UNIVERSIDADE FEDERAL DE ALFENAS

ISIS ALVES

INOCULAÇÃO COM FUNGOS MICORRÍZICOS ARBUSCULARES NA PRODUÇÃO DE MUDAS DE ESPÉCIE ARBÓREA

Alfenas/MG
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Dissertação apresentada como parte dos requisitos para obtenção do Título de Mestre em Ciências Ambientais pela Universidade Federal de Alfenas/UNIFAL-MG. Área de concentração: Tecnologias Ambientais Aplicadas.
Orientador: Prof. Dr. Romero Francisco Vieira Carneiro
Coorientadora: Dra. Kamila Rezende Dázio de Souza

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A Banca examinadora abaixo-assinada aprova a Dissertação apresentada como parte dos requisitos para a obtenção do título de Mestre em Ciências Ambientais pela Universidade Federal de Alfenas. Área de concentração: Ciências Ambientais.

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RESUMO

O cedro rosa é uma espécie que compõe a lista de espécies vulneráveis a extinção e possui grande potencial para recuperação florestal e de solos contaminados. Dessa forma, o objetivo deste estudo foi avaliar a resposta de mudas de cedro à inoculação com fungos micorrizícos arbusculares (FMA) em diferentes doses de fósforo (P) e turnos de irrigação. O experimento foi conduzido em delineamento em blocos ao acaso (DBC) em esquema fatorial 3x2x2, com três repetições. Os tratamentos constituíram na inoculação de duas espécies de FMA: *Acaulospora longula* (Al) e *Claroideoglomus etunicatum* (Ce), além de um tratamento controle sem a inoculação (Ct); duas doses de fósforo 0 e 30 mg dm^{-3}; e dois turnos de regas sendo uma (1x) ou duas vezes (2x) por semana de reposição da lâmina de irrigação. Avaliou-se a altura, diâmetro do caule, índice de qualidade de Dickson (IQD), biomassa seca da parte aérea (MSPA), biomassa seca da raiz (MSR), biomassa seca total (PMST), acúmulo de P, relação biomassa seca R:PA (raiz parte aérea), taxa de colonização micorrízica e contagem de esporos aos 150 dias após semeadura. Houve diferenças significativas para inoculação, turnos de rega e doses de P sobre as variáveis analisadas. A inoculação com FMAs foi eficiente para o crescimento e qualidade das mudas. O IQD foi um bom indicador do padrão de qualidade de mudas de cedro rosa. Al proporcionou uma taxa de colonização micorrízica e número de esporos maior do que Ce, evidenciando que essas variações são ocorrentes em função a especificidade do fungo inoculado e espécie vegetal trabalhada. As mudas de *C. fissilis* inoculadas com *Claroideglomus etunicatum*, sem inserção da dose de fósforo e submetidas a dois turnos de regas semanais, apresentaram melhor desenvolvimento, indicando maior crescimento e qualidade das mudas.

Palavras-chave: Índice de qualidade de Dickson. biomassa seca. taxa de colonização micorrízica. Meliaceae.

ABSTRACT

Pink cedar is a species that makes up the list of species vulnerable to extinction and has great potential for forest and contaminated soil recovery. Thus, the aim of this study was to evaluate the response of cedar seedlings to inoculation with arbuscular mycorrhizal fungi (AMF) at different doses of phosphorus (P) and irrigation shifts. The experiment was conducted in a randomized block design (DBC) in a 3x2x2 factorial scheme, with three replications. The treatments consisted of the inoculation of two species of AMF: *Acaulospora longula* (Al) and *Claroideoglomus etunicatum* (Ce), in addition to a control treatment without the inoculation (Ct); two doses of phosphorus 0 and 30 mg dm-3; and two irrigation shifts, once (1x) or twice (2x) a week to replace the irrigation blade. Height, stem diameter, Dickson quality index (DQI), dry shoot biomass (MSPA), dry root biomass (MSR), total dry biomass (PMST), P accumulation, dry biomass ratio were evaluated A: PA (aerial part root), mycorrhizal colonization rate and spore count at 150 days after sowing. There were significant differences for inoculation, irrigation shifts and P doses on the analyzed variables. The inoculation with FMAs was efficient for the growth and quality of the seedlings. The DQI was a good indicator of the quality standard of pink cedar seedlings. Al provided a rate of mycorrhizal colonization and a number of spores greater than Ce, showing that these variations are occurring due to the specificity of the inoculated fungus and plant species. The seedlings of *C. fissilis* inoculated with *Claroideglomus etunicatum*, without inserting the dose of phosphorus and submitted to two weekly watering shifts, showed better development, indicating greater growth and quality of the seedlings.

Keywords: Dickson's quality score. dry biomass. mycorrhizal colonization rate. Meliaceae.

LISTA DE ABREVIATURAS E SIGLAS

AM	Micorriza Arbuscular
DBC	Delineamento em Blocos Casualizados
DC	Diâmetro do Coleto
ECM	Ectomicorriza
FMA	Fungos Micorrízicos Arbusculares
H	Altura da Parte Aérea
IQD	Índice de Qualidade de Dickson
P	Fósforo
PMSPA	Peso de Matéria Seca da Parte Aérea
PMSR	Peso de Matéria Seca das Raízes
PMST	Peso de Matéria Seca Total
RPM	Rotações por Minuto

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1 INTRODUÇÃO

A associação simbiótica entre fungos e raízes de plantas, denominada micorriza, está intimamente ligada ao desenvolvimento vegetal nos mais variados ecossistemas terrestres. As micorrizas aumentam a capacidade da planta em extrair nutrientes menos solúveis do solo, podendo aumentar a produção primária do vegetal principalmente em solos pobres (RICKLEFS, 2010). Dessa maneira, a formação da micorriza pelos fungos micorrízicos arbusculares (FMA) possui papel importante na manutenção da diversidade e produtividade dos ecossistemas vegetais terrestres (SOUZA *et al*., 2010). Neste contexto, a inoculação com FMAs potencializa a oferta de mudas e assim contribui positivamente com o processo de restauração florestal, tanto no aspecto da sobrevivência quanto no aumento de diversidade de espécies vegetais envolvidas nesse processo (NEUENKAMP *et al*., 2019).

A capacidade dos FMAs em aumentar a absorção de nutrientes do solo (potássio, cálcio, magnésio, enxofre, zinco e principalmente o fósforo) (SILVA *et al*., 2017) favorece o crescimento da planta. Com isso, aumenta a capacidade de resistência da planta ao ataque de patógenos (tombamento, podridões radiculares e murchas vasculares), bem como a sobrevivência em ambientes sob estresses (RICKLEFS, 2010). A simbiose estabelecida com FMAs ainda no viveiro, promove melhorias na qualidade das mudas produzidas, favorecendo a tolerância das mesmas ao transplantio para o campo (CARMO *et al*., 2016; SILVA *et al*., 2017).

Embora os efeitos dos FMAs sobre o crescimento e nutrição vegetal, e sobre o controle de patógenos seja conhecido, sua utilização prática e em larga escala ainda não está amplamente estabelecida. Uma das principais desvantagens está relacionada à produção de inóculo, visto que os FMAs só se desenvolvem através da planta hospedeira, limitando sua comercialização e multiplicação. Do ponto de vista econômico, principalmente para a produção de mudas, as associações micorrízicas tornam-se cada vez mais atraentes, visando minimizar gastos com fertilizantes minerais, pesticidas e também irrigação (SANTANA, 2017). No entanto, é necessário considerar que os efeitos benéficos de FMAs sobre as mudas pode variar de acordo com a espécie do fungo utilizada e das características da espécie colonizada (CARMO *et al*., 2016; GOETTEN; MORETTO; STÜRMER, 2016; VAN GEEL *et al*., 2016).

O adequado manejo de irrigação é de extrema necessidade para a formação de mudas de qualidade. Tanto o déficit quanto o excesso hídrico, podem ser prejudiciais para a formação e fitossanidade das mesmas, bem como, influenciar sobre os recursos ambientais e econômicos investidos, pois a ausência de um manejo racional da água, resulta sobretudo em desperdício de energia. Assim sendo, a forma de administrar a lâmina d’água correspondente à capacidade de campo em vaso (capacidade máxima do solo contido naquele recipiente em reter água) é um parâmetro que pode auxiliar a operacionalização de todo o sistema de irrigação, visando garantir a produção de mudas com fornecimento adequado da lâmina, sem gerar excessos, prejudicar as plantas e comprometer o custo de produção ou ainda utilizar de maneira exacerbada um recurso natural gerando com isso um impacto ambiental considerável.

A dosagem de fósforo também deve ser utilizada de modo bastante criterioso, visto que os benefícios da inoculação micorrízica tendem a diminuir com o aumento nas doses de adubação fosfatada, conforme verificado por Carneiro *et al*. (2002), onde os melhores efeitos ocorrem em doses baixas ou intermediárias, proporcionando teores de P classificados entre baixos e médios, o que pode ser obtido em substratos arenosos com as doses entre 25 a 60 mg dm^{-3}. É importante salientar que os gastos com soluções nutritivas, também demandam um percentual considerável dos custos, e ao utilizar dosagens menores e associadas a inoculação, tem-se a possibilidade de gerar economia de custos variáveis no viveiro, além da minimização dos impactos ambientais que ocorrem através das adubações.

A espécie arbórea *Cedrela fissilis* Vell., de acordo com a Lista Nacional Oficial de Espécies da Flora Ameaçadas de Extinção, está classificada na categoria vulnerável à extinção (MMA, 2014). Espécie pertencente à família Meliaceae, conhecida popularmente como cedro rosa, possui ocorrência em diversas formações florestais brasileiras. Apresenta considerável contribuição ao setor econômico do país, por possuir diversas utilidades como: construção civil, movelaria, marcenaria, extração de óleo essencial, movimentando constantemente o comércio madeireiro e de cosméticos (LORENZI, 2014). Do ponto de vista ambiental, o cedro apresenta utilização para recuperação florestal, matas ciliares e também é promissor para a recuperação de solos contaminados por metais pesados. Assim, a busca por tecnologias que potencializem a qualidade das mudas no viveiro, podem minimizar a vulnerabilidade de espécies vegetais à extinção.

Diante da importância da espécie *Cedrela fissilis* do ponto de vista econômico, ambiental e dos desafios da utilização de fungos micorrízicos arbusculares na produção de mudas de espécies lenhosas, o presente trabalho teve como objetivo avaliar a influência da inoculação com fungos micorrízicos arbusculares, doses de fósforo e, turnos de rega sobre o crescimento e a qualidade de mudas de *Cedrela fissilis*.

2 REVISÃO DE LITERATURA

2.1 FUNGOS MICORRÍZICOS ARBUSCULARES

Os FMAs pertencentes ao Filo Glomeromycota e classe Glomeromycetes, são organismos biotróficos obrigatórios que estabelecem relação simbiótica mutualista com raízes de angiospermas, gimnospermas, além de alguns representantes das briófitas e pteridófitas (SOUZA *et al*., 2010).

Sua distribuição encontra-se atualmente disseminada em quatro ordens (Archaeosporales, Diversisporales, Glomerales e Paraglomerales), 13 famílias (Acaulosporaceae, Ambisporaceae, Archaeosporaceae, Dentiscutataceae, Diversisporaceae, Entrophosporaceae, Geociphonaceae, Gigasporaceae, Glomeraceae, Pacisporaceae, Paraglomeraceae, Racocetraceae e Scutellosporaceae) e 19 gêneros (OEHL; SOUZA; SIEVERDING, 2008), cerca de 300 espécies foram reconhecidas, com pelo menos 50% já descritas no Brasil (GOTO *et al*., 2010).

Duas formas principais de micorrizas são conhecidas: a ectomicorriza (ECM) e a micorriza arbuscular (AM). Os fungos ECM encontram-se normalmente associados às plantas lenhosas. Eles formam uma densa cobertura em volta das partes externas de pequenas raízes e penetram nos espaços entre as células da camada cortical da raiz. A maioria dos ECM pertencem às divisões Basidiomycota (cogumelos típicos) e Ascomycota (moreias). Os FMAs penetram nas paredes celulares dos tecidos das raízes e formam vesículas ou estruturas ramificadas em contato íntimo com as membranas das células das raízes. As AM são formadas somente por fungos na divisão taxonômica Glomeromycota e são associadas a maioria dos vegetais superiores, incluindo também plantas de interesse agrícola (RICKLEFS, 2010).

Estas simbioses apresentam importante função biológica no processo de ciclagem de nutrientes e na manutenção da qualidade do solo. As hifas funcionam como extensão das raízes das plantas, uma vez que, devido à sua grande capacidade de ramificação, exploram o solo, realizando absorção de água e nutrientes minerais, que são transferidos para as plantas por meio de estruturas intracelulares, efêmeras, denominadas arbúsculos (HOFFMAN; LUCENA, 2006). Além disso, como os fungos secretam enzimas e ácidos (íons de hidrogênio) no solo circundante, as micorrizas são mais eficientes do que as raízes das plantas por si só em função a extração de certos nutrientes inorgânicos do solo. As micorrizas podem também proteger as raízes das plantas contra doenças ao excluir fisicamente patógenos ou produzir antibióticos, como toxinas antibacterianas (RICKLEFS, 2010).

2.1.1 Fungos Micorrízicos: *Acaulospora longula* e *Claroideglomu etunicatum*

O FMA *Acaulospora longula* pertence a ordem Diversisporales, família Acaulosporaceae e gênero *Acaulospora* Gerd. & Trappe emmend. Berch. Já o FMA *Claroideglomu etunicatum*, concerne a ordem Glomerales, da família Glomeraceae e gênero *Glomus* Tul. & Tul. (CAVALCANTE; GOTO, MAIA, 2009).

De acordo com Bagyaraj (1991), os FMAs possuem ampla distribuição e não apresentam especificidade quanto ao hospedeiro, indicando que os requerimentos nutricionais não são específicos. Com isso, é importante salientar, que uma espécie de planta pode ser colonizada por qualquer espécie de FMA, porém resultados distintos poderão ser encontrados em meio aos efeitos da simbiose em função das combinações entre solo, fungo e hospedeiro (COSTA *et al*., 2001).

Em função a grande diversidade de espécies dos FMA existentes, a inoculação com fungos micorrízicos arbusculares é bastante variada. A alternância no tipo de solo onde é realizado o plantio, bem como, as variações ambientais são condições que podem influenciar o desenvolvimento das plantas de acordo com o aumento na absorção nutricional (BALOTA, MACHINESKI e STENZEL, 2011). O manejo adequado dos FMA contribui para aumentar a produtividade em diferentes tipos de solos, incluindo solos com baixos índices de produção comercial. A micorrização possibilita melhor utilização e conservação dos nutrientes disponíveis no solo pelas plantas que se adaptam mais facilmente ao serem introduzidas em diferentes ambientes (ARNALDO FILHO; NOGUEIRA, 2007).

Segundo estudo realizado em Recife, em que se testou duas tecnologias para produção de FMA, as plantas de sorgo inoculadas com *Claroideglomu etunicatum*, e *Acaulospora longula* em função a composição de substratos distintos, locais e ciclos de produção analisados apresentaram resultados satisfatórios em função a eficiência analisada (SANTANA, 2017).

2.1.2 Benefícios dos FMA

Diversos benefícios podem ser obtidos através da utilização dos FMA, como na produção de mudas, recuperação de áreas degradadas, estabelecimento de plantas micropropagadas, tolerância a estresse hídrico, controle biológico e outros (SANTANA, 2017).

Os FMAs aumentam a resistência das plantas por meio da associação simbiótica estabelecida, possibilitando melhor utilização de nutrientes disponíveis no solo. A capacidade dos FMA em aumentar a absorção de nutrientes do solo (potássio, cálcio, magnésio, zinco e principalmente o fósforo); favorece o crescimento da planta, principalmente em solos pobres, aumentando a capacidade de resistência ao ataque de patógenos (tombamento, podridões radiculares e murchas vasculares) bem como a propensão em sobreviver em ambientes estressados. A simbiose estabelecida com fungos micorrízicos arbusculares favorece também a tolerância dos vegetais ao transplantio para o campo em várias situações (CARNEIRO *et al.*, 2004).

2.1.3 Problematização

Um dos principais desafios para o uso dos FMAs em larga escala está relacionado à produção de inóculo, visto que os FMA só se desenvolvem através da planta hospedeira, dificultando com isso sua multiplicação e consequentemente o estabelecimento de protocolos para comercialização, podendo esta ser a chave para o uso sustentável desses organismos. Segundo Santana (2017), é interessante a multiplicação dos FMA em larga escala para a diminuição dos custos de produção em função ao uso de fertilizantes fosfatados, mas também é importante atentar-se para a manutenção da qualidade e biodiversidade do solo.

Administrar as doses de fósforo e turnos de rega também é um grande obstáculo a ser vencido em meio a temática em questão, por este fato experimentos que evidenciem a melhor dosagem de adubação a ser aplicada, bem como, lâmina de irrigação, podem facilitar a produção e o desenvolvimento de espécies vulneráveis a extinção.

2.2 *SORGHUM BICOLOR* L.- SORGO

O cultivo de leguminosas forrageiras e gramíneas tem apresentado elevado percentual de colonização das raízes e número de propágulos infecciosos de FMA, o que favorece o uso dessas plantas nos programas de multiplicação dos FMAs (CARNEIRO *et al.*, 2012).

Neste sentido, *Sorghum bicolor* L. foi utilizado inicialmente como planta hospedeira neste estudo para multiplicação dos FMA. Essa é uma espécie pertencente à família das gramíneas, conhecida popularmente como milho-zaburro (PEREIRA, 2008). A planta de sorgo pode alcançar de três a cinco metros de altura, com colmos suculentos, eretos e dispostos em forma de touceiras. As folhas dessa gramínea são lineares, entrecruzando-se, com vinte e cinco a cinquenta milímetros de largura e cinquenta a cem centímetros de comprimento.

Assim sendo visto que *Sorghum bicolor* L. adapta-se bem em solos médios e arenosos, com melhores resultados de semeadura de meados de outubro a meados de dezembro e período de formação de sessenta a noventa dias (PEREIRA, 2008) o mesmo foi utilizado como planta hospedeira para desenvolvimento do substrato inóculo utilizado neste estudo.

2.3 *CEDRELA FISSILIS* VELL.

Em função ao grande avanço populacional, desmatamentos, atividades agropecuárias e ações antrópicas de modo geral, as espécies arbóreas tendem a estar cada vez mais vulneráveis a extinção. Desta forma, em meio aos benefícios advindos da micorrização, entende-se que contribuições significativas podem ser advindas desta simbiose mutualista, alterando este cenário. *Cedrela fissilis* Vell. conhecida popularmente como cedro rosa, de acordo com a Lista Nacional Oficial de Espécies da Flora Ameaçadas de Extinção, está classificada na categoria vulnerável a extinção (MMA, 2014). Espécie pertencente à família Meliaceae, possui ocorrência em diversas formações florestais brasileiras (Rio Grande do Sul até Minas Gerais). E apresenta características das florestas semidecíduas e menos frequente na Floresta ombrófila densa como a pluvial da costa atlântica (LORENZI, 2014).

2.3.1 Características principais e utilidades da *Cedrela fissilis* Vell.

Trata-se de uma espécie caducifólia, com altura variando entre vinte e trinta e cinco metros, tronco de sessenta a noventa centímetros. As folhas são compostas de sessenta a cem centímetros de comprimento e folíolos de oito a quatorze centímetros (LORENZI, 2014).

O cedro é uma espécie de crescimento médio, intermediária na sucessão (secundária tardia) compondo o grupo das espécies não pioneiras, regenerando-se preferencialmente em clareiras ou bordas de mata. Possui ciclo de vida longo, correspondente em média de vinte e cinco a cem anos, e sua regeneração ocorre por banco de plântulas efêmero (MARTINS; LAGO, 2008).

É uma planta hermafrodita, que floresce durante os meses de agosto e setembro. Os frutos possuem formatos similar a uma pera que se abrem por ocasião de deiscência, alojando em média de trinta a cem sementes viáveis. O poder germinativo das sementes geralmente ultrapassa 80% e a germinação ocorre entre doze e dezoito dias após a semeadura (LORENZI, 2014). *Cedrela fissilis* Vell. é uma espécie florestal heliófila na fase adulta, medianamente tolerante às baixas temperaturas e parcialmente umbrófila no estádio juvenil (CARVALHO, 1994).

Essa é uma espécie rara, e por possuir coloração semelhante ao mogno, produz uma madeira bastante apreciada comercialmente. É também considerada uma madeira mais leve, que possibilita um uso bastante diversificado (CARVALHO, 1994).

A madeira é largamente empregada em compensados, esculturas e obras de talha, moveis em geral, marcenaria, construção civil e outros. É empregada para o paisagismo de parques, grandes jardins e também compõe projetos de reflorestamento heterogêneos de áreas degradadas e de preservação permanente (LORENZI, 2014).

O cedro apresenta utilização para recuperação florestal, matas ciliares, e também é promissor para a recuperação de solos contaminados por metais pesados. Além disso, apresenta considerável contribuição ao setor econômico do país, por possuir diversas utilidades como: construção civil, movelaria, marcenaria, extração de óleo essencial, movimentando constantemente o comércio madeireiro e de cosméticos (LORENZI, 2014).

2.3.2 Principais problemas ocorrentes na espécie estudada

Um dos maiores problemas enfrentados na produção e plantio de mudas do cedro estão relacionadas ao ataque das gemas ocasionados por *Hypsipyla grandella* conhecida popularmente como broca-do-cedro, causando deformações e até a morte em várias plantas. A broca constitui fator limitante para cultivo da *Cedrela fissilis* Vell. pois ainda não foi encontrada uma solução eficaz para o controle da praga. Quando os ataques ocorrem atingem as gemas apicais e os sucessivos ataques aos ponteiros paralisam o desenvolvimento do cedro (MARTINS; LAGO, 2008).

É difícil obter-se o controle da broca, porém alguns métodos podem ser implementados ou mesmo exercidos com o intuito de minimizar a proliferação da praga. Dentre os métodos tem-se o controle químico, biológico, mas nenhuma técnica isolada apresentou total eficácia em meio ao combate (PEREIRA *et al*., 2016).

Cedrela fissilis Vell., de acordo com a Lista Nacional Oficial de Espécies da Flora Ameaçadas de Extinção, está classificada na categoria vulnerável à extinção (MMA, 2014). A espécie estudada possui diversas utilidades como: construção civil, movelaria, marcenaria, extração de óleo essencial, movimentando constantemente o comércio madeireiro e de cosméticos (LORENZI, 2014). Do ponto de vista ambiental, o cedro apresenta utilização para recuperação florestal, matas ciliares e também é promissor para a recuperação de solos contaminados por metais pesados. Assim, a busca por tecnologias que potencializem a qualidade das mudas no viveiro, podem minimizar a vulnerabilidade de espécies vegetais à extinção e acelerar o processo de produção, visto que o crescimento da referida é lento.

3 JUSTIFICATIVA

A associação simbiótica entre fungo e as raízes das plantas, proporciona às plantas aumentarem a captação de nutrientes do solo, estimulando, com isso, o crescimento vegetal, protegendo-as de doenças e tornando-as mais resistentes com relação a possibilidade de desenvolver-se e sobreviver em ambientes estressados (RICKLEFS, 2010). Do ponto de vista econômico, as associações micorrízicas tornam-se cada vez mais atraentes, já que contribuem para minimizar gastos com insumos, como fertilizantes minerais, pesticidas e também irrigação (SANTANA, 2017). E através de uma adubação fosfatada intermediária e uma dosagem adequada da lâmina de irrigação, tem-se influências positivas em meio ao crescimento e qualidade na produção de mudas. Assim, em função aos benefícios trazidos pela micorrização pesquisas desenvolvidas visando promover a multiplicação de espécies, através da inoculação com Fungos Micorrízicos Arbusculares fazem-se necessárias.

4 OBJETIVOS

4.1 OBJETIVO GERAL

Avaliar a influência da inoculação com fungos micorrízicos arbusculares, doses de fósforo e, turnos de rega sobre o crescimento e a qualidade de mudas de *Cedrela fissilis*.

4.2 OBJETIVOS ESPECÍFICOS

a) Avaliar a taxa de crescimento da espécie selecionada e qualidade das mudas através do índice de qualidade de Dickson;
b) Realizar a avaliação dos parâmetros agronômicos das mudas de *Cedrela fissilis* Vell.;
c) Avaliar a taxa de colonização e contagem dos esporos de FMAs;
d) Analisar a influência de diferentes combinações entre fungos micorrízicos arbusculares, doses de fósforo e turnos de rega.

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ARTICLE - Arbuscular mycorrhizal fungi, phosphorus and irrigation shifts in the growth and quality of *Cedrela fissilis* Vell. seedlings

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ABSTRACT

(i) Aim of the study: Evaluate the response of *C. fissilis* seedlings to inoculation with arbuscular mycorrhizal fungi (AMF) at different phosphorus (P) doses and irrigation shifts.

(ii) Area of study: Botanic Garden, Poços de Caldas – MG, Brazil

(iii) Material and Methods: The experiment was conducted in a randomized block design in a 3x2x2 factorial scheme: the inoculation of two AMF species (*Acaulospora longula* and *Claroideoglomus etunicatum*), and the control without inoculation (Ct); two P doses (0 and 30 mg dm^{-3} of soil); and two irrigation shifts (once or twice a week). Height, stem diameter, shoot dry biomass, roots dry biomass, root: shoot dry biomass ratio, Dickson's quality index, P accumulation, mycorrhizal colonization rate and spore count, were evaluated 150 days after sowing.

(iv) Main results: There were influences of inoculation, irrigation shifts and P doses on seedlings. AMF inoculation was beneficial for the seedling growth and quality, whereas the DQI was a good indicator of *C. fissilis* seedlings quality. The inoculated AMF species differently influenced the growth parameters of seedlings.

(v) Research highlights: The higher growth and quality of *C. fissilis* seedlings in the substrate soil: sand (1:1) is superior when they are inoculated with *Claroideoglomus etunicatum*, without the addition of P and the irrigating twice a week.

Keywords: Meliaceae; dry matter; Dickson quality index; irrigation shifts; mycorrhizal colonization rate

1. INTRODUCTION

The symbiotic association between arbuscular mycorrhizal fungi and plant roots, named mycorrhiza, is closely associated with plant development in different ecosystems. This association has a biological function of favouring fungi and plants since there are nutrient cycling and soil quality maintenance (Smith *et al*., 2018). In this symbiotic association, plants provide shelter and essential compounds (carbohydrates, amino acids, etc.) for the fungi, which increase the availability of nutrients such as phosphorus and nitrogen for plants (Silva *et al*., 2017; Chen *et al*., 2017), as the established AMF increases the volume of soil explored by plants roots. This leads to greater absorption of water and mineral nutrients, as well as the aggregation of soil particles, further contributing to its physical quality (Kumar *et al*., 2016).

The absorption of soil nutrients by AMF (potassium, calcium, magnesium, sulphur, zinc, and especially phosphorus) (Silva *et al*., 2017) favours plant growth. AMF intensifies the plant capacity to resist the attack of pathogens (Wu *et al*., 2021), as well as to survive in stressful environments (Abdel-Salam *et al*., 2018; Chandrasekaran *et al*., 2019). In forest restoration, it benefits the seedling production by supplying vigorous seedlings with greater tolerance to transplantation in the field (Carmo *et al*., 2016; Silva *et al*., 2017).

Although the effects of AMF on plant growth, nutrition, and tolerance are known, their practical and large-scale use is not yet well established. One of the main challenges is related to inoculum production, since its development is mainly through a host plant, limiting their commercialization and multiplication. From an economic viewpoint, especially for seedlings production, mycorrhizal associations are becoming increasingly attractive, decreasing expenses with mineral fertilizers, pesticides, and irrigation (de Santana *et al*., 2014). Due to its beneficial effects on plants' primary production, AMF inoculation aiming at the increased production of quality seedlings have become common. However, since the inoculation efficiency depends on the inoculated AMF species, the plant species and the edaphoclimatic characteristics *(Carmo et al*., 2016; Van Geel *et al*., 2016; Goetten *et al*., 2016), studies elucidating the best combinations between AMF and plants are needed to optimize the production of forest seedlings.

Adequate irrigation management is necessary for the development of quality seedlings. Both the deficit and the excess of water can be harmful to plant development and health, as well as to the AMF. Furthermore, the absence of water management results, above all, in energy waste and environmental damage. Therefore, managing the irrigation based on the field capacity in a pot can help the operationalization of the entire irrigation system, aiming to guarantee the seedlings' production with adequate water supply, avoiding harming the plants and compromising the production and its costs.

In the search for the maximum benefit of AMF inoculation, it is needed to also consider phosphorus (P) dosage, which must be used carefully, as the benefits of mycorrhizal inoculation tend to decrease with the increasing availability of P, leading to different impacts depending on the species of AMF (Carmo *et al*., 2016; Chen *et al*., 2017; Essahibi *et al*., 2019). It is worth noting that the AMF inoculation lowers dosages and costs concerning nutritive solutions, henceforward its costs.

According to the Official National List of Endangered Flora Species, *Cedrela fissilis* Vell. is classified as vulnerable to extinction (Ministério do Meio Ambiente, 2014). This tree species belongs to the Meliaceae family, occurs in several Brazilian forest formations. It is economically important for, among others: civil construction, furniture, carpentry, essential oil extraction, timber and cosmetics industries in general (Lorenzi, 2014). From an environmental perception, *C. fissilis* is employed in riparian forests recovery and, potentially, for the recovery of soils contaminated with heavy metals. Thus, the search for technologies that enhance the quality of the seedlings in the nursery can diminish its vulnerability to extinction, in addition to contribute to the production process as a whole.

Given the economic and environmental importance of *C. fissilis*, and the challenges of using AMF in the production of woody species seedlings, there was an interest in arising knowledge about the management conditions for obtaining *C. fissilis* high-quality seedlings, contributing to lesser use of external inputs. Thus, the present study aimed to evaluate the influence of arbuscular mycorrhizal fungi inoculation, phosphorus doses, and irrigation shifts on the growth and quality of *Cedrela fissilis* seedlings.

2. MATERIAL AND METHODS

The experiment was conducted at the “Laboratório de Manejo Vegetal e Cultivo in Vitro” (Vegetable Management and In Vitro Cultivation Laboratory) of the Jardim Botânico Foundation (Poços de Caldas – MG). The plants were grown on wooden benches with a lighting system of 32W/127V fluorescent lamps at a height of 90 cm from the pots. The photoperiod was 16 hours of light/8 hours of darkness.

2.1 Obtention of AMF and experimental substrate

Two species of arbuscular mycorrhizal fungi (AMF) were used: *Claroideglomus etunicatum* and *Acaulospora longula*. Initially, they were inoculated in *Sorghum bicolor* L. for multiplication. For this, three sorghum plants were grown in a 5000 mL plastic container with an autoclaved substrate composed of dystrophic red Latosol and sand in a 1:1 ratio, with five replicates.

Fungi multiplication lasted four months. Then, the sorghum plants were removed from the plastic containers, and the lasting substrate, which contained AMF propagules, was used as an inoculum for the subsequent step. In the inoculum substrate obtained, 200 spores of *Acaulospora longula* (Al) and 13 spores of *Claroideoglomus etunicatum* (Ce) were counted per 10 g of substrate.

2.2 Plant material, experimental conduction, and design

C. fissilis seeds were obtained from a tree of origin with mature fruits, in the municipality of Poços de Caldas (21°42'58.1"S 46°35'31.5"W) and are properly registered (access code nº221 and acquisition code nº2212018). Afterwards, seed processing was proceeded, with storage at -5ºC until experimental conduction.

For the experiment, it was performed the sterilization by autoclaving of 1.8 L pots containing a mixture of soil and sand (1:1). The chemical characteristics of the substrate used was: pH: 5.9; Organic Matter: 15 g dm^{-3}; P (Melich I): 14 mg dm^{-3}; K: 0.5 $mmol_c$ dm^{-3}; Ca: 15 $mmol_c$ dm^{-3}; Mg: 2 $mmol_c$ dm^{-3}; H + Al: 19 $mmol_c$ dm^{-3}, Al: 0 $mmol_c$ dm^{-3}, H: 19 $mmol_c$ dm^{-3}; B: 0.31 mg dm^{-3}; Cu: 0.4 mg dm^{-3}; Fe: 31 mg dm^{-3}; Mn: 13 mg dm^{-3} and Zn: 0.8 mg dm^{-3}. The field capacity of the substrate in the pot was determined according to gravimetric method (Teixeira *et al*., 2017). Thereafter, irrigation shifts were established, restoring the substrate humidity to 60% of the field capacity.

The substrate containing the AMF was used for the inoculation of the experimental substrate so that three conditions of inoculation were obtained: Ce (autoclaved substrate and inoculated with 10g of inoculum substrate containing *Claroideglomus etunicatum*), Al (autoclaved substrate and inoculated with 10g of inoculum substrate containing *Acaulospora longula*) and Ct (only autoclaved substrate without no inoculation). Furthermore, two P doses were established: 0 and 30 mg P dm^{-3} substrate; and two irrigation shifts: 1x (once a week) and 2x (twice a week).

After substrate preparation, *C. fissilis* seeds were sowed. Based on the substrate's chemical analysis and the results of a previous experiment (Carneiro *et al*., 2011), a micronutrient solution was applied to all pots, composed of 0.5 mg of boron (B); 1.5 mg of copper (Cu); 3.0 mg of manganese (Mn) and 5.0 mg of zinc (Zn) per dm^3 of the substrate, and reagents (P.A.): H_3BO_3; $CuCl_2.2H_2O$; $MnCl_2$. $4H_2O$ and $ZnSO_4.7H_2O$.

Concerning P doses, the pots received solutions containing 30 mg dm^{-3} P or without it (0 mg P). Thus, for the 30 mg dm^{-3} P solution, the reagents KH_2PO_4, K_2SO_4, and KCl were used, so that the K and S dosages were kept at 100 and 15 mg dm^{-3}, respectively (Carneiro *et al*., 2011). On the other hand, for the 0 mg P treatment, there was no addition of the KH_2PO_4, so that the only source of P was what was originally in the substrate.

At 12 days after sowing, all individuals had already germinated. At 15 days post-sowing, the plants were thinned, eliminating surpluses and leaving only the most vigorous and central seedling in each pot. Then, seedlings were placed in trays positioned on wooden benches.

The experiment was conducted in a randomized block design in a 3x2x2 factorial scheme, consisting of three inoculation conditions: inoculated with *A. longula* (Al), inoculated with *C. etunicatum* (Ce), and control without inoculation (Ct); two P doses 0 (0 mg P) and 30 (30 mg P) mg dm^{-3} of soil; and two irrigation shifts, once (1x) or twice (2x) a week; with three replicates, totalling 36 experimental plots. Each experimental plot consisted of one seedling. The experiment was conducted for 150 days after germination, by when the plants were collected and separated into shoots and roots and, subsequently, submitted to analysis.

2.3 Biometric analysis of seedlings

Plants' height was evaluated with a measuring tape (measured from the base to the apex of the stem) and the stem diameter with a calliper (measured at the stem's base). Shoot and root dry biomass were obtained by drying in an oven with forced air circulation at 65°C until constant weight. The root dry biomass/ shoot dry biomass ratio was also calculated.

2.4 Dickson Quality Index

The Dickson Quality Index (DQI) was determined as a function of the shoot height, the stem diameter, seedlings total dry biomass, shoot and root dry biomass, using the formula (Dickson *et al*., 1960):

$$\mathrm{DQI} = \frac{\text{Seedling Total Dry Biomass (g)}}{\text{Height(cm)/Stem Diameter (mm)} + \text{Shoot Dry Biomass (g) / Root Dry Biomass (g)}}$$

2.5 Microbiological parameters analysis

For the AMF spore density quantification (*A. longula* and *C. etunicatum*), samples of 50 mL of the substrate were collected in each pot, with the method of settling and wet sieving (Gerdemann & Nicolson 1963). The samples were transferred to a 2,000 mL beaker, where 1L of drinking water was added. The suspension was stirred with a glass stick until it was dispensed through the sieves (750, 250, 100, and 45 µm).

The material retained in the smallest sieve was centrifuged in 50 mL tubes for 3 minutes, 3000 RPM. The supernatant was discarded, and a 50% sucrose solution was added, followed by second centrifugation for 2 minutes, 2000 RPM. The supernatant from each tube was dispensed separately in a sieve (45 µm) and washed to remove excess sucrose. The material was transferred to Petri dishes for later spore counting.

To analyse the rate of mycorrhizal colonization of seedlings in both inoculated AMF, the method described by Giovanetti and Mosse (1980) was used. Samples of one gram of fine roots were collected, in all pots, in the portions: upper, middle and tips of the root system. Then the samples were conditioned in 50% ethanol, until analysis. For clarification, the roots were placed in a 50 mL beaker, immersed in a 10% KOH solution and heated in a water bath at 60 to 90ºC for 15 to 30 minutes, and then washed in water twice.

For staining, the roots were transferred to 50 mL beakers and immersed in a 5% trypan blue solution, heated in a water bath, followed by the same clarification step. The samples were placed in a Petri dish to calculate the colonization percentage (Giovanetti & Mosse 1980). Root segments containing fungal structures were evaluated with a stereomicroscope.

2.6 Phosphorus accumulation in shoots

This determination was carried out according to standard leaf analysis protocols. Thus, shoot dry biomass samples were submitted to nitric perchloric digestion and the P content in the samples was determined by metavanadate colorimetry (P total) and molybdenum blue colorimetry (P soluble in acetic acid) (Malavolta *et al*., 1997). The P accumulation was obtained by the product between P content (mg P g^{-1} shoot dry biomass) and shoot biomass.

2.7 Data analysis

Pearson's correlation analysis ($p \leq 0.05$) was performed between all variables (Supplementary Table S1) so that the parameters highly correlated by Pearson's coefficient ($r > 0.7$, $p \leq 0.05$) were removed to avoid redundancy in multivariate analysis. Then, to analyse the relationship between the response variables with the treatments, the principal component analysis (PCA) was carried out (Jolliffe & Cadima 2016), where the coefficients for each component were established from a correlation matrix. The data obtained were tested for the assumptions of the analysis of variance and then subjected to comparisons between means by the Scott-Knott test ($p \leq 0.05$).

3. RESULTS

In the principal component analysis showed that the inoculants promoted very different behaviours in *C. fissilis* seedlings, and the height and spore variables contributed most to the variation of the data in this experiment. Thus, Dickson Quality Index (DQI) and Total dry biomass (TDB) were more closely related to *C. etunicatum,* whereas plant height and the number of spores were more interconnected to *A. longula*. The component 1 (PC1) explained 50.7% of the total variation of the data, while component 2 (PC2) explained 15.2% of the data variation, totalling 65.9% for the two components together (Figure 1).

[Insert Figure 1 here]

For all parameters, the occurrence of significance for the interactions was identified first, initially of the triple interactions, then double interactions, and lastly the occurrence of the effect of the isolated factors. Regarding plant height and DQI, there was a triple interaction between the factors, while for the stem diameter there was a double interaction between P doses and irrigation shifts (Figure 2).

The plants that received 30 mg P, were irrigated once a week and inoculated with *A. longula* (Al), presented higher height in comparison to *C. etunicatum* (Ce) and no inoculation (Ct), respectively 21% and 49.5%. Plants inoculated with Ce, at the same conditions mentioned, grew 36.8% more than Ct plants. In contrast, plants irrigated 1x and under 0 mg P, as well as those irrigated 2x and under either 30 mg P or 0 mg P, did not show plant height responsiveness to the inoculants (Figure 2A),

When evaluating the P doses, according to each inoculum and irrigation shift, it was observed that the plants inoculated with Al and Ce irrigated 1x showed greater height when treated with 30 mg of P. For the seedlings irrigated 2x there was no significant difference in height for P doses. In the Ct treatment, the plants under irrigation 2x and with 30 mg P, were taller than those under 0 mg P. On the other hand, under irrigation performed 1x, there was no influence of P on plant height (Figure 2A). As for the irrigation shifts performed comparatively with inoculants and P doses, it is worth highlighting that in the irrigation 2x, the inoculants Al and Ce under 0 mg P provided higher plants. Furthermore, under 30 mg P, plants inoculated with Ce were not influenced by the irrigation shift, however, those inoculated with Al showed greater height when irrigated 1x. Ct plants, on the other hand, showed greater height under 2x and 0 mg P, with no response to the irrigation regimen under 30 mg P (Figure 2A).

The P application increased the diameter of *C. fissilis* seedlings in interaction with the irrigation shifts, where for the 1x, plants under 30 mg P had a stem diameter about 17% larger than those under 0 mg P. As for plants submitted to 2x, they did not show differences between P doses. In contrast, when comparing the irrigation shifts within each level of P, it was found that plants under 0 mg P exhibited a stem diameter 7.7% larger when under 2x than under 1x, whereas under 30 mg P, there was no significant response (Figure 2B).

Concerning the DQI, when comparing the inoculants within each P dose and irrigation shift, it was found that plants under 2x, 0 mg P, and Ce showed higher quality, reaching 25% and 35% higher than those inoculated with Al and Ct, respectively. In contrast, in 1x, with 30 mg P or 0 mg P, as well as those under 2x with 30 mg P, the inoculants did not influence the seedling quality (Figure 2C). When evaluating P within each inoculum and irrigation shift, it was found that plants under 2x, Ce, and 0 mg P showed higher DQI than those under 30 mg P and other inoculants. It is worth noting that plants inoculated with Al and Ct, with 30mg P or 0 mg P, were not affected by the irrigation shifts on the seedlings' quality. On the other hand, when comparing irrigation shifts in each inoculum and P dose, plants inoculated with Ce and with 30 mg P did not suffer the influence of the irrigation shift, while under 0 mg P, the seedlings under 2x showed higher quality than those under 1x (Figure 2C).

[Insert Figure 2 here]

For the shoot dry biomass (Figure 3A), there was only a double interaction between the inoculum and phosphorus doses. It was observed that plants under 30 mg dm^{-3} inoculated with Ce and Al showed higher values of SDB than Ct plants, thus indicating a positive effect of inoculation. This difference represented a 44% increase in the biomass of the inoculated plants in comparison to Ct. However, there was no difference between the inoculants for plants under 0 mg P. Additionally, Al also had the highest rate of colonization for the same P dose. Regarding the P influence within each inoculum, there was no influence in the shoot dry biomass of inoculated plants (Figure 3A).

Assessing the root dry biomass, a triple interaction was obtained between inoculation, irrigation shifts, and P doses (Figure 3B). Plants with 1x, 30 mg P and Ce and Al showed higher root dry biomass than Ct plants. On the other hand, there was no influence of inoculation for root dry biomass of plants under 0 mg P and an irrigation shift.

In plants under 2x and 30 mg of P, the highest root dry biomass was found in Al, while in plants under 2x and 0 mg P the largest root dry biomass was found in plants inoculated with Ce, followed by Al and Ct. These results show the positive effect of mycorrhizal inoculation. Thus, plants under 2x, 0 mg P, and Ce showed a 40.8% increase in root dry biomass in comparison to Al, and 65.5% in comparison to Ct. In general, plants under 2x, Ce, and 0 mg P had a higher root dry biomass than those under 30 mg P. For the other inoculants, there was no influence of P on root biomass accumulation (Figure 3B).

It was possible to perceive the influence of AMF on *C. fissilis* root/ shoot dry biomass ratio (Figure 3C). When observing the result obtained in samples inoculated and without P addition, there was a greater relationship in plants inoculated with Ce and Al than in the control, indicating that the AMF provided greater root growth, even without P addition, in comparison to non-inoculated plants. In plants under 30 mg P, the highest ratio was observed in Ct and Al plants, followed by Ce plants. Inoculation with Ce promoted a higher root/ shoot ratio without P addition, while in plants inoculated with Al there was no interference from P. In Ct, it was possible to verify a higher root/ shoot ratio in plants under 30 mg P (Figure 3C).

[Insert Figure 3 here]

For the analysis of spore count (Figure 4A), mycorrhizal colonization rate (MCR) (Figure 4B), and P accumulation in shoots (PAS), only double interactions were observed (Figure 4C). Sporulation was significantly influenced by irrigation and inoculation with FMA. For inoculation with Al in 1x, there was an increase of 47% in comparison to Ce, and 51.2% in 2x. It was noticed that in Ct no spores were found in any of the samples, indicating that there was no contamination during the experiment (Figure 4A). It is also important to emphasize that plants under 2x showed greater sporulation, with a variation of 29 to 41 spores (50 cm3 soil^{-1}), contrasting to the result found for 1x that corresponds to 18 to 34 spores (50 cm3 solo^{-1}). The Ct did not present any significant difference between the irrigation shifts (Figure 4A).

Regarding mycorrhizal colonization, there was a positive effect of inoculation with Al and Ce and P doses. For the application of 30 mg dm^{-3} P to the soil, mycorrhizal colonization rate ranged from 35 to 75%, and Al value 53% higher than Ce. As for 0 mg dm^{-3}, it was found that Al had an mycorrhizal colonization rate of 34% higher than Ce, ranging from 38 to 58%. When comparing each P dose with the inoculum, it was also noticed that Al and Ce did not show a significant difference between the dosages (Figure 4B).

Higher P accumulation was obtained in plants that received a single irrigation shift and were grown with the addition of 30 mg P than those under 0 mg P. Plants irrigated 2x showed no difference between doses (Figure 4C). For the irrigation shifts compared to P doses, it is clear that under 0 mg P and 2x there was a greater PAS than in 1x. Under 30 mg P, there was no significant difference between shifts (Figure 4C).

[Insert Figure 4 here]

4. DISCUSSION

Inoculation with AMF promotes greater growth and quality of *C. fissilis* seedlings, that differ between the species used, depending on P dose and irrigation shift. The higher growth and quality of *Toona ciliata* seedlings inoculated with different AMF species were related to the greater amount of nutrients absorbed by these seedlings (Carmo *et al,*. 2016; Silva *et al*., 2017), which may have contributed to their best physiological performance. Thus, the greater growth of inoculated seedlings in this study can be attributed to the better physiological performance of well-nourished seedlings.

AMF also optimizes plant use of water resources, which in theory allows the use of irrigation shifts that provide savings in water resources without compromising plant production (Ronga *et al*., 2019). Inoculated plants have greater roots hydraulic conductance and higher water status (Abdel-Salam *et al*., 2018) generally provided by the production of compatible solutes, which promote osmotic adjustment (Khalil & El-Ansary 2020). AMF can also lead to roots' increased production of phytohormones (indoleacetic acid, abscisic acid, methyl jasmonate, brassinosteroids) which are related, among others, to greater tolerance to water deficit (Liu *et al*., 2016). Inoculation with AMF, besides promoting a good production of plant biomass, increases plants' robustness and produces more homogeneous seedlings.

In woody species, inoculation with AMF provided seedlings with greater height, stem diameter, and shoot dry biomass, producing more vigorous seedlings. Also, it allowed lower application of fertilizers, thus saving resources (Goetten *et al*., 2016), mainly by increasing the P and other nutrients efficiency use in comparison to uninoculated plants. In the present study, inoculation with Ce allowed P greater use, since seedlings without P addition and inoculated with Ce showed higher dry biomass and DQI compared to the other treatments. In contrast, in the condition of low P availability, inoculation with Ce only optimized P use, making it necessary to irrigate twice a week. This suggests that P and hydraulic optimization does not occur concurrently in *C. fissilis* seedlings.

The Dickson quality index is used to evaluate the quality of seedlings, being established that the higher the value, the higher the quality of the seedling, and 0.20 as the minimum value for such (Hunt, 1990). When evaluating the quality of seedlings through DQI, permissible for morphological parameters, *C. fissilis* benefited from the use of Ce, under two irrigation shifts and without the addition of P, with a DQI corresponding to 0.71, therefore, it is clear that plants under this treatment, hereby showed the highest quality (Figure 2 C). The higher quality of seedlings through the DQI, reflecting the favouring of plant growth, increases its capacity to resist pathogens attack, water deficits, and survival to the transplantation process, especially under environmental stresses.

Woody species inoculated with AMF also showed a higher growth and nutritional status that allowed than to be successfully used in revegetation (Goetten *et al*., 2016). Inoculation with AMF on seedlings leads to higher liquid photosynthetic rate, transpiratory rate, and stomatal conductance, which also allows a higher content of nitrogen, soluble sugars, and proteins (Shi *et al*., 2016). AMF are also linked to greater root activity, so that greater allocation of carbohydrates to the roots allows root growth and, consequently, greater soil exploration, and water and nutrients exploration (Liu *et al*., 2016; Chen *et al*., 2017).

The interaction between plants and AMF has been related to the higher production of root dry biomass (Chen *et al*., 2017). Thus, in this study, when realizing that the microorganisms provided greater root growth without P addition, exhibiting greater root increment when inoculated with Ce, compared to Al and Ct, as well as other results obtained, there is a clear understanding of the benefits arising from mycorrhization. Furthermore, there were advantages of establishing irrigation shifts and water depth control appropriately for the seedlings, in detriment to the field capacity in pots and carrying out phosphate fertilization as indicated for plants inoculated with AMF.

Some studies reported the benefit of inoculation with AMF for the production of quality seedlings (Carmo *et al*., 2016; Goetten *et al*., 2016; Silva *et al*., 2017), including related to the optimization of resources (Goetten *et al*., 2016). However, the variability in specificity between plant-AMF is often reported (Goetten *et al*., 2016; Shi *et al*., 2016). Thus, the mycorrhizal dependence of plant species on AMF can vary according to plant species and inoculated AMF (Tawaraya, 2003; Chen *et al*., 2017). Therefore, in the context of ecological restoration, inoculation with AMF allows the faster growth of several plant species, however, it is dependent on the macro and micro symbiont species involved, on management and inoculation time (Neuenkamp *et al*., 2019). Hence, there is a need for studies to elucidate the best combinations between plants and AMF species, where the benefits of inoculation on plant growth and production are maximized (Chen *et al*., 2017), especially those that describe the specific abiotic conditions.

In the present study, the response variation between the inoculated AMF was evident, so that while Ce led to greater accumulation of biomass and DQI, inoculation with Al provided greater plant height, showing that inoculation with these AMF probably triggered different physiological processes in the seedlings. Regarding the spore count, it was evident that the two species of inoculated AMF showed positive resourcefulness, however, Al stood out. The fact that the AMF had a higher number of spores for Al does not mean that plants inoculated with the aforementioned have greater growth, quality, or even resistance. This is possible because a specific species of this fungus is capable of forming a symbiosis with several plant species of different genera and families, and with different growth profiles.

The influence of irrigation shifts was also quite evident in this work, mainly for root growth for inoculated plants in the absence of phosphorus. According to Lima *et al*. (2016), with the reduction of irrigation intensities, the seedlings tend to decrease in the amount of shoot and root dry biomass. Water availability in soil can influence both the P availability and AMF activity (García *et al*., 2008). In this way, it seems that the adequate water availability not only is related with higher P availability for absorption but also to higher water status and gas exchanges (Chen *et al*., 2017; Abdel-Salam *et al*., 2018; Khalil & El-Ansary 2020). Thus, the seedlings have higher nutritional status for growth and development. On the other hand, seedlings under water deprivation have lower biomass accumulation e lower survival (Hailemariam *et al*., 2017; Mai *et al*., 2018). From that, for the *C. fissilis* seedlings from our study, two irrigation shifts reflected in higher root biomass, contributing also for higher total biomass.

The P dose used in this study is considered intermediate (Carneiro *et al*., 2002), and the best effects of AMF occur with phosphate fertilizers with low or intermediate dosages, ranging from 25 to 60 mg P dm^{-3}, which justifies the results obtained regarding shoot dry biomass, showing greater weighs when applying a dose of 30 mg P dm^{-3}.

Mycorrhizal inoculation is an environmental technology that can be carried out by the rural producer at low costs, bringing sustainability to their management system, valuing their products and services. The use of microbial inoculants can be considered one of the most viable alternatives to improve agricultural sustainability in the short and long term (Chen *et al*., 2018). Thus, by employing such conditions in a practical way and on large-scale cultivation, there are significant gains in producing seedlings with greater chances of survival and lower environmental impacts (Goetten *et al*., 2016). However, given the variations in interactions among mycorrhizas and plants, soil and environmental conditions and the benefits of AMF inoculation may vary, depending on farm conditions (Rillig *et al*., 2020; Gupta & Abbott, 2021).

Nevertheless, dosing the water depth used and knowing the ideal amount of phosphate fertilizer that should be added to produce *C. fissilis* seedlings considerably reduces expenses. Additionally, it contributes to the environment and minimizes possible environmental impacts, through the adequate use of natural resources, the non-pollution of water bodies, soil, etc., leading to more sustainable and economically viable production.

The growth and quality of *Cedrela fissilis* Vell. seedlings in soil: sand (1:1) substrate is superior when they are inoculated with *Claroideoglomus etunicatum*, without adding phosphorus, and irrigating twice a week. The production of *C. fissilis* seedlings can be carried out with the inoculation of *C. etunicatum*, allowing savings of inputs by reducing additional phosphorus demand, however, there is a need to irrigate about twice a week. The inoculation with *C. etunicatum* and *A. longula* optimizes the obtaining of *C. fissilis* seedlings with higher quality, however, there is no concomitant optimization of the amount of P applied neither the irrigation regimen. Depending on the resources available, it is possible to choose the best AMF.

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Figures and legends

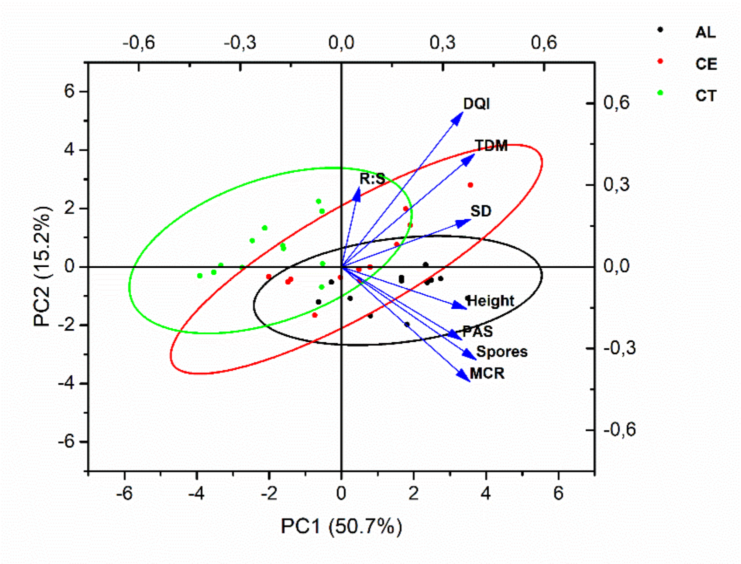

Figure 1: Principal component analysis showing the relationship between treatments and variables analysed in the experiment: plant height (H), stem diameter (SD), Dickson Quality Index (DQI), total dry biomass (TDB), root: shoot dry biomass (R:S), number of spores (Spores), mycorrhizal colonization rate (MCR) and P accumulation in shoots (PAS). The two main components explained 65.9% of the data variation.

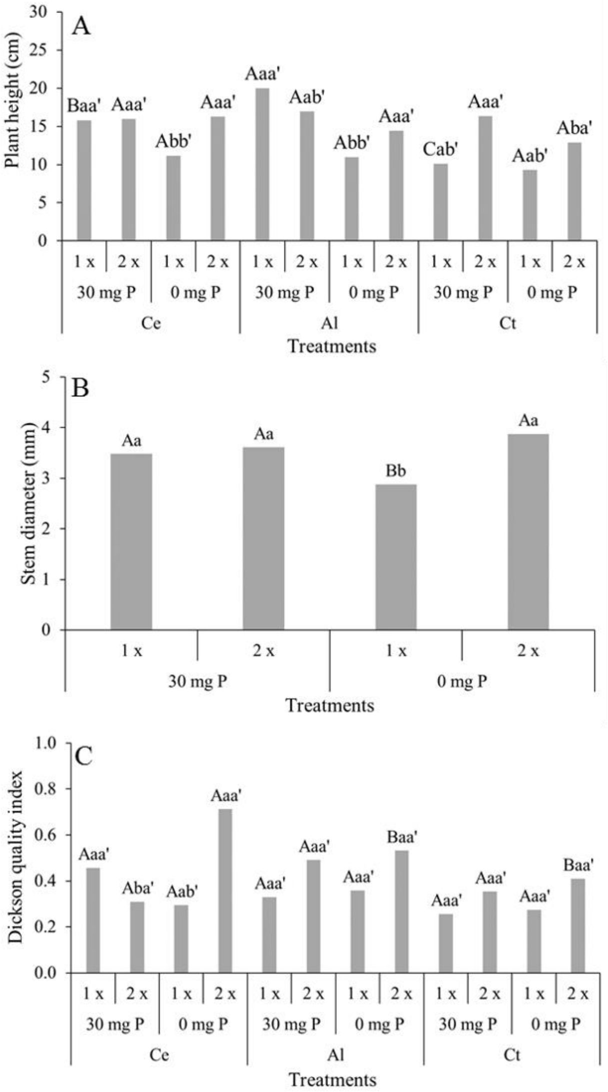

Figure 2: Plant height (A), stem diameter (B), and Dickson Quality Index (C) of *Cedrela fissilis* plants under three inoculation conditions (*Claroideglomus etunicatum* - Ce, *Acaulospora longula* - Al and uninoculated - Ct), two P levels (30 mg dm^{-3} and 0 mg dm^{-3}) and two irrigation shifts (once - 1x or twice - 2x a week). In the triple interaction, for the variables height and DQI, uppercase letters compare inoculants within each P level and each irrigation shift, lowercase letters compare the P levels within each inoculum and irrigation shift, and lowercase letters in apostrophe compare the irrigation shifts within each inoculum and P level. For the double interaction, in the variable stem diameter, the capital letters compare the P levels within each irrigation shift, while the lower letters compare the shifts of irrigation within each P level. Means followed by the same letter do not differ, according to the Scott-Knott test ($p \leq 0.05$).

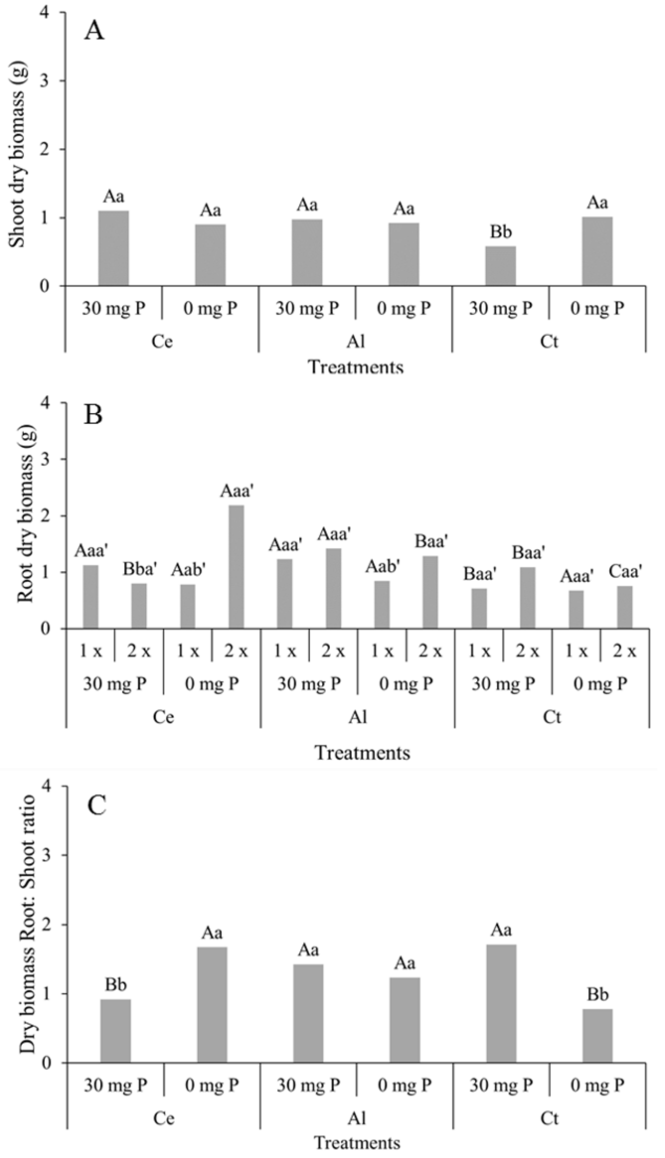

Figure 3: Shoot dry biomass (A), root dry biomass (B), R:S dry biomass ratio (C), and total dry biomass (D) of *Cedrela fissilis* plants under three inoculation conditions (*Claroideglomus etunicatum* - Ce, *Acaulospora longula* - Al and not inoculated - Ct), two P levels (30 mg dm^{-3} and 0 mg dm^{-3}) and two irrigation shifts (once - 1x or twice - 2x a week). In the triple interaction, for the variables root dry biomass and total dry biomass, capital letters compare inoculants within each P dose and each irrigation shift, lower case letters compare the P doses within each inoculum and irrigation shift, and lower-case letters followed by apostrophe compare irrigation shifts within each inoculum and P level. For the double interaction, in the variables shoot dry biomass and R:S dry biomass ratio, the capital letters compare inoculum within each P dose, while the lowercase letters compare the irrigation shifts within each inoculum. Averages followed by the same letter do not differ, according to the Scott-Knott test ($p \leq 0.05$).

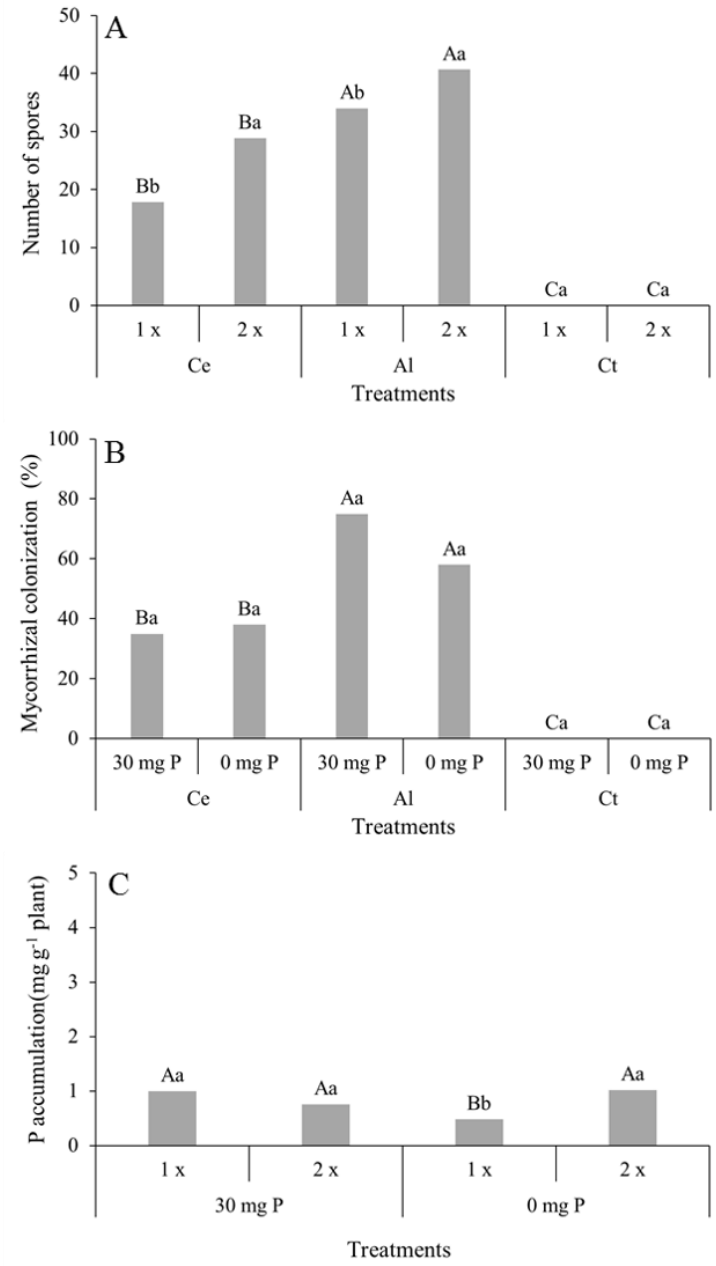

Figure 4: Number of spores (A), mycorrhizal colonization rate (B), and P accumulation in *Cedrela fissilis* plants under three inoculation conditions (*Claroideglomus etunicatum* - Ce, *Acaulospora longula* - Al and uninoculated - Ct), two P levels (30 mg dm^{-3} and 0 mg dm^{-3}) and two shifts of irrigation (once - 1x or twice - 2x a week). For double interactions, in the spore and P accumulation variables, capital letters compare the P doses within each irrigation shift, while the lower letters compare the irrigation shifts within each inoculum. In the colonization variable, the capital letters compare the inoculants within each P level, while the lower letters compare the P doses within each inoculum. Averages followed by the same letter do not differ, according to the Scott-Knott test ($p \leq 0.05$).

SUPPLEMENTARY MATERIAL

Pearson's correlation analysis (Table S1) showed that height had a direct correlation with stem diameter, spores, mycorrhizal colonization rate (MCR), shoot dry biomass (SDB), root dry biomass (RDB), total dry biomass (TDB), and P accumulation in shoots (PAS). An inverse correlation was observed between the R:S dry biomass ratio and the DQI. Stem diameter showed a direct correlation with spores, MCR, SDB, RDB, TDB, DQI, and PAS. The variables R:S and SDB also had an inverse correlation with the stem diameter. MCR showed a direct correlation with DQI, an index that indicates the quality standard of the seedlings and P accumulation. SDB and TDB had a direct correlation with DQI and PAS. Furthermore, RDB showed a direct correlation with DQI. PAS was directly correlated with seedling height, the number of spores, and MCR. Amid the indications evidenced by Pearson's correlation, it was possible to perceive the effects of the mycorrhizal influence on *C. fissilis* seedlings.

		Height	Diameter	Spores	MCR	R:S	SDB	RDB	TDB	IQD	P Accum.
Height	Corr. Pearson	1	0,5748*	0,5038*	0,5114*	0,1249	0,3539*	0,5108*	0,5313*	0,2688	0,5805*
	Significance	--	0,0002	0,0017	0,0014	0.4679	0.0342	0,0015	0,0009	0,1129	0,0002
Diameter	Corr. Pearson	0,5747*	1	0,4079*	0,3502*	-0,0391	0,4964*	0,4108*	0,5364*	0,6439*	0,6066*
	Significance	0.0002	--	0,0135	0,0363	0,8211	0,0021	0,0128	0,0007	0,0000	0,0001
Spores	Corr. Pearson	0,5038*	0,4079*	1	0,9383*	0,0889	0,2866*	0,4899*	0,4824*	0,4118*	0,4949*
	Significance	0.0017	0,0135	--	0	0,6059	0,0901	0,0024	0,0029	0,0126	0,0022
MCR	Corr. Pearson	0,5114*	0,3502*	0,9383*	1	0,0936	0,2277	0,4329*	0,4131*	0,3208	0,5005*
	Significance	0.0014	0,0363	0	--	0,5871	0,1817	0,0084	0,0123	0,0565	0,0019
R:S	Corr. Pearson	0.1249	-0,0391	0,0889	0,0936	1	-0,5229*	0,5051*	0,0755	0,1785	-0,1020
	Significance	0.4679	0,8211	0,6059	0,5871	--	0,0011	0,0017	0,6615	0,2975	0,5537
SDB	Corr. Pearson	0,3539*	0,4964*	0,2866	0,2277	-0,5229*	1	0,3805*	0,7752*	0,6111*	0,3705*
	Significance	0.0342	0,0021	0,0901	0,1817	0,0011	--	0,0221	0,0000	0,0000	0,0261
RDB	Corr. Pearson	0,5108*	0,4108*	0,4899*	0,4329*	0,5051*	0,3805*	1	0,8791*	0,8183*	0,2699
	Significance	0.0014	0,0128	0,0024	0,0084	0,0017	0,0221	--	0,0000	0,0000	0,1115
TDB	Corr. Pearson	0,5313*	0,5364*	0,4824*	0,4131*	0,0755	0,7752*	0,8791*	1	0,8739*	0,3752*
	Significance	0.0009	0,0007	0,0029	0,0123	0,6615	0,0000	0,0000	--	0,0000	0,0241
DQI	Corr. Pearson	0,2688	0,6439*	0,4118*	0,3208*	0,1785	0,6111*	0,8183*	0,8739*	1	0,3158
	Significance	0,1129	0,0000	0,0126	0,0565	0,2975	0,0000	0,0000	0,0000	--	0.0606
PAS	Corr. Pearson	0,5805*	0,6066*	0,4949*	0,5005*	-0,1020	0,3705*	0,2699	0,3752*	0,3158	1
	Significance	0,0002	0,0001	0,0022	0,0019	0,5537	0,0261	0,1115	0,0241	0.0606	--

Table S1: Pearson's correlation between the variables analysed in *C. fissilis* seedlings submitted to inoculation with AMF, P doses and irrigation shifts.

* significant correlations ($p \leq 0{,}05$)