	3.58 a	1.92
T3	0.18	0.54 b	3.51 c	15.39	15.77	7.08	0.08	0.07 b	0.93 b	1.83 b	2.44 b	1.07
Control	0.50	0.86 b	9.94 b	14.97	14.99	8.25	0.03	0.20 ab	2.13 a	1.87 b	3.46 a	1.54
Mean	0.27	1.89	9.20	16.07	15.81		0.05	0.19	1.60	2.54	3.16	
CV(%)	7.55						7.21					
			S (mg)						**S (mg)**			
TW	0.13 ns	5.81 a	20.55 a	22.60 ns	16.36 ns	13.09	0.04 ns	0.29 ns	1.58 a	2.39 a	2.20 a	1.30 ns
T3	0.24	0.59 b	4.27 c	19.84	17.08	8.40	0.07	0.06	0.83 b	1.31 b	1.59 b	0.77
Control	0.51	0.94 b	12.74 b	19.49	15.53	9.84	0.02	0.15	1.44 a	1.53 b	1.99 ab	1.03
Mean	0.29	2.45	12.52	20.64	16.32		0.04	0.17	1.28	1.74	1.93 ns	
CV(%)	9.03						16.16					

The uppercase letters indicate statistical difference between phosphate doses, lowercase letters between inoculation treatments and "ns" represents no significant difference, according to Tukey test ($p < 0.05$). Table cells filled with "-" represent missing value due to plant sample limitation.

Table 7 – Impacts of wild-type and transformed *Talaromyces aculeatus* on shoot (left) and root (right) micronutrients content of *Eucalyptus grandis* submitted to different P doses. Experiment conducted in greenhouse for 60 days.

Treatments	P (mg kg $^{-1}$)												P (mg kg $^{-1}$)											
	35		70		140		215		290		Mean		35		70		140		215		290		Mean	
	Cu (mg)												Cu (mg kg^{-1})											
TW	0.001	ns	0.033	a	0.151	a	0.100	ns	0.079	ns	0.07		0.0005	ns	0.0030	a	0.0198	a	0.0225	a	0.0167	a	0.01	
T3	0.001		0.004	b	0.024	c	0.106		0.090		0.05		0.0009		0.0010	b	0.0091	c	0.0141	b	0.0154	ab	0.01	
Control	0.003		0.005	b	0.074	b	0.103		0.076		0.05		0.0003		0.0015	b	0.0149	b	0.0160	b	0.0124	b	0.01	
Mean	0.00		0.01		0.08		0.10		0.08				0.0006		0.0018		0.0146		0.0175		0.0148			
CV(%)	12.17												8.56											
	Fe (mg)												Fe (mg)											
TW	0.003	ns	0.192	a	0.518	a	0.614	a	0.515	ns	0.37		0.25	ab	2.38	a	7.74	a	8.63	a	8.22	ns	5.44	
T3	0.006		0.022	b	0.127	c	0.470	b	0.557		0.24		0.63	a	0.67	b	5.15	b	4.49	b	7.34		3.66	
Control	0.020		0.031	b	0.266	b	0.512	ab	0.574		0.28		0.20	b	1.54	a	5.94	b	3.85	b	6.82		3.67	
Mean	0.01		0.08		0.30		0.53		0.55				0.36		1.53		6.28		5.66		7.46			
CV(%)	10.32												8.29											
	Zn (mg)												Zn (mg)											
TW	0.004	ns	0.193	a	0.390	a	0.397	b	0.485	b	0.29		0.010	ns	0.049	a	0.202	a	0.321	a	0.310	b	0.18	
T3	0.009		0.031	b	0.159	c	0.921	a	0.470	b	0.32		0.012		0.026	b	0.149	b	0.181	b	0.379	a	0.15	
Control	0.013		0.021	b	0.244	b	0.406	b	0.676	a	0.27		0.003		0.032	ab	0.205	a	0.170	b	0.218	c	0.13	
Mean	0.01		0.08		0.26		0.57		0.54				0.01		0.04		0.19		0.22		0.30			
CV(%)	9.41												8.11											
	Mn (mg)												Mn (mg)											
TW	0.05	ns	1.79	a	1.70	a	1.57	ns	1.48	ns	1.32		0.003	ns	0.014	a	0.043	ns	0.045	ns	0.060	a	0.03	
T3	0.09		0.25	b	0.73	b	1.41		1.53		0.80		0.005		0.005	b	0.045		0.039		0.033	b	0.03	
Control	0.20		0.32	b	1.01	b	1.41		1.52		0.89		0.002		0.012	a	0.038		0.033		0.038	b	0.02	
Mean	0.11		0.79		1.15		1.46		1.51				0.00		0.01		0.04		0.04		0.04			
CV(%)	8.21												9.34											
	B (mg)												B (mg)											
TW	0.002	ns	0.039	a	0.094	a	0.105	a	0.091	a	0.07		0.002	ns	0.010	a	0.027	a	0.032	a	0.033	a	0.02	
T3	0.004		0.007	b	0.028	c	0.088	ab	0.094	a	0.04		0.002		0.004	b	0.015	b	0.028	ab	0.021	b	0.01	
Control	0.006		0.010	b	0.058	b	0.078	b	0.068	b	0.04		0.001		0.008	ab	0.029	a	0.021	b	0.037	a	0.02	
Mean	0.00		0.02		0.06		0.09		0.08				0.00		0.01		0.02		0.03		0.03			
CV(%)	6.91												10.73											

The letters indicate statistical difference and “ns” represents no significant difference, according to Tukey test ($p < 0.05$). Table cells filled with “-” represent missing value due to plant sample limitation.

Zn, 68.61 % of Mn and 63.90 % of B (Tables 6 and 7). On roots, the changes were limited to increases of 33.82 % of Ca, 32.91 % of Cu and 30.41 % of Fe (Tables 6 and 7).

In contrast, *T. aculeatus* (T3) inoculation caused considerable losses on nutrients content, in both shoot and root tissues (Tables 6 and 7). On shoot, the reductions were about 234.28 % of N, 206.02 % of P, 225.00 % of K, 253.03 % of Ca, 183.34 % of Mg, 198.00 % of S, 203.50 % of Cu, 109.62 % of Fe, 53.54 % of Zn and 107.68 % of B (Table 5). On roots, it was observed decreases of 120.13 % of K, 48.06 % of Ca, 129.11 % of Mg, 72.83 % of S, 63.63 % of Cu, 38.10 % of Zn and 93.18 % of B (Tables 6 and 7).

Considering 215 mg kg^{-1}, *T. aculeatus* (TW) only enhanced B on shoot, but on root it caused important increments on almost all nutrients (Tables 6 and 7). However, T3 inoculation only increased Zn on shoot and caused a loss Ca on root (Tables 6 and 7).

On the final dose, changes on nutrients content were more significant on root than on shoot (Tables 6 and 7). On shoot, wild-type fungus inoculation caused increases of B and loss of Zn, but on roots the increments included Ca, Cu, Zn and Mn (Tables 6 and 7). Alternatively, on shoot, transformed fungus caused reduction on Zn and increment on B, while on roots, it caused a rise on Zn and losses on N, K, Ca, Mg and B (Tables 6 and 7).

Considering total content, inoculation impacts were stronger on second and third P dose (70 and 140 mg kg^{-1}). On the first dose, only the wild-type fungus caused a significant effect on plant, which was a decrease of Ca (Table 8). Alternatively, on the second dose, wild-type enhanced all analyzed nutrients, which included rises of 623.16 % of P, 298.10 % of K, 445.99 % of Ca, 328.86 % of Mg, 456.56 % of S, 472.12 of Cu, 63.67 % of Fe, 354.60 % of Zn, 450.25 % of Mn and 175.25 % of B (Table 8). In contrast, transformed fungus caused only decreases of 127.57 % of Fe and 76.38 % of B (Table 8).

On the next dose, 140 mg kg^{-1}, wild fungus also increased significantly contents of all nutrients, except for nitrogen. The increments were about 171.56 % of P, 38.14 % of K, 51.68 % of Ca, 31.90 % of Mg, 56.02 % of S, 92.06 % of Cu, 33.17 % of Fe, 31.85 % of Zn, 66.53 % of Mn and 40.64 % of B (Table 8). On the other hand, transformed fungus caused reductions on all measured nutrients, except for Fe. The losses on nutrients content were of 147.79 % of P, 201.37 % of K, 209.54 % of Ca, 171.98 % of Mg, 177.55 % of S, 165.49 % of Cu, 46.08 % of Zn, 35.07 % of Mn and 102.59 % of B (Table 8).

On the fourth P dose, inoculation effects were weaker than on two previous ones for both fungi (Table 8). Wild-type fungus caused increases of Mg, Fe, Zn and B, while the

Table 8 – Impacts of wild-type and transformed *Talaromyces aculeatus* T3 on the total nutrients content of *Eucalyptus grandis* submitted to different P doses. Experiment conducted in greenhouse for 60 days.

Treatments	P (mg kg $^{-1}$)						P (mg kg $^{-1}$)					
	35	**70**	**140**	**215**	**290**	**Mean**	**35**	**70**	**140**	**215**	**290**	**Mean**
	N (mg)						**Cu (mg)**					
TW	-	-	227.80 ns	288.25 ns	236.45 ns	250.83	0.002 ns	0.036 a	0.170 a	0.122 ns	0.096 ns	0.09
T3	-	-	-	237.56	226.32	231.94	0.002	0.005 b	0.033 c	0.120	0.105	0.05
Control	-	-	183.42	243.27	238.47	221.72	0.004	0.006 b	0.089 b	0.119	0.088	0.06
Mean	-	-	205.61	256.36	233.75		0.00	0.02	0.10	0.12	0.10	
CV(%)	9.02						9.65					
	P (mg)						**Fe (mg)**					
TW	0.08 ns	3.83 a	20.17 a	16.10 ns	14.06 ns	10.85	0.25 ns	2.58 a	8.26 a	9.24 a	8.74 ns	5.81
T3	0.21	0.35 b	3.00 c	15.16	15.68	6.88	0.63	0.69 c	5.27 b	4.96 b	7.90	3.89
Control	0.30	0.53 b	7.43 b	15.46	14.68	7.68	0.22	1.57 b	6.20 b	4.36 b	7.39	3.95
Mean	0.20	1.57	10.20	15.57	14.81		0.37	1.61	6.58	6.19	8.01	
CV(%)	12.41						7.81					
	K (mg)						**Zn (mg)**					
TW	1.09 ns	25.51 a	137.53 a	163.30 ns	147.63 ns	95.01	0.016 ns	0.241 a	0.592 a	0.718 b	0.795 ns	0.47
T3	1.55	3.97 b	33.04 c	158.82	158.21	71.12	0.021	0.057 b	0.307 c	1.103 a	0.850	0.47
Control	3.02	6.41 b	99.56 b	134.28	166.32	81.92	0.016	0.053 b	0.449 b	0.576 c	0.893	0.40
Mean	1.89	11.96	90.04	152.13	157.39		0.02	0.12	0.45	0.80	0.85	
CV(%)	6.41						6.71					
	Ca (mg)						**Mn (mg)**					
TW	0.39 b	10.61 a	50.77 a	63.45 ns	72.03 ns	39.45	0.063 ns	1.803 a	1.744 a	1.618 ns	1.540 ns	1.35
T3	0.49 ab	1.43 b	10.81 c	53.89	70.13	27.35	0.091	0.252 b	0.775 c	1.449	1.562	0.83
Control	1.11 a	1.94 b	33.47 b	52.81	80.58	33.98	0.205	0.328 b	1.047 b	1.447	1.563	0.92
Mean	0.66	4.66	31.68	56.72	74.25		0.12	0.79	1.19	1.50	1.56	
CV(%)	9.91						6.52					
	Mg (mg)						**B (mg)**					
TW	0.18 ns	4.56 a	15.91 a	21.77 a	20.25 ns	12.53	0.004 ns	0.049 a	0.122 a	0.137 a	0.124 ns	0.09
T3	0.26	0.62 b	4.44 c	17.23 b	18.21	8.15	0.006	0.010 c	0.043 c	0.116 ab	0.115	0.06
Control	0.53	1.06 b	12.06 b	16.84 b	18.46	9.79	0.007	0.018 b	0.086 b	0.098 b	0.105	0.06
Mean	0.32	2.08	10.80	18.61	18.97		0.01	0.03	0.08	0.12	0.11	
CV(%)	5.57						6.42					
	S (mg)											
TW	0.17 ns	6.10 a	22.13 a	24.99 ns	18.56 ns	14.39						
T3	0.31	0.66 b	5.11 c	21.15	18.67	9.18						
Control	0.53	1.10 b	14.18 b	21.02	17.53	10.87						
Mean	0.34	2.62	13.81	22.39	18.25							
CV(%)	7.61											

The letters indicate statistical difference and "ns" represents no significant difference, according to Tukey test ($p < 0.05$). Table cells filled with "-" represent missing value due to plant sample limitation.

transformed fungus only enhanced Fe (Table 8). Finally, on the highest P dose, there was no significant effect on any nutrient content for both inoculated fungi (Table 8).

Nutrient utilization efficiency

Nutrient utilization efficiency calculations revealed that the most important alterations due to fungal inoculation occurred on the second and third P doses, especially on second one (Tables 9). On the first dose, the only significant effects were decreases of 437.00 % and 146.27 % on Zn, caused by wild-type and transformed *T. aculeatus* inoculation, respectively (Table 9).

Considering the second dose, wild-type fungus treatment presented higher nutrient utilization efficiency for all considered nutrients (Tables 9). In contrast, there was no difference between control group and T3 treatment on nutrient utilization efficiency for this dose (Tables 9).

On the third P dose, wild-type only caused a reduction on P utilization efficiency, while T3 fungus reduced significantly utilization efficiency for all considered nutrients (Tables 9).

Some significant alterations on nutrient utilization efficiency were also observed on the fourth dose for both fungi inoculation (Tables 9). Wild-type enhanced utilization efficiency of N, K, Mg, S, Cu and Mn (Tables 9). On the other hand, T3 fungus only caused losses on Fe and Zn (Table 9).

Finally, on the highest P dose, the only significant alteration occurred on B utilization efficiency, when both wild-type and transformed fungus caused reductions (Table 9).

P availability on soil and plant growth

Soil analyses using samples collected in intervals during experiment conduction confirmed the expected differences on P content and remaining P between the five P doses applied (Figure 4). According to results, P content on soil varied in both directions until 45 days and suffered a reduction in the last 15 days, particularly on the three highest doses (Figure 4). However, remaining P assumed a clear crescent behavior from 30 days of experiment for all P doses applied (Figure 4).

Table 9 – Comparison of macronutrient and micronutrient utilization efficiency index (E) of three different treatments (wild-type, transformed T3 and control) of *Eucalyptus grandis* submitted to different P doses. Experiment conducted in greenhouse for 60 days.

Treatments	P (mg kg $^{-1}$)				
	35	70	140	215	290
			N (mg)		
TW	-	-	367690 ns	397429 a	463312 ns
T3	-	-	-	322700 ab	449277
Control	-	-	298887	313765 b	475303
CV(%)	9.60				
			P (mg)		
TW	59604 ns	794292 a	3971525 b	7271956 ns	7841598 ns
T3	96373	225895 b	1711191 c	5409297	6498025
Control	141712	454551 b	7359363 a	5144463	8426817
CV(%)	15.78				
			K (mg)		
TW	4701 ns	118583 a	586116 a	698990 a	743027 ns
T3	13239	19897 b	153656 b	482101 b	632746
Control	13442	36360 b	553764 a	567184 b	672685
CV(%)	7.54				
			Ca (mg)		
TW	13704 ns	322173 a	1710034 a	1808313 ns	1524738 ns
T3	42403	54701 b	469954 b	1437543	1423594
Control	35670	121564 b	1637609 a	1475221	1402538
CV(%)	12.43				
			Mg (mg)		
TW	30918 ns	679698 a	5037022 a	5251726 a	5415504 ns
T3	82511	127241 b	1144380 b	4446179 b	5502837
Control	74644	220823 b	4510019 a	4517916 b	6096324
CV(%)	8.50				
			S (mg)		
TW	31668 ns	495159 a	3718951 a	4571416 a	5901170 ns
T3	66411	119902 b	1000974 b	3618180 b	5332567
Control	76075	212272 b	3958747 a	3648555 b	6415679
CV(%)	9.63				

	P (mg kg $^{-1}$)				
	35	70	140	215	290
			Cu (mg)		
TW	4587679 ns	83043960 a	472460609 a	932893792 a	1142729056 ns
T3	8791533	17184459 b	152645067 b	663675381 b	975299648
Control	11297504	36513211 b	625920634 a	654081528 b	1312928904
CV(%)	12.95				
			Fe (mg)		
TW	28828 ns	1184285 a	9912010 a	12396335 ab	12569039 ns
T3	39101	120181 b	961950 b	15601405 b	12675524
Control	272588	151757 b	8833372 a	17722005 a	15091063
CV(%)	11.25				
			Zn (mg)		
TW	448253 ns	12563758 a	135428236 a	160993029 a	139272876 ns
T3	977430	1407917 b	16771005 b	70569266 b	118652834
Control	2407123	4410570 b	121344629 a	132174162 a	124863468
CV(%)	10.27				
			Mn (mg)		
TW	115354 ns	429476858 a	48904495 a	70563300 a	71315230 ns
T3	236932	323247 b	6580014 b	52810037 b	64388112
Control	191047	716445 b	53437570 a	52525546 b	72388959
CV(%)	12.91				
			B (mg)		
TW	1940914 ns	61856070 a	659839620 a	838577685 a	886459498 b
T3	3904729	7953924 b	120342761 b	661989477 b	868783719 b
Control	5647173	13930966 b	629481164 a	771824213 ab	1060347438 a
CV(%)	7.60				

The letters indicate statistical difference and "ns" represents no significant difference, according to Tukey test ($p < 0.05$). Table cells filled with "-" represent missing value due to plant sample limitation.

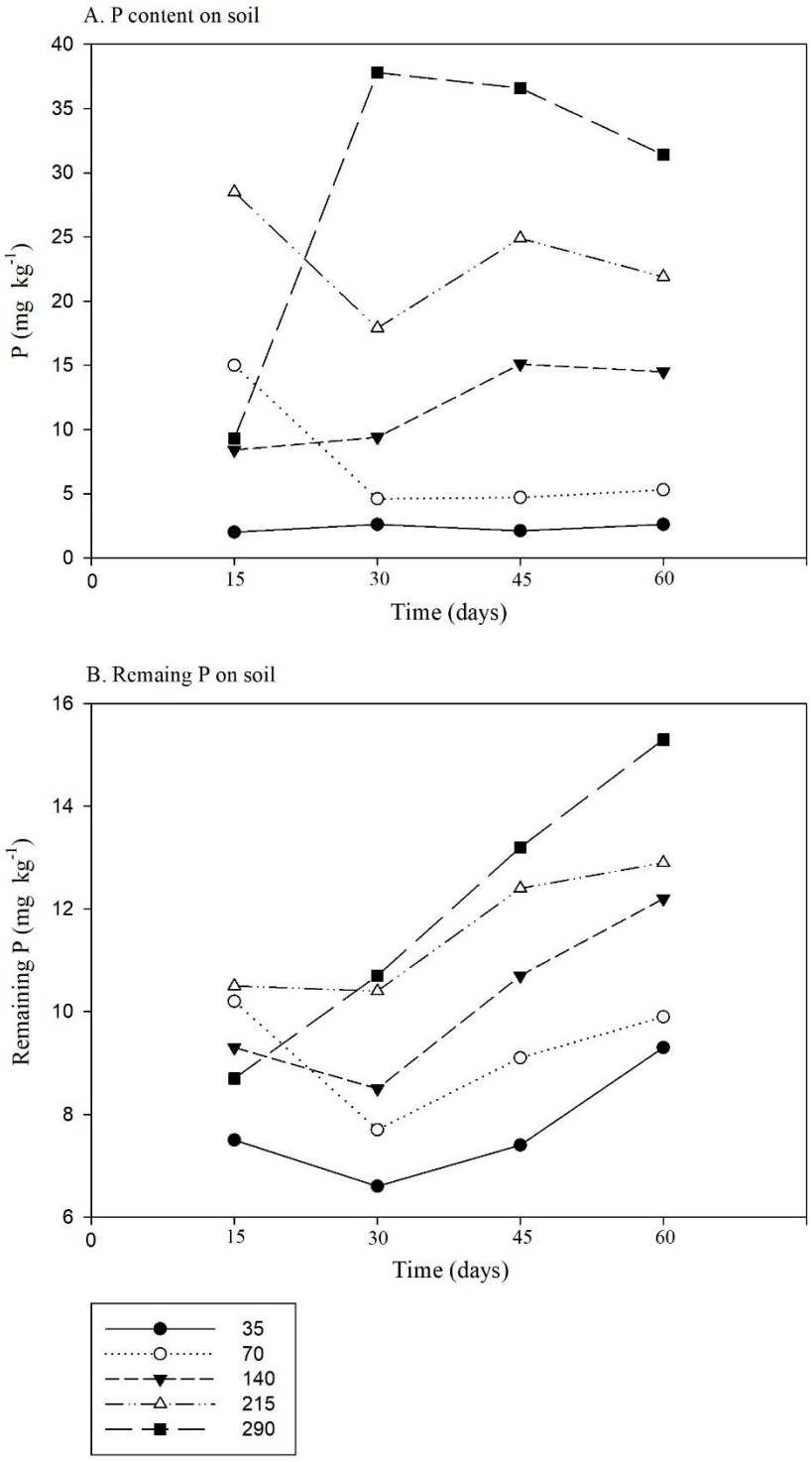

Figure 4 – P content and remaining P on soil during experiment time. The experiment was conducted in greenhouse, during 60 days. Soil samples were collected and analyzed in intervals of 15 days.

Regression

All curves for plant growth express significant interaction between the factors: phosphorous doses and fungal treatment (Figure 5). The differences in the models are more expressive on the phosphorous interval of 35 to 200 mg kg^{-1}.

Considering regression curves for shoot nutrient content, factors interaction was significant to all response variables. Overall, the models expressed clearly how wild-type maximized the plant nutrient acquisition, while the transformed fungus reduced it, particularly in the phosphorous interval of 35 to 200 mg kg^{-1} (Figure 6).

On root nutrient content, interactions between factors were also significant for all response variables, except for P root content (Figure 7). The pattern suggested by curve models was different from shoot content because the curves values remain distinct from each other in the whole interval modeled (35 to 290 mg kg^{-1}) (Figure 8). Generally, the curves clearly illustrated how wild *T. aculeatus* was able to maximize nutrient content on the roots, while T3 mostly reduced it (Figure 7).

Finally, on the total nutrient content regression curves, the pattern observed was similar to shoot nutrient content (Figure 8). All response variables were affected by factors interaction and the interval of 50 to 200 mg kg^{-1} presented more differences between treatments models for the majority of variables (Figure 8). Curve models also indicated high values resulted from wild-type fungus treatment, followed by control group and lowest values from T3 treatment, except for rare and specific conditions in which T3 overcame the other treatments, such as Cu and Zn (Figure 8).

Regression equations which originated the curves are available on supplementary material, in the last section of this chapter (Tables 8, 9 and 10).

Discussion

The initial objective was obtaining a transformed fungus which would have the same performance on plant interaction, but it did not happen in the experiment. Although this fact compromises the intention to measure fungal colonization, effects on plant development from T3 inoculation were importantly concentrated in the same P interval that positive effects from the wild-type fungus were stronger. It might suggest a nutritional interval, particularly related

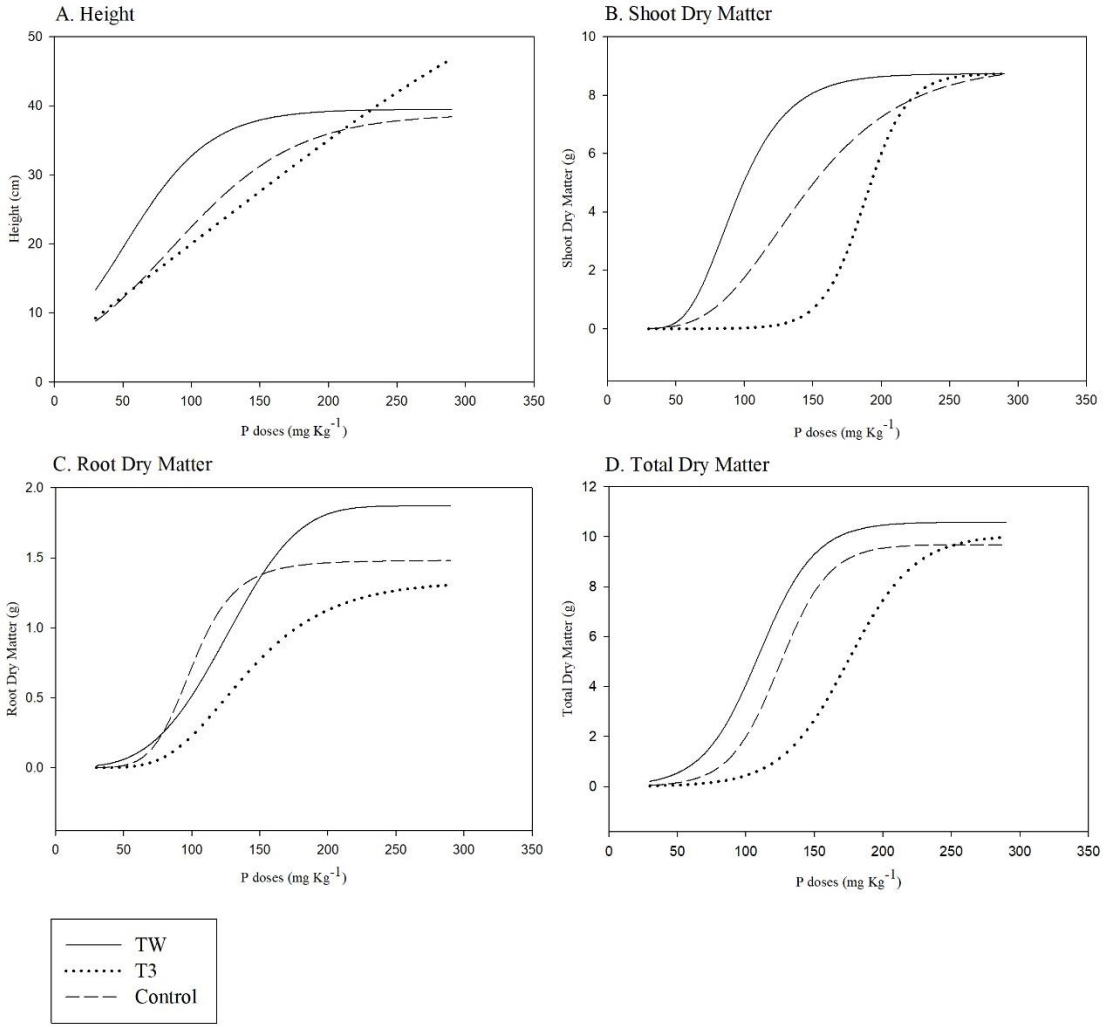

Figure 5 – Regression curves for measured plant growth features resulted from different P doses on soil and *Talaromyces aculeatus* inoculation (wild-type and transformed fungi) on plants roots. The experiment was conducted in greenhouse, during 60 days.

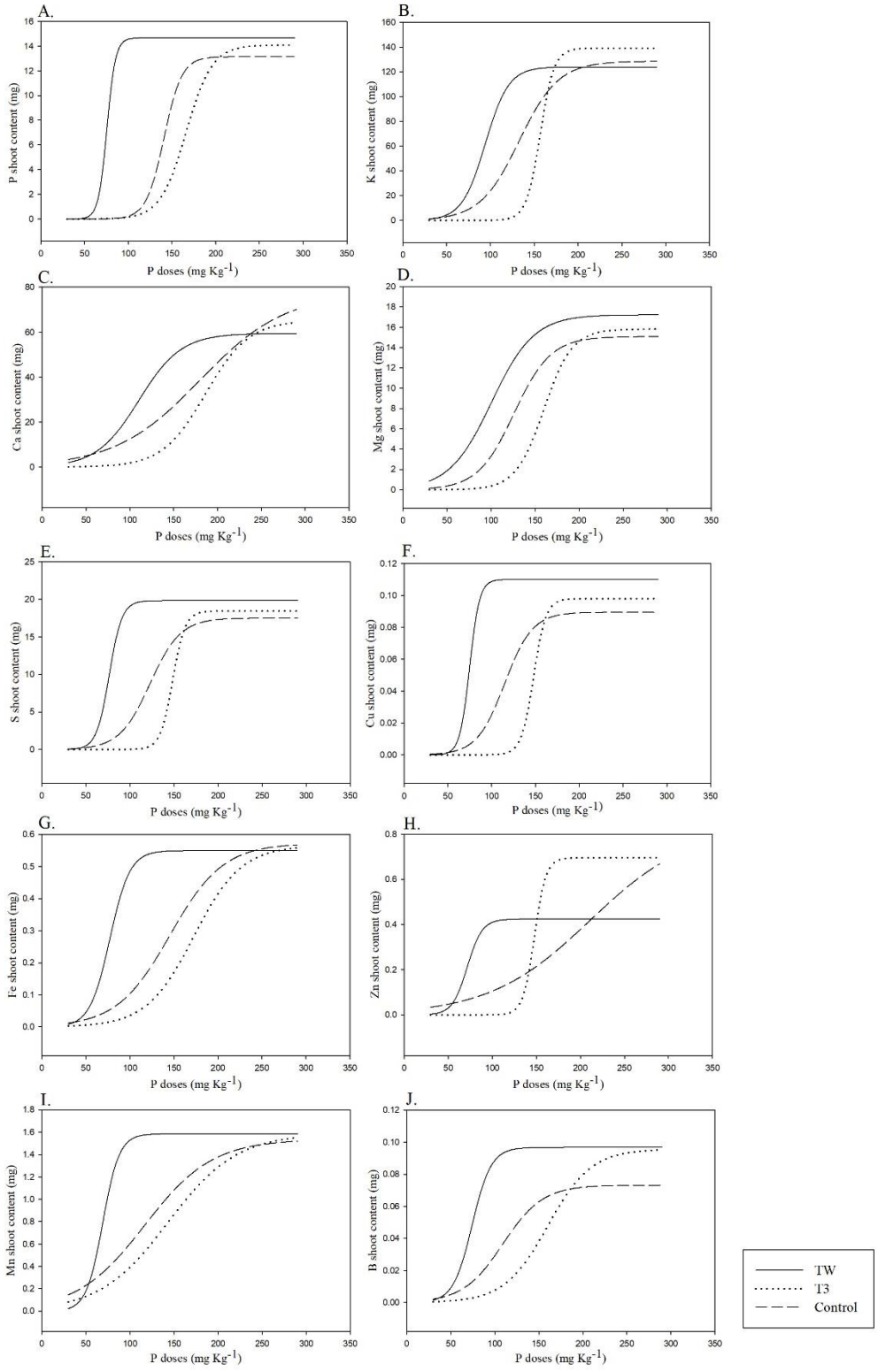

Figure 6 – Regression curves for shoot nutrients content resulted from the interaction between different P doses on soil and *Talaromyces aculeatus* inoculation (wild-type and transformed fungi) on plants roots. The experiment was conducted in greenhouse, during 60 days.

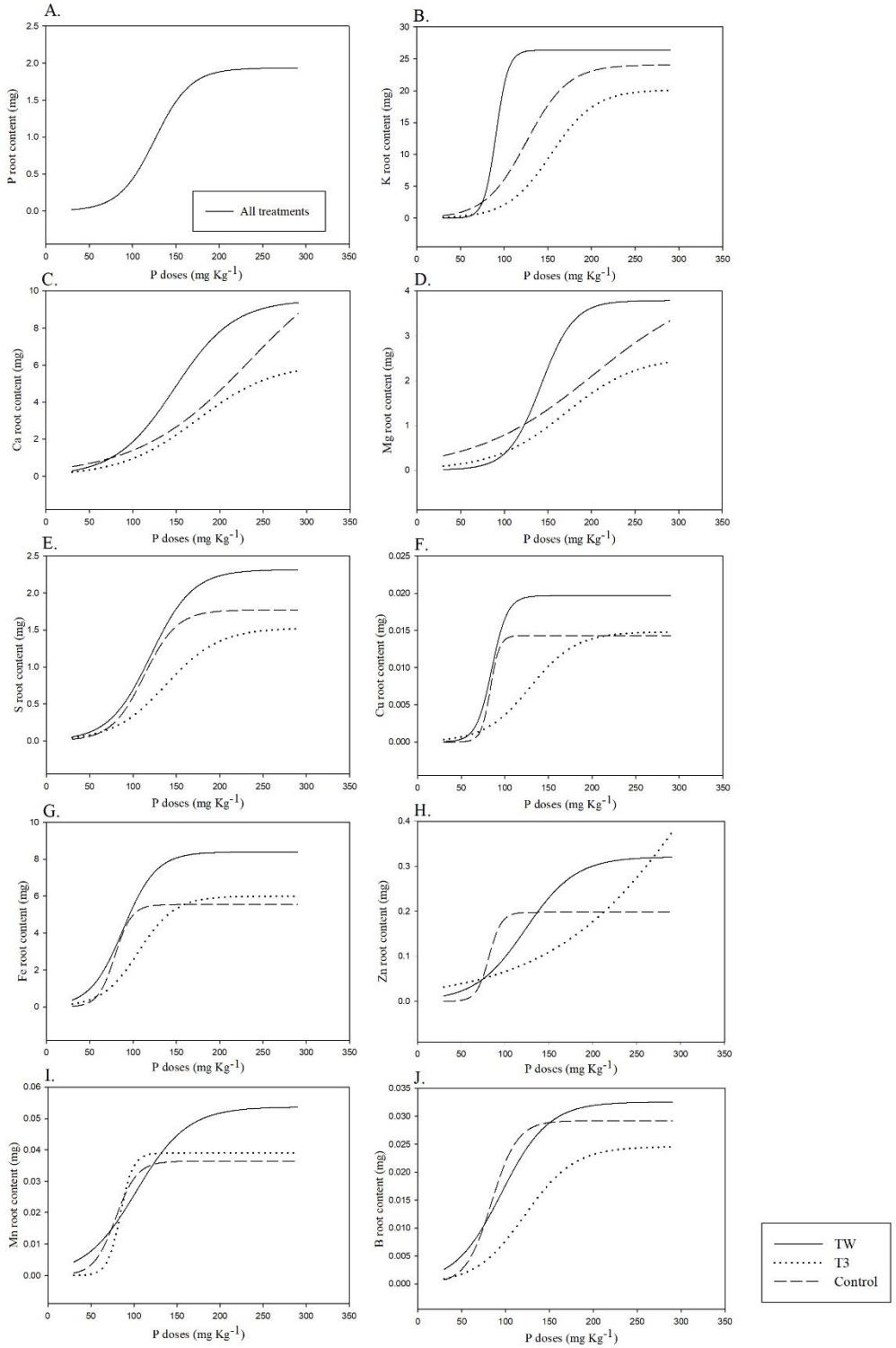

Figure 7 – Regression curves for root nutrients content resulted from the interaction between different P doses on soil and *Talaromyces aculeatus* inoculation (wild-type and transformed fungi) on plants roots. The experiment was conducted in greenhouse, during 60 days.

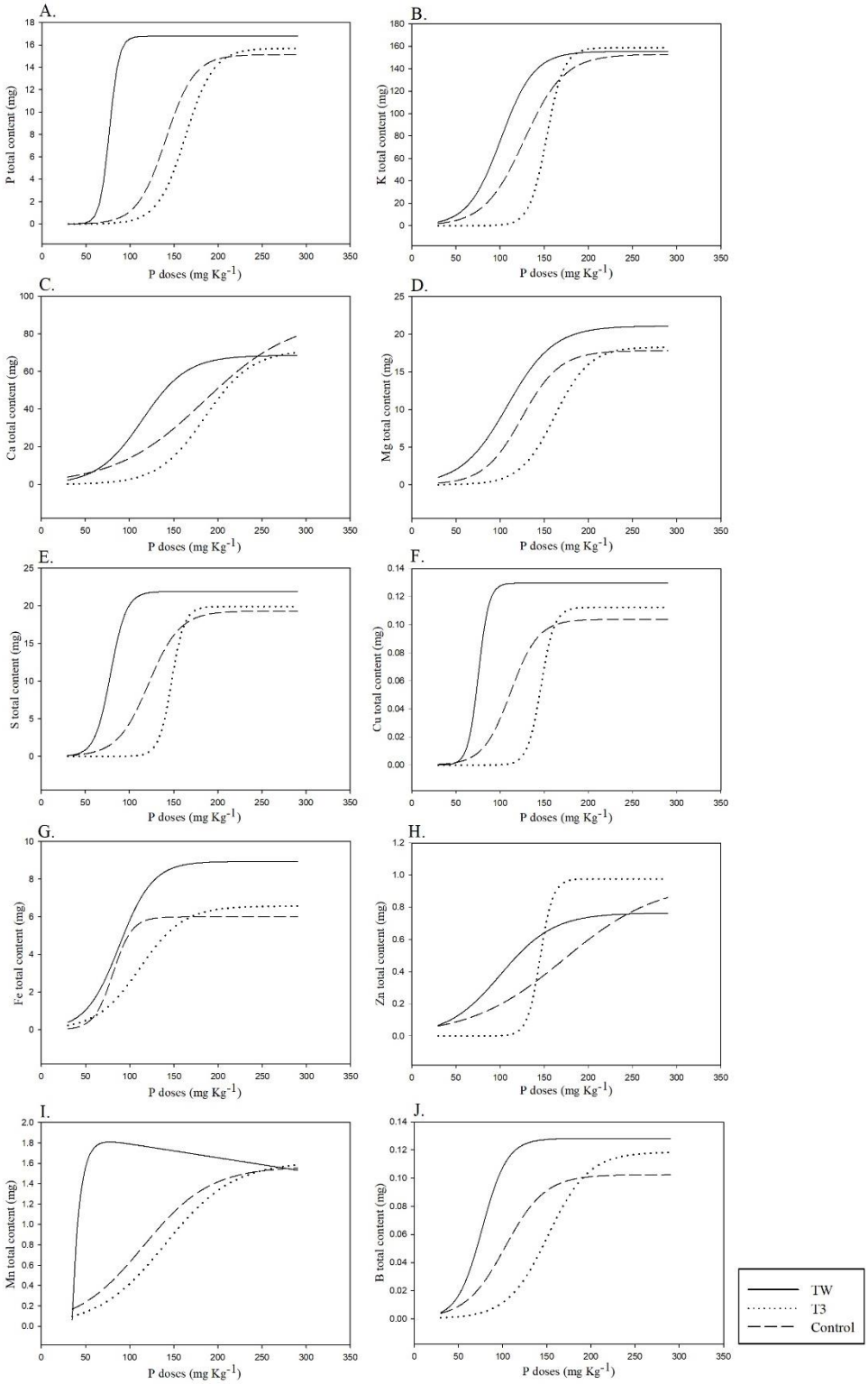

Figure 8 – Regression curves for total nutrients content resulted from the interaction between different P doses on soil and *Talaromyces aculeatus* inoculation (wild-type and transformed fungi) on plants roots. The experiment was conducted in greenhouse, during 60 days.

to P availability, where interactions establishment between plants and microorganisms are more favorable to happen.

Additionally, it was already reported that even a plant growth promoting fungi can compete with plants in nutritional limiting situations (Vinci et al., 2018). In this study, specifically with P variation on soil, it was noticed that both fungi, with different interactional outcomes, had their impact depending on P availability on soil.

Regulatory factors related to phosphate stress response might have played an important role on colonization allowance by fungi on this study. It was already demonstrated that the main transcriptional regulator of phosphate stress response (PHR1) is also directly involved on defense repression. Thus, on phosphate deprivation situation, the plant could prioritize dealing to stress condition over plant defenses (Castrillo et al., 2017).

Similarly, transcriptomics analyses showed that *Colletotrichum tofieldiae* interaction with *Arabidopsis* responded to phosphate availability. While plant defense responses were activated during phosphate sufficient conditions, the interaction between the beneficial fungus and plant was favored when phosphate was limited (Hacquard et al., 2016). Additionally, the transference of P from fungal hyphae by *C. tofieldiae* to plant roots only occurred during P starvation (Hacquard et al., 2016). On the other hand, in the interaction between *Arabidopsis* and *C. incanum*, a pathogen closely related to *C. tofieldiae,* the plant defense responses were not dependent to P status, they were retained independently of P availability (Hacquard et al., 2016).

Our results clearly pointed out that dependency of P status in the soil to fungal interaction establishment and consequent effects. Although, it was not possible to define the alterations that T3 suffered due to transformation process, which led the fungus to harm plant development on lower doses of phosphorous instead of causing benefits, the interaction establishment was concentered when P was less available, as it happened to wild-type fungus. The transformed fungus possibly had important genes interrupted or it was overexpressing the red protein, which might had compromised its outcomes from plant interaction. On the other hand, genes that might be important to interaction establishment possibly were not altered due to transformation, once plant still permitted had effects from T3 presence on the low doses of P.

Another point to be investigated in future studies is that variation in responses is a particularity to phosphorous or if similar effects would happen with other nutrients variation on soil. Considering that *T. aculeatus* had major impacts on micronutrients acquisition, which

were not limited on this study, future studies with limiting concentrations of these elements may reveal even stronger results in terms of plant growth promotion.

Considering the promotion mechanisms tested, wild-type fungus differed significantly from T3 fungus on indoleacetic acid production capacity and, especially, on P solubilization potential (Table 2). Although means of IAA production were statistically different, the values were very close: 11.06 µg mL $^{-1}$ to wild-type fungus and 13.09 µg mL $^{-1}$ to T3. Therefore, it might not have played an important role on the drastic outcomes observed on greenhouse experiment.

On the other hand, differences on P solubilization parameters were dramatic between the fungi, and they might represent important physiological alterations on T3 due to interruption important genes or overexpression of the inserted ones, as a result of transformation. The lower values of pH measured from T3 samples are connected with its higher capacity on organic acids production, evidenced by titratable acidity data (Table 2). It suggests potential deflection of carbon to acid organic metabolic pathways.

Additionally, wild-type fungus negative values on solubilized P suggest immobilization of P by it and its biomass were significantly greater to three of the four tested phosphates (Table 2). Therefore, solubilization experiment *in vitro* pointed out differences in access and utilization of P by the two fungi. Once that on greenhouse experiment it was used calcium phosphate to offer different P doses to soil, the diverse outcomes due to fungal inoculation might be related with their interaction with plants in response to P availability for them.

It's important to notice that, on P solubilization experiment, wild fungus manifested lower values when compared to transformed fungus, but it converted almost all medium in metabolites and probably removed P from the offered sources and incorporated on its biomass. On greenhouse situation, the wild fungus increased the plant access to phosphorous, evidenced for increment on plant shoot tissues on 70 and 140 mg kg^{-1} doses (Table 4). Therefore, on greenhouse experiment, wild fungus was better solubilizer than T3, despite of the results from *in vitro* experiment.

Nutrient content for T3 treatment were considerably lower than the other treatments, it happened because plants were considerably smaller, so even with larger nutrient concentrations, their nutrient content was lower (Figures 7 and 8). Moreover, T3 nutrients content on doses 215 and 290 mg kg^{-1} surpassed the other treatments because plant

development level between treatments has closer values, so it can reflect the higher concentration of these nutrients, such as Cu, Zn and B (Figures 7 and 8).

Decreases on plant morphological features might be explained by a toxic accumulation effect from some micronutrients, which caused development problems to plants, especially on doses 70 and 140 mg kg^{-1} of P. Root growth on the dose 70 mg kg^{-1} was compromised probably due to Zn accumulation (Table 4). On the other hand, on dose 140 mg kg^{-1}, deleterious effect on plant development might be related to significant increases on Zn and Mn on shoot and or increases on concentrations of Fe, Zn and Mn on roots (Table 4).

Plants using strategies like accumulation of Zn to cause toxic effects on microbial pathogens and insects attack were already reported, particularly in plants species capable to accumulate metals (Cabot et al., 2019). Moreover, despite of no information specifically about plants, hypothesis of strategies of plants sequestering Zn to starve pathogens is plausible, considering the similarities between Zn and Fe dynamics and importance to pathogenicity, and also the fact that in animal systems that strategy was already observed (Cabot et al., 2019). The outcomes observed on T3 treatment in low P doses, particularly higher absorption of micronutrients, could be related to a possible unknow plant mechanism to cope with a harmful interaction with T3.

The capacity of *T. aculeatus* to increase nutrient concentration and plant growth already was reported to sorghum. The isolate presented heavy metal tolerance and its inoculation on plant increased concentrations of P, As, Cu, Pb and Zn. The growth promotion mechanisms manifested by the fungal isolate included production of IAA, siderophores, phytases and phosphatases (Babu et al., 2014).

Augmentations on K content might be related to the fact of growth promoting fungi are able not only to release P, but they can efficiently release K, such as *Aspergillus niger* (Wang et al., 2018). Assay conducted *in vitro* indicated increase of 127.59 % in potassium release, which is consequence of organic acids action produced by the fungus, such as oxalic, tartaric and citric acid (Wang et al., 2018).

Similarly, fungi from *Aspergillus* and *Penicillium* genera were reportedly able to release Mn and Zn through organic acid action (Anitha et al., 2015; Mohanty et al., 2016; Gaind, 2016). In this study, T3 especially increased the concentration of micronutrients like Zn and Mn in plant tissues, then it might be related to its capacity to produce organic acids, which was confirmed on P solubilization experiment, resulting on higher availability of the minerals to direct plant access. Moreover, the higher Fe concentrations in plants observed on

inoculated treatments, especially on T3 treatment, are related to fungal capabilities of producing siderophores, which are molecules able to chelate Fe and deliver to plant in its soluble form.

Conclusions

The different doses of phosphorus in the soil significantly impacted the interaction between *T. aculeatus* and *E. grandis*, suggesting an interval in which the plant allows the fungus to colonize and thus its effects are more significant for its development. The dependence on phosphorus status for the establishment of interactions between plants and fungi, and the concentration of effects in situations of phosphorus limitation, indicate the potential action of adaptive systems to deal with the lack of the element, which are related to the plant defensive responses.

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Supplementary material

Table 8 – Regression equations for plant morphological features.

Measured Variable	Treatment	Equation			R^2
Height	TW	$y = a/(1 + be^{-cx})$	a	39.03	0.9956
			b	23.31	
			c	0.05	
	T3	$y = a(b - e^{-cx})$	a	55.96	0.9871
			b	6.32	
			c	0.01	
	Control	$y = a/(1 + be^{-cx})$	a	38.86	0.9644
			b	6.61	
			c	0.02	
SDM	TW	$y = a/(1 + be^{-cx})$	a	8.71	0.9989
			b	200.41	
			c	0.05	
	T3	$y = a/(1 + be^{-cx})$	a	8.79	0.9996
			b	2070.56	
			c	0.04	
	Control	$y = a/(1 + be^{-cx})$	a	8.54	0.9950
			b	139.52	
			c	0.04	
RDM	TW	$y = a/(1 + be^{-cx})$	a	1.88	0.9996
			b	199.98	
			c	0.04	
	T3	$y = a/(1 + be^{-cx})$	a	1.28	0.9981
			b	288.35	
			c	0.04	
	Control	$y = a/(1 + be^{-cx})$	a	1.46	0.9084
			b	1765.65	
			c	0.07	
TDM	TW	$y = a/(1 + be^{-cx})$	a	10.58	0.9989
			b	168.85	
			c	0.05	
	T3	$y = a/(1 + be^{-cx})$	a	10.06	0.9997
			b	1122.53	
			c	0.04	
	Control	$y = a/(1 + be^{-cx})$	a	9.68	0.9825
			b	759.70	
			c	0.06	

Table 9 – Regression equations for shoot nutrients content.

Measured Variable	Treatment	Equation			R^2
P	TW	$y = a/(1 + be^{-cx})$	a	14.67	0.9028
			b	4595455.51	
			c	0.20	
	T3	$y = a/(1 + be^{-cx})$	a	14.09	0.9994
			b	71230.61	
			c	0.07	
	Control	$y = a/(1 + be^{-cx})$	a	13.14	0.9943
			b	990150.94	
			c	0.10	
K	TW	$y = a/(1 + be^{-cx})$	a	124.03	0.997
			b	798.80	
			c	0.08	
	T3	$y = a/(1 + be^{-cx})$	a	139.24	0.9991
			b	207084714.14	
			c	0.13	
	Control	$y = a/(1 + be^{-cx})$	a	128.65	0.9852
			b	304.83	
			c	0.04	
Ca	TW	$y = a/(1 + be^{-cx})$	a	59.47	0.9924
			b	97.13	
			c	0.04	
	T3	$y = a/(1 + be^{-cx})$	a	65.32	0.9998
			b	1914.27	
			c	0.04	
	Control	$y = a/(1 + be^{-cx})$	a	77.51	0.9915
			b	40.31	
			c	0.02	
Mg	TW	$y = a/(1 + be^{-cx})$	a	17.23	0.9918
			b	66.13	
			c	0.04	
	T3	$y = a/(1 + be^{-cx})$	a	15.83	0.9989
			b	21624.29	
			c	0.06	
	Control	$y = a/(1 + be^{-cx})$	a	15.08	0.9994
			b	441.87	
			c	0.05	
S	TW	$y = a/(1 + be^{-cx})$	a	19.84	0.9464
			b	39056.81	
			c	0.14	
	T3	$y = a/(1 + be^{-cx})$	a	18.46	0.9879
			b	1674217702.57	
			c	0.14	
	Control	$y = a/(1 + be^{-cx})$	a	17.52	0.9721
			b	1217.68	
			c	0.06	

Measured Variable	Treatment	Equation			R^2
Cu	TW	$y = a/(1 + be^{-cx})$	a	0.11	0.7988
			b	563173.00	
			c	0.18	
	T3	$y = a/(1 + be^{-cx})$	a	0.10	0.9852
			b	1413669516.96	
			c	0.14	
	Control	$y = a/(1 + be^{-cx})$	a	0.09	0.9547
			b	1427.37	
			c	0.06	
Fe	TW	$y = a/(1 + be^{-cx})$	a	0.55	0.9765
			b	910.30	
			c	0.09	
	T3	$y = a/(1 + be^{-cx})$	a	0.57	0.9996
			b	612.64	
			c	0.04	
	Control	$y = a/(1 + be^{-cx})$	a	0.57	0.9988
			b	124.38	
			c	0.03	
Zn	TW	$y = a/(1 + be^{-cx})$	a	0.42	0.9628
			b	4648.15	
			c	0.12	
	T3	$y = a/(1 + be^{-cx})$	a	0.70	0.8258
			b	7420587149.75	
			c	0.15	
	Control	$y = a/(1 + be^{-cx})$	a	0.84	0.9791
			b	40.06	
			c	0.02	
Mn	TW	$y = a/(1 + be^{-cx})$	a	1.58	0.9870
			b	1625.62	
			c	0.11	
	T3	$y = a/(1 + be^{-cx})$	a	1.59	0.9974
			b	39.67	
			c	0.03	
	Control	$y = a/(1 + be^{-cx})$	a	1.54	0.9979
			b	20.82	
			c	0.03	
B	TW	$y = a/(1 + be^{-cx})$	a	0.10	0.9861
			b	998.76	
			c	0.09	
	T3	$y = a/(1 + be^{-cx})$	a	0.10	0.9949
			b	654.11	
			c	0.04	
	Control	$y = a/(1 + be^{-cx})$	a	0.07	0.9847
			b	124.90	
			c	0.04	

Table 10 – Regression equations for root nutrients content.

Measured Variable	Treatment	Equation			R^2
P	All	$y = a/(1 + be^{-cx})$	a	1.93	0.9998
			b	436.88	
			c	0.05	
K	TW	$y = a/(1 + be^{-cx})$	a	26.37	0.8307
			b	483280.05	
			c	0.14	
	T3	$y = a/(1 + be^{-cx})$	a	20.16	0.9996
			b	468.44	
			c	0.04	
	Control	$y = a/(1 + be^{-cx})$	a	24.08	0.9346
			b	203.94	
			c	0.04	
Ca	TW	$y = a/(1 + be^{-cx})$	a	9.51	0.9914
			b	76.34	
			c	0.03	
	T3	$y = a/(1 + be^{-cx})$	a	6.10	0.9847
			b	52.25	
			c	0.02	
	Control	$y = a/(1 + be^{-cx})$	a	12.56	0.9626
			b	37.08	
			c	0.02	
Mg	TW	$y = a/(1 + be^{-cx})$	a	3.79	0.9896
			b	1935.50	
			c	0.05	
	T3	$y = a/(1 + be^{-cx})$	a	2.54	0.9922
			b	61.25	
			c	0.02	
	Control	$y = a/(1 + be^{-cx})$	a	4.25	0.8795
			b	19.20	
			c	0.01	
S	TW	$y = a/(1 + be^{-cx})$	a	2.32	0.9937
			b	148.89	
			c	0.04	
	T3	$y = a/(1 + be^{-cx})$	a	1.53	0.9869
			b	94.17	
			c	0.03	
	Control	$y = a/(1 + be^{-cx})$	a	1.77	0.9668
			b	393.24	
			c	0.05	

Measured Variable	Treatment	Equation			R^2
Cu	TW	$y = a/(1 + be^{-cx})$	a	0.02	0.9679
			b	14603.10	
			c	0.11	
	T3	$y = a/(1 + be^{-cx})$	a	0.01	0.9964
			b	140.85	
			c	0.04	
	Control	$y = a/(1 + be^{-cx})$	a	0.01	0.9811
			b	8387288.58	
			c	0.19	
Fe	TW	$y = a/(1 + be^{-cx})$	a	8.38	0.9973
			b	105.50	
			c	0.05	
	T3	$y = a/(1 + be^{-cx})$	a	5.99	0.8771
			b	158.69	
			c	0.05	
	Control	$y = a/(1 + be^{-cx})$	a	5.54	0.8522
			b	3617.39	
			c	0.10	
Zn	TW	$y = a/(1 + be^{-cx})$	a	0.32	0.9961
			b	74.68	
			c	0.03	
	T3	$y = a/(1 + be^{-cx})$	a	1.11	0.9523
			b	47.60	
			c	0.01	
	Control	$y = a/(1 + be^{-cx})$	a	0.20	0.9694
			b	100954.89	
			c	0.14	
Mn	TW	$y = a/(1 + be^{-cx})$	a	0.05	0.9687
			b	32.06	
			c	0.03	
	T3	$y = a/(1 + be^{-cx})$	a	0.04	0.9337
			b	77945.92	
			c	0.13	
	Control	$y = a/(1 + be^{-cx})$	a	0.04	0.9832
			b	541.79	
			c	0.08	
B	TW	$y = a/(1 + be^{-cx})$	a	0.03	0.9960
			b	35.24	
			c	0.04	
	T3	$y = a/(1 + be^{-cx})$	a	0.02	0.9321
			b	77.14	
			c	0.04	
	Control	$y = a/(1 + be^{-cx})$	a	0.03	0.8504
			b	309.48	
			c	0.07	

Table 10 – Regression equations for total nutrients content.

Measured Variable	Treatment	Equation			R^2
P	TW	$y = a/(1 + be^{-cx})$	a	16.78	0.9330
			b	1576659.62	
			c	0.19	
	T3	$y = a/(1 + be^{-cx})$	a	15.71	0.9995
			b	28373.15	
			c	0.06	
	Control	$y = a/(1 + be^{-cx})$	a	15.14	0.9971
			b	5848.41	
			c	0.06	
K	TW	$y = a/(1 + be^{-cx})$	a	155.33	0.9940
			b	230.65	
			c	0.05	
	T3	$y = a/(1 + be^{-cx})$	a	158.64	0.9993
			b	7258147.41	
			c	0.10	
	Control	$y = a/(1 + be^{-cx})$	a	152.73	0.9795
			b	281.32	
			c	0.04	
Ca	TW	$y = a/(1 + be^{-cx})$	a	68.60	0.9919
			b	93.03	
			c	0.04	
	T3	$y = a/(1 + be^{-cx})$	a	71.52	0.9999
			b	1085.37	
			c	0.04	
	Control	$y = a/(1 + be^{-cx})$	a	88.66	0.9718
			b	38.92	
			c	0.02	
Mg	TW	$y = a/(1 + be^{-cx})$	a	21.09	0.9915
			b	63.42	
			c	0.04	
	T3	$y = a/(1 + be^{-cx})$	a	18.30	0.9992
			b	3905.42	
			c	0.05	
	Control	$y = a/(1 + be^{-cx})$	a	17.82	0.9963
			b	327.57	
			c	0.05	
S	TW	$y = a/(1 + be^{-cx})$	a	21.90	0.9547
			b	6817.95	
			c	0.11	
	T3	$y = a/(1 + be^{-cx})$	a	19.91	0.9910
			b	756961880.73	
			c	0.14	
	Control	$y = a/(1 + be^{-cx})$	a	19.29	0.9817
			b	974.44	
			c	0.06	

Measured Variable	Treatment	Equation			R^2
Cu	TW	$y = a/(1+be^{-cx})$	a	0.13	0.8428
			b	346938.27	
			c	0.17	
	T3	$y = a/(1+be^{-cx})$	a	0.11	0.9890
			b	273822773.38	
			c	0.13	
	Control	$y = a/(1+be^{-cx})$	a	0.10	0.9553
			b	1354.11	
			c	0.06	
Fe	TW	$y = a/(1+be^{-cx})$	a	8.94	0.9966
			b	105.74	
			c	0.05	
	T3	$y = a/(1+be^{-cx})$	a	6.57	0.8903
			b	94.55	
			c	0.04	
	Control	$y = a/(1+be^{-cx})$	a	5.99	0.8719
			b	1858.62	
			c	0.09	
Zn	TW	$y = a/(1+be^{-cx})$	a	0.76	0.9836
			b	28.74	
			c	0.03	
	T3	$y = a/(1+be^{-cx})$	a	0.98	0.9622
			b	600030852.57	
			c	0.14	
	Control	$y = a/(1+be^{-cx})$	a	0.96	0.9486
			b	25.41	
			c	0.02	
Mn	TW	$y = a + br^x + cx$	a	1.93	0.9998
			b	-119.53	
			c	0.00	
			r	0.89	
	T3	$y = a/(1+be^{-cx})$	a	1.62	0.9981
			b	39.19	
			c	0.03	
	Control	$y = a/(1+be^{-cx})$	a	1.57	0.9979
			b	21.11	
			c	0.03	
B	TW	$y = a/(1+be^{-cx})$	a	0.13	0.9902
			b	238.18	
			c	0.07	
	T3	$y = a/(1+be^{-cx})$	a	0.12	0.9907
			b	732.52	
			c	0.04	
	Control	$y = a/(1+be^{-cx})$	a	0.10	0.9970
			b	97.87	
			c	0.04	

CAPÍTULO 3:

PLANT GROWTH PROMOTION POTENTIAL OF *Talaromyces aculeatus* FOR DIFFERENT EUCALYPT SPECIES.

Abstract

The indiscriminate use of chemical fertilizers and pesticides represent not only damages the environment, but also high economic costs. The market of biofertilizers is constantly growing and it offers an opportunity to apply alternative methods that potentially can increase productivity with reduced cultivation costs and lower levels of negative environmental impacts. Soil microorganisms can benefit vegetal development in different ways, such as increase on nutrient uptake, suppression of pathogens and rise on responses to diverse abiotic stresses. Several species of Plant Growth Promoting Fungi (PGPF) already had their abilities to benefit plants through different promoting mechanisms demonstrated and characterized, but some approaches were not explored or still rarely contemplated. The aim of this study is to investigate the capacity of *Talaromyces aculeatus* to promote growth of six different eucalypt species. Eucalypt seedlings from each species were obtained through seed germination. The plants were cultivated in vases with 3 dm^3 of soil of oxisol. Two treatments (inoculated and non-inoculated), with four repetitions each, were kept for 60 days in greenhouse for each one of the six species. After this period, the plants had their height and dry matter measured, micro and micronutrients analysis were executed and the data were submitted to variance analysis and plant nutrient use efficiency were calculated. The species manifested different growth measures and nutritional gains. Overall, *Eucalyptus urograndis* and *Eucalyptus grandis* were the species mostly benefited by *T. aculeatus* inoculation, represented specially by increments of 32.21 % on *E. urograndis* total dry matter and higher nutrient acquisition by *E. grandis* when inoculated. Additionally, *Eucalyptus urophylla* and *Corymbia torelliana* were especially impacted on root tissues by *T. aculeatus* inoculation, increasing dry matter and nutrients acquisition. Although *Eucalyptus saligna* and *Eucalyptus cloeziana* were not influenced by fungal inoculation, there were increases on nutrient acquisition. Therefore, *T. aculeatus* impacted differently the six species evaluated. According to data, even plants from the same genus may establish interaction with PGPF differently, and as a result, distinct outcomes might be manifested from their relationship.

Introduction

The plantation sector of forest species has an important role in world economy, especially because it provides feedstock to originate diverse products with great demand and value on the world market, which include products like cellulose, charcoal, paper and wood

panels. In Brazil, the sector is in constant growth and it is very important to national economy, representing 10.2 % of value added on Brazil's GDP (Gross Domestic Product) on the last decade (IBA, 2021).

The plantations in Brazil are concentrated especially on the southeast region and *Eucalyptus* genus is the most planted due to its diversity of species, high adaptability, wood use versatility and productivity (IBA, 2021). However, as in any activity, there are some challenges to achieve higher levels of productivity, such as low fertility levels on soil and plant pathogens. Since there is a crescent concern about to find ways to reduce use of chemical fertilizers and pesticides, the interest for biofertilizers is rising because they offer diverse benefits to plant cultivation and represent economic advantages without damaging the environment (Ahkami et al., 2017; Naik et al., 2019).

Fungi have a special position as beneficial microorganisms because they play important roles on crucial soil processes, such as decomposition of organic matter, soil structuration, nutrients cycling and pathogen suppression (Yedidia et al., 2001; Singh et al., 2006; Dini-Andreote and Raaijmakers, 2018). Moreover, diverse studies have shown their potential to benefit plant through several promotion mechanisms, such as phosphate solubilization, phytohormones and siderophores production, among others (Gupta et al., 2015; Rodriguez et al. 2019).

Despite of the considerable amount of information about benefits resulted from microorganism's application on agricultural activities, many aspects of microbial ecology remain unclear, particularly the level of specificity to them with different plant species. For example, it was reported that some plant growth promoting rhizobacteria can manifest specificity on cultivar level, which is a challenge to develop products to general use (Rodriguez et al. 2019). The available data for plant growth promoting fungi (PGPF) is very scarce on that subject, mainly when it is about eucalypt interaction with them.

Studies have already demonstrated considerably different outcomes when the same PGPF is applied to plants with certain phylogenetic distance, but if the fungi performance with plants closely related would result on uniform outcomes, it was not properly approached by them (Wooliver et al., 2018; Baron et al., 2020).

The PGPF *Talaromyces aculeatus* on this work was isolated from the rhizosphere of *Eucalyptus grandis* from the silviculture sector of Universidade Federal de Viçosa, in 2016, as part of a study of *E. grandis* growth promotion for master dissertation (Reis 2018). Posteriorly, the isolate was identified using molecular tools and selected to this study due its

capacity of *E. grandis* growth promotion and desirable features, such as rapid growth and efficient sporulation.

On the literature, the fungus *T. aculeatus* already had mentions as plant growth promoter, but not related with eucalypt species (Babu et al., 2014; Istina et al., 2015; Efthymiou et al., 2018; Raymond et al., 2018). Most of the studies available explored its efficient capabilities as a producer of diverse metabolites and enzymes (Lee et al., 2013; Zhang et al., 2017; Krishnamurthy et al., 2018).

Therefore, the aim of this study is to investigate *Talaromyces aculeatus* capabilities to promote growth of six different eucalypt species. This study hypothesizes that *T. aculeatus* is able to establish interaction with different eucalypt species and that the interaction outcomes from closely related plant species are similar.

Materials and Methods

The experiment was conducted in a greenhouse located at Universidade Federal de Viçosa, Minas Gerais. Six species (*Eucalyptus grandis*, *Eucalyptus urophylla*, *Eucalyptus cloeziana*, *Eucalyptus urograndis*, *Eucalyptus saligna and Corymbia torelliana)* were inoculated with the fungus *Talaromyces aculeatus.* The genus *Corymbia* is closely related to *Eucalyptus*, all belonging to the tribe Eucalypteae, which includes around 860 species distributed in seven genera and its members are commonly referred to the descriptive name “eucalypt”.

The experiment consisted of 12 treatments represented by each one of the six selected species inoculated with *T. aculeatus* and its control group (plant without inoculation). The experiment was constructed in a completely randomized design with four replications to each treatment.

The plants were cultivated during 60 days in plastic vases with 3.0 dm^{-3} of Latossolo Vermelho-amarelo distrófico (Red-yellow oxisol - chemical and physical analysis on Table 1) collected from the Viçosa region (coordinates: 20° 46’ 009’’ S 42° 52’ 43.4’’ W). The soil fertilization was made according to Novais et al. (1991), however using only half of the amounts recommended: 150 mg kg^{-1} ($Ca(H_2PO_4)_2$) of P, 75 mg kg^{-1} of K (KCl), 80 mg kg^{-1} of Ca ($CaCl_2$), 20 mg $kg^{-1}$of S, 2.00 mg $kg^{-1}$of Zn ($ZnSO_4 . 7 H_2O$), 1.83 mg kg^{-1} of Mn ($MnCl_2 . 4 H_2O$), 0.775 mg kg^{-1} of Fe ($FeCl_2 \cdot 4 H_2O$), 0.665 mg kg^{-1} of Cu ($CuSO_4 . 5 H_2O$), 0.405 mg kg^{-1} of B (H_3BO_3), 0.075 mg kg^{-1} of Mo ($NaMoO_4 . 2 H_2O$) e 20 mg kg^{-1} of Mg ($MgCl_2$). Reduction on nutrients offer is to amplify the chances of growth promotion process to happen,

Table 1 - Physical and chemical soil composition from Viçosa region, Minas Gerais.

Mineral Fraction (%)										
Fine sand		**Coarse sand**		**Silt**		**Clay**		**Texture**		
9.2		14.1		4.2		72.4		Very Clayey		
Chemical Analysis										
pH	**P**	**K^+**	**H+Al**	**Al^{3+}**	**Ca^{2+}**	**Mg^{2+}**	**CEC**	**V**	**M**	**OM**
H_2O	mg dm^{-3}		$cmol_c$ dm^{-3}					%		dag Kg^{-1}
4.92	0.4	0	3.4	0.39	0.22	0.03	3.65	6.8	60.9	1.6

considering fungal effects might be lower or even insignificant on soils with abundant nutrients availability. The exception was N, which was kept the original recommendation of 100 mg kg^{-1} ($(NH_4)_2SO_4$ and NH_4NO_3) to avoid compromising seedlings initial growth.

The seeds were germinated in sterile sand, after superficial disinfection. The process includes the seeds immersion in 70% alcohol for 1 minute, then they were washed in distilled and autoclaved water, followed by immersion in 20% H_2O_2 and after seven minutes, successively washed. The seeds were inserted in the washed and autoclaved sand contented on plastic recipient in the laboratory. After germination the plants were transferred to greenhouse. Once a week the plants received Clark nutritive solution on full force (Clark, 1975) and the necessary amount of distilled water to maintain the sand field capacity in 60%. Water addition was controlled based on sand recipient's weight, then it was possible to measure water loss and replenish it. Four weeks later the plants were transplanted, one per vase, and each one received on the roots three 6 mm discs of mycelium from *T. aculeatus* colony.

The irrigation was made twice per day and after the 60 days of experimentation, the plants were collected and the following variables were evaluated: shoot, root and total dry matter, plant height, nutrients concentration and content (Donagema et al., 2011).

The quantification of nutrients was performed on Soil Science Department (DPS) at Federal University of Viçosa. Nitrogen quantification was made through sulfuric digestion and application of Kjeldahl method. All the other nutrients were submitted to nitropercloric digestion and analyzed on Plasma ICP-OES-Optima 8300 (Sarruge & Haag, 1974).

The efficiency of macro and micronutrients utilization was evaluated according to Siddiqi and Glass (1981). The index (E) was calculated using the formula: (E) = (Total Dry Matter)^2/(total nutrient content). Content was calculated based on the concentration values considering the total dry matter of plants. It was not possible to measure N of some treatments due to plant material limitation and it was represented on the tables with "-". The data was submitted to ANOVA ($p < 0.05$) on R 4.2.1 software Rstudio.

Results

Talaromyces aculeatus inoculation on radicular systems of different eucalypt species resulted in different outcomes to plants development and nutrients acquisition (Tables 2 to 7).

Table 2 – Impact of *Talaromyces aculeatus* inoculation on morphological aspects of eucalypt species. Experiment conducted in greenhouse during 60 days.

Species	Height (cm)		SDM (g)		RDM (g)		TDM (g)	
Eucalyptus grandis								
Control	44.83	b	15.36	b	3.97	ns	19.34	ns
T. aculeatus	48.67	a	16.50	a	3.71		20.22	
CV(%)	4.85		4.46		9.30		3.46	
Eucalyptus urophylla								
Control	51.00	b	11.18	ns	1.70	b	12.88	ns
T. aculeatus	56.38	a	12.55		2.56	a	15.11	
CV(%)	6.91		13.20		18.71		14.12	
Eucalyptus urograndis								
Control	49.25	ns	13.71	b	3.02	b	16.73	b
T. aculeatus	53.63		18.26	a	3.86	a	22.12	a
CV(%)	14.80		15.59		16.73		12.64	
Eucalyptus cloeziana								
Control	14.67	ns	1.36	ns	0.50	ns	1.86	ns
T. aculeatus	16.75		1.60		0.56		2.16	
CV(%)	15.60		19.29		19.88		16.55	
Eucalyptus saligna								
Control	43.5	ns	17.02	ns	3.84	ns	20.86	ns
T. aculeatus	48.67		14.53		4.32		18.85	
CV(%)	7.42		18.78		18.94		17.74	
Corymbia torelliana								
Control	45.67	b	7.75	ns	0.21	b	7.96	ns
T. aculeatus	51.75	a	7.83		0.35	a	8.18	
CV(%)	8.51		7.85		26.80		7.74	

The letters indicate statistical difference and “ns” represents no significant difference, according to ANOVA Test F ($p < 0.05$). SDM – Shoot Dry Matter, RDM – Root Dry Matter and TDM – Total Dry Matter.

Table 3 – Impact of *Talaromyces aculeatus* inoculation on nutrients concentrations in the shoot of eucalypt species. Experiment conducted in greenhouse during 60 days.

Species	N	P	K	Ca	Mg	S	Cu	Fe	Zn	Mn	B
	dag kg $^{-1}$						mg kg $^{-1}$				
Eucalyptus grandis											
Control	1.45 b	0.20 ns	1.46 b	0.93 ns	0.20 ns	0.12 b	5.36 b	45.90 b	32.97 b	743.42 b	23.00 ns
T. aculeatus	1.66 a	0.22	1.85 a	0.90	0.20	0.17 a	6.30 a	53.78 a	45.72 a	876.20 a	22.62
CV(%)	8.55	14.83	13.41	9.31	8.13	18.38	10.47	9.55	19.35	11.28	10.24
Eucalyptus urophylla											
Control	1.77 ns	0.21 ns	1.77 ns	0.71 ns	0.20 ns	0.15 ns	4.98 ns	50.75 ns	30.97 ns	654.05 b	19.71 b
T. aculeatus	1.80	0.23	1.79	0.76	0.20	0.18	6.12	59.03	28.39	810.15 a	27.69 a
CV(%)	7.77	11.84	7.04	18.32	12.50	18.91	19.75	19.98	19.02	12.22	19.01
Eucalyptus urograndis											
Control	1.72 a	0.23 a	1.72 ns	0.85 a	0.19 a	0.14 ns	7.59 a	47.89 ns	26.81 ns	741.73 a	18.77 ns
T. aculeatus	1.26 b	0.16 b	1.37	0.59 b	0.15 b	0.11	5.34 b	39.83	21.86	556.59 b	15.88
CV(%)	18.74	19.39	19.37	19.28	14.65	18.55	19.94	17.39	18.20	19.27	14.30
Eucalyptus cloeziana											
Control	2.95 ns	0.24 ns	1.70 ns	0.77 ns	0.32 ns	0.24 b	10.46 ns	57.01 ns	35.74 b	447.81 b	31.22 ns
T. aculeatus	2.85	0.22	1.79	0.66	0.34	0.26 a	9.64	52.96	46.88 a	569.34 a	29.82
CV(%)	18.47	8.36	8.35	13.52	7.77	4.75	12.27	13.20	15.57	15.89	12.61
Eucalyptus saligna											
Control	1.49 ns	0.17 ns	1.36 b	0.77 b	0.18 b	0.13 ns	5.51 ns	54.41 ns	27.07 b	809.98 ns	17.86 b
T. aculeatus	1.75	0.17	1.66 a	0.99 a	0.21 a	0.17	5.09	63.23	51.36 a	806.96	25.24 a
CV(%)	13.63	15.26	12.87	15.34	11.80	19.87	19.47	23.10	33.91	11.60	19.08
Corymbia torelliana											
Control	2.87 ns	0.21 ns	1.65 ns	0.64 ns	0.20 b	0.21 ns	6.99 ns	58.22 ns	82.66 ns	791.42 ns	27.69 ns
T. aculeatus	2.72	0.24	1.90	0.69	0.23 a	0.22	5.65	56.59	80.61	788.08	21.89
CV(%)	5.91	9.16	15.29	11.60	8.41	14.07	18.52	16.70	12.76	12.34	17.37

The letters indicate statistical difference and “ns” represents no significant difference, according to ANOVA F Test ($p < 0.05$).

Table 4 – Impact of *Talaromyces aculeatus* inoculation on nutrients concentrations in the root of eucalypt species. Experiment conducted in greenhouse during 60 days.

Species	N	P	K	Ca	Mg	S	Cu	Fe	Zn	Mn	B
	dag kg $^{-1}$						mg kg $^{-1}$				
Eucalyptus grandis											
Control	0.83 b	0.13 ns	1.75 ns	0.39 ns	0.21 b	0.10 b	8.25 ns	2474.85 ns	47.54 ns	166.88 b	14.59 b
T. aculeatus	0.96 a	0.12	1.85	0.44	0.24 a	0.13 a	7.98	2287.48	46.64	224.12 a	19.53 a
CV(%)	9.20	9.80	5.06	12.14	11.21	17.43	9.74	18.06	18.70	18.35	18.58
Eucalyptus urophylla											
Control	0.85 ns	0.11 ns	1.44 ns	0.37 ns	0.21 ns	0.11 ns	7.66 ns	2407.60 ns	43.83 ns	254.62 ns	15.32 ns
T. aculeatus	0.83	0.10	1.36	0.38	0.19	0.10	6.23	2188.03	37.32	276.93	16.20
CV(%)	9.17	13.26	9.26	9.06	15.61	17.25	18.85	18.60	17.45	18.45	15.61
Eucalyptus urograndis											
Control	0.79 ns	0.10 ns	1.37 ns	0.43 ns	0.16 ns	0.09 ns	7.90 ns	1636.07 ns	53.61 ns	193.18 ns	11.86 b
T. aculeatus	0.79	0.09	1.24	0.41	0.17	0.08	7.54	1993.23	47.02	195.00	17.46 a
CV(%)	14.14	16.81	15.31	7.31	18.77	10.05	16.88	19.95	20.03	18.86	21.40
Eucalyptus cloeziana											
Control	-	0.13 ns	1.31 ns	0.38 ns	0.17 ns	0.12 ns	9.06 ns	5039.85 ns	47.54 ns	124.27 ns	35.75 ns
T. aculeatus	-	0.12	1.31	0.33	0.17	0.11	8.76	5306.91	55.51	130.36	32.10
CV(%)	-	11.84	8.59	14.72	6.06	14.42	6.31	17.90	19.48	12.57	18.21
Eucalyptus saligna											
Control	0.88 ns	0.09 b	1.21 b	0.48 ns	0.16 b	0.08 b	6.98 ns	1074.53 b	35.10 b	135.60 ns	13.15 b
T. aculeatus	0.93	0.11 a	1.34 a	0.46	0.22 a	0.09 a	7.28	1547.35 a	72.32 a	155.71	14.24 a
CV(%)	11.48	13.47	6.29	14.72	16.60	10.19	13.82	18.73	36.43	16.41	4.91
Corymbia torelliana											
Control	1.10 ns	0.10 ns	1.23 ns	0.36 ns	0.13 ns	0.09 ns	6.33 ns	4212.81 a	84.71 ns	182.73 ns	16.20 ns
T. aculeatus	1.20	0.10	1.24	0.30	0.13	0.09	6.20	2292.46 b	47.14	187.14	15.91
CV(%)	14.53	10.67	8.21	19.53	19.52	20.04	13.18	33.02	56.49	18.12	20.80

The letters indicate statistical difference and “ns” represents no significant difference, according to ANOVA F Test ($p < 0.05$). Table cells filled with “-” represent missing value due to plant sample limitation.

Table 5 – Impact of *Talaromyces aculeatus* inoculation on nutrients content in the shoot of eucalypt species. Experiment conducted in greenhouse during 60 days.

Species	N	P	K	Ca	Mg	S	Cu	Fe	Zn	Mn	B
						mg					
Eucalyptus grandis											
Control	223.06 b	30.79 ns	224.31 b	142.95 ns	30.34 ns	18.43 b	0.08 b	0.71 b	0.51 b	11.43 b	0.35 b
T. aculeatus	274.01 a	37.14	305.33 a	148.96	33.00 ns	26.38 a	0.10 a	0.89 a	0.71 a	14.93 a	0.42 a
CV(%)	11.73	16.74	16.82	9.65	9.33	18.15	13.26	13.50	17.85	14.88	12.31
Eucalyptus urophylla											
Control	198.77 ns	22.80 b	198.84 ns	79.17 b	22.01 ns	16.73 ns	0.06 ns	0.66 b	0.34 ns	7.78 b	0.23 b
T. aculeatus	225.22	29.57 a	222.95	103.66 a	25.24	19.48	0.07	0.79 a	0.36	10.80 a	0.32 a
CV(%)	15.14	15.75	12.50	17.95	15.12	14.67	16.78	11.87	17.49	19.21	17.31
Eucalyptus urograndis											
Control	234.94 ns	31.91 ns	235.42 ns	116.58 ns	25.66 ns	18.61 b	0.10 ns	0.66 ns	0.37 ns	10.14 ns	0.24 b
T. aculeatus	229.62	29.19	248.57	102.55	27.34	22.28 a	0.10	0.65	0.40	10.08	0.29 a
CV(%)	8.24	9.43	12.62	8.28	9.10	14.01	7.55	11.25	15.35	11.04	12.77
Eucalyptus cloeziana											
Control	34.97 b	2.77 b	24.79 b	10.44 ns	4.41 ns	3.58 ns	0.01 b	0.08 ns	0.05 ns	0.68 b	0.04 ns
T. aculeatus	50.22 a	4.02 a	31.05 a	11.98	5.41	4.20	0.02 a	0.08	0.07	0.99 a	0.05
CV(%)	18.52	22.37	15.24	17.74	18.71	16.93	19.74	13.49	20.50	24.98	20.94
Eucalyptus saligna											
Control	247.69 ns	28.22 ns	229.11 ns	144.10 ns	29.76 ns	20.69 ns	0.09 a	0.91 ns	0.54 b	13.68 ns	0.30 ns
T. aculeatus	252.42	27.92	241.17	143.65	30.67	22.13	0.07 b	1.06	0.85 a	11.76	0.32
CV(%)	10.34	11.25	16.29	16.69	16.08	8.91	14.01	17.62	25.51	19.70	13.07
Corymbia torelliana											
Control	222.35 ns	16.46 ns	142.60 ns	54.81 ns	15.57 ns	16.30 ns	0.05 ns	0.45 ns	0.64 ns	6.16 ns	0.21 ns
T. aculeatus	213.36	18.57	148.75	54.41	17.82	16.77	0.05	0.44	0.63	6.10	0.17
CV(%)	10.91	11.74	8.60	10.44	13.44	15.83	16.29	15.53	12.60	11.84	18.73

The letters indicate statistical difference and "ns" represents no significant difference, according to ANOVA F Test ($p < 0.05$).

Table 6 – Impact of *Talaromyces aculeatus* inoculation on nutrients content in the root of eucalypt species. Experiment conducted in greenhouse during 60 days.

Species	N	P	K	Ca	Mg	S	Cu	Fe	Zn	Mn	B
						mg					
Eucalyptus grandis											
Control	33.20 ns	4.72 ns	71.86 ns	15.57 ns	8.20 ns	4.08 ns	0.03 ns	8.40 ns	0.19 ns	0.66 ns	0.07 ns
T. aculeatus	35.54	4.54	65.05	16.33	10.72	4.82	0.03	8.51	0.17	0.83	0.07
CV(%)	11.73	4.37	11.92	10.86	11.14	16.08	12.19	12.39	19.70	17.61	9.96
Eucalyptus urophylla											
Control	14.42 b	1.91 b	24.49 b	6.32 b	3.55 b	1.91 b	0.01 ns	3.63 b	0.07 ns	0.46 b	0.03 b
T. aculeatus	21.28 a	2.53 a	34.63 a	8.63 a	4.79 a	2.68 a	0.02	4.80 a	0.08	0.63 a	0.04 a
CV(%)	18.86	15.49	17.20	16.75	16.41	19.60	16.72	17.72	13.35	17.61	18.07
Eucalyptus urograndis											
Control	25.54 b	2.88 ns	38.21 ns	12.93 ns	4.77 ns	2.57 ns	0.02 ns	4.95 ns	0.16 ns	0.58 ns	0.04 b
T. aculeatus	34.31 a	3.01	40.08	14.46	5.51	3.22	0.03	6.35	0.17	0.61	0.07 a
CV(%)	20.14	17.21	7.09	9.75	14.12	20.01	18.41	17.53	14.47	12.27	33.76
Eucalyptus cloeziana											
Control	-	0.72 ns	7.22 ns	1.63 ns	0.90 ns	0.54 ns	0.004 ns	2.44 ns	0.02 ns	0.07 ns	0.02 ns
T. aculeatus	-	0.71	7.24	1.80	0.86	0.53	0.005	2.92	0.03	0.07	0.02
CV(%)	-	16.55	14.85	15.13	16.21	11.16	21.76	17.46	23.23	19.31	32.86
Eucalyptus saligna											
Control	34.05 ns	3.68 b	49.97 ns	16.59 ns	6.80 b	3.11 b	0.03 ns	4.41 b	0.13 b	0.47 b	0.05 ns
T. aculeatus	39.00	5.38 a	51.87	16.51	10.19 a	4.32 a	0.03	6.21 a	0.31 a	0.58 a	0.06
CV(%)	16.52	20.12	11.53	15.70	21.66	18.46	17.51	23.44	44.26	14.31	16.54
Corymbia torelliana											
Control	2.19 b	0.20 b	2.46 b	0.71 b	0.27 b	0.18 b	0.001 b	0.85 ns	0.01 ns	0.04 b	0.003 b
T. aculeatus	4.13 a	0.34 a	4.28 a	0.99 a	0.46 a	0.32 a	0.002 a	0.78	0.01	0.06 a	0.01 a
CV(%)	33.46	29.55	29.72	21.27	31.41	31.99	30.30	15.11	37.92	26.70	31.88

The letters indicate statistical difference and "ns" represents no significant difference, according to ANOVA F Test ($p < 0.05$). Table cells filled with "-" represent missing value due to plant sample limitation.

Table 7 – Alterations (in percentage) caused by *Talaromyces aculeatus* inoculation on total nutrient content of eucalypt species. Experiment conducted in greenhouse during 60 days.

Species	N	P	K	Ca	Mg	S	Cu	Fe	Zn	Mn	B
	%										
E. grandis	20.80 *	17.38	25.06 *	4.27	9.11	39.67 *	15.84 *	3.14	27.34 *	30.36 *	11.05 *
E. urophyla	15.16	29.46 *	14.62	31.12 *	16.69	18.89	24.79 *	30.17 *	5.42	38.61 *	38.97 *
E. urograndis	1.32	-7.45	5.49	-6.34	7.93	13.00	-1.26	24.83	10.10	-0.33	20.70 *
E. cloeziana	-	35.39 *	19.63	14.15	18.07	14.66	25.85 *	19.03 *	16.80	31.03 *	14.52
E. saligna	3.43	-5.44	5.01	-0.33	11.77	11.13	-17.03 *	36.54 *	72.65 *	-12.80	9.76
C. torelliana	-3.12	13.62	5.52	-0.21	15.52	3.69	-16.28	-1.46	-2.55	-0.49	-18.00

The presence of "*" indicates statistical difference according to ANOVA F Test ($p < 0.05$). Table cells filled with "-" represent missing value due to plant sample limitation.

One of the most impacted specie by fungal inoculation was *E. urograndis,* which presented significant increments on shoot and root dry matter, 33.18 % and 27.81 % respectively (Tables 2 to 7). However, shoot nutrients concentrations were higher on control treatment and only few nutrients suffered increases due to inoculation considering nutrients content, particularly S and B on shoot and N and B on root (Tables 2 to 7). Consequently, the nutrient efficiency utilization indexes were significantly higher for all the nutrients on *T. aculeatus* treatment (Table 8 and 9).

Alterations on *E. grandis* included slight increases on plant height and shoot dry matter, but also significant increases on concentration and content for most of nutrients, especially micronutrients on plant shoot (Tables 2 to 7). The efficiency utilization for most of nutrients did not indicate significant difference between treatments, but the exceptions were higher utilization efficiency for K, S and Mn on control group and for Fe on inoculated group (Table 8 and 9).

The effects caused by *T. aculeatus* inoculation on *E. urophylla* were especially remarkable on roots, which represented an increase of 50.59 % on root dry matter (Table 2). Considering nutrients content there were significant increases on all root nutrients, except for Cu and Zn (Table 6). On shoot, the contents of P, Ca, Fe, Mn and B also significantly increased (Table 5).

Similarly, inoculation on *C. torelliana* resulted to positive effects concentrated on plant roots development, presenting an increment of 66.66 % on root dry matter (Table 2). On root, the content increased for all considered nutrients, except for Fe and Zn (Table 6). Additionally, utilization efficiency was significantly higher on inoculated treatment for S, Fe, Zn and Mn (Table 8 and 9).

Despite of no effects on plant height or dry matter, *E. saligna* presented increases on K, Ca and Mg on shoot concentrations, and also P, K, Mg, S, Fe, Zn and B on root concentrations (Tables 3 and 4). Considering nutrient content, the inoculation effects were stronger on root, which included increased on P, Mg, S, Fe, Zn and Mn, while on shoot only Cu and Zn increased significantly (Table 5 and 6). Additionally, the nutrient utilization efficiency was higher on control treatment to Zn and B (Table 8 and 9).

Plant height or dry matter also were not affected by *T. aculeatus* inoculation on *E. cloeziana*, but there were few increases on shoot nutrients content, which included N, P, K, Cu and Mn (Table 5). Considering total nutrient content, Fe was also significantly higher on inoculated treatment (Table 7).

Table 8 – Influence of *Talaromyces aculeatus* inoculation on macronutrient utilization efficiency index (E) of different eucalypt species. Experiment conducted in greenhouse during 60 days.

Species	N		P		K		Ca		Mg		S	
Eucalyptus grandis												
Control	1456005	ns	10786579	ns	1257909	a	2368288	ns	9687684	ns	16606434	a
T. aculeatus	1325508		9852517		1109024	b	2478321		9787190		13025195	b
CV(%)	7.01		13.60		8.42		6.08		7.35		14.26	
Eucalyptus urophylla												
Control	743794	ns	7227138	ns	745345	ns	1732335	b	5863375	ns	9001404	ns
T. aculeatus	838988		7832632		963911		2277581	a	6982291		11341383	
CV(%)	8.78		11.58		18.44		19.16		14.22		16.97	
Eucalyptus urograndis												
Control	1155905	b	8067211	b	936012	b	2160516	b	9814467	b	11859305	b
T. aculeatus	1730818	a	14114172	a	1515240	a	3828085	a	14074095	a	17936929	a
CV(%)	20.72		27.93		24.09		29.01		18.38		20.84	
Eucalyptus cloeziana												
Control	-		657485	ns	107698	ns	283402	ns	655024	ns	864392	ns
T. aculeatus	-		808021		108213		304614		670707		901172	
CV(%)	-		26.12		18.18		13.78		11.16		15.72	
Eucalyptus saligna												
Control	1365799	ns	12131709	ns	1716014	ns	2744677	ns	10822316	ns	16081741	ns
T. aculeatus	1364329		9819261		1327270		2464034		9472797		15301219	
CV(%)	16.45		21.51		20.68		18.87		18.86		23.43	
Corymbia torelliana												
Control	281629	ns	3826894	ns	436237	ns	1142861	ns	4007452	ns	3564788	b
T. aculeatus	308804		3541689		437035		1215270		3662238		4322886	a
CV(%)	9.76		12.06		8.83		11.64		7.58		12.00	

The letters indicate statistical difference and “ns” represents no significant difference, according to ANOVA F Test ($p < 0.05$). Table cells filled with “-” represent missing value due to plant sample limitation.

Table 9 – Influence of *Talaromyces aculeatus* inoculation on micronutrient utilization efficiency index (E) of different eucalypt species. Experiment conducted in greenhouse during 60 days.

Species	Cu		Fe		Zn		Mn		B	
Eucalyptus grandis										
Control	3234450652	ns	37717060	b	540695108	ns	30850590	a	889653253	ns
T. aculeatus	3084023659		46347264	a	464075374		26052132	b	885378872	
CV(%)	7.25		12.97		11.03		10.05		9.47	
Eucalyptus urophylla										
Control	2464323761	ns	34967099	ns	462799915	ns	20322696	ns	592721007	ns
T. aculeatus	3000862956		36172427		531438197		20178050		565199461	
CV(%)	19.16		15.90		21.61		18.66		14.91	
Eucalyptus urograndis										
Control	2294556689	b	50387745	b	566919347	b	26191129	b	961340540	b
T. aculeatus	3680109068	a	67083833	a	769723802	a	41077708	a	1294271806	a
CV(%)	24.35		19.19		16.84		24.46		16.56	
Eucalyptus cloeziana										
Control	229715592	ns	1422135	ns	44498457	ns	4544440	ns	59412546	ns
T. aculeatus	202460786		1400316		44079752		3852527		58385567	
CV(%)	24.82		29.29		14.12		21.11		14.79	
Eucalyptus saligna										
Control	3270509312	ns	74263079	ns	587670938	a	27138832	ns	1108639619	a
T. aculeatus	2941042683		54129990		355133055	b	29192749		764820108	b
CV(%)	19.15		28.27		32.56		18.42		21.14	
Corymbia torelliana										
Control	1300473891	ns	42976749	b	103270868	b	10215367	b	296929681	ns
T. aculeatus	1457551123		54230936	a	115632038	a	12217194	a	362328588	
CV(%)	13.90		14.62		6.59		11.08		15.34	

The letters indicate statistical difference and “ns” represents no significant difference, according to ANOVA F Test ($p < 0.05$).

Discussion

It is clear, considering the data, that plant's species can play an important role on the capacity of the fungus to promote its growth. Each specie responded differently not only on the way the plant is impacted by the fungal inoculation, but also in the extent of this impact.

Overall, *E. grandis* was positively affected especially on shoot nutrients concentrations, while *E. urophylla* had major positive impacts on root nutrients content. Additionally, *E. cloeziana* presented more significant gains on shoot nutrients content and *C. torelliana*, due to root increase, manifested several gains on root nutrients content.

Based on the plant growth and root nutrient content (Table 2 and Table 4), *C. torelliana*, only specie from another genus, manifested some significant positive effects on plant development and nutrient acquisition mediated by the interaction with *T. aculeatus*. While the other species, all from *Eucalyptus* genus, responded differently between themselves, even some of them with weaker effects.

Considering nutrient utilization efficiency, *E. urograndis* was the only one which positively increased efficiency for all nutrients, reinforcing the differences on *T. aculeatus* growth promotion capacities, even between species from the same genus (Tables 8 and 9). Despite of considerable increases on nutrients acquisition by *E. grandis* with fungal inoculation, the growth was not proportional to gains of elements such as K, S, Fe and Mn, consequently the index of utilization was greater for control group. It might be associated with the plant developmental phase, particularly it happened to *E. grandis*, in which higher amounts of certain nutrients will not directly be translated to dry matter increment immediately.

Eucalypt species use mechanisms to survive on soil with nutritional limitation, which includes regulation of uptake, storage and internal cycling. Age and size of the trees are intimately linked to plants distribution of nutrients and storage, and also there is differences between eucalypt species on nutrients dynamics. Early stages of eucalypt growth are characterized by low hates of nutrient redistribution and high rates of nutrient accumulation (Grove et al., 1996).

Finally, even without significant alterations on nutrients concentrations, *C. torelliana* roots developed consistently better with fungal inoculation, which reflects on higher E for S, Fe, Zn and Mn (Tables 8 and 9). It might be related to stimulation of root growth by the action of phytohormones like IAA.

Root development is direct related to B uptake by the plants, especially considering that increases in the numbers of lateral roots can enhance plant capability to access water and ions from soil (Shireen et al., 2018). *Talaromyces aculeatus* inoculation caused increases on B uptake to all species, except to *E. cloeziana*. The mechanism possibly involved on these increments might be lateral roots growth stimulation by IAA production by the fungus.

Talaromyces aculeatus is able to promote growth of sorghum, increasing concentration of nutrients and it also presents heavy metal tolerance. The nutrients increased by *T. aculeatus* inoculation were P, As, Cu, Pb and Zn. The fungal is capable to produce IAA, siderophores, phytases and phosphatases (Babu et al., 2014).

Higher concentrations of K on *E. grandis* and *E. saligna* in inoculated treatments can be related to the fact of growth promoting fungi being capable to release K from soil through organic acids production, then the element could have its availability increased and consequently the plant would have easier access to it (Wang et al., 2018). Additionally, Zn and Mn can also have their availability amplified through the same mechanism, then the species on this study, especially *E. grandis*, *E. cloeziana* and *E. saligna* could have been benefited from isolate's capabilities of solubilizing these elements (Anitha et al., 2015; Mohanty et al., 2016; Gaind 2016).

Facts mentioned above might indicate variation on growth promotion success considering different plant species and points out that not necessarily plants closely related would respond similarly, or even better than less related ones.

These findings indicate the same direction pointed out by the few studies currently available and related to the theme. For example, the same PGPF when applied to different species of plant, from distinct genera, impacted them differently (Baron et al., 2020). Another study, despite of it did not consider PGPF specifically, but dark septate endophytes and non-filamentous fungi, highlighted the influence of the plant phylogeny to soil and microbiome feedbacks (Wooliver et al., 2018).

Some of the relative low results in terms of growth promotion might also be related to soil fertility level, since it potentially affects PGPF and plants interactions (De Santiago et al., 2013; Li et al., 2015). Thus, different soil conditions might make the benefits caused by the fungus inoculation more expressive to some plant species. It can include limitation of certain nutrients, which can impact considerably interaction outcomes (Vince et al., 2018), or even more appropriate conditions to the specie's minimum physiological needs.

Particularly, *E. cloeziana* developed poorly in the conditions set to the experiment and the seedlings took a long time to establish themselves and start to grow. Therefore, it probably needed more time to treatments differentiate even more. Future studies must include nutritional conditions which can amplify the manifestation of growth promotion mediated by *T. aculeatus*, then its full growth potential can be explored for different eucalypt species.

Conclusions

Talaromyces aculeatus was able to influence the growth and nutrient acquisition of the plant species used in this study, but the interaction results are different among them, both in intensity and in the way they are affected. Nutritional conditions probably played an important role in the results observed, therefore, under different nutritional conditions, the fungal effects might be maximized, for example, conditions with micronutrients limitation.

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CONCLUSÃO GERAL

Este trabalho demonstrou como as diferentes dosagens de fósforo são influentes no estabelecimento e resultado da interação entre o fungo promotor de crescimento *Talaromyces aculeatus* e *Eucalyptus grandis*. Além disso, o estudo elucidou que a interação de *T. aculeatus* com diferentes espécies de eucalipto apresenta resultados diferentes tanto na forma como as espécies são afetadas pela inoculação do fungo, quanto na intensidade em que o fungo produz esses efeitos nas plantas.

O conhecimento mais profundo desses aspectos que possuem grande influência na promoção de crescimento vegetal é fundamental para o melhor entendimento das interações entre fungos promotores de crescimentos e plantas, e ainda, essas informações contribuem para otimizar a criação de produtos biotecnológicos e suas aplicações nas atividades agrícolas e cultivos florestais.