ANÁLISES PROTEÔMICA E HORMONAL ASSOCIADAS A SUBCULTURAS *in vitro* E ENRAIZAMENTO *ex vitro* DE BROTAÇÕES DE *Cedrela fissilis* VELL. (MELIACEAE)

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UNIVERSIDADE ESTADUAL DO NORTE FLUMINENSE DARCY RIBEIRO - UENF

CAMPOS DOS GOYTACAZES - RJ
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Aos meus pais, João e Francisca pelo amor, confiança e exemplo de vida.
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SUMÁRIO

RESUMO

OLIVEIRA, Tadeu dos Reis de; D.Sc.; Universidade Estadual do Norte Fluminense Darcy Ribeiro. Março de 2021. Análises proteômica e hormonal associadas a subculturas *in vitro* e enraizamento *ex vitro* de brotações de *Cedrela fissilis* Vell. (Meliaceae).

Os ciclos sucessivos de subculturas desempenham um papel essencial na multiplicação *in vitro* de plântulas em grande escala. O presente estudo teve como objetivo investigar o efeito de ciclos sucessivos de subcultura no desenvolvimento de brotações *in vitro* e no enraizamento *ex vitro*, bem como na alteração do conteúdo endógeno de hormônios, metabolismo de poliaminas (PAs) e perfil proteômico em *Cedrela fissilis*. Para avaliar o desenvolvimento das brotações *in vitro* bem como o enraizamento *ex vitro*, os segmentos nodais apicais e cotiledonares foram excisados de plântulas germinadas *in vitro* e utilizados como explantes. As brotações obtidas a partir destes dois tipos de explante foram multiplicadas em quatro ciclos de subculturas sucessivas com intervalo de 45 dias cada. Ao final de cada subcultura, foram analisados a indução, o número e o comprimento médio de brotações. Para analisar o desenvolvimento das raízes, as brotações de cada subcultura foram submetidas ao enraizamento *ex vitro* e, após 45 dias, foram avaliadas a indução, o número e o comprimento médio de raízes. As mudas enraizadas foram transferidas para a casa de vegetação e foi avaliada a taxa de sobrevivência após 90 dias. Amostras dos segmentos nodais apicais e cotiledonares utilizados como explantes iniciais, e das brotações oriundas destes explantes foram

coletadas para análise do conteúdo hormonal, metabolismo de PAs, e proteômica comparativa para avaliar o desenvolvimento das brotações e o potencial de enraizamento *ex vitro* destas brotações, comparando-se a primeira e quarta subculturas. Verificou-se que o aumento do número de subculturas diminuiu significativamente o crescimento e desenvolvimento das brotações *in vitro* da primeira para a quarta subcultura. Esta redução no crescimento das brotações foi acompanhado de redução no conteúdo de putrescina livre (Put), PAs livres totais, ácido indol-3-acético (AIA), e aumento no conteúdo de ácido abscísico (ABA), ácido 12-oxofitodienoico (OPDA), ácido jasmônico (JA) e ácido salicílico (SA) nos explantes da quarta subcultura, comparativamente com a primeira. A atividade da ornitina descarboxilase foi maior que a arginina descarboxilase. Para o enraizamento *ex vitro*, verificou-se que o número de subcultura também afetou o potencial de desenvolvimento de raízes adventícias. A redução no enraizamento *ex vitro* em brotações na quarta subcultura comparativamente a primeira foi acompanhada da diminuição no conteúdo endógeno de AIA, ABA, OPDA e PAs livres totais, e de aumento no conteúdo de JA, jasmonoil-isoleucina, ácido trans-cinâmico e SA. Adicionalmente, verificou-se redução no acúmulo de proteínas relacionadas com as subunidades do centro de reação dos fotossistemas I e II, metabolismo energético, assimilação de nitrogênio, rota de PAs, e respostas ao ABA e JA, as quais podem ser relevantes para o enraizamento de brotações. Por outro lado, proteínas relacionadas a ferimentos mostraram aumento em brotações na quarta subcultura, e podem estar relacionadas à diminuição no potencial de enraizamento de brotações. Este é o primeiro trabalho que mostra o efeito da subcultura e as alterações hormonais e moleculares associadas à redução do potencial de desenvolvimento de brotações *in vitro* e do enraizamento *ex vitro* em *C. fissilis*. Estes trabalhos demonstram dados hormonal e proteômico relaventes sobre a competência para o desenvolvimento *in vitro* de brotações e seu potencial para o enraizamento *ex vitro* e podem ajudar a melhorar a produção de mudas em larga escala para a espécie em estudo.

Palavras-chave: Micropropagação, Proteômica, Hormônios, Subculturas *in vitro*; Enraizamento *ex vitro*.

ABSTRACT

OLIVEIRA, Tadeu dos Reis de; D.Sc.; Universidade Estadual do Norte Fluminense Darcy Ribeiro. March, de 2021. Proteomic and hormonal analyzes associated to the *in vitro* subcultures and *ex vitro* rooting of *Cedrela fissilis* Vell. (Meliaceae) shoots.

Successive cycles of subcultures play an essential role for the *in vitro* multiplication of plantlets in large-scale. The present study aimed to investigate the effect of successive subculture cycles on the development of *in vitro* shoots and on the *ex vitro* rooting, as well as on the alteration on endogenous contents of hormones, polyamine (PA) metabolism, and proteomic profile in *Cedrela fissilis*. The apical and cotyledonary nodal segments were excised from seedlings germinated *in vitro* and used as explants to evaluate the *in vitro* shoot development and the *ex vitro* rooting. The shoots obtained from two types of explants were multiplied in four successive subcultures, at an interval of 45 days each. At the end of each subculture cycle, the induction, number, and average length of shoots were analyzed. To evaluate the root development, the shoots from each subculture cycle were subjected to *ex vitro* rooting and after 45 days, the induction, number and average length of roots were evaluated. The rooted plantlets were transferred to the greenhouse, and the survival rate was evaluated after 90 days. Samples of apical and cotyledonary nodal segments used as initial explants, and shoots from these type of explants were collected for analysis of hormonal content, PA metabolism, and comparative proteomics to evaluate the *in vitro* shoot development and the *ex vitro* rooting

potential comparing the first and fourth subcultures. The increase in the number of subcultures significantly decreased the *in vitro* shoot development as compared to the first to the fourth subculture. This reduction in growth of shoots was accompanied by a reduction in the content of free putrescine, total free PAs, indole-3-acetic acid (IAA), and an increase in the contents of abscisic acid (ABA), 12-oxo-phytodienoic acid (OPDA), jasmonic acid (JA) and salicylic acid (SA) in the explants at the fourth subculture compared to the first. Ornithine decarboxylase activity was greater than arginine decarboxylase. To *ex vitro* rooting we found that the number of subcultures also affected the potential of adventitious roots development. The reduction in *ex vitro* rooting in shoots in the fourth subculture compared to the first was accompanied by a decrease in the endogenous content of IAA, ABA, OPDA and total free PAs, and an increase in the contents of JA, jasmonoyl-isoleucine, trans-cinnamic acid and SA. Additionally, we verified a reduction in the accumulation of proteins related to the subunits of the reaction centers of photosystem I and II, energy metabolism, nitrogen assimilation, via PAs, and responses to ABA and JA, which may be relevant for the rooting of shoots. In contrast, wounding-related proteins showed an increase in shoots at the fourth subculture and may be related to a decrease in the rooting potential of the shoots. This is the first work showing the effect of subculture and the hormonal and molecular changes associated to reduction on potential for *in vitro* shoot development and *ex vitro* rooting in *C. fissilis.* These works show relevant hormonal and proteomic data about the competence for the *in vitro* shoot development and their potential for *ex vitro* rooting and can be useful to improve the production in large-scale of plantlets for this species.

Keywords: Micropropagation; Proteomics; Hormones; Subcultures *in vitro*; *Ex vitro* rooting.

1. INTRODUÇÃO

Dentre as técnicas de cultura de tecidos, a propagação *in vitro* ou micropropagação, apresenta-se como uma opção para a multiplicação em várias espécies, incluindo arbóreas (Thorpe, 2007; Zeng et al., 2019; Oliveira et al., 2020). A regeneração *in vitro* é um método de propagação assexuada que possibilita o fornecimento de mudas de plantas com rápido crescimento, livres de vírus e sem restrições sazonais, com potencial para aplicação em proteção ecológica, produção de celulose e utensílios para fins comerciais (Zeng et al., 2019).

A propagação *in vitro* compreende várias fases, desde a escolha do explante, indução e multiplicação das brotações, seguido de enraizamento e aclimatização das mudas, sendo necessário a adequação das condições em cada fase para cada espécie em estudo. Em espécies arbóreas, estudos têm mostrado a capacidade de regeneração de diferentes tipos de explantes, e a seleção de explantes de segmentos nodais tem sido mais utilizada, apresentando resultados promissores na multiplicação, como mostrado para *Tectona grandis* (Tiwari et al., 2002), *Populus tremula* (Huang e Dai, 2011), *Cabralea canjerana* (Rocha et al., 2007), *Cedrela fissilis* (Nunes et al., 2002; Aragão et al., 2016; Aragão et al., 2017; Oliveira et al., 2020) e *Cariniana legalis* (Lerin et al., 2019).

Na fase de multiplicação das brotações são realizadas sucessivas subculturas. No entanto, com o aumento do número de subculturas pode ocorrer redução do potencial da capacidade de regeneração em algumas espécies

arbóreas, incluindo a perda da capacidade de formação de novas brotações (Rocha et al., 2007). Ademais, pode ocorrer a redução do potencial de enraizamento em resposta à diminuição do comprimento dos propágulos e, possivelmente, sendo o tamanho dos propágulos um dos fatores relacionados ao sucesso do enraizamento de brotações (Moura et al., 2012). Os fatores bioquímicos e moleculares associados à redução da capacidade de regeneração, em resposta ao número sucessivo de ciclos de subculturas, ainda não são bem compreendidos em plantas (Moura et al., 2012). O entendimento destes fatores é relevante para otimizar e melhorar a produção em larga escala de plantas, especialmente para espécies arbóreas. Estudos têm mostrado que a variedade no potencial regenerativo entre diferentes tipos de explantes é atribuída a alterações fisiológicas e hormonais, incluindo diferenças no conteúdo de poliaminas (PAs) (Sethi et al., 1988; Zhang e Jiang, 1997), proteínas (Tian et al., 2003; Tang e Newton, 2005) e hormônios (Wang et al., 2015).

As PAs são importantes moléculas associadas ao desencolvimento de plantas (Kusano et al., 2008) devido à sua interação com outras moléculas que possibilitam uma relação chave com o metabolismo de aminoácidos, nitrogênio e fixação do carbono (Majumdar et al., 2016). As PAs têm mostrado extensa relação em processos fisiológicos em plantas, incluindo o envolvimento na morfogênese *in vitro*, como organogênese (Saldanha et al., 2006) e desenvolvimento de brotações (Aragão et al., 2017; Oliveira et al., 2020). Dentre as PAs, a putrescina tem sido alvo de estudos em espécies arbóreas, e tem sido mostrado o envolvimento desta PA na regulação da morfogênese *in vitro* (Aragão et al., 2016), bem como na modulação de proteínas relacionadas ao metabolismo de PAs e relacionadas ao desenvolvimento das brotações *in vitro* (Oliveira et al., 2020).

As proteínas são moléculas essenciais para o crescimento e desenvolvimento em plantas (Takáč et al., 2011), além de apresentarem papel importante na regeneração *in vitro* em espécies arbóreas (Aragão et al., 2017; Oliveira et al., 2020). Abordagens recentes em estudos proteômicos em plantas possibilitaram a identificação e caracterização de proteínas em diferentes condições e suas relações com o crescimento e desenvolvimento vegetal (Baginsky, 2009; Takáč et al., 2011; Almeida et al., 2020; Oliveira et al., 2020). Estas estratégias possibilitam estudar comparativamente diversos processos fisiológicos relacionados ao crescimento de plantas (Yin et al., 2008; Jorrín-Novo et

al., 2009). Adicionalmente, estes estudos possibilitam avanços no entendimento do papel de proteínas associadas com a morfogênese *in vitro* de espécies arbóreas, bem como relacionar o envolvimento de proteínas com outras moléculas, como por exemplo PAs, associadas ao crescimento *in vitro*, bem como o envolvimento de hormônios relacionados com o enraizamento (Aragão et al., 2017; Lerin et al., 2019; De Almeida et al., 2020; Oliveira et al., 2020). Há escassez de dados na literatura sobre alterações no perfil de proteínas associados ao efeito do número de subculturas em *C. fissilis*.

Uma grande variedade de hormônios vegetais atua de forma sinérgica ou antagônica na regulação do crescimento e desenvolvimento, afetando de forma significativa várias vias de respostas, como melhoria de produção de plantas e em respostas a estresses (Woodward e Bartel, 2005; De Jong et al., 2009; Wang et al., 2010). Estas moléculas são adicionadas ao meio de cultura para controlar o crescimento e desenvolvimento de plantas e são fundamentais para modulação e entendimento da capacidade da regeneração das plantas *in vitro* (Giri et al., 2004; Sauer et al., 2013). Dentre estas, as auxinas demonstram ser essenciais no desenvolvimento *in vitro*, bem como no processo de enraizamento, por serem um dos principais sinalizadores relacionados à competência e regeneração em plantas juntamente com as citocininas (Giri et al., 2004; Tanaka et al., 2006), além de apresentar relevância no enraizamento de plantas lenhosas (De Almeida et al., 2020). As auxinas também podem interagir com outros hormônios, incluído ácido abscísico (ABA) (Albacete et al., 2008), ácido jasmônico (JA) (Gutierrez et al., 2012) e ácido salicílico (SA) (Zhang et al., 2007). A relação de auxinas com ABA pode ocorrer no processo de enraizamento. Estes hormônios atuam como sinalizadores para promover ou inibir o crescimento celular em resposta ao estresse (Zhao et al., 2014). As plantas usam ácido abscísico para interromper o crescimento das raízes laterais (Rugini e Verma, 1982; Aloufa, 2007; Zhao et al., 2014). Além disso, o ABA está envolvido na síntese de proteínas antioxidantes e de defesa contra espécies reativas de oxigênio (ROS) (Gomez-Cadenas et al., 2015). Os Jasmonatos, como ácido 12-oxo-fitodienoico (OPDA), JA e jasmonoil-isoleucina (JA-Ile) são hormônios de crescimento envolvidos na sinalização de respostas a estresses bióticos e abióticos e apresentam estreita relação no processo de crescimento e desenvolvimento, incluindo a melhoria do sistema radicular (Wang et al., 1999; Besson et al., 2010). Isso demonstra que estes hormônios podem estar associados ao

efeito das subculturas *in vitro* em espécies arbóreas. Além disso, o SA e seu precursor ácido trans-cinâmico (t-CA) (Sendon et al., 2011) são hormônios relacionados à regeneração de plantas, devido à sua relação antagônica direta com JA (Caarls et al., 2015), sendo potencialmente uma referência para estudos da regeneração de brotações *in vitro* e enraizamento *ex vitro*. Deste modo, estudos relacionados ao conteúdo endógeno de PAs, hormônios e proteínas diferencialmente acumulados são importantes para elucidar os efeitos do número de subculturas sobre a capacidade de regeneração de brotações *in vitro* e no enraizamento *ex vitro*, sendo fundamentais para entendimento e melhoria da produção de mudas em larga escala de espécies arbóreas.

C. fissilis (Meliaceae) é uma espécie arbórea de ocorrência natural na América do Sul, com ocorrência em grande parte do território nacional (Barstow, 2018). A madeira desta espécie está dentre as mais desejáveis, fornecendo insumos para diversos setores industriais, incluindo a construção naval, civil e moveleira (Carvalho, 2003; Ruschel et al., 2003; Valério et al., 2008). Devido ao desmatamento e à exploração não controlada, esta espécie encontra-se entre as ameaçadas de extinção, classificada como vulnerável (Barstow, 2018), sendo importante a busca por formas de propagação alternativas às convencionais para a produção de mudas desta espécie com o intuito de conservação.

A propagação *in vitro* é uma alternativa viável para a propagação de indivíduos de forma rápida e independente da estação do ano (Nunes et al., 2002). Estudos iniciais de propagação *in vitro* foram realizados nesta espécie utilizando segmentos nodais como fonte de explantes (Nunes et al., 2002). Recentemente, estudos têm apontando melhoria nos protocolos de regeneração de brotações *in vitro* utilizando a adição de PAs e o uso de lâmpadas de diodo emissores de luz (LED) com diferentes espectros de luz (Aragão et al., 2017; Oliveira et al., 2020). No entanto, ainda não foram aprofundados estudos relacionados ao potencial da capacidade regenerativa das brotações *in vitro* ao longo de subculturas. Ademais, pouco se sabe sobre os efeitos causados pelo número de subculturas no desenvolvimento das brotações *in vitro* e no processo de enraizamento *ex vitro*.

Nesse sentido, estudos visando elucidar os mecanismos moleculares e bioquímicos associados a capacidade de regeneração de brotações *in vitro* e enraizamento *ex vitro*, como PAs, hormônios e proteínas diferencialmente acumulados são fundamentais em plantas lenhosas, como *C. fissilis*.

2. REVISÃO BIBLIOGRÁFICA

2.1. Morfogênese *in vitro*

A propagação *in vitro*, através da cultura de tecidos vegetais, é utilizada para propagação vegetativa de plantas, por meio de culturas assépticas de células, tecidos, órgãos ou plantas inteiras sob condições nutricionais e ambientais adequadas (Thorpe, 2007; Oliveira et al., 2020). A propagação *in vitro* de células e tecidos em plantas é possível devido à pluripotencialidade das células vegetais (Phillips e Garda, 2019). A pluripotência se refere à capacidade de múltiplas respostas organogênicas, como formação de brotações, raízes e calos, quando mantidos em meios nutritivos e condições adequadas, resultando na formação de uma planta completa (Grafi, 2004; Thorpe, 2007; Phillips e Garda, 2019).

A propagação *in vitro* se divide em várias fases, iniciando com a escolha do explante, indução e multiplicação das brotações, enraizamento das brotações e aclimatização das mudas. Neste processo, a resposta morfogenética *in vitro* é dependente de vários fatores, como o genótipo e o tipo de explante, os reguladores de crescimento vegetal, e as condições de cultura (Phillips e Garda, 2019).

Como fontes de explantes podem ser utilizadas células e meristemas (Aasim et al., 2008), embriões zigóticos (Zuraida et al., 2017) e somáticos (Quiroz-Figueroa et al., 2002), bem como segmentos foliares (Siwach e Gill, 2014), caulinares (Nunes et al., 2002; Moura et al., 2012; Aragão et al., 2016; Aragão et al., 2017; Oliveira et

al., 2020) e radiculares (Rathore et al., 2014). Em resposta ao estímulo químico e ambiental adequado, ocorre a desdiferenciação e rediferenciação de células destes explantes, possibilitando a geração de novos órgãos, como brotações, raízes ou embriões somáticos, ou ainda a indução do desenvolvimento de órgãos preexistentes, como gemas axilares (Giri et al., 2004; Vogel, 2005; Phillips e Garda, 2019; Oliveira et al., 2020). Em geral, para espécies arbóreas, a grande maioria dos trabalhos na literatura utiliza segmentos nodais como fonte de explante para a indução de brotações *in vitro* (Tiwari et al., 2002; Rocha et al., 2007; Raposo et al., 2010; Moura et al., 2012; Pijut et al., 2012).

Além do tipo de explante e das condições ideais de cultura, os reguladores de crescimento, como auxinas e citocininas exercem função fundamental na sinalização e estímulo para a divisão celular e formação de novos tecidos e órgãos (El-Showk et al., 2013). Destaca-se a importância destes sinalizadores no processo de organogênese e enraizamento (Fett-Neto et al., 2001; Nunes et al., 2002). Em um trabalho utilizando *C. fissilis,* destaca-se o uso da citocinina benziladenina (BA) para a formação de novas brotações a partir do desenvolvimento de gemas axilares (Nunes et al., 2002; Aragão et al., 2016). Segundo Tanaka et al. (2006) a auxina produzida no meristema apical controla negativamente a biossíntese de citocinina em gemas axilares. Quando efetuada a retirada do meristema apical, a biossíntese de citocinina é aumentada nas gemas laterais, alterando o balanço de auxina e citocinina e, consequentemente, promovendo o desenvolvimento das brotações.

O enraizamento das brotações é fundamental para a produção de mudas (Lall et al., 2006; Moura et al., 2012). A fase de enraizamento pode ser realizada *in vitro*, em meio de cultura semissólido, seguido de aclimatização das brotações enraizadas em substrato. No enraizamento *ex vitro*, as brotações propagadas *in vitro* podem ser tratadas ou não com auxinas, e são transferidas diretamente para o substrato, ocorrendo simultaneamente o enraizamento das brotações e aclimatização das mudas (Yan et al., 2009). O enraizamento *ex vitro* favorece a redução de etapas no processo de propagação, e consequentemente, redução de custos comparativamente ao enraizamento *in vitro* (Xu et al., 2008; Ranaweera et al., 2013; Phillips e Garda, 2019).

Para a produção em larga escala na propagação *in vitro*, as brotações de plantas passam pelo processo de multiplicação (Tiwari et al., 2002; Raposo et al., 2010; Moura et al., 2012). Em estudos anteriores mostram os efeitos das

subculturas na inibição do desenvolvimento de brotações *in vitro*, comprometendo também o desenvolvimento de raízes adventícias (Tiwari et al., 2002; Rocha et al., 2007; Flôres et al., 2011; Moura et al., 2012). Entretanto, pouco se conhece ainda sobre as alterações bioquímicas e moleculares relacionadas à competência para o desenvolvimento das brotações *in vitro* associadas ao enraizamento *ex vitro* destas brotações.

2.2. Influência dos ciclos de subculturas *in vitro* no crescimento das brotações e desenvolvimento de mudas

A subcultura é a subdivisão de material estabelecido *in vitro* e sua transferência para o novo meio e subsequente incubação em condições controladas (Carvalho et al., 2011), sendo utilizado para a multiplicação de brotações *in vitro*. Estudos demonstram que a competência ou capacidade para o desenvolvimento da organogênese pode ser influenciada pelo número de subculturas, desencadeando alterações no crescimento e desenvolvimento de brotações em *Plathymenia reticulata* (Moura et al., 2012).

Amplos esforços vêm sendo abordados para otimização de protocolos visando o aumento do número de propágulos e consequentemente melhorando a produção de plântulas em espécias arbóreas (Rocha et al., 2007; Flôres et al., 2011; Moura et al., 2012). Embora haja suplementação de reguladores de crescimento e variações entre os meios de cultura tenham favorecido o alongamento de brotações, os resultados ainda precisam ser otimizados devido ao escasso conhecimento do conteúdo endógeno dos hormônios e seus efeitos no processo de subcultura *in vitro* (Nunes et al., 2002; Rocha et al., 2007; Flôres et al., 2011; Aragão et al., 2016; Oliveira et al., 2020).

A capacidade de desenvolvimento *in vitro* de brotações tem sido monitorada em algumas espécies, visando avaliar quantas subculturas podem ser realizadas sem ocorrer a redução ou perda da capacidade de formação de brotações (Moura et al., 2012). Algumas espécies apresentam diferenças no potencial de regeneração, sendo a competência para a regenerar brotações *in vitro* menor em espécies lenhosas, como *Eucalyptus urophylla*, *Pyrus communis* e *Tectona grandis* (Raposo et al., 2010; Vujović et al., 2012; Mendonça et al., 2020).

Tendo em vista as alterações no crescimento e desenvolvimento decorrente das subculturas é de grande importância estudar os fatores que podem estar associados com a redução da capacidade regenerativa das brotações *in vitro* e indução do enraizamento *ex vitro* em espécies arbóreas. Neste sentido, abordagens bioquímicas e moleculares, como o conteúdo de hormônios e proteômica comparativa, fornecerão informações relevantes para a morfogênese em espécies arbóreas.

2.3. PAs e seus efeitos no crescimento e desenvolvimento *in vitro* de plantas

PAs são moléculas alifáticas e policatiônicas com cadeias carbônicas contendo dois ou mais grupos amino ($-NH_3^+$), estando presentes em todos os organismos vivos (Lenis et al., 2017). Devido a esta característica, as PAs podem ligar-se a complexos aniônicos carregados negativamente, tais como DNA, RNA, ATP, proteínas, fosfolipídios e polissacarídeos (Martin-Tanguy, 2001; Lenis et al., 2017). Nas plantas, as três principais PAs estudadas são a putrescina (Put), espermidina (Spd) e espermina (Spm), sendo consideradas importantes para o crescimento e desenvolvimento (Kusano et al., 2008).

A biossíntese e catabolismo de PAs relacionam-se com várias outras moléculas, como aminoácidos, ocupando posições chave na ligação do metabolismo de nitrogênio e fixação de carbono, sendo determinantes para o crescimento de plantas (Majumdar et al., 2016). Em plantas, a biossíntese de Put pode ocorrer em duas vias independentes, reguladas pelas enzimas arginina descarboxilase (ADC) e ornitina descarboxilase (ODC). A primeira rota se inicia com a ação da ADC, atuando na descarboxilação da arginina; a segunda inicia-se com a conversão do aminoácido ornitina em Put pela enzima ODC. A conversão de Put em Spd, e posteriormente em Spm, ocorre por adição de radicais aminopropil oriundos da S-adenosilmetionina (SAM), pela ação da enzima SAM descarboxilase (SAMDC). O catabolismo de Put, Spd e Spm ocorre por ação das enzimas diamino oxidase (DAO) e PA oxidase (PAO) (Minocha et al., 2004; Kuznetsov et al., 2006; Moschou et al., 2012). A mudança no metabolismo de PAs, bem como a razão de PAs, varia dependendo da espécie, órgão, tecido e estágio de desenvolvimento (Kuznetsov et al., 2006; Kosov et al., 2011).

Estudos esclarecem a interação do metabolismo de PAs com várias moléculas, desempenhando papeis fundamentais para a sinalização e desenvolvimento de plantas (Turano et al., 1997; Majumdar et al., 2016). As PAs atuam na divisão, diferenciação, proliferação e morte celular programada (Seiler e Raul, 2005), promovendo o desenvolvimento de processos morfogenéticos *in vitro* em plantas, como a organogênese (Saldanha et al., 2006) e desenvolvimento de brotações (Aragão et al., 2016; Aragão et al., 2017) e fotomorfogênese (Oliveira et al., 2020).

Em *C. fissilis* foi mostrado que a suplementação de BA ao meio de cultura resultou no aumento do conteúdo endógeno de Put e, consequentemente, promoveu maior número de brotações por explantes oriundos de segmentos nodais apicais (Aragão et al., 2016). Outro estudo mostra que a adição de Put ao meio de cultura induziu aumento significativo no alongamento das brotações oriundas de segmentos nodais cotiledonares de *C. fissilis* cultivados em 2,5 mM de Put (Aragão et al., 2017). Estes autores verificaram que a adição de Put promove alterações significativas na abundância de proteínas específicas, que podem estar relacionadas com o maior alongamento das brotações. Outro estudo mostrou que o uso de lâmpadas LED melhorou o alongamento das brotações e aumentou o conteúdo endógeno de Put livre, além de modular a abundância de proteínas relacionadas relevantes para esta resposta morfogênica (Oliveira et al., 2020).

Diante da importância de PAs na morfogênese *in vitro*, estudos relacionados ao metabolismo e influência destas moléculas à capacidade de regeneração são relevantes para elucidar o seu envolvimento no crescimento e desenvolvimento de brotações *in vitro*, bem como no enraizamento *ex vitro* sob ciclos sucessivos de subculturas.

2.4. Estudos proteômicos envolvidos na regeneração *in vitro* de plantas

As proteínas são principais componentes determinantes da estrutura e das funções celulares (Baginsky, 2009), e relacionam-se com diferentes papeis no crescimento e desenvolvimento e vias metabólicas em plantas (Takáč et al., 2011). As abordagens de estudos sobre o proteoma visando comparar conjunto de proteínas sintetizadas pelo genoma de um organismo em um dado momento ou

condição (Wasinger et al., 1995), têm possibilitado a identificação e caracterização de proteínas diferencialmente abundantes em diversas condições de desenvolvimento, relacionadas com o crescimento e desenvolvimento em plantas (Hochholdinger et al., 2006; Baginsky, 2009; Takáč et al., 2011).

A proteômica de plantas tem possibilitado estudos comparativos na maturação de sementes, germinação e embriogênese somática (Yin et al., 2008; Jorrín-Novo et al., 2009), bem como na morfogênese e fotomorfogênese *in vitro* em espécies arbóreas (Aragão et al., 2017; Lerin et al., 2019; Oliveira et al., 2020). Em *Vigna radiata* foram observadas proteínas diferencialmente abundantes associadas com a aquisição da competência morfogenética em explantes responsivos comparados aos não responsivos à organogênese, indicando a relevância de proteínas envolvidas no metabolismo de carboidratos e nitrogênio, de reserva, e associadas com estresse (Ghosh e Pal, 2013). Durante a aquisição da competência, proteínas envolvidas com resposta ao estresse, metabolismo e divisão celular foram diferencialmente abundantes em culturas nodulares de *Vriesea reitzii* (Corredor-Prado et al., 2016). As principais proteínas reguladas neste processo foram proteínas de choque térmico de 22 kDa, chaperona dnaJ 50, S-adenosilmetionina sintetase e proteínas semelhantes a 14-3-3. Estas proteínas foram reguladoras do desenvolvimento da organogênese através da modulação de vias específicas de resposta ao estresse, ao metabolismo e à divisão celular, promovendo a aquisição da competência morfogênica em *V. reitzii.* (Corredor-Prado et al., 2016). Estudos utilizando diferentes espectros de luz artificial mostraram que as lâmpadas LED afetaram significativamente o crescimento de brotações *in vitro* e o acúmulo de proteínas em comparação à lâmpada fluorescente. Além disso, o acúmulo de proteína argininosuccinato sintetase foi associado ao aumento no conteúdo de Put e ao maior alongamento de brotações em *C. fissilis* sob lâmpada LED com a combinação de azul e vermelho (Oliveira et al., 2020). Em *Cariniana legalis*, a combinação dos espectros de azul baixo e vermelho distante promoveu um aumento no alongamento das brotações *in vitro* bem como acúmulo diferencial de proteínas relacionadas à homeostase e organização celular, como chaperonina, tubulinas e proteínas relacionadas ao estresse (Lerin et al., 2019).

Além do desenvolvimento de brotações, estudos proteômicos têm sido realizados durante o enraizamento adventício. Em estudo comparativo de uma

espécie recalcitrante ao enraizamento (*Eucalyptus globulus*), e uma espécie de fácil enraizamento (*E. grandis*) foram identificadas proteínas diferencialmente abundantes envolvidas com o estresse oxidativo, metabolismo energético e fotossíntese (De Almeida et al., 2020). Além disso, algumas proteínas podem atuar como reguladores positivos do enraizamento adventício, como as proteínas relacionadas ao metabolismo do amido, com maior acúmulo em *E. grandis* quando comparadas a *E. globulus* (De Almeida et al., 2020).

Embora vários estudos mostrem a interação de PAs e proteínas diferencialmente acumuladas na resposta ao desenvolvimento da morfogênese *in vitro* e no enraizamento adventício, poucos são os trabalhos relacionando o efeito do número de subculturas *in vitro* e alterações proteômicas em espécies arbóreas. Neste sentido, é importante a realização de estudos com esta abordagem que permitam relacionar alterações na abundância diferencial de proteínas associadas a capacidade de regeneração de brotações e no enraizamento *ex vitro* em *C. fissilis* durante sucessivas subculturas.

2.5. Rede de interação hormonal na regeneração *in vitro* de plantas

Os hormônios vegetais desempenham papel crucial no controle do crescimento e desenvolvimento das plantas. Eles servem como mediadores de programas de desenvolvimento e integram sinalização para regular e otimizar o crescimento e desenvolvimento das plantas (Sauer et al., 2013). Em nível celular, atua em estímulos para processos celulares, como divisão, alongamento e diferenciação, dando origem ao crescimento de novos órgãos, como raízes adventícias (Swarup et al., 2002), e controlando a formação de frutos (De Jong et al., 2009), dominância apical (Woodward e Bartel, 2005) e respostas abióticas (Wang et al., 2010).

O estudo pioneiro de Skoog e Miller (1957) demostrou a importância dos reguladores de crescimento na promoção do desenvolvimento e regeneração da morfogênese *in vitro* através do balanço de auxina e citocinina adicionados ao meio de cultura. Nas plantas, a síntese de auxina ocorre nos tecidos jovens, especialmente no meristema apical, sendo transportada de forma polar ao longo do caule, possibilitando diferença no balanço da mesma nos tecidos (Ljung et al.,

2001). Este fato pode estar associado à capacidade de regeneração das brotações sob ciclos sucessivos de subculturas (Moura et al., 2012), uma vez que o balanço de auxinas endógenas tem sido relacionado à competência para regeneração em plantas (Giri et al., 2004; Tanaka et al., 2006), bem como o envolvimento fundamental no enraizamento de plantas lenhosas (De Almeida et al., 2020).

Dentre as auxinas, o ácido indol-3-acético (AIA) está associado ao crescimento e desenvolvimento das raízes (Kramer e Ackelsberg, 2015). Adicionalmente, a suplementação de auxinas, como ácido indol-3-butírico (AIB) desempenha papel essencial no enraizamento adventício e é geralmente o mais utilizado devido à sua maior capacidade de induzir raízes e maior estabilidade à luz em comparação com AIA (Pacurar et al., 2014). O conteúdo endógeno de auxina é importante para o desenvolvimento de raízes adventícias em estacas caulinares, devido à sua relação com a citocinina (Phillips e Garda, 2019). O equilíbrio hormonal adequado da relação entre auxina e citocinina favorável à reprogramação dos tecidos para estimular a divisão celular necessária ao desenvolvimento de raízes adventícias (Gutierrez et al., 2012; Legué et al., 2014). Além disso, o AIA é associado à interferência entre diferentes reguladores de crescimento, como ácido abscísico (ABA) (Albacete et al., 2008), ácido jasmônico (JA) (Gutierrez et al., 2012) e ácido salicílico (SA) (Zhang et al., 2007). Neste contexto, a análise de conteúdo endógeno de auxina permite entender e relacionar o efeito do conteúdo endógeno de hormônios presente nos explantes utilizados para a morfogênese *in vitro* e no enraizamento *ex vitro.*

O ABA é um hormônio que atua na inibição da germinação e respostas ao estresse (Tardieu et al., 2010). Na propagação *in vitro*, estudos demonstram a relação de ABA com a inibição do crescimento, podendo ocasionar mudanças fisiológicas ou gerar diminuições nos parâmetros de crescimentos no material propagado (Souza et al., 2009). O ABA pode interagir com as auxinas no processo de enraizamento, com efeito inibitório na indução e crescimento de raízes em plantas (Rugini e Verma, 1982; Aloufa, 2007). Desta forma, destaca-se a relevância de estudar o conteúdo endógeno de ABA para verificar seu efeito na multiplicação *in vitro* de brotações e no enraizamento em arbóreas para melhor compreender a relação deste hormônio de crescimento com a capacidade regenerativa de brotações ou raízes.

Os jasmonatos, incluindo ácido 12-oxo-fitodienoico (OPDA), JA e jasmonoil-isoleucina (JA-Ile) são considerados hormônios de crescimento de plantas envolvidos principalmente nos processos de sinalização e defesa relacionados com estresses bióticos e abióticos (Wang et al., 1999; Besson et al., 2010). O JA pertence a um grupo de ácidos graxos bioativos, também chamado de oxilipinas, o qual está envolvido em diversos processos de desenvolvimento, como aumento da habilidade da planta de responder a situações de estresse, melhoria no desenvolvimento do sistema radicular (Wang et al., 1999; Besson et al., 2010). Um estudo anterior relacionou interações entre JA e auxinas em plantas. A suplementação de auxina na germinação *in vitro* de sementes de *Lagenaria siceraria* induziu a biossíntese do JA, e esta resposta foi associada com o aumento da habilidade da planta de responder às situações de estresse (Silva et al., 2007). Neste sentido, é possível que ocorra uma interação entre auxina e JA na cultura *in vitro* durante a multiplicação de brotações, assim como no desenvolvimento de raízes adventícias, sendo relevante elucidar a interligação das vias hormonais nestes processos morfogenéticos.

O SA assim como o ácido trans-cinâmico (t-CA), um composto das reações iniciais da via fenilpropanoide e precursor do SA (Sendon et al., 2011), são encontrados em plantas e também são considerados como hormônios relacionados a múltiplos processos, assim como defesa da planta contra respostas a diferentes condições de estresse abiótico e biótico (Wang et al., 2010; Khan et al., 2019). Estudos demonstram que SA apresenta relação antagônica ao JA (Caarls et al., 2015). Neste sentido, a análise do conteúdo endógeno de JA, SA, e seus precursores, é relevante para entender a interação entre estes compostos e seus efeitos no crescimento e desenvolvimento de brotações *in vitro* sob ciclos sucessivos de subculturas e no enraizamento *ex vitro*.

Deste modo, estudos relacionados à influência dos hormônios com a capacidade de regeneração são de grande importância para elucidar o envolvimento e a rede de interações entre estes hormônios, como AIA, ABA, JA, OPDA, JA-Ile, t-CA, SA e PAs, no processo de regeneração de brotações *in vitro* e no enraizamento *ex vitro* de brotações submetidas a sucessivos ciclos de subculturas.

2.6. Espécie em estudo

C. fissilis (Meliaceae), também conhecida como cedro-rosa, é uma espécie encontrada em diferentes países da América do Sul, e possui ampla distribuição no território brasileiro, com ocorrência nas cinco regiões do território nacional (Figura 1) (Barstow, 2018).

Figura 1: Mapa da distribuição de *C. fissilis* no Brasil. Os pontos vermelhos indicam os locais de ocorrência natural da espécie. Imagem adaptada de fontes: Barstow (2018) e IBGE (https://www.ibge.gov.br/).

Esta espécie apresenta porte arbóreo com potencial de atingir até 40 metros de altura, com densidade básica média de 431,06 m^{-3}, e sua madeira está dentre as mais valorizadas pelos setores primários da economia brasileira, fornecendo matéria-prima para as indústrias naval, construção civil, moveleira e insumo para formulação de óleos essenciais (Carvalho, 2003; Ruschel et al., 2003; Valério et al., 2008). Devido à importância da madeira, esta espécie tem sido intensamente explorada, e encontra-se ameaçada de extinção na categoria vulnerável, sendo incluída na lista vermelha da "International Union for Conservation of Nature" (IUCN) (Barstow, 2018). *C. fissilis* encontra-se no Apêndice III da "Convention on

International Trade in Endangered Species of wild fauna and flora” (CITES) a qual recomenda a expansão da população desta espécie visando sua proteção (Barstow, 2018). Várias tentativas foram feitas para realizar plantações massivas de cedros, porém com pouco sucesso em decorrência do ataque pelo inseto *Hypsipyla grandella*. Este inseto na sua fase larval ataca as brotações, matando o meristema apical e consequentemente, afeta o desenvolvimento da parte aérea da árvore, principalmente na fase juvenil (Muellner et al., 2010; Chavarro-Rodríguez et al., 2013).

Estudos de propagação *in vitro* foram desenvolvidos para esta espécie, sendo estabelecido as melhores condições para as várias etapas, como a obtenção de brotações a partir de segmentos nodais, e enraizamento *in vitro* (Nunes et al., 2002). Recentemente, foi mostrado o efeito da adição de citocinina no crescimento das brotações e no conteúdo endógeno de PAs e carboidratos (Aragão et al., 2017). Também foi demonstrado que diferentes espectros de luz fornecidos por lâmpadas LED afetam significativamente o alongamento das brotações e promovem aumento do conteúdo endógeno de Put livre, além de modular a abundância de proteínas (Oliveira et al., 2020). No entanto, pouco se sabe sobre os efeitos do número de subculturas no desenvolvimento das brotações *in vitro* e no processo de enraizamento *ex vitro* destas brotações para esta espécie.

Em algumas espécies arbóreas, o efeito do subcutivo resultou em redução na capacidade de desenvolvimento de parte aérea e radicular (Tiwari et al., 2002; Rocha et al., 2007). Entretanto, ainda não se sabe como os fatores bioquímicos e moleculares estão relacionados à redução da capacidade de regeneração *in vitro* e, ou com a redução do potencial de desenvolvimento de raízes adventícias. Nesse sentido, a busca por moléculas marcadoras e essenciais para elucidar os mecanismos moleculares e bioquímicos associados à capacidade de regeneração de brotações *in vitro* e, ao enraizamento *ex vitro*, como PAs, hormônios e proteínas diferencialmente acumulados, são fundamentais para espécies lenhosas, como *C. fissilis*, visando ampliar a produção de mudas em larga escala.

3. OBJETIVOS

3.1. Objetivo geral

Estudar o efeito dos ciclos de subculturas sucessivas no desenvolvimento *in vitro* e no enraizamento *ex vitro* das brotações de *C. fissilis*, e alterações no metabolismo de PAs, conteúdo de hormônios e proteínas diferencialmente abundantes.

3.2. Objetivos específicos

- Avaliar o efeito do número de ciclos de subculturas no desenvolvimento *in vitro* de brotações de *C. fissilis* e alterações no conteúdo endógeno de hormônios vegetais e no metabolismo de PAs;

- Analisar o efeito do ciclo de subculturas das brotações *in vitro* sobre o enraizamento *ex vitro* e alterações no conteúdo endógeno de hormônios vegetais, metabolismo de PAs e perfil proteômico.

4. TRABALHOS

4.1. ALTERATION ON HORMONE CONTENTS DURING SUBCULTURE CYCLES AFFECT *in vitro* SHOOT DEVELOPMENT OF *Cedrela fissilis* VELL. (MELIACEAE)

RESUMO

A competência para o desenvolvimento *in vitro* de brotações é relevante à propagação em larga escala. O presente estudo teve como objetivo investigar o efeito de sucessivos ciclos de subcultura *in vitro* no desenvolvimento de brotações em *Cedrela fissilis* e as alterações nos conteúdos endógenos de hormônios vegetais e metabolismo de poliaminas (PAs). Os ciclos de subculturas afetaram o desenvolvimento das brotações *in vitro*, diminuindo significativamente o crescimento das brotações na primeira comparativamente à quarta subcultura. O conteúdo de putrescina (Put) livre e PAs livres totais foi maior em explantes na primeira subcultura comparativamente à quarta, sendo relevante para o maior crescimento das brotações. A atividade ODC foi maior que o da arginina descarboxilase, sugerindo que a ODC é via enzimática preferencial para a

biossíntese de Put nesta espécie. O maior conteúdo de ácido indol-3-acético na primeira subcultura é relevante para o desenvolvimento de brotações, enquanto um aumento no conteúdo de ácido abscísico, ácido 12-oxofitodienoico, ácido jasmônico e ácido salicílico nos explantes na quarta subcultura comparado à primeira sugere o envolvimento desses hormônios na redução do potencial de crescimento de brotações durante os ciclos sucessivos de subculturas *in vitro*. Este é o primeiro trabalho que mostra a relação do conteúdo hormonal nos explantes utilizados para o desenvolvimento de brotações e o número de subculturas *in vitro* para esta espécie. Os dados representam um entendimento relevante da competência para o desenvolvimento *in vitro* de brotações em nível hormonal e podem ajudar a melhorar a produção de mudas em larga escala dessa espécie lenhosa.

ABSTRACT

The competence for *in vitro* development of shoots is relevant for propagation on a large-scale. The present study aimed to investigate the effects of the number of subculture cycles on *in vitro* development of *C. fissilis* shoots and alterations on endogenous contents of plant hormones and polyamines (PAs) metabolism. The subculture cycles affected the *in vitro* shoot development, decreasing significantly the growth of shoots from the first compared to the fourth subculture. The contents of free putrescine (Put) and total free PAs were significantly higher in explants at the first subculture compared to the fourth, being relevant for the higher growth of shoots. The ODC activity was higher than arginine decarboxylase, suggesting that the ODC pathway is the preferentially enzyme for Put biosynthesis in this species. A higher content of indole-3-acetic acid at the first subculture is relevant for the development of shoots, while an increase on contents of abscisic acid, 12-oxo phytodienoic acid, jasmonic acid, and salicylic acid in the explants at the fourth subculture compared to the first suggests the involvement of these hormones on the reduction of shoot growth potential during *in vitro* successive subculture cycles. This

is the first work showing the relationship of hormone contents of the explant used for shoot development under successive *in vitro* subculture cycles for this species. The data represent a relevant understanding of competence to *in vitro* shoot development at the hormonal content and may help to improve the large-scale plantlet production of this woody species.

1. INTRODUCTION

Several factors are associated with the *in vitro* acquisition of morphogenic competence and can affect significantly the plant regeneration, including the type of explant, culture medium composition, plant growth regulators (PGRs), light, and successive subculture cycles (Rocha et al., 2007; Duclercq et al., 2011; Shemer et al., 2015; Oliveira et al., 2020). Among the factors, the subculture cycles is important for increasing the multiplication of shoots in woody species (Tiwari et al., 2002; Rocha et al., 2007; Flôres et al., 2011), being applied to increase the number of propagules obtained in micropropagation systems, essential for large-scale production of plantlets (Rocha et al., 2007; Pastelín Solano et al., 2019). On *in vitro* propagation process, plants show differences on potential for regeneration, being the competence to regenerate shoots *in vitro* lower in woody species compared to others, such as the *Eucalyptus urophylla, Pyrus communis,* and *Tectona grandis* (Raposo et al., 2010; Vujović et al., 2012; Mendonça et al., 2020).

In woody species, the shoot induction by nodal segments containing pre-existing axillary meristems has been used to improve responses of *in vitro* propagation (Xavier and Otoni, 2009). Thus, apical and cotyledonary nodal segments are the type of explants that contain pre-existing axillary buds, being used for the development of *in vitro* shoots in some tree species (Aragão et al., 2016; Lerin et al., 2019; Oliveira et al., 2020). The axillary meristems present in these types of explants are determined for vegetative growth, requiring optimal stimuli and conditions for *in vitro* growth (Aragão et al., 2017; Oliveira et al., 2020). The use of this type of explant for *in vitro* culture of tree species provides advantages on

plantlets production, such as the ease for *in vitro* growth, genetic fidelity, speed in the propagation process and is considered more simple compared to other systems, e.g. as somatic embryogenesis (Xavier and Otoni, 2009).

The ability of explant for shoot formation is controlled by multiple endogenous and environmental factors (Li et al., 2009). The interaction of several signaling compounds is necessary for the function of metabolic network and physiological processes underlying the successful growth of the plant (Naseem et al. 2012), among which the plant hormones play a fundamental role (Su et al., 2011; Koike et al., 2017). Several molecules are related to promotion of *in vitro* morphogenesis in plants, including polyamines (PAs) and plant hormones (Cassells and Curry, 2001; Da Costa et al., 2013; Aragão et al., 2017).

Among them, auxins play a central role in shoot formation, in which the endogenous auxin distribution appears to be determining (Koike et al., 2017). Some studies show that auxin can interact with other hormones, such as abscisic acid (ABA), jasmonic acid (JA), jasmonoyl-isoleucine (JA-Ile) and salicylic acid (SA), in response to stress and injury (Stenzel et al., 2003; Zhang et al., 2007; Albacete et al., 2008; Gutierrez et al., 2012; Zhang et al., 2015). In addition, it has been shown that the crosstalk between indole-3-acetic acid (IAA) and ABA during abiotic stress conditions plays an important role in improving the shoot/root growth (Albacete et al. 2008). However, little is known on the effects of ABA on the *in vitro* shoot development during the successive subculture cycles.

JA is another hormone with important responses on plant development (Gutierrez et al., 2012). A crosstalk between IAA and JA plays an important role in the regulation of JA homeostasis in etiolated hypocotyls in *Arabidopsis thaliana* (Gutierrez et al., 2012). Furthermore, in plants the injuries caused by cutting have a relationship with JA production, in which JA can influence the stress-induced cell reprogramming (Da Costa et al., 2013), being considered a potential hormone for studies of competence on *in vitro* shoot development. Moreover, 12-oxo-phytodienoic acid (OPDA), an intermediary of JA biosynthesis, is also involved in signaling, inducing defense and wounding response (Stenzel et al., 2003). Although the precise functions of OPDA in physiological events in plants, their involvement on shoot development during the successive subculture cycles is still largely unknown.

In addition, PAs can interact with other molecules and participate on signaling, cell division and differentiation (Kevers et al., 2000; Vuosku et al., 2006; Kusano et al., 2008). These molecules promotes the division of cells by the transition through the G1/S and G2/M phases of cell cycle (Weiger and Hermann, 2014). In plants, we can highlight the involvement of the putrescine (Put) with cell division, whereas the spermidine (Spd) and spermine (Spm) appears to be more related with cellular differentiation (Santa-Catarina et al., 2006; Aragão et al., 2017). Some studies showed the involvement of PAs on *in vitro* development of shoots in woody species (Aragão et al., 2016; Aragão et al., 2017; Lerin et al., 2019; Oliveira et al., 2020). In this sense, PAs are candidates for the regulation of *in vitro* development of shoots under successive subculture cycles. Moreover, Put is synthesized directly from ornithine decarboxylase (ODC), or indirectly from arginine decarboxylase (ADC) (Majumdar et al., 2013). The existence of two pathways for Put biosynthesis in some plant species may be related to their different contributions to development and tissue-specific processes (Bais and Ravishankar, 2002; Vuosku et al., 2006). Possibly, the Put biosynthesis can occur in a preferential way depending on the stimulus or tissues used (Carbonell and Blázquez, 2009; Majumdar et al., 2016). However, the action of biosynthetic enzyme ODC on Put biosynthesis pathway in woody species, especially when influenced by successive subculture cycles, is not well understood.

The hormonal and molecular mechanisms controlling the competence of *in vitro* morphogenesis during successive subculture cycles are not fully understood in woody species. Among the woody species, *Cedrela fissilis* stands out because of its high economic value for the commercial wood production (Cusatis et al., 2013) and has been included in the Red List of Endangered Species by the International Union for Conservation of Nature (IUCN), classified as vulnerable (Barstow, 2018). An *in vitro* propagation system has been successfully developed for this species (Nunes et al., 2002). Recently, the development of shoots has been improved by the addition of PAs (Aragão et al., 2017) and by the use of different light spectra from light-emitting diodes (LED) lamps (Oliveira et al., 2020). However, little is known about the effects of successive subculture cycles on *in vitro* shoot development of this species. In this sense, the analysis of endogenous contents of plant hormones on explants during the number of subculture cycles can provide relevant knowlegdement about the competence for *in vitro* development of shoots

for this species. Thus, the present study aimed to investigate the effects of the number of subculture cycles on *in vitro* development of *C. fissilis* shoots and alterations on endogenous contents of plant hormones and PAs metabolism.

2. MATERIALS AND METHODS

2.1. Plant material

Mature seeds of *C. fissilis* obtained from Caiçara Comércio de Sementes LTDA located in Brejo Alegre (21º10'S and 50º10'W), São Paulo State, Brazil, were germinated *in vitro*. Sixty-day-old seedlings were used as the source of apical and cotyledonary nodal segment explants to obtain the shoots at each subculture cycle.

2.2. *In vitro* germination

In vitro germination was performed according to Oliveira et al. (2020). First, the seeds were surface disinfected and were then transferred to glass test tubes (15 x 2.5 cm; Laborglas; São Paulo, Brazil) containing 10 mL of MS (Murashige and Skoog, 1962) culture medium (M519; Phytotechnology Lab, Lenexa, USA) supplemented with 20 g L^{-1} sucrose (Vetec; Rio de Janeiro, Brazil) and 2 g L^{-1} Phytagel (Sigma-Aldrich; St. Louis, USA). The seeds were incubated in a culture room at 25 ± 2 °C under a 16-h photoperiod (55 µmol m^{-2} s^{-1}) using T10 40 W fluorescent lamps (Osram, Munich, Germany) for culture period of 60 days. Sixty-day-old seedlings were used as a source of explants (cotyledonary and apical nodal segments) for the first experiment.

2.3. *In vitro* multiplication of shoots during successive subculture cycles

The explants (apical and cotyledonary nodal segments) obtained from 60-day-old seedlings were inoculated into glass test tubes (15 x 2.5 cm; Laborglas) containing 10 mL of MS culture medium supplemented with 20 g L^{-1} sucrose, 2 g L^{-1} Phytagel, and 2.5 µM 6-benzyladenine (BA; Sigma-Aldrich), according to Oliveira et al. (2020). The pH of the culture medium was adjusted to 5.7 before an

autoclaving at 121 °C for 15 min. The explants were incubated in a culture room at 25 ± 2 °C under a 16-h photoperiod using fluorescent lamps (55 µmol m^{-2} s^{-1}) for a culture period of 45 days. This step corresponded to the shoots obtained in the first subculture cycle using nodal segments obtained from 60-day-old seedlings as explants. For the second, third, and fourth subculture cycles, the shoots grown from apical and cotyledonary nodal segments after 45 days of incubation were excised (~2 cm), cutting the leaves and apical meristem to obtain the explants, which were then used in the various subcultures. The explants were inoculated in the same culture medium again under the same conditions used for the first subculture. From the first to the fourth subculture cycles, shoot induction (%), length (cm) and number of shoots per explant were evaluated after 45 days of rooting. Each treatment (subculture cycle) was composed of eight replicates, with five explants per replicate. For PA analysis, explants from cotyledonary nodal segments without leaves were collected after 45 days of culture across four subculture cycle. For hormonal analysis, explants from cotyledonary nodal segments without leaves were collected after 45 days of culture in the first and fourth subculture cycles. Samples of the segments used in the PA and hormonal analyses were macerated with liquid nitrogen until a fine powder was obtained, which then stored at −80 °C until assessment. Samples of shoots for hormonal analysis were lyophilized for storage until assessment.

2.4. Free PA determination

Free PA were determined according to Santa-Catarina et al. (2006), using three biological replicate samples (200 mg fresh matter (FM) each) of explants from cotyledonary nodal segments after 45 days of growth in MS culture medium in each subculture cycle (1, 2, 3 and 4). The samples were ground in 1.2 mL of 5% perchloric acid (Merck; Darmstadt, Germany), incubation at 4 °C for 1 h, and centrifuged for 20 min at 20,000 × *g* at 4 °C. The supernatant containing free PAs was obtained, followed by derivatization with dansyl chloride (Merck) and identification by high-performance liquid chromatography (HPLC; Shimadzu, Kyoto, Japan) using a 5-µm C_{18} reverse-phase column (Shimadzu Shin-pack CLC ODS). The HPLC column gradient was created by adding increasing volumes of absolute acetonitrile (Merck) to a 10% aqueous acetonitrile solution at pH 3.5 adjusted with hydrochloric acid (Merck). The absolute acetonitrile concentration was maintained at 65% for the first

10 min, increased from 65 to 100% between 10 and 13 min, and maintained at 100% between 13 and 21 min; the mobile phase was added at a flow rate of 1 mL min^{-1} at 40 °C. The PA concentration was determined using a fluorescence detector at 340 nm (excitation) and 510 nm (emission). The peak areas and retention times of the samples were measured through comparisons with the standard PAs Put, Spd, and Spm (Sigma-Aldrich).

2.5. Determination of ADC and ODC activities

ODC and ADC activities were determined according to De Oliveira et al. (2017), with some modifications, using three biological replicate samples (500 mg FM per sample) of explants from cotyledonary nodal segments after 45 days of growth in MS culture medium during the first and fourth subculture cycles. Each sample was homogenized in an ice-cold mortar with liquid nitrogen and transferred to 500 µL of extraction buffer containing 50 mM Tris–HCl (Invitrogen; Carlsbad, USA) (pH 8.5), 0.5 mM pyridoxal-5-phosphate (Sigma-Aldrich), 0.1 mM ethylenediaminetetraacetic acid (EDTA; Sigma-Aldrich) and 5 mM dithiothreitol (DTT; Bio-Rad, Hercules, USA). The sample mixtures were vortexed and centrifuged for 20 min at 13,000 × *g* at 4 °C, and the supernatant was used for ADC and ODC enzymatic assays. A reaction mixture containing 100 µL of protein extract, 8.3 µL of extraction buffer, 12 mM unlabeled L-Arg or L-Orn, and 25 mCi of either L-[^{14}C(U)]-Arg (specific activity 274.0 mCi $mmol^{-1}$; PerkinElmer; Waltham, USA) or L-[1-^{14}C]-Orn (specific activity 57.1 mCi$mmol^{-1}$; PerkinElmer) was used. For blank samples,100 µL of extraction buffer was used. The reaction mixtures were incubated in glass tubes fitted with rubber stoppers and filter paper discs (GE Healthcare; Piscataway, USA) soaked in 2 N KOH (Vetec). The material was incubated at 37 °C at 120 rpm in an orbital shaker for 90 min, and the reaction was stopped by adding 200 µL of 5% (v/v) perchloric acid, followed by further incubation for 15 min under the same conditions. Filter paper containing $^{14}CO_2$ was immersed in 1 mL of scintillation fluid (PerkinElmer). Radioactivity was then measured using a scintillation counter (Tri-Carb2910TR; PerkinElmer). Protein content was measured using the Bradford method (Bradford, 1976) with bovine serum albumin as the standard. The specific enzymatic activities of ADC and ODC were expressed as nmol $^{14}CO_2$ mg $protein^{-1}$ h^{-1}.

2.6. Plant hormone analysis

Plant hormones analysis was performed according to Durgbanshi et al. (2005) with a few modifications, using three biological replicate samples (30 mg dry matter (DM) for each sample) of explants from cotyledonary nodal segments obtained from cotyledonary nodal segments after 45 days of growth in MS culture medium in the first and fourth subculture cycles. Before extraction, a mixture containing 50 ng of [$^{2}H_{6}$]-ABA, [C_{13}]-SA, dihydrojasmonic acid and 5 ng of [$^{2}H_{2}$]-IAA (all from Sigma-Aldrich) was added to each sample as an internal standard. [$^{2}H_{2}$]-IAA was used to determine IAA contents, [$^{2}H_{6}$]-ABA was used to determine ABA contents [^{13}C]-SA was used to determine SA and trans-cinnamic acid (t-CA) contents, and dihydrojasmonic acid was used to determine JA, OPDA and jasmonoyl-isoleucine (JA-Ile) contents. The samples were immediately homogenized in 2 mL of ultrapure water in a ball mill (MillMix20; Domel; Železniki, Slovenija). After centrifugation at 4,000 × *g* and 4 °C for 10 min, the supernatants were recovered and the pH was adjusted to 3.0 with 30% (v/v) acetic acid (Labkem; Barcelona, Spain). The water extract was partitioned twice against 2 mL of diethyl ether (Labkem) and the organic layer was recovered and evaporated under vacuum in a centrifuge concentrator (Speed Vac; Jouan, Saint-Herblain, France). Once dried, the samples were resuspended in a 10% (v/v) methanol (Fisher Scientific; Loughborough, UK) solution via gentle sonication. The resulting solution was filtered through 0.22-µm polytetrafluoroethylene membrane syringe filters (PTFE 13-mm diameter; Kinesis Ltd; Cambridgeshire, UK) and directly transferred to vials for mass spectrometry analysis.

LC-electrospray ionization (ESI)-MS/MS analysis was performed using an Acquity Ultra-High Performance Liquid Chromatography (UHPLC) system (Waters; Milford, USA) coupled to a tandem Xevo TQ-XS triple quadrupole mass spectrometer (Waters) using an orthogonal Z-Spray ESI interface operated in negative-ion mode. Chromatographic separations were carried out in a reversed-phase C_{18} column (50 × 2.1 mm, 1.6-µm particle size; Phenomenex Luna Omega; Madrid, Spain) at a flow rate of 300 µL min^{-1} with a column temperature of 40 °C. A binary gradient was used for elution: mobile phase A consisted of ultrapure water and 0.1% acetic acid, and mobile phase B consisted of 99.9% (v/v) methanol (Fisher Scientific) and 0.1% (v/v) acetic acid. Gradient elution was performed sequentially

as follows: maintenance of 10% B for 2 min, followed by ramping from 10 to 90% B at 6 min, and a decreased to 10% B at 7 min, after which 10% B was maintained until the end of the run at 8 min. The drying gas and the nebulizing gas were nitrogen (Praxair; Valencia, Spain). The cone gas flow was set to 250 L h^1, and the desolvation gas flow was set to 1200 L h^{-1}. For operation in tandem MS (MS/MS) mode, the collision gas was 99.995% pure argon (Praxair). The cone voltage and collision energies were adjusted depending on the compound under investigation, as described by Durgbanshi et al. (2005) with few modifications. The desolvation gas temperature was 650 °C, the source temperature was 150 °C, and the capillary voltage was 2 kV. The mass spectrometer was operated in multiple reaction monitoring (MRM) mode. Masslynx v4.1 software was used for mass spectral acquisition, and growth regulators were measured through comparisons with the internal standard for each deuterium-labeled growth regulator.

2.7. Statistical analysis

The shoot development was performed using a completely randomized design. The data on shoots induction, the numbers and lengths of shoots, free PAs, ADC/ODC enzyme activities and plant hormones were analyzed by analysis of variance (ANOVA) ($P < 0.05$) followed by the Student-Newman-Keuls (SNK) test (Sokal and Rohlf, 1995) in the R Statistical software (R core team, 2017).

3. RESULTS

3.1. Effect of subculture cycles on the *in vitro* development of shoots

The subcultures significantly affected the *in vitro* shoots development from apical and cotyledonary nodal segments used as explants (Fig. 1).

A higher induction of shoots obtained from cotyledonary and apical nodal segments was observed at the first subculture, following the decrease until the third subculture to apical nodal segments and fourth subculture to cotyledonary nodal segments (Fig. 1a).

The number of shoots per explant was higher at the first and second subculture in shoots obtained from both type (cotyledonary and apical nodal

segments). A reduction until the fourth subculture was observed for shoots obtained from cotyledonary nodal segments, whereas those obtained from apical nodal segments, resulting in a reduction occurred at the third subculture, keeping the same result at the fourth subculture (Fig. 1b).

A decrease in the length of shoots was observed with the increase in the number of subcultures from the two types of explants, starting a reduction in the third subculture for shoots from apical nodal segments and from the second subculture for shoots from cotyledonary nodal segments (Fig. 1c).

Segments obtained after each subculture cycle showed a reduction in the size of shoots and leaves development (Fig. 2). From these results, the PAs, ADC and ODC enzymes, and hormone analysis were measured in explants obtained from cotyledonary nodal segments used during the subcultures.

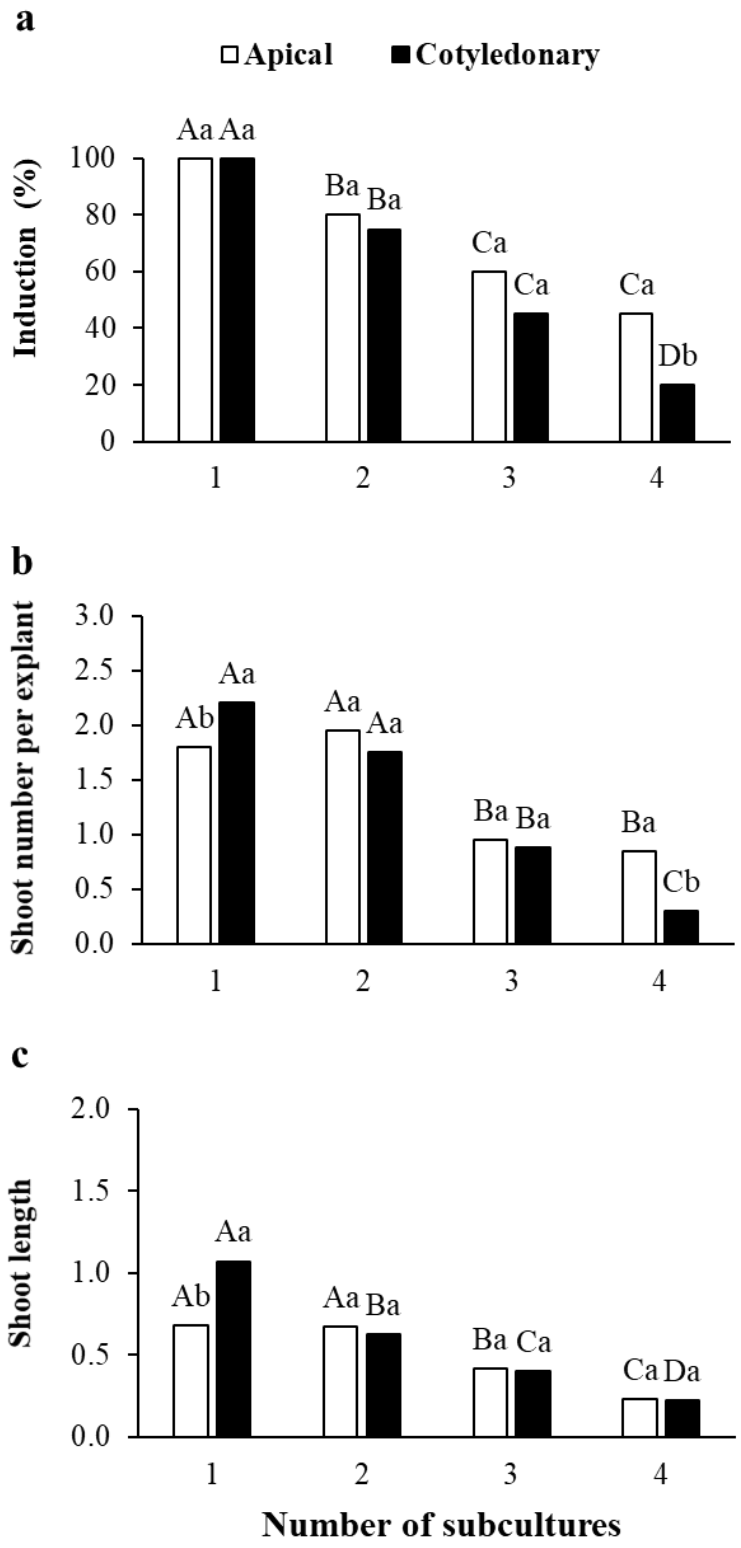

Fig. 1 – Effects of the number of subculture cycles and type of initial explant (apical or cotyledonary nodal segments) from *Cedrela fissilis* on induction of shoots (**a**), number of shoots per explant (**b**), and length of shoots (**c**). Means followed by different letters are significantly different ($P < 0.05$) according to the SNK test. Capital letters denote statistical difference between different subculture cycles in each type of initial explant (apical or cotyledonary nodal segments). Lowercase letters represent statistical difference between the type of initial explants (apical or cotyledonary nodal segments) in each subculture cycle. CV = Coefficient of variation. (n =8; CV for shoot induction = 23.31%; CV for number of shoots = 22.12%; CV for length of shoots = 30.68%).

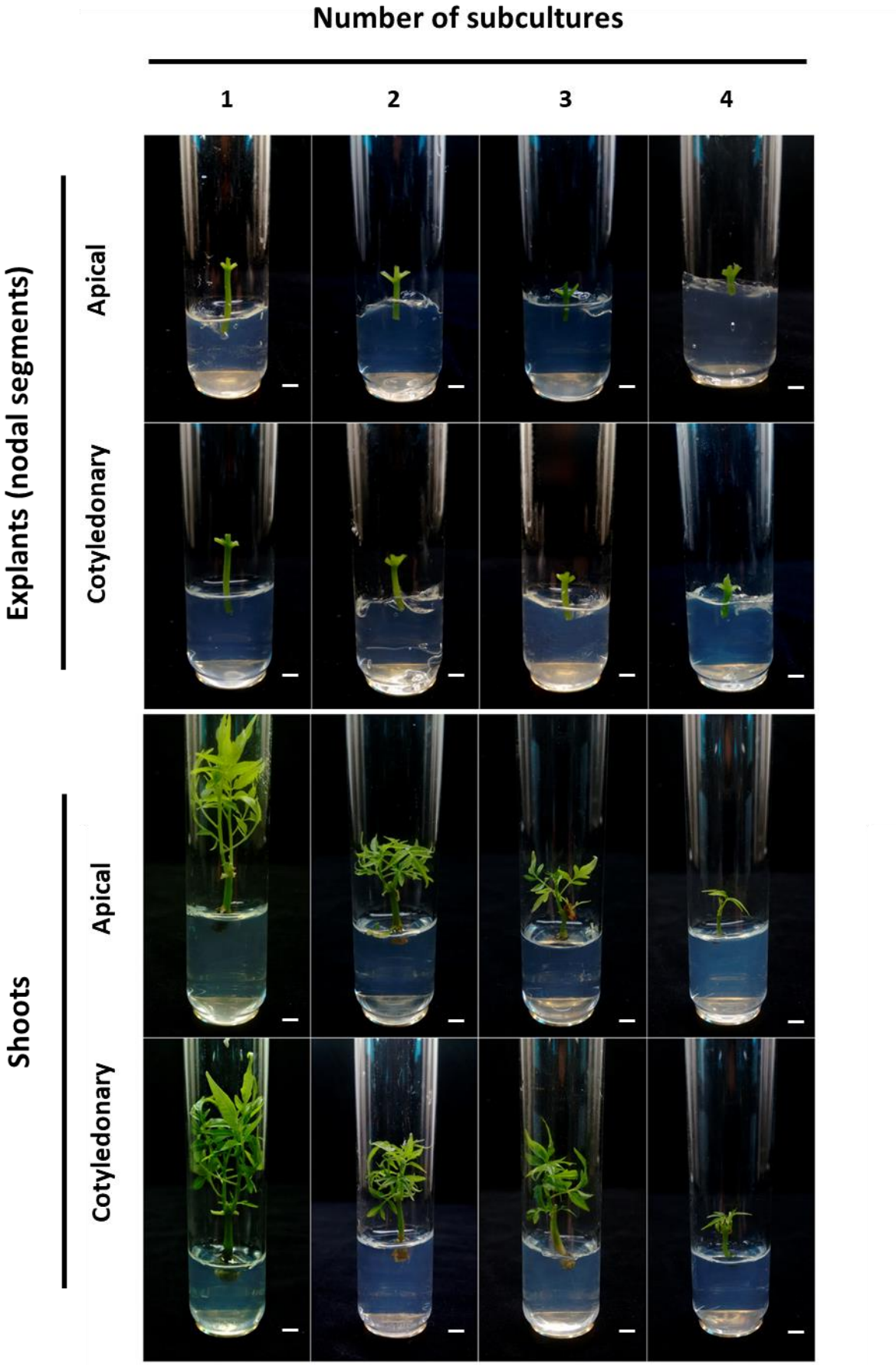

Fig. 2 - Morphological aspects of the explants used to each subculture cycle and the shoots obtained *in vitro* in *Cedrela fissilis*. *Bars = 1cm*.

3.2. Effect of subculture cycles on the endogenous contents of free PAs

Significant differences in the endogenous PAs contents during subculture cycles of cotyledonary nodal segments were observed (Fig. 3). A significant higher content of endogenous free Put was observed in the explants at the first subculture cycle, decreasing significantly at the second subculture, and maintaining similar values until the fourth subculture (Fig. 3a). Free Spd (Fig. 3b) and Spm (Fig. 3c), and total free PAs (Fig. 3d) contents were highest in explants at the first and second subcultures, decreasing significantly until the fourth subculture.

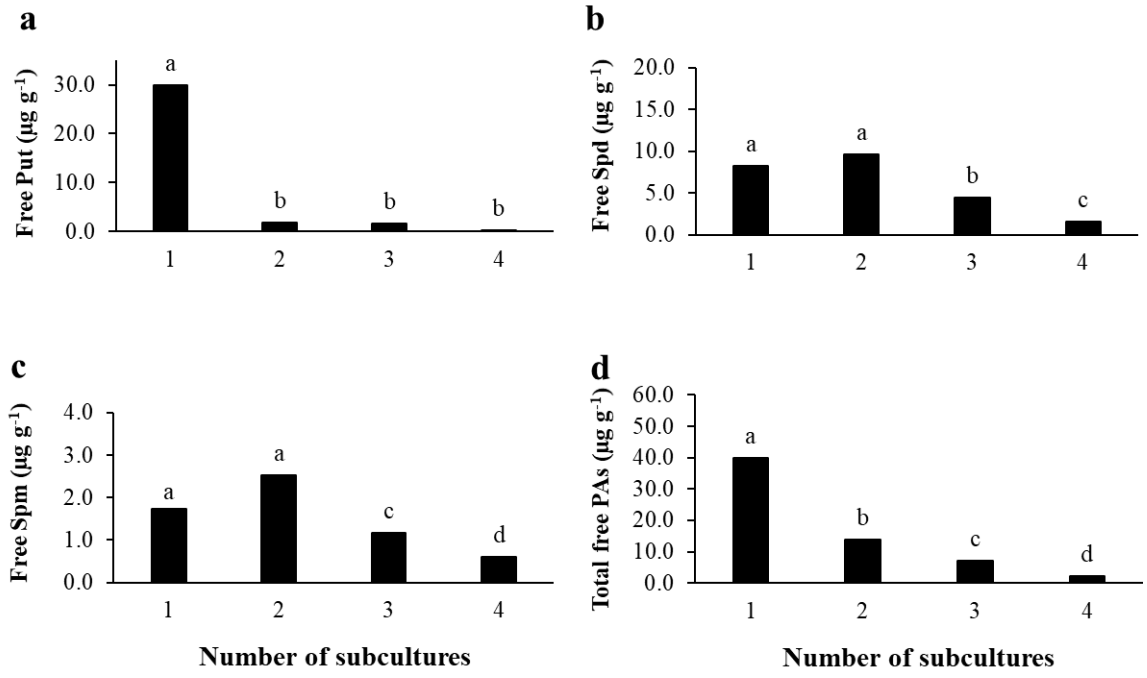

Fig. 3 - Endogenous contents (µg g^{-1}) of free Put (**a**), Spd (**b**), Spm (**c**) and total free PAs (**d**) in explants from cotyledonary nodal segments of *Cedrela fissilis* used in the four subculture cycles. Means followed by *different letters* are significantly different ($P < 0.05$) according to the SNK test. CV = Coefficient of variation. (n =3; CV of Put =14.66%; CV of Spd = 12.04%; CV of Spm = 15.72%; CV of total free PAs = 10.05%).

3.3. Effect of subculture cycles on ADC and ODC enzymes activities

Explants of cotyledonary nodal segments at first subuculture showed a significant higher enzymatic activity of ODC compared to the fourth (Fig. 4), and could be related to the higher Put contents observed at the first subculture (Fig. 3a). On the other hand, the activity of ADC did not show significantly differences in the explants between first and fourth subcultures (Fig. 4). Moreover, the ODC activity was significantly higher compared to ADC activity in cotyledonary nodal segments

at the first subculture cycle (Fig. 4), suggesting that the higher ODC is the relevant for Put synthesis.

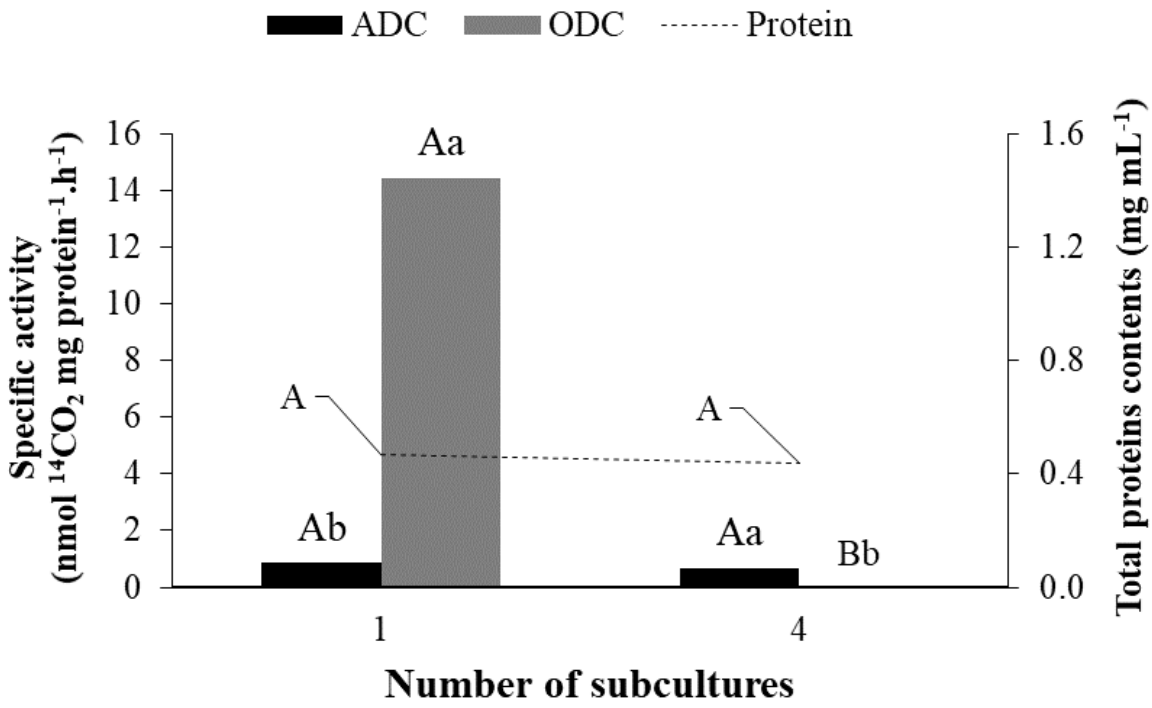

Fig. 4 - Enzymatic activities of arginine decarboxylase (ADC) and ornithine decarboxylase (ODC) in the explants from cotyledonary nodal segments of *Cedrela fissilis* used for the first and fourth subculture cycles. Means followed by *different letters* are significantly different ($P < 0.05$) according to the SNK test. *Capital letters* show statistical difference comparing the two subculture cycles (first and fourth). *Lowercase letters* show significant difference between the two types of enzymatic activities (ADC and ODC) in each subculture cycle. (n =3; Coefficient of variation = 15.04%).

3.4. Effect of subculture cycles on the endogenous contents of IAA, ABA, OPDA, JA, JA-Ile, t-CA and SA

Endogenous contents of IAA, ABA, OPDA, JA, JA-Ile and SA were measured in cotyledonary nodal segments in the first and fourth subculture cycles (Fig. 5). The endogenous content of IAA (Fig. 5a) was significantly higher in cotyledonary nodal segments in the first subculture compared to the first, while the endogenous contents of ABA (Fig. 5b), JA (Fig. 5c), OPDA (Fig. 5e) and SA (Fig. 5f) occurred on fourth subculture. However, the endogenous contents of JA-Ile (Fig. 5d) showed no significant difference when comparing the first and fourth subcultures.

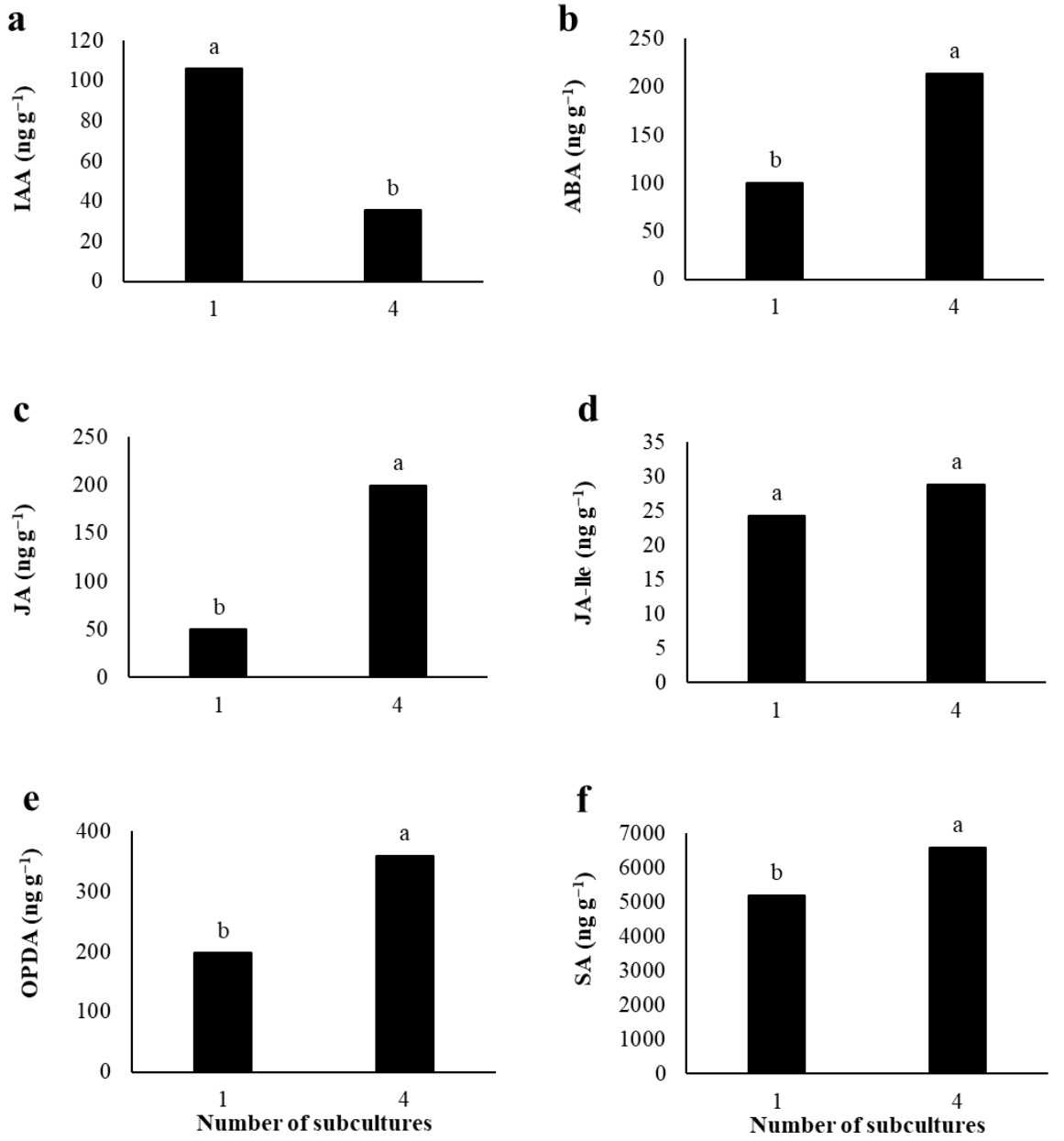

Fig. 5 - Endogenous contents (ng g^{-1} DM) of IAA (**a**), ABA (**b**), JA (**c**), JA-Ile (**d**), OPDA (**e**) and SA (**f**) in the explants from cotyledonary nodal segments of *Cedrela fissilis* at the first and fourth subculture cycle. Means followed by *different letters* are significantly different ($P < 0.05$) according to the SNK test. CV = Coefficient of variation. (n =3; CV of IAA = 17.29%; CV of ABA = 14.31%; CV of JA = 12.43%; CV of JA-Ile = 8.61%; CV of OPDA = 21.17%; CV of SA= 11.27%).

4. DISCUSSION

In this work, we can observe that the subcultures affected negatively the *in vitro* development of shoots, reducing significantly the induction of shoots, as well as the number and length of shoots using explants from apical and cotyledonary nodal segments (Figs. 1a, b and c). A decrease in the *in vitro* multiplication rate during subcultures was reported in some tree species, such as *Cabralea canjerana* (Rocha et al., 2007), *Luehea divaricate* (Flôres et al., 2011), *T. grandis* (Tiwari et al., 2002; Raposo et al., 2010). Other studies also show a decline in shoot multiplication rate in four contemporary fruit rootstocks, i.e. cherry rootstocks Gisela 5 and Gisela 6 (*Prunus cerasus* × *Prunus canescens*), plum rootstock (*Prunus salicina* × *Prunus spinosa*) and pear rootstock (*P. communis*) (Vujović et al., 2012). Furthermore, the decrease in the capacity of shoots development in *C. fissilis* throughout subcultures could be associated with the reduced size of shoots (less than 0.5 cm) at third and fourth subculture. *Plathymenia reticulata* shoots with a length below 0.5 cm can negatively affect the *in vitro* propagation process (Moura et al., 2012). It has been know that the effects of excision or wounding caused by the number of subcultures can be one of the factors related to wounding-induced reprogramming in shoots cells and metabolic re-adjustment (Da Costa et al., 2013). In plants, the regeneration of shoots starts at cut sites of tissues, and hardly occurs in intact or uncut plants (Iwase et al., 2015), being the wounding one of the factors inducing of numerous cellular responses, including the production of plant hormones, loss of cell-to-cell communication and interruption of long-distance signaling (Melnyk et al., 2015; Ikeuchi et al., 2016). In this sense, possibly the successive excision of explants until the fourth subculture can affected the endogenous hormonal content in response to the wounding, leading to a reduction in the regeneration capacity of *C. fissilis* shoots observed at fourth subculture compared to the first one.

Some molecules are candidates for involvement in the regulation of development under successive subcultures of *in vitro* shoots. Among these molecules are PAs, as they could interact with other molecules and participate of metabolism signaling, cell division and differentiation (Kevers et al., 2000; Vuosku

et al., 2006; Kusano et al., 2008). Our results suggest that free Put is essential for *in vitro* shoot development in *C. fissilis*, once a higher content of free Put on explants at first subculture is directly associated to the higher growth of shoots compared to the fourth subculture (Fig. 3a). Studies show that Put can induce the transition through the G1/S and G2/M cell cycle phases, stimulating the cell division (Weiger and Hermann, 2014) and elongation of shoots (Aragão et al., 2017). In addition, it is known that the co-existence of ADC and ODC in the Put biosynthetic pathway may relate to their differential contribution to development processes and tissue specificity, e.g., ODC is particularly active in cell proliferation and ADC is involved in embryo and organ differentiation, stress response (Kevers et al., 2000; Vuosku et al., 2006; De Oliveira et al., 2017). In our work, the ADC and ODC are active in the cotyledonary nodal segments, and the higher activity on the ODC enzyme compared to the ADC in the first subculture (Fig. 4) seems to be decisive for improving the development of shoots (Figs. 1 and 2), resulting in higher free Put contents (Fig. 3a). Possibly, the ODC enzyme actively participates in the responses to cell proliferation in the cotyledonary nodal segments and consequently improving the potential of shoot development in *C. fissilis*. Relatively little is known about these pathways in non-model species, such as the *C. fissilis*, and to the best of our knowledge, this is the first report that shows the activity of the ODC enzyme for this species from cotyledonary nodal segments.

The increase in the number of *in vitro* subculture cycles also affected the hormonal content of the explants (Fig. 5). The higher content of endogenous IAA was observed in explants at the first subculture cycle compared to fourth (Fig. 5a) indicating the relevance of this hormone on *in vitro* shoot development for *C. fissilis*. Some studies show the importance of the endogenous auxin content and its relationship with cytokinin, i.e. an appropriate hormonal balance auxin/cytokinin ratio favorable to the reprogramming of tissues to stimulate the cell division necessary for the development of shoots (Su et al., 2011; Koike et al., 2017). A study with *Carapichea ipecacuanha* shows the dynamics of plant hormones during shoots formation, being endogenous auxin accumulated in the basal region of segments whereas cytokinins accumulated in the middle region (Koike et al., 2017). Thus, the distribution of auxin, not cytokinins, determined where shoots were formed (Koike et al., 2017). Therefore, we suggest that the IAA is linked to improving the shoots development at the first subculture, and the reduction in the content is related

to a reduction in the development potential of the shoots at the fourth subculture cycle in *C. fissilis*. Analyses of ABA during development of shoots from internodes on *Fargesia yunnanensis* revealed that ABA is negatively related to internode elongation (Shuguang et al., 2016). Besides little is known about the involvement of ABA on *in vitro* shoot development in plants, our results suggest that an increase in its contents (Fig. 5b) negatively regulates the *in vitro* growth of shoots in *C. fissils* (Fig. 1).

Some studies have shown that JA and its conjugate form, JA-Ile, have been implicated in responses abiotic (Balfagón et al., 2019) and an transient JA increase plays an important role in the wound responses (Ahkami et al., 2009). Our results show the negative effects of JA content (Fig. 5c), and also OPDA (Fig. 5e), an intermediate in the biosynthesis of jasmonates (Stenzel et al., 2003), in the growth of *C. fissilis* shoots from explants of cotyledonary nodal segments at fourth subculture compared to the first. Our results suggest that OPDA plays an essential role for JA biosynthesis induced by the increase in subcultures (Figs. 5c and 5d). Besides OPDA plays a role in signaling and inducing defense and wounding responses (Stintzi et al., 2001; Taki et al., 2005) and point to the positive feedback response between OPDA and JA (Stenzel et al., 2003), its precise functions are still largely unknown in physiological events. We suggest a negative regulation role of jasmonates (OPDA, JA) in the potential of shoot development in *C. fissilis* linked to the possible wounding response resulting from subcultures.

SA also showed an increase at the fourth subculture in cotyledonary nodal segments (Fig. f) and could be related to the decrease on the potential of *C. fissilis* shoot development. SA is a hormone involved in various responses to biotic and abiotic stresses (Wani et al., 2017). In studies evaluating the callus induction from different cultivars and organs as explants in *Hordeum vulgare*, the endogenous content of JA and SA exhibited higher levels in immature embryos, showing the relationship of these two hormones to *in vitro* regeneration (Hisano et al., 2016). In our work, the lower endogenous content of JA and SA in the explants of cotyledonary nodal segments at the first subculture compared to the fourth suggests the negative effect on the development of shoots in *C. fissilis.*

This work is highlighted the network of hormones and PAs to the capacity of *in vitro* shoot development under successive subculture in *C. fissilis*. From our

knowledge, it is the first work reporting these alterations for this hardwood endangered species from the Brazilian Atlantic Forest.

5. CONCLUSION

The subculture cycles decreased the potential of shoot development during four successive subcultures, which could be due to the stress caused by the wounding due excisions of explants during subcultures, changing the endogenous content of PAs and hormones. The higher development of *in vitro* shoots at the first subculture could be related to the higher contents of free Put and IAA in the explants. In addition, the higher ODC activity in explants at the first subculture was associated to the higher endogenous Put contents, and the higher growth of shoots compared. On the other hand, the lower growth of shoots at the fourth subculture was associated to a higher content of ABA, JA, OPDA and SA in the explants of *C. fissilis*. This work is the first report showing the effects of subcultures cycle on development of shoots associated with PAs and hormones in *C. fissilis*, providing relevant insights on relationship of these compounds on *in vitro* shoot development during successive subculture.

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4.2. *In vitro* SUBCULTURE CYCLES AFFECT ROOTING COMPETENCE VIA CHANGES IN THE PROTEOMIC PROFILE AND HORMONAL CONTENT IN *Cedrela fissilis* VELL. (MELIACEAE) SHOOTS[1]

RESUMO

O número de ciclos de subcultura desempenha um papel essencial na multiplicação clonal em grande escala e na produção de mudas somáticas. Nosso objetivo foi investigar os efeitos de ciclos de subcultura de brotações *in vitro* sobre o enraizamento *ex vitro* e alterações endógenas no conteúdo de hormônios vegetais, metabolismo de poliamina (PA) e perfis proteômicos de *Cedrela fissilis*.

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Oliveira, TDR; Sousa, KR; Aragão, VPM; Oliveira, LF; Floh, EIS; Balfagón, D; Gómez-Cadenas, A; Silveira, V; Santa-Catarina, C. (2020). *In vitro* subculture cycles affect rooting competence via changes in the proteomic profile and hormonal content in *Cedrela fissilis* Vell. (Meliaceae) shoots. Journal of Proteome Research (Submetido).

O número de ciclos de subcultura diminuiu o enraizamento *ex vitro* das brotações na quarta subcultura em relação à primeira. Essa redução no desenvolvimento da raiz adventícia (RA) foi associada à diminuições nos conteúdos endógenos de ácido indol-3-acético, ácido abscísico (ABA), ácido 12-oxo-fitodienoico, putrescina (Put) e espermina. Em contraste, aumentos nos conteúdos de ácido jasmônico (JA), jasmonoil-isoleucina, ácido trans-cinâmico e ácido salicílico foram associados negativamente com o desenvolvimento de RA em brotações do quarto ciclo de subcultura. Além disso, a diminuição do enraizamento no quarto ciclo da subcultura foi associada à redução no acúmulo de algumas proteínas relacionadas às subunidades do centro de reação, metabolismo energético, assimilação de nitrogênio, vias de PA e ABA e JA. Em contraste, proteínas relacionadas à ferimentos mostraram aumentos nas brotações na quarta subcultura e podem estar relacionadas à diminuição no desenvolvimento de RA. A enzima ornitina descarboxilase atua preferencialmente na via de biossíntese de Put durante o enraizamento em brotações de *C. fissilis*. Esses resultados aumentam a compreensão dos mecanismos hormonais e moleculares relacionados ao potencial de desenvolvimento de RA em brotações sob sucessivos ciclos de subculturas e podem ajudar a melhorar a produção de mudas em larga escala nesta espécie.

ABSTRACT

The number of subculture cycles plays an essential role in the large-scale clonal multiplication and production of somatic plantlets. We aimed to investigate the effects of *in vitro* shoot subculture cycles on *ex vitro* rooting and endogenous alterations in the plant hormone contents, polyamine (PA) metabolism and proteomic profiles of *Cedrela fissilis*. The number of subculture cycles decreased the *ex vitro* rooting of shoots in the fourth subculture relative to the first. This reduction in adventitious root (AR) development was associated with decreases in the endogenous contents of indole-3-acetic acid, abscisic acid (ABA), 12-oxo phytodienoic acid, putrescine (Put) and spermine. In contrast, increases in the

contents of jasmonic acid (JA), jasmonoyl-isoleucine, trans-cinnamic acid and salicylic acid were negatively associated with AR development in shoots from the fourth subculture cycle. In addition, the decrease in rooting in the fourth subculture cycle was associated with reductions in the accumulation of some proteins related to reaction center subunits, energy metabolism, nitrogen assimilation, PA pathways, and ABA and JA. In contrast, wounding-related proteins showed increases in shoots in the fourth subculture and could be related to the decrease in AR development. The ornithine decarboxylase enzyme preferentially functions in the Put biosynthesis pathway during rooting in *C. fissilis* shoots. These results increase the understanding of hormonal and molecular mechanisms related to potential AR development in shoots under successive subculture cycles and may help to improve large-scale plantlet production in this species.

1. INTRODUCTION

Clonal multiplication is an important process in plant propagation in which plant cells become competent to recognize a signal that leads to a specific developmental trajectory, such as the development of new organs (Zeng et al., 2019). Several factors are associated with the *in vitro* acquisition of morphogenic competence and significantly affect plant regeneration, including the explant type, culture medium composition, plant growth regulators (PGRs), plant genotype and subculture cycles (Duclercq et al., 2011; Shemer et al., 2015). The number of subculture cycles is an important factor for increasing the number of propagules obtained in micropropagation systems and plays an essential role in the clonal multiplication and production of plantlets on a large scale (Rocha et al., 2007; Pastelín Solano et al., 2019). However, species present differences in their competence to produce new organs with increasing number of subculture cycles (Rocha et al., 2007; Hamad and Taha, 2008). In addition, increases in recalcitrance due to the oxidative damage induced by wounding may explain reductions in the *in vitro* propagation potential under some circumstances (Papadakis et al., 2001;

Singh et al., 2017). Wounding induces numerous cellular responses, including the production of plant hormones, loss of cell-to-cell communication and disruption of long-distance signaling, and can be considered a trigger of both cell division and damaging oxidative bursts (Cassells and Curry, 2001; Ikeuchi et al., 2016).

It has been shown that the reduction in the root formation capacity increases with the number of subculture cycles, which affects adventitious root (AR) regeneration, one of the fundamental steps for micropropagation, and consequently results in low plantlet production (Tiwari et al., 2002; Rocha et al., 2007). The ability to form ARs is a heritable quantitative trait controlled by multiple endogenous and environmental factors (Li et al., 2009), among which plant hormones play a central role (Druege et al., 2019). The interaction of several signaling compounds is necessary for the function of the network of metabolic and physiological processes underlying the successful growth of the plant (Naseem et al., 2012).

Among plant hormones, auxins play a central role in root induction and are often applied exogenously to promote the development of ARs on stem cuttings of difficult-to-root species (De Almeida et al., 2020). Auxins can interact with other hormones, such as abscisic acid (ABA), jasmonic acid (JA) and salicylic acid (SA) (Zhang et al., 2007; Albacete et al., 2008; Gutierrez et al., 2012). There is considerable evidence that the crosstalk between indole-3-acetic acid (IAA) and ABA during abiotic stress conditions plays an important role in improving root growth (Albacete et al., 2008). Beyond the evidence showing that ABA activates the expression of genes encoding antioxidant enzymes (Gomez-Cadenas et al., 2015), little is known about the involvement and effects of ABA in *ex vitro* rooting. In addition, the crosstalk between IAA and JA plays an important role in the regulation of AR formation in etiolated hypocotyls, in which IAA controls AR initiation by negatively regulating JA signaling (Gutierrez et al., 2012). Furthermore, JA has been related to injuries induced by cuts in isolated plant tissues, in which it influences stress-induced reprogramming and is one of the factors associated with hormonal variation (Da Costa et al., 2013). An intermediate in JA biosynthesis, 12-oxo-phytodienoic acid (OPDA), is also involved in signaling and induces defense and wounding responses independently (Stenzel et al., 2003), although the precise functions of OPDA in physiological events, including rooting, are still largely unknown. SA is another plant hormone involved in root development and shows a

potential role in AR formation in some species (Gutierrez et al., 2012; Yang et al., 2013).

In addition, polyamines (PAs) modulate the morphogenetic response and can be involved in rooting competence (Kusano et al., 2008; Ahkami et al., 2009; Wang et al., 2020). PAs are low-molecular-weight aliphatic compounds (Lenis et al., 2017) that are essential for several processes involved in plant growth (Kevers et al., 2000; Vuosku et al., 2006; Kusano et al., 2008). Among PAs, the diamine putrescine (Put) is synthesized directly from ornithine decarboxylase (ODC) or from arginine decarboxylase (ADC) via two additional synthesis steps (Bais and Ravishankar, 2002). The coexistence of ADC and ODC in the Put biosynthetic pathway in some plant species may be related to their different contributions to development and tissue-specific processes (Vuosku et al., 2006). In plants, some studies have indicated that specific PAs are associated with different processes; e.g., spermidine (Spd) and spermine (Spm) have been associated with cell differentiation, whereas Put is more closely related to cell division (Santa-Catarina et al., 2006; Aragão et al., 2017). Although significant progress has been made in understanding the regulation of PA metabolism and signal transduction (Tun et al., 2006; Lasanajak et al., 2014; Majumdar et al., 2016), little is known about the roles of these compounds in shoots and AR development (Acosta et al., 2005), especially under successive subculture cycles of *in vitro* shoots and *ex vitro* rooting in woody species.

In addition to plant hormone analysis, a proteomic approach can provide information related to the protein profile during morphogenetic responses (Hochholdinger et al., 2006; Takáč et al., 2011), such as *in vitro* shoot and AR development (Aragão et al., 2017; De Almeida et al., 2020). The application of 2.5 mM Put improved *in vitro* shoot development in *C. fissilis* and induced an alteration in the abundance of proteins, primarily consisting of metabolic and cellular proteins associated with cell division (Aragão et al., 2017). During AR development, proteomic studies have revealed roles of several proteins involved in different biological pathways (mainly oxidative stress, energy metabolism and photosynthesis) (De Almeida et al., 2020). In addition, some proteins that can act as positive regulators of ARs, such as hydrogen peroxide or starch metabolism-related proteins, have been shown to exhibit higher accumulation in *Eucalyptus grandis* than in *Eucalyptus globulus*, which are species with different rooting potentials (De Almeida et al., 2020).

The hormonal and molecular mechanisms controlling the competence of morphogenesis during successive *in vitro* subculture cycles are still poorly understood in woody plants, such as *Cedrela fissilis*. This species is a native woody tree from the Brazilian Atlantic Forest, and because of its high economic value for commercial wood production, *C. fissilis* has been included in the Red List of Endangered Species by the International Union for Conservation of Nature (IUCN), in which it is classified as vulnerable (Barstow, 2018). In this context, the assessment of changes in the endogenous contents of plant hormones and proteomic profiles during increasing numbers of subculture cycles can provide important information about the AR competence of this woody plant. Thus, the aim of this work was to study the effects of the number of subculture cycles on *in vitro* shoot development and *ex vitro* rooting in *C. fissilis* and the roles that these effects on endogenous hormones, free PAs and key proteins play in rooting competence.

2. MATERIALS AND METHODS

2.1. Plant material

Mature seeds of *C. fissilis* obtained from Caiçara Comércio de Sementes LTDA located in Brejo Alegre (21°10'S and 50°10'W), São Paulo State, Brazil, were germinated *in vitro* according to Oliveira et al. (2020). Sixty-d-old seedlings were used as the source of apical and cotyledonary nodal segment explants to obtain the shoots that were used for rooting in each subculture cycle.

2.2. *In vitro* germination

In vitro germination was performed according to Oliveira et al. (2020). First, the seeds were surface disinfected and were then transferred to glass test tubes (15 x 2.5 cm; Laborglas; São Paulo, Brazil) containing 10 mL of MS (Murashige and Skoog, 1962) culture medium (M519; Phytotechnology Lab, Lenexa, USA) supplemented with 20 g L^{-1} sucrose (Vetec; Rio de Janeiro, Brazil) and 2 g L^{-1} Phytagel (Sigma-Aldrich; St. Louis, USA). The pH of the culture medium was

adjusted to 5.7 before being autoclaved at 121 °C for 15 min. The seeds were incubated in a culture room at 25 ± 2 °C under a 16-h photoperiod using fluorescent lamps (55 µmol/m^{-2}/s) for a culture period of 60 days. Sixty-d-old seedlings were used as a source of explants (cotyledonary and apical nodal segments) for the experiment.

2.3. *In vitro* multiplication of shoots during successive subculture cycles

The explants (apical and cotyledonary nodal segments) obtained from 60-d-old seedlings were inoculated into glass test tubes (15 x 2.5 cm; Laborglas) containing 10 mL of MS culture medium supplemented with 20 g L^{-1} sucrose, 2 g L^{-1} Phytagel, and 2.5 µM 6-benzyladenine (BA; Sigma-Aldrich), according to Oliveira et al. (2020). The pH of the culture medium was adjusted to 5.7 before autoclaving at 121 °C for 15 min. The explants were incubated in a culture room at 25 ± 2 °C under a 16-h photoperiod using fluorescent lamps (55 µmol/m^{-2}/s) for a culture period of 45 days. This step corresponded to the shoots obtained in the first subculture cycle using nodal segments obtained from 60-d-old seedlings as explants. For the second, third, and fourth subculture cycles, the shoots grown from apical and cotyledonary nodal segments after 45 days of incubation were excised (~ 2 cm), cutting samples of the leaves and apical meristem to obtain the explants, which were then used in the various subcultures. The explants were inoculated in the same culture medium again under the same conditions used for the first subculture. Intervals of 45 days were maintained between each cycle under the same conditions of incubation for the growth of shoots. After 45 days of incubation, at the end of each subculture cycle, shoots were collected and used for *ex vitro* rooting.

2.4. Effect of subculture cycles on *ex vitro* adventitious rooting of micropropagated shoots

To study the effect of subculture cycles on *ex vitro* rooting, shoots (2 cm) obtained from apical and cotyledonary nodal segments after 1, 2, 3 and 4 subculture cycles were used. The shoots were removed from the culture medium and transferred to disposable polypropylene plastic cups (50 mL; Orleans, Santa Catarina, Brazil) containing Basaplant® (Artur Nogueira, São Paulo, Brazil) substrate and vermiculite (Mineração Pedra Lavrada LTDA; Atibaia, São Paulo,

Brazil) in a 2:1 (v/v) proportion. The plastic cups were maintained in plastic trays (50 x 60 x 10 cm) covered with plastic film to maintain high humidity for 15 days. Thereafter, the humidity level was gradually reduced through perforation of the plastic film, and after 25 days, the plastic film was removed. The explants were maintained in a culture room at 25 ± 2 °C under a photoperiod of 16 h light at 55 µmol/m^{-2}/s for 45 days.

From the first to the fourth subculture cycles, root induction (%), the number of roots per explant, and the length (cm) of roots were evaluated after 45 days of rooting. Each treatment (subculture cycle) was composed of eight replicates, with five shoots per replicate. For PA analyses, shoots from cotyledonary nodal segments with leaves were collected after 45 days of culture across four subculture cycles. For hormone and comparative proteomic analyses, shoots from cotyledonary nodal segments with leaves were collected after 45 days of culture in the first and fourth subculture cycles. Samples of the shoots used in the proteomic and PA analyses were macerated with liquid nitrogen until a fine powder was obtained, which was then stored at −80 °C until assessment. Samples of shoots for hormonal analysis were lyophilized for storage until assessment.

After rooting, the plantlets were transplanted to disposable polypropylene plastic cups (250 mL) containing Basaplant® substrate and vermiculite in a 2:1 (v/v) proportion and were maintained under a 16 h photoperiod using fluorescent lamps at 55 µmol/m^{-2}/s at 25 ± 2 °C for 15 days. Then, the rooted plantlets were transferred to a greenhouse with relative air humidity higher than 85% and temperatures between 20 and 30 °C (measured using an Extech RHT10 USB Datalogger; Extech, Waltham, USA). After 90 days, plantlet survival (%) was analyzed.

2.5. Free PA determination

Free PA contents were determined according to Santa-Catarina et al. (2006) using three biological replicate samples (200 mg fresh matter (FM) each) of shoots obtained from cotyledonary nodal segments after 45 days of growth in MS culture medium in each subculture cycle (1, 2, 3 and 4). The samples were ground in 1.2 mL of 5% perchloric acid (Merck; Darmstadt, Germany), incubated at 4 °C for 1 h, and centrifuged for 20 min at 20,000 × *g* at 4 °C. The supernatant containing free PAs was obtained, followed by derivatization with dansyl chloride (Merck) and identification by high-performance liquid chromatography (HPLC; Shimadzu, Kyoto,

Japan) using a 5-µm C_{18} reverse-phase column (Shimadzu Shin-pack CLC ODS). The HPLC column gradient was created by adding increasing volumes of absolute acetonitrile (Merck) to a 10% aqueous acetonitrile solution at pH 3.5, adjusted with hydrochloric acid (Merck). The absolute acetonitrile concentration was maintained at 65% for the first 10 min, increased from 65 to 100% between 10 and 13 min, and maintained at 100% between 13 and 21 min; the mobile phase was added at a flow rate of 1 mL min^{-1} at 40 °C. The PA concentration was determined using a fluorescence detector at 340 nm (excitation) and 510 nm (emission). The peak areas and retention times of the samples were measured through comparisons with the standard PAs Put, Spd, and Spm (Sigma-Aldrich).

2.6. Determination of ADC and ODC activities

ODC and ADC activities were determined according to De Oliveira et al. (2017) with some modifications using three biological replicate samples (500 mg FM per sample) of shoots obtained from cotyledonary nodal segments after 45 days of growth in MS culture medium during the first and fourth subculture cycles. Each sample was homogenized in an ice-cold mortar with liquid nitrogen and transferred to 500 µL of extraction buffer containing 50 mM Tris–HCl (Invitrogen; Carlsbad, USA) (pH 8.5), 0.5 mM pyridoxal-5-phosphate (Sigma-Aldrich), 0.1 mM ethylenediaminetetraacetic acid (EDTA; Sigma-Aldrich) and 5 mM dithiothreitol (DTT; Bio-Rad, Hercules, USA). The sample mixtures were vortexed and centrifuged for 20 min at 13,000 × *g* at 4 °C, and the supernatant was used for the ADC and ODC enzymatic assays. A reaction mixture containing 100 µL of protein extract, 8.3 µL of extraction buffer, 12 mM unlabeled L-Arg or L-Orn, and 25 mCi of either L-[^{14}C(U)]-Arg (specific activity 274.0 mCi $mmol^{-1}$; PerkinElmer; Waltham, USA) or L-[1-^{14}C]-Orn (specific activity 57.1 mCi$mmol^{-1}$; PerkinElmer) was used. For blank samples, 100 µL of extraction buffer was used. The reaction mixtures were incubated in glass tubes fitted with rubber stoppers and filter paper discs (GE Healthcare; Piscataway, USA) soaked in 2 N KOH (Vetec). The material was incubated at 37 °C at 120 rpm in an orbital shaker for 90 min, and the reaction was stopped by adding 200 µL of 5% (v/v) perchloric acid, followed by further incubation for 15 min under the same conditions. Filter paper containing $^{14}CO_2$ was immersed in 1 mL of scintillation fluid (PerkinElmer). Radioactivity was then measured using a scintillation counter (Tri-Carb2910TR; PerkinElmer). Protein content was measured

using the Bradford method (Bradford, 1976) with bovine serum albumin as the standard. The specific enzymatic activities of ADC and ODC were expressed as nmol $^{14}CO_2$ mg protein^{-1} h^{-1}.

2.7. Plant hormone analysis

Plant hormone analysis was performed according to Durgbanshi et al. (2005) with slight modification using three biological replicate samples (30 mg dry matter (DM) for each sample) of shoots obtained from cotyledonary nodal segments after 45 days of growth in MS culture medium in the first and fourth subculture cycles. Before extraction, a mixture containing 50 ng of [$^2H_6$]-ABA, [C_{13}]-SA, dihydrojasmonic acid and 5 ng of [$^2H_2$]-IAA (all from Sigma-Aldrich) was added to each sample as an internal standard. [$^2H_2$]-IAA was used to determine IAA contents, [$^2H_6$]-ABA was used to determine ABA contents, and [^{13}C]-SA was used to determine SA and trans-cinnamic acid (t-CA) contents, and dihydrojasmonic acid was used to determine JA, OPDA and jasmonoyl-isoleucine (JA-Ile) contents. The samples were immediately homogenized in 2 mL of ultrapure water in a ball mill (MillMix20; Domel; Železniki, Slovenija). After centrifugation at 4,000 × *g* and 4 °C for 10 min, the supernatants were recovered, and the pH was adjusted to 3.0 with 30% (v/v) acetic acid (Labkem; Barcelona, Spain). The water extract was partitioned twice against 2 mL of diethyl ether (Labkem), and the organic layer was recovered and evaporated under vacuum in a centrifuge concentrator (Speed Vac; Jouan, Saint-Herblain, France). Once dried, the samples were resuspended in a 10% (v/v) methanol (Fisher Scientific; Loughborough, UK) solution via gentle sonication. The resulting solution was filtered through 0.22-µm polytetrafluoroethylene membrane syringe filters (PTFE 13-mm diameter; Kinesis Ltd; Cambridgeshire, UK) and directly transferred to vials for mass spectrometry analysis.

LC-electrospray ionization (ESI)-MS/MS analysis was performed using an Acquity Ultra-High Performance Liquid Chromatography (UHPLC) system (Waters; Milford, USA) coupled to a tandem Xevo TQ-XS triple quadrupole mass spectrometer (Waters) using an orthogonal Z-Spray ESI interface operated in negative-ion mode. Chromatographic separations were carried out in a reversed-phase C_{18} column (50 × 2.1 mm, 1.6-µm particle size; Phenomenex Luna Omega; Madrid, Spain) at a flow rate of 300 µL min^{-1} with a column temperature of 40 °C. A binary gradient was used for elution: mobile phase A consisted of ultrapure water

and 0.1% acetic acid, and mobile phase B consisted of 99.9% (v/v) methanol (Fisher Scientific) and 0.1% (v/v) acetic acid. Gradient elution was performed sequentially as follows: maintenance of 10% B for 2 min, followed by ramping from 10 to 90% B at 6 min, and a decrease to 10% B at 7 min, after which 10% B was maintained until the end of the run at 8 min. The drying gas and the nebulizing gas were both nitrogen (Praxair; Valencia, Spain). The cone gas flow was set to 250 L h^{-1}, and the desolvation gas flow was set to 1200 L h^{-1}. For operation in tandem MS (MS/MS) mode, the collision gas was 99.995% pure argon (Praxair). The cone voltage and collision energies were adjusted depending on the compound under investigation, as described by Durgbanshi et al. (2005), with few modifications. The desolvation gas temperature was 650 °C, the source temperature was 150 °C, and the capillary voltage was 2 kV. The mass spectrometer was operated in multiple reaction monitoring (MRM) mode. Masslynx v4.1 software was used for mass spectral acquisition, and growth regulators were measured through comparisons with the internal standard for each deuterium-labeled growth regulator.

2.8. Protein extraction and digestion

Comparative proteomic analyses were performed with three biological replicate samples (300 mg FM per sample) of shoots obtained from cotyledonary nodal segments grown in MS culture medium from the first and fourth subculture cycles. Total protein extraction was performed according to Damerval et al. (1986) with some modifications. The samples were first macerated to a fine powder with liquid nitrogen. Then, the samples were resuspended in 1 mL of chilled solution containing 10% (w/v) trichloroacetic acid (TCA; Sigma-Aldrich) in acetone (Merck) and 20 mM DTT (GE Healthcare, Piscataway, USA), and the mixture was vortexed for 5 min at 4 °C and kept at − 20 °C for 1 h, followed by centrifugation at 16,000 x *g* for 20 min at 4 °C. The resulting pellets were washed three times with cold acetone containing 20 mM DTT and centrifuged for 5 min in each wash step. The pellets were air dried, resuspended in buffer containing 7 M urea (GE Healthcare), 2 M thiourea (GE Healthcare), 2% Triton X-100 (GE Healthcare), 1% DTT (GE Healthcare), and 1 mM phenylmethanesulfonyl fluoride (PMSF; Sigma-Aldrich), vortexed, and incubated on ice for 30 min. Following incubation, the samples were centrifuged at 16,000 × *g* for 20 min at 4 °C. The supernatants containing total

proteins were collected, and the protein concentration was measured using a 2-D Quant Kit (GE Healthcare).

Before the digestion step, the extracted proteins (100 µg from each biological replicate) were first precipitated using methanol/chloroform (Nanjo et al., 2012). Then, the samples were resuspended in a solution of 7 M urea (GE Healthcare) and 2 M thiourea (GE Healthcare), and protein digestion was performed with trypsin (50 ng μL^{-1}; V5111, Promega, Madison, USA) using the filter-aided sample preparation (FASP) method (Burrieza et al., 2019). The digested samples were transferred to Total Recovery Vials (Waters) for mass spectrometry analysis.

Nano-LC-electrospray ionization (ESI)-MS/MS analysis was performed using a nanoAcquity UPLC system (Waters) coupled to a Synapt G2-Si mass spectrometer (Waters). First, a chromatography step was performed by loading 1 µg of the digested samples according to Oliveira et al. (2020) to normalize the relative protein quantification results. To ensure standardized molar values for all conditions, the normalization among samples was based on stoichiometric measurements of the total ion counts (TICs) from MSE scouting runs prior to the analyses using ProteinLynx Global SERVER v. 3.0 (PLGS; Waters). After sample normalization, the peptide mixtures were separated via liquid chromatography using a nanoAcquity UPLC 5 µm C18 trap column (180 µm × 20 mm; Waters) at 5 µL min^{-1} for 3 min, followed by a nanoAcquity HSS T3 1.8 µm analytical reverse-phase column (75 µm × 150 mm; Waters) at 400 nL min^{-1} at 45 °C. A binary gradient of mobile phase A containing water (Tedia; Fairfield, USA) and 0.1% formic acid (Sigma-Aldrich) and mobile phase B containing absolute acetonitrile (Sigma-Aldrich) and 0.1% formic acid was used for peptide elution. Gradient elution started with 7% B, followed by 7 to 40% B until 91.12 min, 40 to 99.9% B until 92.72 min, holding at 99.9% B until 106 min, then a decrease to 7% B until 106.1 min and holding at 7% B until the end of the run at 120 min. Mass spectrometry was performed with the following settings: positive and resolution mode (V mode), 35,000 full width at half maximum ion mobility separation (IMS), data-independent acquisition (DIA) mode ($HDMS^E$). IMS was performed using a wave velocity of 600 m s^{-1}. The helium and IMS gas flow rates were 180 and 90 mL/min, respectively. The transfer collision energy was ramped from 19 to 55 V in high-energy mode, with cone and capillary voltages of 30 and 2750 V, respectively, and a source temperature of 70 °C. For the time of flight (TOF) parameters, the scan time was set

to 0.5 s in continuum mode, with a mass range of 50 to 2000 Da. Human [Glu^{1}]-fibrinopeptide B (Sigma-Aldrich; 100 fmol μL^{-1}) was used as an external standard, and lock mass acquisition was performed every 30 s. Mass spectral acquisition was performed for 90 min using MassLynx v4.0 software.

2.9. Proteomic data analysis

Spectral processing and database searching were performed using the ProteinLynx Global Server (PLGS; version 3.0.2) (Waters). The Apex3D parameters were set to a low-energy threshold of 150 counts, an elevated-energy threshold of 50 counts, and an intensity threshold of 750 counts. In addition, the analysis settings included the following: one missed cleavage, minimum fragment ion per peptide equal to 3, minimum fragment ions per protein equal to 7, minimum peptide per protein equal to 2, automatic peptide and fragment tolerance, a fixed modification of carbamidomethyl and variable modifications of oxidation and phosphoryl. The false discovery rate (FDR) was set to a maximum of 1%. Protein identification was performed against a nonredundant protein databank for *C. fissilis* generated by transcriptome sequencing and de novo assembly (Oliveira et al., 2020). Comparative label-free quantification analysis was performed using ISOQuant software v.1.7 (Distler et al., 2014). The following parameters were used to identify proteins: a 1% FDR, a peptide score greater than six, a minimum peptide length of six amino acids, and at least two peptides per protein were required for label-free quantitation using the TOP3 approach, followed by the multidimensional normalized process within ISOQuant. The mass spectrometry proteomic data have been deposited with the ProteomeXchange (Deutsch et al., 2019) Consortium via the PRIDE (Perez-Riverol et al., 2019) partner repository with the dataset identifier PXD023173.

To ensure the quality of the results after data processing, only the proteins that were present or absent (for unique proteins) in all three runs of biological replicates were considered in the differential accumulation analysis using Student's *t*-test (two-tailed; $P < 0.05$). Differentially accumulated proteins were considered to be up-accumulated if the $\log_2$ value of their fold change (FC) was greater than 0.6 and down-accumulated if the $\log_2$ value of their FC was lower than -0.6 according to Student's *t*-test. Finally, the proteins were subjected to BLASTp searches against the Nonredundant (nr) Green Plants/Viridiplantae Protein

Sequences database using OmicsBox software (https://www.biobam.com/omicsbox) for high-throughput functional annotation (Götz et al., 2008).

2.10. Statistical analysis

The rooting of shoots was performed using a completely randomized design. The data on root induction, the numbers and lengths of roots, plantlet survival, free PAs, ADC/ODC enzyme activities and plant hormones were analyzed by analysis of variance (ANOVA) ($P < 0.05$) followed by the Student-Newman-Keuls (SNK) test (Sokal and Rohlf, 1995) in the R statistical environment (R core team, 2017).

3. RESULTS

3.1. Effect of subculture cycles on the *ex vitro* adventitious rooting of micropropagated shoots

The number of subcultures significantly affected the rooting of the shoots, resulting in a significant reduction in the development of ARs on shoots from apical and cotyledonary nodal segments (Figs. 1 and 2).

Shoots obtained from cotyledonary nodal segments showed a significant reduction in the induction of *ex vitro* rooting (Fig. 1a) and the number of roots formed (Fig. 1b) in the third and fourth subcultures, whereas those obtained from apical nodal segments showed a significant reduction in both parameters beginning in the second subculture (Fig. 1). In addition, a significant decrease in the length of roots on shoots from the two types of explants was observed with an increase in the number of subcultures (Fig. 1c).

A comparison of shoots from the apical and cotyledonary nodal segments used for *ex vitro* rooting revealed a significant difference in the percentage of root induction (Fig. 1a) and number of roots per shoot (Fig. 1b) beginning in the second subculture cycle. In addition, shoots from cotyledonary nodal segments presented a significantly higher percentage of root induction and number of roots per shoot than shoots from apical nodal segments (Fig. 1a). Moreover, shoots obtained from

cotyledonary nodal segments showed longer roots than those initiated from apical nodal segments in the first, second and third subculture cycles (Fig. 1c).

The highest survival rates of the plantlets were obtained with shoots from the first subculture, with no significant difference between the two types of explants used (Fig. 1d). In the second subculture, the survival rate was significantly higher for shoots initiated from cotyledonary nodal segments than those initiated from apical nodal segments, while in the other subculture cycles (third and fourth), a significant decline in plantlet survival occurred among the shoots from both types of explants used (Fig. 1d). Therefore, shoots generated from cotyledonary nodal segments collected in the first and fourth subculture cycles were used for PA enzyme activity, proteomic and hormonal analyses.

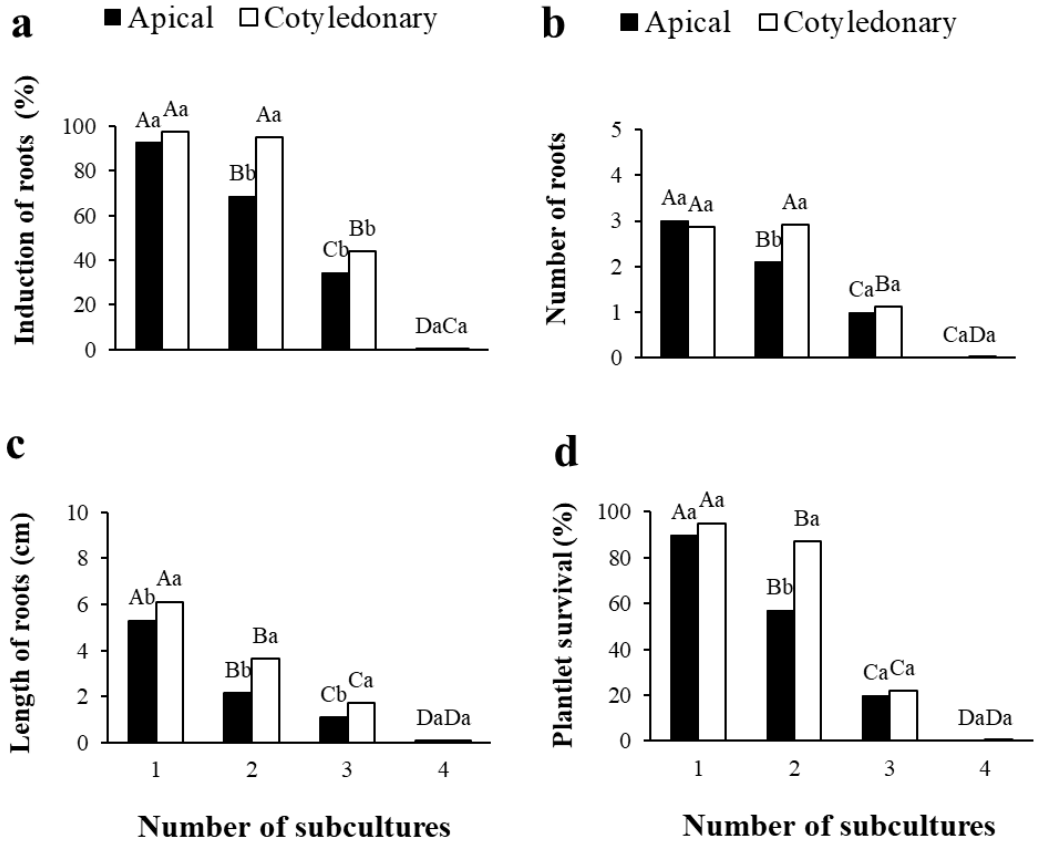

Fig. 1 - Effects of the number of subculture cycles and the type of initial explant (apical or cotyledonary nodal segments) on the *ex vitro* rooting of micropropagated *Cedrela fissilis* shoots. The induction of roots (**a**), number of roots per shoot (**b**), length of roots (**c**), and plantlet survival throughout the subculture cycle (**d**) were analyzed in the shoots of *C. fissilis* during four subculture cycles at 45-day intervals in each subculture. Means followed by *different letters* are significantly different ($P < 0.05$) according to the SNK test. *Capital letters* show significant differences between subculture cycles according to the initial explant type (apical or cotyledonary nodal segments). *Lowercase letters* show significant differences between initial explant types (apical or cotyledonary nodal segments) in each subculture cycle. CV = coefficient of variation. (n = 8; CV for the induction of roots = 10.4%; CV for the number of roots = 16.3%; CV for the length of roots = 14.5%; CV for plantlet survival = 14.3%).

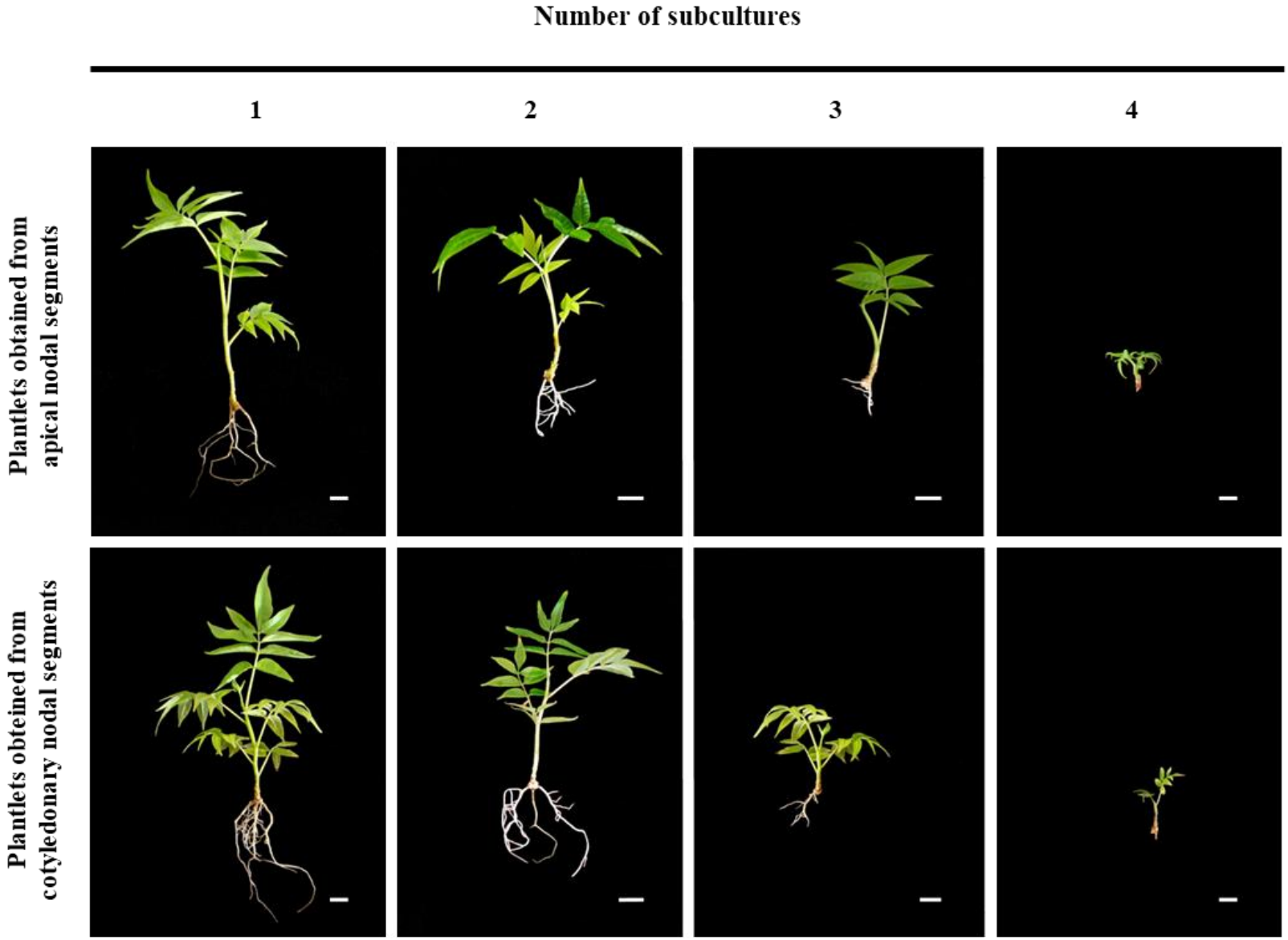

Fig. 2 - Morphological aspects of the plantlets and adventitious root development in the shoots of *Cedrela fissilis* initiated from apical and cotyledonary nodal segments during four subculture cycles. *Bars = 1 cm*.

3.2. Effect of subculture cycles on the endogenous contents of free PAs

The number of subculture cycles significantly affected the endogenous contents of PAs in shoots from cotyledonary nodal segments (Fig. 3). The contents of free Put (Fig. 3a), Spd (Fig. 3b) and Spm (Fig. 3c) decreased significantly in shoots from the first to the second subculture cycles. The endogenous contents of total free PAs were significantly higher in the first subculture, when greater rooting was observed, and they then significantly decreased in shoots with increasing subculture cycles, when the rooting potential also decreased (Fig. 3d).

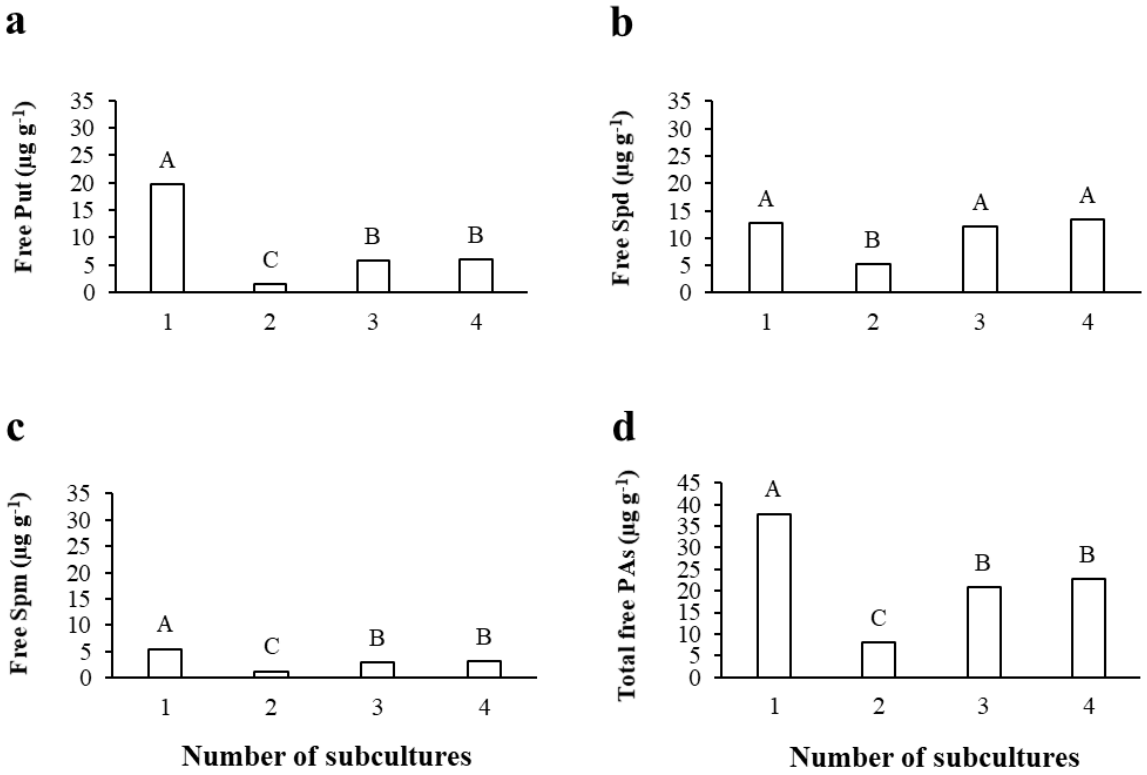

Fig. 3 - Endogenous contents (µg g^{-1} FM) of free Put (**a**), Spd (**b**), Spm (**c**) and total free PAs (**d**) in shoots from cotyledonary nodal segments of *Cedrela fissilis* during four subculture cycles. Means followed by *different letters* show significant differences ($P < 0.05$) according to the SNK test. CV = coefficient of variation. (n = 3; CV for Put = 7.56%; CV for Spd = 5.41%; CV for Spm = 11.45; CV for total free PAs = 4.63%).

3.3. Effect of subculture cycles on ADC and ODC enzymes activities

Because of the results regarding endogenous PA contents, especially for free Put, enzyme activity analysis was performed on shoots generated from cotyledonary nodal segments from the first and fourth subculture cycles. A significant reduction in ODC enzyme activity was observed in the fourth subculture cycle relative to the first (Fig. 4), resulting in a significant reduction in the free Put content (Fig. 3a). The

activity of the ADC enzyme did not show significant differences between the first and fourth subculture cycles (Fig. 4), indicating that ODC predominantly functions in Put synthesis in this species.

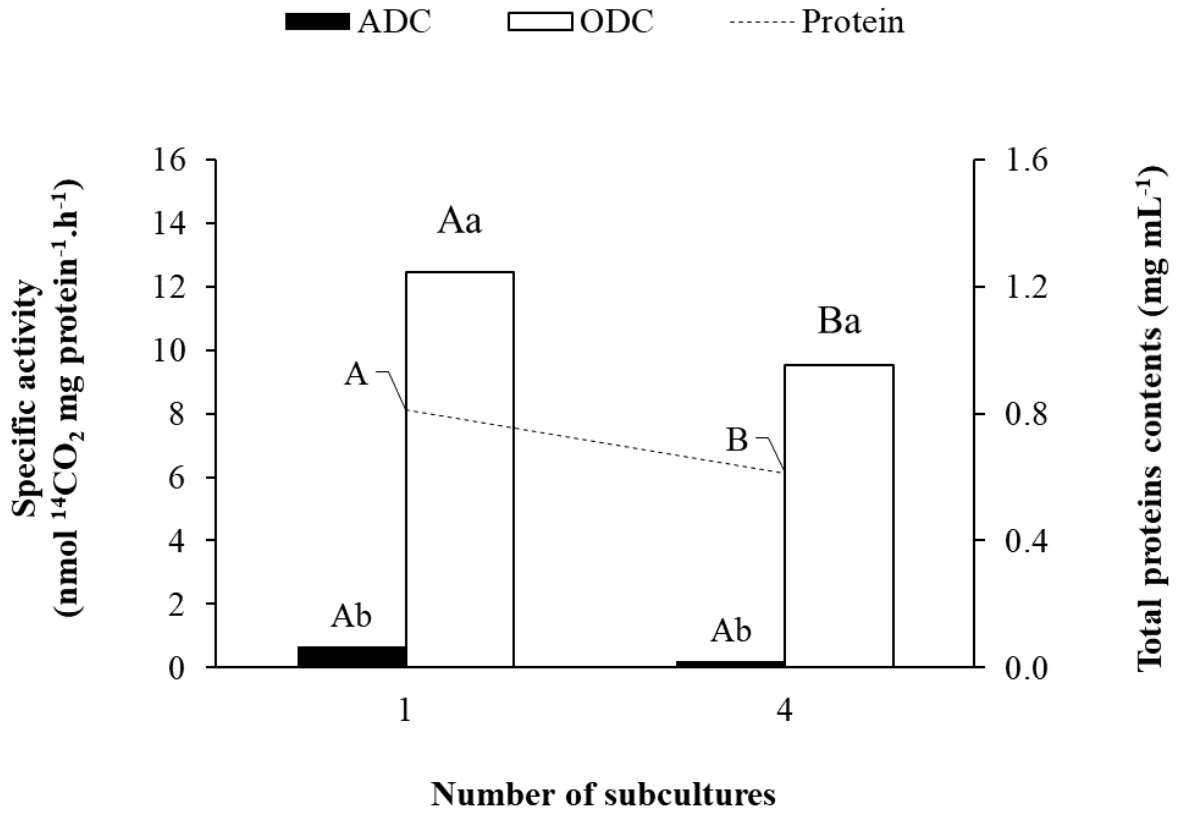

Fig. 4 - Enzymatic activities of arginine decarboxylase (ADC) and ornithine decarboxylase (ODC) in the shoots of *Cedrela fissilis* obtained from cotyledonary nodal segments in the first and fourth subculture cycles. Means followed by *different letters* show significant differences ($P < 0.05$) according to the SNK test. *Capital letters* show significant differences between subculture cycles (first and fourth) for each enzyme (ADC or ODC). *Lowercase letters* show significant differences comparing the activities of enzymes (ADC and ODC) in each subculture cycle (first or fourth). CV = coefficient of variation. (n =3; CV = 23.04%).

3.4. Effect of subculture cycles on the endogenous contents of IAA, ABA, OPDA, JA, JA-Ile, t-CA and SA

Similar to the ADC and ODC analysis, the endogenous contents of IAA, ABA, OPDA, JA, JA-Ile, t-CA and SA were measured in shoots obtained from cotyledonary nodal segments in the first and fourth subculture cycles (Figs. 5 and 6). Decreases in the endogenous contents of IAA (Fig. 5a), ABA (Fig. 5b) and OPDA (Fig. 5c) were observed in shoots from cotyledonary nodal segments from the fourth subculture cycle relative to those from the first, showing that successive subculture reduces the contents of these hormones, which are necessary for AR formation. In contrast, the endogenous contents of JA (Fig. 6a), JA-Ile (Fig. 6b), t-CA (Fig. 6c)

and SA (Fig. 6d) were significantly higher in shoots from cotyledonary nodal segments from the fourth subculture than in those from the first, showing that these hormones could be related to the reduction in the rooting potential under successive subculture in this species.

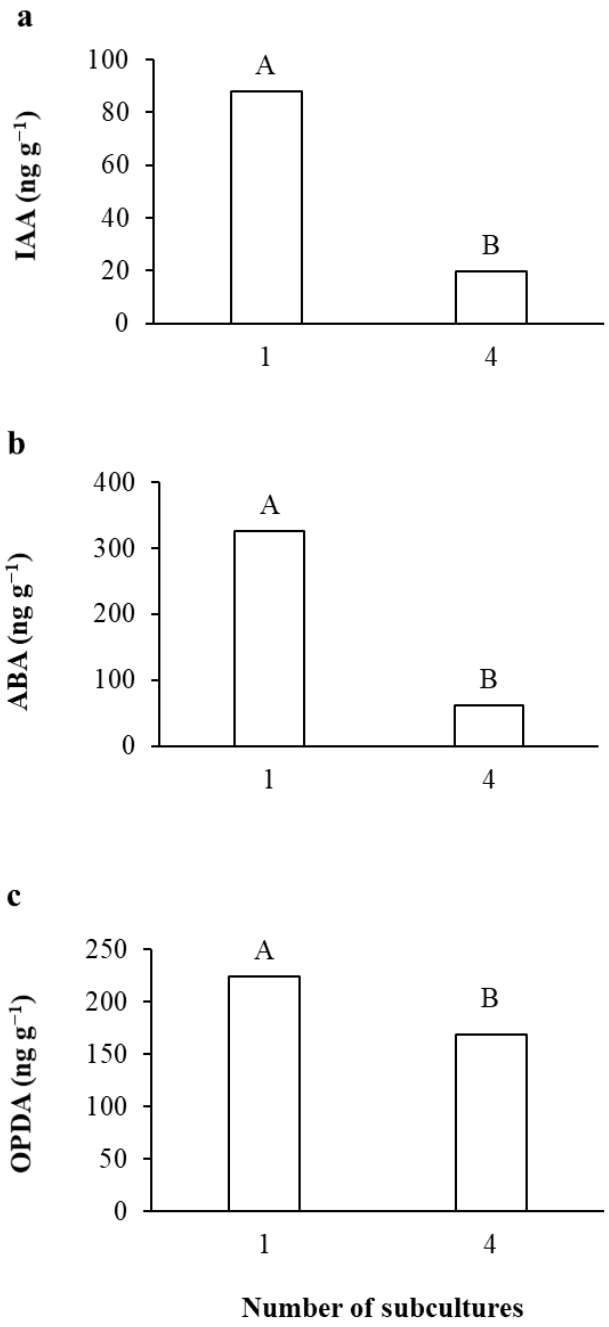

Fig. 5 - Endogenous contents (ng g^{-1}) of IAA (**a**), ABA (**b**) and OPDA (**c**) in the shoots of *Cedrela fissilis* obtained from cotyledonary nodal segments at the first and fourth subculture cycles. Means followed by *different letters* show significant differences ($P < 0.05$) according to the SNK. CV = coefficient of variation. (n =3; CV for IAA = 19.56%; CV for ABA = 4.33%; CV for OPDA = 18.78%).

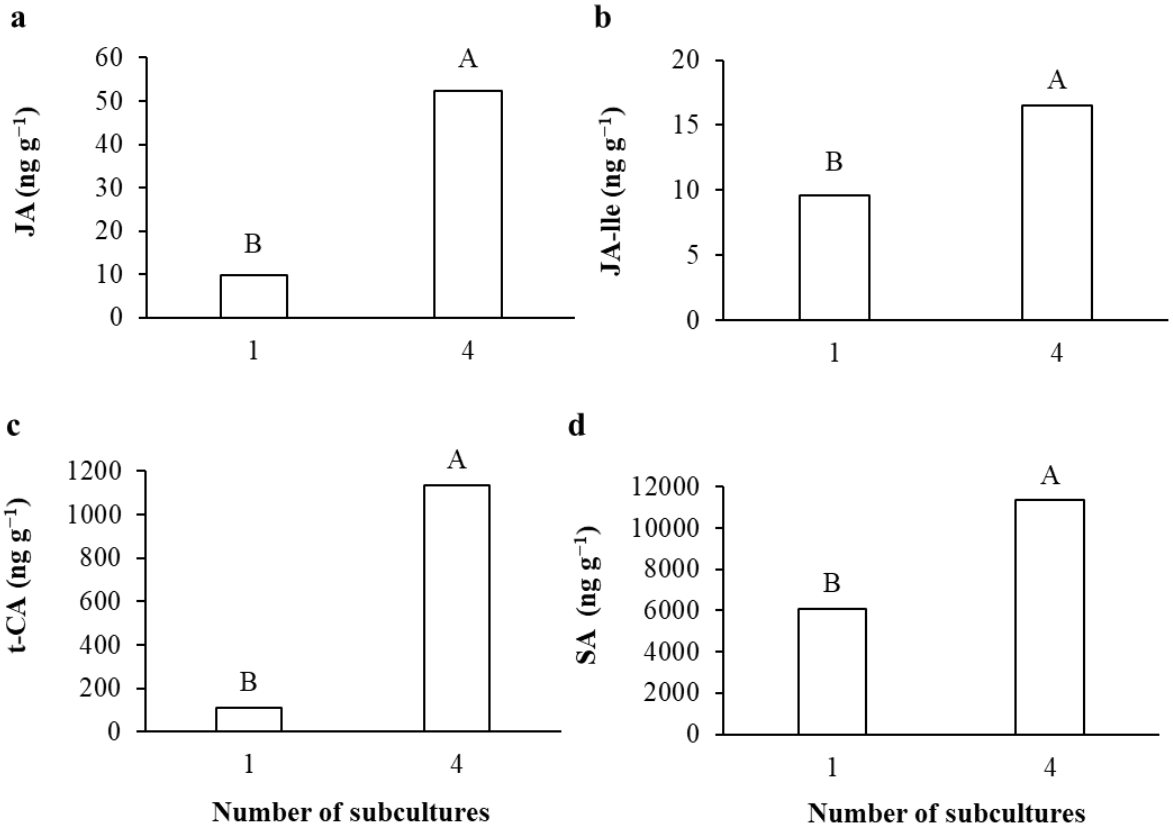

Fig. 6 - Endogenous contents (ng g^{-1}) of JA (**a**), JA-Ile (**b**), t-CA (**c**) and SA (**d**) in the shoots of *Cedrela fissilis* obtained from cotyledonary nodal segments in the first and fourth subculture cycles. Means followed by *different letters* show significant differences ($P < 0.05$) according to the SNK. CV = coefficient of variation. (n =3; CV for JA = 17.46%; CV for JA-Ile = 6.69%; CV for t-CA = 5.54%; CV for SA = 13.27%).

3.5. Effect of subculture cycles on the proteomic profile

Similar to the hormone analysis, proteomic analysis was performed using shoots obtained from cotyledonary nodal segments from the fourth subculture and the first subculture for comparison between subculture cycles. A total of 858 proteins were identified (Supplementary table). Among these proteins, 321 were identified as differentially accumulated proteins (DAPs) in terms of their relative abundance, including 86 up-and 218 down-accumulated proteins (Table 1). In addition, 15 proteins were unique to the shoots from the first subculture, and 2 proteins were unique to the shoots from the fourth subculture (Table 1).

The 321 DAPs were classified into five groups according to biological processes: photosynthesis, energy metabolism, nitrogen compound metabolic process, response to stimulus, and response to stress (Fig. 7). Among these proteins, some were of particular note due to their relevance to plant growth,

showing functions related to the maintenance of the photosystems, energy balance, and CO_2 fixation. In addition, we identified important antioxidant enzymes and proteins that play a role in PA and hormone metabolism (Table 1), possibly modulating the competence for AR formation in *C. fissilis*.

Some proteins related to photosynthesis were down-accumulated in shoots from the fourth subculture relative to those from the first subculture (Fig. 7). Among these proteins, several photosystem I (PSI) reaction center subunit proteins (Ce_fissilis.001790.1, Ce_fissilis.003800.3, Ce_fissilis.004692.1, Ce_fissilis.018999.1 and Ce_fissilis.015428.1) were down-accumulated in the shoots from the fourth subculture relative to those from the first (Table 1). Moreover, in PSII, the photosystem II 22 kDa protein, chloroplastic (CP22; Ce_fissilis.000704.1), the photosystem II CP43 reaction center protein (CP-43; Ce_fissilis.006118.1) and the photosystem II CP47 reaction center protein (CP-47; Ce_fissilis.001288.2) were down-accumulated in the shoots from the fourth subculture relative to those from the first subculture (Table 1).

In the energy metabolism biological process group (Fig. 7), proteins associated with carbon metabolism, such as glucose-6-phosphate isomerase, cytosolic (PGI; Ce_fissilis.016775.1), were down-accumulated in the shoots from the fourth subculture relative to those from the first subculture (Table 1). In addition, proteins associated with the assimilation of CO_2 in plants were down-accumulated in the shoots from the fourth subculture relative to those from the first (Table 1); these proteins included two ribulose bisphosphate carboxylase/oxygenase activase 1, chloroplastic, proteins (RuBisCO; Ce_fissilis.018513.1 and Ce_fissilis.018684.1), as well as the ribulose bisphosphate carboxylase small chain, chloroplastic (Ce_fissilis.000071.1), and the ribulose bisphosphate carboxylase small chain, chloroplastic (Ce_fissilis.000411.1). Furthermore, a large number of proteins related to carbohydrates were also down-accumulated in the shoots from the fourth subculture relative to those from the first subculture (Table 1), such as phosphoglycerate kinase 2, chloroplastic (Ce_fissilis.007229.1), phosphoglycerate kinase, cytosolic (Ce_fissilis.016667.1), phosphoglucomutase, cytoplasmic (Ce_fissilis.009200.1; Ce_fissilis.011316.1), a putative glucose-6-phosphate 1-epimerase (Ce_fissilis.000392.1), transketolase-2, chloroplastic

(Ce_fissilis.005337.1), and sedoheptulose-1,7-bisphosphatase, chloroplastic (Ce_fissilis.007537.1) (Table 1).

In the nitrogen compound metabolic process group (Fig. 7), proteins related to ammonium fixation, such as glutamine synthetase cytosolic isozyme 2 (Ce_fissilis.008404.1), glutamine synthetase leaf isozyme, chloroplastic (Ce_fissilis.008156.1), and glutamate dehydrogenase 1 (GDH; Ce_fissilis.009844.1), were down-accumulated in shoots from the fourth subculture relative to those from the first subculture (Table 1). Furthermore, methionine synthetase and 5-methyltetrahydropteroyltriglutamate-homocysteine methyltransferase (MET; Ce_fissilis.001903.1 and Ce_fissilis.012853.1), involved in the production of methionine, a precursor of Spd and Spm, were down-accumulated in the shoots from the fourth subculture relative to those from the first (Table 1).

Among the proteins related to stress responses (Fig. 7), some important antioxidant enzymes were identified, such as L-ascorbate peroxidase, cytosolic (APX; Ce_fissilis.017763.1), monodehydroascorbate reductase (MDHAR; Ce_fissilis.000462.1) and 2-Cys peroxiredoxin BAS1-like, chloroplastic (Prxs 2-Cys; Ce_fissilis.010608.1), which were down-accumulated in the shoots from the fourth subculture (when AR formation was reduced) relative to those from the first subculture (Table 1). In addition, some wounding-induced proteins were up-accumulated in the shoots from the fourth subculture relative to those from the first and were related to the responses to stimuli (Fig. 7); these proteins included caffeoyl-CoA O-methyltransferase 1 (CCoAOMT; Ce_fissilis.006261.1) and two peroxidase 4 proteins (POX4; Ce_fissilis.008801.1 and Ce_fissilis.015659.1) (Table 1).

Additionally, proteins related to plant hormones were identified and could be related to the reduction in AR formation observed in the fourth subculture relative to the first. Among these proteins, cysteine-rich repeat secretory protein 38 (CRRSP-38; Ce_fissilis.014551.1 and Ce_fissilis.006103.1), which is involved in the response to ABA, was up-accumulated in shoots from the fourth subculture relative to those from the first (Table 1). Additionally, two isoforms of auxin-binding protein ABP19a related to auxin-activated signaling pathway, the auxin-binding protein ABP19a (Ce_fissilis.009318.1 and Ce_fissilis.010810.1) were down-accumulated in shoots from the fourth subculture relative to those from the first, and the reduction

in accumulation could be related to the reduction in the potential for AR formation in this species (Table 1). Allene oxide cyclase, chloroplastic (AOC; Ce_fissilis.001385.1) protein, related to JA biosynthesis, was identified as a protein unique to shoots from the fourth subculture (Table 1) and could be involved in the reduction in AR formation observed in this species.

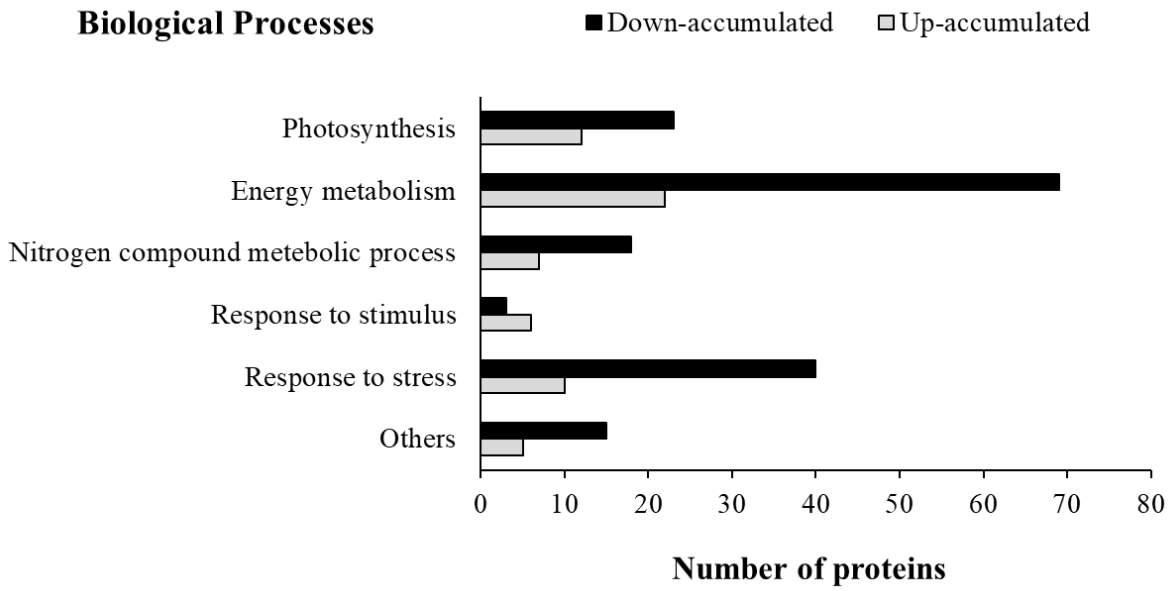

Fig. 7 - Functional classification of the biological processes of differentially accumulated proteins identified in the shoots of *Cedrela fissilis* obtained from cotyledonary nodal segments from the first and fourth subculture cycles.

Table 1. Differentially abundant proteins identified of *Cedrela fissilis* comparing between shoots from the fourth subculture (S4) with shoots from the first subculture (S1).

Accession	Reported peptide	Max score	Description	Differential accumulation S4/S1
			Photosynthesis	
Ce_fissilis.001790.1	12	35644.27	Photosystem I reaction center subunit II, chloroplastic	DOWN
Ce_fissilis.003800.3	6	7346.311	Photosystem I reaction center subunit III, chloroplastic	DOWN
Ce_fissilis.004692.1	4	4473.067	Photosystem I reaction center subunit V, chloroplastic	DOWN
Ce_fissilis.018999.1	3	9799.956	Photosystem I reaction center subunit VI-2, chloroplastic	DOWN
Ce_fissilis.015428.1	4	2993.098	Photosystem I reaction center subunit XI, chloroplastic	DOWN
Ce_fissilis.000704.1	9	5838.552	Photosystem II 22 kDa protein, chloroplastic	DOWN
Ce_fissilis.006118.1	11	7067.999	Photosystem II CP43 reaction center protein	DOWN
Ce_fissilis.001288.2	8	1987.76	Photosystem II CP47 reaction center protein	DOWN
			Energy metabolism	
Ce_fissilis.016775.1	6	1936.058	Glucose-6-phosphate isomerase, cytosolic	DOWN
Ce_fissilis.018513.1	24	25107.98	Ribulose bisphosphate carboxylase/oxygenase activase 1, chloroplastic	DOWN
Ce_fissilis.018684.1	35	41411.96	Ribulose bisphosphate carboxylase/oxygenase activase 1, chloroplastic	DOWN
Ce_fissilis.011316.1	8	1430.092	Phosphoglucomutase, chloroplastic	DOWN
Ce_fissilis.000071.1	10	17167.75	Ribulose bisphosphate carboxylase small chain, chloroplastic	DOWN
Ce_fissilis.000411.1	11	13973.5	Ribulose bisphosphate carboxylase small chain, chloroplastic	DOWN
Ce_fissilis.016667.1	15	20359.93	Phosphoglycerate kinase, cytosolic	DOWN
Ce_fissilis.007229.1	22	35709.38	Phosphoglycerate kinase 2, chloroplastic	DOWN
Ce_fissilis.009200.1	25	5037.207	Phosphoglucomutase, cytoplasmic	DOWN
Ce_fissilis.000392.1	3	2229.426	Putative glucose-6-phosphate 1-epimerase	DOWN
Ce_fissilis.005337.1	15	3715.861	Transketolase-2, chloroplastic	DOWN
Ce_fissilis.007537.1	17	15298.04	Sedoheptulose-1,7-bisphosphatase, chloroplastic	DOWN
			Nitrogen compound metebolic process	
Ce_fissilis.008404.1	6	4275.998	Glutamine synthetase cytosolic isozyme 2	DOWN
Ce_fissilis.008156.1	13	13830.39	Glutamine synthetase leaf isozyme, chloroplastic	DOWN
Ce_fissilis.009844.1	9	2258.954	Glutamate dehydrogenase 1	DOWN
Ce_fissilis.001903.1	24	10632.16	5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase 1	DOWN
Ce_fissilis.012853.1	12	3968.484	5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase	DOWN
			Response to stress	
Ce_fissilis.017763.1	13	13161.51	L-ascorbate peroxidase, cytosolic	DOWN
Ce_fissilis.000462.1	17	7484.855	Monodehydroascorbate reductase, chloroplastic/mitochondrial	DOWN
Ce_fissilis.010608.1	13	17993.16	2-Cys peroxiredoxin BAS1-like, chloroplastic	DOWN
			Auxin-activated signaling pathway	
Ce_fissilis.009318.1	6	17822.08	Auxin-binding protein ABP19a	DOWN
Ce_fissilis.010810.1	5	7272.733	Auxin-binding protein ABP19a	DOWN
			Response to stimulus	
Ce_fissilis.006261.1	12	5783.515	Caffeoyl-CoA O-methyltransferase 1	UP
Ce_fissilis.008801.1	15	18668.3	Peroxidase 4	UP
Ce_fissilis.015659.1	13	10832.72	Peroxidase 4	UP
			Response to abscisic acid	
Ce_fissilis.014551.1	9	7215.392	Cysteine-rich repeat secretory protein 38	UP
Ce_fissilis.006103.1	5	1806.976	Cysteine-rich repeat secretory protein 38	UP
			Jasmonic acid biosynthetic	
Ce_fissilis.001385.1	2	573.7998	Allene oxide cyclase, chloroplastic	Unique S4

Proteins were deemed up-accumulated if the $\log_2$ value of the fold change (FC) was greater than 0.6 and down-accumulated if the $\log_2$ value of the FC was less than -0.6, as determined by Student's t-test (two-tailed; $P < 0.05$).

4. DISCUSSION

The number of subculture cycles is an important factor in increasing the production of plantlets during *in vitro* propagation (Juncker and Favre, 1989; Rocha et al., 2007; Pastelín Solano et al., 2019). The number of subculture cycles was shown to affect *ex vitro* rooting in *C. fissilis*, resulting in significant decreases in root induction, root numbers and lengths, and resulting plantlet survival rates (Figs. 1 and 2). Similarly, the number of subculture cycles has been associated with a reduction in the regeneration capacity during *in vitro* propagation in some tree species. In *Cabralea canjerana* and *Tectona grandis*, the maximum average numbers of shoots per explant were obtained in the first and second subculture cycles (1.6 and 1.7 shoots per explant, respectively), and the multiplication rate then decreased in the following subculture cycles, directly affecting the clonal propagation of these species (Rocha et al., 2007; Raposo et al., 2010). In addition, the increase in the number of subculture cycles affected the length of shoots (~ 0.5 cm in the fourth subculture) and, consequently, the development of ARs in *C. fissilis* (Figs. 1 and 2). Similar results were observed in *Plathymenia reticulata*, in which a reduction in rooting was observed in response to a reduced length of shoots in the fourth subculture (0.66 cm), resulting in inadequate conditions for AR induction and development (Moura et al., 2012). However, little is known about the factors controlling the AR formation potential in shoots under successive subculture cycles. It has been established that wounding due to the excision of explants during successive subculture cycles can trigger stress in the plant and increase tissue differentiation, which induces the physiological aging of tissues (Leakey and Storeton-West, 1992; Iwase et al., 2015; Ikeuchi et al., 2016). A study comparing cuttings of different ages from *Pisum sativum* showed that older cuttings were slower to respond to wounding than younger cuttings and showed reduced AR induction (Rasmussen et al., 2014). Moreover, the injuries caused by cutting can lead to stress-induced reprogramming of shoot cell fate and metabolic readjustment, which is one of the factors related to hormonal variation (Da Costa et al., 2013). In this context, wounding associated an increase in the number of subculture cycles in *C. fissilis* may affect the hormonal contents of shoots, reducing the potential for AR development.

The plant hormone auxin is important for the development of ARs on stem cuttings, and an appropriate hormonal balance of the auxin/cytokinin ratio is necessary to reprogram tissues to stimulate the cell division necessary for AR development (Gutierrez et al., 2012; Legué et al., 2014). The reduction in rooting with increasing subculture cycles was associated with a reduction in endogenous IAA contents in the shoots of *C. fissilis* (Fig. 5a). Due to the reduction in the IAA content with the increase in the number of subculture cycles and its relationship with AR formation, we expected to identify differentially accumulated proteins related to IAA signaling. The reduction in the accumulation of two isoforms of the auxin-binding protein ABP19a (Ce_fissilis.009318.1 and Ce_fissilis.010810.1) observed in shoots from the fourth subculture cycle relative to those from the first (Table 1) could be related to the reduction in IAA content observed in *C. fissilis*. It has been shown that ABP19a protein up-accumulation during the induction phase in *E. grandis* is associated with the auxin-induced signaling pathway for AR development in this easy-to-root species (De Almeida et al., 2020). Considering that ABP1 and ABP19a are in the same family, the ABP19a protein probably acts as an auxin-binding molecule similar to the well-known protein ABP1, modulating quick responses to auxin (Tromas et al., 2009). In this context, we suggest that the decrease in the accumulation of auxin-binding protein ABP19a in shoots from the fourth subculture could induce a decrease in the IAA content and may be linked to the reduced AR formation potential observed in *C. fissilis* in this subculture cycle.

In addition, IAA has been indicated to engage in crosstalk with other plant hormones, such as ABA (Albacete et al., 2008), JA (Gutierrez et al., 2012) and SA (Zhang et al., 2007). Similar to IAA, the content of endogenous ABA was higher in the shoots of *C. fissilis* from the first subculture than in those from the fourth subculture (Fig. 5b). While ABA is known to play a role in the maturation and dormancy of seeds, little is know about its involvement in rooting. It has been shown that crosstalk between IAA and ABA during abiotic stress conditions plays an important role in improving the growth of roots in *Solanum lycopersicum* plants cultivated under high salinity, when increased IAA and ABA contents are observed (Albacete et al., 2008). In this context, our results suggest a possible interaction between IAA and ABA in AR development from the shoots of *C. fissilis* under successive subculture cycles. Moreover, the endogenous contents of JA (Fig. 6a) and JA-Ile (Fig. 6b), the active conjugated form of

JA, were higher in shoots from the fourth subculture relative to those from the first, which could be related to the lower AR development observed. In *Arabidopsis thaliana*, crosstalk between IAA and JA has been observed to play an important role in the regulation of AR formation in etiolated hypocotyls, in which IAA controls AR initiation by negatively regulating JA signaling (Gutierrez et al., 2012). In *Petunia hybrida*, the excision of cuttings leads to a fast, transient increase in endogenous JA, showing the involvement of this hormone in the wound response during AR formation (Ahkami et al., 2009). Through proteomic analysis, we identified one of the main enzymes related to JA biosynthesis, allene oxide cyclase, chloroplastic (AOC; Ce_fissilis.001385.1), which was unique to the shoots from the fourth subculture (Table 1). This protein gives rise to OPDA, the first biologically active compound in the JA pathway (Schaller et al., 2008). The decreased OPDA content in shoots from the fourth subculture (Fig. 5c) could be related to the increases in JA and JA-Ile contents (Figs. 6a and 6b). Studies have shown that OPDA plays a role in wounding response-related signaling that is independent of JA (Stintzi et al., 2001; Taki et al., 2005), indicating a positive feedback response between OPDA and JA (Stenzel et al., 2003). Our results clearly showed an increase in the endogenous content of JA in shoots from the fourth subculture relative to those from the first subculture, and the presence of the AOC protein could play an essential role in the production of JA induced by the increase in subculture cycles via a reduction in the OPDA content (Figs. 5c, d and e). In this context, we suggest that jasmonates (OPDA, JA and JA-Ile) may negatively regulate the rooting of *C. fissilis* shoots, which could be linked to the wounding response resulting from successive subculture cycles.

In addition, studies have shown that SA could play a role in AR development. The AR formation potential of two *A. thaliana* mutants deficient in SA biosynthesis (*eds5-1* and *eds5-2*) was found to be lower than that of the wild type, showing that SA is a positive regulator of adventitious rooting (Gutierrez et al., 2012). Similar results were observed in *Phaseolus radiatus,* in which hypocotyl cuttings showed a significant improvement in AR formation after exogenous SA treatments (Yang et al., 2013). However, SA was shown to inhibit IAA-induced AR formation in the shoots of *Malus* Jork 9 during the first few days posttreatment, possibly as a result of SA-enhanced IAA decarboxylation (De Klerk et al., 2011). Similarly, we observed lower IAA and higher SA contents in the shoots of *C. fissilis* in the fourth subculture (Figs. 5a and 6d). It is

possible that SA enhances IAA decarboxylation due to the higher accumulation of SA in the shoots of *C. fissilis* under successive subculture cycles. The increase in SA content in shoots from the fourth subculture may be linked to the higher content of t-CA, one of the first compounds in the phenylpropanoid pathway and a precursor of SA (Sendon et al., 2011). In our work, the lowest t-CA and SA contents in shoots were identified in the first subculture cycle (Figs. 6c and 6d), when greater rooting was observed, while the higher contents of these compounds in the fourth subculture cycle were related to a reduction in the rooting potential of *C. fissilis* shoots (Fig. 1).

Changes in the endogenous contents of free PAs have been shown to be determinants of AR induction in *in vitro*-propagated shoots from *Juglans regia* (Heloir et al., 1996). Recently, cuttings of *Taxus chinensis* var. mairei with an increased potential for AR formation were found to show a higher free Put content in the basal stem portion of rooted cuttings as well as a higher IAA content relative to cuttings with a low rate of AR formation (Fei and Tang, 2018). In our work, the number of subculture cycles decreased the AR induction potential and the root length and number per shoot as well as the plantlet survival rate (Fig. 1), which could be related to the decreases in the endogenous contents of free Put (Fig. 3a), Spd (Fig. 3c) and total free PAs (Fig. 3d). Studies have shown that Put stimulates the cell division and elongation of shoots (Kuznetsov et al., 2002; Aragão et al., 2017) since Put can induce the G1/S to G2/M cell cycle phase transition (Weiger and Hermann, 2014). Thus, the reduction in endogenous free Put contents could be directly associated with the reduction in *ex vitro* rooting in the shoots of *C. fissilis*, suggesting that Put plays an important role in this process. In addition, the significantly higher activity of ODC relative to the ADC enzyme (Fig. 4) suggests that ODC is the main enzyme affecting Put biosynthesis during AR induction in *C. fissilis* shoots, an observation that is made in this species for the first time to our knowledge. It has been reported that the ODC pathway is preferably used for Put biosynthesis during the growth of embryogenic cell lines from *A. angustifolia* (De Oliveira et al., 2017). During the development of *Lycopersicon esculentum*, the localization of ODC and ADC mRNAs in shoots and roots indicates that both pathways show cell-type specialization, with ODC expression specifically localizing to mitotically active cells (e.g., meristem–procambium), while ADC mRNA localizes to differentiated and elongating tissues in both shoots and roots (Acosta et al., 2005). The differential subcellular localization of ODC observed in mitotically active cells of *L. esculentum* may

indicate a specific feature of the Put biosynthetic pathway in the growth and development of *C. fissilis* roots.

In addition to the observed effects on hormones, the increase in the number of *in vitro* subculture cycles affected the abundance of proteins in the shoots of *C. fissilis* (Table 1). The reduction in the AR regeneration capacity with increasing subculture cycles (Figs. 1 and 2) could be related to the down-accumulation of proteins in the shoots of *C. fissilis* from the fourth subculture relative to those from the first (Fig. 7). These decreased proteins included several proteins related to PSI and PSII complexes (Table 1). These proteins are associated with photosynthesis, which plays a fundamental role in energy transduction by trapping light energy and converting it into biochemical energy (Amunts and Nelson, 2008). The decreases in accumulation of these proteins may affect current net photosynthesis and the initial carbohydrate reserves in the leaves of shoots, which have been shown to be important determinants of high AR formation in the stem base of leafy cuttings (Druege et al., 2019).

During rooting, high inputs of energy and carbon skeletons are necessary to support cell division, new root meristem establishment and AR formation (Ahkami et al., 2009; Da Costa et al., 2013). Proteins involved in carbon metabolism have been previously related to adventitious rooting in *A. thaliana* (Sorin et al., 2006), *Chrysanthemum morifolium* (Liu et al., 2013) and *Vigna radiata* seedlings (Li et al., 2017). Among the identified proteins, PGI (Ce_fissilis.016775.1) was down-accumulated in shoots from the fourth subculture relative to those from the first (Table 1), suggesting that this protein is necessary for rooting in *C. fissilis*. Higher accumulation of the PGI protein during rooting has been observed in *E. grandis*, an easy-to-root species, than in *E. globulus,* a difficult-to-root species, suggesting that this protein is a positive regulator of AR formation (De Almeida et al., 2020). These studies provided evidence that this protein may play a positive role in the development of roots in *C. fissilis*, indicating the decrease in its accumulation could be related to the reduction in the *ex vitro* rooting of shoots observed in the fourth subculture. Moreover, some proteins related to carbohydrate metabolism (Ce_fissilis.007229.1; Ce_fissilis.009200.1; Ce_fissilis.000392.1; Ce_fissilis.005337.1 and Ce_fissilis.007537.1) were down-accumulated in shoots from the fourth subculture relative to those from the first (Table 1). These proteins are involved in the Calvin cycle and carbohydrate metabolic processes, such as the synthesis of starch and sucrose in

plants (Malinova et al., 2014). Studies assessing the effects on carbohydrate dynamics on rooting in two *Eucalyptus* species with differences in recalcitrance pointed to a key role of a higher proportion of carbohydrate allocation at the base of shoot cuttings, increasing energy and carbon availability for new AR formation and therefore contributing to overcoming rooting recalcitrance (Ruedell et al., 2013).

Nitrogen is a key element in plant growth, nutrition and reproduction and an essential building block of nucleic acids and proteins (Stitt and Schulze, 1994; Bernard and Habash, 2009), and glutamine synthetase (GS) plays a major role in fixing ammonium to produce the amino acid glutamine (Bernard and Habash, 2009). Two GS proteins (Ce_fissilis.008404.1 and Ce_fissilis.008156.1) showed lower accumulation in the shoots of *C. fissilis* from the fourth subculture relative to those from the first (Table 1), suggesting the relevance of this protein to adventitious rooting in this species. Comparative proteomic analyses conducted during AR formation in a rooting-recalcitrant species (*E. globulus*) and an easily rooting species (*E. grandis*) showed lower accumulation of GS in *E. globulus*, which was related to lower AR formation (Corrêa et al., 2005). Similarly, the reduced accumulation of GS in *C. fissilis* shoots in the fourth subculture could be related to the lower *ex vitro* rooting potential observed. In addition, the down-accumulation of 5-methyltetrahydropteroyltriglutamate-homocysteine methyltransferase (MET; Ce_fissilis.001903.1 and Ce_fissilis.012853.1) proteins in shoots from the fourth subculture relative to those from the first (Table 1) could be related to reduced rooting. MET is a critical enzyme that catalyzes the transfer of a methyl group leading to the production of methionine, which may be the substrate for PA biosynthesis (Garras et al., 1991). Our results revealed the highest endogenous contents of total free PAs and free Spm (Fig. 3) in the first subculture. Thus, we suggest a relationship between the MET protein and methionine metabolism that may influence the biosynthesis of free Spm from methionine, thus conferring a higher capacity for rooting in the shoots of *C. fissilis* during the first subculture.

Wounding during the process of shoot multiplication can be a trigger for cell division (Sangwan et al., 1992). Our results showed that some wound-related proteins were up-accumulated in the shoots from the fourth subculture relative to those from the first subculture (Table 1; Fig. 7). Similar results were found for CCoAOMT (Ce_fissilis.006261.1), which plays a role in the synthesis of feruloylated polysaccharides, producing compounds such as monolignols, the building units of

lignin, and other compounds implicated in plant development and interactions with the environment (Pinçon et al., 2001). An increase in the accumulation of lignin inhibits the growth of *A. thaliana* (Zhou et al., 2009). Thus, the increase in accumulation of CCoAOMT observed in shoots from the fourth subculture (Table 1) could be linked to increased synthesis of feruloylated polysaccharides and lignin, which are required for defense mechanisms and adaptive responses to wounding but may reduce the AR formation potential of *C. fissilis* shoots.

In addition, plant cells can detect mechanical disturbances and initiate a defense reaction in response (Muday and Brown-Harding, 2018). Other wound-induced proteins, such as POX, have been shown to exhibit increases in activity or mRNA levels upon mechanical wounding in several plants, such as *Oryza sativa* (Hiraga et al., 2000; Ito et al., 2000), *Nicotiana tabacum* (Hiraga et al., 2000) and *Ipomoea batatas* (Kim et al., 1999). Two POXs were found to accumulate in the shoots from the fourth subculture relative to those from the first (Ce_fissilis.008801.1 and Ce_fissilis.015659.1) (Table 1). Adventitious rooting is marked by lower POX activities during AR induction and initiation in microcuttings of *Bacopa monnieri* (Goel et al., 2018), while no apparent correlation between the activity of POX and the ability of the cuttings to form roots has been found in *Populus nigra*, *P. alba* or *P. tremula* (Güneş, 2000). We suggest that up-accumulation of POX proteins may be related to wounding induction due to increasing subculture cycles and may activate self-defense systems to restore damaged tissues, resulting in a reduced rooting potential in *C. fissilis*.

Due to the decrease in the endogenous content of ABA and its role in activating the expression of genes encoding antioxidant enzymes (Gomez-Cadenas et al., 2015), we expected to observe a change in the abundance of proteins related to the antioxidant system. The association of proteins related to hydrogen peroxide (H_2O_2) production with morphogenic processes is well established (Libik-Konieczny et al., 2012; Ghosh and Pal, 2013). Among the proteins related to the stress response (Fig. 7), some antioxidant enzymes, such as APX (Ce_fissilis.017763.1) and MDHAR (Ce_fissilis.000462.1), showed increased accumulation in shoots with increasing subculture cycles, with higher accumulation in the fourth subculture than in the first (Table 1). These enzymes play a crucial role during organ regeneration in plants (Ghosh and Pal, 2013), in addition to being important enzymes related to stress responses, such as wounding responses (Hiraga et al., 2001). In addition, the Prxs 2-

Cys protein (Ce_fissilis.010608.1) is part of the 2-Cys peroxyredoxin family of enzymes, which are widely distributed among all organisms (Rouhier and Jacquot, 2005). Transgenic antisense *A. thaliana* plants with reduced levels of Prx 2-Cys show developmental retardation and transient damage to the photosynthetic membrane, suggesting that Prxs 2-Cys is important for seedling development (Baier and Dietz, 1999). In our study, the down-accumulation of Prxs 2-Cys in the shoots from the fourth subculture relative to those from the first subculture (Table 1) indicates an association between the higher accumulation of this protein and the higher AR formation potential of shoots from the first subculture.

Two isoforms of cysteine-rich repeat secretory protein 38 (CRRSP-38; Ce_fissilis.014551.1 and Ce_fissilis.006103.1) exhibited higher accumulation in shoots from the fourth subculture than in those from the first. These proteins belong to the large family of CRRSPs (Chen, 2001) and are related to responses to ABA and JA in *A. thaliana* and *O. sativa* (Xin et al., 2005; Jiang et al., 2007), although CRRSP-38 is poorly characterized. In *Populus deltoides*, the phosphorylated protein CRRSP-38 has been detected in the leaf-stem-root apoplast and is related to cell wall and carbohydrate metabolism (Pechanova et al., 2010). It is possible that the increased accumulation of these proteins associated with an increased number of subculture cycles could be related to the reduced AR development potential of the shoots of *C. fissilis.*

Taken together, the data support an initial working model of the roles of key proteins and hormonal alterations in the regulation of rooting competence (Fig. 8). This work is the first to report the subculture effect on AR development competence in *C. fissilis* shoots and its relationship with endogenous hormones, PA contents and differentially accumulating proteins.

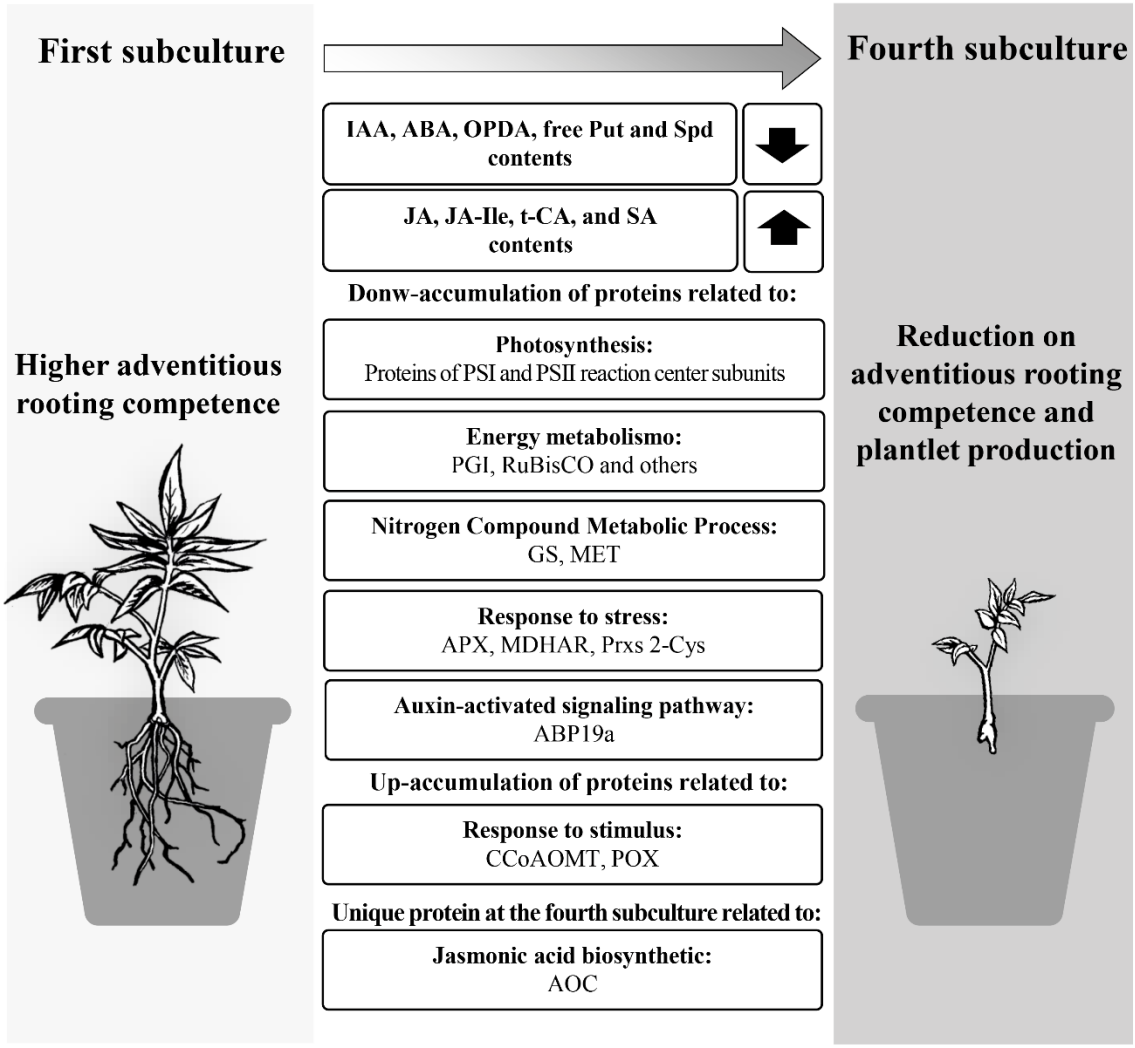

Fig. 8 - Proposed working model representing the positive and negative regulators of the rooting potential based on comparisons of differentially accumulated proteins and plant hormones in shoots of *Cedrela fissilis* from the first and fourth subculture cycles. A loss of the energy transduction capacity for photosynthesis and the maintenance of PSII results from the decreased abundance of essential proteins for electron transfer and the removal of damaged PSII proteins. In turn, these proteins influence the capacity for biochemical energy conversion necessary for energy metabolism, triggering imbalances in resource accumulation and distribution during rooting, influenced by the decreased accumulation of essential proteins involved in processes such as the Calvin–Benson cycle and CO_2 fixation and of PGI and RuBisCO, among others. The increased accumulation of key proteins in nitrogen assimilation and mobilization could be related to the modulation of PA signaling pathways (mainly Put and Spm). Moreover, an increasing number of subculture cycles increased the accumulation of wound-related proteins in the fourth subculture, as observed for CCoAOMT and some peroxidase isoforms involved in adaptive responses to wounding. On the other hand, essential antioxidant enzymes for H_2O_2 detoxification, such as APX, MDHAR and Prxs 2-Cys proteins showed greater accumulation in shoots from the first subculture, and they could be associated with the low rooting potential of shoots in the fourth subculture cycle. Endogenous IAA, ABA, and OPDA contents decreased, while the endogenous contents of JA, JA-Ile, t-CA and SA increased in shoots from the first subculture relative to those from the fourth subculture cycle, showing a possible relationship of these homonyms with rooting competence. Some proteins related to these hormones were identified, as the auxin-binding protein ABP19a exhibited decreased accumulation in shoots from the fourth subculture, which might decrease the content of IAA. This IAA modulation may in turn influence CRRSP-38 and AOC, which are proteins associated with the auxin signaling pathway and responses to ABA and JA, respectively, suggesting that these proteins play a role in adventitious rooting competence in *C. fissilis*.

5. CONCLUSION

Increasing subculture cycles decreased the potential for AR development. The reduction in *ex vitro* rooting was associated with decreases in the endogenous contents of IAA, ABA, 12-oxo phytodienoic acid and free Put and Spm. On the other hand, the increases in JA, JA-Ile, t-CA and SA contents observed in shoots from the fourth subculture could negatively affect AR development. The AOC protein could increase JA accumulation in shoots from the fourth subculture, resulting in a reduction in the AR development potential during successive subculture cycles. Additionally, a decrease in the accumulation of the ABP19a protein could be linked to a decreased IAA content and a consequently reduced AR formation potential in shoots from the fourth subculture. In addition, the decreased accumulation of proteins related to maintenance and energy transduction in PSI and PSII and Calvin–Benson cycle, CO_2 fixation, nitrogen assimilation and metabolism pathways and antioxidant enzyme protein levels could be related to the reduced AR development potential in shoots from the fourth subculture relative to those from the first. The increased accumulation of wound-related proteins and some POX isoform proteins in shoots from the fourth subculture may be related to the lower AR formation potential relative to that of shoots from the first subculture. The ODC enzyme (rather than ADC) plays he predominant role in Put synthesis during adventitious rooting in *C. fissilis*. This work is the first to report the effect of subculture cycles on AR development potential in the shoots of *C. fissilis* and its crosstalk with endogenous plant hormones contents and the protein profile.

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5. RESUMO E CONCLUSÕES

No presente trabalho foram obtidas informações inéditas e de relevância para propagação *in vitro* de brotações e enraizamento *ex vitro* em *C. fissilis.* Quatro subcultivos foram realizados avaliando-se os parâmetros de crescimento das brotações juntamente com análises hormonais e proteômicas, visando identificar os efeitos da redução da capacidade de desenvolvimento de brotações *in vitro* e do enraizamento *ex vitro* com o aumento do número de subculturas.

No primeiro capítulo foi mostrado que os ciclos sucessivos de subculturas *in vitro* afetam significativamente o desenvolvimento de brotações *in vitro*. Verificou-se a redução dos parametros analizados, como a indução, o número e o comprimento das brotações com o aumento do número de subculturas. A partir destes dados, foram elaboradas análises bioquímicas em explantes utilizados para os subcultivos para elucidação dos fatores associados à competência sob influência das subculturas *in vitro*. Foram mostradas alterações hormonais e metabólicas de PAs nos explantes, as quais estão relacionadas com a redução da competência do desenvolvimento *in vitro* das brotações. O conteúdo de Put e AIA foi mais elevado nos explantes da primeira subcultura comparativamente à quarta, e relevante para o maior desenvolvimento das brotações. Além disso, a atividade da ODC foi significativamente maior em explantes da primeira subcultura, e pode estar relacionada com o maior conteúdo de Put e, consequentemente, com o maior crescimento observado. Adicionalmente, a ODC parece atuar como precursor preferencial nesta espécie na via de biossíntese de Put. Por outro lado, houve

um aumento no conteúdo de ABA, OPDA, JA e SA nos explantes na quarta subcultura, sugerindo o envolvimento desses hormônios na redução do potencial de crescimento de brotações durante as subculturas *in vitro*. Esse é o primeiro trabalho a descrever a redução da competência do desenvolvimento de brotações sob ciclos sucessivos de subculturas *in vitro* em *C. fissilis* e seu envolvimento com PAs e hormônios.

A partir destas informações obtidas pode-se sugerir novos estudos a fim de compreender o complexo processo de competência no desenvolvimento de brotações *in vitro*, como a proteômica comparativa. Estudos neste sentido, podem permitir a identificação de proteinas que possam ser utilizadas como marcadores da competência ao desenvolvimento *in vitro* a partir do explantes. Estudos associados com alterações histológicas e ultraestruturais são fundamentais para entender como a subcultura afeta a organização estrutural das células e tecidos durante o desenvolvimento *in vitro* das brotações.

No segundo capítulo foi mostrado que o desenvolvimento *ex vitro* de raízes adventícias nas brotações foi afetado pelas subculturas, ocorrendo redução significativa na indução, número e comprimento das raízes com o aumento das subculturas. Os resultados demonstram diminuição no conteúdo endógeno de AIA, ABA, OPDA e PAs nas brotações da primeira comparativamente à quarta subcultura, demonstrando que estes compostos são importantes para o enraizamento das brotações. Por outro lado, os teores de JA, JA-Ile, t-CA e SA foram maiores nas brotações da quarta subcultura, comparativamente à primeira, podendo atuar negativamente no enraizamento. A análise proteômica mostrou que várias proteínas diminuíram seu acúmulo em brotações da primeira para a quarta subcultura, principalmente relacionadas às subunidades do centro de reação dos fotossistemas, metabolismo energético, assimilação de nitrogênio, via de PAs, proteínas que desempenham papéis nas respostas ao ABA e JA, sendo relevantes para o enraizamento de brotações. Em contraste, proteínas relacionadas à ferimentos mostraram um acúmulo em brotações na quarta subcultura, e podem estar relacionadas a diminuição no enraizamento de brotações. Este é o primeiro trabalho que mostra a relação do conteúdo hormonal e alterações proteômicas em brotações obtidas sob sucessivas subculturas e utilizadas para o enraizamento *ex vitro*. Os resultados deste trabalho apresentam dados importantes para o potencial de enraizamento *ex vitro* em nível hormonal e proteômico. Estes dados podem ajudar a melhorar a produção de mudas em larga escala para a espécie em estudo, além de ajudar nos programas de

reflorestamentos e conservação desta espécie ameaçada de extinção. Estudos futuros envolvendo análises histomorfológicas são importantes para visualizar possíveis alterações estruturais durante o desenvolvimento radicular adventício.

Este estudo agrega conhecimento científico-tecnológico relevante para os estudos em andamento no grupo de pesquisa com espécies arbóreas.

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APÊNDICE

Apêndice – Capítulo 2: *In vitro* subculture cycles affect rooting competence via changes in the proteomic profile and hormonal content in *Cedrela fissilis* Vell. (Meliaceae) shoots.

Supplementary table**:** Complete list of all identified proteins of *Cedrela fissilis* comparing between shoots from the fourth subculture (S4) with shoots from the first subculture (S1).

Accession	Reported Peptide	Max score	Description	Normalized Total Ion Count (Tic)						Average		t-Test	Log_2 of Fold Change	Differential accumulation
				S1-1	S1-2	S1-3	S4-1	S4-2	S4-3	S1	S4	S4/S1	S4/S1	S4/S1
Ce_fissilis.015866.1	15	35693.34	Secoisolariciresinol dehydrogenase	165756	192412	213760	355512	383882	298745	190643	346047	0.0056	0.86	UP
Ce_fissilis.002414.1	14	22731.44	Alcohol dehydrogenase 2	241541	247849	270286	412051	386681	407403	253225	402045	0.0002	0.67	UP
Ce_fissilis.006379.1	8	21868.53	Osmotin-like protein OSML13	136237	169990	203450	340624	230771	388953	169892	320116	0.0413	0.91	UP
Ce_fissilis.005206.1	12	19619.02	Membrane steroid-binding protein 1	100904	111676	125685	191989	242417	207211	112755	213872	0.0036	0.92	UP
Ce_fissilis.008801.1	15	18668.30	Peroxidase 4	129384	129478	139730	317326	336299	341352	132864	331659	0.0000	1.32	UP
Ce_fissilis.003326.2	14	17892.33	Polygalacturonase inhibitor	81202	91769	105479	445133	419515	359453	92817	408034	0.0003	2.14	UP
Ce_fissilis.011438.1	4	17104.35	Malate dehydrogenase, cytoplasmic	8115	8309	9009	36772	28960	25019	8477	30251	0.0033	1.84	UP
Ce_fissilis.002902.1	9	16551.78	Kunitz trypsin inhibitor 2	259679	250870	272762	456655	503247	495456	261104	485119	0.0001	0.89	UP
Ce_fissilis.009721.1	3	16392.30	Putative phosphatidylglycerol/phosphatidylinositol transfer protein DDB_G0282179	131439	78454	85136	144596	170011	164802	98343	159803	0.0287	0.70	UP
Ce_fissilis.012486.1	7	16058.08	Proteasome subunit beta type-1	92711	93687	95028	129400	183765	153226	93809	155464	0.0173	0.73	UP
Ce_fissilis.006148.1	7	15844.09	Glyceraldehyde-3-phosphate dehydrogenase, cytosolic	16317	15606	13086	41994	34160	31080	15003	35745	0.0036	1.25	UP
Ce_fissilis.002712.1	16	14444.26	Probable fructokinase-4	131706	119980	138033	215397	239822	220805	129906	225341	0.0005	0.79	UP
Ce_fissilis.003482.2	9	11591.75	Endochitinase	30353	36329	50473	200401	207793	219974	39051	209389	0.0000	2.42	UP
Ce_fissilis.013185.1	17	11114.49	Heat shock cognate 70 kDa protein	38631	30433	34303	107460	133249	117781	34456	119497	0.0004	1.79	UP
Ce_fissilis.015659.1	13	10832.72	Peroxidase 4	94814	87019	89751	286100	291085	294098	90528	290427	0.0000	1.68	UP
Ce_fissilis.005901.1	8	9765.91	Putative methyltransferase DDB_G0268948	37486	33004	39054	185625	199722	152521	36515	179289	0.0005	2.30	UP
Ce_fissilis.015956.1	9	9087.88	Chalcone--flavonone isomerase	36183	39884	47401	134696	161339	133317	41156	143117	0.0005	1.80	UP
Ce_fissilis.012362.1	5	9037.21	Epidermis-specific secreted glycoprotein EP1	107451	112274	117171	187482	188199	176176	112299	183952	0.0001	0.71	UP
Ce_fissilis.009901.1	12	8216.84	Proteasome subunit alpha type-4	71518	77136	80916	118212	151842	126351	76523	132135	0.0061	0.79	UP
Ce_fissilis.006915.1	7	8196.52	Probable NAD(P)H dehydrogenase (quinone) FQR1-like 1	53553	63240	61928	89141	104180	83218	59574	92180	0.0093	0.63	UP
Ce_fissilis.000745.2	12	7409.71	Alpha-galactosidase 1	33532	31093	34921	84628	96719	58888	33182	80078	0.0139	1.27	UP

Continuação

Ce_fissilis.014551.1	9	7215.39	Cysteine-rich repeat secretory protein 38	13096	12481	14847	82606	89148	86145	13475	85966	0.0000	2.67	UP
Ce_fissilis.012697.1	14	7211.35	Heat shock cognate 70 kDa protein 2	8585	7415	7188	25432	32976	29167	7729	29192	0.0006	1.92	UP
Ce_fissilis.003219.1	6	7080.16	Probable chalcone--flavonone isomerase 3	35850	37396	40266	101510	103736	86133	37837	97126	0.0005	1.36	UP
Ce_fissilis.007193.1	14	6846.00	Glucan endo-1,3-beta-glucosidase	48494	54249	65213	121849	117406	107107	55986	115454	0.0008	1.04	UP
Ce_fissilis.006770.1	14	6438.14	Enolase	11249	12102	11561	23920	19800	18848	11637	20856	0.0043	0.84	UP
Ce_fissilis.009238.1	20	6170.13	Pyruvate decarboxylase 1	87771	92745	91437	179714	225084	164499	90651	189766	0.0056	1.07	UP
Ce_fissilis.006261.1	12	5783.52	Caffeoyl-CoA O-methyltransferase 1	37661	46954	44002	116236	122500	87673	42872	108803	0.0040	1.34	UP
Ce_fissilis.008054.1	5	5386.71	NADP-dependent malic enzyme 4, chloroplastic	5840	5059	4042	113678	52934	58743	4980	75119	0.0223	3.91	UP
Ce_fissilis.012383.1	20	5146.70	Mitochondrial-processing peptidase subunit alpha	59166	71721	99066	117777	143738	112601	76651	124705	0.0342	0.70	UP
Ce_fissilis.000573.1	2	4912.92	Wound-induced protein WIN2	14185	8776	19423	51315	50369	73527	14128	58404	0.0056	2.05	UP
Ce_fissilis.008304.1	11	4840.92	Alpha-galactosidase 3	120787	135013	143930	209541	210001	190376	133243	203306	0.0017	0.61	UP
Ce_fissilis.013077.2	14	4711.28	Aspartic proteinase A1	95041	108886	116138	181086	172680	170013	106688	174593	0.0006	0.71	UP
Ce_fissilis.016449.1	2	4596.62	Ribulose-phosphate 3-epimerase, cytoplasmic isoform	43360	48508	55228	68378	97229	69710	49032	78439	0.0425	0.68	UP
Ce_fissilis.010325.1	10	4558.45	Berberine bridge enzyme-like 26	2515	27455	29507	100276	109884	106349	19826	105503	0.0007	2.41	UP
Ce_fissilis.015598.1	10	4484.10	Heat shock 70 kDa protein	5994	5464	5914	14172	18722	16298	5791	16397	0.0013	1.50	UP
Ce_fissilis.018028.1	3	4335.86	Cysteine proteinase inhibitor	9997	16908	25089	37732	31635	30672	17331	33346	0.0306	0.94	UP
Ce_fissilis.000421.1	10	4237.89	Pectinesterase 2	6754	7200	7905	115596	103129	121860	7286	113528	0.0000	3.96	UP
Ce_fissilis.013923.2	4	4226.45	Peroxidase 15	475	2707	5220	20061	21593	20591	2801	20748	0.0002	2.89	UP
Ce_fissilis.016439.2	5	3875.26	Stem-specific protein TSJT1	18701	23006	15382	109372	52882	107355	19030	89869	0.0191	2.24	UP
Ce_fissilis.011362.1	17	3568.74	UDP-glucose 6-dehydrogenase 1	49047	46503	46146	103791	115811	112130	47232	110577	0.0001	1.23	UP
Ce_fissilis.014796.1	4	3508.93	Succinate dehydrogenase subunit 5, mitochondrial	29667	30507	33096	46958	50765	46345	31090	48023	0.0006	0.63	UP
Ce_fissilis.017860.1	13	3465.71	Pyruvate decarboxylase 1	37513	42013	46363	81138	95716	83456	41963	86770	0.0010	1.05	UP
Ce_fissilis.010805.1	14	3315.25	Protein DNA-DAMAGE INDUCIBLE 1	58396	61303	57401	88944	113793	92817	59033	98518	0.0072	0.74	UP
Ce_fissilis.012147.1	5	3233.30	Nuclear transport factor 2B	43431	46866	43288	71299	75308	63228	44528	69945	0.0024	0.65	UP
Ce_fissilis.006369.1	6	3177.62	Cysteine protease RD19A	32616	29045	34965	48582	67241	51019	32208	55614	0.0185	0.79	UP
Ce_fissilis.018656.2	4	3107.01	Aspartate aminotransferase, cytoplasmic	40408	42872	47964	74413	79363	74221	43748	75999	0.0003	0.80	UP
Ce_fissilis.017631.1	8	2913.80	3-isopropylmalate dehydrogenase, chloroplastic	41028	46918	48794	63148	95419	70427	45580	76331	0.0376	0.74	UP
Ce_fissilis.002863.1	3	2754.27	Germin-like protein subfamily 1 member 13	7202	7252	9626	22945	27164	23268	8027	24459	0.0005	1.61	UP
Ce_fissilis.013176.1	6	2710.22	L-lactate dehydrogenase A	26707	28594	33452	52263	48152	55619	29584	52011	0.0016	0.81	UP
Ce_fissilis.008583.1	3	2601.22	Peroxidase 53	51274	52825	57973	186899	148421	120202	54024	151841	0.0073	1.49	UP

Continuação

Ce_fissilis.013981.1	7	2289.52	Mitochondrial outer membrane protein porin 2	17667	19527	29266	62671	54606	72280	22153	63186	0.0028	1.51	UP
Ce_fissilis.018551.1	7	2061.99	Ubiquitin receptor RAD23c	39561	44887	57464	73206	69529	79257	47304	73997	0.0114	0.65	UP
Ce_fissilis.017206.1	4	1915.62	Short-chain dehydrogenase reductase 3b	21879	23476	25482	48807	33223	41214	23612	41081	0.0194	0.80	UP
Ce_fissilis.014293.1	11	1899.97	Probable acetyl-CoA acetyltransferase, cytosolic 2	29906	31768	42121	76267	88111	64781	34598	76386	0.0057	1.14	UP
Ce_fissilis.006103.1	5	1806.98	Cysteine-rich repeat secretory protein 38	18569	13151	13753	50050	62017	38254	15158	50107	0.0078	1.72	UP
Ce_fissilis.018507.1	7	1780.72	Probable pectinesterase/pectinesterase inhibitor 41	20572	18511	19657	103120	87380	84256	19580	91585	0.0003	2.23	UP
Ce_fissilis.010676.1	8	1695.36	Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex 2, mitochondrial	50290	52198	53418	138513	117379	107038	51969	120977	0.0018	1.22	UP
Ce_fissilis.006464.1	3	1654.08	Cysteine proteinase RD21A	21462	24266	25321	87357	67210	52803	23683	69123	0.0108	1.55	UP
Ce_fissilis.014369.1	2	1617.27	NAC domain-containing protein 2	29656	31305	33467	45031	53506	58602	31476	52379	0.0070	0.73	UP
Ce_fissilis.018301.1	3	1507.09	Probable cysteine protease RD21C	23612	24386	25250	33667	40496	38371	24416	37511	0.0032	0.62	UP
Ce_fissilis.016610.3	3	1481.76	12-oxophytodienoate reductase 1	18714	21415	24742	51123	52859	49896	21624	51293	0.0001	1.25	UP
Ce_fissilis.012560.1	2	1448.94	Endochitinase EP3	13651	13797	15769	69579	40785	45190	14405	51851	0.0140	1.85	UP
Ce_fissilis.017212.1	2	1433.21	Pathogenesis-related protein 1	23592	12599	16112	40539	29505	56555	17434	42199	0.0435	1.28	UP
Ce_fissilis.014524.1	3	1389.76	Proliferating cell nuclear antigen	30525	34096	36507	47493	51750	56184	33709	51809	0.0041	0.62	UP
Ce_fissilis.004398.2	6	1316.02	Ubiquitin receptor RAD23c	18473	20443	25233	34124	31470	34086	21383	33227	0.0057	0.64	UP
Ce_fissilis.005821.1	8	1227.50	Cysteine synthase	11960	14308	14183	17613	22368	23799	13484	21260	0.0183	0.66	UP
Ce_fissilis.011695.1	2	1178.50	Acyl-coenzyme A oxidase 4, peroxisomal	19367	21936	20115	28228	32081	35464	20473	31924	0.0068	0.64	UP
Ce_fissilis.013421.1	5	1117.34	Probable inactive purple acid phosphatase 27	19538	20910	26595	41948	45635	47257	22348	44947	0.0011	1.01	UP
Ce_fissilis.003483.1	3	1099.69	Basic endochitinase A	6396	6656	10534	86107	54111	57179	7862	65799	0.0049	3.07	UP
Ce_fissilis.003624.1	2	1077.91	PITH domain-containing protein 1	6653	6356	7331	10082	11199	10524	6780	10602	0.0009	0.64	UP
Ce_fissilis.007644.1	3	1053.68	26S proteasome non-ATPase regulatory subunit 8 homolog A	12130	11813	14160	19439	26944	22783	12701	23055	0.0107	0.86	UP
Ce_fissilis.000459.1	6	984.02	Probable pectinesterase/pectinesterase inhibitor 40	29375	31972	33563	49593	44289	53099	31637	48994	0.0036	0.63	UP
Ce_fissilis.011010.1	8	972.37	Peroxidase 72	17127	13782	15346	39620	48759	46973	15418	45117	0.0006	1.55	UP
Ce_fissilis.016477.2	6	918.46	UBP1-associated protein 2C	22376	23077	27085	34841	41072	40812	24179	38909	0.0042	0.69	UP
Ce_fissilis.010412.1	3	892.61	Lactoylglutathione lyase	15238	16923	16163	40661	40119	41334	16108	40705	0.0000	1.34	UP
Ce_fissilis.018749.1	3	878.50	3-oxoacyl-[acyl-carrier-protein] synthase III, chloroplastic	9888	10290	10873	15094	19560	17663	10350	17439	0.0059	0.75	UP
Ce_fissilis.002037.1	3	853.31	NADPH-dependent aldehyde reductase-like protein, chloroplastic	24040	25455	25146	38879	42915	38869	24880	40221	0.0004	0.69	UP

Continuação

Ce_fissilis.002297.1	2	792.97	Prohibitin-3, mitochondrial	17382	17492	17561	35404	52654	41280	17478	43112	0.0072	1.30	UP
Ce_fissilis.010786.1	5	726.68	Glutathione hydrolase 3	9950	11731	9802	49522	56142	36513	10494	47393	0.0031	2.18	UP
Ce_fissilis.008123.1	3	641.53	calcium-binding EF hand family protein	13792	14014	16037	24592	23886	35972	14614	28150	0.0273	0.95	UP
Ce_fissilis.015744.1	3	591.79	Homoserine kinase	13366	13702	15673	26211	28993	18240	14247	24481	0.0362	0.78	UP
Ce_fissilis.002664.1	3	520.08	Hexokinase-2, chloroplastic	13732	14169	17082	109177	134656	94973	14994	112935	0.0011	2.91	UP
Ce_fissilis.000871.1	2	441.72	-	15736	13273	16526	24519	27215	27968	15178	26567	0.0014	0.81	UP
Ce_fissilis.002419.1	2	432.07	Probable glucan 1,3-beta-glucosidase A	11010	15089	18344	43308	39617	44582	14814	42502	0.0004	1.52	UP
Ce_fissilis.018570.1	2	425.34	Small nuclear ribonucleoprotein-associated protein B'	8636	11786	16560	18283	26712	27343	12327	24113	0.0339	0.97	UP
Ce_fissilis.015339.1	2	771.31	Cytochrome b561 and DOMON domain-containing protein At4g12980				37005	48103	45499	0	43535	-	-	Unique S4
Ce_fissilis.001385.1	2	573.80	Allene oxide cyclase, chloroplastic				134407	134613	134138	0	134386	-	-	Unique S4
Ce_fissilis.016967.1	15	48269.02	Beta carbonic anhydrase 1, chloroplastic	434545	407810	405120	96873	82561	74105	415825	84513	0.0000	-2.30	DOWN
Ce_fissilis.011041.1	35	41507.72	ATP synthase subunit beta, chloroplastic	613610	608995	604338	307952	395720	317207	608981	340293	0.0007	-0.84	DOWN
Ce_fissilis.018684.1	35	41411.96	Ribulose bisphosphate carboxylase/oxygenase activase 1, chloroplastic	792522	758981	729058	415377	438603	347621	760187	400534	0.0004	-0.92	DOWN
Ce_fissilis.007229.1	22	35709.38	Phosphoglycerate kinase 2, chloroplastic	369015	351631	334133	135457	166498	133491	351593	145149	0.0001	-1.28	DOWN
Ce_fissilis.001790.1	12	35644.27	Photosystem I reaction center subunit II, chloroplastic	273873	292725	275874	107678	127764	96508	280824	110650	0.0001	-1.34	DOWN
Ce_fissilis.012078.1	11	30421.37	Actin	20233	18585	21134	11283	15039	11232	19984	12518	0.0070	-0.67	DOWN
Ce_fissilis.007079.1	16	27533.34	Fructose-bisphosphate aldolase 2, chloroplastic	454531	445769	412333	190802	213676	202432	437544	202304	0.0001	-1.11	DOWN
Ce_fissilis.013959.1	12	25179.43	Tubulin beta-4 chain	62982	55187	59651	27688	37953	30633	59273	32091	0.0020	-0.89	DOWN
Ce_fissilis.018513.1	24	25107.98	Ribulose bisphosphate carboxylase/oxygenase activase 1, chloroplastic	85355	81743	78520	31034	33913	25938	81873	30295	0.0001	-1.43	DOWN
Ce_fissilis.013782.1	27	23575.88	Epidermis-specific secreted glycoprotein EP1	479547	460808	447968	257913	272391	252775	462774	261027	0.0000	-0.83	DOWN
Ce_fissilis.014748.1	10	22210.85	ADP-ribosylation factor 2	190097	224126	210958	133735	101486	88720	208394	107980	0.0038	-0.95	DOWN
Ce_fissilis.000008.2	6	21505.68	Chlorophyll a-b binding protein 8, chloroplastic	243258	253314	280171	147908	166578	129420	258914	147969	0.0020	-0.81	DOWN
Ce_fissilis.016769.1	5	20839.32	Chlorophyll a-b binding protein, chloroplastic	23160	21419	24622	10352	13184	11291	23067	11609	0.0008	-0.99	DOWN
Ce_fissilis.016667.1	15	20359.93	Phosphoglycerate kinase, cytosolic	171840	141528	118719	64582	58474	54082	144029	59046	0.0056	-1.29	DOWN
Ce_fissilis.016012.1	11	19872.50	Glyceraldehyde-3-phosphate dehydrogenase, cytosolic	104826	96415	80649	60861	52835	56793	93964	56830	0.0076	-0.73	DOWN
Ce_fissilis.015772.1	9	19632.06	Peroxiredoxin-2E, chloroplastic	243619	258097	277410	120101	140131	120957	259709	127063	0.0004	-1.03	DOWN
Ce_fissilis.010608.1	13	17993.16	2-Cys peroxiredoxin BAS1-like, chloroplastic	324392	332567	335857	237724	216284	179605	330939	211204	0.0023	-0.65	DOWN

Continuação

Ce_fissilis.009318.1	6	17822.08	Auxin-binding protein ABP19a	214138	314948	292660	129871	70266	71423	273915	90520	0.0073	-1.60	DOWN
Ce_fissilis.005917.1	7	17782.12	Epidermis-specific secreted glycoprotein EP1	247161	248452	243448	157720	151258	153686	246354	154221	0.0000	-0.68	DOWN
Ce_fissilis.015714.1	21	17657.50	Peroxisomal (S)-2-hydroxy-acid oxidase	219458	193472	206554	90104	84623	70825	206495	81851	0.0002	-1.34	DOWN
Ce_fissilis.000071.1	10	17167.75	Ribulose bisphosphate carboxylase small chain, chloroplastic	122663	114751	132978	45274	43758	61591	123464	50207	0.0007	-1.30	DOWN
Ce_fissilis.011301.1	15	16399.44	Chlorophyll a-b binding protein CP26, chloroplastic	171970	168026	181533	111187	122061	96308	173843	109852	0.0016	-0.66	DOWN
Ce_fissilis.007537.1	17	15298.04	Sedoheptulose-1,7-bisphosphatase, chloroplastic	184892	182493	195942	92188	93770	87406	187776	91121	0.0000	-1.04	DOWN
Ce_fissilis.015764.1	5	15171.62	Uncharacterized protein At5g01610	200527	205004	213492	157931	112217	125993	206341	132047	0.0062	-0.64	DOWN
Ce_fissilis.006734.1	12	14933.76	31 kDa ribonucleoprotein, chloroplastic	173775	170033	187351	97330	109158	81536	177053	96008	0.0011	-0.88	DOWN
Ce_fissilis.002484.1	6	14567.86	Tubulin beta-3 chain	26268	29648	30434	15519	17970	15575	28783	16355	0.0012	-0.82	DOWN
Ce_fissilis.006082.1	11	14124.48	Tubulin alpha-3 chain	61315	66522	67843	20072	23875	19140	65227	21029	0.0001	-1.63	DOWN
Ce_fissilis.000411.1	11	13973.50	Ribulose bisphosphate carboxylase small chain, chloroplastic	618811	570682	462346	238078	239109	217684	550613	231624	0.0024	-1.25	DOWN
Ce_fissilis.013135.1	24	13897.33	NADP-dependent malic enzyme	208429	201905	171480	72187	72468	62769	193938	69141	0.0005	-1.49	DOWN
Ce_fissilis.008156.1	13	13830.39	Glutamine synthetase leaf isozyme, chloroplastic	163821	157590	144231	41996	43061	33839	155214	39632	0.0001	-1.97	DOWN
Ce_fissilis.017763.1	13	13161.51	L-ascorbate peroxidase, cytosolic	240544	235928	207164	144497	168727	127717	227879	146980	0.0069	-0.63	DOWN
Ce_fissilis.015459.1	8	12576.02	Oxygen-evolving enhancer protein 3-2, chloroplastic	121063	111805	143100	63052	66248	57653	125323	62318	0.0028	-1.01	DOWN
Ce_fissilis.013019.1	9	11507.43	Protein of unknown function, DUF642	165245	173961	178222	61470	71337	64426	172476	65744	0.0000	-1.39	DOWN
Ce_fissilis.017003.1	15	11365.07	Glucan endo-1,3-beta-glucosidase	258467	294951	281910	174593	182608	156386	278443	171196	0.0012	-0.70	DOWN
Ce_fissilis.001428.1	4	11357.39	Ferredoxin-1, chloroplastic	227418	234959	245533	130594	141501	6795	235970	92963	0.0303	-1.34	DOWN
Ce_fissilis.001903.1	24	10632.16	5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase 1	188537	164836	105003	47145	33827	32989	152792	37987	0.0105	-2.01	DOWN
Ce_fissilis.005797.1	8	9877.96	Uncharacterized protein At2g37660, chloroplastic	133770	136853	118891	85649	70601	86258	129838	80836	0.0029	-0.68	DOWN
Ce_fissilis.018999.1	3	9799.96	Photosystem I reaction center subunit VI-2, chloroplastic	111168	129549	140422	85616	80374	69992	127047	78660	0.0075	-0.69	DOWN
Ce_fissilis.016050.2	6	9019.64	Glycine cleavage system H protein, mitochondrial	191819	187427	201974	89564	117880	70436	193740	92627	0.0022	-1.06	DOWN
Ce_fissilis.002280.1	14	8854.28	Formate dehydrogenase, mitochondrial	232452	226121	154244	91776	55518	62131	204272	69809	0.0080	-1.55	DOWN
Ce_fissilis.001130.2	10	8803.94	Fructose-bisphosphate aldolase, cytoplasmic isozyme 1	12305	13424	14988	5580	5032	4462	13572	5024	0.0005	-1.43	DOWN
Ce_fissilis.004112.2	19	8460.36	Guanine nucleotide-binding protein subunit beta-like protein	96917	78826	79102	48690	46162	52151	84948	49001	0.0045	-0.79	DOWN
Ce_fissilis.006726.1	6	7656.51	Ribulose-phosphate 3-epimerase, chloroplastic	159092	152646	158272	100148	113952	92779	156670	102293	0.0011	-0.62	DOWN
Ce_fissilis.014689.1	17	7484.86	Monodehydroascorbate reductase, chloroplastic/mitochondrial	124278	107011	96601	33733	30023	25878	109297	29878	0.0007	-1.87	DOWN

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Ce_fissilis.003800.3	6	7346.31	Photosystem I reaction center subunit III, chloroplastic	157220	127875	134074	10236	9453	10937	139723	10209	0.0001	-3.77	DOWN
Ce_fissilis.006997.1	10	7300.91	Tubulin beta-1 chain	11034	10868	12310	2348	1909	1490	11404	1916	0.0001	-2.57	DOWN
Ce_fissilis.012369.1	17	7299.15	Glutamate--glyoxylate aminotransferase 2	76281	77356	73797	43922	55397	46316	75811	48545	0.0017	-0.64	DOWN
Ce_fissilis.010810.1	5	7272.73	Auxin-binding protein ABP19a	114220	134683	155525	60884	53802	40492	134809	51726	0.0034	-1.38	DOWN
Ce_fissilis.015248.1	7	7267.04	Chlorophyll a-b binding protein CP29.2, chloroplastic	192072	205456	167566	59593	73987	62801	188365	65460	0.0005	-1.52	DOWN
Ce_fissilis.006118.1	11	7068.00	Photosystem II CP43 reaction center protein	186256	182128	190726	44181	104300	82330	186370	76937	0.0035	-1.28	DOWN
Ce_fissilis.000974.1	6	6912.65	Chlorophyll a-b binding protein 13, chloroplastic	85561	78681	76313	36020	46860	41846	80185	41575	0.0008	-0.95	DOWN
Ce_fissilis.012161.1	6	6897.78	NADP-dependent malic enzyme	159169	139569	112157	7085	2959	4887	136965	4977	0.0006	-4.78	DOWN
Ce_fissilis.002025.1	11	6860.33	NADPH-dependent alkenal/one oxidoreductase, chloroplastic	79368	97178	101036	24339	24129	20065	92528	22844	0.0005	-2.02	DOWN
Ce_fissilis.014532.1	23	6692.28	Pyrophosphate--fructose 6-phosphate 1-phosphotransferase subunit alpha	141074	132285	102777	38452	76864	35330	125378	50215	0.0131	-1.32	DOWN
Ce_fissilis.009061.1	4	6649.39	Xyloglucan endotransglucosylase/hydrolase protein 22	35593	36084	41700	17392	20214	20563	37792	19390	0.0011	-0.96	DOWN
Ce_fissilis.014659.1	14	6536.37	Aconitate hydratase, cytoplasmic	88260	64775	39730	19716	18860	12374	64255	16983	0.0291	-1.92	DOWN
Ce_fissilis.014222.2	25	6526.94	Stromal 70 kDa heat shock-related protein, chloroplastic	143982	142191	126787	58830	63032	56524	137653	59462	0.0002	-1.21	DOWN
Ce_fissilis.000704.1	9	5838.55	Photosystem II 22 kDa protein, chloroplastic	172927	197397	193948	125339	128899	103324	188091	119187	0.0034	-0.66	DOWN
Ce_fissilis.016675.1	6	5754.57	Probable UDP-arabinopyranose mutase 2	43010	44404	40478	21331	15737	15658	42631	17575	0.0003	-1.28	DOWN
Ce_fissilis.013669.1	4	5522.44	Zeaxanthin epoxidase, chloroplastic	73453	68468	75062	33894	41551	38097	72328	37847	0.0003	-0.93	DOWN
Ce_fissilis.000342.1	5	5474.70	MLP-like protein 31	94483	101062	100972	58627	28104	27070	98839	37934	0.0045	-1.38	DOWN
Ce_fissilis.014972.1	12	5199.08	Malate dehydrogenase, glyoxysomal	51797	63043	60291	18370	12781	14140	58377	15097	0.0003	-1.95	DOWN
Ce_fissilis.002212.1	11	5134.99	Trans-resveratrol di-O-methyltransferase	76008	59033	45661	17133	16185	14997	60234	16105	0.0074	-1.90	DOWN
Ce_fissilis.009200.1	25	5037.21	Phosphoglucomutase, cytoplasmic	118333	107845	98248	48750	56074	52092	108142	52306	0.0008	-1.05	DOWN
Ce_fissilis.011648.1	8	4972.70	Glyceraldehyde-3-phosphate dehydrogenase, cytosolic	72359	48551	39142	27973	22630	24281	53351	24962	0.0471	-1.10	DOWN
Ce_fissilis.001261.1	11	4959.30	Elongation factor Tu, mitochondrial	56678	54297	39743	29132	26781	24194	50239	26702	0.0127	-0.91	DOWN
Ce_fissilis.008153.1	3	4822.66	Perakine reductase	33739	35106	29905	19258	9956	12756	32917	13990	0.0039	-1.23	DOWN
Ce_fissilis.001101.1	12	4807.91	Phosphoglycolate phosphatase 1B, chloroplastic	72070	73200	84448	34406	41214	26089	76573	33903	0.0019	-1.18	DOWN
Ce_fissilis.015082.1	9	4792.48	L-ascorbate oxidase homolog	99432	96166	93025	46569	40052	41437	96207	42686	0.0000	-1.17	DOWN
Ce_fissilis.000055.1	13	4773.15	Eukaryotic initiation factor 4A-14	107502	109824	93238	44942	48036	38628	103521	43869	0.0005	-1.24	DOWN

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Ce_fissilis.000080.2	16	4725.48	6-phosphogluconate dehydrogenase, decarboxylating 2	33323	36738	21539	13036	8824	8594	30534	10151	0.0134	-1.59	DOWN
Ce_fissilis.016174.1	10	4701.88	3-oxoacyl-[acyl-carrier-protein] synthase I, chloroplastic	114983	104362	103555	68550	47077	42782	107633	52803	0.0034	-1.03	DOWN
Ce_fissilis.003105.1	7	4531.36	Chlorophyll a-b binding protein CP24 10A, chloroplastic	77088	74799	70621	38594	58062	44238	74169	46965	0.0111	-0.66	DOWN
Ce_fissilis.013330.1	16	4507.60	Aconitate hydratase, cytoplasmic	42899	41994	29188	21549	24429	22293	38027	22757	0.0276	-0.74	DOWN
Ce_fissilis.004692.1	4	4473.07	Photosystem I reaction center subunit V, chloroplastic	114205	135876	157629	59041	31077	42552	135903	44224	0.0036	-1.62	DOWN
Ce_fissilis.008404.1	6	4276.00	Glutamine synthetase cytosolic isozyme 2	58878	48227	36324	20473	17026	24834	47810	20778	0.0172	-1.20	DOWN
Ce_fissilis.002416.1	8	4143.21	Alcohol dehydrogenase class-3	79539	72849	72012	40956	44103	46994	74800	44018	0.0005	-0.76	DOWN
Ce_fissilis.004767.2	7	4071.97	Major allergen Pru av 1	59995	56297	54511	15918	12006	17848	56935	15257	0.0001	-1.90	DOWN
Ce_fissilis.016779.2	8	4025.45	Rhodanese-like domain-containing protein 4, chloroplastic	40301	47807	50351	15111	17289	12849	46153	15083	0.0007	-1.61	DOWN
Ce_fissilis.015713.1	10	4002.97	(S)-2-hydroxy-acid oxidase GLO1	82104	71303	84127	61049	44856	38041	79178	47982	0.0168	-0.72	DOWN
Ce_fissilis.012853.1	12	3968.48	5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase	79323	67321	35714	17871	10925	10015	60786	12937	0.0225	-2.23	DOWN
Ce_fissilis.001873.1	15	3924.92	Pyrophosphate--fructose 6-phosphate 1-phosphotransferase subunit beta	82881	76594	67858	18754	15076	10773	75778	14868	0.0002	-2.35	DOWN
Ce_fissilis.011703.1	10	3907.99	Serine--glyoxylate aminotransferase	90099	86423	91405	34795	34000	37864	89309	35553	0.0000	-1.33	DOWN
Ce_fissilis.006733.1	4	3808.88	RNA-binding protein CP31B, chloroplastic	50455	51593	57037	23056	18238	20415	53028	20570	0.0002	-1.37	DOWN
Ce_fissilis.014757.2	12	3790.76	Isocitrate dehydrogenase [NAD] regulatory subunit 1, mitochondrial	53889	50324	46732	27395	24311	25372	50315	25692	0.0004	-0.97	DOWN
Ce_fissilis.013329.1	19	3724.28	Aconitate hydratase 1	49826	41338	29061	16045	18856	17133	40075	17345	0.0202	-1.21	DOWN
Ce_fissilis.005337.1	15	3715.86	Transketolase-2, chloroplastic	109750	82245	59341	26636	24931	22967	83779	24845	0.0157	-1.75	DOWN
Ce_fissilis.005743.2	4	3660.98	3-hydroxyacyl-[acyl-carrier-protein] dehydratase FabZ	58668	60280	62922	34992	21461	19666	60623	25373	0.0021	-1.26	DOWN
Ce_fissilis.013635.2	10	3579.89	Glutathione hydrolase 1	149425	132265	136195	47807	66969	10598	139295	41791	0.0049	-1.74	DOWN
Ce_fissilis.014335.1	3	3484.51	Ubiquitin-conjugating enzyme E2-17 kDa	41480	51705	50991	20046	7840	7940	48059	11942	0.0023	-2.01	DOWN
Ce_fissilis.010530.1	5	3449.17	NADPH-dependent oxidoreductase 2-alkenal reductase	30288	29548	33603	12690	14984	18051	31146	15242	0.0013	-1.03	DOWN
Ce_fissilis.015887.1	4	3418.95	Ras-related protein RABA2a	83569	78068	81626	35772	35987	44330	81088	38696	0.0002	-1.07	DOWN
Ce_fissilis.000495.1	4	3393.79	Tryptophan synthase alpha chain, chloroplastic	172871	174338	188271	20533	25281	16116	178493	20644	0.0000	-3.11	DOWN
Ce_fissilis.018593.1	3	3375.23	-	58730	57436	66540	19983	13321	23536	60902	18946	0.0005	-1.68	DOWN
Ce_fissilis.000462.1	5	3325.33	Mitochondrial phosphate carrier protein 3, mitochondrial	69072	70251	74568	42073	23191	25632	71297	30299	0.0026	-1.23	DOWN
Ce_fissilis.003765.1	7	3181.72	Bark storage protein A	40653	36743	36262	23104	23271	22146	37886	22840	0.0005	-0.73	DOWN
Ce_fissilis.010887.1	8	3169.69	Aspartyl protease family protein At5g10770	47730	45647	59408	32412	22261	21725	50928	25466	0.0099	-1.00	DOWN

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Ce_fissilis.001171.1	4	3156.65	Peptide methionine sulfoxide reductase B5	27221	23339	25392	17254	15873	15730	25317	16286	0.0018	-0.64	DOWN
Ce_fissilis.015257.1	8	3148.14	GDSL esterase/lipase At5g33370	54276	52030	59330	18226	18308	18942	55212	18492	0.0001	-1.58	DOWN
Ce_fissilis.011841.1	4	3113.49	-	57127	59617	71638	35566	26253	40760	62794	34193	0.0098	-0.88	DOWN
Ce_fissilis.015700.1	10	3062.04	DNA damage-repair/toleration protein DRT100	36710	40948	39153	26828	26171	22737	38937	25245	0.0015	-0.63	DOWN
Ce_fissilis.015428.1	4	2993.10	Photosystem I reaction center subunit XI, chloroplastic	99249	115599	116057	43967	42618	43393	110302	43326	0.0003	-1.35	DOWN
Ce_fissilis.011909.1	9	2976.00	Glutamate-1-semialdehyde 2,1-aminomutase, chloroplastic	27202	29524	31850	13432	10320	11666	29525	11806	0.0004	-1.32	DOWN
Ce_fissilis.002539.1	6	2903.92	Malate dehydrogenase [NADP], chloroplastic	88560	90912	91921	50692	50353	48240	90464	49762	0.0000	-0.86	DOWN
Ce_fissilis.016304.2	10	2859.11	Probable lactoylglutathione lyase, chloroplastic	34710	31815	29045	16932	22852	19059	31857	19614	0.0068	-0.70	DOWN
Ce_fissilis.006334.1	5	2857.30	MLP-like protein 423	49874	51813	54725	21206	21846	21097	52138	21383	0.0000	-1.29	DOWN
Ce_fissilis.005604.1	5	2811.24	Fructose-1,6-bisphosphatase, cytosolic	40584	41814	42454	23204	22502	20901	41618	22202	0.0000	-0.91	DOWN
Ce_fissilis.007803.1	13	2804.76	Sorbitol dehydrogenase	83406	85593	88259	57539	58092	45438	85753	53689	0.0018	-0.68	DOWN
Ce_fissilis.014865.1	6	2778.56	Probable aldo-keto reductase 1	19237	18981	19062	2714	3191	2026	19093	2644	0.0000	-2.85	DOWN
Ce_fissilis.008930.1	6	2629.06	Formamidase	55899	48538	41032	21007	20057	23046	48490	21370	0.0035	-1.18	DOWN
Ce_fissilis.011400.1	5	2563.15	Protein MET1, chloroplastic	37517	35667	31645	6651	7797	7667	34943	7372	0.0001	-2.24	DOWN
Ce_fissilis.000562.1	8	2490.66	Eukaryotic translation initiation factor 5A	47181	51413	52880	30433	36053	31224	50491	32570	0.0019	-0.63	DOWN
Ce_fissilis.002030.1	3	2413.33	GTP-binding nuclear protein Ran-3	48114	37032	33358	11187	8092	7334	39501	8871	0.0026	-2.15	DOWN
Ce_fissilis.007320.1	8	2369.63	Catalase isozyme 3	101563	102460	91680	70272	47649	49071	98568	55664	0.0061	-0.82	DOWN
Ce_fissilis.007983.1	9	2317.42	S-adenosylmethionine synthase	31762	32866	22000	16715	15694	13578	28876	15329	0.0193	-0.91	DOWN
Ce_fissilis.018860.1	4	2271.27	14-3-3 protein 7	13434	12415	13846	8734	8936	6897	13232	8189	0.0029	-0.69	DOWN
Ce_fissilis.009844.1	9	2258.95	Glutamate dehydrogenase 1	42097	36003	28744	15462	12676	12003	35615	13380	0.0051	-1.41	DOWN
Ce_fissilis.001892.1	9	2247.37	Glycerate dehydrogenase HPR, peroxisomal	59268	62819	76766	41236	39183	34832	66284	38417	0.0079	-0.79	DOWN
Ce_fissilis.008775.1	9	2240.76	ATP synthase gamma chain, chloroplastic	69687	65949	63791	14774	12669	10031	66476	12491	0.0000	-2.41	DOWN
Ce_fissilis.000392.1	3	2229.43	Putative glucose-6-phosphate 1-epimerase	46744	49481	53668	15046	22826	16342	49964	18071	0.0005	-1.47	DOWN
Ce_fissilis.002699.1	2	2123.00	L-ascorbate oxidase homolog	9981	9839	11065	3415	4928	5162	10295	4502	0.0010	-1.19	DOWN
Ce_fissilis.004527.1	5	2096.26	Monodehydroascorbate reductase	66745	49121	38552	19840	16212	6912	51473	14321	0.0150	-1.85	DOWN
Ce_fissilis.001288.2	8	1987.76	Photosystem II CP47 reaction center protein	62236	45906	25972	4737	7008	6054	44705	5933	0.0210	-2.91	DOWN
Ce_fissilis.015174.1	5	1958.47	Elongation factor 1-gamma	57701	44415	31751	10256	9852	11693	44622	10600	0.0106	-2.07	DOWN
Ce_fissilis.016645.1	10	1950.60	30S ribosomal protein S1, chloroplastic	30344	35811	41024	22907	19896	21167	35726	21323	0.0109	-0.74	DOWN

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Ce_fissilis.016775.1	6	1936.06	Glucose-6-phosphate isomerase, cytosolic	85807	87139	79805	57205	52879	55064	84250	55049	0.0003	-0.61	DOWN
Ce_fissilis.001377.2	8	1934.28	NADP-dependent glyceraldehyde-3-phosphate dehydrogenase	24824	19414	16443	2730	2959	2269	20227	2653	0.0020	-2.93	DOWN
Ce_fissilis.017877.1	8	1829.28	Flagellar radial spoke protein 5	19212	20789	20479	13240	8001	8236	20160	9826	0.0043	-1.04	DOWN
Ce_fissilis.018886.1	15	1786.90	NAD-dependent malic enzyme 62 kDa isoform, mitochondrial	49167	54480	47670	36359	30115	32638	50439	33037	0.0032	-0.61	DOWN
Ce_fissilis.002047.1	8	1773.63	Probable nucleoredoxin 1	160620	112664	96450	47505	45138	22138	123245	38261	0.0153	-1.69	DOWN
Ce_fissilis.011024.1	8	1759.94	Succinate dehydrogenase [ubiquinone] flavoprotein subunit 1, mitochondrial	36020	33423	27026	24978	22043	15880	32156	20967	0.0417	-0.62	DOWN
Ce_fissilis.001377.3	10	1739.48	NADP-dependent glyceraldehyde-3-phosphate dehydrogenase	48017	42335	36311	10371	12430	10923	42221	11241	0.0008	-1.91	DOWN
Ce_fissilis.016304.1	7	1688.10	Probable lactoylglutathione lyase, chloroplastic	26087	23911	21829	6512	8173	7849	23943	7511	0.0002	-1.67	DOWN
Ce_fissilis.003137.2	7	1668.54	Hydroxyacylglutathione hydrolase cytoplasmic	73768	74550	70024	27113	21942	20266	72781	23107	0.0000	-1.66	DOWN
Ce_fissilis.010053.1	6	1650.88	Aspartic proteinase nepenthesin-1	48837	53583	59306	31515	30236	25324	53908	29025	0.0022	-0.89	DOWN
Ce_fissilis.000870.2	7	1629.11	HIPL1 protein	53508	39437	45014	28809	31031	29753	45986	29864	0.0176	-0.62	DOWN
Ce_fissilis.003758.1	8	1598.04	Uncharacterized oxidoreductase At4g09670	29260	27406	26396	10990	10356	11471	27688	10939	0.0000	-1.34	DOWN
Ce_fissilis.005604.2	6	1555.37	Fructose-1,6-bisphosphatase, cytosolic	23469	25800	25664	11269	11207	11914	24978	11463	0.0001	-1.12	DOWN
Ce_fissilis.010261.1	19	1505.76	Puromycin-sensitive aminopeptidase	55551	45817	33214	15610	16961	16160	44861	16244	0.0115	-1.47	DOWN
Ce_fissilis.015354.1	4	1495.84	1-aminocyclopropane-1-carboxylate oxidase	33182	40384	45094	14289	13196	13075	39553	13520	0.0017	-1.55	DOWN
Ce_fissilis.014432.1	5	1476.26	Probable cinnamyl alcohol dehydrogenase 1	24210	22470	22516	8875	12931	15898	23066	12568	0.0077	-0.88	DOWN
Ce_fissilis.011254.1	8	1476.01	3-oxoacyl-[acyl-carrier-protein] synthase II, chloroplastic	35678	34615	36039	14146	15976	12496	35444	14206	0.0000	-1.32	DOWN
Ce_fissilis.000440.1	2	1475.66	Thioredoxin M-type, chloroplastic	40727	43767	46532	12770	16640	14718	43675	14709	0.0001	-1.57	DOWN
Ce_fissilis.015339.2	3	1472.26	Cytochrome b561 and DOMON domain-containing protein At4g12980	46141	48521	55682	26196	27663	26642	50115	26834	0.0013	-0.90	DOWN
Ce_fissilis.017918.1	3	1452.88	Early nodulin-like protein 1	41972	40585	42534	27018	33067	19274	41697	26453	0.0194	-0.66	DOWN
Ce_fissilis.011316.1	8	1430.09	Phosphoglucomutase, chloroplastic	29243	31968	32175	10279	8796	8586	31128	9220	0.0000	-1.76	DOWN
Ce_fissilis.014480.1	3	1425.47	Peptide methionine sulfoxide reductase	29974	33109	37921	13951	14958	20281	33668	16397	0.0047	-1.04	DOWN
Ce_fissilis.001617.1	9	1385.09	D-3-phosphoglycerate dehydrogenase 2, chloroplastic	32693	35876	35213	19870	22514	21133	34594	21172	0.0004	-0.71	DOWN
Ce_fissilis.015098.1	5	1379.61	ATP synthase delta chain, chloroplastic	20426	18779	23412	5613	8652	8292	20872	7519	0.0013	-1.47	DOWN
Ce_fissilis.008692.1	5	1370.30	Rhodanese-like domain-containing protein 9, chloroplastic	18492	21131	22770	6304	6056	7181	20798	6514	0.0004	-1.67	DOWN
Ce_fissilis.012804.1	6	1363.35	Pyruvate kinase, cytosolic isozyme	15990	13465	9892	7268	7841	7903	13115	7671	0.0377	-0.77	DOWN
Ce_fissilis.008442.1	7	1361.81	T-complex protein 1 subunit delta	48880	45328	27429	16730	14292	11363	40546	14129	0.0179	-1.52	DOWN

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Ce_fissilis.013403.1	7	1360.83	Calcium sensing receptor, chloroplastic	56736	62156	62132	26053	31912	27925	60341	28630	0.0002	-1.08	DOWN
Ce_fissilis.006659.1	2	1359.67	Rieske (2Fe-2S) domain-containing protein	23747	25808	25960	9840	11594	11317	25172	10917	0.0001	-1.21	DOWN
Ce_fissilis.010661.1	11	1355.93	ATP-dependent zinc metalloprotease FTSH, chloroplastic	34132	36259	31998	10353	8398	8251	34130	9001	0.0001	-1.92	DOWN
Ce_fissilis.000870.1	7	1329.54	HIPL1 protein	9808	10311	10207	2852	3476	3376	10108	3235	0.0000	-1.64	DOWN
Ce_fissilis.008180.1	11	1320.01	Glucose-1-phosphate adenylyltransferase large subunit 1	38077	41005	41589	4165	3416	4764	40224	4115	0.0000	-3.29	DOWN
Ce_fissilis.013279.1	5	1317.22	Probable aquaporin PIP1-2	35329	35337	44614	13019	17391	13425	38427	14612	0.0022	-1.39	DOWN
Ce_fissilis.013568.1	14	1312.96	Alpha-glucosidase	48180	45046	41062	20028	18578	16733	44763	18446	0.0003	-1.28	DOWN
Ce_fissilis.009026.1	7	1281.16	Long chain acyl-CoA synthetase 7, peroxisomal	37692	35127	28608	19469	10021	5928	33809	11806	0.0104	-1.52	DOWN
Ce_fissilis.006965.1	3	1250.55	Proteasome subunit alpha type-7-B	19967	22992	15378	12918	7364	7549	19446	9277	0.0239	-1.07	DOWN
Ce_fissilis.016194.1	5	1208.35	Succinate-semialdehyde dehydrogenase (acetylating)	36367	30329	23224	16190	15253	16279	29973	15907	0.0210	-0.91	DOWN
Ce_fissilis.009686.1	2	1189.53	Chlorophyll a-b binding protein, chloroplastic	38918	34880	43445	4425	9532	7018	39081	6992	0.0004	-2.48	DOWN
Ce_fissilis.014129.2	6	1175.70	3-oxo-Delta(4,5)-steroid 5-beta-reductase	25962	23301	17564	5225	4300	4102	22276	4542	0.0021	-2.29	DOWN
Ce_fissilis.007876.3	2	1164.68	Protein SUPPRESSOR OF QUENCHING 1, chloroplastic	28351	32652	30495	14133	18524	16011	30499	16222	0.0013	-0.91	DOWN
Ce_fissilis.014130.1	8	1151.91	Protein transport protein SEC23	41363	42457	39058	22745	24710	15874	40959	21110	0.0023	-0.96	DOWN
Ce_fissilis.001633.1	8	1142.94	Glycerol kinase	71804	61400	54985	27865	22501	21005	62730	23791	0.0019	-1.40	DOWN
Ce_fissilis.009303.1	4	1128.66	Probable cinnamyl alcohol dehydrogenase 9	31769	26451	24583	4827	5478	7811	27601	6039	0.0008	-2.19	DOWN
Ce_fissilis.019092.1	7	1120.88	Probable mitochondrial-processing peptidase subunit beta, mitochondrial	35997	31230	20624	6213	6269	6505	29284	6329	0.0072	-2.21	DOWN
Ce_fissilis.011248.1	2	1087.20	Probable ribose-5-phosphate isomerase 3, chloroplastic	25172	28311	37444	18321	11846	19460	30309	16542	0.0347	-0.87	DOWN
Ce_fissilis.000319.1	9	1071.09	Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A beta isoform	19269	16859	19083	7058	8003	6747	18404	7269	0.0002	-1.34	DOWN
Ce_fissilis.001088.1	6	1065.02	D-3-phosphoglycerate dehydrogenase 3, chloroplastic	9758	10447	10663	3005	3706	3013	10289	3241	0.0000	-1.67	DOWN
Ce_fissilis.010335.1	5	1064.81	NAD-dependent malic enzyme 59 kDa isoform, mitochondrial	34969	32937	24592	17686	12264	14386	30833	14779	0.0106	-1.06	DOWN
Ce_fissilis.011383.1	6	1045.94	Protein HOTHEAD	36304	40214	43534	16611	19889	17909	40017	18137	0.0007	-1.14	DOWN
Ce_fissilis.011778.1	6	1031.34	Cytochrome c1-1, heme protein, mitochondrial	17611	18378	14860	8509	7880	7879	16950	8089	0.0012	-1.07	DOWN
Ce_fissilis.005519.1	9	1018.50	T-complex protein 1 subunit eta	21840	21735	19536	12456	12604	12350	21037	12470	0.0003	-0.75	DOWN
Ce_fissilis.015795.1	2	1009.37	Fruit protein pKIWI502	18189	22686	19633	10976	6524	6454	20169	7985	0.0037	-1.34	DOWN
Ce_fissilis.016593.1	3	1004.87	Patellin-5	20882	15338	10079	6673	6611	6890	15433	6724	0.0493	-1.20	DOWN

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Ce_fissilis.002433.1	11	985.80	Tol-Pal system protein TolB	34273	30454	27313	20329	21168	19120	30680	20206	0.0075	-0.60	DOWN
Ce_fissilis.018123.1	3	952.53	ATP synthase subunit gamma, mitochondrial	22847	17099	17796	7348	6679	8678	19247	7568	0.0036	-1.35	DOWN
Ce_fissilis.005225.2	2	947.93	Metallo-hydrolase/oxidoreductase superfamily protein	24507	25346	27281	10501	12584	15988	25711	13024	0.0021	-0.98	DOWN
Ce_fissilis.009294.1	3	940.68	Ferritin-3, chloroplastic	30299	30248	23800	19652	21921	13788	28116	18454	0.0408	-0.61	DOWN
Ce_fissilis.000738.2	3	936.06	Plasma membrane-associated cation-binding protein 1	21188	18949	14490	2300	3182	2575	18209	2686	0.0014	-2.76	DOWN
Ce_fissilis.009003.1	3	921.83	Argininosuccinate synthase, chloroplastic	25323	22724	14556	8499	7385	5502	20868	7129	0.0150	-1.55	DOWN
Ce_fissilis.002776.1	4	890.29	Ribose-phosphate pyrophosphokinase 4	25487	23479	21465	19695	11601	13254	23477	14850	0.0341	-0.66	DOWN
Ce_fissilis.003143.1	3	835.33	Fasciclin-like arabinogalactan protein 2	31373	34236	34112	18225	12372	19545	33240	16714	0.0023	-0.99	DOWN
Ce_fissilis.006652.1	7	802.18	2-hydroxyacyl-CoA lyase	91354	76843	63268	30457	37470	38276	77155	35401	0.0079	-1.12	DOWN
Ce_fissilis.006965.2	3	791.59	Proteasome subunit alpha type-7	10151	11784	8560	4799	2273	3235	10165	3436	0.0048	-1.56	DOWN
Ce_fissilis.011818.1	2	790.39	Thaumatin-like protein 1	23256	17922	23897	5485	6338	6451	21692	6091	0.0012	-1.83	DOWN
Ce_fissilis.012926.1	3	784.19	Glyoxylate/succinic semialdehyde reductase 2, chloroplastic	16721	16708	19406	10761	9848	8703	17612	9770	0.0019	-0.85	DOWN
Ce_fissilis.015758.1	3	781.52	Guanosine nucleotide diphosphate dissociation inhibitor 1	20621	16052	14200	7781	9087	7929	16958	8266	0.0112	-1.04	DOWN
Ce_fissilis.013842.1	2	737.61	Red chlorophyll catabolite reductase, chloroplastic	17342	21635	25062	16691	12091	10235	21346	13005	0.0472	-0.71	DOWN
Ce_fissilis.001841.2	2	730.27	Beta-hexosaminidase 2	32674	36530	37103	8155	5489	5202	35436	6282	0.0001	-2.50	DOWN
Ce_fissilis.008596.1	7	728.15	Uncharacterized protein At5g02240	18675	17486	17028	2435	2704	5116	17730	3418	0.0001	-2.37	DOWN
Ce_fissilis.017503.1	3	721.95	Pathogen-related protein	12838	13022	13047	7238	7556	7556	12969	7450	0.0000	-0.80	DOWN
Ce_fissilis.005581.1	4	705.69	UDP-glycosyltransferase 74F2	26060	20506	21470	6339	4852	6588	22678	5926	0.0007	-1.94	DOWN
Ce_fissilis.015895.1	2	683.92	Uncharacterized protein ycf39	22865	23921	25249	11118	13447	12975	24011	12513	0.0003	-0.94	DOWN
Ce_fissilis.014909.1	6	670.15	Delta-aminolevulinic acid dehydratase 1, chloroplastic	32847	40795	33204	20570	21489	8549	35615	16869	0.0188	-1.08	DOWN
Ce_fissilis.014409.1	5	644.45	Serine carboxypeptidase-like 50	21090	22068	23457	18631	9647	12257	22205	13512	0.0343	-0.72	DOWN
Ce_fissilis.018077.1	3	631.69	4-coumarate--CoA ligase-like 5	18549	15427	12690	6100	7180	6359	15555	6546	0.0064	-1.25	DOWN
Ce_fissilis.012940.1	5	630.19	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 9, mitochondrial	24105	28515	37459	19563	11861	12134	30026	14519	0.0293	-1.05	DOWN
Ce_fissilis.009048.1	3	622.78	ATP-dependent zinc metalloprotease FTSH 2, chloroplastic	63627	58440	37495	15061	11464	9740	53187	12088	0.0072	-2.14	DOWN
Ce_fissilis.008641.1	3	616.74	Glutathione reductase, chloroplastic	34844	31205	30732	15186	15307	11646	32260	14046	0.0005	-1.20	DOWN
Ce_fissilis.012347.1	4	610.35	Proteasome subunit beta type-4	17646	15668	14142	4889	9526	11577	15819	8664	0.0323	-0.87	DOWN
Ce_fissilis.008109.1	2	601.64	Alpha-galactosidase	36035	30899	32922	20664	19567	24692	33285	21641	0.0057	-0.62	DOWN

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Ce_fissilis.013858.1	6	555.82	Bifunctional aspartokinase/homoserine dehydrogenase 2, chloroplastic	21939	16856	15156	10917	12257	6704	17984	9959	0.0383	-0.85	DOWN
Ce_fissilis.016211.2	2	552.70	Flavone 3'-O-methyltransferase 1	17652	16403	15027	9885	8083	7769	16360	8579	0.0015	-0.93	DOWN
Ce_fissilis.017998.1	4	533.03	Probable endo-1,3(4)-beta-glucanase ARB_01444	17146	17295	17458	8466	9069	8185	17300	8573	0.0000	-1.01	DOWN
Ce_fissilis.013173.1	2	482.07	Pyruvate kinase isozyme A, chloroplastic	11836	9502	8056	5444	5015	5353	9798	5271	0.0151	-0.89	DOWN
Ce_fissilis.014384.1	2	462.06	Clavaminate synthase-like protein At3g21360	5257	5687	6321	2087	2275	2474	5755	2278	0.0005	-1.34	DOWN
Ce_fissilis.013775.1	2	455.85	Galactokinase	35354	29179	28163	12968	12933	12750	30899	12884	0.0013	-1.26	DOWN
Ce_fissilis.007062.1	2	383.34	Prostaglandin reductase-3	19891	17622	15267	8556	9586	5452	17593	7865	0.0059	-1.16	DOWN
Ce_fissilis.004594.1	2	381.57	Chloroplastic lipocalin	24194	25739	30238	18601	16463	11002	26723	15355	0.0172	-0.80	DOWN
Ce_fissilis.000916.1	2	372.94	Thylakoid lumenal 29 kDa protein, chloroplastic	17015	17392	16760	9449	11035	9639	17055	10041	0.0002	-0.76	DOWN
Ce_fissilis.001602.1	3	367.33	Leucine aminopeptidase	17032	17082	15825	8591	7386	8290	16646	8089	0.0001	-1.04	DOWN
Ce_fissilis.016159.1	5	352.52	Acylamino-acid-releasing enzyme	37446	46421	24600	13835	14091	14360	36156	14096	0.0253	-1.36	DOWN
Ce_fissilis.014397.1	2	316.55	Trans-resveratrol di-O-methyltransferase	23913	24039	22565	9962	8875	7000	23505	8612	0.0001	-1.45	DOWN
Ce_fissilis.007385.1	2	314.99	Acetyl-coenzyme A synthetase, chloroplastic/glyoxysomal	14563	11342	12346	5588	6983	6792	12750	6454	0.0038	-0.98	DOWN
Ce_fissilis.009377.1	17	20874.55	ATP synthase subunit beta, mitochondrial	8511	9137	8189				8612	0	-	-	Unique S1
Ce_fissilis.002760.1	7	4546.71	Eukaryotic initiation factor 4A-15	12543	15119	12836				13499	0	-	-	Unique S1
Ce_fissilis.010582.1	4	3473.39	Ras-related protein RABA1f	3943	1518	1772				2411	0	-	-	Unique S1
Ce_fissilis.001393.2	5	3023.18	Ras-related protein Rab7	9600	9237	10779				9872	0	-	-	Unique S1
Ce_fissilis.015325.1	4	1672.52	MLP-like protein 34	15659	14936	10507				13701	0	-	-	Unique S1
Ce_fissilis.013494.1	3	1281.98	Glucose-1-phosphate adenylyltransferase small subunit 2, chloroplastic	44921	35923	27424				36089	0	-	-	Unique S1
Ce_fissilis.007855.1	4	1265.99	Serine/threonine-protein phosphatase PP2A catalytic subunit	18883	19999	20009				19630	0	-	-	Unique S1
Ce_fissilis.013260.1	3	1253.48	1-aminocyclopropane-1-carboxylate oxidase	11285	13489	14062				12945	0	-	-	Unique S1
Ce_fissilis.001377.1	5	1210.60	NADP-dependent glyceraldehyde-3-phosphate dehydrogenase	10664	7949	7563				8725	0	-	-	Unique S1
Ce_fissilis.000324.1	4	975.56	Protochlorophyllide reductase, chloroplastic	7332	7123	4944				6467	0	-	-	Unique S1
Ce_fissilis.015533.1	8	909.80	Granule-bound starch synthase 1, chloroplastic/amyloplastic	28370	25922	21338				25210	0	-	-	Unique S1
Ce_fissilis.001089.1	3	465.98	Granule-bound starch synthase 1, chloroplastic/amyloplastic	9536	8859	7953				8782	0	-	-	Unique S1
Ce_fissilis.013799.1	2	437.88	Major allergen Pru ar 1	11608	11605	13026				12080	0	-	-	Unique S1
Ce_fissilis.014048.1	2	405.25	Elongation factor G-2, chloroplastic	7804	2995	1887				4229	0	-	-	Unique S1

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Ce_fissilis.001285.1	2	382.27	Protoporphyrinogen oxidase 1, chloroplastic	18160	15892	19461				17837	0	-	-	Unique S1
Ce_fissilis.003786.1	10	97629.25	Major allergen Pru ar 1	736084	725325	648291	759222	769990	679533	703233	736248	0.4527	0.07	UNCHANGED
Ce_fissilis.006015.1	8	69983.10	Germin-like protein subfamily 1 member 14	385800	396690	406225	996467	755877	534546	396238	762297	0.0518	0.94	UNCHANGED
Ce_fissilis.014428.1	28	63179.46	Actin-7	516710	511748	505074	427465	416619	360717	511178	401600	0.0064	-0.35	UNCHANGED
Ce_fissilis.002071.1	28	61425.23	Perakine reductase	717334	611835	549719	435230	447279	414156	626296	432222	0.0177	-0.54	UNCHANGED
Ce_fissilis.014996.1	29	59490.09	Tubulin beta-8 chain	126290	129476	130519	92034	102543	93096	128762	95891	0.0008	-0.43	UNCHANGED
Ce_fissilis.012078.2	25	54324.66	Actin-97	41178	42118	40956	47152	53955	56095	41417	52401	0.0156	0.34	UNCHANGED
Ce_fissilis.003787.1	11	54019.82	Major allergen Pru av 1	282619	292277	286938	424960	461122	364971	287278	417017	0.0100	0.54	UNCHANGED
Ce_fissilis.004456.1	9	53717.44	Universal stress protein PHOS32	228715	234653	247261	202649	180255	150700	236876	177868	0.0211	-0.41	UNCHANGED
Ce_fissilis.013959.2	25	52554.23	Tubulin beta-4 chain	75482	79751	80938	58991	79965	156167	78724	98374	0.5427	0.32	UNCHANGED
Ce_fissilis.015095.1	5	51531.97	Peroxidase 15	494151	550751	528119	614074	543447	397966	524340	518496	0.9334	-0.02	UNCHANGED
Ce_fissilis.015306.1	23	47013.53	Basic 7S globulin	457878	430788	414742	466958	443597	448586	434469	453047	0.2680	0.06	UNCHANGED
Ce_fissilis.017405.1	17	45492.89	Peroxidase 12	290276	291687	321781	382167	398286	385740	301248	388731	0.0015	0.37	UNCHANGED
Ce_fissilis.016689.1	28	44263.96	Tubulin beta-2 chain	27970	30080	30363	28937	32635	27713	29471	29761	0.8697	0.01	UNCHANGED
Ce_fissilis.004609.1	14	43199.26	Oxygen-evolving enhancer protein 2, chloroplastic	491324	514538	513734	347837	389325	349968	506532	362376	0.0007	-0.48	UNCHANGED
Ce_fissilis.013923.1	7	42793.38	Peroxidase 15	463671	470639	493033	700188	565709	626814	475781	630903	0.0177	0.41	UNCHANGED
Ce_fissilis.005614.1	20	41262.20	Oxygen-evolving enhancer protein 1, chloroplastic	650137	649426	641491	441599	484253	410611	647018	445488	0.0007	-0.54	UNCHANGED
Ce_fissilis.017405.2	13	39814.04	Peroxidase 12	330089	345973	372722	442056	451840	389517	349594	427805	0.0273	0.29	UNCHANGED
Ce_fissilis.015704.1	11	39191.86	Chlorophyll a-b binding protein 21, chloroplastic	389643	361690	391065	321839	412268	387732	380799	373946	0.8227	-0.03	UNCHANGED
Ce_fissilis.015536.1	14	38547.45	Malate dehydrogenase, mitochondrial	309037	335196	334130	401387	429570	355438	326121	395465	0.0406	0.28	UNCHANGED
Ce_fissilis.013557.1	23	36103.32	Fructose-bisphosphate aldolase 6, cytosolic	477711	495465	503548	550436	559376	552547	492241	554119	0.0016	0.17	UNCHANGED
Ce_fissilis.016597.2	33	34380.16	Probable L-gulonolactone oxidase 6	693076	552338	483481	402375	411213	399216	576299	404268	0.0496	-0.51	UNCHANGED
Ce_fissilis.017401.1	19	33153.85	Tubulin beta-6 chain	163440	171236	176864	146089	161860	135409	170513	147786	0.0576	-0.21	UNCHANGED
Ce_fissilis.016865.1	29	33078.20	ATP synthase subunit beta, mitochondrial	469188	509190	457527	441157	436030	379982	478635	419056	0.0763	-0.19	UNCHANGED
Ce_fissilis.014854.1	26	32680.38	Enolase	362897	362200	355435	346502	370695	333073	360177	350090	0.4210	-0.04	UNCHANGED
Ce_fissilis.009112.1	17	32227.40	Malate dehydrogenase	425408	451379	446598	408706	451151	398988	441128	419615	0.2956	-0.07	UNCHANGED
Ce_fissilis.014995.2	22	32116.56	Tubulin beta-1 chain	23185	23304	23613	26812	30896	26366	23367	28025	0.0323	0.26	UNCHANGED
Ce_fissilis.006085.3	19	31835.51	Tubulin alpha chain	276047	274836	282525	194348	222999	144177	277803	187175	0.0173	-0.57	UNCHANGED
Ce_fissilis.002854.1	12	31708.72	Thaumatin-like protein	254062	278485	289357	267789	266664	246085	273968	260179	0.3352	-0.07	UNCHANGED
Ce_fissilis.018100.2	18	26807.32	Basic 7S globulin	338765	372772	437745	490472	584894	394639	383094	490002	0.1604	0.36	UNCHANGED

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Ce_fissilis.008983.1	12	26516.88	Triosephosphate isomerase, cytosolic	264609	238457	244778	181889	165482	182097	249281	176489	0.0016	-0.50	UNCHANGED
Ce_fissilis.019019.1	9	24743.18	Alcohol dehydrogenase class-P	169182	208684	189115	293711	271897	240472	188994	268693	0.0143	0.51	UNCHANGED
Ce_fissilis.006083.1	8	24695.37	Tubulin alpha-3 chain	107047	104501	107139	80653	105750	92194	106229	92866	0.1413	-0.19	UNCHANGED
Ce_fissilis.002415.1	20	24336.52	Alcohol dehydrogenase	209112	237081	211739	278864	279519	257417	219311	271934	0.0102	0.31	UNCHANGED
Ce_fissilis.009802.1	10	23195.09	Chlorophyll a-b binding protein 151, chloroplastic	228626	216589	218204	170819	217199	201305	221140	196441	0.1552	-0.17	UNCHANGED
Ce_fissilis.014429.1	15	22861.44	Actin	69967	73141	69974	67738	73420	74872	71027	72010	0.7054	0.02	UNCHANGED
Ce_fissilis.008984.1	15	22589.47	Triosephosphate isomerase, cytosolic	255903	266216	244756	226855	225915	255400	255625	236057	0.1638	-0.11	UNCHANGED
Ce_fissilis.004298.1	20	21627.45	(+)-neomenthol dehydrogenase	247061	253617	284536	234506	175913	161601	261738	190673	0.0474	-0.46	UNCHANGED
Ce_fissilis.004456.2	4	21477.03	Universal stress protein PHOS32	157602	128866	169728	147954	107690	118160	152065	124601	0.1835	-0.29	UNCHANGED
Ce_fissilis.006084.1	15	21309.25	Tubulin alpha chain	167339	184737	187036	189931	227323	179778	179704	199010	0.2872	0.15	UNCHANGED
Ce_fissilis.015325.2	11	20644.54	MLP-like protein 31	165837	163066	169583	151320	154511	140455	166162	148762	0.0202	-0.16	UNCHANGED
Ce_fissilis.013782.2	12	19473.01	Epidermis-specific secreted glycoprotein EP1	299111	273360	258082	361372	402674	330657	276851	364901	0.0216	0.40	UNCHANGED
Ce_fissilis.005132.1	15	19176.45	Triosephosphate isomerase, chloroplastic	150667	164124	180323	144164	144071	136678	165038	141638	0.0587	-0.22	UNCHANGED
Ce_fissilis.009261.1	16	19117.43	Cysteine synthase	157078	155190	175396	194648	202017	185802	162555	194156	0.0166	0.26	UNCHANGED
Ce_fissilis.014515.1	26	18086.55	ATP synthase subunit alpha, mitochondrial	287585	294741	314489	289642	317853	285336	298938	297610	0.9235	-0.01	UNCHANGED
Ce_fissilis.015096.1	6	18004.90	Peroxidase 15	263138	282403	282378	398338	381289	322445	275973	367357	0.0186	0.41	UNCHANGED
Ce_fissilis.006840.1	13	17074.98	Tubulin beta-1 chain	41046	43605	44794	35303	39816	32349	43149	35823	0.0397	-0.27	UNCHANGED
Ce_fissilis.011648.2	13	16902.39	Glyceraldehyde-3-phosphate dehydrogenase GAPC1, cytosolic	118824	100493	86103	83118	66419	70069	101806	73202	0.0562	-0.48	UNCHANGED
Ce_fissilis.018837.2	11	16710.90	Peptidyl-prolyl cis-trans isomerase	265644	308028	296674	229877	200173	190732	290115	206927	0.0086	-0.49	UNCHANGED
Ce_fissilis.004249.1	11	16177.17	Reactive Intermediate Deaminase A, chloroplastic	216181	204283	168550	198084	205027	200981	196338	201364	0.7456	0.04	UNCHANGED
Ce_fissilis.005602.1	12	15942.21	Beta carbonic anhydrase 4	142892	141906	136121	120238	128416	113954	140306	120869	0.0143	-0.22	UNCHANGED
Ce_fissilis.010564.1	24	15448.00	V-type proton ATPase subunit B2	198227	208787	223860	165114	184467	150895	210291	166825	0.0238	-0.33	UNCHANGED
Ce_fissilis.018100.1	13	15389.22	Basic 7S globulin	237669	240231	275048	345258	377940	358874	250983	360691	0.0020	0.52	UNCHANGED
Ce_fissilis.008713.1	29	15091.53	Leucine aminopeptidase 2, chloroplastic	188446	192233	199952	182764	232972	215534	193544	210423	0.3263	0.12	UNCHANGED
Ce_fissilis.008811.1	13	14595.99	Esterase	454121	434702	412049	350761	303631	312630	433624	322341	0.0041	-0.43	UNCHANGED
Ce_fissilis.009132.1	12	14301.84	Isoflavone reductase homolog PCBER	161093	156929	173491	141553	145442	105295	163838	130763	0.0735	-0.33	UNCHANGED
Ce_fissilis.017826.2	13	14251.00	Quinone oxidoreductase	165335	148361	159310	138971	160997	150349	157669	150106	0.4017	-0.07	UNCHANGED
Ce_fissilis.001741.1	15	14206.96	Probable cysteine protease RD21B	197556	204248	208054	274912	286227	250813	203286	270651	0.0035	0.41	UNCHANGED
Ce_fissilis.014356.1	9	14117.00	Translationally-controlled tumor protein homolog	241553	265651	304563	251683	248233	222438	270589	240785	0.2205	-0.17	UNCHANGED
Ce_fissilis.010974.1	15	14070.02	20 kDa chaperonin, chloroplastic	250524	259191	269841	219847	217395	186183	259852	207808	0.0130	-0.32	UNCHANGED

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Ce_fissilis.005977.1	18	14011.77	2,3-dimethylmalate lyase	177481	173324	183064	171821	165335	152402	177956	163186	0.0812	-0.13	UNCHANGED
Ce_fissilis.013763.1	13	13740.38	Fructose-bisphosphate aldolase 3, chloroplastic	178581	155517	157943	153712	186892	174356	164014	171654	0.5629	0.07	UNCHANGED
Ce_fissilis.012880.1	6	13672.60	Nucleoside diphosphate kinase 1	160219	177376	185157	209766	209213	184045	174251	201008	0.0759	0.21	UNCHANGED
Ce_fissilis.009300.1	10	13593.90	Mitochondrial outer membrane protein porin of 34 kDa	295484	261219	271730	237183	251068	214993	276144	234415	0.0460	-0.24	UNCHANGED
Ce_fissilis.009079.1	20	13419.56	3-ketoacyl-CoA thiolase 2, peroxisomal	346530	333804	303854	226020	272313	217885	328063	238739	0.0134	-0.46	UNCHANGED
Ce_fissilis.001215.1	4	12793.81	Leucine-rich repeat protein 1	321339	324758	318812	370345	390811	312323	321637	357826	0.1995	0.15	UNCHANGED
Ce_fissilis.011699.1	34	12561.54	V-type proton ATPase catalytic subunit A	179432	180883	189679	192845	230170	204386	183331	209134	0.0880	0.19	UNCHANGED
Ce_fissilis.002927.1	10	12141.41	Calmodulin-related protein	145756	146455	160845	161042	177646	121299	151019	153329	0.9009	0.02	UNCHANGED
Ce_fissilis.018575.1	23	12083.12	Aldehyde dehydrogenase family 2 member B4, mitochondrial	245769	243229	274396	246364	260584	240392	254465	249113	0.6698	-0.03	UNCHANGED
Ce_fissilis.013983.1	12	12034.37	Elongation factor 1-beta 1	114173	109592	122150	85069	103590	92765	115305	93808	0.0298	-0.30	UNCHANGED
Ce_fissilis.011006.1	6	11995.08	40S ribosomal protein S12	127362	134810	143378	114817	128286	106871	135183	116658	0.0758	-0.21	UNCHANGED
Ce_fissilis.015424.1	10	11852.73	Cytochrome b6-f complex iron-sulfur subunit, chloroplastic	95532	104697	113278	53106	79750	77012	104502	69956	0.0251	-0.58	UNCHANGED
Ce_fissilis.013428.3	16	11739.50	Delta-1-pyrroline-5-carboxylate dehydrogenase 12A1, mitochondrial	81157	82938	84497	66798	67358	66169	82864	66775	0.0001	-0.31	UNCHANGED
Ce_fissilis.006747.1	6	11598.04	Stem-specific protein TSJT1	90864	85083	85499	71815	65526	73319	87149	70220	0.0050	-0.31	UNCHANGED
Ce_fissilis.009906.2	28	11510.51	UTP--glucose-1-phosphate uridylyltransferase	150194	159856	158622	141601	147467	136130	156224	141733	0.0315	-0.14	UNCHANGED
Ce_fissilis.007253.1	29	11443.78	RuBisCO large subunit-binding protein subunit alpha, chloroplastic	189907	200064	198913	161231	181639	177052	196295	173307	0.0299	-0.18	UNCHANGED
Ce_fissilis.015546.1	18	11361.43	Calreticulin-1	160081	163120	167891	144500	154790	117998	163697	139096	0.0929	-0.23	UNCHANGED
Ce_fissilis.001833.1	31	11151.08	Chaperonin 60 subunit beta 1, chloroplastic	192033	201863	201892	134573	141144	128424	198596	134714	0.0002	-0.56	UNCHANGED
Ce_fissilis.011946.2	8	11076.54	Acidic endochitinase	209054	214553	208470	217432	194507	228199	210693	213379	0.8038	0.02	UNCHANGED
Ce_fissilis.013428.1	12	11033.86	Probable aldehyde dehydrogenase	67100	70417	76976	67182	71168	73159	71498	70503	0.7839	-0.02	UNCHANGED
Ce_fissilis.000745.1	13	10932.03	Alpha-galactosidase 1	87539	87826	100404	111987	115959	113907	91923	113951	0.0074	0.31	UNCHANGED
Ce_fissilis.009947.2	8	10571.43	Probable NAD(P)H dehydrogenase (quinone) FQR1-like 1	155876	154795	169959	127274	154424	127058	160210	136252	0.0809	-0.23	UNCHANGED
Ce_fissilis.015325.3	7	10554.73	MLP-like protein 31	154399	150676	158987	156658	161836	133596	154687	150697	0.6806	-0.04	UNCHANGED
Ce_fissilis.001535.1	4	10402.59	Cold shock domain-containing protein 4	87940	96382	102653	100572	107758	99662	95658	102664	0.2316	0.10	UNCHANGED
Ce_fissilis.004732.1	21	10326.80	Aldehyde dehydrogenase family 2 member B7, mitochondrial	184031	188200	193049	244013	238170	223181	188427	235121	0.0023	0.32	UNCHANGED
Ce_fissilis.001324.1	10	9916.13	Probable phospholipid hydroperoxide glutathione peroxidase	231723	250030	274621	307835	320906	265262	252125	298001	0.0931	0.24	UNCHANGED
Ce_fissilis.016614.1	3	9830.97	60S acidic ribosomal protein P1-1	62886	54652	54631	58806	47919	32220	57390	46315	0.2478	-0.31	UNCHANGED
Ce_fissilis.010970.1	13	9806.59	Elongation factor 1-delta	128520	127493	134848	116296	145129	134984	130287	132136	0.8430	0.02	UNCHANGED

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Ce_fissilis.005796.1	5	9735.20	Uncharacterized protein At2g37660, chloroplastic	75865	82872	86649	72574	75635	70559	81796	72923	0.0637	-0.17	UNCHANGED
Ce_fissilis.015021.1	3	9584.43	Glycine-rich RNA-binding protein 3, mitochondrial	101956	107571	107027	137604	112551	116326	105518	122161	0.1060	0.21	UNCHANGED
Ce_fissilis.017791.1	11	9569.32	Uncharacterized isomerase BH0283	77063	83901	97093	97468	109865	104885	86019	104073	0.0589	0.27	UNCHANGED
Ce_fissilis.010535.1	12	9549.91	14-3-3 protein 4	81694	88403	96767	93041	94243	82601	88955	89962	0.8688	0.02	UNCHANGED
Ce_fissilis.002621.1	9	9470.61	Glutathione S-transferase F9	112249	105906	91589	86976	90269	84169	103248	87138	0.0644	-0.24	UNCHANGED
Ce_fissilis.012685.1	23	9425.28	Protein disulfide-isomerase	133828	133846	142267	155044	153518	128880	136647	145814	0.3627	0.09	UNCHANGED
Ce_fissilis.019038.1	11	9411.33	Peroxidase 4	170055	154523	137309	163529	182511	169552	153962	171864	0.1787	0.16	UNCHANGED
Ce_fissilis.010199.1	11	9067.33	Basic secretory protease	85802	95231	104427	109792	106173	103099	95153	106355	0.1215	0.16	UNCHANGED
Ce_fissilis.013030.1	8	9049.64	Chitinase 4	58396	77428	95242	70689	29694	69677	77022	56686	0.3023	-0.44	UNCHANGED
Ce_fissilis.001259.1	19	8784.40	Elongation factor Tu, chloroplastic	258183	263465	259582	272117	166829	240294	260410	226413	0.3373	-0.20	UNCHANGED
Ce_fissilis.018863.1	11	8756.90	Thiol protease aleurain	65066	80222	87389	126474	98381	95258	77559	106704	0.0706	0.46	UNCHANGED
Ce_fissilis.017963.1	17	8666.98	Selenium-binding protein 2	122885	131940	148197	106866	112263	97188	134341	105439	0.0285	-0.35	UNCHANGED
Ce_fissilis.012710.1	7	8664.35	Peroxiredoxin-2	94328	111589	121662	152506	157899	137007	109193	149138	0.0170	0.45	UNCHANGED
Ce_fissilis.016893.2	20	8621.48	Probable UDP-arabinopyranose mutase 2	73197	75176	78198	79764	68838	64671	75524	71091	0.4017	-0.09	UNCHANGED
Ce_fissilis.012992.1	13	8568.64	Proteasome subunit alpha type-2-A	107653	107440	116872	131244	159365	143373	110655	144661	0.0175	0.39	UNCHANGED
Ce_fissilis.013029.1	10	8517.44	Chitinase 4	40600	79161	110594	132352	103043	119489	76785	118295	0.1315	0.62	UNCHANGED
Ce_fissilis.018166.1	10	8425.13	CBS domain-containing protein CBSX3, mitochondrial	116497	116322	108666	137017	123516	114353	113828	124962	0.1905	0.13	UNCHANGED
Ce_fissilis.017826.1	5	8314.59	2-haloacrylate reductase	42890	34493	38557	26036	25460	27136	38647	26211	0.0073	-0.56	UNCHANGED
Ce_fissilis.016026.1	8	8300.11	Endo-1,3	77132	75939	82753	89424	108588	98534	78608	98849	0.0268	0.33	UNCHANGED
Ce_fissilis.014343.1	10	8298.63	Proteasome subunit alpha type-5	66178	69836	72281	99420	115680	87000	69432	100700	0.0212	0.54	UNCHANGED
Ce_fissilis.002694.1	3	8242.47	60S acidic ribosomal protein P0-1	35959	43659	37916	39312	28054	30705	39178	32690	0.1895	-0.26	UNCHANGED
Ce_fissilis.014363.1	13	8180.73	Short-chain dehydrogenase TIC 32, chloroplastic	133401	141298	139569	166322	181484	133798	138089	160535	0.1908	0.22	UNCHANGED
Ce_fissilis.010970.2	9	8180.42	Elongation factor 1-delta	37971	35513	35144	24289	29417	28295	36209	27334	0.0077	-0.41	UNCHANGED
Ce_fissilis.000008.1	2	8167.57	Chlorophyll a-b binding protein 3, chloroplastic	142756	153784	158315	118221	134359	99547	151619	117376	0.0364	-0.37	UNCHANGED
Ce_fissilis.001312.1	16	8147.00	Aldehyde dehydrogenase family 7 member B4	87745	100151	112905	94213	86444	83885	100267	88181	0.2008	-0.19	UNCHANGED
Ce_fissilis.015141.1	14	8071.97	Plastid-lipid-associated protein, chloroplastic	143964	152206	163092	129110	110945	90758	153087	110271	0.0259	-0.47	UNCHANGED
Ce_fissilis.006337.1	6	8063.80	Ras-related protein RABD2a	22141	21992	24418	22415	21716	31602	22850	25244	0.5059	0.14	UNCHANGED
Ce_fissilis.002282.2	15	7992.82	Caffeic acid 3-O-methyltransferase	139372	144291	151540	160856	152238	149632	145068	154242	0.1344	0.09	UNCHANGED
Ce_fissilis.007184.1	5	7947.89	Eukaryotic translation initiation factor 6-2	40017	46362	44801	46045	35353	34306	43727	38568	0.2875	-0.18	UNCHANGED

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Ce_fissilis.010325.2	8	7946.14	Berberine bridge enzyme-like 24	115692	108473	104705	137133	164763	137406	109623	146434	0.0193	0.42	UNCHANGED
Ce_fissilis.002774.1	19	7926.57	Phosphoribulokinase, chloroplastic	192848	250071	288419	224644	218828	221781	243780	221751	0.4727	-0.14	UNCHANGED
Ce_fissilis.003679.1	7	7855.57	Ras-related protein RABE1c	59786	55026	56815	50069	40803	43385	57209	44752	0.0157	-0.35	UNCHANGED
Ce_fissilis.007862.1	5	7844.66	Fasciclin-like arabinogalactan protein 6	231579	243145	271862	211107	201404	219637	248862	210716	0.0434	-0.24	UNCHANGED
Ce_fissilis.016136.1	13	7798.58	Pyruvate dehydrogenase E1 component subunit beta-1, mitochondrial	93531	96074	101415	130329	155274	152915	97007	146173	0.0040	0.59	UNCHANGED
Ce_fissilis.009651.3	18	7788.47	Cysteine synthase, chloroplastic/chromoplastic	48912	51839	58059	34962	39234	35583	52937	36593	0.0056	-0.53	UNCHANGED
Ce_fissilis.016893.1	20	7783.23	Probable UDP-arabinopyranose mutase 1	64971	64327	67998	63766	69879	71436	65766	68360	0.3748	0.06	UNCHANGED
Ce_fissilis.009086.1	7	7739.23	Proteasome subunit beta type-6	76295	79061	88932	91495	89712	93117	81429	91441	0.0648	0.17	UNCHANGED
Ce_fissilis.003035.4	2	7668.05	6,7-dimethyl-8-ribityllumazine synthase, chloroplastic	94218	112559	104472	90143	62217	59867	103749	70742	0.0408	-0.55	UNCHANGED
Ce_fissilis.014541.1	14	7576.45	Adenosine kinase 2	122866	116166	129536	152299	197642	184064	122856	178002	0.0169	0.53	UNCHANGED
Ce_fissilis.016025.1	10	7467.80	Endo-1,3	160132	149124	164682	129083	153207	132024	157979	138105	0.0891	-0.19	UNCHANGED
Ce_fissilis.001130.1	11	7459.40	Fructose-bisphosphate aldolase, cytoplasmic isozyme 1	11062	20433	12286	13569	13679	13730	14593	13659	0.7667	-0.10	UNCHANGED
Ce_fissilis.005239.1	18	7459.15	Alanine aminotransferase 2, mitochondrial	98099	109992	102020	127237	146069	137305	103371	136870	0.0066	0.40	UNCHANGED
Ce_fissilis.011776.1	8	7445.53	Glutelin type-D 1	83336	87948	98081	105549	143301	116449	89789	121766	0.0565	0.44	UNCHANGED
Ce_fissilis.005535.1	7	7361.82	Peroxidase 15	258486	236682	242843	164315	197690	186900	246004	182968	0.0059	-0.43	UNCHANGED
Ce_fissilis.004006.1	11	7302.73	Malate dehydrogenase 1, mitochondrial	72573	72250	83698	56383	67997	62471	76174	62284	0.0511	-0.29	UNCHANGED
Ce_fissilis.008921.1	9	7215.96	Late embryogenesis abundant protein Lea14-A	140877	131866	127614	121953	118885	93364	133452	111401	0.0892	-0.26	UNCHANGED
Ce_fissilis.016894.1	13	7202.92	Probable UDP-arabinopyranose mutase 2	33938	36146	35627	32139	31146	26225	35237	29837	0.0501	-0.24	UNCHANGED
Ce_fissilis.001196.1	11	7175.73	Ras-related protein Rab7	43616	48210	54197	61445	63965	58312	48674	61241	0.0224	0.33	UNCHANGED
Ce_fissilis.002282.1	11	7151.52	Caffeic acid 3-O-methyltransferase	54077	71317	65115	81036	72913	57531	63503	70493	0.4590	0.15	UNCHANGED
Ce_fissilis.004152.1	10	7123.14	Tropinone reductase-like 3	84355	86935	90048	98698	94720	92623	87113	95347	0.0274	0.13	UNCHANGED
Ce_fissilis.002464.1	8	7108.80	Nascent polypeptide-associated complex subunit alpha-like protein	97885	122191	104517	117994	122042	91841	108198	110626	0.8486	0.03	UNCHANGED
Ce_fissilis.004061.1	13	7079.98	Malate dehydrogenase, glyoxysomal	45080	47838	47734	44965	34143	34564	46884	37891	0.0695	-0.31	UNCHANGED
Ce_fissilis.007245.1	9	7022.30	Enoyl-[acyl-carrier-protein] reductase [NADH], chloroplastic	106748	128558	128545	108400	94042	98015	121284	100152	0.0664	-0.28	UNCHANGED
Ce_fissilis.002370.1	18	7000.32	ATP-dependent zinc metalloprotease FTSH 2, chloroplastic	79504	83916	80383	64462	61417	42777	81268	56219	0.0223	-0.53	UNCHANGED
Ce_fissilis.018055.1	16	6966.97	Pectinesterase 3	138652	141671	140510	166326	166347	149035	140278	160570	0.0254	0.19	UNCHANGED
Ce_fissilis.000574.1	5	6960.19	Pathogenesis-related protein PR-4B	58512	63702	75287	95668	84229	93747	65834	91214	0.0141	0.47	UNCHANGED
Ce_fissilis.016182.1	9	6930.16	Tropinone reductase homolog At2g29360	141474	148635	154192	124345	120636	117711	148100	120897	0.0028	-0.29	UNCHANGED

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Ce_fissilis.001268.1	7	6808.53	Putative 4-hydroxy-4-methyl-2-oxoglutarate aldolase 2	111476	92070	124556	154358	172024	165187	109367	163856	0.0071	0.58	UNCHANGED
Ce_fissilis.016683.1	6	6773.37	Aspartyl protease AED3	114426	117259	123496	124850	165644	126446	118394	138980	0.2048	0.23	UNCHANGED
Ce_fissilis.015457.1	9	6726.90	Protein usf	117228	121117	111113	101219	94186	100102	116486	98503	0.0078	-0.24	UNCHANGED
Ce_fissilis.002001.1	15	6662.43	Glutathione reductase, cytosolic	123628	111173	98259	144775	142247	112265	111020	133096	0.1585	0.26	UNCHANGED
Ce_fissilis.008361.1	11	6657.32	Proteasome subunit alpha type-1-B	78390	81153	82620	93202	87685	82115	80721	87667	0.1130	0.12	UNCHANGED
Ce_fissilis.005032.1	18	6553.11	DNA damage-repair/toleration protein DRT102	99460	115392	109313	127078	81867	79419	108055	96121	0.5016	-0.17	UNCHANGED
Ce_fissilis.009699.1	22	6471.23	Chaperonin CPN60-2, mitochondrial	210102	202035	170239	155872	149531	134238	194125	146547	0.0259	-0.41	UNCHANGED
Ce_fissilis.016317.1	11	6440.24	Glyoxylate/succinic semialdehyde reductase 1	52803	60074	59165	35709	53529	41710	57347	43649	0.0745	-0.39	UNCHANGED
Ce_fissilis.009062.1	3	6357.10	Probable xyloglucan endotransglucosylase/hydrolase protein 23	44832	44446	49572	44302	52621	50936	46283	49287	0.3773	0.09	UNCHANGED
Ce_fissilis.016305.1	12	6313.06	Putative lactoylglutathione lyase	133341	139377	140482	119721	155940	116205	137733	130622	0.6106	-0.08	UNCHANGED
Ce_fissilis.018219.2	14	6200.87	2-alkenal reductase (NADP(+)-dependent)	82418	90417	98304	97587	96469	91615	90380	95224	0.3822	0.08	UNCHANGED
Ce_fissilis.017980.1	9	6129.77	Proteasome subunit beta type-5-B	50577	48422	45705	57920	63174	56684	48235	59259	0.0107	0.30	UNCHANGED
Ce_fissilis.016597.1	4	6041.94	Probable L-gulonolactone oxidase 6	19581	16426	15072	26264	26687	18540	17026	23831	0.0835	0.49	UNCHANGED
Ce_fissilis.016375.2	6	6002.76	Glycine-rich RNA-binding protein	73864	82468	101289	93783	73415	83352	85874	83516	0.8253	-0.04	UNCHANGED
Ce_fissilis.002720.1	8	5896.88	40S ribosomal protein SA	91013	94407	96551	66996	81185	65664	93990	71282	0.0122	-0.40	UNCHANGED
Ce_fissilis.017839.2	12	5838.66	2-keto-3-deoxy-L-rhamnonate aldolase	115159	121376	119574	104503	99479	102352	118703	102111	0.0021	-0.22	UNCHANGED
Ce_fissilis.012930.1	14	5757.93	Malate dehydrogenase, chloroplastic	74296	79987	91112	71050	87848	90141	81798	83013	0.8836	0.02	UNCHANGED
Ce_fissilis.016375.3	6	5735.42	Glycine-rich RNA-binding protein GRP1A	54733	61108	75054	92413	72343	82134	63631	82297	0.0889	0.37	UNCHANGED
Ce_fissilis.003226.1	16	5666.07	Ketol-acid reductoisomerase, chloroplastic	129515	128559	122211	107011	103261	95260	126762	101844	0.0039	-0.32	UNCHANGED
Ce_fissilis.012678.1	16	5665.35	Pyruvate kinase, cytosolic isozyme	181493	143786	164549	103057	127636	100887	163276	110527	0.0191	-0.56	UNCHANGED
Ce_fissilis.018219.3	8	5544.32	2-alkenal reductase (NADP(+)-dependent)	75808	80916	85709	109296	118403	103336	80811	110345	0.0048	0.45	UNCHANGED
Ce_fissilis.003525.1	2	5460.69	Ras-related protein RABD2a	55625	54665	62167	44143	56108	49053	57486	49768	0.1398	-0.21	UNCHANGED
Ce_fissilis.016815.1	7	5456.44	Stress-response A/B barrel domain-containing protein UP3	81348	87107	97753	67052	69182	67137	88736	67791	0.0125	-0.39	UNCHANGED
Ce_fissilis.017536.1	10	5333.58	Protein DJ-1 homolog D	124917	133152	138169	166014	178778	170492	132079	171761	0.0018	0.38	UNCHANGED
Ce_fissilis.017834.2	8	5258.22	Bifunctional epoxide hydrolase 2	59451	62987	68856	85731	75753	71984	63765	77823	0.0464	0.29	UNCHANGED
Ce_fissilis.003571.1	6	5236.26	MLP-like protein 43	84679	76030	90170	58640	68797	57862	83626	61766	0.0157	-0.44	UNCHANGED
Ce_fissilis.017423.1	2	5176.14	Cytochrome b559 subunit alpha	292055	326914	328741	290351	306670	167931	315903	254984	0.2506	-0.31	UNCHANGED
Ce_fissilis.014222.1	25	5158.91	Stromal 70 kDa heat shock-related protein, chloroplastic	59533	55349	58584	67216	72017	64581	57822	67938	0.0159	0.23	UNCHANGED

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Ce_fissilis.000080.1	17	5146.38	6-phosphogluconate dehydrogenase, decarboxylating 2	63218	71625	47969	48905	38988	31200	60937	39698	0.0692	-0.62	UNCHANGED
Ce_fissilis.008398.2	8	5117.54	Tubulin alpha-3 chain	27665	31127	35271	22108	28712	24359	31354	25060	0.0982	-0.32	UNCHANGED
Ce_fissilis.013697.2	5	5086.18	Photosynthetic NDH subunit of lumenal location 5, chloroplastic	82043	85984	88725	104795	76291	89899	85584	90328	0.6047	0.08	UNCHANGED
Ce_fissilis.003688.1	7	5042.04	6-phosphogluconate dehydrogenase, decarboxylating 1	34657	36512	31872	35701	23798	26433	34347	28644	0.2130	-0.26	UNCHANGED
Ce_fissilis.009745.2	17	5008.49	Probable cinnamyl alcohol dehydrogenase 1	56969	60006	69799	81713	95324	86775	62258	87938	0.0098	0.50	UNCHANGED
Ce_fissilis.003917.1	16	4943.88	Quinone oxidoreductase PIG3	72102	75241	79584	89845	113522	85849	75642	96405	0.0801	0.35	UNCHANGED
Ce_fissilis.005087.1	4	4919.78	Glutaredoxin	51406	55568	68046	40454	44971	40922	58340	42115	0.0355	-0.47	UNCHANGED
Ce_fissilis.007805.1	4	4895.79	Nucleoside diphosphate kinase 2, chloroplastic	37293	40512	43867	32674	30973	29702	40557	31116	0.0106	-0.38	UNCHANGED
Ce_fissilis.001679.1	9	4844.60	3-isopropylmalate dehydratase small subunit 3	46190	48422	53517	60626	52355	51030	49376	54670	0.2261	0.15	UNCHANGED
Ce_fissilis.010338.1	12	4804.20	Aspartyl protease AED3	93586	99139	118769	123037	122610	146729	103831	130792	0.0710	0.33	UNCHANGED
Ce_fissilis.007698.1	13	4729.33	Methylmalonate-semialdehyde dehydrogenase [acylating], mitochondrial	108118	97773	110021	113192	119635	103290	105304	112039	0.3307	0.09	UNCHANGED
Ce_fissilis.018333.1	6	4718.63	2-alkenal reductase (NADP(+)-dependent)	66580	73395	85013	77349	95663	95154	74996	89389	0.1493	0.25	UNCHANGED
Ce_fissilis.009745.1	14	4681.35	Cinnamyl alcohol dehydrogenase 1	88992	92818	102281	93515	102169	88309	94697	94664	0.9956	0.00	UNCHANGED
Ce_fissilis.005115.2	4	4678.39	Ubiquitin-60S ribosomal protein L40	100026	104802	111685	104458	103120	98285	105504	101955	0.4108	-0.05	UNCHANGED
Ce_fissilis.018295.1	7	4671.38	Putative allene oxide cyclase	89038	89411	90711	121051	129644	134510	89720	128402	0.0006	0.52	UNCHANGED
Ce_fissilis.011298.1	7	4601.87	Thioredoxin superfamily protein	70520	72816	85561	87072	92509	84312	76299	87964	0.0909	0.21	UNCHANGED
Ce_fissilis.013598.1	18	4586.33	D-3-phosphoglycerate dehydrogenase 1, chloroplastic	74181	69837	93491	60106	64442	55141	79170	59896	0.0677	-0.40	UNCHANGED
Ce_fissilis.000738.1	4	4582.80	Plasma membrane-associated cation-binding protein 1	88641	88734	81576	76258	79659	70862	86317	75593	0.0372	-0.19	UNCHANGED
Ce_fissilis.007829.1	8	4579.44	Plastid-lipid-associated protein 6, chloroplastic	69710	71187	85864	68360	61084	55687	75587	61710	0.0935	-0.29	UNCHANGED
Ce_fissilis.010672.2	8	4574.26	Superoxide dismutase [Mn], mitochondrial	84880	91075	94344	84949	68886	71468	90100	75101	0.0581	-0.26	UNCHANGED
Ce_fissilis.012598.1	13	4559.39	NADPH-dependent alkenal/one oxidoreductase, chloroplastic	65969	64782	68717	56888	61543	40493	66490	52975	0.1057	-0.33	UNCHANGED
Ce_fissilis.011057.1	7	4478.11	Enoyl-[acyl-carrier-protein] reductase [NADH], chloroplastic	81401	84489	87072	72829	50970	70386	84321	64728	0.0510	-0.38	UNCHANGED
Ce_fissilis.009651.1	13	4465.04	Cysteine synthase, chloroplastic/chromoplastic	29364	30859	33700	21618	23421	24484	31308	23175	0.0059	-0.43	UNCHANGED
Ce_fissilis.016930.1	11	4394.12	Fumarylacetoacetase	56888	59322	60049	73544	62322	65153	58753	67006	0.0780	0.19	UNCHANGED
Ce_fissilis.017019.1	12	4335.93	14-3-3-like protein	24872	24459	30158	31148	29180	31736	26496	30688	0.1030	0.21	UNCHANGED
Ce_fissilis.019047.1	2	4328.50	PLAT domain-containing protein 2	69124	68832	76252	85416	98015	88910	71403	90780	0.0123	0.35	UNCHANGED
Ce_fissilis.015261.1	10	4216.10	Heat shock 70 kDa protein	66410	62149	68113	63257	79271	69436	65557	70655	0.3646	0.11	UNCHANGED

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Ce_fissilis.015561.1	8	4186.77	Proteasome subunit beta type-2-A	86856	100409	103438	94816	98640	94740	96901	96065	0.8814	-0.01	UNCHANGED
Ce_fissilis.001378.1	9	4162.09	Mitochondrial import receptor subunit TOM40-1	80517	83079	85463	78550	89742	75895	83019	81396	0.7352	-0.03	UNCHANGED
Ce_fissilis.016186.1	10	4159.35	Adenosine kinase 2	91365	79193	72656	77218	83909	88931	81071	83353	0.7413	0.04	UNCHANGED
Ce_fissilis.018219.1	9	4144.22	2-alkenal reductase (NADP(+)-dependent)	23466	26079	28539	28112	32206	31426	26028	30581	0.0776	0.23	UNCHANGED
Ce_fissilis.007403.1	13	4132.93	Probable fructokinase-6, chloroplastic	74802	68712	77272	59922	52672	54513	73596	55702	0.0059	-0.40	UNCHANGED
Ce_fissilis.013077.1	15	4103.43	Aspartic proteinase A1	59605	60202	64072	73465	44078	53133	61293	56892	0.6433	-0.11	UNCHANGED
Ce_fissilis.016024.1	10	4099.23	Class V chitinase	117452	130690	138772	169476	185790	190000	128971	181756	0.0039	0.49	UNCHANGED
Ce_fissilis.003482.1	5	4021.10	Endochitinase	34682	50101	82426	91348	83323	109964	55736	94879	0.0722	0.77	UNCHANGED
Ce_fissilis.011945.1	11	3997.40	Succinate--CoA ligase [ADP-forming] subunit beta, mitochondrial	113468	116706	133163	137810	151264	136076	121112	141717	0.0567	0.23	UNCHANGED
Ce_fissilis.000472.1	5	3976.27	Putative ATP synthase protein YMF19	92004	96957	101102	75344	54178	65145	96688	64889	0.0088	-0.58	UNCHANGED
Ce_fissilis.007218.1	3	3976.11	Kunitz trypsin inhibitor 2	36533	42007	47804	48910	46238	49128	42115	48092	0.1521	0.19	UNCHANGED
Ce_fissilis.006549.1	4	3920.42	Elicitor-responsive protein 3	25107	22786	22726	19752	24300	13945	23540	19333	0.2459	-0.28	UNCHANGED
Ce_fissilis.001213.1	6	3900.45	GDSL esterase/lipase 2	54564	55547	54131	52260	63370	59185	54748	58272	0.3413	0.09	UNCHANGED
Ce_fissilis.018656.1	13	3896.69	Aspartate aminotransferase, cytoplasmic	58595	61090	56587	90819	89430	79529	58758	86593	0.0018	0.56	UNCHANGED
Ce_fissilis.005415.1	11	3875.32	Glycerophosphodiester phosphodiesterase GDPDL3	125987	126807	127392	106310	109968	92567	126729	102948	0.0110	-0.30	UNCHANGED
Ce_fissilis.001060.1	6	3835.84	GTP-binding protein SAR1A	32032	35798	34878	28226	25871	23302	34236	25800	0.0097	-0.41	UNCHANGED
Ce_fissilis.007536.1	19	3817.77	Heat shock 70 kDa protein, mitochondrial	162057	158578	151336	208680	195248	184102	157324	196010	0.0076	0.32	UNCHANGED
Ce_fissilis.017138.1	8	3771.46	Protein STRICTOSIDINE SYNTHASE-LIKE 4	89820	90167	98216	93083	96053	86021	92734	91719	0.8142	-0.02	UNCHANGED
Ce_fissilis.009062.2	3	3760.96	Probable xyloglucan endotransglucosylase/hydrolase protein 25	57676	68821	75967	36291	22409	60829	67488	39843	0.0902	-0.76	UNCHANGED
Ce_fissilis.000685.1	16	3748.81	Dihydroxy-acid dehydratase, chloroplastic	106567	107898	108395	71278	85705	75786	107620	77590	0.0022	-0.47	UNCHANGED
Ce_fissilis.001041.1	6	3748.17	Peptidyl-prolyl cis-trans isomerase CYP19-3	33664	39786	39936	30346	31278	29787	37795	30470	0.0256	-0.31	UNCHANGED
Ce_fissilis.010088.1	5	3737.78	GDSL esterase/lipase At5g45920	41848	44938	48032	39883	43316	34883	44939	39360	0.1394	-0.19	UNCHANGED
Ce_fissilis.009542.1	10	3694.39	Gamma carbonic anhydrase 1, mitochondrial	46014	46720	52557	58584	68765	75556	48430	67635	0.0230	0.48	UNCHANGED
Ce_fissilis.003446.1	9	3684.90	Mitochondrial outer membrane protein porin of 36 kDa	53803	57333	62411	79489	95849	78835	57849	84724	0.0116	0.55	UNCHANGED
Ce_fissilis.006144.1	4	3631.89	Fasciclin-like arabinogalactan protein 1	71355	76594	81915	52622	64720	55514	76622	57619	0.0162	-0.41	UNCHANGED
Ce_fissilis.017211.1	2	3624.08	Pathogenesis-related protein 1	45014	22073	24604	13937	16128	24840	30564	18302	0.1996	-0.74	UNCHANGED
Ce_fissilis.016289.1	6	3607.44	Proteasome subunit alpha type-3	60256	60877	57514	49546	53966	55020	59549	52844	0.0272	-0.17	UNCHANGED
Ce_fissilis.004297.1	5	3597.70	(+)-neomenthol dehydrogenase	12407	13345	14683	22159	20414	13120	13478	18564	0.1485	0.46	UNCHANGED

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Ce_fissilis.014568.1	3	3596.77	60S acidic ribosomal protein P2B	34826	32929	42052	45298	77576	43676	36603	55517	0.1720	0.60	UNCHANGED
Ce_fissilis.011918.1	7	3554.14	Cathepsin B-like protease 2	71606	72483	78340	66403	77436	71791	74143	71877	0.5852	-0.04	UNCHANGED
Ce_fissilis.005482.1	4	3514.17	ATP synthase subunit delta', mitochondrial	50617	64588	68488	98461	74445	37948	61231	70285	0.6486	0.20	UNCHANGED
Ce_fissilis.017755.1	3	3488.34	Proteasome subunit beta type-7-B	29540	31133	32070	31753	20622	22427	30914	24934	0.1652	-0.31	UNCHANGED
Ce_fissilis.006288.1	5	3464.55	Protein transport protein SEC13 homolog B	35333	30820	35541	28433	32785	39789	33898	33669	0.9530	-0.01	UNCHANGED
Ce_fissilis.017339.1	8	3450.80	Glutathione S-transferase F6	46411	43152	37375	38201	37206	29962	42313	35123	0.1242	-0.27	UNCHANGED
Ce_fissilis.006370.1	6	3447.64	Cysteine proteinase 15A	85809	67600	76818	59726	71149	72625	76743	67833	0.2515	-0.18	UNCHANGED
Ce_fissilis.018172.1	9	3425.34	Proteasome subunit alpha type-6	64884	61754	58476	54527	48663	49626	61705	50939	0.0142	-0.28	UNCHANGED
Ce_fissilis.009977.1	13	3423.42	Glyceraldehyde-3-phosphate dehydrogenase A, chloroplastic	109628	107123	106654	104363	95083	84241	107801	94562	0.0878	-0.19	UNCHANGED
Ce_fissilis.001988.1	11	3390.18	Probable 6-phosphogluconolactonase 4, chloroplastic	53814	46295	50706	59731	62379	62830	50271	61647	0.0089	0.29	UNCHANGED
Ce_fissilis.019066.2	14	3387.05	Gamma aminobutyrate transaminase 1, mitochondrial	54631	52954	42235	43126	42890	47512	49940	44509	0.2621	-0.17	UNCHANGED
Ce_fissilis.008517.2	10	3374.35	Metalloendoproteinase 3-MMP	70510	68360	74808	58454	60468	74375	71226	64433	0.2731	-0.14	UNCHANGED
Ce_fissilis.004237.1	12	3350.50	Transaldolase	50590	57109	53449	62007	48887	41477	53716	50790	0.6661	-0.08	UNCHANGED
Ce_fissilis.013797.1	3	3335.32	Probable aldo-keto reductase 1	54853	52156	41270	42489	35156	57523	49427	45056	0.6044	-0.13	UNCHANGED
Ce_fissilis.016813.1	7	3328.48	Gamma carbonic anhydrase 1, mitochondrial	21550	21779	27343	21011	20938	22417	23558	21455	0.3426	-0.13	UNCHANGED
Ce_fissilis.000498.1	12	3323.63	2-methylacyl-CoA dehydrogenase, mitochondrial	45986	43529	48544	54164	66923	62963	46020	61350	0.0192	0.41	UNCHANGED
Ce_fissilis.013227.1	18	3285.33	Polyadenylate-binding protein 2	52738	74754	55254	56178	62807	56546	60915	58510	0.7578	-0.06	UNCHANGED
Ce_fissilis.005619.1	12	3258.05	Beta-glucosidase BoGH3B	85773	79823	76306	64360	64206	61995	80634	63520	0.0040	-0.34	UNCHANGED
Ce_fissilis.018908.1	9	3252.32	Bifunctional L-3-cyanoalanine synthase/cysteine synthase D1	35700	38291	42361	31913	29435	23114	38784	28154	0.0310	-0.46	UNCHANGED
Ce_fissilis.017753.1	3	3191.01	Isoflavone reductase homolog TP7	20301	20176	21343	7970	22259	20156	20606	16795	0.4418	-0.30	UNCHANGED
Ce_fissilis.002549.1	5	3181.57	Tropinone reductase homolog	92284	97616	97739	74559	69425	63566	95880	69183	0.0019	-0.47	UNCHANGED
Ce_fissilis.018888.1	4	3165.90	Thioredoxin M4, chloroplastic	57545	50474	63793	45420	46609	37573	57271	43201	0.0422	-0.41	UNCHANGED
Ce_fissilis.011805.1	7	3132.19	NADH-cytochrome b5 reductase-like protein	36285	38667	39323	35061	38546	33638	38092	35748	0.2459	-0.09	UNCHANGED
Ce_fissilis.011909.2	11	3132.04	Glutamate-1-semialdehyde 2,1-aminomutase, chloroplastic	40021	44239	47870	31376	37420	37882	44043	35559	0.0515	-0.31	UNCHANGED
Ce_fissilis.013698.1	5	3091.49	Peptidyl-prolyl cis-trans isomerase CYP20-3, chloroplastic	121610	132176	116905	80173	99335	90178	123564	89895	0.0092	-0.46	UNCHANGED
Ce_fissilis.005744.1	3	3028.55	3-hydroxyacyl-[acyl-carrier-protein] dehydratase FabZ	66354	68235	74665	62782	34037	51670	69751	49497	0.0813	-0.49	UNCHANGED
Ce_fissilis.012931.1	12	3016.85	Photosystem II stability/assembly factor HCF136, chloroplastic	57441	58021	61536	36211	48236	42755	58999	42400	0.0110	-0.48	UNCHANGED

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Ce_fissilis.012358.1	7	3009.89	26S proteasome non-ATPase regulatory subunit 4 homolog	53318	54338	63456	59270	69506	63205	57037	63994	0.1882	0.17	UNCHANGED
Ce_fissilis.018880.1	3	3008.91	Nectarin-1	95201	100188	113707	125861	103909	135127	103032	121632	0.1596	0.24	UNCHANGED
Ce_fissilis.007375.1	6	2975.60	Branched-chain amino acid aminotransferase 2, chloroplastic	62026	66161	68389	44982	42915	42460	65525	43452	0.0004	-0.59	UNCHANGED
Ce_fissilis.010244.1	4	2943.41	Lactoylglutathione lyase	81265	83483	80071	61391	56809	47133	81606	55111	0.0036	-0.57	UNCHANGED
Ce_fissilis.018774.1	7	2930.59	14-3-3-like protein D	37065	34888	40655	51825	47899	55647	37536	51790	0.0070	0.46	UNCHANGED
Ce_fissilis.015062.2	11	2921.03	Hypersensitive-induced response protein 1	50596	64038	75830	43346	53645	40794	63488	45928	0.1013	-0.47	UNCHANGED
Ce_fissilis.018746.1	9	2898.33	Adenylosuccinate synthetase 2, chloroplastic	56305	57489	60870	39921	48653	33214	58221	40596	0.0196	-0.52	UNCHANGED
Ce_fissilis.018223.1	14	2885.31	6-phosphogluconate dehydrogenase, decarboxylating 3, chloroplastic	49357	45143	41119	27626	33484	49889	45206	37000	0.3106	-0.29	UNCHANGED
Ce_fissilis.002939.2	4	2882.40	Peptide methionine sulfoxide reductase B2, chloroplastic	17810	16200	20798	18544	17798	18056	18270	18133	0.9249	-0.01	UNCHANGED
Ce_fissilis.004964.1	14	2851.52	Tol-Pal system protein TolB	77029	73654	77436	60720	80329	60761	76040	67270	0.2570	-0.18	UNCHANGED
Ce_fissilis.002293.1	8	2838.64	Peroxiredoxin-2F, mitochondrial	54723	60393	71205	80967	99706	78301	62107	86325	0.0432	0.48	UNCHANGED
Ce_fissilis.014022.1	7	2835.28	Succinate--CoA ligase [ADP-forming] subunit alpha-1, mitochondrial	75505	79793	88426	69565	81264	83022	81241	77951	0.5935	-0.06	UNCHANGED
Ce_fissilis.003687.1	4	2829.09	Cyanate hydratase	33109	33285	38518	25998	27424	19758	34971	24393	0.0230	-0.52	UNCHANGED
Ce_fissilis.008924.1	2	2820.59	Late embryogenesis abundant protein, group 2	29692	30218	31351	25983	51825	43078	30420	40295	0.2639	0.41	UNCHANGED
Ce_fissilis.011671.2	4	2812.89	Profilin-4	90287	92916	82415	106160	92668	81954	88540	93594	0.5465	0.08	UNCHANGED
Ce_fissilis.014837.1	12	2799.01	Protochlorophyllide reductase, chloroplastic	69699	68146	62685	50694	44939	54270	66843	49968	0.0081	-0.42	UNCHANGED
Ce_fissilis.010897.1	4	2793.56	Heme-binding protein 2	50838	51389	60568	31472	51751	46614	54265	43279	0.1843	-0.33	UNCHANGED
Ce_fissilis.002523.1	8	2761.38	Basic 7S globulin	58876	68498	77484	52090	63439	51163	68286	55564	0.1290	-0.30	UNCHANGED
Ce_fissilis.005981.1	12	2756.78	Epoxide hydrolase A	48341	57906	69582	58746	46183	57591	58610	54173	0.5779	-0.11	UNCHANGED
Ce_fissilis.018743.1	3	2707.55	Peptidyl-prolyl cis-trans isomerase FKBP12	33324	37588	27937	33698	27641	42352	32950	34563	0.7676	0.07	UNCHANGED
Ce_fissilis.017714.1	17	2685.67	Succinate-semialdehyde dehydrogenase, mitochondrial	87196	77767	76065	65467	78241	66045	80343	69918	0.1265	-0.20	UNCHANGED
Ce_fissilis.004017.1	5	2678.72	Ras-related protein Rab7	11657	11728	12831	14165	11986	13599	12072	13250	0.1939	0.13	UNCHANGED
Ce_fissilis.014710.1	7	2651.42	Probable nitronate monooxygenase	51137	55491	58557	49448	49354	49875	55062	49559	0.0634	-0.15	UNCHANGED
Ce_fissilis.003264.1	10	2611.09	Formate--tetrahydrofolate ligase	66507	73308	74271	62696	80351	69384	71362	70811	0.9275	-0.01	UNCHANGED
Ce_fissilis.013307.1	6	2609.34	Glutamine synthetase nodule isozyme	19477	18492	20483	17114	23427	28679	19484	23073	0.3497	0.24	UNCHANGED
Ce_fissilis.012378.2	7	2597.74	Mitochondrial carnitine/acylcarnitine carrier-like protein	20057	34135	22392	20013	42071	33591	25528	31892	0.4583	0.32	UNCHANGED
Ce_fissilis.004356.1	11	2589.70	Secoisolariciresinol dehydrogenase	46844	50607	63315	81020	72502	68994	53589	74172	0.0284	0.47	UNCHANGED

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Ce_fissilis.005689.1	17	2586.55	LL-diaminopimelate aminotransferase, chloroplastic	54771	50203	50936	43351	38113	33878	51970	38447	0.0118	-0.43	UNCHANGED
Ce_fissilis.006429.1	6	2582.16	Aconitate hydratase 2, mitochondrial	11207	10257	7482	12456	17413	12013	9649	13961	0.1045	0.53	UNCHANGED
Ce_fissilis.004297.2	3	2571.42	(+)-neomenthol dehydrogenase	23118	25956	30038	17594	20025	30625	26371	22748	0.4638	-0.21	UNCHANGED
Ce_fissilis.012740.1	13	2557.55	T-complex protein 1 subunit theta	58826	53230	50349	44181	37478	31849	54135	37836	0.0200	-0.52	UNCHANGED
Ce_fissilis.007214.1	14	2556.64	Beta-galactosidase 9	71623	97374	96984	99919	108997	84042	88660	97652	0.4676	0.14	UNCHANGED
Ce_fissilis.014602.1	6	2546.91	Isocitrate dehydrogenase [NAD] catalytic subunit 5, mitochondrial	40006	33244	30935	23988	25234	28071	34729	25764	0.0395	-0.43	UNCHANGED
Ce_fissilis.000840.1	8	2546.02	Acetylornithine deacetylase	55214	50461	54353	44496	54379	47324	53343	48733	0.2329	-0.13	UNCHANGED
Ce_fissilis.011103.1	3	2541.06	Expansin-like A1	43623	42659	50707	38310	44412	34906	45663	39209	0.1616	-0.22	UNCHANGED
Ce_fissilis.015745.1	8	2523.39	Protein of unknown function, DUF642	58602	61773	63056	62492	61658	67727	61144	63959	0.2908	0.06	UNCHANGED
Ce_fissilis.018441.1	17	2511.39	Dipeptidyl aminopeptidase BIII	62949	60647	66306	75355	72655	80095	63301	76035	0.0095	0.26	UNCHANGED
Ce_fissilis.004789.1	9	2505.98	S-adenosylmethionine synthase 1	45560	48630	30967	37152	30024	28963	41719	32046	0.1837	-0.38	UNCHANGED
Ce_fissilis.004204.1	10	2502.27	Alcohol dehydrogenase-like 7	106987	113509	121785	93465	98014	96066	114094	95848	0.0152	-0.25	UNCHANGED
Ce_fissilis.000218.1	3	2450.32	-	23157	25217	20105	30628	38577	31813	22826	33673	0.0198	0.56	UNCHANGED
Ce_fissilis.017338.1	10	2426.74	Mitochondrial outer membrane protein porin 4	44340	49087	55283	70908	80685	73729	49570	75107	0.0040	0.60	UNCHANGED
Ce_fissilis.001601.1	10	2422.32	Mitochondrial dicarboxylate/tricarboxylate transporter DTC	60976	60860	71458	56772	67385	65245	64431	63134	0.7994	-0.03	UNCHANGED
Ce_fissilis.000235.1	8	2408.59	UDP-glucose 6-dehydrogenase 1	11072	11525	10853	18135	20310	9568	11150	16004	0.2135	0.52	UNCHANGED
Ce_fissilis.006783.1	11	2395.76	Betaine aldehyde dehydrogenase 1, chloroplastic	44061	44927	45875	41008	42729	33303	44954	39013	0.1138	-0.20	UNCHANGED
Ce_fissilis.018195.2	3	2338.37	Probable 3-hydroxyisobutyrate dehydrogenase, mitochondrial	25807	27602	29588	35728	22749	29373	27666	29283	0.6998	0.08	UNCHANGED
Ce_fissilis.011761.2	5	2335.39	Ras-related protein RABH1b	18529	18904	21643	19429	16361	16821	19692	17537	0.1907	-0.17	UNCHANGED
Ce_fissilis.016121.1	5	2316.84	Enoyl-CoA delta isomerase 3	45843	47097	53893	82672	57628	48604	48944	62968	0.2523	0.36	UNCHANGED
Ce_fissilis.014316.2	4	2313.27	-	22016	23444	28939	41876	28005	34405	24800	34762	0.0927	0.49	UNCHANGED
Ce_fissilis.000463.1	7	2310.29	Mitochondrial phosphate carrier protein 3, mitochondrial	56081	44129	99267	78648	69723	77464	66492	75278	0.6321	0.18	UNCHANGED
Ce_fissilis.015839.1	8	2308.95	Subtilisin-like protease SBT1.4	48673	50117	46761	34442	32376	39164	48517	35327	0.0041	-0.46	UNCHANGED
Ce_fissilis.014757.1	8	2303.25	Isocitrate dehydrogenase [NAD] regulatory subunit 1, mitochondrial	31133	31123	31529	32799	25765	32705	31262	30423	0.7374	-0.04	UNCHANGED
Ce_fissilis.011572.1	8	2274.86	Dihydrolipoyllysine-residue acetyltransferase component 4 of pyruvate dehydrogenase complex, chloroplastic	36328	38862	41925	35143	41980	30189	39038	35771	0.4363	-0.13	UNCHANGED
Ce_fissilis.014984.2	6	2257.94	S-formylglutathione hydrolase	36147	34467	39044	29962	27218	22674	36553	26618	0.0167	-0.46	UNCHANGED
Ce_fissilis.009612.1	21	2252.51	Probable alpha-mannosidase At5g13980	54732	58042	58068	68178	76489	68627	56947	71098	0.0083	0.32	UNCHANGED

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Ce_fissilis.008229.1	8	2209.79	Glyceraldehyde-3-phosphate dehydrogenase GAPCP1, chloroplastic	22150	19931	21373	19429	12217	10567	21151	14071	0.0646	-0.59	UNCHANGED
Ce_fissilis.009374.1	4	2209.06	LysM domain-containing GPI-anchored protein 2	37810	39405	43854	52933	35680	38098	40356	42237	0.7575	0.07	UNCHANGED
Ce_fissilis.014143.1	9	2190.27	Porphobilinogen deaminase, chloroplastic	59812	74083	68895	49584	54208	48368	67597	50720	0.0204	-0.41	UNCHANGED
Ce_fissilis.003225.1	7	2177.83	Carbonic anhydrase, chloroplastic	123822	129644	126268	111751	116023	93623	126578	107133	0.0514	-0.24	UNCHANGED
Ce_fissilis.011254.2	7	2164.52	3-oxoacyl-[acyl-carrier-protein] synthase II, chloroplastic	29793	30477	31612	21025	20653	22123	30627	21267	0.0002	-0.53	UNCHANGED
Ce_fissilis.009533.1	4	2156.85	Universal stress protein A-like protein	30054	22710	18334	25453	12287	17284	23699	18341	0.3560	-0.37	UNCHANGED
Ce_fissilis.015110.1	4	2130.68	Cytochrome b5 isoform E	19768	24080	24793	16704	17896	19322	22880	17974	0.0481	-0.35	UNCHANGED
Ce_fissilis.016779.1	7	2128.73	Rhodanese-like domain-containing protein 4, chloroplastic	41986	47338	47356	36069	40760	35005	45560	37278	0.0301	-0.29	UNCHANGED
Ce_fissilis.018164.1	2	2122.01	Cyclase-like protein 2	35461	34226	42818	26963	30870	34466	37502	30766	0.1224	-0.29	UNCHANGED
Ce_fissilis.017834.1	6	2093.78	Epoxide hydrolase A	51431	68272	54706	87566	60402	56715	58136	68228	0.4112	0.23	UNCHANGED
Ce_fissilis.013718.1	9	2092.10	Probable 3-hydroxyisobutyrate dehydrogenase-like 1, mitochondrial	59027	49208	59420	43978	42961	48250	55885	45063	0.0435	-0.31	UNCHANGED
Ce_fissilis.013475.1	2	2086.72	S-norcoclaurine synthase 2	49865	52311	55330	65621	60670	60979	52502	62423	0.0116	0.25	UNCHANGED
Ce_fissilis.016105.1	3	2079.31	Ubiquitin-conjugating enzyme E2 36	32594	33350	31900	36108	41238	35054	32615	37466	0.0681	0.20	UNCHANGED
Ce_fissilis.004804.1	5	2025.36	Aromatic aminotransferase ISS1	28539	26009	30406	26966	26994	23233	28318	25731	0.2207	-0.14	UNCHANGED
Ce_fissilis.006751.1	16	2023.82	Alpha-L-arabinofuranosidase 1	55006	53011	56153	50225	56687	58029	54723	54980	0.9254	0.01	UNCHANGED
Ce_fissilis.004604.1	6	2002.70	Protein SRC2	44187	44917	48519	44391	54057	48952	45874	49133	0.3519	0.10	UNCHANGED
Ce_fissilis.014540.1	8	1998.38	Ras-related protein RABB1c	18391	19075	21043	17709	20934	13997	19503	17547	0.4156	-0.15	UNCHANGED
Ce_fissilis.003520.1	8	1996.24	Thiamine thiazole synthase, chloroplastic	30423	32876	35041	23532	29782	18953	32780	24089	0.0634	-0.44	UNCHANGED
Ce_fissilis.003154.2	8	1978.60	4-hydroxy-tetrahydrodipicolinate reductase 2, chloroplastic	25626	28241	30753	27103	35395	35740	28207	32746	0.2276	0.22	UNCHANGED
Ce_fissilis.015643.1	13	1974.35	Aspartate aminotransferase, chloroplastic	38189	36588	35319	29429	27338	23021	36698	26596	0.0080	-0.46	UNCHANGED
Ce_fissilis.003443.1	5	1961.07	Heme-binding-like protein At3g10130, chloroplastic	19232	21087	19716	20254	15592	15690	20012	17179	0.1582	-0.22	UNCHANGED
Ce_fissilis.005711.1	8	1952.85	Protease Do-like 1, chloroplastic	42962	38040	41718	29641	27003	26077	40907	27574	0.0019	-0.57	UNCHANGED
Ce_fissilis.000798.1	7	1933.65	Cinnamoyl-CoA reductase 1	34947	35625	28434	33689	36980	43918	33002	38196	0.2422	0.21	UNCHANGED
Ce_fissilis.009611.1	26	1927.81	Probable alpha-mannosidase At5g13980	51273	58094	54179	74063	78682	74928	54515	75891	0.0009	0.48	UNCHANGED
Ce_fissilis.011070.2	5	1874.45	RNA-binding protein CP29B, chloroplastic	23551	26836	27146	26962	33079	21304	25844	27115	0.7412	0.07	UNCHANGED
Ce_fissilis.016610.2	7	1863.72	12-oxophytodienoate reductase 2	33291	37929	37822	76231	50685	52151	36347	59689	0.0502	0.72	UNCHANGED
Ce_fissilis.018173.1	8	1844.33	Importin subunit alpha-2	33923	35693	36634	25451	32855	36864	35417	31723	0.3430	-0.16	UNCHANGED
Ce_fissilis.004268.1	5	1841.19	Stem-specific protein TSJT1	76651	85841	94230	89367	98370	87096	85574	91611	0.3807	0.10	UNCHANGED

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Ce_fissilis.017944.1	11	1837.71	Alpha-xylosidase 1	113995	117430	110057	129655	128394	106921	113827	121657	0.3655	0.10	UNCHANGED
Ce_fissilis.000393.1	6	1824.77	3-oxoacyl-[acyl-carrier-protein] reductase, chloroplastic	67320	60075	65740	78655	92731	73312	64379	81566	0.0501	0.34	UNCHANGED
Ce_fissilis.012548.1	7	1816.09	Eukaryotic translation initiation factor 3 subunit F	19438	27096	27801	31871	35796	38840	24778	35502	0.0330	0.52	UNCHANGED
Ce_fissilis.002231.1	5	1807.01	-	13917	17372	17062	20265	19785	21020	16117	20357	0.0217	0.34	UNCHANGED
Ce_fissilis.001068.1	16	1789.07	Dihydrolipoyllysine-residue acetyltransferase component 2 of pyruvate dehydrogenase complex, mitochondrial	61977	61331	64003	57248	47200	51528	62437	51992	0.0258	-0.26	UNCHANGED
Ce_fissilis.012496.1	4	1783.98	DNA repair RAD52-like protein 2, chloroplastic	29295	24627	29167	28628	37795	38699	27696	35041	0.1084	0.34	UNCHANGED
Ce_fissilis.004200.1	4	1781.93	2-keto-3-deoxy-L-rhamnonate aldolase	42543	22789	25430	32139	30510	26984	30254	29878	0.9557	-0.02	UNCHANGED
Ce_fissilis.004271.1	9	1779.88	Aldehyde oxidase GLOX1	48273	49113	51386	67433	65126	57655	49591	63405	0.0111	0.35	UNCHANGED
Ce_fissilis.017165.1	6	1773.92	Flowering locus K homology domain	45647	42484	50945	38971	50025	43220	46359	44072	0.6031	-0.07	UNCHANGED
Ce_fissilis.011090.1	3	1762.63	Protein FATTY ACID EXPORT 2, chloroplastic	18738	19095	24454	20110	23945	22782	20762	22279	0.5230	0.10	UNCHANGED
Ce_fissilis.011707.1	4	1756.66	Serine/threonine-protein phosphatase PP2A catalytic subunit	21796	8674	8589	19854	16465	18446	13020	18255	0.3090	0.49	UNCHANGED
Ce_fissilis.007583.1	7	1746.29	Adenine phosphoribosyltransferase 1, chloroplastic	39456	39400	42440	56441	42446	39242	40432	46043	0.3555	0.19	UNCHANGED
Ce_fissilis.012274.1	2	1742.21	Aquaporin PIP2-7	46934	42849	49328	32000	36140	41050	46370	36397	0.0366	-0.35	UNCHANGED
Ce_fissilis.018641.1	4	1741.75	Polyadenylate-binding protein RBP45	37748	42070	45159	42432	34384	43075	41659	39964	0.6558	-0.06	UNCHANGED
Ce_fissilis.008309.1	5	1725.23	Proteasome subunit beta type-3-A	26467	27856	30575	44387	21993	21662	28299	29347	0.8972	0.05	UNCHANGED
Ce_fissilis.017020.1	8	1721.19	Dihydrolipoyllysine-residue acetyltransferase component 5 of pyruvate dehydrogenase complex, chloroplastic	56867	57652	58042	72573	75491	73462	57520	73842	0.0001	0.36	UNCHANGED
Ce_fissilis.009832.1	9	1713.17	Thiosulfate/3-mercaptopyruvate sulfurtransferase 1, mitochondrial	51373	46688	49020	45409	44931	41347	49027	43896	0.0512	-0.16	UNCHANGED
Ce_fissilis.010249.1	6	1711.36	Probable calcium-binding protein CML13	19337	19046	21814	22059	26127	20603	20066	22930	0.2007	0.19	UNCHANGED
Ce_fissilis.018544.1	6	1686.49	Probable fructokinase-7	6734	6069	6945	7232	12145	9749	6583	9709	0.0962	0.56	UNCHANGED
Ce_fissilis.010435.1	9	1681.30	3-isopropylmalate dehydratase large subunit, chloroplastic	42826	44393	48570	36347	45684	41649	45263	41227	0.2759	-0.13	UNCHANGED
Ce_fissilis.003179.1	7	1668.78	Serine carboxypeptidase II-3	31303	28960	33977	21546	23120	21532	31413	22066	0.0037	-0.51	UNCHANGED
Ce_fissilis.001984.1	14	1644.55	NADH dehydrogenase [ubiquinone] iron-sulfur protein 1, mitochondrial	51684	44699	48266	29267	36991	37087	48217	34448	0.0138	-0.49	UNCHANGED
Ce_fissilis.016851.1	8	1643.16	Nitrile-specifier protein 5	20826	21902	20239	20284	11714	16111	20989	16036	0.1210	-0.39	UNCHANGED
Ce_fissilis.018233.1	10	1631.27	Diaminopimelate epimerase, chloroplastic	51986	58601	57501	49540	47817	40733	56029	46030	0.0418	-0.28	UNCHANGED
Ce_fissilis.012967.2	7	1630.36	Glutathione S-transferase L3	31215	32992	36083	37160	44476	34630	33430	38755	0.1795	0.21	UNCHANGED

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Ce_fissilis.018158.1	6	1626.21	Omega-amidase, chloroplastic	23230	22280	22606	19203	19621	21511	22705	20112	0.0273	-0.17	UNCHANGED
Ce_fissilis.015284.1	8	1625.44	Glutelin type-A 2	26940	28885	31231	34726	40636	44011	29019	39791	0.0225	0.46	UNCHANGED
Ce_fissilis.017820.1	6	1617.11	Adenosylhomocysteinase 1	33796	24270	10888	6939	7758	4535	22985	6411	0.0691	-1.84	UNCHANGED
Ce_fissilis.009153.1	10	1616.69	Aspartate-semialdehyde dehydrogenase	21234	22268	23853	20484	24692	28618	22452	24598	0.4337	0.13	UNCHANGED
Ce_fissilis.009610.1	22	1610.35	Alpha-mannosidase At3g26720	53173	51698	51414	56050	60855	82727	52095	66544	0.1539	0.35	UNCHANGED
Ce_fissilis.010160.2	2	1604.69	Uncharacterized protein OsI_027940	43519	46825	54373	56148	48671	48264	48239	51028	0.5345	0.08	UNCHANGED
Ce_fissilis.014280.2	7	1597.59	Serine/threonine-protein phosphatase PP2A-3 catalytic subunit	21239	23678	25297	21339	16850	20410	23405	19533	0.0987	-0.26	UNCHANGED
Ce_fissilis.014865.2	4	1591.32	Probable aldo-keto reductase 1	6437	5419	3510	4270	4096	4405	5122	4257	0.3725	-0.27	UNCHANGED
Ce_fissilis.001837.1	10	1562.64	Omega-hydroxypalmitate O-feruloyl transferase	30867	31735	31212	50535	38389	49652	31271	46192	0.0190	0.56	UNCHANGED
Ce_fissilis.014181.1	5	1555.42	Polyadenylate-binding protein 8	22101	24207	25669	23483	23360	20974	23992	22606	0.3525	-0.09	UNCHANGED
Ce_fissilis.015355.1	3	1545.99	Succinate dehydrogenase subunit 6, mitochondrial	21922	20434	21113	13632	16629	27621	21156	19294	0.6855	-0.13	UNCHANGED
Ce_fissilis.017454.1	8	1523.73	Protein disulfide isomerase-like 1-4	37283	39376	38902	46810	50912	41596	38520	46439	0.0459	0.27	UNCHANGED
Ce_fissilis.018430.1	2	1513.11	Protein disulfide-isomerase	32216	46403	53946	57028	74032	67514	44188	66191	0.0526	0.58	UNCHANGED
Ce_fissilis.002060.1	6	1501.27	Probable glucan endo-1,3-beta-glucosidase A6	30044	31808	35103	32971	31619	29323	32318	31304	0.6081	-0.05	UNCHANGED
Ce_fissilis.001167.2	5	1497.31	Thylakoid lumenal 15 kDa protein 1, chloroplastic	24889	26555	22248	14057	19379	15516	24564	16317	0.0151	-0.59	UNCHANGED
Ce_fissilis.015954.1	9	1489.83	Gamma carbonic anhydrase-like 1, mitochondrial	47971	48750	52245	53918	57278	51687	49655	54294	0.0906	0.13	UNCHANGED
Ce_fissilis.016765.1	8	1486.44	(R,S)-reticuline 7-O-methyltransferase	53454	58350	68387	53318	52324	37255	60064	47632	0.1418	-0.33	UNCHANGED
Ce_fissilis.014152.1	6	1484.60	Aldose 1-epimerase	38541	38800	40519	26616	24969	29583	39287	27056	0.0012	-0.54	UNCHANGED
Ce_fissilis.002488.1	9	1484.04	Enolase 1, chloroplastic	25683	29890	33277	39890	42410	43823	29617	42041	0.0074	0.51	UNCHANGED
Ce_fissilis.017696.1	3	1480.29	Endo-1,3	16485	28143	25265	32005	11692	16399	23297	20032	0.6681	-0.22	UNCHANGED
Ce_fissilis.000204.1	6	1478.70	Beta-hexosaminidase 1	57227	61003	59801	59702	45927	55141	59343	53590	0.2428	-0.15	UNCHANGED
Ce_fissilis.003449.2	6	1462.54	Enoyl-[acyl-carrier-protein] reductase, mitochondrial	16815	17280	20696	16670	18183	18431	18264	17761	0.7270	-0.04	UNCHANGED
Ce_fissilis.018172.2	6	1460.48	Proteasome subunit alpha type-6	26474	29920	28170	28084	29647	28342	28188	28691	0.6728	0.03	UNCHANGED
Ce_fissilis.015941.1	2	1452.24	Single-stranded DNA-binding protein WHY1, chloroplastic	34514	35979	40183	32575	34687	34077	36892	33779	0.1609	-0.13	UNCHANGED
Ce_fissilis.006615.1	7	1447.38	GDSL esterase/lipase 2	39453	36906	42129	74878	44467	33614	39496	50986	0.4080	0.37	UNCHANGED
Ce_fissilis.002002.1	6	1434.01	Uncharacterized protein At1g32220, chloroplastic	32966	37760	45910	34329	27060	30623	38879	30671	0.1304	-0.34	UNCHANGED
Ce_fissilis.008042.1	3	1409.86	5'-methylthioadenosine/S-adenosylhomocysteine nucleosidase 1	35232	38298	43669	34083	26949	19720	39066	26917	0.0655	-0.54	UNCHANGED
Ce_fissilis.017776.1	7	1406.09	Octicosapeptide/Phox/Bem1p family protein	29525	32035	34695	37693	47794	44147	32085	43211	0.0282	0.43	UNCHANGED

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Ce_fissilis.010160.1	3	1401.07	Uncharacterized protein Osl_027940	40264	49788	62124	34860	41231	46889	50725	40993	0.2489	-0.31	UNCHANGED
Ce_fissilis.009689.2	6	1386.34	Ras-related protein RABB1b	16982	17515	19462	23615	28757	14275	17987	22216	0.3816	0.30	UNCHANGED
Ce_fissilis.014316.1	2	1365.62	-	20589	18974	26955	23282	23373	15767	22172	20807	0.7168	-0.09	UNCHANGED
Ce_fissilis.011270.1	5	1354.27	Protein GrpE	40741	45623	51444	42843	47308	32919	45936	41023	0.4030	-0.16	UNCHANGED
Ce_fissilis.004678.1	6	1339.31	2-dehydro-3-deoxyphosphooctonate aldolase 1	14808	15536	18676	16231	18222	15756	16340	16736	0.7919	0.03	UNCHANGED
Ce_fissilis.011506.1	2	1338.66	ferredoxin-related	7552	7623	9742	10873	14671	21215	8306	15586	0.0789	0.91	UNCHANGED
Ce_fissilis.003162.1	3	1330.90	Non-symbiotic hemoglobin 1	52729	54295	31067	40367	16535	22884	46030	26595	0.1334	-0.79	UNCHANGED
Ce_fissilis.003776.1	7	1322.53	UDP-D-apiose/UDP-D-xylose synthase 2	25884	26873	26409	20829	20962	21164	26389	20985	0.0001	-0.33	UNCHANGED
Ce_fissilis.011004.1	8	1321.99	UDP-glucose 4-epimerase GEPI48	31750	35486	38174	46734	40720	50861	35137	46105	0.0346	0.39	UNCHANGED
Ce_fissilis.016601.1	13	1320.59	Glucose-6-phosphate isomerase 1, chloroplastic	43926	41801	45195	37810	51354	44142	43641	44435	0.8535	0.03	UNCHANGED
Ce_fissilis.002901.3	2	1305.52	Non-classical arabinogalactan protein 30	40124	43248	57981	46567	44181	53985	47117	48244	0.8657	0.03	UNCHANGED
Ce_fissilis.016925.1	3	1301.82	Inosine-5'-monophosphate dehydrogenase 2	31965	32933	33513	47003	47206	42365	32804	45525	0.0015	0.47	UNCHANGED
Ce_fissilis.006939.1	3	1291.73	Bifunctional nitrilase/nitrile hydratase NIT4A	15970	11552	9744	6036	9498	9692	12422	8408	0.1418	-0.56	UNCHANGED
Ce_fissilis.018179.1	4	1288.20	Probable plastid-lipid-associated protein 13, chloroplastic	30291	32475	33386	29012	28409	29830	32051	29084	0.0421	-0.14	UNCHANGED
Ce_fissilis.014897.1	4	1281.62	L-idonate 5-dehydrogenase	16988	16942	15408	18244	14836	20880	16446	17987	0.4461	0.13	UNCHANGED
Ce_fissilis.011997.1	2	1281.01	PsbP-like protein 1, chloroplastic	18567	22039	30283	15463	14386	16301	23630	15383	0.0791	-0.62	UNCHANGED
Ce_fissilis.010680.1	5	1269.92	NADH--cytochrome b5 reductase 1	17457	18130	21632	26963	21794	23562	19073	24106	0.0651	0.34	UNCHANGED
Ce_fissilis.017280.1	5	1265.30	Ankyrin repeat domain-containing protein 2B	57513	59218	60355	65040	85597	67458	59029	72698	0.1048	0.30	UNCHANGED
Ce_fissilis.012520.1	7	1263.58	Red chlorophyll catabolite reductase, chloroplastic	40023	46672	48388	44048	40535	32914	45028	39166	0.2316	-0.20	UNCHANGED
Ce_fissilis.004962.1	2	1250.50	Phosphoglycerate mutase family protein	6039	6043	5735	4729	7019	5568	5939	5772	0.8170	-0.04	UNCHANGED
Ce_fissilis.002481.1	2	1230.06	NADH dehydrogenase [ubiquinone] iron-sulfur protein 7, mitochondrial	36462	41650	39672	35486	29863	38756	39261	34702	0.2038	-0.18	UNCHANGED
Ce_fissilis.018187.1	3	1184.12	Putative glucose-6-phosphate 1-epimerase	17535	13912	9571	12711	22594	15367	13673	16891	0.4385	0.30	UNCHANGED
Ce_fissilis.009651.2	4	1180.20	Cysteine synthase, chloroplastic/chromoplastic	17685	22947	26591	13574	17030	16930	22407	15845	0.0807	-0.50	UNCHANGED
Ce_fissilis.016896.1	6	1166.18	Spermidine coumaroyl-CoA acyltransferase	29878	32307	34817	46106	43722	37718	32334	42515	0.0240	0.39	UNCHANGED
Ce_fissilis.013745.1	5	1159.01	Far upstream element-binding protein 2	19701	27156	29183	36968	38184	38640	25347	37931	0.0126	0.58	UNCHANGED
Ce_fissilis.003027.1	6	1125.46	Aspartic proteinase A1	50400	48501	59027	77800	68412	44517	52643	63576	0.3534	0.27	UNCHANGED
Ce_fissilis.001894.1	6	1121.08	Erlin-2-B	27095	27827	25855	24178	23313	24076	26926	23856	0.0085	-0.17	UNCHANGED
Ce_fissilis.015356.1	3	1110.49	Chloroplast envelope quinone oxidoreductase homolog	52859	52088	53422	38043	35149	57330	52790	43507	0.2539	-0.28	UNCHANGED

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Ce_fissilis.012967.1	3	1085.30	Glutathione S-transferase L3	22473	23270	23723	21155	21637	15784	23155	19525	0.1303	-0.25	UNCHANGED
Ce_fissilis.007814.1	8	1061.14	Probable N-acetyl-gamma-glutamyl-phosphate reductase, chloroplastic	40383	38563	35431	37388	40416	32909	38126	36904	0.6650	-0.05	UNCHANGED
Ce_fissilis.015635.1	5	1052.95	Spermidine synthase	38768	43324	45369	36712	29880	32200	42487	32931	0.0269	-0.37	UNCHANGED
Ce_fissilis.014783.1	7	1041.92	2-oxoisovalerate dehydrogenase subunit beta 1, mitochondrial	44193	45159	44847	54184	61968	52135	44733	56096	0.0195	0.33	UNCHANGED
Ce_fissilis.016498.1	8	1037.62	Subtilisin-like protease SBT1.7	43269	48786	51576	42618	50903	43198	47877	45573	0.5588	-0.07	UNCHANGED
Ce_fissilis.002700.1	3	1028.58	L-ascorbate oxidase homolog	7564	8024	7526	5622	4920	5443	7705	5328	0.0008	-0.53	UNCHANGED
Ce_fissilis.019119.1	3	1023.90	UDP-glycosyltransferase 88B1	17971	19038	19967	15525	15128	11274	18992	13976	0.0272	-0.44	UNCHANGED
Ce_fissilis.015542.1	3	1022.45	Phospholipid hydroperoxide glutathione peroxidase 1, chloroplastic	8921	10841	12794	10470	9622	11805	10852	10632	0.8727	-0.03	UNCHANGED
Ce_fissilis.010928.1	5	1018.18	Monothiol glutaredoxin-S17	18172	23902	15739	16375	25227	14481	19271	18695	0.8950	-0.04	UNCHANGED
Ce_fissilis.002924.1	9	1003.25	Beta-galactosidase 8	41302	41913	44348	25687	35297	26627	42521	29204	0.0141	-0.54	UNCHANGED
Ce_fissilis.015290.2	4	998.13	3-hydroxyisobutyryl-CoA hydrolase-like protein 3, mitochondrial	22244	21518	26321	17543	18898	15652	23361	17364	0.0274	-0.43	UNCHANGED
Ce_fissilis.015933.1	4	991.87	Probable acetyl-CoA acetyltransferase, cytosolic 2	17061	15596	17264	14781	14534	11460	16640	13592	0.0626	-0.29	UNCHANGED
Ce_fissilis.016916.1	11	983.21	Non-specific phospholipase C3	35871	24660	33089	26274	28690	24420	31206	26462	0.2568	-0.24	UNCHANGED
Ce_fissilis.015802.1	5	977.88	Probable serine protease EDA2	19443	18169	19162	18479	23480	21808	18925	21256	0.1999	0.17	UNCHANGED
Ce_fissilis.004086.1	2	969.53	Non-specific lipid-transfer protein-like protein At2g13820	36510	36925	43922	47642	38077	36408	39119	40709	0.7271	0.06	UNCHANGED
Ce_fissilis.016574.1	4	955.61	Probable carboxylesterase 17	17209	19759	22128	18881	25718	15048	19699	19882	0.9599	0.01	UNCHANGED
Ce_fissilis.011838.1	3	952.38	Tropinone reductase homolog At5g06060	20134	20191	22504	12161	15000	14887	20943	14016	0.0046	-0.58	UNCHANGED
Ce_fissilis.019104.1	6	947.69	Farnesylcysteine lyase	24326	27175	30281	38036	41333	32900	27261	37423	0.0275	0.46	UNCHANGED
Ce_fissilis.017489.2	4	946.96	RNA-binding protein CP33, chloroplastic	23575	25310	25124	16629	26240	13939	24670	18936	0.2033	-0.38	UNCHANGED
Ce_fissilis.014484.1	2	945.61	Uncharacterized protein At4g14100	23771	25968	29975	23726	26385	26697	26571	25602	0.6606	-0.05	UNCHANGED
Ce_fissilis.005782.1	5	939.33	Protein TIC 40, chloroplastic	18000	22659	22106	25450	26471	18624	20922	23515	0.4170	0.17	UNCHANGED
Ce_fissilis.014569.1	4	936.12	2-alkenal reductase (NADP(+)-dependent)	42403	44661	52619	40437	37187	32630	46561	36752	0.0629	-0.34	UNCHANGED
Ce_fissilis.018735.1	3	933.68	Thioredoxin reductase 2	16257	19482	20330	25763	18101	27246	18690	23703	0.1804	0.34	UNCHANGED
Ce_fissilis.000114.1	11	912.14	Ultraviolet-B receptor UVR8	35589	34766	35489	38007	43133	43954	35281	41698	0.0269	0.24	UNCHANGED
Ce_fissilis.016477.1	4	909.75	UBP1-associated protein 2C	11794	17583	18353	19455	15567	13807	15910	16276	0.8971	0.03	UNCHANGED
Ce_fissilis.018592.1	2	905.59	LysM domain-containing GPI-anchored protein 1	21809	21858	28177	21826	30075	18789	23948	23563	0.9277	-0.02	UNCHANGED
Ce_fissilis.013351.1	2	903.46	UDP-glycosyltransferase 73C5	35867	34650	42225	34111	57244	45867	37580	45741	0.3132	0.28	UNCHANGED
Ce_fissilis.014596.1	6	899.80	Cell division protein FtsZ homolog 2-1, chloroplastic	27828	30516	34496	28808	33768	27154	30946	29910	0.7278	-0.05	UNCHANGED

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Ce_fissilis.012734.1	9	885.07	Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A beta isoform	37846	44585	50992	38970	46220	35901	44474	40363	0.4466	-0.14	UNCHANGED
Ce_fissilis.006732.1	2	881.44	Glutathione S-transferase DHAR3, chloroplastic	15855	18782	20943	13234	21882	19866	18527	18327	0.9502	-0.02	UNCHANGED
Ce_fissilis.014129.1	3	867.43	3-oxo-Delta(4,5)-steroid 5-beta-reductase	16537	13679	11437	15712	16319	19680	13884	17237	0.1564	0.31	UNCHANGED
Ce_fissilis.008397.1	6	866.47	Peroxidase 73	23028	26418	27160	31827	37312	42023	25535	37054	0.0230	0.54	UNCHANGED
Ce_fissilis.012774.2	5	865.69	Probable polygalacturonase	25877	21533	24419	14830	16766	18855	23943	16817	0.0145	-0.51	UNCHANGED
Ce_fissilis.018977.1	4	865.08	Fructose-1,6-bisphosphatase, chloroplastic	18747	19373	20661	12870	18697	13101	19594	14889	0.0770	-0.40	UNCHANGED
Ce_fissilis.010352.1	2	864.02	Protein of unknown function (DUF674)	13965	16595	20003	18089	14713	13034	16854	15279	0.5300	-0.14	UNCHANGED
Ce_fissilis.009843.1	5	858.98	Glutamate dehydrogenase 2	23478	20530	16722	14866	15643	12607	20244	14372	0.0529	-0.49	UNCHANGED
Ce_fissilis.019077.1	4	856.76	Bifunctional epoxide hydrolase 2	18698	19537	21070	18287	12778	10797	19768	13954	0.0683	-0.50	UNCHANGED
Ce_fissilis.018517.1	8	848.82	1-deoxy-D-xylulose 5-phosphate reductoisomerase, chloroplastic	30291	29799	33117	29995	29379	26441	31069	28605	0.1774	-0.12	UNCHANGED
Ce_fissilis.018922.1	3	835.44	Proline iminopeptidase	30639	28869	25224	27226	28369	24890	28244	26828	0.4966	-0.07	UNCHANGED
Ce_fissilis.018852.1	3	832.86	PsbP domain-containing protein 1, chloroplastic	8613	10650	10674	6098	7234	7484	9979	6939	0.0195	-0.52	UNCHANGED
Ce_fissilis.010986.2	5	827.41	Histone deacetylase 5	22930	21477	20684	21044	26420	25356	21697	24273	0.2193	0.16	UNCHANGED
Ce_fissilis.017395.1	8	825.45	Beta-D-xylosidase 4	26689	31823	36497	26725	30484	28607	31670	28605	0.3695	-0.15	UNCHANGED
Ce_fissilis.002384.1	2	820.50	Peptidyl-prolyl cis-trans isomerase FKBP16-3, chloroplastic	37473	40536	46921	29832	33760	27687	41643	30426	0.0274	-0.45	UNCHANGED
Ce_fissilis.002366.1	2	816.66	ACT domain-containing protein ACR12	32181	35428	34477	32942	27375	20456	34029	26924	0.1301	-0.34	UNCHANGED
Ce_fissilis.017570.1	2	810.45	Short-chain dehydrogenase TIC 32, chloroplastic	24739	25427	21806	23935	15881	17685	23991	19167	0.1464	-0.32	UNCHANGED
Ce_fissilis.004398.1	4	806.02	Ubiquitin receptor RAD23c	6813	7885	11410	6180	4860	6949	8703	5996	0.1489	-0.54	UNCHANGED
Ce_fissilis.008783.1	3	788.64	Bifunctional aspartate aminotransferase and glutamate/aspartate-prephenate aminotransferase	16554	16392	16908	13357	24992	15211	16618	17853	0.7496	0.10	UNCHANGED
Ce_fissilis.011695.2	4	787.28	Acyl-coenzyme A oxidase 4, peroxisomal	11831	11064	11450	8759	8081	12801	11448	9880	0.3519	-0.21	UNCHANGED
Ce_fissilis.017444.1	3	787.07	Carbamoyl-phosphate synthase small chain, chloroplastic	15690	15111	16062	16158	19789	24466	15621	20137	0.1355	0.37	UNCHANGED
Ce_fissilis.006579.1	3	786.61	Coatomer subunit delta	13435	14446	16497	10966	12328	10059	14793	11118	0.0301	-0.41	UNCHANGED
Ce_fissilis.013952.1	4	785.21	Naringenin,2-oxoglutarate 3-dioxygenase	14029	13209	9306	16352	16327	18507	12181	17062	0.0399	0.49	UNCHANGED
Ce_fissilis.007894.1	3	784.73	Actin-related protein 4	9211	9280	9063	7121	7634	9485	9185	8080	0.2000	-0.18	UNCHANGED
Ce_fissilis.008972.1	5	767.74	ATP-dependent (S)-NAD(P)H-hydrate dehydratase	17808	18679	21549	24117	26797	25993	19345	25636	0.0104	0.41	UNCHANGED
Ce_fissilis.008343.1	2	755.16	Adenylylsulfatase HINT1	33404	34370	39448	42509	53031	51888	35740	49143	0.0248	0.46	UNCHANGED

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Ce_fissilis.011003.1	3	743.87	Enoyl-CoA hydratase 2, peroxisomal	13545	17601	19010	16943	23819	25173	16719	21978	0.1575	0.39	UNCHANGED
Ce_fissilis.017817.1	7	741.63	Histidinol dehydrogenase, chloroplastic	16685	17529	18374	20205	15907	15842	17529	17318	0.8964	-0.02	UNCHANGED
Ce_fissilis.007298.1	3	734.94	Uncharacterized protein At2g34460, chloroplastic	20981	23773	19400	16182	14237	13250	21385	14556	0.0114	-0.55	UNCHANGED
Ce_fissilis.016181.3	2	734.80	Tropinone reductase homolog At2g29170	17469	19592	20229	19404	14846	10687	19097	14979	0.1955	-0.35	UNCHANGED
Ce_fissilis.018214.2	6	729.10	Serine carboxypeptidase-like 46	16924	19680	22766	28369	28528	26415	19790	27771	0.0118	0.49	UNCHANGED
Ce_fissilis.017159.1	5	719.11	Subtilisin-like protease SBT1.7	18423	19251	22011	16392	16986	14037	19895	15805	0.0441	-0.33	UNCHANGED
Ce_fissilis.007256.1	7	715.50	Protein transport protein SEC31 homolog B	31886	30130	37700	26980	32028	18940	33239	25983	0.1779	-0.36	UNCHANGED
Ce_fissilis.001012.1	2	708.57	Probable plastid-lipid-associated protein 4, chloroplastic	22365	25622	23835	21849	13417	13669	23941	16312	0.0595	-0.55	UNCHANGED
Ce_fissilis.015448.1	7	698.99	Beta-fructofuranosidase, soluble isoenzyme I	37546	37984	41968	39598	35714	35611	39166	36974	0.3182	-0.08	UNCHANGED
Ce_fissilis.015290.1	4	694.10	3-hydroxyisobutyryl-CoA hydrolase-like protein 3, mitochondrial	19800	23019	28422	19642	32408	28865	23747	26971	0.5186	0.18	UNCHANGED
Ce_fissilis.009974.1	2	691.79	V-type proton ATPase subunit C	32723	33928	35099	36757	20411	31972	33917	29713	0.4394	-0.19	UNCHANGED
Ce_fissilis.007317.1	5	688.45	Lysosomal Pro-X carboxypeptidase	50610	51726	54851	65710	51223	61630	52396	59521	0.1882	0.18	UNCHANGED
Ce_fissilis.010376.1	2	678.16	Serine hydroxymethyltransferase 4	18805	11539	9056	6868	6281	5114	13133	6087	0.0766	-1.11	UNCHANGED
Ce_fissilis.017100.1	2	676.33	Tropinone reductase homolog At5g06060	12288	12204	15111	13696	11185	18356	13201	14412	0.6274	0.13	UNCHANGED
Ce_fissilis.013945.1	4	675.55	Aminoacylase-1	19401	21504	20747	18352	22275	20043	20551	20223	0.8123	-0.02	UNCHANGED
Ce_fissilis.018290.1	5	669.34	Probable pyridoxal 5'-phosphate synthase subunit PDX1	35384	35188	37304	38370	38749	36236	35959	37785	0.1519	0.07	UNCHANGED
Ce_fissilis.010976.1	3	662.86	Tropinone reductase homolog At5g06060	7152	8055	9287	5726	4824	8861	8165	6470	0.2842	-0.34	UNCHANGED
Ce_fissilis.014101.1	3	657.48	Dihydropyrimidine dehydrogenase (NADP(+)), chloroplastic	14338	15106	17356	12016	11445	10966	15600	11475	0.0125	-0.44	UNCHANGED
Ce_fissilis.005099.1	5	652.78	Acetylglutamate kinase, chloroplastic	21299	18465	25456	15580	19765	17024	21740	17456	0.1453	-0.32	UNCHANGED
Ce_fissilis.018079.3	2	651.89	Protein DJ-1 homolog B	21836	22122	27264	26304	30324	29133	23740	28587	0.0851	0.27	UNCHANGED
Ce_fissilis.000227.1	3	649.62	Gamma-glutamyl hydrolase 2	23156	21552	10476	7893	10544	9158	18395	9198	0.0861	-1.00	UNCHANGED
Ce_fissilis.004702.1	2	641.71	ABC transporter I family member 6, chloroplastic	5700	7495	10292	8334	5499	8161	7829	7331	0.7741	-0.09	UNCHANGED
Ce_fissilis.012174.1	2	634.62	Single-stranded DNA-binding protein WHY2, mitochondrial	10660	10280	11043	11230	18163	14731	10661	14708	0.1148	0.46	UNCHANGED
Ce_fissilis.015731.1	6	632.49	Ureidoglycolate hydrolase	21419	23811	26437	20173	17866	19421	23889	19153	0.0416	-0.32	UNCHANGED
Ce_fissilis.015435.1	3	630.47	Fructose-1,6-bisphosphatase, chloroplastic	41105	41448	40458	32896	53423	29038	41004	38452	0.7531	-0.09	UNCHANGED
Ce_fissilis.003393.1	2	626.26	Biotin carboxyl carrier protein of acetyl-CoA carboxylase	16891	18430	20379	19576	21098	15886	18567	18853	0.8841	0.02	UNCHANGED
Ce_fissilis.012884.1	2	626.20	Macro domain-containing protein XCC3184	11844	13393	14515	12722	14007	17611	13251	14780	0.4080	0.16	UNCHANGED

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Ce_fissilis.000786.1	2	622.04	Reticulon-4-interacting protein 1, mitochondrial	27611	27674	29160	17831	20061	21018	28148	19637	0.0014	-0.52	UNCHANGED
Ce_fissilis.000430.1	2	613.37	Universal stress protein PHOS32	23839	27799	30417	29524	25247	27495	27352	27422	0.9768	0.00	UNCHANGED
Ce_fissilis.006173.1	2	601.45	60S acidic ribosomal protein P2B	13157	13701	17470	28581	17437	16032	14776	20683	0.2318	0.49	UNCHANGED
Ce_fissilis.018903.1	2	596.61	Ferredoxin-2, mitochondrial	13411	9791	16567	12454	15110	14385	13256	13983	0.7480	0.08	UNCHANGED
Ce_fissilis.017736.1	3	589.97	Probable cinnamyl alcohol dehydrogenase	10566	11452	11773	17645	15160	14130	11264	15645	0.0166	0.47	UNCHANGED
Ce_fissilis.015885.1	2	576.37	Shikimate O-hydroxycinnamoyltransferase	9535	11006	11483	10278	6937	9867	10674	9027	0.2432	-0.24	UNCHANGED
Ce_fissilis.005583.2	2	572.96	Biotin carboxyl carrier protein of acetyl-CoA carboxylase 1, chloroplastic	12033	14770	18011	27637	24442	17706	14938	23261	0.0705	0.64	UNCHANGED
Ce_fissilis.005050.1	2	570.54	SAL1 phosphatase	34016	34996	39407	25413	30497	29609	36139	28506	0.0287	-0.34	UNCHANGED
Ce_fissilis.019012.1	2	568.93	Electron transfer flavoprotein subunit alpha, mitochondrial	19188	21006	23588	27198	32649	29708	21261	29851	0.0133	0.49	UNCHANGED
Ce_fissilis.015165.1	2	566.51	4-diphosphocytidyl-2-C-methyl-D-erythritol kinase, chloroplastic/chromoplastic	19927	20991	23427	18331	26429	21458	21448	22072	0.8204	0.04	UNCHANGED
Ce_fissilis.017422.1	3	562.54	Acetyl-coenzyme A carboxylase carboxyl transferase subunit beta, chloroplastic	21726	23217	26731	20959	24474	20244	23891	21892	0.3693	-0.13	UNCHANGED
Ce_fissilis.012355.1	3	561.66	Bifunctional dTDP-4-dehydrorhamnose 3,5-epimerase/dTDP-4-dehydrorhamnose reductase	13379	14855	10807	12341	14430	10707	13014	12492	0.7609	-0.06	UNCHANGED
Ce_fissilis.014513.1	2	555.43	Cytochrome c oxidase subunit 2	29805	25540	32977	21475	22589	26269	29441	23444	0.0821	-0.33	UNCHANGED
Ce_fissilis.016326.1	2	540.63	5'-nucleotidase SurE	15763	20740	24927	18414	22766	21256	20477	20812	0.9147	0.02	UNCHANGED
Ce_fissilis.013080.1	2	537.71	UPF0603 protein At1g54780, chloroplastic	15367	18710	17768	13426	12781	10069	17282	12092	0.0222	-0.52	UNCHANGED
Ce_fissilis.011754.1	2	536.36	Pyruvate kinase 1, cytosolic	27618	17649	12450	8310	10240	6683	19239	8411	0.0768	-1.19	UNCHANGED
Ce_fissilis.009657.1	3	525.66	Cyclase-associated protein 1	15444	15498	13879	8582	12956	15846	14940	12461	0.3185	-0.26	UNCHANGED
Ce_fissilis.009742.1	4	510.85	Peptidyl-prolyl cis-trans isomerase CYP38, chloroplastic	35020	27671	28502	24701	24524	22244	30398	23823	0.0553	-0.35	UNCHANGED
Ce_fissilis.017200.1	2	508.59	Acetolactate synthase 1, chloroplastic	24465	17790	13709	9810	13412	11096	18655	11439	0.0946	-0.71	UNCHANGED
Ce_fissilis.014150.1	2	505.04	Berberine bridge enzyme-like 13	20730	18030	20168	14071	20924	13902	19642	16299	0.2449	-0.27	UNCHANGED
Ce_fissilis.012275.1	2	504.34	Probable aquaporin PIP1-2	28002	27346	33046	15091	20633	26900	29465	20875	0.0899	-0.50	UNCHANGED
Ce_fissilis.008066.1	3	494.37	Putative 3,4-dihydroxy-2-butanone kinase	7072	6215	8386	10837	9838	9666	7224	10113	0.0167	0.49	UNCHANGED
Ce_fissilis.000754.1	2	489.94	Heparanase-like protein 1	20610	21525	22256	25068	23564	22240	21463	23624	0.0843	0.14	UNCHANGED
Ce_fissilis.002115.2	4	488.96	Beta-fructofuranosidase, insoluble isoenzyme CWINV1	26807	24202	7237	11983	34937	6985	19415	17968	0.8977	-0.11	UNCHANGED
Ce_fissilis.015584.1	2	488.42	Alpha-amylase	26738	29319	26037	24399	21552	20842	27365	22264	0.0259	-0.30	UNCHANGED
Ce_fissilis.018632.1	3	485.96	-	11511	11529	13763	13722	11479	13633	12267	12945	0.5531	0.08	UNCHANGED

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Ce_fissilis.001953.1	2	477.15	PsbP domain-containing protein 6, chloroplastic	10051	10499	13374	7738	9231	9531	11308	8833	0.1038	-0.36	UNCHANGED
Ce_fissilis.018696.1	4	467.19	Xylulose kinase 2	11766	12840	12706	11224	11491	11920	12437	11545	0.0864	-0.11	UNCHANGED
Ce_fissilis.005135.1	2	438.46	Aldehyde dehydrogenase family 2 member C4	21512	12775	12118	18885	19090	21332	15468	19769	0.2411	0.35	UNCHANGED
Ce_fissilis.013426.1	3	432.75	Aspartyl protease family protein 2	32354	30949	30976	25664	28851	24794	31426	26436	0.0193	-0.25	UNCHANGED
Ce_fissilis.014077.1	2	428.97	Putative dihydroflavonol 4-reductase	10515	10937	12490	19108	16503	11978	11314	15863	0.1038	0.49	UNCHANGED
Ce_fissilis.013488.1	2	420.67	Vacuolar protein sorting-associated protein 26A	197340	204674	108585	120073	127150	84737	170200	110653	0.1506	-0.62	UNCHANGED
Ce_fissilis.002033.1	4	385.96	Monocopper oxidase-like protein SKU5	23697	22568	23828	10898	12942	23184	23364	15675	0.1145	-0.58	UNCHANGED
Ce_fissilis.012882.1	2	385.86	Protein ASPARTIC PROTEASE IN GUARD CELL 1	17061	18503	19537	13088	14165	17847	18367	15033	0.1071	-0.29	UNCHANGED
Ce_fissilis.006611.2	2	385.54	Uncharacterized protein At4g13200, chloroplastic	18906	19955	22927	25099	28086	22480	20596	25221	0.0837	0.29	UNCHANGED
Ce_fissilis.018432.1	2	366.32	ATP-dependent Clp protease proteolytic subunit-related protein 4, chloroplastic	11620	11946	11626	9318	10886	10660	11731	10288	0.0450	-0.19	UNCHANGED
Ce_fissilis.014176.1	2	326.50	Peptide methionine sulfoxide reductase A4, chloroplastic	9866	12200	15581	14601	15542	19499	12549	16547	0.1484	0.40	UNCHANGED
Ce_fissilis.002306.1	3	324.35	Subtilisin-like protease SBT1.6	21824	21656	23509	13978	16211	14081	22330	14757	0.0013	-0.60	UNCHANGED
Ce_fissilis.011457.1	3	284.08	Enhancer of mRNA-decapping protein 4	11535	17128	9941	6892	4468	9937	12868	7099	0.0989	-0.85807521	UNCHANGED

Proteins were deemed up-accumulated if the $\log_2$ value of the fold change (FC) was greater than 0.6 and down-accumulated if the $\log_2$ value of the FC was less than -0.6, as determined by Student's t-test (two-tailed; $P < 0.05$).