

EFEITO DA MEMBRANA MICROPOROSA E MEIO DE CULTURA NA
GERMINAÇÃO E CRESCIMENTO *IN VITRO* DE PLÂNTULAS DE *Dalbergia nigra*
(VELL.) ALLEMÃO EX. BENTH. E ALTERAÇÕES NO CONTEÚDO ENDÓGENO
DE ETILENO, CO₂, POLIAMINAS E PERFIL PROTEÔMICO

RENAN CARRARI DOS SANTOS

CAMPOS DOS GOYTACAZES – RJ
FEVEREIRO 2023

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RENAN CARRARI DOS SANTOS

Tese apresentada ao Centro de Biociências e
Biotecnologia, da Universidade Estadual do
Norte Fluminense Darcy Ribeiro, como parte
das exigências para obtenção do título de
Doutor em Biotecnologia Vegetal.

Orientadora: Profa. Dra. Claudete Santa-Catarina

CAMPOS DOS GOYTACAZES – RJ

FEVEREIRO 2023

FICHA CATALOGRÁFICA

UENF - Bibliotecas

Elaborada com os dados fornecidos pelo autor.

S237 Santos, Renan Carrari dos.

Efeito da membrana microporosa e meio de cultura na germinação e crescimento *in vitro* de plântulas de *Dalbergia nigra* (Vell.) Allemão ex. Benth. e alterações no conteúdo endógeno de etileno, CO₂, poliaminas e perfil proteômico / Renan Carrari dos Santos. - Campos dos Goytacazes, RJ, 2023.

97 f. : il.

Inclui bibliografia.

Tese (Doutorado em Biotecnologia Vegetal) - Universidade Estadual do Norte Fluminense Darcy Ribeiro, Centro de Biociências e Biotecnologia, 2023.

Orientadora: Claudete Santa Catarina.

1. Endangered species. 2. Gas exchange. 3. In vitro germination. 4. Leaf abscission. 5. Plant proteomics. I. Universidade Estadual do Norte Fluminense Darcy Ribeiro. II. Título.

CDD - 660.6

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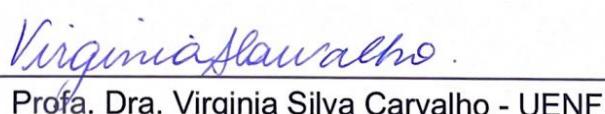
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Aprovada em 16 de fevereiro de 2023

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DEDICATÓRIA

A **Deus** toda honra e glória!

A meus pais **Domingos Roberto e Sandra** por estarem sempre ao meu lado, me dando força em cada dificuldade e vibrando com cada conquista!

A todos os meus **verdadeiros amigos**, que apesar da distância ou falta de contato contínuo, sempre seremos amigos, independentemente do tempo ou distância.

AGRADECIMENTOS

Durante esses quatro anos de incontáveis aprendizados, aprendi que não existe uma fórmula que nos leve ao sucesso, à realização profissional e pessoal. Existem opções e concretas são apenas as escolhas que fiz, escolhas que guiadas e abençoadas por Deus, me trouxeram a este momento único e inesquecível. Por isso, só tenho a agradecer a todos que passaram pelo meu caminho e que com certeza deixaram um pouco de si. Os momentos de alegria serviram para me permitir acreditar na beleza da vida e os momentos difíceis serviram para um crescimento pessoal único. É muito difícil transformar sentimentos em palavras, mas serei eternamente grato a vocês, pessoas imprescindíveis para a realização e conclusão deste trabalho;

Agradeço primeiramente a Deus pela força imensurável que sempre recebo para prosseguir na minha caminhada, não me deixando desistir nos momentos mais difíceis, permanecendo de pé e sempre forte;

Ao meu pai Domingos Risse dos Santos e à minha mãe Sandra Maria Carrari, por todo incentivo e apoio, pelo exemplo de homem e mulher a ser sempre seguido. Eu os amo muito!

As minhas irmãs Sabrina Carrari e Roberta Carrari por fazerem parte da minha vida e sem perceberem foram essenciais para essa caminhada;

À minha família por ser suporte, abraços, conforto, consolo e a melhor de todas as torcidas;

À Universidade Estadual do Norte Fluminense Darcy Ribeiro (UENF) por ser espaço de construção humana permanente;

Ao Programa de Pós-Graduação em Biotecnologia Vegetal (PGBV), de maneira especial aos docentes que contribuíram para minha formação profissional e pessoal;

A secretaria Margareth, por ser sempre solícita, atenciosa e incentivadora;

A todos os membros do grupo de pesquisa em Biotecnologia Vegetal, em especial ao professor Vanildo Silveira e ao Pós-doutorando Vitor Batista, pela disponibilidade em nos ajudar com as análises de proteômica. Aos professores

Eliemar Campostrini, Jurandi Gonçalves e ao Pós-doutorando Edinaldo pela ajuda com as análises fisiológicas.

Um agradecimento mais que especial a minha orientadora Claudete Santa-Catarina, por todo o conhecimento compartilhado, todos os conselhos e incentivos. Por toda a orientação durante o projeto. Serei eternamente grato!

Aos membros da banca pelos ensinamentos e oportunidade de aprendizagem;

A minha grande amiga Rosana Gobbi Vetorazzi, pela amizade e companheirismo, por toda ajuda durante estes quatro anos. Aos colegas e aos ex-colegas de laboratório, agradeço pela companhia diária, trocas de experiências e momentos de descontração. Serei eternamente grato a vocês, o meu eterno amor e gratidão!

A todos aqueles que direta ou indiretamente contribuíram para a realização deste trabalho. Meu muito obrigado!

RESUMO

CARRARI-SANTOS, Renan, D.Sc. Universidade Estadual do Norte Fluminense Darcy Ribeiro, fevereiro de 2023. **Efeito da membrana microporosa e meio de cultura na germinação e crescimento *in vitro* de plântulas de *Dalbergia nigra* (Vell.) Allemão Ex. Benth. e alterações no conteúdo endógeno de etileno, CO₂, poliaminas e perfil proteômico.** Orientadora: Profa. Dra. Claudete Santa-Catarina

Espécies arbóreas veem sendo intensamente exploradas ao longo dos anos para a utilização da madeira, resultando na redução das áreas de ocorrência natural e na ameaça de extinção de várias espécies, como a *Dalbergia nigra*. O uso de biotecnologias, como o cultivo *in vitro*, apresenta potencial de aplicação para a propagação e preservação de espécies ameaçadas de extinção, por apresentar vantagens comparativamente aos métodos convencionais de propagação. O meio de cultura e as condições de cultivo são fatores relevantes para o sucesso da resposta morfogênica *in vitro*, e podem induzir alterações no conteúdo de poliaminas (PAs) e no perfil proteômico, permitindo a obtenção da resposta *in vitro*. Estudos iniciais de propagação *in vitro* foram realizados para *D. nigra*, e demonstraram que as plântulas e brotações apresentam clorose e abscisão foliar após 30 dias de incubação, sugerindo que a espécie apresenta sensibilidade ao etileno que pode ser produzido no cultivo convencional com uso de tampas sem membranas microporosas. Nesse sentido objetivou-se avaliar a influência de tampas de vedação e meios de cultura na germinação *in vitro* de *D. nigra* e no teor endógeno de etileno, CO₂, PAs, índice fotossintético e perfil proteômico. Foram utilizadas tampas de vedação de polipropileno sem e com membranas microporosas, e dois meios de cultura (MS e WPM) em duas concentrações salinas (50 e 100%). Após 45 dias, foi determinado a germinação (%), matéria fresca e seca (g), abscisão foliar (%), eficiência fotoquímica (Fv/Fm), índice de verde e índice fotossintético (PI), conteúdo de etileno, CO₂, PAs e perfil proteômico. O uso de tampas com membrana possibilitou germinação significativamente maior comparado às tampas sem membrana, exceto para o meio MS. O tipo de tampa não afetou significativamente a matéria fresca e seca e índice de verde. O uso de

tampas sem membrana promoveu maior abscisão foliar nas plântulas, em todos os tratamentos, sendo significativamente superior em plântulas mantidas no meio de cultura MS (80%), o qual apresentou também conteúdo significativamente superior de etileno. A restrição das trocas gasosas resultou em um aumento no nível de CO₂ em todos os tratamentos sem o uso de membranas microporosas. O conteúdo de PAs livres totais e Putrescina livre não foram afetados significativamente pelo tipo de tampa, mas foram afetados pelos tratamentos com meios de cultura. Um total de 545 proteínas foram identificadas, das quais 101 foram diferencialmente acumuladas. Destas, 38 proteínas foram up e 43 down-acumuladas, e 10 proteínas foram únicas no tratamento com membrana e 10 proteínas únicas no tratamento sem membrana. As proteínas identificadas foram classificadas de acordo com o processo biológico que estão envolvidas. Dentre as proteínas, a presença da proteína aspartato aminotransferase, relacionada à biossíntese de etileno, em plântulas cultivadas em frascos contendo meio de cultura MS sem membrana foi associada ao maior acúmulo de etileno e, consequentemente, maior abscisão foliar. Proteínas relacionadas à fotossíntese e ao metabolismo do carbono acumuladas em mudas cultivadas em frascos com membranas microporosas foram afetadas pelas trocas gasosas e associadas a um maior índice fotossintético. Os resultados obtidos neste estudo são inéditos para *D. nigra* e são importantes para otimizar a germinação *in vitro* e crescimento das plântulas nesta espécie.

ABSTRACT

CARRARI-SANTOS, Renan, D.Sc. Universidade Estadual do Norte Fluminense Darcy Ribeiro, fevereiro de 2023. **Effect of microporous membrane and culture medium on germination and *in vitro* growth of *Dalbergia nigra* (Vell.) Alemão Ex. Benth. and alterations in the endogenous content of ethylene, CO₂, polyamines and proteomic profile.** Advisor: Profa. Dra. Claudete Santa-Catarina

Tree species from Brazilian biomes have been intensively exploited over the years for the use of wood, resulting in the reduction of areas of natural occurrence and threat of extinction of several species, such as *D. nigra*. The use of biotechnologies, as in vitro culture, presents potential application for the propagation of endangered species, showing advantages over conventional propagation methods, such as controlled growth conditions, reduced production cycle and disease-free plants. However, the success of the morphogenic response depends on several factors, such as the culture medium and the culture conditions. These factors are of fundamental importance for the in vitro morphogenic response, being able to induce biochemical alterations, such as, for example, in the PAs content and/or in its proteomic profile, allowing to obtain the *in vitro* response. Initial *in vitro* propagation studies were carried out for *D. nigra* and demonstrated that seedlings and shoots show chlorosis and leaf abscission after 30 days of incubation. Given the observed characteristics, regarding *in vitro* behavior, apparently the species is sensitive to ethylene, which can be produced in conventional cultivation using lids without membranes. In this sense, the objective of this work was to evaluate the influence of sealing lids and culture media on the *in vitro* germination of *D. nigra* and on the endogenous content of ethylene, CO₂, PAs, photosynthetic index and proteomic profile. Polypropylene lids with and without microporous membrane and two culture media (MS and WPM) at two saline concentrations (50 and 100%) were used. After 45 days, germination (%), fresh and dry matter (g), leaf abscission (%), photochemical efficiency (Fv/Fm), green index and photosynthetic index (PI), ethylene content, CO₂, PAs were evaluated and proteomic profile. The use of lids with a membrane allowed significantly higher germination compared to lids without

a membrane, except for the MS medium. The type of cover did not significantly affect the fresh and dry mass and the green index. The use of lids without a membrane promoted greater leaf abscission in the seedlings, in all treatments, being significantly higher in the seedlings kept in MS culture medium (80%), which also had a significantly higher ethylene content. The restriction of gas exchange resulted in an increase in the CO₂ content in all treatments without the use of porous membranes. The levels of total free PAs and free Putrescine were not significantly affected by the sealing caps, but were affected by the treatments with culture media, with significantly higher levels in seedlings obtained in WPM medium with 50% saline concentration. Seedlings obtained in MS culture medium with a salt concentration of 50% and with lids with membranes showed higher levels of spermidine and spermine, in comparison with lids without a membrane. A total of 545 proteins were identified, of which 101 were differentially accumulated. Of these, 38 proteins were accumulated upwards and 43 downwards, and 10 proteins were unique in the membrane treatment and 10 proteins were unique in the no-membrane treatment. The identified proteins were classified according to the biological process in which they are involved. Among the proteins, the presence of aspartate aminotransferase protein, related to ethylene biosynthesis, in seedlings grown in flasks containing MS culture medium without a membrane was associated with greater ethylene accumulation and, consequently, greater leaf abscission. Photosynthesis- and carbon metabolism-related proteins accumulated in seedlings grown in culture flasks with microporous membranes were affected by gas exchange and associated with a higher photosynthetic index. The results obtained in this study are unprecedented for *D. nigra* and are important to optimize in vitro germination and seedling growth of this species.

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LISTA DE ABREVIATURAS

ACC	1- Aminocyclopropane 1-carboxylic acid
ACO	ACC oxidase
ACS	ACC- synthase
BA	Benzyladenine
DAPs	Differentially accumulated proteins
DM	Dry matter
DTT	Dithiothreitol
FM	Fresh matter
GSI	Germination speed index
IUCN	International Union for Conservation of Nature
MS	Murashige and Skoog
PAs	Polyamines
Put	Putrescine
SAM	S-adenosyl-L-methionine
Spd	Spermidine
Spm	Spermine
TCA	Trichloroacetic acid
WPM	Woody Plant Medium

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1. INTRODUÇÃO

1.1. Mata Atlântica

A Mata Atlântica compreendia uma área original de 1,5 milhões de km² (Tabarelli et al. 2010) e sua extensão cobria total e/ou parcialmente 17 estados brasileiros, abrangendo a costa litorânea leste desde o Rio Grande do Norte até o Rio Grande do Sul. Nos últimos anos, a Mata Atlântica têm sido intensamente explorada, sendo reduzida a 15,2% da área original, somados aos remanescentes florestais preservados e os fragmentos naturais acima de 3 hectares (Fundação SOS Mata Atlântica 2022).

Dentre os fatores que levaram à redução da área florestal, encontra-se principalmente, o desmatamento, a expansão da agricultura e a ocupação populacional desordenada (Ferreira et al. 2019). Estas práticas, fizeram com que, atualmente, a Mata Atlântica fosse constituída em sua maioria por remanescentes florestais menores que 100 hectares (Fig.1) (Fundação SOS Mata Atlântica 2022).

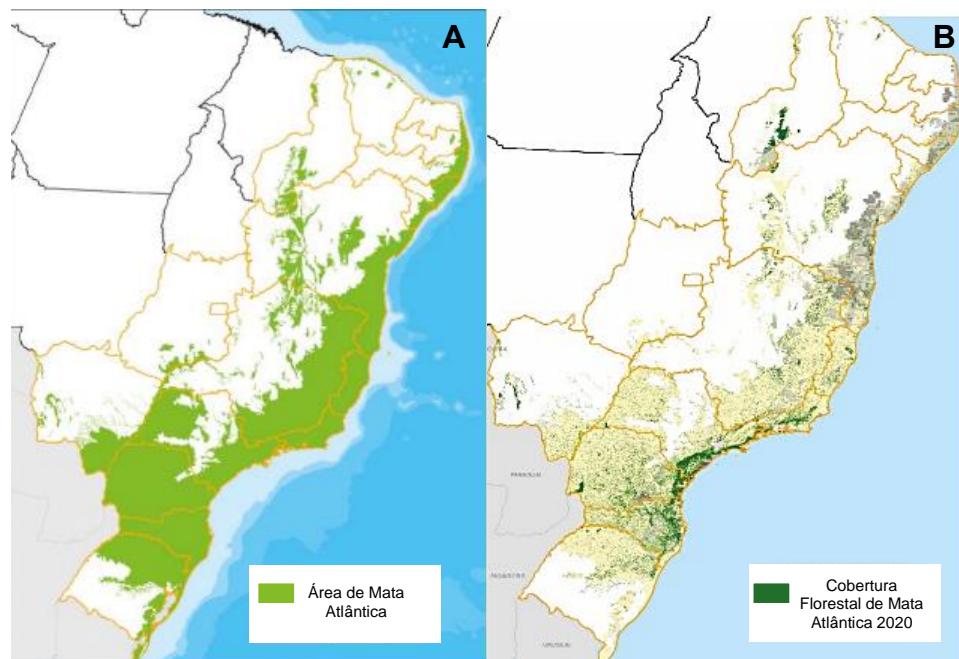


Figura 1. Mapas ilustrando a cobertura florestal original (**A**) e a cobertura florestal em 2021 da Mata Atlântica (**B**). Adaptado de Atlas dos remanescentes da Mata Atlântica período 2020 - 2021. Fonte: SOS Mata Atlântica (2023).

Entre os anos de 2020 – 2021 foram desmatados 216,42 Km² de remanescentes florestais nos 17 estados de Mata Atlântica, um aumento de 66% na taxa de desmatamento, em relação ao período anterior (2019-2020), sendo o valor mais alto desde 2015 quando ocorreu uma redução no desmatamento (Fundação SOS Mata Atlântica 2022).

O Estado do Rio de Janeiro era coberto integralmente pela Mata Atlântica, e atualmente, a cobertura está restrita a menos de 21% da área original (Fundação SOS Mata Atlântica 2022). Adicionalmente, na região Norte Fluminense, o bioma sofreu perturbações, causada pela extração indiscriminada de madeira e a substituição de florestas por áreas agrícolas (de Abreu et al. 2014) o que levou a diminuição e a fragmentação dos remanescentes florestais em áreas inferiores a 100 hectares, localizados em sua maioria em propriedades privadas (Carvalho et al. 2006). Municípios localizados no Norte Fluminense, como Campos dos Goytacazes, apresentam aproximadamente 3% de remanescentes florestais em consequência do intenso desmatamento no início do século XIV para implementação de monocultura de cana-de-açúcar (Carvalho et al. 2006).

Em virtude da grande biodiversidade e dos níveis de ameaça existentes neste bioma, a Mata Atlântica foi indicada como um dos 36 *hotspots* globais de biodiversidade (Costa et al. 2017; Rezende et al. 2018). Os *hotspots* são locais do planeta que concentram espécies endêmicas ameaçadas (Hopper et al. 2016) e que o bioma tem área inferior a 30% da cobertura vegetal original (Myers et al. 2000).

A fragmentação da cobertura original da Mata Atlântica, tem impactos graves na diversidade genética de espécies arbóreas, bem como em organismos que estão associados aos ecossistemas florestais (Tambarussi et al. 2015). Assim, a conservação e a recuperação da Mata Atlântica representam um grande desafio, uma vez que as intervenções necessárias para implementar estas ações deparam-se com entraves, destacando-se a fragmentação do bioma, o pouco conhecimento a respeito da biologia reprodutiva das espécies florestais nativas, a baixa viabilidade de sementes após pouco tempo de armazenamento e a baixa disponibilidade de mudas (Rodrigues et al. 2009; Haddad et al. 2015). Portanto, programas de conservação e estudos relacionados a propagação de espécies arbóreas e o uso

de metodologias alternativas de propagação em especial em espécies ameaçadas de extinção, tornam-se de grande importância para preservação dos remanescentes e a reposição das espécies em áreas impactadas.

1.2. Espécie de estudo: *Dalbergia nigra*

A espécie *Dalbergia nigra* (Vell.) Allemão Ex. Benth, conhecida popularmente como jacarandá da Bahia, é uma leguminosa de ocorrência no bioma Mata Atlântica com distribuição nos Estados da Bahia, Espírito Santo, Minas Gerais, Rio de Janeiro e São Paulo (Fig. 2) (Carvalho 2003).



Figura 2. Mapa da distribuição de *Dalbergia nigra* no Brasil. Os pontos verdes indicam os locais de ocorrência natural da espécie. (Fonte: Carvalho 2003).

A floração de *D. nigra* ocorre entre os meses de setembro a janeiro. É uma planta hermafrodita e a polinização é feita por abelhas e insetos pequenos. Os frutos são do tipo sâmara elíptica ou oblonga, plana, membranácea, indeiscente, com 3 a 8 cm de comprimento e 18 a 22 mm de largura, estipe com 3 a 5 mm de

comprimento, em geral com uma semente, mas podendo também conter até três sementes centrais. As sementes são castanhas, lisas, reniformes, achataadas e pequenas, de testa delgada, membranáceas e não apresentam dormência. A dispersão dos frutos e sementes é do tipo anemocórica. (Carvalho 2003). A espécie produz sementes com comportamento ortodoxo, podendo ser armazenadas por um período de até dois anos em geladeira a $6^{\circ}\text{ C} \pm 2$, apresentando queda de 50% na capacidade germinativa (Aguiar et al. 2010).

A espécie se popularizou no Brasil, e no comércio exterior, devido a qualidade de sua madeira. As décadas de 60 e 70 representaram dois dos períodos de maior exportação da madeira desta espécie (Aguiar et al. 2010). O uso da madeira de *D. nigra* são numerosos, contudo o mais frequente emprego é na confecção de móveis finos, na carpintaria decorativa de peças artesanais e na confecção de instrumentos musicais (Martinelli 2013). Tais fatos associados à exploração indiscriminada da espécie e fragmentação de seu habitat, levou esta espécie a condição de vulnerabilidade à extinção, contribuindo para a devastação do estoque genético da espécie (Carvalho 2003; Martinelli 2013). Segundo critérios da *International Union for Conservation of Nature* (IUCN), *D. nigra* é classificada na categoria vulnerável (IUCN 2022). Nesse sentido, estudos que visam identificar formas e estratégias de propagação e conservação da espécie são fundamentais e devem ser investigados com urgência.

1.3. Propagação de espécies arbóreas

A reprodução sexuada é a forma mais utilizada para a produção comercial de mudas de espécies arbóreas nativas. Entretanto, existem espécies que, devido à ausência de estudos em relação à biologia reprodutiva, apresentam dificuldades para a aplicação em programas de produção de mudas. (Debnath 2004). Dentre os aspectos limitantes para a propagação de espécies nativas podemos citar o longo período de desenvolvimento até atingirem a fase de reprodução, dificultando a sua propagação e perpetuação; a floração esporádica entre anos, influenciada por variações climáticas; e a baixa viabilidade das sementes de algumas espécies que

pode dificultar a regeneração natural e a disponibilidade de mudas para programas de reflorestamento (Carvalho 2003; Rodrigues et al. 2009).

A utilização de sementes é a maneira mais usual de propagação de espécies arbóreas nativas, sendo também considerada mais fácil e econômica do que a propagação vegetativa (Rego et al. 2009) porém, a dificuldade em se obter sementes viáveis, associado a floração e frutificação supra anual, com quantidade de sementes variável ao longo dos anos, são fatores que limitam a produção de mudas (Carvalho 2003). Dessa forma, a propagação vegetativa vem ao encontro de superar as dificuldades da propagação seminífera, possibilitando assim auxiliar no resgate e conservação de recursos florestais (Dias et al. 2012a; Abiyu et al. 2016).

Estudos iniciais mostraram que a miniestaqueia pode ser utilizada como método de propagação vegetativa em *D. nigra*, a partir de matrizes de origem seminal. No entanto, os resultados indicaram desuniformidade no enraizamento de estacas, variando de 67 à 80% (dos Santos et al. 2021). Esses resultados sugerem que essas variações na taxa de enraizamento podem ser efeito do genótipo (Dias et al. 2012b).

A capacidade na promoção do enraizamento em arbóreas é intrínseco, podendo ser influenciado por uma série de fatores internos, tais como a condição fisiológica, idade da estaca, estado fitossanitário da planta-doadora, equilíbrio hormonal, a existência de barreiras anatômicas ao enraizamento, a presença de folhas e brotos e a época do ano, bem como por fatores externos associados as condições ambientais durante o enraizamento, como disponibilidade de água, temperatura, luz e o substrato (Dias et al. 2012a; Owusu et al. 2014). Diante do exposto, a micropropagação constitui uma alternativa adequada e viável comparativamente aos métodos convencionais de propagação de espécies florestais nativas (Debnath 2004).

A cultura de tecidos vegetais, se refere as técnicas nas quais explantes, como células, tecidos ou órgãos, podem ser cultivados em um meio nutritivo, em condições assépticas e controladas (Ostrolucká et al. 2004). Dentre as técnicas da cultura de tecidos vegetais a micropropagação consiste na multiplicação *in vitro* de plantas livres de doenças, sob condições controladas, no qual possibilita a produção

em larga escala de mudas sadias em um curto espaço de tempo, com a utilização de uma área relativamente pequena se comparada com os métodos convencionais de propagação (Giri et al. 2004; González-Olmedo et al. 2005).

A micropropagação apresenta vantagens sobre a propagação vegetativa *ex vitro* pela possibilidade de obter taxa mais elevada de multiplicação, menor necessidade de espaço físico, ausência de pragas e doenças, além de oferecer técnicas mais seguras, à medida que se obtenha um maior controle dos fatores envolvidos (Pacurar et al. 2014). No Brasil, pesquisas referentes a micropropagação de arbóreas nativas ainda contemplam poucas espécies, os quais destinam-se principalmente aos genótipos considerados de difícil multiplicação pelos métodos tradicionais de propagação, como é o caso de *D. nigra* (Simões et al. 2022; Pessanha et al. 2022), *Cordia trichotoma* (Fick et al. 2007), *Ocotea porosa* (Pelegrini et al. 2011), *Cedrela fissilis* (Aragão et al. 2016, 2017a; Oliveira et al. 2020), e *Cariniana legalis* (Aragão et al. 2017a; Lerin et al. 2019).

A micropropagação é um método de reprodução vegetativa, por meio do qual, reguladores de crescimento vegetais e condições ambientais controladas são utilizados, a fim de induzirem divisões celulares e obter a produção de mudas (Ostrolucká et al. 2004). A micropropagação pode ser realizada via embriogênese somática (Ahn et al. 2019) e organogênese (Lerin et al. 2019), a qual inclui a proliferação de gemas axilares (Amirchakhmaghi et al. 2019). A micropropagação via organogênese se divide em cinco etapas principais: a) a escolha da planta-matriz e obtenção do explante para introdução *in vitro*, b) seguido do estabelecimento do material vegetal *in vitro*, c) da multiplicação e alongamento das brotações, e por último, d) o enraizamento *in vitro* ou *ex vitro* das brotações e e) aclimatização das mudas para que as plantas desenvolvam sua capacidade fotossintética e sejam capazes de sobreviver em condições *ex vitro* (González-Olmedo et al. 2005).

As respostas das plantas à micropropagação são variáveis, e dependem da espécie, época de coleta, tipo de explante utilizado, meio de cultura, balanço hormonal e condições de cultivo. No entanto, a variabilidade genética entre as espécies e dentro da mesma espécie, pressupõe o ajuste de metodologia para cada

material genético, mesmo quando a finalidade da propagação for a mesma (Wendling et al. 2006).

Para espécies arbóreas, devido à dificuldade de assepsia e indução de respostas utilizando explantes já diferenciados e desenvolvidos, como segmentos nodais e foliares, o uso de sementes para a germinação *in vitro* tem sido amplamente utilizado para obtenção de explantes em condições assépticas, como mostrado para *Cariniana legalis* (Aragão et al. 2017a; Lerin et al. 2021b), *Cedrela fissilis* (Aragão et al. 2016; Oliveira et al. 2020) e *D. nigra* (Santos et al. 2020; Simões et al. 2022; Pessanha et al. 2022). Desta forma, plântulas obtidas da germinação *in vitro* são utilizadas como fonte de explantes, especialmente segmentos nodais contendo gemas axilares, como é o caso de estudos em arbóreas.

Para a germinação *in vitro*, a composição do meio de cultura é um fator importante para algumas espécies, o meio de cultura WPM (Wood Plant Medium) (Lloyd and McCown 1980) possibilitou germinação significativamente superior comparativamente ao meio de cultura MS (Murashige and Skoog 1962), como observado para *C. legalis* (Aragão et al. 2017a). No entanto, para *D. nigra* apesar de não ter diferenças significativas na porcentagem de germinação entre estes dois meios de cultura, morfologicamente as plântulas obtidas no meio de cultura WPM apresentaram-se mais vigorosas, com folhas verdes e bem desenvolvidas, menor senescência e maior comprimento comparativamente às plântulas obtidas no meio MS (Pessanha et al. 2022).

Para o desenvolvimento de brotações a partir de segmentos nodais o uso de reguladores de crescimento, principalmente citocinina, e seu balanço com auxina, são fundamentais (Wendling et al. 2006; Simões et al. 2022; Pessanha et al. 2022). Além dos reguladores de crescimento, estudos mostram que os meios de cultura MS e WPM também são fatores determinantes para o desenvolvimento das brotações, conforme verificado para *D. nigra* (Sartor et al. 2013; Pessanha et al. 2022). Com o objetivo de desenvolver uma metodologia eficiente para o desenvolvimento de brotações *in vitro* de *D. nigra*, vários tecidos vem sendo utilizados como fonte de explantes, como segmentos nodais, apical e cotiledonar (Pessanha et al. 2022), e segmento nodal e segmento cotiledonar (Simões et al.

2022). Em ambos os trabalhos o desenvolvimento das brotações *in vitro* foram induzidas com o uso de reguladores de crescimento, especialmente o uso de citocinina, como o benziladenina (BA), e o segmento cotiledonar foi o explante que apresentou os melhores resultados para esta espécie.

Além da composição do meio de cultura e do explante utilizado, fatores extrínsecos, como a qualidade e quantidade de luz utilizada em cada fase do processo de micropropagação, são importantes para a resposta morfogênica *in vitro*. Em *C. legalis* o uso de auxinas, citocininas e giberelinas não apresentaram efeito significativo sobre o crescimento das brotações (Aragão et al. 2017a), enquanto o espectro de luz influenciou significativamente esta resposta *in vitro* (Lerin et al. 2019). Entretanto, estudos do efeito da qualidade e espectro da luz sobre o desenvolvimento das brotações ainda não foram realizados para *D. nigra*. Ademais, o desenvolvimento das brotações sob o efeito de luz também pode estar associado a alterações bioquímicas importantes para o crescimento e desenvolvimento vegetal, como por exemplo, no conteúdo de poliaminas (PAs) e perfil proteômico (Lerin et al. 2019). Estudos neste sentido foram desenvolvidos em *C. fissilis* e *C. legalis*, e mostram que alterações no conteúdo de PAs e na abundância de proteínas estão associadas com a morfogênese *in vitro* (Aragão et al. 2016, 2017a; Lerin et al. 2019; Oliveira et al. 2020).

No entanto, não há relatos relacionados com PAs e o perfil proteômico para esta espécie, bem como sobre o efeito de tipo de vedação das tampas dos frascos de cultura sobre a germinação *in vitro* e o crescimento das plântulas para a obtenção de explantes, especialmente relacionado com senescência e produção de etileno.

1.4. Trocas gasosas e membranas permeáveis a gases no cultivo *in vitro*

A cultura de tecido vegetal é realizada comumente em frascos de cultivo selados, o que influencia significativamente o acúmulo de gases como etileno e CO₂ no interior desses recipientes (Xiao et al. 2011; Saldanha et al. 2012; Martins et al. 2015). Esse ambiente *in vitro*, é caracterizado por alta umidade relativa do ar e altas concentrações de etileno (Xiao et al. 2011).

O cultivo *in vitro* requer a vedação dos frascos, a fim de evitar a contaminação da cultura, ou seja, a entrada de microrganismos nos recipientes. Algumas das vedações utilizadas no cultivo *in vitro* como tampas rígidas de polipropileno, filmes PVC e tampas metálicas, limitam as trocas gasosas entre o ambiente *in vitro* e *ex vitro*, causando problemas em algumas espécies durante o cultivo *in vitro* e na fase de aclimatização (Kozai et al. 2005; Alvarez et al. 2012). Devido a vedação dos frascos, às taxas de transpiração e fotossíntese são baixas, além de ocorrer dificuldades na absorção de água e nutrientes (Xiao et al. 2011).

Uma forma de melhorar as trocas gasosas é o uso de membranas permeáveis a gases, reduzindo a umidade relativa do ar e aumentando a transpiração e a efetiva absorção de água e nutrientes pela planta. Adicionalmente, o uso destas membranas pode favorecer a manutenção da concentração de CO₂ adequada para a realização da fotossíntese e contribuir para a redução das concentrações de etileno no interior dos frascos (Kozai and Kubota 2001; Xiao et al. 2011; da Silva et al. 2022). Portanto, o emprego de membranas permeáveis a gases é uma alternativa que pode melhorar as condições ambientais *in vitro* para a resposta morfogenética, proporcionando aumento das trocas gasosas entre o ambiente e o interior dos frascos (Saldanha et al. 2012).

Diferentes tipos de membranas, como MilliSeal® (MilliSeal, Nihon Millipore Ltda., Yonezawa, Japan), MilliWrap® (Millipore Corporation, USA), disco transparente de polipropileno (Courtaulds Films, Bridgewater, Somerset, UK), membranas de Teflon (Flora Laboratories; Austrália), Suncap® (Sigma, USA) e discos TQPL® (TQPL Supplies, UK) vem sendo disponibilizados comercialmente para uso no cultivo *in vitro* a fim de proporcionar uma melhoria nas trocas gasosas (Zobayed 2006). Espécies florestais, como *Eucalyptus tereticornis* (Sha Valli Khan et al. 2002) e *Azadirachta indica* (Rodrigues et al. 2012), já foram propagadas *in vitro* com sucesso a partir do uso de membranas MilliSeal®. No entanto, membranas MilliSeal® apresentam alto custo e são de difícil aquisição no Brasil (Saldanha et al. 2012).

Nesse sentido, uma alternativa é a utilização de membranas preparadas artesanalmente, constituídas por duas ou mais camadas de fita microporosa, com

uma camada de fita vedante rosca de politetrafluoretileno entre as duas camadas (Saldanha et al. 2012). Esta membrana é viável, propiciando as trocas gasosas e diminuindo a incidência de etileno no interior dos recipientes (Saldanha et al. 2012), sendo utilizada em diferentes estudos *in vitro* (Iarema et al. 2012; Saldanha et al. 2012; Martins et al. 2015; da Silva et al. 2022). No entanto, não há relatos na literatura relacionados com o efeito de tipo de vedação das tampas dos frascos de cultura sobre a germinação e o crescimento das plântulas de *D. nigra*, bem como o acúmulo de etileno no interior dos frascos.

1.5. Interação entre Etileno e Poliaminas (PAs) em plantas

O etileno é um fitohormônio gasoso e volátil, de molécula química simples (C_2H_4), responsável por desencadear uma variedade de respostas fisiológicas e morfológicas nas plantas, tais como a inibição da expansão celular, a promoção de senescência em folhas e flores, a indução da maturação e abscisão dos frutos, e a resistência a patógenos e ao ataque de insetos (Bleecker and Kende 2000).

Adicionalmente, as PAs são substâncias importantes na regulação da morfogênese em plantas. PAs são moléculas poliaciônicas alifáticas, carregadas positivamente, e, portanto, ligam-se a moléculas carregadas negativamente, como ácidos nucléicos, fosfolipídios e proteínas. As principais PAs encontradas em células vegetais são a pustrecina (Put), espermidina (Spd) e espermina (Spm). Essas aminas estão presentes em todos os compartimentos da célula vegetal e atuam em diversos processos fundamentais da célula como a replicação, transcrição, tradução, estabilização da membrana, divisão e expansão celular (Alcázar et al. 2016; Rakesh et al. 2021), estando relacionadas a vários processos essenciais para o crescimento e desenvolvimento da planta (Santa-Catarina et al. 2006; Alcázar et al. 2010; Reis et al. 2016; Aragão et al. 2016), incluindo o desenvolvimento de brotações a partir de segmentos nodais em arbóreas, como *C. fissilis* (Aragão et al., 2016, 2017b, 2023). Embora estudos mostrem a influência das PAs na germinação de sementes (Dias et al. 2009b; Aragão et al. 2015) e nas respostas morfogênicas *in vitro*, como na embriogênese zigótica e somática (Santa-Catarina et al. 2006, 2007; Dutra et al. 2013) e no desenvolvimento de brotações

(Aragão et al. 2016, 2017b) ainda não está elucidado o efeito da interação entre etileno e PAs na morfogênese *in vitro* em *D. nigra*.

Embora os dois grupos, etileno e PAs, possuam aparentemente funções biológicas opostas, existe uma relação de síntese entre os dois, uma vez que utilizam o mesmo precursor (SAM) (Dias et al. 2010b). Estas duas vias são competitivas, e a produção de PAs pode inibir ação do etileno, desempenhando um importante papel na regulação da regeneração *in vitro*, promovendo o alongamento e a divisão celular (Hu et al. 2006). A principal via para biossíntese do etileno tem como precursor central o aminoácido metionina, a qual é convertida a S-adenosil metionina (SAM), e este por sua vez é convertida a ácido aminociclopropano carboxílico (ACC), precursor na síntese de etileno. As principais enzimas moduladoras desta rota são a SAM-sintetase, ACC-sintase (ACCS) e ACC-oxidase (ACCO) (Bleecker e Kende, 2000). O aminoácido metionina também é precursor na síntese de PAs via SAM, com papel fundamental na doação de grupos aminopropil para a síntese de espermidina (Spd) a partir de putrescina (Put) pela ação da enzima Spd sintase, e da espemina (Spm) a partir Spd, pela ação da Spm sintase (Fig.3) (Martin-Tanguy 2001; Kusano et al. 2008).

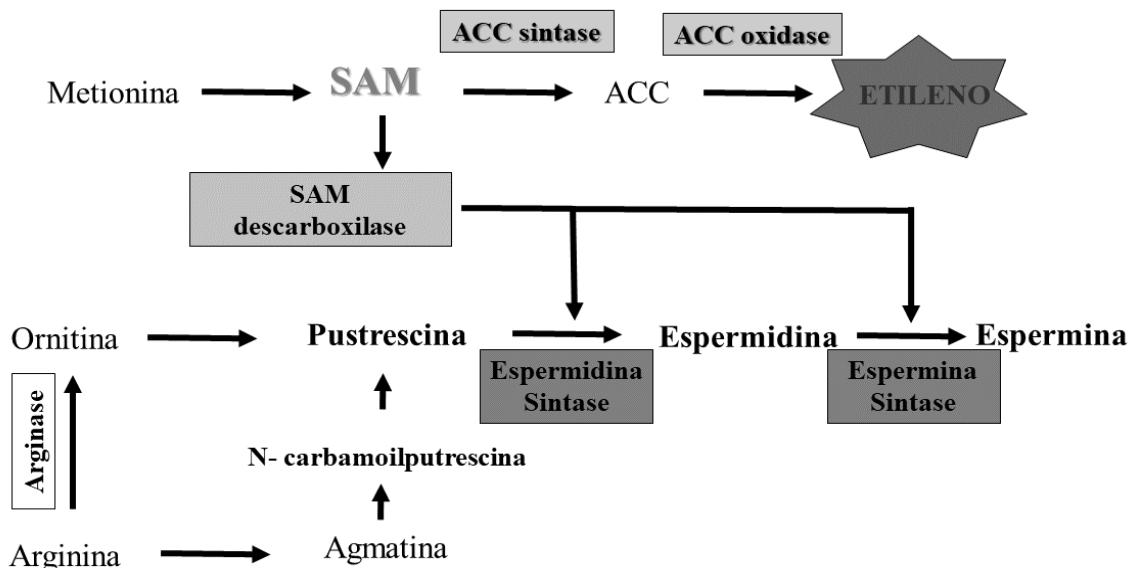


Figura 3. Vias de síntese do etileno e poliaminas (PAs) interligadas pela S-adenosil metionina (SAM). Adaptado: (Martin-Tanguy 2001)

Observada a importância da cultura de tecidos e a influência das trocas gasosas, do etileno e das PAs na morfogênese *in vitro*, faz-se necessário um estudo do efeito das trocas gasosas promovidas pelas tampas de vedação sobre o conteúdo de etileno e PAs, e como estes fatores afetam o processo morfogênico *in vitro* em *D. nigra*.

1.6 A abordagem proteômica no cultivo *in vitro*

Estudos proteômicos permitem a identificação de proteínas diferencialmente acumuladas que estejam associadas com os diferentes eventos fisiológicos que ocorrem nas células, tecidos e órgãos em um determinado processo de desenvolvimento fisiológico (Kormučák et al. 2006). Neste sentido, a análise proteômica permite medições qualitativas e quantitativas de um grande número de proteínas que podem fornecer mudanças no perfil de proteínas durante o crescimento e desenvolvimento vegetal (Chen e Harmon 2006). Atualmente diversas são as aplicações da proteômica, tais como, estudos do acúmulo diferencial de proteínas, de modificações pós-traducionais, de interação proteína-proteína, estrutural e da função das proteínas (Dias et al. 2010a).

Vários mecanismos estão envolvidos na regulação da síntese proteica, durante a qual ocorrem modificações pós-transcpcionais e pós-traducionais que podem regular a expressão espacial e temporal e a conformação de proteínas (Fig.4). Essas alterações modulam classes distintas de proteínas que bioquimicamente e estruturalmente, podem desempenhar diferentes papéis nas vias metabólicas e na composição proteômica de um organismo (Balbuena et al. 2011).

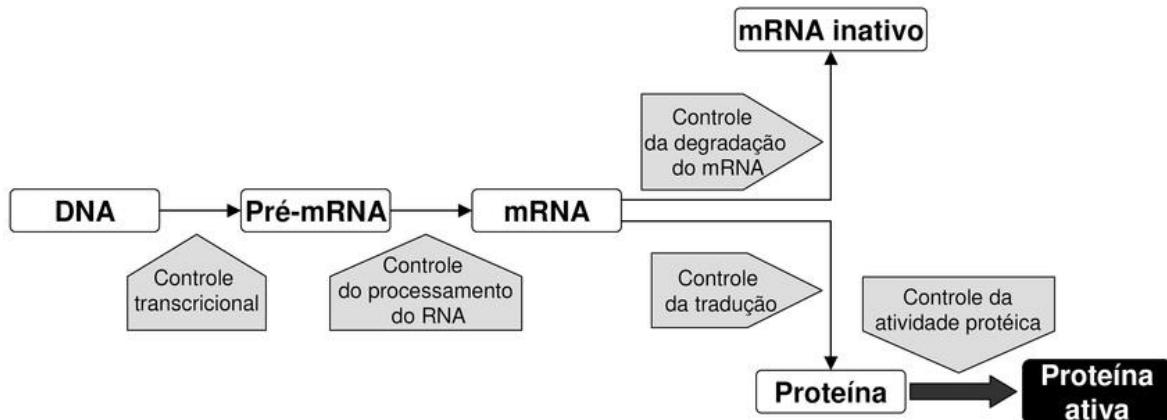


Figura 4. Mecanismos de controle que atuam na síntese proteica onde um único gene dá origem à múltiplas conformações proteicas e diferentes funções. Adaptado: (Balbuena et al. 2011).

Dentre as diversas técnicas de proteômica que foram surgindo a partir dos anos 2000, a eletroforese bidimensional (2-DE), o *shotgun* e a top-down, são as mais utilizadas (Schlüter et al. 2009). Portanto, há várias técnicas e critérios para classificar a abundância de proteínas em estudos de proteômica.

A proteômica comparativa é a estratégia que permite a elaboração de perfis proteicos ao longo de diferentes estádios de desenvolvimento, garantindo a identificação de proteínas estádio-específico, cuja expressão possa ser usada como marcadores do desenvolvimento (Dias et al. 2010a). Estudos com proteômica comparativa em plantas já vem sendo realizados a fim de avaliar as proteínas diferencialmente acumuladas durante os processos de embriogênese somática (Rode et al. 2012; Reis et al. 2016) e na morfogênese *in vitro* via desenvolvimento de brotações (Ghosh and Pal 2013; Aragão et al. 2017b; Lerin et al. 2019).

Estudos iniciais realizados com o desenvolvimento *in vitro* de brotações em *D. nigra* mostraram que alterações na abundância de proteínas envolvidas no metabolismo central, na homeostase redox, na manutenção das taxas fotossintéticas e no fluxo de carbono durante as condições de fotorrespiração foram associadas ao maior crescimento das brotações *in vitro* em meio de cultura contendo BA comparativamente à condição sem BA (Pessanha et al. 2022). Estes autores mostraram que o uso de BA é importante para o desenvolvimento das

brotações nesta espécie via a modulação do acúmulo de proteínas diferencialmente acumuladas.

Estudos proteômicos também têm revelado uma associação entre PAs e o metabolismo de proteínas nas respostas morfogenéticas *in vitro*. A Put foi a principal responsável por desencadear mudanças de proteínas relacionadas a embriogênese somática, proteínas como: arabinogalactanas, peroxidases, heat shock proteins (HSPs), glutationa transferase, proteínas abundantes na embriogênese tardia como as proteínas LEA (late embryogenesis abundant), e proteínas 14-3-3, as quais desempenham funções importantes para o crescimento e desenvolvimento vegetal, e contribuiu para a formação de embriões somáticos durante o tratamento de maturação em cana de açúcar (Reis et al. 2016). No desenvolvimento de brotações em *C. fissilis*, a adição de Put no meio de cultura alterou o perfil proteômico de proteínas relacionadas a divisão celular, contribuindo para o desenvolvimento das brotações (Aragão et al. 2017b). Adicionalmente, o acúmulo de proteínas específicas e sua interação com PAs foi mostrado no desenvolvimento *in vitro* de brotações de *C. legalis* sob efeito de diferentes espectros de luz (Lerin et al. 2019).

Embora vários estudos mostrem a interação de PAs e proteínas diferencialmente acumuladas na resposta ao desenvolvimento da morfogênese *in vitro*, não existem trabalhos relacionando o efeito do uso de membrana microporosa nas tampas e do meio de cultura na germinação *in vitro* de espécies arbóreas. Neste sentido, é importante a realização de estudos com esta abordagem, que permitam relacionar alterações na abundância diferencial de proteínas associadas ao desenvolvimento das plântulas de *D.nigra*.

2. OBJETIVOS

2.1 Objetivo geral

- O objetivo geral foi avaliar o efeito de tampas microporosas e meios de cultura na germinação *in vitro* e nas alterações bioquímicas e fisiológicas em *D. nigra*.

2.2 Objetivos específicos

- Determinar a influência de diferentes tampas de vedação (tampas de polipropileno com e sem membranas microporosas) e meios de cultura (MS e WPM) em diferentes concentrações salinas (50 e 100%) na germinação *in vitro*.
- Quantificar os níveis endógenos de etileno, PAs, CO₂ e do perfil de proteínas diferencialmente acumuladas nas plântulas cultivadas em diferentes meios de cultura com diferentes tampas de vedação.
- Avaliar as taxas fotossintéticas durante o crescimento de plântulas de *D. nigra* cultivadas em frascos com diferentes tampas de vedação e meios de cultura.

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3. TRABALHO: Microporous membrane and culture medium affect in vitro seedling development of *Dalbergia nigra* (Vell.) ex Benth. (Fabaceae) by modulation of the protein profile and accumulation of ethylene and CO₂

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*Este artigo foi submetido à Revista Plant Cell, Tissue and Organ Culture (PCTOC)

Abstract

The use of sealing lids with microporous membranes (MM) is relevant to understanding the role of gas exchange on changes in ethylene and polyamine (PA) contents and proteome profiles during seedling growth. This study aimed to evaluate the influence of sealing lids and culture media, Murashige and Skoog (MS) and woody plant medium (WPM), on the in vitro seed germination and seedling development of *Dalbergia nigra* and on the endogenous content of ethylene, CO₂, PAs, photosynthetic index, and protein profile. Sealing lids with MM allowed higher germination of seeds. Higher CO₂ and leaf abscission were observed in seedlings grown in all culture media without an MM. The contents of total free PAs and free putrescine were significantly affected by the different culture media but not by the

MM. A total of 545 proteins were identified, of which 101 were differentially accumulated (DAP) in seedlings grown in MS culture medium with MM compared to those grown without MM. Among DAPs, the presence of aspartate aminotransferase protein, related to ethylene biosynthesis, in seedlings grown in culture tubes containing MS culture medium without an MM was associated with greater ethylene accumulation and, consequently, greater leaf abscission. Photosynthesis- and carbon metabolism-related proteins accumulated in seedlings grown in MS culture medium sealed with MM were affected by gas exchange and associated with a higher photosynthetic index. This is the first study showing the effects of gas exchange promotion on in vitro seed germination and seedling development in *D. nigra* and its interaction with ethylene and protein accumulation and is relevant for optimizing in vitro micropropagation studies in this endangered woody species from the Brazilian rainforest.

Key message

The type of sealing lids and culture media affects in vitro seedling growth of *Dalbergia nigra* by accumulation of ethylene and changes in the protein profile

Keywords:

Endangered species. Gas exchange. In vitro germination. Leaf abscission. Plant proteomics. Polyamines.

Abbreviations

ACC 1- Aminocyclopropane 1-carboxylic acid

ACO ACC oxidase

ACS ACC- synthase

BA Benzyladenine

DAPs Differentially accumulated proteins

DM Dry matter

DTT Dithiothreitol

FM	Fresh matter
GSI	Germination speed index
MS	Murashige and Skoog
PAs	Polyamines
Put	Putrescine
SAM	S-adenosyl-L-methionine
Spd	Spermidine
Spm	Spermine
TCA	Trichloroacetic acid
WPM	Woody Plant Medium

Introduction

Plant tissue culture is a biotechnological tool that has been widely used for the in vitro multiplication of many economically important or endangered plant species (Oseni et al. 2018). Conventional in vitro propagation techniques are carried out in sealed vials with lids that allow limited gas exchange (Skrebsky et al. 2004). The internal atmosphere of these flasks consists of high relative humidity, varying concentrations of CO₂, and potential accumulation of ethylene and other gases (Kozai and Kubota 2001). Changing the microenvironment of the culture flask to a condition that benefits gas exchange can reduce the amount of ethylene and the relative humidity and increase the concentration of CO₂ inside the culture flasks, which can stimulate photosynthesis (Kozai and Kubota 2001).

In this sense, the use of lids containing microporous membranes (MM) is an alternative for improving gas exchange and reducing the accumulation of ethylene inside flasks (Saldanha et al. 2012) and is used in different in vitro culture studies (Iarema et al. 2012; Martins et al. 2015; Walter et al. 2019; Oliveira Junior et al. 2022; Rocha et al. 2022). Ethylene triggers a variety of physiological and morphological responses in plants, such as inhibition of cell expansion, senescence in leaves and flowers, induction of fruit maturation and abscission (Bleecker and Kende 2000). Some studies have shown that woody species, such as *Azadirachta indica*, have high accumulation of ethylene when grown in vitro using fully sealed containers,

leading to remarkable yellowing, culminating in leaf senescence and a lower percentage of shoots compared to shoots grown in flasks with lids that allow gas exchange (Rodrigues et al. 2012). In the ethylene biosynthesis pathway, the amino acid methionine is its central precursor, which is converted into S-adenosylmethionine (SAM), which in turn is converted into 1-aminocyclopropane 1-carboxylic acid (ACC), a precursor of ethylene, by the action of the main enzymes SAM-sintase, ACC-sintase (ACS) and ACC-oxidase (ACCO) (Bleecker and Kende 2000). Methionine is also a precursor in the synthesis of polyamines (PAs) via SAM, with a fundamental role in the donation of aminopropyl groups for the synthesis of spermidine (Spd) from putrescine (Put) by the action of the enzyme Spd synthase and spermine (Spm) from Spd by the action of Spm synthase (Kusano et al. 2008).

Although ethylene and PAs apparently have opposite biological functions, there is a synthetic relationship between the two phytohormones, as they use the same precursor (Dias et al. 2010). These two pathways are competitive, and the production of PAs can inhibit the action of ethylene, playing an important role in the regulation of in vitro regeneration and promoting cell elongation and division (Kuznetsov et al. 2006; Hu et al. 2006). Although studies have shown the influence of PAs on in vitro morphogenic responses, including shoot development (Aragão et al. 2016, 2017b; Lerin et al. 2019) and the interaction between PAs and ethylene during in vitro morphogenesis in *Passiflora cincinnata* (Dias et al. 2010), the effect of the interaction between ethylene and PAs in the in vitro culture of *Dalbergia nigra* has not yet been elucidated.

Proteomic studies allow the identification of proteins associated with different physiological events that occur in cells, tissues and organs (Kormuták et al. 2006), allowing the qualitative and quantitative measurements of a large number of proteins during plant growth and development (Chen and Harmon 2006). Thus, plant proteome studies can be used as an important tool in the study of morphogenic processes in vitro, allowing us to understand the profile of proteins at different stages of plant development, including in vitro shoot development (Ghosh and Pal 2013; Aragão et al. 2016, 2017b; Lerin et al. 2019). However, there are still few studies

showing the relationship of the proteomic profile in seedlings germinated in vitro (Ebstrup et al. 2005; Dai et al. 2007).

D. nigra (Fabaceae), popularly known as Jacarandá-da-Bahia, is a native endangered tree from the Atlantic Forest biome. Due to its high-quality wood, this species is under severe exploitation for use in civil construction and the manufacturing of luxury furniture and musical instruments, such as pianos (Lorenzi 1992). As a result of this exploitation and lack of reforestation programs, this species was included as vulnerable on the International Union for Conservation of Nature red list (IUCN 2022). Some studies related to seed germination have been developed for *D. nigra* under different conditions, including different temperatures and substrates (Salomão et al. 1996; Ferraz-Grande and Takaki 2001; Guedes et al. 2011; Matos et al. 2014; Regnier 2019; Medeiros Simões et al. 2021). Recently, a disinfection protocol for in vitro germination of seeds has been developed for *D. nigra* (Santos et al. 2020). In addition, micropropagation studies have already been carried out with this species as an alternative method for plant propagation, using in vitro germinated seedlings as a source of nodal segment explants for shoot development (Pessanha et al. 2022). The authors have shown the positive effects of benzyladenine (BA) on in vitro shoot development from nodal segments by modulating the endogenous Put content and differential accumulation of specific proteins. In addition, the use of Murashige and Skoog (MS) culture medium resulted in seedlings with leaves that were less developed compared to leaves from seedlings grown in woody plant medium (WPM) using conventional sealing lids of culture tubes (Pessanha et al. 2022).

In this way, sealing lids containing MM and the type of culture medium effects on germination and seedling growth, together with knowledge of the biochemical and physiological changes, are essential to improve the quality of explant sources for optimization of in vitro propagation of *D. nigra*. In this sense, we aimed to evaluate the effect of MM and culture media on in vitro morphogenesis and on biochemical and physiological changes during *D. nigra* seedling growth.

Materials and methods

Plant material

D. nigra seeds were obtained in October 2019 from Arbocenter Comércio de Sementes, located in Birigui, SP.

Preparation of sealing lids with microporous membrane

Rigid polypropylene lids (Bioplast®; Sarandi, RS, Brazil), commonly used in plant tissue culture, were used to prepare sealing lids with microporous membranes (Fig. 1a). The microporous membrane was homemade according to Saldanha et al. (2012), using two layers of microporous tape Missner® (Blumenau, Brazil), with a layer of Amanco® polytetrafluoroethylene (PTFE; Joinville, Brazil) inserted between the microporous tape. The lids were perforated (1 cm diameter) to insert the microporous membranes (Fig. 1b).

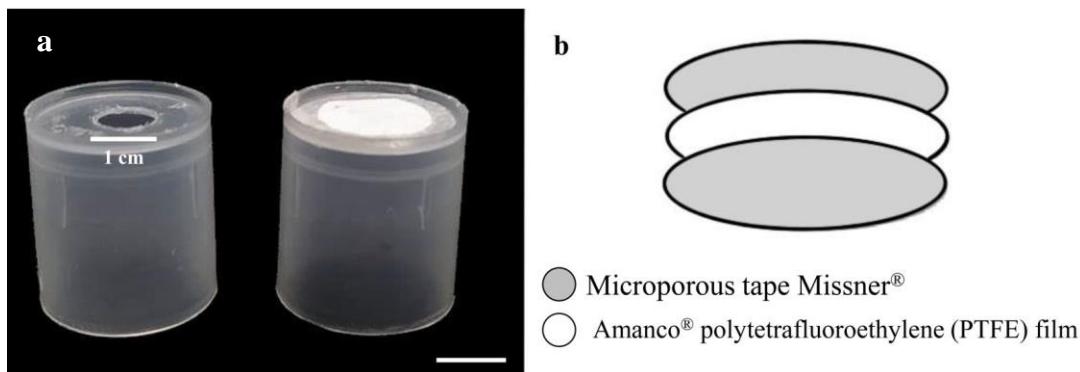


Figure 1. Sealing lids showing homemade microporous membranes (a) used for in vitro germination and seedling development and scheme of membrane layers (b). Bars = 1.0 cm

Effect of sealing lids and culture media on in vitro germination and seedling growth

For germination in vitro, seeds were first surface-disinfested before in vitro transference, according to the methodology of Pessanha et al. (2022). First, seeds were washed in water with three drops of neutral detergent and then rinsed ten times in running water. Next, seeds were immersed in 70% alcohol for one min and then placed in 100% bleach containing Derosal® (Bayer S.; Leverkusen, Germany)

fungicide ($200 \mu\text{L L}^{-1}$) for 30 min. In a laminar flow chamber, the seeds were washed five times with autoclaved distilled water.

After surface disinfection, the seeds were transferred to flask tubes (25x150 mm) containing 10 mL of MS (Murashige and Skoog 1962) (M519; Phytotechnology Lab, Lenexa, USA) or WPM (Lloyd and McCown 1980) (L449, Phytotechnology Lab, Lenexa, USA) culture media, both with 50 and 100% saline concentrations. All culture media treatments were 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, solidified with Phytagel® and autoclaved at 121 °C at 1.5 atm for 20 min. After transferring to the culture medium, seeds were kept in a growth room with a photoperiod of 16 h, light intensity of $55 \mu\text{mol m}^{-2} \text{s}^{-1}$ and temperature of 25 ± 2 °C. After transference of seeds to the treatments, the flasks with sealing lids were sealed with plastic film type PVC. Each treatment consisted of four replicates, with 50 seeds each.

The number of germinated seeds was evaluated daily to calculate the germination speed index (GSI) (Maguire 1962). After 45 days of in vitro incubation, germination (%), fresh (FM) and dry matter (DM) (g), seedling height (cm), leaf abscission (%), photochemical efficiency by the maximum quantum yield of photosystem II (Fv/Fm), photosynthetic index (PI), green intensity, PAs, ethylene (C_2H_4) and CO_2 accumulation were evaluated.

Analysis of FM and DM

FM and DM were obtained by weighing the samples on an analytical balance (AYU220, Shimadzu; Quioto, Japan). For the DM, the samples were kept at 70 °C for 72 h. Six replicates, with five plants each, were used.

Analysis of leaf abscission

Leaf abscission was evaluated by quantifying the number of seedlings with the leaf dropped on the surface of the culture medium inside the flasks to calculate the leaf abscission (%). Six replicates, with five seedlings each, were used. The results were expressed as percentages (%), according to the following equation:

$$\text{Leaf abscission (\%)} = \frac{\text{number of seedlings with leaf abscission}}{\text{number total of seedlings}} \times 100$$

Analysis of the maximum quantum yield of photosystem II (Fv/Fm) and photosynthetic index (PI)

The evaluation of photochemical efficiency, maximum quantum yield of photosystem II (PSII) (Fv/Fm) and photosynthetic index (PI) [PI=RC/ABS x FV/F0 x ET/(TR-ET)] was performed on the third most developed leaf of the seedling by means of a nonmodulated fluorimeter Pocket PEA Chlorophyll Fluorimeter (Hansatech Instruments Ltda; Kings Lynn, Norfolk, England). To proceed with the evaluation, the seedlings at 45 days of germination in test tubes were kept for 20 min in the dark for the adaptation of the chloroplasts to the dark with the purpose of greater precision of the PSII reaction centers. For the analyses, the equipment focused a light beam of 3.500 µmol of photons m⁻² s⁻¹ with the aid of three light-emitting diodes with a length of 650 nm. For this analysis, six replicates were used for each treatment, with each replicate consisting of five seedlings.

Green color intensity

The intensity of green color was evaluated in the 45-day-old seedlings germinated in vitro using a SPAD (502 Chlorophyll Meter®; Minolta, Japan) in the third leaflet of the leaves of the seedlings with the largest area size. For this analysis, six replicates for each treatment were used, with each replicate consisting of five leaves. Three readings were performed on each leaflet.

Analysis of ethylene and CO₂ emissions

Analysis of ethylene and CO₂ emissions was performed according to Burg and Burg (1962), with some modifications. For the analysis, 5 mL of the air inside the culture tubes containing the 45-day-old seedlings in the different treatments was obtained using a plastic syringe. The gas sample was injected into a Trace 1310 gas chromatograph (Thermo Fischer Scientific®, Italy) equipped with a 1 mL loop,

Porapak Q packed column and flame ionization detector and methanator. The analysis conditions were as follows: 120 kPa pressure in the injector and temperatures of the oven, injector, detector and methanator at 80, 150, 300 and 360 °C, respectively. The methanator was used for CO₂ measurement only. For each of the compounds analyzed (ethylene and CO₂), a calibration curve was performed using standard gas (0 - 100 µM ethylene and 0 - 1000 mM CO₂). For each treatment, four replicates were used, and each replicate consisted of four culture flask tubes containing three plants. Two readings were taken per vial. The results were expressed in µL kg⁻¹ h⁻¹. The following equation was used for both measurements:

$$\frac{[(\Delta \times 10) \times (\text{empty volume space (mL)})]}{[\text{seedling weight (kg)} \times \text{in vitro storage time (h)}]} = \mu\text{L kg}^{-1} \text{ h}^{-1}$$

where:

(Δ) = Accumulated value of ethylene or CO₂

Free polyamine (PA) determination

Free PAs were determined according to Aragão et al. (2017b). Samples (200 mg FM each sample, in three biological replicates) were macerated in 1.2 mL of 5% perchloric acid (Merck, Darmstadt, Germany). After 1 h of incubation at 4 °C, the samples were centrifuged for 20 min at 20,000 × g at 4 °C. The supernatant was collected, and free PAs were analyzed from the supernatant by dansylating with dansyl chloride (Merck). Then, PAs were identified and quantified by high-performance liquid chromatography (HPLC; Shimadzu, Kyoto, Japan) using a reversed-phase (5 µm) C18 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 (pH 3.5) adjusted with hydrochloric acid (Merck). The absolute gradient of acetonitrile was programmed to 65% in the first 10 min, increasing from 65 to 100% between 10 and 13 min and maintained at 100% between 13 and 21 min at a flow rate of 1 mL min⁻¹ at 40 °C. A fluorescence detector at 340 nm (excitation) and 510 nm (emission) was used to detect PA peaks.

Peak areas and retention times of PAs were measured by comparison with standard Put, Spd and Spm PAs (Sigma–Aldrich).

Protein extraction and digestion

Proteins were extracted from three biological replicates (300 mg FM each sample) of 45-day-old seedlings grown on MS with 100% saline concentration in flasks sealed with and without a microporous membrane. Proteins were extracted using the trichloroacetic acid (TCA) (Sigma–Aldrich)/acetone (Sigma–Aldrich) method according to Damerval et al. (1986), with modifications. Initially, the samples were pulverized in liquid nitrogen using a ceramic mortar and pestle. The resulting powder was resuspended in 1 mL of chilled solution containing 10% (w/v) TCA in acetone (TCA/acetone) with 20 mM dithiothreitol (DTT; GE Healthcare, Piscataway, USA) and vortexed for 30 min at 8 °C. Next, the samples were centrifuged at 16,000 × g for 30 min at 4 °C. The resulting pellets were washed three times with cold acetone plus 20 mM DTT and centrifuged for 5 min each time at 4 °C. The pellets were air dried and resuspended in buffer containing 7 M urea (GE Healthcare), 2 M thiourea (GE Healthcare), 2% Triton X-100 (GE Healthcare), 1% DTT, and 1 mM phenylmethylsulfonyl fluoride (PMSF; Sigma–Aldrich), vortexed for 30 min at 8 °C, and centrifuged for 20 min at 16,000 × g at 4 °C. The supernatants were collected, and the protein concentrations were determined using a 2-D Quant Kit (GE Healthcare).

Before the trypsin digestion step, protein samples were precipitated using the methanol/chloroform methodology (Nanjo et al. 2012). After protein precipitation, the samples were resuspended in 7 M urea/2 M thiourea solution. Aliquots of 100 µg of protein were subjected to tryptic digestion using the filter-aided sample preparation (FASP) methodology (Reis et al. 2021). Next, the peptides were resuspended in 100 µL of solution containing 95% of 50 mM ammonium bicarbonate, 5% acetonitrile and 0.1% formic acid. The resulting peptides were quantified with the A₂₀₅ nm protein and peptide method using a NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific). The samples were transferred to Total Recovery Vials (Waters, Manchester, UK) for mass spectrometry analysis.

Mass spectrometry analysis

Mass spectrometry was performed using a nanoAcquity UPLC connected to a Q-TOF SYNAPT G2-Si instrument (Waters) (Passamani et al. 2018). Runs consisted of three biological replicates of 1 µg of peptide samples. During separation, samples were loaded onto the nanoAcquity UPLC M-Class Symmetry C18 5 µm trap column (180 µm × 20 mm) at 5 µL min⁻¹ for 3 min and then onto the nanoAcquity M-Class HSS T3 1.8 µm analytical reversed-phase column (75 µm × 150 mm) at 400 nL min⁻¹, with a column temperature of 45 °C. For peptide elution, a binary gradient was used, with mobile phase A consisting of water (Tedia, Fairfield, Ohio, USA) and 0.1% formic acid (Sigma–Aldrich) and mobile phase B consisting of acetonitrile (Sigma Aldrich) and 0.1% formic acid. The gradient elution started at 7% B, ramped from 7 to 40% B until 91.12 min, ramped again from 40 to 99.9% B until 92.72 min, remained at 99.9% B until 106.00 min, decreased to 7% B until 106.1 min, and finally remained at 7% B until the end of run, at 120 min. Mass spectrometry was performed in positive and resolution mode (V mode), with 35,000 full widths at half maximum and ion mobility separation (IMS), and in data-independent acquisition mode (HDMS^E). The ion mobility wave was set to a velocity of 600 m s⁻¹, and the helium and IMS gas flows were 180 and 90 mL min⁻¹, respectively. The transfer collision energy ramped from 19 to 55 V in high-energy mode; the cone and capillary voltages were 30 and 2750 V, respectively; and the source temperature was 70 °C. Regarding the time of flight (TOF) parameters, the scan time was set to 0.5 s in continuum mode with a mass range of 50 – 2000 Da. Human [Glu¹]-fibrinopeptide B at 100 fmol µL⁻¹ was used as an external calibrant, and lock mass acquisition was performed every 30 s. Mass spectra were acquired by Mass Lynx version 4.1 software.

Proteomics data analysis

Spectra processing and database search conditions were performed using ProteinLynx Global Server (PLGS 3.0.2) (Waters). PLGS 3.0.2 was processed by the following parameters: Apex3D of 150 counts for low-energy threshold, 50 counts for elevated-energy threshold, and 750 counts for intensity threshold; two missed

cleavages; minimum fragment ions per peptide equal to three; minimum fragment ions per protein equal to seven; minimum peptides per protein equal to two; fixed modifications of carbamidomethyl (C) and variable modifications of oxidation (M) and phosphoryl (STY); and the false discovery rate (FDR) was set to a maximum of 1%. We used the *Arachis hypogaea* protein databank from UniProtKB ID UP000289738 (<http://www.uniprot.org>) for protein identification, as it is the largest databank with proximity to *D. nigra*. Label-free quantification analysis was performed using ISOQuant 1.7 (Distler et al. 2014). Briefly, the following parameters were used to identify proteins: FDR 1%, a peptide score greater than six, a minimum peptide length of six amino acids, and at least two peptides per protein were considered for label-free quantitation using the TOP3 approach, followed by the multidimensional normalized process within ISOQuant1.7. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE (Perez-Riverol et al. 2022) partner repository with the dataset identifier PXD039009. To ensure the quality of the results after data processing, only the proteins that were either present or absent (for unique proteins) in all three biological replicates were considered for differential accumulation analysis. Data were analyzed using Student's t test (two tailed). Proteins with ANOVA ($P < 0.05$) were considered up-accumulated if the \log_2 value of the fold change (FC) was greater than 0.60 and down-accumulated if the \log_2 value of the FC was less than -0.60. Functional annotations were performed using OmicsBox software version 1.0.34 and UniProtKB (<http://www.uniprot.org>). The predicted interaction networks of DAPs were constructed using *Glycine max* homologs of *A. hypogaea* identified through STRING search, with confidence > 0.7 , followed by downstream analysis in Cytoscape (version 3.8.2) (Shannon et al. 2003).

Statistical analysis

The experiment followed a completely randomized design in a 4×2 factorial arrangement, with four concentrations of culture media (MS and WPM both with 50 and 100% saline concentration) and two types of sealing lids (with and without microporous membrane). Data were analyzed using analysis of variance (ANOVA)

($P < 0.05$) followed by Tukey's test using the R software environment (R Main Team 2014).

Results

Effect of culture media and sealing lids on in vitro germination and seedling growth

Higher in vitro germination was observed in treatments using sealing lids with MM, without significant differences between culture media. Moreover, in treatments with lids without MM, a statistically significant difference between the culture media was observed, being significantly higher in the MS treatment (73%) and significantly lower (41%) in the WPM and $\frac{1}{2}$ WPM treatments (Fig. 2a). The GSI values were significantly affected for seeds incubated in WPM culture medium compared to the use of MM (Fig. 2b). The greatest seedling length was observed in seedlings germinated in culture tubes sealed with lids without an MM (Fig. 2c). Regarding leaf abscission, a lower percentage was observed in seedlings germinated in culture tubes sealed with an MM (Fig. 2d).

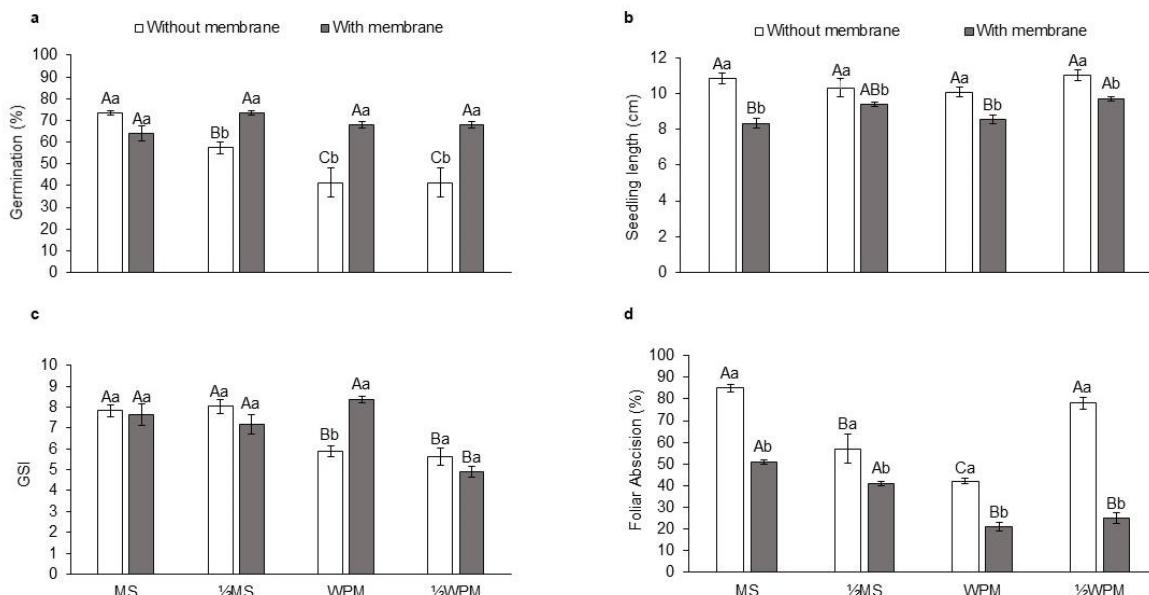


Figure 2. Germination (a), germination speed index (GSI) (b), length (cm) of seedlings (c), and foliar abscission (d) in *Dalbergia nigra* seedlings after 45 days of incubation in culture tubes containing MS, $\frac{1}{2}$ MS, WPM and $\frac{1}{2}$ WPM culture media sealed with lids with or without a microporous membrane (MM). The capital letters show significant differences between the

culture tubes sealed with two types of lids (with or without an MM) in the different culture media. The lowercase letters show significant differences between the two types of sealing lids (with and without an MM) in the same culture medium. Different letters denote significant differences according to Tukey's test ($p \leq 0.05$). CV = coefficient of variation. ($n = 8$; CV of germination = 3.72%; CV of length = 8.69%; CV of GSI = 13.46%; CV of foliar abscission = 11.60%)

Morphologically, the seedlings grown in the culture tubes sealed with MM showed well-developed green leaves and lower leaflet abscission (Fig. 3).

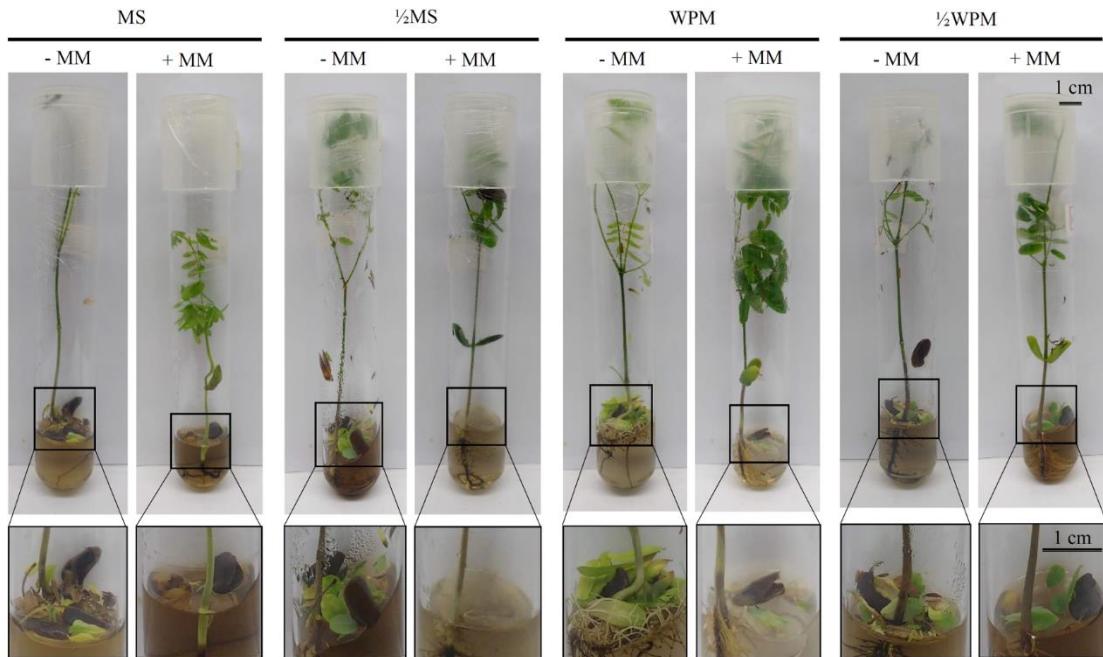


Figure 3. Morphological aspects of *Dalbergia nigra* seedlings at 45 days of in vitro incubation in culture tubes containing MS, ½MS, WPM and ½WPM culture media sealed with lids with and without microporous membranes. Bars = 1.0 cm

In addition to the seedling length being higher under culture tubes sealed without MM (Fig. 2b), the use of MM and culture media did not significantly affect the DM and FM of *D. nigra* seedlings among the different culture media used (Fig. 4). These results show that the height of seedlings does not affect the overall quality of seedlings, as the weights are not significantly different (Fig. 4).

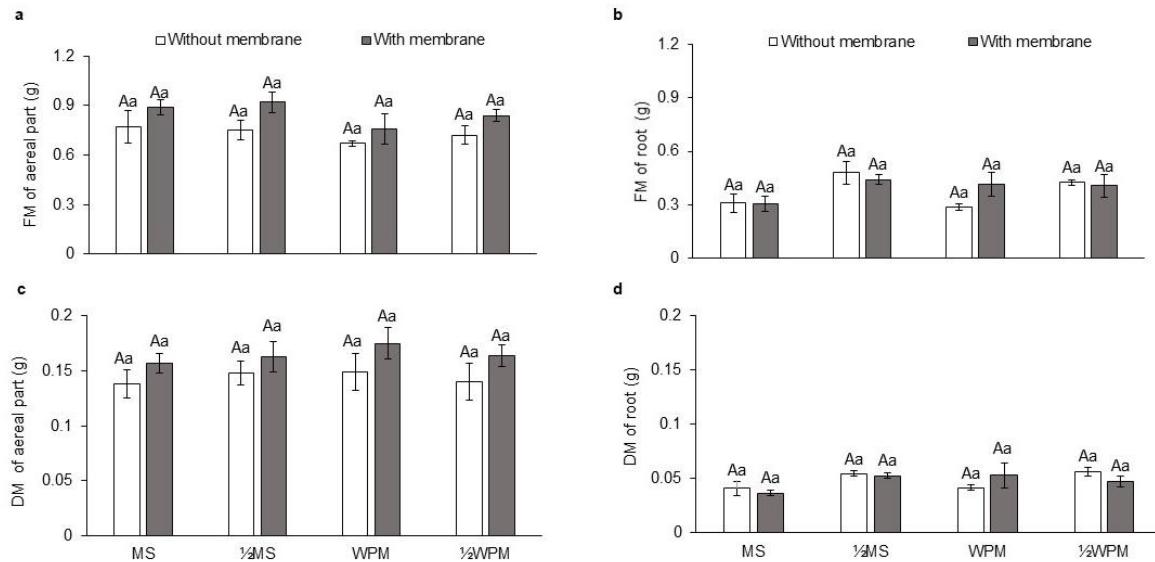


Figure 4. Fresh and dry mass (g) of shoots (**a, b**) and roots (**c, d**) in *D. nigra* seedlings after 45 days of incubation in culture tubes containing MS, ½MS, WPM and ½WPM culture media and sealed with lids with or without a microporous membrane (MM). The capital letters show a significant difference comparing the sealing caps (with or without MM) in the different culture media. Small letters show significant differences between the two types of sealing caps (with and without MM) in the same culture medium. Different letters denote significant differences according to Tukey's test ($p \leq 0.05$). FM = fresh mass. DM= Dry mass. CV = coefficient of variation ($n = 6$; CV of shoot FM = 17.19%; CV of root FM = 32.96%; CV of shoot DM = 17.52%, CV of root DM = 23.82%)

Effect of culture media and sealing lids on ethylene and CO₂ accumulation

The culture media and MM affected ethylene accumulation. The highest levels of ethylene at 45 days of incubation were observed within culture tubes sealed with lids without MM in MS and ½MS culture media. The lower accumulation of ethylene was observed for WPM and ½WPM culture media, without significant differences comparing the sealing lids with or without MM (Fig. 5a). The CO₂ accumulation inside the culture tubes was significantly influenced by the gas exchange capacity by the use of MM but was not affected by the culture media treatments. Restriction of gas exchange in sealed culture tubes with lids without MM resulted in increased CO₂ levels in all culture media treatments (Fig. 5b).

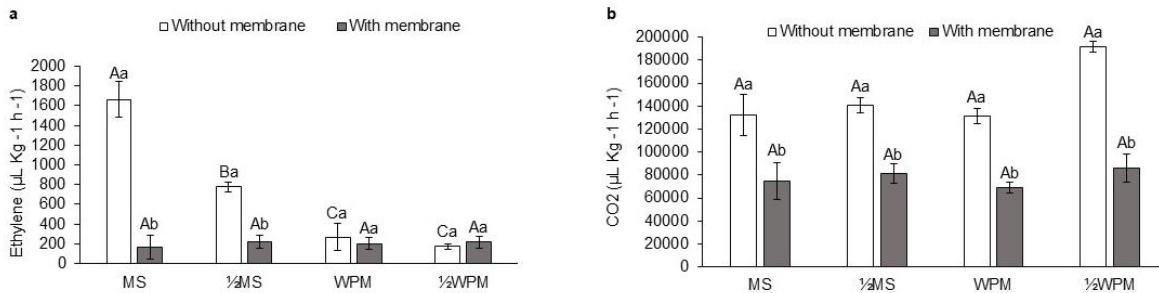


Figure 5. Ethylene (a) and CO_2 (b) accumulation ($\mu\text{L kg}^{-1} \text{h}^{-1}$) within the culture tubes containing *Dalbergia nigra* seedlings after 45 days of incubation in MS, ½MS, WPM and ½WPM culture media, sealed with lids with or without a microporous membrane (MM). The capital letters show significant differences comparing the sealing lids (with or without an MM) in the different culture media. The lowercase letters show significant differences between the two types of sealing lids (with and without MM) in the same culture medium. Different letters denote significant differences according to Tukey's test ($p \leq 0.05$). CV = coefficient of variation. ($n = 4$; CV of accumulated ethylene = 23.58%; CV of accumulated CO_2 = 22.10%)

Effects of culture media and sealing lids on physiological parameters during seedling development

There was a significant effect of the type of sealing lid on the culture tubes with or without an MM, but not for the culture media treatments, on the Fv/Fm ratio (Fig. 6a). Seedlings under treatments of culture medium using MM showed an Fv/Fm ratio greater than 0.75 (between 0.77 and 0.78), indicating good functioning of photosystem II (PSII), while seedlings grown under treatments without MM showed an Fv/Fm ratio lower than 0.75 (from 0.65 to 0.69). The PI showed a similar result to the Fv/Fm, showing higher values in seedlings grown in all culture media using lids containing MM compared without MM. Lower PI values were observed in seedlings incubated in ½MS, WPM ½WPM treatments without MM (Fig. 6b). On the other hand, in addition to the differences in Fv/Fm and PI, the seedling green color index (SPAD) was not significantly influenced by the use of sealing lids containing MM. The green color index value was only reduced in seedlings grown in ½WPM without MM compared to other culture media treatments (Fig. 6c).

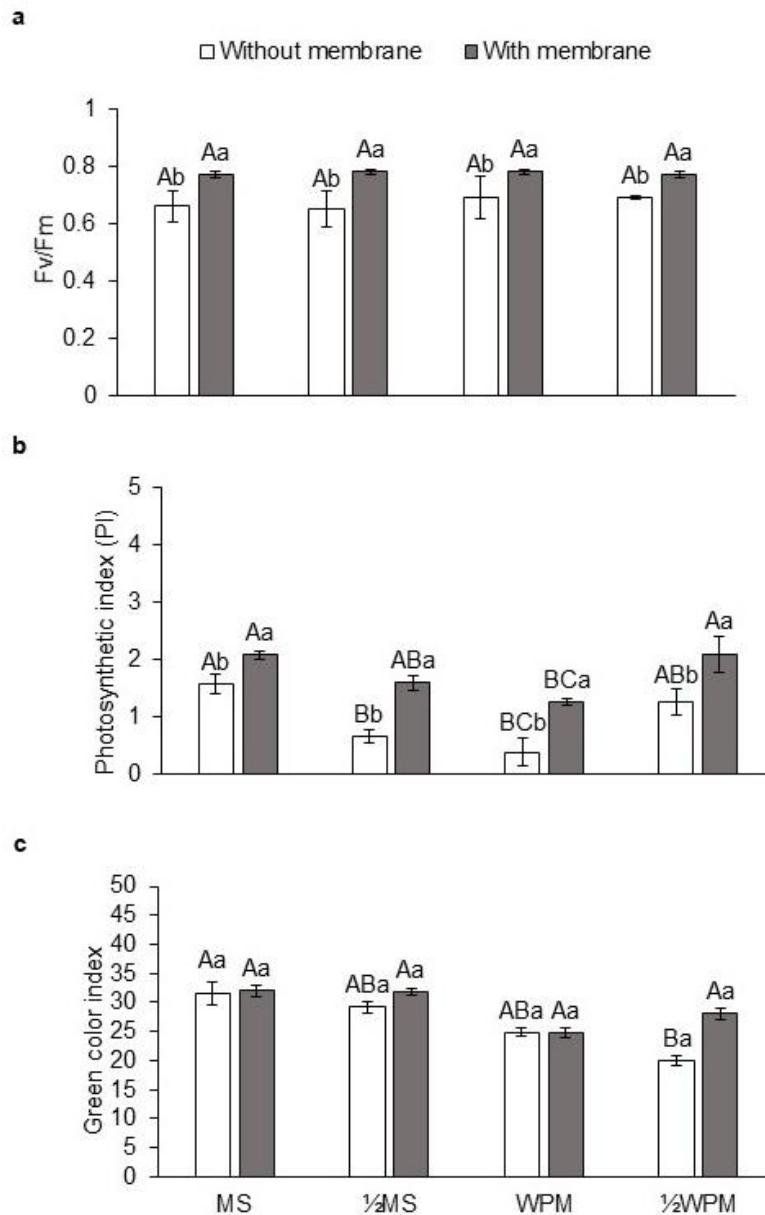


Figure 6. Fv/Fm (a), photosynthetic index (PI) (b) and green color (SPAD) index (c) in *D. nigra* seedlings after 45 days of incubation in culture tubes containing MS, 1/2MS, WPM and 1/2WPM culture media and sealed with lids with or without a microporous membrane (MM). The capital letters show a significant difference comparing the type of sealing lids (with or without MM) in the different culture media. The lowercase letters show significant differences between the two types of sealing lids (with and without an MM) in the same culture medium. Different letters denote significant differences according to Tukey's test ($p \leq 0.05$). CV= coefficient of variation ($n = 6$; CV of Fv/Fm = 3.72%; CV of photosynthetic index (PI) = 26.97%; CV of green color index = 13.46%)

Effect of culture media and sealing lids on the content of free PAs in seedlings

The contents of total free PAs (Fig. 7a) and free Put (Fig. 7b) were significantly affected by the culture media treatments, with higher values in seedlings incubated in $\frac{1}{2}$ WPM. However, the type of sealing lid did not significantly influence the total free PA and Put contents (Figs. 7a, b). Seedlings grown in $\frac{1}{2}$ MS and sealed with lids without an MM showed higher contents of free Spd (Fig. 7c) and Spm (Fig. 7d) than seedlings grown in culture tubes with an MM.

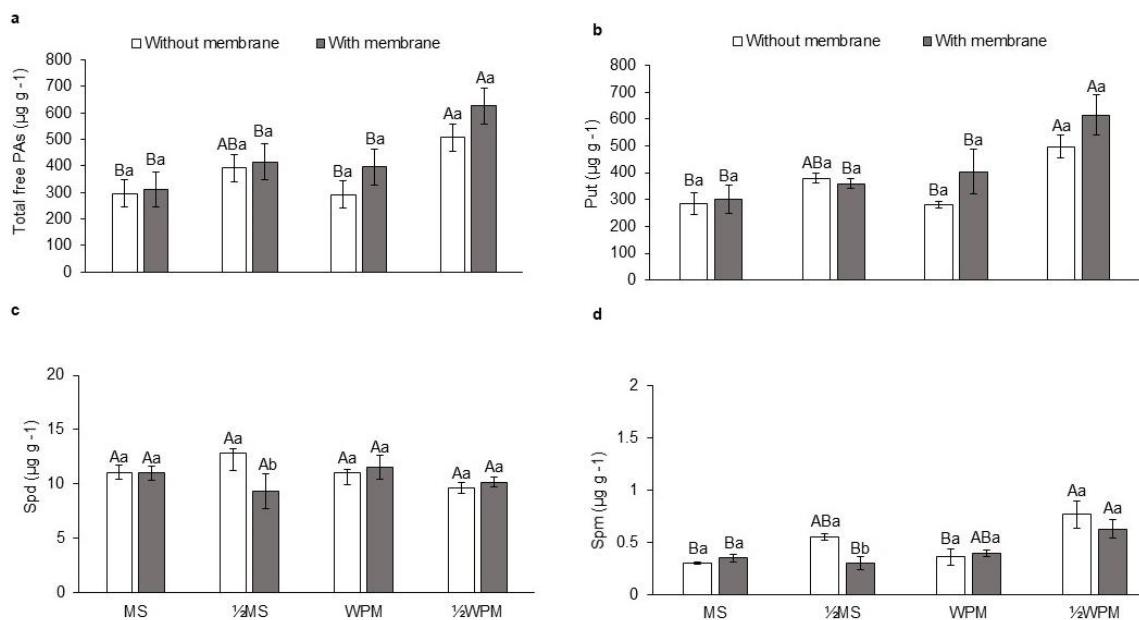


Figure 7. Endogenous contents ($\mu\text{g g}^{-1}$ fresh mass) of total free PAs (a), Put (b), Spd (c) and Spm (d) in *Dalbergia nigra* seedlings after 45 days of incubation in culture tubes containing MS, $\frac{1}{2}$ MS, WPM and $\frac{1}{2}$ WPM culture media sealed with lids with or without a microporous membrane (MM). The capital letters show significant differences comparing the type of sealing lids (with or without MM) in the different culture media. The lowercase letters show significant differences between the two types of sealing lids (with and without MM) in the same culture medium. Different letters denote significant differences according to Tukey's test ($p \leq 0.05$). CV= coefficient of variation ($n = 3$; CV of total PAs = 21.56%; CV of Put = 22.03%; CV of Spd = 14.30%; CV of Spm = 14.30%)

Effects of the sealing lids on the differential accumulation of proteins (DAPs) during in vitro seedling growth

Proteomic analysis was performed comparing the effect of sealing lids with and without MM on in vitro seedling growth of *D. nigra* using seedlings from MS

culture medium that presented the greatest accumulation of ethylene and leaf abscission between the evaluated culture media (Fig. 5a).

A total of 545 proteins were identified, of which 101 were DAPs. Of these, 38 proteins were up- and 43 were down-accumulated in seedlings grown in culture tubes sealed with lids containing MM (MSCM) compared to those seedlings without (MSN). In addition, 10 proteins were unique to seedlings grown in culture tubes sealed with lids containing MM (MSCM), and 10 proteins were unique to seedlings grown in culture tubes sealed with lids without MM (MSN) (Fig. 8; Supplementary Table 1).

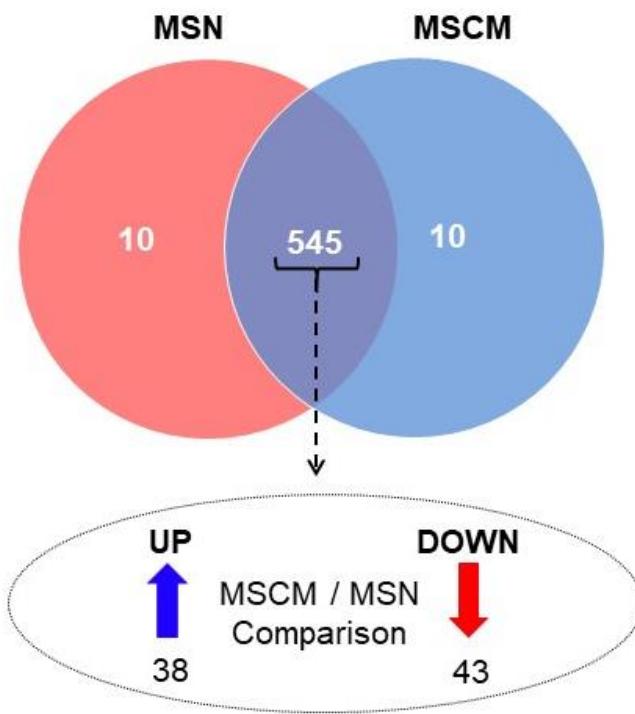


Figure 8. Venn diagram of proteins identified in 45-day-old seedlings of *Dalbergia nigra* grown in culture tubes containing MS culture medium sealed with a microporous membrane (MSCM) compared to seedlings grown without a membrane (MSN)

Among the DAPs, some were highlighted and discussed according to their relevance in the growth and development of seedlings. Proteins linked to plant growth and development by translation elongation factor activity, such as elongation

factor 1-alpha (A0A444Y7Y0), were unique to seedlings grown in tube cultures sealed with lids containing MM (Supplementary Table 1).

Proteins involved in metabolic processes, such as malate dehydrogenase (A0A445BC02), malate synthase (A0A445EQQ4), 1,4-alpha-D-glucan glucanohydrolase (A0A444YG83) and lipoxygenase (A0A445A943), were up-accumulated in seedlings grown under MM compared to those grown without MM, showing higher accumulation in seedlings under gas exchange conditions (Supplementary Table 1).

Proteins related to photosynthesis and carbon metabolism and fixation, such as the RuBisCO_large domain-containing protein, was unique (A0A445BHJ0) and up-accumulated (A0A445EET8) in seedlings grown in culture tubes sealed with lids containing MM compared to those without, showing the effects of gas exchange promotion on this protein accumulation. In addition, the ribulose bisphosphate carboxylase small chain (A0A444X9E3) and PAP_fibrillin domain (A0A445DJV5) were also up-accumulated in seedlings grown with MM compared to those without MM (Supplementary Table 1).

Some proteins unique to seedlings grown without an MM were identified, including proteins related to the photosynthesis process, such as photosystem I (A0A445BZ80), and proteins related to metabolic processes, such as the PsbP domain (A0A445CDV1) and aspartate aminotransferase (A0A444YV53) (Supplementary Table 1), with these proteins present only in seedlings grown under less gas exchange treatment. Moreover, aspartate aminotransferase (A0A444YV53) has a relationship with ethylene biosynthesis that directly depends on the pathway derived from the aspartate amino acid.

Otherwise, some proteins were down-accumulated in seedlings grown in culture tubes with lids with MM compared to those grown in culture tubes with lids without, such as the protein D-fructose-1,6-bisphosphate 1-phosphohydrolase (A0A444YX51), related to metabolic processes and carbon metabolism, and the chlorophyll a-b binding protein chloroplastic (A0A444XLS8), related to photosynthesis and metabolic processes (Supplementary Table 1).

The network of KEGG pathway enrichment by STRING for DAPS resulted in the enrichment of biological processes of carbon metabolism, metabolic pathways, carbon fixation in photosynthetic organisms and photosynthesis (Fig. 9).

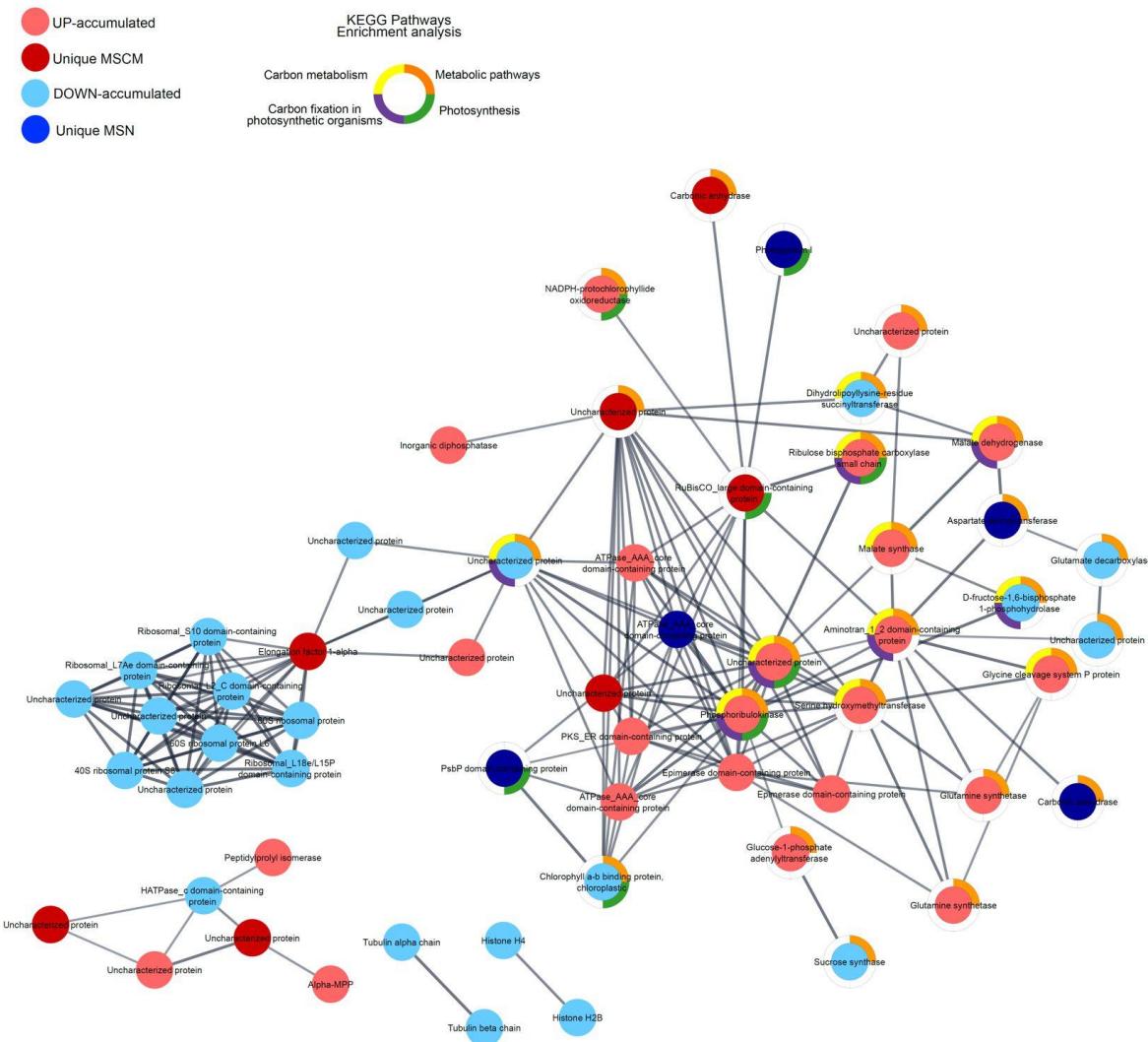


Figure 9. Protein–protein interaction network between DAPS identified in *Dalbergia nigra* seedlings at 45 days of incubation in culture tubes containing MS culture medium sealed with a microporous membrane compared to seedlings grown without a membrane

In the predicted protein–protein interaction network, the identified proteins ribulose bisphosphate carboxylase small chain (A0A444X9E3), phosphoribulokinase (A0A444WVJ5) and NADPH-protochlorophyllide oxidoreductase (A0A445A3V0) related to the biological processes enriched were up-accumulated in seedlings grown in culture tubes sealed with lids containing MM and

showed interactions with several other proteins identified (Fig. 9). Moreover, the photosynthesis and carbon metabolism processes RuBisCO_large domain-containing protein (A0A445BHJ0), unique in seedlings grown in culture tubes sealed with MM, showed direct interaction with several up-accumulated proteins, such as NADPH-protochlorophyllide oxidoreductase (A0A445A3V0), ribulose bisphosphate carboxylase small chain (A0A444X9E3), phosphoribulokinase (A0A444WVJ5) and aminotran_1_2 domain-containing protein (A0A444Z767) (Fig. 9). The aminotran_1_2 domain-containing protein (A0A444Z767), which was up-accumulated in seedlings under the use of an MM, showed an interaction with several up-accumulated proteins related to metabolic processes, carbon metabolism and carbon fixation, such as malate dehydrogenase (A0A445BC02), serine hydroxymethyltransferase (A0A444ZVK9), glycine cleavage system P protein (A0A444YVR3) and malate synthase (A0A445EQQ4) (Fig. 9). In the group of metabolic processes, the protein aspartate aminotransferase (A0A444YV53), responsible for the synthesis of amino acids and directly linked to ethylene biosynthesis that occurs from the aspartate pathway, accumulated exclusively in seedlings grown in the treatment without an MM (Supplementary Table 1) and showed interaction with the up-accumulated proteins malate dehydrogenase (A0A445BC02) and aminotran_1_2 domain-containing protein (A0A444Z767) and with down-accumulated glutamate decarboxylase (A0A445DQ85) proteins in seedlings grown in culture tubes with an MM (Fig. 9).

Taken together, it was observed that gas exchange promoted by the use of MM resulted in lower ethylene and CO₂ concentrations within the culture tubes, resulting in reduced leaf abscission of seedlings but increasing the PI and Fv/Fm ratio, as well as inducing the accumulation of proteins related to photosynthesis, metabolic processes, carbon metabolism and carbon fixation (Figure 10). Moreover, the presence of proteins related to metabolic processes related to ethylene synthesis only in seedlings grown in MS culture medium without MM was related to an increase in ethylene synthesis (Figure 10).

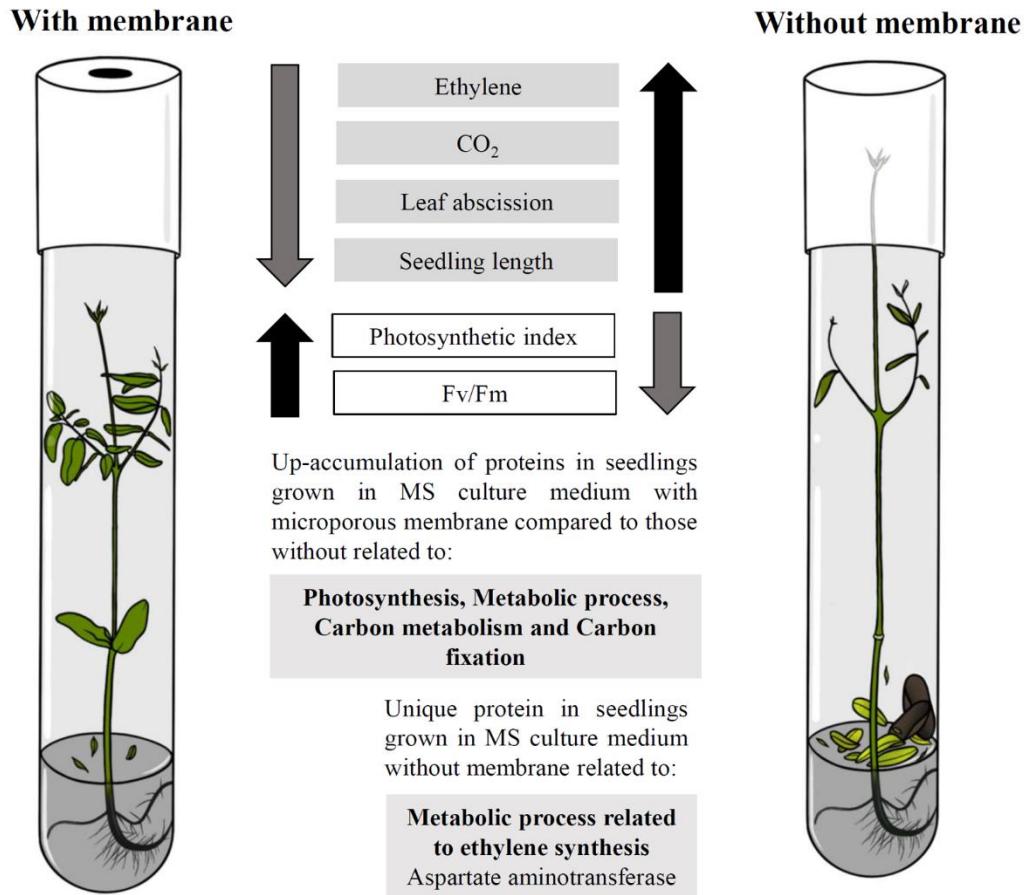


Figure 10. Scheme showing the main alterations in the development and biochemical changes of *Dalbergia nigra* seedlings germinated in culture tubes containing MS culture medium sealed with lids with and without microporous membranes

Discussion

In vitro seed germination is an alternative for obtaining aseptic explants for micropropagation studies, as it is used in the in vitro propagation of tree species such as *Cedrela fissilis* (Aragão et al. 2016; Reis de Oliveira et al. 2022; Oliveira et al. 2020), *Cariniana legalis* (Aragão et al. 2017a; Lerin et al. 2021a,b) and *D. nigra* (Pessanha et al. 2022). Sealing the culture tubes with lids containing MM allowed significantly higher germination, showing a better morphological aspect of the seedlings, especially those obtained in the culture medium in WPM and ½WPM. Likewise, the in vitro germination of *D. nigra* seeds cultured in full strength WPM medium resulted in seedlings with greener leaves and lower senescence compared to those from MS (Pessanha et al. 2022). The best development of seedlings grown

in WPM (Lloyd and McCown 1980) culture medium is due to the different proportions of nutrient balances formulated for better growth of woody species. This culture medium contains approximately four times less macronutrient salts related to nitrogen, such as NH_4^+ and NO_3^- , than MS culture medium (Phillips and Garda 2019). In vitro germination studies with other tree species, such as *C. legalis* (Aragão et al. 2017a), *Chionanthus retusus* (Tar et al. 2018), and *Campomanesia phaea* (Demétrio et al. 2021), have also shown that the WPM culture medium was better for seedling development than the MS culture medium. However, the best culture medium for in vitro seed germination varies according to species. In contrast, in *Amburana acreana*, the best germination was obtained in MS medium at 50% strength of macro salts (Fermino Junior and Scherwinski-Pereira 2012).

The use of MM resulted in a shorter length of *D. nigra* seedlings but did not significantly affect the FM and DM values in all culture media. The lower length of seedlings in gas exchange conditions can be related to the fact that the use of an MM allows an increase in evapotranspiration, which can change the physical properties of the culture medium, attributed to water loss (Ivanova and van Staden 2010). This alteration can induce a concentration of mineral salts and carbohydrates and promote a reduction in the osmotic potential of the culture medium (Cui et al. 2010). Consequently, plants can reduce their vegetative growth to conserve and redistribute resources, increasing their chance of survival (Skirycz and Inzé 2010). In accordance with our results, other studies also reported that the use of MM resulted in lower shoot elongation in in vitro culture (Casanova et al. 2008; Ivanova and van Staden 2010; Martins et al. 2015). These results suggest that in addition to lower seedling length under conditions with gas exchange promotion, no significant effects on seedling growth were observed once FM and DM were not affected significantly using MM and culture media.

The use of MM can improve environmental conditions in vitro by providing an increase in gas exchange between the external environment and within the culture flasks, leading to lower leaf abscission, lower senescence and lower incidence of chlorosis in leaves (Batista et al. 2013). In the present work, the seedlings obtained in all culture media sealed with an MM showed lower leaf abscission. On in vitro

propagation of forest species, such as *A. indica*, the use of lids containing MM allowed the development of more vigorous shoots, with a lower rate of leaf abscission, yellowing, chlorosis and leaf senescence in relation to those cultured in flasks without membranes (Rodrigues et al. 2012). These characteristics are associated with a lower accumulation of ethylene gas inside the culture flasks (Trevisan and Mendes 2005; Martins et al. 2015). The correlation of high ethylene levels and leaf abscission in in vitro culture has been demonstrated for shoot development of *Citrus australasica* (Mahmoud et al. 2020). Ethylene triggers a wide variety of physiological and morphological responses in plants, such as inhibition of cell expansion and induction of leaf senescence and abscission, resulting in detrimental effects on the development of in vitro morphogenesis (Iqbal et al. 2017). Culture tubes sealed with lids without MM showed significantly higher accumulation of ethylene when used with MS culture medium. Consequently, leaves of *D. nigra* seedlings maintained in these culture media became visually yellower but without differences in the green color index. Yellowing and chlorosis of leaves are classic symptoms of ethylene emission in vitro (Iqbal et al. 2017). In addition, some studies have shown that the effects of ethylene on seedling growth (Syu et al. 2014; Wang et al. 2020) are not related to leaf abscission. Ethylene treatment increased the chlorophyll contents and relieved the leaf yellowing phenotype of salt stress in seedlings of *Arabidopsis* (Wang et al. 2020). In this sense, *D. nigra* seedlings grown under MS culture media without MM showed significantly higher accumulation of ethylene, which can induce higher leaf abscission. However, ethylene accumulation did not affect the green color intensity of leaves when comparing the *D. nigra* seedlings grown under MS with and without MM. Moreover, the higher accumulation of ethylene and leaf abscission observed in *D. nigra* seedlings grown under MS culture medium (at both 50 and 100% strength of salts) compared to WPM may have also occurred in response to salt concentration, since the amount of mineral salt in the MS medium is higher than that in the WPM (Silva et al. 2019). Mineral salts in the culture medium have a nutritive effect during in vitro growth, which enhances cell growth and morphogenesis due to their osmotic properties (George et al. 2007). Salt reduction is generally beneficial for the in vitro growth of woody species due to

increased water availability and decreased osmotic pressure (Assis et al. 2012), as observed for *Anacardium occidentale* (Thimmappaiah et al. 2002; Assis et al. 2012).

Studying the interrelationships between plant hormones and organic compounds has gained more interest as hormonal pathways interact in complex information transfer networks, in which a variety of stimuli trigger multiple responses, thus constituting a network of cross-talk signals (Chow 2003). Ethylene and PAs are involved in plant growth and development and are interrelated by their biosynthetic pathways and their physiological roles (Harpaz-Saad et al. 2012; Rakesh et al. 2021). As the biosynthesis of PAs and ethylene share common intermediates, some studies have shown competition between the pathways (Kaur-Sawhney et al. 2003; Puga-Hermida et al. 2006). In addition to the effects on reducing ethylene accumulation within the culture tube environment, the use of sealing lids with MM did not significantly affect the endogenous contents of PAs during seedling development of *D. nigra*, suggesting that the biosynthesis of these two molecules was not in competition in this study. Studies with in vitro cultures of *Passiflora edulis* and *P. flavicarpa* showed that high concentrations of Spd and Spm added to the culture medium did not reduce ethylene emission, while lower exogenous concentrations of Spd and Spm were accompanied by reduced levels of ethylene in cultures of *P. cincinnata* (Dias et al. 2009). However, PA addition to culture medium reduced ethylene in *Capsicum annuum* (Batista et al. 2013), showing that the concentration and response are species dependent.

The CO₂ content was influenced by the type of MM used but not by the culture medium treatments, with greater accumulation within culture tubes sealed without an MM. These results are similar to those found by da Silva et al. (2022) for *Vitis* spp. These authors observed that the microenvironment without a membrane showed a greater accumulation of CO₂ compared to the microenvironment with a membrane. The use of sealing lids with gas permeable membranes allows exchanges of CO₂ between the external environment and the interior of the culture flasks, resulting in better morphological and physiological responses of seedlings grown in vitro (Trevisan and Mendes 2005; Xiao and Kozai 2006). In *Billbergia zebrina* seedlings grown in vitro, the lower accumulation of CO₂ observed in culture

flasks sealed with MM positively affected growth, anatomy, physiology and survival rate when transferred to the ex vitro environment (Martins et al. 2015). The positive effect of MM is mainly due to the better exchange of CO₂ and the prevention of ethylene accumulation inside the culture flasks (Rodrigues et al. 2012) and has a positive effect on the quality of seedlings grown in vitro (Ribeiro et al. 2009; Iarema et al. 2012; Saldanha et al. 2012; Martins et al. 2015; Rocha et al. 2022). The low gas exchange between the external and internal environments in culture flasks sealed without MM conventionally used for in vitro culture can reduce nutrient uptake and photosynthetic performance of plants (Xiao et al. 2011), since CO₂ is the raw material for photosynthesis and its concentration directly affects photosynthetic efficiency and chlorophyll synthesis (Yusuke Onoda, Kouki Hikosaka 2005). In this study, the photosynthetic index (Fv/Fm) and the functioning of photosystems I and II (PI) by photosynthetic index showed a positive effect on seedlings of *D. nigra* grown in culture tubes sealed with an MM. It has been shown that a weaker gas exchange capacity in culture flasks without MM reduces the photosynthetic capacity and delays the physiological metabolism process (Tholen et al. 2007). Moreover, ethylene reduces the potential photochemical efficiency of PSII (Fv/Fm), resulting in decreased CO₂ assimilation in leaves of *Glycine max* L. (Wullschleger et al. 1992; Fan et al. 2013). Thus, the accumulation of ethylene within the culture tubes with MM can affect the Fv/Fm values in *D. nigra* seedlings but not the chlorophyll content.

The use of proteomics as a tool for understanding biochemical and molecular aspects has been important in in vitro morphogenesis studies (Lerin et al. 2019; Pessanha et al. 2022). This approach was applied in the present work to understand the differential accumulation of proteins during the development of *D. nigra* seedlings in vitro in culture tubes sealed with and without an MM under MS culture medium. The MS culture medium without an MM resulted in lower seed germination, higher ethylene accumulation within the culture tubes and consequently higher leaf abscission in *D. nigra*. We found that proteins up-accumulated in seedlings grown on MS culture sealed with lids containing MM, such as lipoxygenase protein (A0A445A943). This protein produces signaling molecules involved in several functions, including growth and development and seed germination (Viswanath et al.

2020). Another protein related to plant development and growth is elongation factor 1-alpha (A0A444Y7Y0), which is unique in seedlings grown under lids with MM, which promotes higher gas exchange. This protein functions as a promoter of GTP-dependent binding of aminoacyl-tRNA to the ribosome during protein biosynthesis (White et al. 2019). Thus, the accumulation of these proteins in seedlings grown in culture tubes sealed with lids containing MM was affected by gas exchange and can play an important role in seedling metabolism, as it affects growth and development in *D. nigra*. The protein related to photosynthesis and carbon fixation and metabolism, RuBisCO containing large domains, was also found to be unique (A0A445EET8) and up-accumulated (A0A445EET8) in seedlings grown in culture tubes sealed with MM, which may be associated with higher Fv/Fm and PI values when compared to treatment without membrane in *D. nigra*, suggesting the accumulation of this protein in response to membrane use and gas exchange promotion.

On the other hand, the protein aspartate aminotransferase (A0A444YV53) was unique in seedlings grown in culture tubes sealed with lids without an MM. This protein is related to ethylene biosynthesis and may be directly associated with the higher accumulation of ethylene found in seedlings maintained in this treatment, consequently inducing higher leaf abscission. Ethylene biosynthesis depends directly on the Asp-derived amino acid pathway in which the precursor of ethylene biosynthesis, ACC, is produced and regulated by Asp metabolism (Lemaire et al. 2013). In *Arabidopsis*, this protein is one of the essential enzymes in the biosynthesis of ethylene from the conversion of oxaloacetate into L-aspartate, L-methionine and SAM (Kwon et al. 2010). Furthermore, aspartate aminotransferase protein activity plays a key role in the metabolic regulation of carbon and nitrogen metabolism (Canovas et al. 2007). It has been shown that this protein can promote greater in vitro growth of *D. nigra* shoots under treatment with benzyladenine, altering nitrogen metabolism and increasing biomass (Pessanha et al. 2022).

Malate dehydrogenase (A0A445BC02) protein, accumulated in seedlings under lids with MM, can be relevant for *D. nigra* seedling development, as this protein plays an important role in plant nutrition, metabolism and cellular energy supply

(Schulze et al. 2002). This protein catalyzes a reversible NAD⁺-dependent dehydrogenase reaction involved in central metabolism and redox homeostasis between organelle compartments (Tomaz et al. 2010) and is also necessary for the maintenance of photosynthetic rates under photorespiratory conditions (Cousins et al. 2008). Thus, the accumulation of malate dehydrogenase (A0A445BC02) protein was affected by gas exchange promoted by treatment with an MM in seedlings of *D. nigra*.

Furthermore, proteins related to carbohydrate metabolic processes accumulated in seedlings grown in culture tubes sealed with lids without MM. The 60S ribosomal protein (A0A444XM11) is a structural constituent of ribosomes and has cytoplasmic translation and biogenesis of large ribosomal subunits with biological functions (Xiao et al., 2019). This protein is responsible for the synthesis of proteins necessary for cell growth, division and development (Barakat et al. 2001). Thus, the greater accumulation of the 60S ribosomal protein (A0A444XM11) may be related to the greater elongation of *D. nigra* seedlings grown under lids without an MM. Similar results were observed for in vitro morphogenesis of *D. nigra*, in which the greater elongation of shoots was related to the greater accumulation of this 60S ribosomal protein (Pessanha et al. 2022).

Taken together, the data support an early working model of the roles of differential accumulation of key proteins and different physiological, biochemical and morphological responses of *D. nigra* seedlings grown on MS culture medium. This work is the first to report the effect of MMs and their relationship with the in vitro germination and seedling development of *D. nigra* and their interaction with ethylene accumulation and changes in the protein profile (Fig. 10). Therefore, the results obtained in this study are important to optimize the in vitro germination step for aseptic explants obtention for shoot multiplication for this species.

Conclusion

The use of lids containing MM affected seed germination and ethylene accumulation depending on the culture medium. Germination rates were greater in the WPM, ½WPM and ½MS culture media in tubes sealed with lids containing MM.

The contents of total free PAs and Put were affected by culture medium treatment but not by MM, while Spd and Spm were affected by MM only in seedlings grown in MS½ culture medium. Lower accumulation of CO₂ and leaf abscission occurred in all culture media treatments when culture tubes were sealed with lids containing an MM. Higher ethylene accumulation was observed in seedlings grown in culture tubes with MS and ½MS sealed with lids without MM. Higher accumulation of proteins involved in the processes of photosynthesis and metabolic pathways was observed in seedlings grown on MS culture medium comparing lids with and without an MM. The presence of aspartate aminotransferase protein only in seedlings grown in culture tubes sealed without an MM was related to the higher ethylene accumulation and higher leaf abscission. The presence of photosynthesis-related proteins in seedlings grown under culture tubes sealed with lids with MM was associated with greater gas exchange, corroborating the higher photosynthetic parameters (Fv/Fm and PI) evaluated. The results observed in this study are relevant for the knowledge of biochemical and physiological alterations promoted by gas exchange on *D. nigra* seed germination and seedling growth and are important to optimize the in vitro propagation steps for this species.

Declaration

Financial interests: The authors declare they have no financial interests.

Conflicts of interest/Competing interests: The authors have no conflicts of interest to declare that are relevant to the content of this article.

Data availability: The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD039009. All identified proteins are available in the supplementary material.

Code availability: PXD039009

Author contributions RC and CSC conceived the study, designed the experiments and wrote the manuscript. RC was responsible for the in vitro culture and performed the statistical analyses. RC, RGV and EC performed the physiological analyses. RC and RGV were responsible for the polyamine analyses. RC, EOAS and JGO were responsible for the analysis of ethylene and CO₂. RC, VBP and VS were responsible for proteomic analyses.

Acknowledgments The authors thank Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and the Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) for funding. This study was also financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brazil (CAPES) - Finance Code 001. RCS, RGV, EOAS and VBP are thankful for the scholarship funding provided by FAPERJ and CAPES.

Funding This research was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (444453/2014-8; 309303/2019-2) and the Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) (E26/202.969/2016; E26/202.533/2019). This study was also financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior—Brazil (CAPES)—Finance Code 001.

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4. CONSIDERAÇÕES FINAIS

O uso de biotecnologias, como o cultivo *in vitro*, apresenta potencial de aplicação para a propagação de espécies ameaçadas de extinção, principalmente as arbóreas de alto valor econômico e ecológico, como a *D. nigra*. Nesse sentido, os resultados obtidos nesse estudo, como a definição do meio de cultura associado ao emprego de tampas com membranas microporosas, são importantes para a melhoria da etapa de germinação *in vitro* para *D. nigra*, e consequentemente, a produção de explantes com qualidade para a etapa de obtenção de explantes e multiplicação de brotações para esta espécie.

Dado as características observadas, no que se refere ao comportamento *in vitro*, a espécie *D. nigra* apresenta sensibilidade ao etileno, produzido no cultivo *in vitro* com o uso de tampas de vedação sem membranas microporosas. Neste sentido, os resultados encontrados nessa pesquisa, contribuem para o entendimento dos aspectos de senescência influenciados pelo acúmulo de etileno em espécies lenhosas propagadas *in vitro*, bem como da influência desse hormônio vegetal sobre os processos morfológicos. Assim, estes resultados colaboram para uma maior compreensão bioquímica no processo germinativo *in vitro* das sementes desta espécie ameaçada de extinção em condições onde é favorecida trocas gasosas com o uso de tampas com membranas microporosas, sendo descrito pela primeira vez para a espécie.

As análises bioquímicas relacionadas ao perfil de PAs e proteínas diferencialmente abundantes, são importantes para a compreensão dos mecanismos que tornam as células competentes para o desenvolvimento da morfogênese *in vitro*. Destaca-se ainda, que este estudo possibilitou a identificação de proteínas específicas que são afetadas pelo uso de tampas de vedação com e sem membranas, associadas à maior acúmulo de etileno na cultura *in vitro* nesta espécie arbórea.

Os resultados apresentados neste trabalho são inéditos e relevantes no que tange a germinação *in vitro* de *D. nigra*, e possibilitaram aumentar o conhecimento bioquímico e molecular relacionado com a germinação *in vitro* e

crescimento de plântulas em espécies arbóreas nativas sob influência de tampas de vedação e meio de cultura. Em continuidade, estudos com o uso de tampas de vedação são necessários para investigar a sua influência no sucesso da resposta morfogênica durante o desenvolvimento das brotações e enraizamento nesta espécie.

5. ANEXO

Table 1. Complete list of all proteins identified in *Dalbergia nigra* comparing seedlings grown in culture tubes containing MS culture medium and sealed with lids with (MSCM) and without microporous membrane (MSN). Proteins were considered up-accumulated if the log₂ value of the fold change (FC) was greater than 0.60 and down-accumulated if the log₂ value of the FC was less than -0.60, as determined by Student's t test (two-tailed; P < 0.05)

	Reported peptides	Max score	Description	Biological Process	Normalized Ion Count						Average		t-test	Log ₂ of fold change	Differential Accumulation
					MS CM1	MS CM 2	MS CM 3	MSN 1	MSN 2	MSN 3	MS CM	MSN			
A0A445DQJ5	19	33600,74	ATPase_AAA_core domain-containing protein		496243	658131	641775	179538	278315	389301	598716	282385	0,016406221	1,0842	UP
A0A445EET8	9	33566,79	RuBisCO_large domain-containing protein		15242	17145	20270	8710	10366	7716	17552	8931	0,006500311	0,9748	UP
A0A444YCV9	23	29075,40	ATPase_AAA_core domain-containing protein		72664	128887	149912	16366	22557	44763	117154	27895	0,022240759	2,0703	UP
A0A444X9E3	10	9453,42	Ribulose bisphosphate carboxylase small chain	photorespiration; reductive pentose-phosphate cycle	56281	53966	55271	35784	35507	23664	55173	31652	0,004374837	0,8017	UP
A0A444YDF4	6	7937,54	Uncharacterized protein		6248	4576	4176	1409	1514	1993	5000	1639	0,006990167	1,6094	UP
A0A445E4X3	18	7001,43	Uncharacterized protein	protein refolding	91571	107666	101171	56147	66940	73787	100136	65625	0,007651217	0,6096	UP
A0A444Z767	9	6710,77	Aminotran_1_2 domain-containing protein	biosynthetic process; L-alanine catabolic process	48069	62112	64407	23105	33110	30640	58196	28951	0,0078505	1,0073	UP
A0A445BC02	12	6249,95	Malate dehydrogenase	malate metabolic process; tricarboxylic acid cycle	49085	61186	68097	24404	37008	44168	59456	35194	0,038895227	0,7565	UP
A0A444WVJ5	8	5284,84	Phosphoribulokinase	reductive pentose-phosphate cycle	152434	188760	167953	88499	110096	101366	169715	99987	0,004706663	0,7633	UP
A0A444ZCD3	6	4370,53	Glutamine synthetase	glutamine biosynthetic process	198939	212437	208683	122195	139925	126992	206686	129704	0,00031802	0,6722	UP
A0A445CWK8	9	3812,04	Aldehd domain-containing protein		48725	53317	59128	32370	37000	31776	53723	33715	0,004317383	0,6722	UP
A0A444YCC5	9	3105,37	Glutamate decarboxylase	glutamate metabolic process	33289	38810	37433	15688	21574	27522	36511	21595	0,017140643	0,7576	UP
A0A445C7B3	6	2430,90	Uncharacterized protein	carbohydrate metabolic process	114228	134845	136348	40389	45767	82576	128474	56244	0,008664649	1,1917	UP
A0A444ZVK9	11	2064,61	Serine hydroxymethyltransferase	glycine biosynthetic process from serine; tetrahydrofolate interconversion	25237	32098	38527	13399	17917	22662	31954	17992	0,040535157	0,8286	UP
A0A445AD56	10	2051,26	Epimerase domain-containing protein		66380	66932	69619	46084	46823	33796	67644	42234	0,004251387	0,6795	UP
A0A445A3V0	5	1905,45	NADPH-protochlorophyllide oxidoreductase	chlorophyll biosynthetic process; photosynthesis	47612	47539	27332	21479	19736	23080	40828	21431	0,046609325	0,9298	UP

A0A445DND4	11	1656,16	C2 NT-type domain-containing protein		50508	62382	45982	34166	28108	34030	52958	32101	0,016838399	0,7222	UP
A0A444XD25	4	1632,66	Uncharacterized protein		1660	3656	2202	772	1004	551	2506	775	0,047042944	1,6924	UP
A0A444Y8G4	3	1630,74	Uncharacterized protein	carbohydrate metabolic process	89751	98751	62506	29292	33635	37360	83669	33429	0,010749082	1,3236	UP
A0A445CE47	3	1565,73	Epimerase domain-containing protein		11513	13399	14185	4026	6635	6822	13032	5828	0,003888137	1,1611	UP
A0A444ZFB8	3	1478,57	Uncharacterized protein	xylan catabolic process	22673	23308	35048	7545	8429	6204	27010	7393	0,00856104	1,8693	UP
A0A445A943	5	1279,54	Lipoxygenase	fatty acid biosynthetic process; oxylipin biosynthetic process	47834	50331	48144	30014	29339	21074	48769	26809	0,001806002	0,8633	UP
A0A445EHL4	8	1220,12	Epimerase domain-containing protein		24553	25534	33769	14184	15445	13289	27952	14306	0,010289308	0,9663	UP
A0A444WXY5	3	1207,95	Inorganic diphosphatase	phosphate-containing compound metabolic process	7956	10477	7522	5017	2937	4639	8652	4198	0,016510723	1,0434	UP
A0A444Y4E5	3	1195,08	Thioredoxin domain-containing protein		38052	51283	24881	8055	3728	11509	38072	7764	0,018878407	2,2939	UP
A0A444YVR3	11	1166,01	Glycine cleavage system P protein	glycine catabolic process	51022	43673	36910	26046	24574	24321	43868	24981	0,010066418	0,8124	UP
A0A445D410	2	958,34	Uncharacterized protein	carbohydrate metabolic process	5551	8764	6880	3168	2683	3619	7065	3156	0,015777448	1,1624	UP
A0A445EEZ5	3	936,91	Epimerase domain-containing protein		56194	41011	51293	30210	27224	23009	49499	26814	0,010073212	0,8844	UP
A0A444ZV87	3	854,35	PKS_ER domain-containing protein		53701	57004	47801	38781	37015	24455	52835	33417	0,020881748	0,6609	UP
A0A444Y8T0	4	808,59	Alpha-MPP	DNA repair; protein processing involved in protein targeting to mitochondrion	107313	81144	94337	51616	47934	60508	94265	53353	0,008307618	0,8212	UP
A0A445ELC9	2	774,88	Glutamine synthetase	glutamine biosynthetic process	41047	53789	57553	12447	17612	34217	50796	21425	0,023590851	1,2454	UP
A0A445EEA1	2	753,86	Uncharacterized protein		26464	26234	35561	12718	16546	16174	29420	15146	0,012450504	0,9579	UP
A0A444YFQ5	7	636,15	Glucose-1-phosphate adenylyltransferase	glycogen biosynthetic process; starch biosynthetic process	26644	22076	19931	12681	15799	13765	22884	14082	0,015639137	0,7005	UP
A0A445EQQ4	3	604,37	Malate synthase	Glyoxylate bypass; Tricarboxylic acid cycle	37346	24208	29963	7849	4012	4566	30505	5476	0,003285018	2,4780	UP
A0A444YG83	4	539,29	1,4-alpha-D-glucan glucanohydrolase	carbohydrate metabolic process; proteolysis	64635	76449	108948	16965	33606	43639	83344	31403	0,027762925	1,4082	UP
A0A445BC65	2	524,04	SOR_SNZ domain-containing protein	pyridoxal phosphate biosynthetic process	22183	20013	23582	9997	12585	15685	21926	12756	0,009199613	0,7815	UP
A0A445BDL3	3	521,93	Peptidylprolyl isomerase	cytokinesis by cell plate formation; protein folding; protein peptidyl-prolyl isomerization	26104	35650	28215	21029	19049	17757	29990	19278	0,024559983	0,6375	UP
A0A445DJV5	2	469,79	PAP_fibrillin domain-containing protein		24527	32968	36201	17893	10842	22770	31232	17168	0,045702277	0,8633	UP
A0A445BHJ0	12	42283,16	RuBisCO_large domain-containing protein		4009	4165	6668	-	-	-	4947	-	-	-	Unique MSCM
A0A444Y7Y0	14	9447,65	Elongation factor 1-alpha		3948	4367	5004	-	-	-	4440	-	-	-	Unique MSCM
A0A444Z1Z5	4	7646,40	Carbonic anhydrase	carbon utilization	120979	146901	173077	-	-	-	146986	-	-	-	Unique MSCM

A0A444WRU8	8	5685,70	NmrA domain-containing protein	lignan biosynthetic process	650	680	1233	-	-	-	854	-	-	-	-	Unique MSCM
A0A444YAR2	2	4697,87	Uncharacterized protein		2572	2419	2797	-	-	-	2596	-	-	-	-	Unique MSCM
A0A445BV01	8	4194,77	Lipoxygenase		28505	64132	81251	-	-	-	57963	-	-	-	-	Unique MSCM
A0A444ZBN3	9	4084,07	Uncharacterized protein		2219	2791	2903	-	-	-	2638	-	-	-	-	Unique MSCM
A0A444XB51	4	3980,03	14_3_3 domain-containing protein		1885	2266	2440	-	-	-	2197	-	-	-	-	Unique MSCM
A0A445BXG3	2	3954,80	Uncharacterized protein		1880	1641	1307	-	-	-	1609	-	-	-	-	Unique MSCM
A0A444ZCN2	5	1700,53	Uncharacterized protein		2035	3033	3849	-	-	-	2972	-	-	-	-	Unique MSCM
A0A444ZW91	17	27066,27	ATPase_AAA_core domain-containing protein		-	-	-	7351	12405	17352	-	12369	-	-	-	Unique MSN
A0A444ZMW4	4	8746,27	PEROXIDASE_4 domain-containing protein	response to oxidative stress	-	-	-	3628	4958	5237	-	4608	-	-	-	Unique MSN
A0A445DPL3	4	7330,51	Carbonic anhydrase	carbon utilization	-	-	-	39382	44133	35263	-	39593	-	-	-	Unique MSN
A0A445BZ80	3	4968,37	Photosystem I	photosynthesis	-	-	-	4177	4156	2931	-	3754	-	-	-	Unique MSN
A0A445CDV1	2	3505,62	PsbP domain-containing protein	photosynthesis	-	-	-	2390	2454	1250	-	2031	-	-	-	Unique MSN
A0A445BUX1	14	2496,46	Lipoxygenase	Fatty acid biosynthesis; Fatty acid metabolism; Lipid biosynthesis; Lipid metabolism; Oxylipin biosynthesis	-	-	-	97	749	297	-	381	-	-	-	Unique MSN
A0A445CM38	7	1865,86	Uncharacterized protein	translation	-	-	-	4353	6222	4951	-	5176	-	-	-	Unique MSN
A0A444YV53	14	1856,60	Aspartate aminotransferase	amino acid metabolic process; biosynthetic process	-	-	-	53366	61020	41883	-	52090	-	-	-	Unique MSN
A0A444YGE5	3	1604,58	MHD domain-containing protein		-	-	-	3686	5954	7676	-	5772	-	-	-	Unique MSN
A0A444YWA9	3	1389,08	Polyadenylate-binding protein		-	-	-	15703	15719	7095	-	12839	-	-	-	Unique MSN
A0A445BAE6	19	35784,74	Uncharacterized protein		39818	39674	33695	105640	107572	96078	37729	103097	8,92799E-05	1,4503	DOWN	
A0A444XG75	16	30593,34	Uncharacterized protein		12731	12935	12265	24352	24911	24563	12643	24608	1,26362E-06	0,9608	DOWN	
A0A445ARH5	4	15591,13	Histone H2B		48514	91068	57071	110017	139877	156823	65551	135572	0,020641255	1,0484	DOWN	
A0A444Y6R0	10	14103,61	Peroxidase	hydrogen peroxide catabolic process; response to oxidative stress	122926	149056	95300	190874	258741	217823	122428	222479	0,016322201	-0,8617	DOWN	
A0A445BU13	13	12114,55	Tubulin alpha chain	microtubule-based process	92632	93640	80540	181537	189193	151006	88937	173912	0,002373915	-0,9675	DOWN	
A0A444XLS8	4	11147,05	Chlorophyll a-b binding protein, chloroplastic	photosynthesis, light harvesting	8413	11100	11977	15565	19766	18241	10496	17857	0,010691032	-0,7666	DOWN	
A0A444ZW25	8	9939,84	Histone H4		22117	36365	37375	64699	88138	77556	31952	76798	0,005875032	-1,2651	DOWN	
A0A444XM11	4	9543,59	60S ribosomal protein	translation	26566	34278	40724	45785	62887	72098	33856	60257	0,038980264	-0,8317	DOWN	
A0A445CS98	4	8786,40	Ribosomal_L7Ae domain-containing protein		33595	29102	27092	56428	62269	46958	29929	55218	0,00648959	-0,8836	DOWN	

A0A445AJN6	16	8469,44	Tubulin beta chain	microtubule-based process	11114	8361	6534	13397	14240	11843	8669	13160	0,04057123 6	- 0,6022	DOWN
A0A445BR63	9	6551,09	NmrA domain-containing protein	lignan biosynthetic process	75012	69865	93210	11476 1	14190 6	10943 8	79362	12203 5	0,02558109 2	- 0,6208	DOWN
A0A444Y3F0	8	6169,96	Uncharacterized protein		60291	73586	53190	97307	10331 3	11374 3	62355	10478 8	0,00521024 6	- 0,7489	DOWN
A0A445B1R3	14	5421,29	5-methyltetrahydropteroylglutamate e-homocysteine S-methyltransferase	methionine biosynthetic process; methylation	90708	11813 7	11749 2	17193 4	20291 9	16358 2	10877 9	17947 8	0,00920684 6	- 0,7224	DOWN
A0A444Y6Y4	9	4831,96	Aconitase domain-containing protein		8635	8753	8663	13507	15176	14191	8684	14291	0,00032187 7	- 0,7188	DOWN
A0A445CFR2	4	3679,75	NmrA domain-containing protein	lignan biosynthetic process	46545	40436	23757	75059	65389	57133	36913	65861	0,02770426 5	- 0,8353	DOWN
A0A444X032	5	3590,71	Germin-like protein		40739	63188	78235	89615	12569 5	14798 8	60721	12109 9	0,04035923 1	- 0,9959	DOWN
A0A445DQ85	11	3539,68	Glutamate decarboxylase	glutamate metabolic process	17493	20368	19637	40649	55930	72246	19166	56275	0,01548112 5	- 1,5539	DOWN
A0A445DIV1	12	3215,44	Peroxidase	hydrogen peroxide catabolic process; response to oxidative stress	109391	11506 6	10912 9	17116 6	19825 0	18705 2	11119 5	18549 0	0,00078182 7	- 0,7382	DOWN
A0A445CPT6	3	2732,52	Uncharacterized protein	translation	27020	22973	22622	47672	42924	37082	24205	42559	0,00553107 3	- 0,8142	DOWN
A0A444WNT6	5	2609,22	Aldo_ket_red domain-containing protein		21564	19349	18949	38030	35936	29320	19954	34429	0,00622229	- 0,7869	DOWN
A0A445AF75	2	2571,92	Ribosomal_S10 domain-containing protein	translation	36939	31582	53733	71698	72881	80274	40751	74951	0,00893660 9	- 0,8791	DOWN
A0A445AB22	11	2131,33	Uncharacterized protein		8913	10100	5958	19583	25768	17115	8324	20822	0,01187055 9	- 1,3228	DOWN
A0A445C543	5	2042,44	Peroxidase	hydrogen peroxide catabolic process; response to oxidative stress	48095	46726	41074	63289	77314	76070	45298	72224	0,00562792 4	- 0,6730	DOWN
A0A444YF68	4	1997,38	Dihydrolipoyllysine-residue succinyltransferase	L-lysine catabolic process to acetyl-CoA via saccharopine; tricarboxylic acid cycle	26512	37863	40327	46390	62593	52187	34900	53723	0,04174957 9	- 0,6223	DOWN
A0A444YJV3	2	1482,45	Ribosomal_L18e/L15P domain-containing protein	translation	28122	30422	23016	40807	45898	46988	27186	44564	0,00390733 1	- 0,7130	DOWN
A0A445BN79	2	1399,02	40S ribosomal protein S8	translation	22212	25951	15497	38838	53297	34685	21220	42273	0,03046554 5	- 0,9943	DOWN
A0A445C521	2	1259,27	Peroxidase	hydrogen peroxide catabolic process; response to oxidative stress	19313	25107	21701	39998	41098	51124	22040	44073	0,00491999 4	- 0,9998	DOWN
A0A444ZH95	2	1203,30	Uncharacterized protein	translation	1997	9461	10593	30017	20532	15777	7350	22108	0,04139	- 1,5887	DOWN
A0A445E2J6	3	1168,79	Uncharacterized protein		7876	11662	11096	14885	18617	15249	10211	16250	0,02259211 9	- 0,6703	DOWN
A0A445CMJ0	10	1138,44	Sucrose synthase	sucrose metabolic process	37707	34208	31406	52655	54006	54274	34441	53645	0,00052869 2	- 0,6393	DOWN
A0A444Z989	3	1078,94	Ribosomal_L2_C domain-containing protein	translation	20639	27399	11462	42824	49580	30430	19833	40945	0,04384698 7	- 1,0458	DOWN
A0A444YJM4	3	1067,29	Uncharacterized protein	translation	17620	16292	17236	25613	28407	24050	17049	26023	0,00254424	- 0,6101	DOWN
A0A445A5G2	5	991,89	Assimilatory sulfite reductase (ferredoxin)		16781	62059	16841	81193	72693	75296	31894	76394	0,04366729 9	- 1,2602	DOWN
A0A445CIL2	13	968,35	Phospholipase D	Lipid degradation; Lipid metabolism	51161	35740	42050	83958	71637	55845	42984	70480	0,04151330 4	- 0,7134	DOWN

A0A444Y1E4	3	958,47	Uncharacterized protein		24999	13923	20701	58801	46150	45154	19875	50035	0,005205627	-1,3320	DOWN
A0A445BF06	4	938,65	S-methyl-5-thioribose kinase	methionine biosynthetic process	24108	28898	26022	39323	44607	36656	26342	40195	0,007013532	-0,6096	DOWN
A0A445BRF2	10	870,66	HATPase_c domain-containing protein		22735	23066	26508	31244	47987	44368	24103	41200	0,030775829	-0,7734	DOWN
A0A445AD05	3	740,65	Pectinesterase	cell wall modification; pectin catabolic process	23155	29336	28362	32804	49069	45249	26951	42374	0,043004492	-0,6529	DOWN
A0A444ZCQ1	2	726,79	Dihydrolipolylysine-residue succinyltransferase	L-lysine catabolic process to acetyl-CoA via saccharopine; tricarboxylic acid cycle	29746	30298	28632	54622	35970	54560	29558	48384	0,039027652	-0,7110	DOWN
A0A444YX51	3	545,95	D-fructose-1,6-bisphosphate 1-phosphohydrolase	carbohydrate metabolic process	13203	18556	25663	32342	40684	39307	19140	37444	0,014554175	-0,9681	DOWN
A0A444X517	2	434,23	60S ribosomal protein L6	translation	8154	7652	5961	16436	17144	11378	7256	14986	0,016139793	-1,0464	DOWN
A0A444ZMTO	2	399,25	Uncharacterized protein	carbohydrate metabolic process	24378	17237	14965	39642	47291	26401	18860	37778	0,048243536	-1,0022	DOWN
A0A445E765	2	373,01	Pectinesterase	cell wall modification; pectin catabolic process	33451	37208	35520	56952	76943	71175	35393	68357	0,005475278	-0,9496	DOWN
A0A445AH34	33	85530,68	Ribulose bisphosphate carboxylase large chain	photorespiration; reductive pentose-phosphate cycle	753638	89878	94796	71348	81775	84930	86679	79351	0,362277434	0,1274	UNCHANGED
A0A445CYP2	10	49375,18	Chlorophyll a-b binding protein, chloroplastic	photosynthesis, light harvesting	545445	58641	44635	51942	71983	54125	52607	59350	0,42440998	-0,1740	UNCHANGED
A0A444XGR5	4	45306,76	9 kDa polypeptide	photosynthetic electron transport in photosystem I	212556	18938	13126	13707	12865	10264	17773	12278	0,104975521	0,5336	UNCHANGED
A0A445DVLO	5	44348,22	9 kDa polypeptide	photosynthetic electron transport in photosystem I	237211	22027	16763	18486	17297	15374	20837	17052	0,172631881	0,2892	UNCHANGED
A0A445BUJ9	26	44346,90	H(+)-transporting two-sector ATPase		330473	39089	36566	22850	23615	26136	36234	24200	0,003940477	0,5823	UNCHANGED
A0A445BJK2	13	42793,44	Uncharacterized protein		369258	46561	47400	22090	30206	39873	43629	30723	0,103469391	0,5060	UNCHANGED
A0A445CL48	18	35041,34	Uncharacterized protein		169203	21923	18675	16330	20379	17415	19173	18041	0,58368977	0,0878	UNCHANGED
A0A444X337	6	33195,30	Uncharacterized protein	photosynthetic electron transport chain	164040	16294	12472	18461	19831	15845	15057	18045	0,161535485	-0,2612	UNCHANGED
A0A444X591	20	31518,32	Uncharacterized protein		86634	14257	13740	11140	17564	15751	12220	14818	0,376773131	-0,2781	UNCHANGED
A0A445DVJ9	14	29761,00	Uncharacterized protein	ATP metabolic process; proton transmembrane transport	262703	26606	28410	19975	18394	20815	27095	19728	0,001625753	0,4578	UNCHANGED
A0A445DA67	17	28979,78	Uncharacterized protein		56143	69957	59740	50792	65358	62147	61947	59432	0,699176579	0,0598	UNCHANGED
A0A445B598	4	26975,39	ATP-synt_ab_N domain-containing protein	ATP metabolic process; proton transmembrane transport	138120	16424	16058	10835	13146	10573	15431	11518	0,027633039	0,4219	UNCHANGED
A0A445C618	3	22503,04	Uncharacterized protein	photosynthetic electron transport in photosystem II	22249	17912	32331	33504	21609	20663	29148	0,102941728	-0,4963	UNCHANGED	
A0A445AJD1	20	22314,71	ATPase AAA_core domain-containing protein		27489	46742	44223	22988	32864	37456	39485	31103	0,320519349	0,3442	UNCHANGED

A0A445C2K4	6	21033,30	Chlorophyll a-b binding protein, chloroplastic	photosynthesis, light harvesting	358131	340078	363810	334487	334439	285825	354006	318250	0,113794345	0,1536	UNCHANGED
A0A445BI73	15	20479,61	Malate dehydrogenase	malate metabolic process; tricarboxylic acid cycle	247174	305950	311008	175818	242053	283073	288044	233648	0,219138737	0,3020	UNCHANGED
A0A445BSX1	4	19723,81	Uncharacterized protein	photosynthetic electron transport in photosystem II	photosystem II	157789	127046	184331	228018	164848	142789	192399	0,074449901	-0,4302	UNCHANGED
A0A445C3D7	13	18001,41	FCP1 homology domain-containing protein	photosystem II assembly; photosystem II stabilization	271384	316359	324239	314591	336040	271462	303994	307364	0,899806046	-0,0159	UNCHANGED
A0A444YFD2	12	17959,14	Uncharacterized protein	photosystem II assembly; photosystem II stabilization	194605	226856	232507	200387	214050	172915	217989	195784	0,259200364	0,1550	UNCHANGED
A0A444WQ31	5	17105,54	Uncharacterized protein	photosynthetic electron transport chain	107525	100169	136754	118233	141979	113462	114816	124558	0,531281865	-0,1175	UNCHANGED
A0A444WPU1	4	16853,77	H(+)-transporting two-sector ATPase		64674	61262	51216	65470	64308	57160	59051	62313	0,534316154	-0,0776	UNCHANGED
A0A445DWQ9	16	16241,78	Glyceraldehyde-3-phosphate dehydrogenase	glucose metabolic process	251753	310102	326651	257299	339935	366472	296169	321235	0,564477624	-0,1172	UNCHANGED
A0A444YPV6	9	16010,29	Uncharacterized protein		395888	400875	377840	297837	327050	295719	391534	306869	0,002330465	0,3515	UNCHANGED
A0A445BCL3	7	15411,64	Peptidyl-prolyl cis-trans isomerase	protein folding; protein peptidyl-prolyl isomerization	323137	325537	294171	297183	273719	280674	314282	283859	0,067919655	0,1469	UNCHANGED
A0A444XDL1	4	15278,80	Nucleoside diphosphate kinase	CTP biosynthetic process; GTP biosynthetic process; nucleoside diphosphate phosphorylation; UTP biosynthetic process	236104	194053	203606	265713	258783	209408	211254	244635	0,200843675	-0,2117	UNCHANGED
A0A445E302	13	15083,90	Fructose-bisphosphate aldolase	glycolytic process	323024	308355	337817	315051	315703	232072	323065	287609	0,289177351	0,1677	UNCHANGED
A0A444YP93	15	14974,65	Fructose-bisphosphate aldolase	glycolytic process	329871	332230	334584	330270	358367	377593	332228	355410	0,168481516	-0,0973	UNCHANGED
A0A444XJB9	6	14851,99	Uncharacterized protein	photosynthetic electron transport chain	206930	268700	203627	248683	338520	274728	226419	287310	0,148318998	0,3436	UNCHANGED
A0A444XI53	6	14780,21	Uncharacterized protein	protein transport; vesicle-mediated transport	8301	41134	61620	4511	26778	102745	37018	44678	0,830590125	-0,2713	UNCHANGED
A0A445A0G3	8	14751,61	Uncharacterized protein	ATP metabolic process; proton transmembrane transport	212159	206470	206907	177268	183987	156550	208512	172601	0,013195784	0,2727	UNCHANGED
A0A444WQJ4	6	14737,65	Uncharacterized protein	photosynthetic electron transport chain	78001	115442	92630	102033	146851	104624	95358	117836	0,283435204	-0,3054	UNCHANGED
A0A445CCR6	15	14596,93	Tubulin beta chain	microtubule-based process	108835	107960	90121	123932	128888	122685	102305	125168	0,0231502	-0,2910	UNCHANGED
A0A445DA98	24	14562,06	Uncharacterized protein		150269	139349	148968	160254	187779	180825	146195	176286	0,02827444	-0,2700	UNCHANGED
A0A444WZ23	11	14247,51	Chlorophyll a-b binding protein, chloroplastic	photosynthesis, light harvesting	143002	181531	180873	123147	148912	144141	168469	138733	0,118362394	0,2802	UNCHANGED
A0A445DA00	8	13952,59	Ribulose bisphosphate carboxylase small chain	photorespiration; reductive pentose-phosphate cycle	850129	722578	556410	666081	572015	487059	709706	575052	0,247451803	0,3035	UNCHANGED
A0A445DY22	16	13690,32	Glyoxalase I		72410	80063	61420	92051	79569	61194	71298	77605	0,579314123	-0,1223	UNCHANGED
A0A444XWW4	7	13430,45	Photosystem I reaction center subunit II	photosynthesis	265836	304952	179765	152147	152934	163762	250184	156281	0,064897646	0,6788	UNCHANGED
A0A445D4N7	9	13230,20	Chlorophyll a-b binding protein, chloroplastic	photosynthesis, light harvesting	102874	153555	166037	96111	134003	145243	140822	125119	0,554423423	0,1706	UNCHANGED
A0A445BRP0	13	13059,01	Catalase	hydrogen peroxide catabolic process; response to oxidative stress	271344	252951	308535	369855	372827	370973	277610	371218	0,004629474	-0,4192	UNCHANGED
A0A444XB67	16	12712,05	Tubulin alpha chain	microtubule-based process	103241	100458	89589	98718	94663	113571	97763	102317	0,55599715	-0,0657	UNCHANGED

A0A445A9Y6	17	12504,4 8	Uncharacterized protein		57999	60754	77357	48256	62843	62045	65370	57715	0,37524954 2	0,1797	UNCHANGED
A0A445BRG5	12	12398,9 5	Delta-tubulin	cell projection organization; microtubule-based process	8027	7534	5773	10582	9710	8837	7111	9710	0,03771561 - 0,4493	-	UNCHANGED
A0A445DXV8	12	12281,4 8	Malic enzyme	malate metabolic process	130218	16257 3	17231 7	14534 9	19485 5	20850 6	15503 6	18290 3	0,29273403 - 0,2385	-	UNCHANGED
A0A445BF91	12	12133,1 6	Tubulin alpha chain	microtubule-based process	5654	5478	4171	6559	6281	4875	5101	5905	0,31499403 - 0,2111	-	UNCHANGED
A0A445BCY2	17	12060,9 3	Elongation factor 1-alpha		268513	29696 6	34030 4	26912 6	35548 4	36929 7	30192 8	33130 2	0,47892887 - 0,1339	-	UNCHANGED
A0A444YZP5	13	11722,7 9	Malic enzyme	malate metabolic process	108234	12352 5	14372 7	86151	11263 8	12084 2	12516 2	10654 4	0,27322955 - 0,2323	-	UNCHANGED
A0A444XW77	23	11476,3 1	ATP synthase subunit beta		216748	23626 8	26810 6	24016 1	25775 4	33101 3	24037 4	27630 9	0,31882729 - 0,2010	-	UNCHANGED
A0A445CBX6	7	11281,7 9	NmrA domain-containing protein	lignan biosynthetic process	84199	88273	10779 1	10577 8	18489 7	16981 1	93421	15349 5	0,07662132 - 0,7164	-	UNCHANGED
A0A445EAT1	5	11151,7 9	Chlorophyll a-b binding protein, chloroplastic	photosynthesis, light harvesting	167766	21815 8	24696 5	20185 0	24918 2	21806 2	21096 3	22303 1	0,67792341 - 0,0803	-	UNCHANGED
A0A445CD99	5	11147,0 5	Chlorophyll a-b binding protein, chloroplastic	photosynthesis, light harvesting	16189	22567	23358	12286	17521	17955	20704	15921	0,17555010 1	0,3790	UNCHANGED
A0A445BQM2	15	10995,0 7	Heat shock protein 70 family protein		18822	22494	23042	25640	31002	29120	21453	28587	0,02553168 - 0,4142	-	UNCHANGED
A0A445D2U1	12	10730,0 4	Malic enzyme	malate metabolic process	28385	38016	40563	20158	30581	33121	35654	27953	0,22908231 - 0,3511	-	UNCHANGED
A0A445CEI5	16	10704,9 6	Phosphoglycerate kinase	glycolytic process	245236	27816 3	31041 6	22208 3	29777 9	31156 7	27793 8	27714 3	0,98224232 - 0,0041	-	UNCHANGED
A0A444Y0B6	6	10560,3 8	Uncharacterized protein		141260	17866 7	18813 4	12958 7	18442 8	20917 1	16935 4	17439 5	0,86359096 - 0,0423	-	UNCHANGED
A0A445E4L8	7	10528,0 4	Uncharacterized protein	translation	133795	10290 4	15233 4	14163 8	18076 9	15222 9	12967 7	15821 2	0,19896842 - 0,2869	-	UNCHANGED
A0A444WUG6	12	10398,5 6	Ferredoxin-NADP reductase, chloroplastic		253155	26354 0	29949 6	16804 0	22362 6	17192 4	27206 4	18786 3	0,02085104 - 0,5343	-	UNCHANGED
A0A445BI82	14	10319,0 5	Malate dehydrogenase	malate metabolic process; tricarboxylic acid cycle	50103	54901	61915	73226	82590	86617	55640	80811	0,00864633 - 0,5384	-	UNCHANGED
A0A445DRP4	6	10248,4 2	NAD(P)H dehydrogenase (quinone)		144216	12186 2	13116 9	19137 2	19094 9	12558 8	13241 6	16930 3	0,18098989 - 0,3545	-	UNCHANGED
A0A445B9L1	10	10208,0 4	Glyceraldehyde-3-phosphate dehydrogenase	glucose metabolic process	45346	61746	68398	34101	54293	52060	58497	46818	0,28057238 - 0,3213	-	UNCHANGED
A0A445B7R8	7	9867,67	Ribulose bisphosphate carboxylase large chain	photorespiration; reductive pentose-phosphate cycle	10073	12059	14033	11267	14499	14238	12055	13335	0,45359289 - 0,1456	-	UNCHANGED
A0A445D9F8	6	9852,21	Uncharacterized protein	photosynthetic electron transport in photosystem II	13407 1	12505 2	75904	12271 9	14501 6	11538 6	11454 6	0,97475097	0,0105	UNCHANGED	
A0A444YMI9	18	9603,35	Uncharacterized protein		92213	78985	65222	12386 0	10824 3	91097	78807	10773 4	0,07765893 - 0,4511	-	UNCHANGED
A0A445EUW8	17	9379,29	Photosystem I P700 chlorophyll a apoprotein A1	photosynthesis	93758	15385 5	12108 7	14518 0	22820 4	13800 6	12290 0	17046 3	0,23163268 - 0,4720	-	UNCHANGED
A0A444ZDX1	17	9366,31	Tubulin beta chain	microtubule-based process	174035	18627 7	17954 4	12926 0	16729 2	19919 3	17995 2	16524 8	0,51327921 - 0,1230	-	UNCHANGED
A0A445C698	7	9313,32	Uncharacterized protein	photosynthetic electron transport in photosystem II; translation	49257	79335	54720	51579	83051	85854	61104	73495	0,43698307 - 0,2664	-	UNCHANGED

A0A444Y8D3	7	9293,85	Uncharacterized protein	glycolytic process	121964	15084 0	17678 2	13830 3	16587 4	18144 8	14986 2	16187 5	0,58480653 2	- 0,1112	UNCHANGED
A0A445BE75	16	9261,18	Vacuolar proton pump subunit B	ATP metabolic process	118343	11545 3	12013 8	10144 6	12011 2	13163 1	11797 8	11772 9	0,97905763 3	0,0030	UNCHANGED
A0A445CD15	11	9251,19	Glyceraldehyde-3-phosphate dehydrogenase	glucose metabolic process	127671	15892 3	14907 4	80543	10705 8	12184 3	14522 3	10314 8	0,05043535 6	0,4936	UNCHANGED
A0A445CIR4	9	8638,53	Uncharacterized protein		5615	5860	5484	6332	7272	7750	5653	7118	0,02725499 5	- 0,3324	UNCHANGED
A0A445D2M1	5	8491,89	Glutaredoxin-dependent peroxiredoxin		77516	14130 0	12720 5	65349	12030 7	12996 3	11534 0	10520 6	0,73493018 3	0,1327	UNCHANGED
A0A445DC02	4	8315,63	ATP synthase subunit alpha, mitochondrial	proton motive force-driven ATP synthesis; proton transmembrane transport	34597	49494	48894	35342	45469	65427	44328	48746	0,68408587 6	- 0,1371	UNCHANGED
A0A445E771	10	8279,66	Pyruvate kinase		9729	11979	15548	13601	30772	19805	12419	21392	0,16557483 6	- 0,7846	UNCHANGED
A0A444XSI4	8	8239,24	Pyruvate kinase		48825	70014	83014	59840	73704	82661	67285	72068	0,70990375 6	- 0,0991	UNCHANGED
A0A445AUQ2	8	8109,03	Uncharacterized protein		64495	59131	50441	64455	58422	61591	58022	61489	0,47946987 7	- 0,0837	UNCHANGED
A0A445ETS2	3	8047,58	Uncharacterized protein	protein transport; vesicle-mediated transport	4142	10161	13537	1291	5233	12877	9280	6467	0,55505101 7	0,5210	UNCHANGED
A0A445CQU6	8	8015,36	Pyruvate kinase		21107	31594	39181	11764	28606	19531	30627	19967	0,21031128 9	0,6172	UNCHANGED
G9HPX8	8	7871,07	Peptidyl-prolyl cis-trans isomerase	protein folding; protein peptidyl-prolyl isomerization	126493	11872 7	13170 6	96974	94057	93710	12564 2	94914	0,0014169	0,4046	UNCHANGED
A0A444YF39	5	7823,69	Uncharacterized protein		50316	87068	10281 4	31420	54583	10035 0	80066	62118	0,52088129 7	0,3662	UNCHANGED
A0A445BDQ8	20	7797,79	Transketolase		175897	19422 9	18021 3	13077 5	13942 0	14579 4	18344 6	13866 3	0,00313006	0,4038	UNCHANGED
A0A445C025	5	7788,78	Cytochrome f	photosynthesis	89987	69379	10990 1	73542	10060 3	10538 4	89755	93176	0,83439201 2	- 0,0540	UNCHANGED
A0A444ZVQ6	17	7707,06	UTP--glucose-1-phosphate uridylyltransferase	UDP-glucose metabolic process	144345	11590 7	13146 2	16482 5	17374 4	17908 8	13057 1	17255 2	0,01036853 9	- 0,4022	UNCHANGED
A0A444X967	6	7702,30	Proteasome subunit alpha type	ubiquitin-dependent protein catabolic process	59426	83077	93972	65438	99425	10166 7	78825	88844	0,55411963 2	- 0,1726	UNCHANGED
A0A0A6ZDN9	8	7631,46	Peptidyl-prolyl cis-trans isomerase	protein folding; protein peptidyl-prolyl isomerization	24474	25958	16040	28841	15921	25074	22158	23279	0,83109151 7	- 0,0712	UNCHANGED
A0A445ED48	15	7618,40	ATP synthase subunit alpha		178414	18105 1	15584 4	13780 1	15324 0	18641 6	17177 0	15915 3	0,48514166 7	0,1101	UNCHANGED
A0A444ZC42	23	7506,72	Uncharacterized protein		143723	17573 5	19256 2	11535 4	12659 7	12499 4	17067 4	12231 5	0,03052917 4	0,4806	UNCHANGED
A0A445CZ80	18	7445,80	Tubulin beta chain	microtubule-based process	27940	26129	15998	37200	38711	30181	23356	35364	0,05766841 3	- 0,5985	UNCHANGED
A0A444ZZ51	14	7312,10	Tubulin beta chain	microtubule-based process	19674	17407	15682	22558	24374	21642	17588	22858	0,02004692 8	- 0,3781	UNCHANGED
A0A444Y8J0	11	7279,40	PfkB domain-containing protein	phosphorylation	48987	79622	84152	45812	84313	13320 5	70920	87777	0,57430312 9	- 0,3076	UNCHANGED
A0A444Y035	24	7170,90	Tr-type G domain-containing protein	translation	130188	14132 3	13714 3	11101 1	11660 5	12483 3	13621 8	11748 3	0,02219175 9	0,2135	UNCHANGED
T2B9M0	12	7141,37	Fructose-bisphosphate aldolase	glycolytic process	160326	15131 7	11734 3	21482 2	15733 3	16139 7	14299 5	17785 1	0,19914162 5	- 0,3147	UNCHANGED
A0A444YRD7	9	7122,84	Tubulin alpha chain	microtubule-based process	24120	24669	13620	17461	17901	14589	20803	16650	0,32931710 8	0,3212	UNCHANGED
A0A445CCX1	14	7108,36	Catalase	hydrogen peroxide catabolic process; response to oxidative stress	137236	11167 5	14023 9	89501	88810	82340	12971 7	86884	0,01015568 5	0,5782	UNCHANGED
A0A445ETU5	9	7093,20	Pectin acetyl esterase	cell wall organization	108312	12523 9	14418 1	16962 6	19306 2	14836 2	12591 1	17035 0	0,05495286 5	- 0,4361	UNCHANGED

A0A445CQ67	14	7032,84	Phosphopyruvate hydratase	glycolytic process	119393	12224 4	11708 3	12988 2	13844 4	11999 1	11957 3	12943 9	0,14934922 9	- 0,1144	UNCHANGED
A0A444YY24	12	6928,21	Phosphopyruvate hydratase	glycolytic process	58977	60118	65480	61838	68871	61692	61525	64134	0,44790659 9	- 0,0599	UNCHANGED
A0A445E6W9	20	6884,67	Transketolase		52956	56451	59622	47170	45027	40338	56343	44178	0,01203866 9	0,3509	UNCHANGED
A0A190CSF1	9	6861,30	Eukaryotic translation initiation factor 4A		61530	50745	50424	68889	44994	57612	54233	57165	0,72634063 5	- 0,0760	UNCHANGED
A0A444XZW0	3	6832,55	Bet_v_1 domain-containing protein	defense response	138711	15259 4	14500 4	15690 3	20225 6	26458 4	14543 6	20791 4	0,11808294 4	- 0,5156	UNCHANGED
A0A444ZRW6	12	6620,81	Serine hydroxymethyltransferase	glycine biosynthetic process from serine; tetrahydrofolate interconversion	107550	12389 7	13205 2	11456 1	12625 8	15433 1	12116 6	13171 7	0,48793032 8	- 0,1204	UNCHANGED
A0A445E4E5	11	6587,70	L-ascorbate peroxidase	cellular response to oxidative stress	125686	17415 5	17707 8	12631	10429 1	18970 2	15897 3	14010 4	0,57019963 7	0,1823	UNCHANGED
A0A445BTG1	13	6427,87	14_3_3 domain-containing protein		73115	52659	59960	69455	56868	54759	61911	60361	0,84711653 2	0,0366	UNCHANGED
A0A445C8P8	7	6393,88	Malic enzyme	malate metabolic process	23245	30096	31973	34768	53752	58354	28438	48958	0,05589967 2	- 0,7837	UNCHANGED
A0A444XV97	10	6312,17	Fructose-bisphosphate aldolase	Glycolysis	191416	24863 7	25736 8	15087 1	19072 9	17505 4	23247 4	17221 8	0,06389051 7	0,4328	UNCHANGED
A0A444Z9I7	9	6271,86	NmrA domain-containing protein	lignan biosynthetic process	176917	19444 6	25644 9	22015 2	24182 2	20707 4	20927 1	22301 6	0,62715090 5	- 0,0918	UNCHANGED
A0A444Y8X9	13	6233,65	Lipoxygenase	fatty acid biosynthetic process; oxylipin biosynthetic process	72090	89849	78641	12086 8	18032 8	69796	12661 2	0,15002434 5	- 0,8592	UNCHANGED	
A0A444Y2I8	14	6202,24	(S)-2-hydroxy-acid oxidase	response to other organism	86576	13683 8	15700 5	75542	10056 5	11096 4	12680 6	95691	0,25489554 3	0,4062	UNCHANGED
A0A444Y6R9	6	6184,22	Plastoquinol--plastocyanin reductase		57812	70500	14849	23447	42026	54386	26774	0,10384943 6	1,0224	UNCHANGED	
A0A445A8H5	2	6139,03	Uncharacterized protein		154266	15806 9	12941 2	15527 9	16947 2	17311 0	14724 9	16595 4	0,14956169 8	- 0,1725	UNCHANGED
A0A445DV66	21	6083,91	Uncharacterized protein		31926	39218	65883	49394	88814	10190 4	45676	80038	0,14249000 2	- 0,8092	UNCHANGED
A0A445BUL7	8	6055,52	Photosystem I	photosynthesis	88707	95551	74000	12404 0	11738 3	96787	86086	11273 7	0,06211512 3	- 0,3891	UNCHANGED
A0A444YNN6	19	5991,23	Uncharacterized protein	cell cycle	65718	85258	68082	54360	75346	84450	73019	71385	0,88735017 4	0,0327	UNCHANGED
A0A444XBL2	4	5987,67	PSI-G	photosynthesis	134825	14843 6	12362 0	14065 2	13698 1	79145	13562 7	11892 6	0,47433226 8	0,1896	UNCHANGED
A0A444XGU2	17	5865,95	Lipoxygenase	fatty acid biosynthetic process; oxylipin biosynthetic process	18110 9	18018 1	13336 2	19361 4	17728 5	17389 0	16808 7	0,77776669 2	0,0490	UNCHANGED	
A0A445AU23	4	5840,53	Gp_dh_C domain-containing protein		13883	19723	18518	9386	12457	15315	17375	12386	0,11352779 5	0,4883	UNCHANGED
A0A444ZBS6	12	5828,85	Adenosylhomocysteinase	one-carbon metabolic process	74778	94903	68258	73040	93073	10921 9	79313	91778	0,39794012 9	- 0,2106	UNCHANGED
A0A445AA41	19	5792,10	Uncharacterized protein	cell cycle	8409	13624	15482	3681	6582	16268	12505	8843	0,44777159 2	0,4998	UNCHANGED
A0A445AV2	22	5788,82	Aconitate hydratase	oxoacid metabolic process	53616	58059	60593	53136	57094	64463	57422	58231	0,84563815 4	- 0,0202	UNCHANGED
A0A445BWU3	17	5743,38	Aconitate hydratase	oxoacid metabolic process	33007	33532	32791	30289	38044	36592	33110	34975	0,47882434 9	- 0,0791	UNCHANGED
A0A444ZRU7	14	5681,03	Adenosylhomocysteinase	one-carbon metabolic process	58979	71903	78999	48361	65542	79149	69961	64351	0,62660721 9	0,1206	UNCHANGED
A0A445BKU9	7	5675,96	Calreticulin	protein folding	168927	19347 9	16000 4	16808 0	20845 8	17868 2	17413 7	18507 3	0,52420991 6	- 0,0879	UNCHANGED
A0A445C3W2	4	5618,19	PsbP domain-containing protein	photosynthesis	244971	27634 5	22259 2	20600 8	21815 4	18656 3	24796 9	20357 5	0,07025577 2	0,2846	UNCHANGED

A0A445AND8	22	5509,69	HATPase_c domain-containing protein		52721	50330	65351	61837	69689	86258	56134	72595	0,127318698	-0,3710	UNCHANGED
A0A445D9X7	8	5469,21	14_3_3 domain-containing protein		35505	32667	39523	33263	29811	29273	35898	30782	0,094964685	0,2218	UNCHANGED
A0A445E0E4	6	5449,50	Rieske domain-containing protein		19690	28911	30087	20355	28502	37491	26229	28783	0,689481563	-0,1340	UNCHANGED
A0A444XBL6	4	5408,10	Chlorophyll a-b binding protein, chloroplastic	photosynthesis, light harvesting	79785	84243	88872	78805	71567	80372	84300	76915	0,121931086	0,1323	UNCHANGED
A0A445CEK0	14	5300,55	Phosphoglycerate kinase	glycolytic process	30536	41389	61615	31475	37307	70757	44513	46513	0,902042997	-0,0634	UNCHANGED
A0A445EN22	4	5201,28	Uncharacterized protein		13214	16772	13617	24112	22912	13702	14534	20242	0,175880302	-0,4779	UNCHANGED
A0A445D6C6	3	5094,34	Uncharacterized protein	photosynthetic electron transport chain	44890	87436	76645	48545	146263	74966	69657	89925	0,559188969	-0,3685	UNCHANGED
A0A445AU1	9	5074,33	Aldehd domain-containing protein		128185	139275	143060	127992	156159	153410	136840	145854	0,418998024	-0,0920	UNCHANGED
A0A444YQY9	21	5042,55	5-methyltetrahydropteroylglutamate-homocysteine S-methyltransferase	methionine biosynthetic process; methylation	93970	144808	162920	123456	140865	201167	133899	155163	0,534314647	-0,2126	UNCHANGED
A0A445AI22	5	4976,72	ATP-synt_ab domain-containing protein		57575	49465	46753	82811	78134	50935	51265	70627	0,137705761	-0,4623	UNCHANGED
A0A445EDG2	3	4925,39	Cytochrome b	photosynthetic electron transport chain; protein stabilization; respiratory electron transport chain	15498	18147	17766	15524	22318	12161	17137	16668	0,887042295	0,0400	UNCHANGED
A0A445E7B3	7	4879,88	Fructose-bisphosphate aldolase	glycolytic process	22219	19485	17380	30782	26257	19274	19694	25438	0,188648097	-0,3692	UNCHANGED
A0A445A9D0	9	4877,34	EF1_GNE domain-containing protein		91216	137486	154408	61104	100161	156329	127703	105864	0,549726252	0,2706	UNCHANGED
A0A445CNW3	12	4852,00	Uncharacterized protein		116526	88145	67096	102253	87067	72965	90589	87429	0,85854475	0,0512	UNCHANGED
A0A444YIX8	8	4743,36	S-adenosylmethionine synthase	one-carbon metabolic process; S-adenosylmethionine biosynthetic process	107157	156655	137885	112423	125064	147853	133899	128446	0,774214143	0,0600	UNCHANGED
A0A445DVL6	2	4705,78	ATP synthase subunit alpha, mitochondrial	proton motive force-driven ATP synthesis; proton transmembrane transport	156838	166230	161408	90984	144622	114683	161492	116763	0,046903058	0,4679	UNCHANGED
A0A445D5H5	12	4702,40	UDP-arabinopyranose mutase	cell wall organization; plant-type cell wall biogenesis; UDP-L-arabinose metabolic process	83103	111317	111430	50527	75274	122678	101950	82826	0,455551602	0,2997	UNCHANGED
A0A445D9V9	12	4701,23	Transaldolase	carbohydrate metabolic process; pentose-phosphate shunt	95695	107650	118787	79641	89205	86781	107377	85209	0,037884374	0,3336	UNCHANGED
A0A445EJD6	6	4640,17	Pentose-5-phosphate 3-epimerase	carbohydrate metabolic process; pentose-phosphate shunt	76129	112626	133173	34217	57885	74533	107309	55545	0,063921601	0,9501	UNCHANGED
A0A445BTV1	5	4609,19	14_3_3 domain-containing protein		32278	25054	26058	33585	36659	29202	27797	33149	0,162300245	-0,2540	UNCHANGED
A0A444YFL6	19	4592,13	HATPase_c domain-containing protein		146777	150807	149651	132786	161943	160938	149079	151889	0,784936441	-0,0269	UNCHANGED
A0A445C5M8	5	4517,31	60S acidic ribosomal protein P0	ribosome biogenesis	88279	93240	99884	101071	139401	104836	93801	115103	0,167540908	-0,2952	UNCHANGED
A0A445CE24	6	4450,88	RRM domain-containing protein		82292	72605	53099	68704	56644	55762	69332	60370	0,400986111	0,1997	UNCHANGED
A0A445CEV9	7	4432,76	Glyceraldehyde-3-phosphate dehydrogenase	glucose metabolic process	116332	105088	149307	171804	200272	171973	123575	181349	0,023901411	-0,5534	UNCHANGED

A0A444YRS0	3	4421,20	Xyloglucan endotransglucosylase/hydrolase	cell wall biogenesis; cell wall organization; xyloglucan metabolic process	18455	28731	35578	34706	74499	11238 9	27588	73865	0,11422030 7	- 1,4208	UNCHANGED	
A0A444YKF2	6	4320,23	Cupin_2 domain-containing protein	carbohydrate metabolic process	98612	96228	78329	12359 6	12893 3	12936 6	91056	12729 8	0,00554921 9	- 0,4834	UNCHANGED	
A0A445BBW8	4	4294,06	14_3_3 domain-containing protein		52938	59551	72071	51995	53785	56391	61520	54057	0,26433234 2	0,1866	UNCHANGED	
A0A445ACY5	7	4217,73	14_3_3 domain-containing protein		12230	13154	13668	15997	15477	16397	13017	15957	0,00411569 7	- 0,2938	UNCHANGED	
A0A445E3Q0	3	4144,12	NmrA domain-containing protein	lignan biosynthetic process	28940	57176	75022	25586	59037	10656 9	53713	63731	0,72994924 2	- 0,2467	UNCHANGED	
A0A445DZ22	4	4126,44	Chlorophyll a-b binding protein, chloroplastic	photosynthesis, light harvesting	74049	10369 5	13251 4	86653	11319 2	79300	10341 9	93049	0,62758916 4	0,1525	UNCHANGED	
A0A445BNQ2	11	4104,04	Lipoxygenase	fatty acid biosynthetic process; oxylipin biosynthetic process	55105	84638	11848 4	50637	81057	86913	86076	72869	0,57202911 7	0,2403	UNCHANGED	
A0A445A3S6	8	4100,01	Malate dehydrogenase	Tricarboxylic acid cycle	131154	15986 3	17815 0	12322	19135 8	21756 5	15638 9	17738 3	0,53871834 4	- 0,1817	UNCHANGED	
A0A444YWV9	8	4019,17	Ornithine transcarbamylase	ubiquitin-dependent protein catabolic process	189320	13292 0	31259	15371 7	81291	52765	11783 3	95925	0,71147465 9	0,2968	UNCHANGED	
A0A444Y2P1	18	3971,62	Uncharacterized protein	protein refolding	113043	18456 0	12870 6	92142	11538 8	12143 4	14210 3	10965 5	0,23896799 1	0,3740	UNCHANGED	
A0A445DA80	7	3957,15	Uncharacterized protein		108250	36003	10515 6	34560	10696 0	32682	83136	58068	0,50151054 6	0,5177	UNCHANGED	
A0A445AQB1	2	3908,88	PSI-K	photosynthesis	94487	76338	89697	43885	76860	96681	86841	72475	0,42862395 7	0,2609	UNCHANGED	
J7LMM2	7	3858,98	Eukaryotic translation initiation factor 5A	positive regulation of translational elongation; positive regulation of translational termination	30669	29437	29774	44121	36996	41225	29960	40780	0,00674472 6	- 0,4448	UNCHANGED	
A0A445BME0	19	3843,25	Lipoxygenase	fatty acid biosynthetic process; oxylipin biosynthetic process	cytoplasm	24743 7	26543 1	17745 0	25504 4	24798 9	24504 3	22682 8	0,54749598 1	0,1114	UNCHANGED	
A0A445E437	15	3804,93	Tr-type G domain-containing protein	translation	67432	10871 3	10551 7	74749	11339 4	14837 4	93887	11217 2	0,50600259 9	- 0,2567	UNCHANGED	
A0A444YRR3	5	3751,29	Gp_dh_N domain-containing protein			65901	11748 1	13513 8	50091	76442	94084	10617 3	73539	0,25187791 6	0,5298	UNCHANGED
A0A444XY32	6	3697,69	GTP-binding nuclear protein	nucleocytoplasmic transport; protein transport	93367	82460	85841	10835	11256 9	71989	87222	97637	0,47671105 3	- 0,1627	UNCHANGED	
Q06H28	3	3679,15	Photosystem I psaH protein	photosynthesis	41052	79332	31057	7495	35422	31090	50480	24669	0,20526401 1	1,0330	UNCHANGED	
A0A445AB33	10	3673,19	Elongation factor Tu			72360	67978	70601	44383	48213	58558	70313	50385	0,01075930 3	0,4808	UNCHANGED
A0A444ZB47	19	3534,18	V-ATPase 69 kDa subunit			80738	93163	82273	92114	87318	10496 4	85391	94799	0,22490226 7	0,1508	UNCHANGED
A0A444XQQ0	6	3515,72	Putative plastid-lipid-associated protein 6			12017	30860	28551	26722	34501	32532	23809	31252	0,30799428 3	- 0,3924	UNCHANGED
A0A444ZQ84	10	3435,34	Uncharacterized protein			99500	93866	89188	88882	87758	98567	94184	91735	0,61860287 8	0,0380	UNCHANGED
A0A445DAS3	5	3413,95	Allene-oxide cyclase	jasmonic acid biosynthetic process		69666	92429	12403 2	98401	13104 8	13468 1	95376	12137 7	0,25395095 1	- 0,3478	UNCHANGED
A0A444YCA5	7	3356,20	Isocitrate dehydrogenase [NADP]	isocitrate metabolic process; tricarboxylic acid cycle		19367	19516	23460	21540	35692	29567	20781	28933	0,13162687 5	- 0,4774	UNCHANGED
A0A444ZM98	7	3337,89	Pyruvate dehydrogenase E1 component subunit beta	acetyl-CoA biosynthetic process from pyruvate		75913	81080	72222	74995	75654	75471	76405	75373	0,70930327 3	0,0196	UNCHANGED
A0A444YW80	5	3303,84	Uncharacterized protein	proteolysis		153665	15497 9	13519 0	15504 2	16697 4	17008 6	14794 5	16403 4	0,11017906 6	- 0,1489	UNCHANGED

A0A445DHP6	3	3281,98	Peroxidase	hydrogen peroxide catabolic process; response to oxidative stress	50727	33908	56600	44697	84102	51064	47078	59954	0,409103132	-0,3488	UNCHANGED
A0A445DJP1	10	3279,48	Aminomethyltransferase	glycine catabolic process	57340	82885	93786	62128	75008	54289	78004	63808	0,315262493	0,2898	UNCHANGED
A0A445EGK3	5	3183,76	Uncharacterized protein	translation	52748	53593	58270	52754	52104	60112	54870	54990	0,970901147	-0,0031	UNCHANGED
A0A444YKT8	5	3161,93	Lipoxygenase domain-containing protein	oxidoreductase activity, acting on single donors with incorporation of molecular oxygen, incorporation of two atoms of oxygen	6323	21064	26921	8977	17391	40505	18102	22291	0,728367514	-0,3003	UNCHANGED
A0A444YJX1	8	3155,10	Uncharacterized protein		44588	42902	56129	54638	63128	52376	47873	56714	0,170009398	-0,2445	UNCHANGED
A0A444X4D8	5	3154,51	Uncharacterized protein	methylation	17089	35488	56230	23691	47514	105658	36269	58954	0,445608697	-0,7008	UNCHANGED
A0A445DAZ1	6	3129,17	6-phosphogluconate dehydrogenase, decarboxylating	D-gluconate metabolic process; pentose-phosphate shunt	19646	26798	27257	23184	31376	39883	24567	31481	0,270688635	-0,3578	UNCHANGED
A0A444X9P4	7	3129,16	Proteasome subunit alpha type	ubiquitin-dependent protein catabolic process	65213	61129	60686	58969	60494	66106	62342	61856	0,861028943	0,0113	UNCHANGED
A0A445AUY4	3	3125,65	Peroxidase	hydrogen peroxide catabolic process; response to oxidative stress	32856	91582	102188	109077	70771	245690	75542	141846	0,311597521	-0,9090	UNCHANGED
A0A445EU47	2	3122,58	Chitinase	cell wall macromolecule catabolic process; chitin catabolic process; defense response; polysaccharide catabolic process	238858	212210	142054	40367	23217	-	197707	31792	-	-	-
A0A445ATT0	8	3096,27	Elongation factor Tu		36266	39782	34905	22494	24489	31732	36984	26238	0,027270261	0,4952	UNCHANGED
A0A445DRB1	5	3086,31	Tyrosinase_Cu-bd domain-containing protein	pigment biosynthetic process	3547	7053	9928	1942	4997	8084	6843	5008	0,512915379	0,4505	UNCHANGED
A0A445EE69	3	3072,35	Uncharacterized protein		23862	61614	38967	32235	26650	34542	41481	31142	0,408881455	0,4136	UNCHANGED
A0A445CH92	2	3050,17	Photosystem II 10 kDa polypeptide, chloroplastic	photosynthesis	30445	68730	77989	32496	62161	49118	59055	47925	0,546045979	0,3013	UNCHANGED
A0A445EW07	6	3049,83	Peptidyl-prolyl cis-trans isomerase	protein folding; protein peptidyl-prolyl isomerization	49699	47686	64785	37985	52220	57916	54057	49374	0,590430359	0,1307	UNCHANGED
A0A445BXJ4	2	3048,08	FAS1 domain-containing protein		14783	64515	61447	13983	56824	104535	46915	58447	0,726303313	-0,3171	UNCHANGED
A0A444YQD3	3	3035,54	NAC-A/B domain-containing protein		48228	74852	73306	48086	66314	79949	65462	64783	0,959736221	0,0150	UNCHANGED
A0A445E5A7	7	3013,80	Ribosomal_S7 domain-containing protein	translation	45073	48406	95767	44473	74923	84610	63082	68002	0,820877342	-0,1083	UNCHANGED
A0A444WRW7	2	2990,32	NmrA domain-containing protein	lignan biosynthetic process	69988	45969	61124	71798	82978	88963	59027	81247	0,06167185	-0,4609	UNCHANGED
A0A445BQJ2	4	2943,37	40S ribosomal protein S12	translation	46719	54442	49148	24971	32017	59127	50103	38705	0,345139266	0,3724	UNCHANGED
A0A444Z1P6	8	2933,37	Glutamate decarboxylase	glutamate metabolic process	20009	23127	22846	18321	28703	35593	21994	27539	0,339543851	-0,3244	UNCHANGED
A0A445BPG2	14	2910,69	Uncharacterized protein	amino acid metabolic process	5740	3765	5614	6047	5798	7185	5039	6343	0,164780158	-0,3320	UNCHANGED
A0A445BK32	8	2870,00	Cysteine synthase	Amino-acid biosynthesis; Cysteine biosynthesis	51606	54246	55561	46095	55179	34943	53804	45406	0,231979319	0,2449	UNCHANGED
A0A444WW71	2	2808,91	Pectin acetylesterase	cell wall organization	16308	44184	38429	14006	13224	11806	32974	13012	0,079154911	1,3415	UNCHANGED
A0A445AV80	4	2795,09	Xyloglucan endotransglucosylase/hydrolase	cell wall biogenesis; cell wall organization; xyloglucan metabolic process	48826	50396	43733	48986	55418	39999	47652	48134	0,926280092	-0,0145	UNCHANGED

A0A445EC07	4	2781,22	NAD(P)-bd_dom domain-containing protein		60587	55825	51134	44957	55522	37907	55849	46128	0,16909656 7	0,2759	UNCHANGED
A0A445CSV5	7	2734,18	Glutamate dehydrogenase	amino acid metabolic process	36022	44176	41073	43639	63307	78711	40424	61885	0,10858490 3	- 0,6144	UNCHANGED
A0A445E3R9	7	2698,60	Glutamate-1-semialdehyde 2,1-aminomutase	chlorophyll biosynthetic process; protoporphyrinogen IX biosynthetic process	93295	88545	89742	69491	65504	61958	90527	65651	0,00066840 9	0,4635	UNCHANGED
A0A445ANY1	9	2663,69	Proteasome subunit alpha type	ubiquitin-dependent protein catabolic process	50571	41768	48163	56580	69848	68103	46834	64843	0,02159718 8	- 0,4694	UNCHANGED
A0A444XPT6	5	2662,75	Putative aldo-keto reductase		23463	18572	19371	30401	24757	18302	20468	24486	0,35105483 3	- 0,2586	UNCHANGED
A0A444ZR23	2	2645,10	PSI subunit V	photosynthesis	33307	12941 9	12948 7	12155 6	14581 8	11903 1	97404	12880 1	0,39742778 9	- 0,4031	UNCHANGED
A0A445DJL0	7	2626,49	Lactoylglutathione lyase		46470	51527	67080	56871	58569	82010	55026	65817	0,35016428 2	- 0,2584	UNCHANGED
A0A445DPG9	12	2602,98	Aldehd domain-containing protein		26696	30031	29108	29985	38038	39902	28612	35975	0,08293608 9	- 0,3304	UNCHANGED
A0A445DA96	6	2581,59	Uncharacterized protein		3110	3553	3139	7679	4858	4462	3267	5666	0,07884045	- 0,7944	UNCHANGED
A0A445EQM5	3	2571,91	Uncharacterized protein		2416	3045	3683	1542	2384	4056	3048	2661	0,66283439 6	0,1961	UNCHANGED
A0A444Y8T2	16	2560,56	Lipoxygenase	fatty acid biosynthetic process; oxylipin biosynthetic process	cytoplasm	20903 8	25208 8	32535 9	32229 6	23822 0	20626 5	29529 2	0,08724921 7	- 0,5176	UNCHANGED
A0A445EPP4	18	2536,19	Sucrose synthase	sucrose metabolic process	100589	14924 3	13100 0	12574 1	15346 0	17022 5	12694 4	14980 9	0,30009334 9	- 0,2389	UNCHANGED
A0A444ZK37	5	2529,76	Proteasome subunit alpha type	ubiquitin-dependent protein catabolic process	17735	30940	35839	19080	33551	39239	28171	30624	0,77655858 3	- 0,1204	UNCHANGED
A0A445C427	6	2519,10	Uncharacterized protein	response to desiccation	27792	31297	43053	23152	44681	60382	34047	42738	0,50011491 9	- 0,3280	UNCHANGED
A0A444ZLL1	2	2500,18	Uncharacterized protein		2576	4172	3767	3859	3280	4772	3505	3970	0,51156572 8	- 0,1798	UNCHANGED
A0A445CNY6	4	2466,37	Epimerase domain-containing protein		33040	34935	38144	25959	24718	25712	35373	25463	0,00298043 9	0,4742	UNCHANGED
A0A445DLN8	8	2463,33	(S)-2-hydroxy-acid oxidase	response to other organism	22675	17623	14641	20579	14723	18118	18313	17807	0,86961157 7	0,0405	UNCHANGED
A0A444XIV6	3	2445,75	Peptidyl-prolyl cis-trans isomerase	protein folding; protein peptidyl-prolyl isomerization	10853	10080	8471	9357	7186	5334	9802	7293	0,13833336 6	0,4266	UNCHANGED
A0A445BMH5	10	2435,77	Lipoxygenase	fatty acid biosynthetic process; oxylipin biosynthetic process	cytoplasm	13315 5	13287 0	19467 8	14838 8	11775 5	14026 1	15360 7	0,60053902 6	- 0,1311	UNCHANGED
A0A444WYK0	4	2430,61	Uncharacterized protein		114441	14126 2	16553 5	14603 4	21003 7	31829 9	14041 3	22479 0	0,18258618 9	- 0,6789	UNCHANGED
A0A444ZRY7	6	2427,91	Uncharacterized protein		92729	97777	91891	85743	10272 5	13969 2	94132	10938 6	0,39522465	- 0,2167	UNCHANGED
A0A445CJK2	5	2415,52	Uncharacterized protein		25419	40571	30186	16559	35499	41498	32059	31185	0,92525301 9	0,0399	UNCHANGED
A0A444ZGF7	2	2340,54	Phosphogluconate dehydrogenase (NADP(+)-dependent, decarboxylating)	D-gluconate metabolic process; pentose-phosphate shunt	12862	23883	34034	11545	18755	29952	23593	20084	0,68817353 4	0,2323	UNCHANGED
A0A444YKD2	2	2336,94	Aldo_ket_red domain-containing protein		37522	25788	21904	42567	26405	16956	28404	28643	0,97976864 5	- 0,0120	UNCHANGED
A0A445DC87	3	2333,90	Uncharacterized protein	proteolysis	25305	26069	29359	69757	45486	40698	26911	51980	0,05081492 2	- 0,9498	UNCHANGED
A0A445DDZ5	2	2330,83	Uncharacterized protein		32127	68760	77419	27736	82322	81889	59435	63982	0,85184292	- 0,1063	UNCHANGED
A0A445E7N6	12	2311,33	Pyr_redox_2 domain-containing protein		109053	86415	83051	11142 7	11580 2	89062	92840	10543 0	0,33985771 3	- 0,1835	UNCHANGED

A0A445AQW6	5	2291,14	Peroxidase	hydrogen peroxide catabolic process; response to oxidative stress	68386	11610 8	11089 3	10745 0	16827 6	11630 0	98462	13067 5	0,25488587 6	- 0,4083	UNCHANGED
A0A444Z225	9	2278,93	Formate dehydrogenase, mitochondrial	formate catabolic process	55299	67803	41645	52323	55719	70170	54916	59404	0,65551601 6	- 0,1133	UNCHANGED
A0A444WQ77	8	2260,35	L-ascorbate peroxidase	cellular response to oxidative stress	99363	77635	39262	76498	48908	46726	72087	57377	0,50309616 9	0,3293	UNCHANGED
A0A445BGZ8	17	2227,60	Uncharacterized protein	protein refolding	39425	45220	40594	37242	46496	42740	41746	42159	0,90404150 3	- 0,0142	UNCHANGED
A0A445EBD9	7	2226,35	CN hydrolase domain-containing protein	polyamine biosynthetic process	77050	81556	87356	97024	95839	96693	81987	96519	0,00841096 4	- 0,2354	UNCHANGED
A0A445AW73	12	2221,09	Uncharacterized protein	cell redox homeostasis	75677	92838	85897	67939	75059	91857	84804	78285	0,49378919 6	0,1154	UNCHANGED
A0A444X279	4	2193,88	Chlorophyll a-b binding protein, chloroplastic	photosynthesis, light harvesting	344330	37323 3	38522 2	26759 5	30268 4	31097 8	36759 5	29375 2	0,01482997 2	0,3235	UNCHANGED
A0A444Y8V2	9	2187,69	Uncharacterized protein	malate metabolic process	45231	50360	51545	40366	46209	57525	49045	48033	0,86038946 5	0,0301	UNCHANGED
A0A445DIV4	3	2169,33	PSI-F	photosynthesis	41001	90281	11560 0	49994	11261 2	12673 2	82294	96446	0,68284493 2	- 0,2289	UNCHANGED
A0A445CPP5	2	2151,11	Chlorophyll a-b binding protein, chloroplastic	photosynthesis, light harvesting	78299	10817 5	12466 5	11925 5	10415 3	70067	10371 3	97825	0,78197585 2	0,0843	UNCHANGED
A0A444X1A7	4	2122,09	Uncharacterized protein	methylation	21792	19691	20802	21712	22212	30500	20762	24808	0,23720181 9	- 0,2569	UNCHANGED
A0A444ZQ13	3	2059,18	Protein kinase domain-containing protein	protein phosphorylation; proteolysis	82060	84428	78350	94405	10426 6	92633	81613	97101	0,01837520 7	- 0,2507	UNCHANGED
A0A444ZU75	3	2015,71	Glutaredoxin-dependent peroxiredoxin		53932	57380	87794	42232	33364	41250	66369	38948	0,06923424 1	0,7689	UNCHANGED
A0A445CN03	2	1947,05	Pept_C1 domain-containing protein	proteolysis; regulation of catalytic activity	84213	68500	11306 8	11111 0	87885	12059 0	88594	10652 8	0,33219203 9	- 0,2660	UNCHANGED
A0A444XRR8	16	1941,06	Chaperonin CPN60-2	protein refolding	27138	28629	26724	23112	26341	21315	27497	23589	0,06873592 9	0,2211	UNCHANGED
A0A445BH25	15	1939,24	Uncharacterized protein	protein refolding	21706	34513	40901	23451	35841	43154	32373	34149	0,83639110 1	- 0,0770	UNCHANGED
A0A445AXK1	6	1938,67	Glyoxalase I		26035	13852	15861	35905	40365	21555	18583	32608	0,10863242 8	- 0,8113	UNCHANGED
A0A444WW04	17	1911,77	Serine hydroxymethyltransferase	glycine biosynthetic process from serine; proteolysis; tetrahydrofolate interconversion	21284	33824	32280	12755	20185	29523	29129	20821	0,25475821 3	0,4844	UNCHANGED
A0A445DB36	4	1898,07	UDP-arabinopyranose mutase	cell wall organization; plant-type cell wall biogenesis; UDP-L-arabinose metabolic process	64242	64649	54222	47858	39925	34784	61038	40856	0,01680062 9	0,5792	UNCHANGED
A0A445D171	7	1897,49	Glutamate decarboxylase	glutamate metabolic process	13692	19395	21371	8705	17047	22162	18153	15971	0,65647696 6	0,1847	UNCHANGED
A0A445D891	8	1888,07	Thiamine thiazole synthase, chloroplastic	thiamine biosynthetic process; thiazole biosynthetic process	40094	32502	29222	30165	29005	30205	33939	29791	0,27015034 8	0,1881	UNCHANGED
A0A445A752	2	1851,12	Dirigent protein	phenylpropanoid biosynthetic process	99133	18580 1	17637 7	12638 6	13573 0	56768	15377 0	10629 5	0,26951837 9	0,5327	UNCHANGED
A0A445E3J7	3	1845,76	RRM domain-containing protein		19039	14354	9127	18772	15306	11323	14173	15134	0,80185322 2	- 0,0946	UNCHANGED
A0A445AVK1	11	1844,89	Aldehd domain-containing protein		43412	44667	42731	42194	30981	26684	43603	33286	0,09112709 6	0,3895	UNCHANGED
A0A445D6R8	2	1825,04	PDZ domain-containing protein		37708	38735	46315	69650	39385	41994	40919	50343	0,40175930 1	- 0,2990	UNCHANGED
A0A444XVP4	8	1824,59	Proteasome subunit alpha type	proteasome-mediated ubiquitin-dependent protein catabolic process	41440	37858	41490	28131	23456	34789	40263	28792	0,03061069 7	0,4838	UNCHANGED

A0A444ZBQ0	6	1819,78	Uncharacterized protein		29512	30894	53151	22988	27581	47916	37853	32828	0,66689141 1	0,2055	UNCHANGED
A0A445ERM0	16	1818,88	Elongation factor G, chloroplastic		59388	82979	10535 0	33739	62081	67962	82572	54594	0,17439064 7	0,5969	UNCHANGED
A0A444XFG9	4	1814,98	Ribosomal_L7Ae domain-containing protein		6564	12812	6637	8042	16667	26843	8671	17184	0,21703313 2	- 0,9868	UNCHANGED
A0A444XKS2	6	1809,48	AAA domain-containing protein	protein catabolic process	18520	20802	24840	14138	21786	27031	21387	20985	0,92784167 4	0,0274	UNCHANGED
A0A445CWF5	14	1807,52	Aspartate aminotransferase	amino acid metabolic process; biosynthetic process	133134	12564 2	94845	90137	10306 5	70741	11787 4	87981	0,11735541 4	0,4220	UNCHANGED
A0A445C511	6	1791,23	Uroporphyrinogen decarboxylase	chlorophyll biosynthetic process; protoporphyrinogen IX biosynthetic process	24634	28587	32387	18018	29503	19447	28536	22323	0,21762828 4	0,3543	UNCHANGED
A0A444ZB48	5	1757,41	Hydroxymethylbilane synthase	peptidyl-pyromethane cofactor linkage; protoporphyrinogen IX biosynthetic process	207465	30217 8	20915 9	25412 6	36151 2	44656 8	23960 1	35406 8	0,14756649 9	- 0,5634	UNCHANGED
A0A444XLM2	2	1750,54	UPF0603 protein		71972	67348	52652	74211	76691	55296	63991	68733	0,62314879 7	- 0,1031	UNCHANGED
A0A444YNV1	6	1748,65	Uncharacterized protein	glycolytic process	18779	28889	43102	15315	30438	51292	30257	32348	0,87612976 6	- 0,0964	UNCHANGED
A0A445B3G3	7	1723,79	Uncharacterized protein		54484	63585	58808	56011	68783	57623	58959	60806	0,72000737 9	- 0,0445	UNCHANGED
A0A445A7Z3	8	1704,69	PSII_BNR domain-containing protein	photosynthesis	45081	46301	44315	33327	31936	28746	45232	31337	0,00070628 2	0,5295	UNCHANGED
A0A445CC17	4	1695,16	AAA domain-containing protein	protein catabolic process	5101	6986	9814	4963	8331	10019	7301	7771	0,82730682 2	- 0,0901	UNCHANGED
A0A445ESM3	9	1686,44	AAA domain-containing protein	protein catabolic process	24602	20189	22341	27779	29570	21879	22377	26409	0,20277239 5	- 0,2390	UNCHANGED
A0A445BP96	6	1686,24	Uncharacterized protein		56137	49312	35485	42068	36108	25653	46978	34610	0,18531334 7	0,4408	UNCHANGED
A0A445BFH3	4	1668,33	40S ribosomal protein	translation	29639	34010	54325	50571	77355	69271	39325	65732	0,07410588 6	- 0,7412	UNCHANGED
A0A444ZCX5	2	1658,06	Fumarylacetoacetate	L-phenylalanine catabolic process; tyrosine catabolic process	23671	19220	17602	27695	22350	16295	20164	22113	0,63152995 5	- 0,1331	UNCHANGED
A0A445DN91	7	1648,00	Guanosine nucleotide diphosphate dissociation inhibitor	protein transport; small GTPase mediated signal transduction	12735	18694	24461	14148	25776	29281	18630	23068	0,47898947 8	- 0,3083	UNCHANGED
A0A445DJL9	5	1641,63	Uncharacterized protein		45362	61697	78261	28043	47210	48372	61774	41208	0,14986369 5	0,5841	UNCHANGED
A0A445BBA7	5	1604,57	PKS_ER domain-containing protein		24843	34741	33012	21767	31999	28346	30865	27370	0,45956874 8	0,1734	UNCHANGED
A0A445BVA9	9	1603,86	Uncharacterized protein	fatty acid biosynthetic process; oxylipin biosynthetic process	2150	6634	9834	605	2972	6346	6206	3307	0,35633403 8	0,9079	UNCHANGED
A0A444XUP8	5	1595,74	Uncharacterized protein		64457	75720	86739	24562	34919	64835	75639	41438	0,06677310 1	0,8682	UNCHANGED
A0A444Y177	4	1593,28	Isocitrate dehydrogenase [NAD] subunit, mitochondrial	carboxylic acid metabolic process; tricarboxylic acid cycle	36715	31265	37444	39479	37425	41145	35141	39350	0,13175001 6	- 0,1632	UNCHANGED
A0A444WX50	12	1591,01	Uncharacterized protein		36395	46376	50556	41279	44382	45130	44442	43597	0,85586101	0,0277	UNCHANGED
A0A445DAI2	9	1588,37	SWIM-type domain-containing protein		45107	56514	61703	46374	66785	72420	54441	61860	0,47004458 3	- 0,1843	UNCHANGED
A0A445BZ81	7	1585,69	NADPH-protochlorophyllide oxidoreductase	chlorophyll biosynthetic process; photosynthesis	39222	35789	32862	53045	59816	39748	35958	50870	0,07314434 7	- 0,5005	UNCHANGED
A0A445A5Z7	2	1582,09	DLH domain-containing protein		99555	10451	11133 0	11048 6	11474 5	11099 2	10513 4	11207	0,13132606	- 0,0923	UNCHANGED

A0A445D0T6	4	1581,61	Proteasome subunit beta	proteasome-mediated ubiquitin-dependent protein catabolic process	26426	29108	33128	22236	26621	41510	29554	30122	0,930849535	-0,0275	UNCHANGED
A0A444Z0T8	6	1562,64	WD_REPEATS_REGION domain-containing protein		41643	41841	26009	49218	59030	38902	36498	49050	0,184092682	-0,4264	UNCHANGED
A0A445CLS5	2	1540,02	S-formylglutathione hydrolase	formaldehyde catabolic process	60234	76418	88750	92431	78868	63627	75134	78309	0,799905923	-0,0597	UNCHANGED
Q45W77	2	1536,13	Ubiquitin-conjugating enzyme 1		22228	23411	22880	18796	14784	27407	22840	20329	0,538723674	0,1680	UNCHANGED
A0A445BAQ8	13	1524,31	Uncharacterized protein	regulation of DNA-templated transcription	18638	20842	28504	22120	28587	24226	22662	24977	0,549210681	-0,1404	UNCHANGED
A0A445D3E3	5	1514,23	Isocitrate dehydrogenase [NADP]	isocitrate metabolic process; tricarboxylic acid cycle	11013	12561	11754	11658	18196	21006	11776	16953	0,138696256	-0,5257	UNCHANGED
A0A445E311	8	1512,24	Aldo_ket_red domain-containing protein		48121	37721	42152	87547	41056	36708	42665	55104	0,494006412	-0,3691	UNCHANGED
A0A444Y9V3	11	1498,68	Serine hydroxymethyltransferase	glycine biosynthetic process from serine; tetrahydrofolate interconversion	8100	11340	14117	9123	10938	16160	11186	12074	0,761541332	-0,1102	UNCHANGED
A0A445CKV5	10	1494,21	Elongation factor Tu		24952	35228	33127	28080	36706	38717	31102	34501	0,494289946	-0,1496	UNCHANGED
A0A445DL53	5	1486,39	AAA domain-containing protein	protein catabolic process	11998	13198	12820	11506	16187	19550	12672	15747	0,262351766	-0,3135	UNCHANGED
A0A444ZMJ2	7	1485,57	Cysteine synthase	cyanide metabolic process; cysteine biosynthetic process from serine	51721	48212	38591	35678	33781	27025	46175	32161	0,041249101	0,5218	UNCHANGED
A0A445CDV5	10	1484,36	Uncharacterized protein	proteolysis	276219	394869	464088	365497	507845	510903	378392	461415	0,318158428	-0,2862	UNCHANGED
A0A445BQ62	12	1467,21	Uncharacterized protein		20517	41694	57590	23151	49134	61027	39934	44437	0,785893389	-0,1542	UNCHANGED
A0A445CNU0	5	1451,08	Proteasome subunit beta	proteolysis involved in protein catabolic process	28212	24962	27726	34402	36074	29811	26966	33429	0,038542876	-0,3099	UNCHANGED
A0A445DM32	9	1449,63	AAA domain-containing protein	proteolysis	59127	55308	56435	50657	48862	47597	56956	49039	0,00532264	0,2159	UNCHANGED
A0A445AVI1	4	1433,15	CYTOSOL_AP domain-containing protein	proteolysis	35175	48828	41275	29873	42615	40917	41759	37802	0,519923377	0,1436	UNCHANGED
A0A445BNR1	4	1427,02	Uncharacterized protein	fatty acid biosynthetic process; oxylipin biosynthetic process	129774	142415	129988	160142	201676	182000	134059	181273	0,020528926	-0,4353	UNCHANGED
A0A445B4C1	6	1420,54	Aspartate aminotransferase	amino acid metabolic process; biosynthetic process	27456	26749	27460	27311	31013	24611	27222	27645	0,832157457	-0,0223	UNCHANGED
A0A445A1D1	3	1414,20	Uncharacterized protein	positive regulation of superoxide dismutase activity	41287	41439	21035	17630	17720	15227	34587	16859	0,060211331	1,0367	UNCHANGED
A0A445BRN9	3	1407,43	Uncharacterized protein		13505	20321	21507	22393	44449	47700	18444	38181	0,076907709	-1,0497	UNCHANGED
A0A445EVF8	3	1402,48	Peroxidase	hydrogen peroxide catabolic process; response to oxidative stress	33512	52031	72767	41133	80779	89296	52770	70403	0,398546711	-0,4159	UNCHANGED
A0A445BW6	2	1386,76	40S ribosomal protein S27	translation	22205	24108	27357	39305	31984	40043	24556	37111	0,013548581	-0,5957	UNCHANGED
A0A444Z2W0	5	1386,72	Phosphoglucomutase (alpha-D-glucose-1,6-bisphosphate-dependent)	glucose metabolic process	31988	43729	44813	31136	44175	54359	40177	43224	0,718557177	-0,1055	UNCHANGED
A0A445AKS9	5	1382,02	PROTEASOME_ALPHA_1 domain-containing protein	ubiquitin-dependent protein catabolic process	6557	10865	11088	7060	11864	15070	9503	11331	0,543317462	-0,2538	UNCHANGED
A0A445B3H6	5	1375,12	Glucose-6-phosphate isomerase	gluconeogenesis; glycolytic process	28635	34195	26906	30816	37653	36713	29912	35061	0,168620572	-0,2291	UNCHANGED

A0A445DTY1	7	1373,97	Pyr_redox_2 domain-containing protein		28213	37088	51713	19709	31045	42400	39004	31051	0,448644668	0,3290	UNCHANGED
A0A445B037	8	1362,78	Aldehd domain-containing protein		37094	40168	45229	33328	33630	35971	40831	34310	0,060475863	0,2510	UNCHANGED
A0A444XDL8	2	1359,00	Uncharacterized protein		17867	24078	21328	16031	25563	23541	21091	21711	0,864474666	-0,0418	UNCHANGED
A0A444ZWP1	5	1333,74	Uncharacterized protein		158679	152984	128221	208540	185520	144965	146628	179675	0,18735421	-0,2932	UNCHANGED
A0A444WS14	5	1330,11	Succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial	electron transport chain; tricarboxylic acid cycle	32482	29376	25268	40130	47859	37809	29042	41933	0,024983743	-0,5299	UNCHANGED
A0A445DRV6	5	1325,08	Guanosine nucleotide diphosphate dissociation inhibitor	protein transport; small GTPase mediated signal transduction	9555	14628	25112	19237	18214	23721	16432	20391	0,462948934	-0,3114	UNCHANGED
A0A445A908	3	1324,43	Uncharacterized protein		38250	42663	42035	45171	64482	56305	40983	55319	0,067676272	-0,4328	UNCHANGED
A0A444WQ52	3	1321,53	Uncharacterized protein		58002	99090	134508	69643	98823	77687	97200	82051	0,558348224	0,2444	UNCHANGED
A0A444X3D0	5	1291,66	Polyadenylate-binding protein		33020	39934	37779	25515	30254	35704	36911	30491	0,147661076	0,2757	UNCHANGED
A0A445C9Y8	8	1287,65	Protein disulfide-isomerase		62642	52340	56235	58560	53664	56031	57072	56085	0,780860991	0,0252	UNCHANGED
A0A445A120	8	1258,73	Sucrose synthase	sucrose metabolic process	20622	30399	33706	21951	37963	41848	28242	33921	0,477038689	-0,2643	UNCHANGED
A0A445A0P3	2	1249,35	Proteasome subunit beta	proteasomal protein catabolic process	34269	51941	35101	32945	60122	42244	40437	45104	0,659895619	-0,1576	UNCHANGED
A0A444YZL3	10	1220,17	Biotin carboxylase	fatty acid biosynthetic process; malonyl-CoA biosynthetic process	25077	21285	23656	24649	23155	22207	23339	23337	0,998669046	0,0001	UNCHANGED
A0A444Y833	4	1219,24	Protein disulfide-isomerase		21736	28594	50835	27752	44917	24710	33721	32459	0,912638601	0,0550	UNCHANGED
A0A445ARD2	6	1204,21	Fumarate hydratase	fumarate metabolic process; tricarboxylic acid cycle	24897	27704	32857	30137	39646	41502	28486	37095	0,11105424	-0,3810	UNCHANGED
A0A445A1J4	3	1195,86	Epimerase domain-containing protein		12430	19426	17464	10948	14190	18189	16440	14442	0,535953372	0,1869	UNCHANGED
D8KXZ7	7	1195,02	Enoyl-ACP reductase 1-2	fatty acid biosynthetic process	66806	63851	58622	49938	54167	58859	63093	54321	0,06714835	0,2160	UNCHANGED
A0A444Z0X0	3	1190,57	Uncharacterized protein	carbohydrate metabolic process	11251	26954	27292	10567	26364	32932	21832	23288	0,872181738	-0,0931	UNCHANGED
A0A444YY54	4	1185,32	Succinate--CoA ligase [ADP-forming] subunit alpha, mitochondrial	tricarboxylic acid cycle	15736	17758	18790	15994	16077	19123	17428	17064	0,803295284	0,0304	UNCHANGED
A0A445EUE2	5	1166,20	VWFA domain-containing protein		23237	26275	24942	25994	26072	31269	24818	27778	0,204436938	-0,1626	UNCHANGED
A0A445CUR8	2	1156,83	PKS_ER domain-containing protein		26046	24677	20825	10961	19053	20345	23849	16786	0,100971155	0,5067	UNCHANGED
A0A444YBV0	4	1154,00	Uncharacterized protein		37947	40901	20749	25490	20791	21414	33199	22565	0,174753128	0,5570	UNCHANGED
A0A444YQ11	3	1144,68	Thioredoxin-dependent peroxiredoxin		37795	43719	63211	-	33329	48308	48242	40818	-	-	-
A0A444ZAS5	5	1138,72	Malate dehydrogenase	malate metabolic process; tricarboxylic acid cycle	8798	14611	24361	5943	11845	17450	15923	11746	0,499016032	0,4390	UNCHANGED
A0A444X9W6	10	1133,97	Uncharacterized protein	carbohydrate metabolic process	25602	27913	25755	34308	36979	27176	26423	32821	0,101483737	-0,3128	UNCHANGED
A0A444ZNC6	5	1109,66	D-fructose-1,6-bisphosphate 1-phosphohydrolase	carbohydrate metabolic process	18004	33522	35830	14085	17839	18798	29119	16907	0,102139969	0,7843	UNCHANGED
A0A445DRQ1	3	1103,47	Inorganic diphosphatase	phosphate-containing compound metabolic process	13070	24280	19545	8082	9367	11642	18965	9697	0,053167075	0,9677	UNCHANGED

A0A444ZLA4	6	1075,50	Phosphoglycerate mutase (2,3-diphosphoglycerate-independent)	glucose catabolic process; glycolytic process	20108	30101	42627	10587	19755	33948	30945	21430	0,369306057	0,5301	UNCHANGED
A0A445CTJ8	2	1072,38	V-type proton ATPase subunit C		17587	33075	41414	18574	37700	49015	30692	35096	0,716536168	-0,1935	UNCHANGED
A0A445D0Z4	3	1070,86	Peptidylprolyl isomerase	protein peptidyl-prolyl isomerization	29657	34035	38193	23065	27414	23293	33962	24591	0,02996505	0,4658	UNCHANGED
A0A0A6ZDS7	4	1056,47	60s ribosomal protein L1	translation	21517	26075	16455	18960	38927	36585	21349	31490	0,214854523	-0,5607	UNCHANGED
A0A445E1H1	5	1052,19	Uncharacterized protein	cell cycle; cell division	61560	59486	62921	77136	63624	59789	61322	66850	0,360305514	-0,1245	UNCHANGED
A0A445C4F8	7	1050,35	Uncharacterized protein	carbohydrate metabolic process	31129	32533	28396	36703	37557	43167	30686	39143	0,023206191	-0,3511	UNCHANGED
A0A444WUM0	4	1049,62	Aconitase domain-containing protein		30173	37628	36603	21428	34747	27265	34801	27813	0,195831257	0,3234	UNCHANGED
A0A445BP55	5	1040,22	Beta-galactosidase	carbohydrate metabolic process	42105	48750	40328	37839	28193	36146	43727	34059	0,069482673	0,3605	UNCHANGED
A0A444WUY4	5	1033,99	Phosphoserine aminotransferase	L-serine biosynthetic process	15860	30350	47796	14626	35001	54520	31335	34716	0,830093158	-0,1478	UNCHANGED
A0A445CA43	3	1032,28	Uncharacterized protein		69759	95691	85447	76331	100467	94575	83633	90458	0,550084676	-0,1132	UNCHANGED
A0A445C2G3	4	1013,17	Iso_dh domain-containing protein	carboxylic acid metabolic process; tricarboxylic acid cycle	20417	25806	24938	23824	28355	23746	23720	25308	0,521239479	-0,0935	UNCHANGED
A0A445DF33	10	1012,91	Citrulline--aspartate ligase	arginine biosynthetic process	35082	37040	33501	27706	29976	39433	35208	32371	0,489783297	0,1212	UNCHANGED
A0A444X199	4	1012,49	Proteasome subunit beta	proteolysis involved in protein catabolic process	8586	20602	19704	7885	33029	24760	16297	21891	0,539464971	-0,4257	UNCHANGED
A0A445AKK6	3	1011,98	Aldo_ket_red domain-containing protein		12220	11309	11780	6144	7927	9501	11769	7857	0,017638828	0,5830	UNCHANGED
A0A444XJA1	2	1008,93	Uncharacterized protein	lipid metabolic process; proteolysis	44284	84769	92042	32961	75020	88352	73698	65444	0,730559877	0,1714	UNCHANGED
A0A444YMB1	6	988,45	S-(hydroxymethyl)glutathione dehydrogenase	ethanol oxidation	31426	36167	31213	32885	30155	31538	32935	31526	0,477162538	0,0631	UNCHANGED
A0A445DEB0	3	987,90	Alpha-galactosidase	carbohydrate metabolic process	21709	22318	19432	30507	26826	18979	21153	25437	0,289413155	-0,2661	UNCHANGED
A0A445B448	3	984,04	Proteasome subunit beta	proteolysis involved in protein catabolic process	35247	34203	38723	59541	51948	32162	36058	47884	0,226134748	-0,4092	UNCHANGED
A0A445CXA1	9	967,14	Glycine cleavage system P protein	glycine catabolic process	26459	31350	22978	21045	27984	23143	26929	24057	0,417652294	0,1627	UNCHANGED
A0A444WWZ9	2	965,31	MitMem_reg domain-containing protein		26539	23194	22653	26256	26494	25763	24128	26171	0,17324373	-0,1172	UNCHANGED
A0A445DUP5	5	958,06	Xylose isomerase	D-xylose metabolic process	7950	7194	10257	16133	8668	17616	8467	14139	0,123844618	-0,7398	UNCHANGED
A0A445CAE3	5	957,68	Uncharacterized protein		22498	35891	30455	21635	39018	52996	29615	37883	0,449294684	-0,3552	UNCHANGED
A0A444Z0A9	2	956,03	Protein FAR1-RELATED SEQUENCE	regulation of DNA-templated transcription	87665	112031	102081	75489	105919	204355	100592	128588	0,517873957	-0,3542	UNCHANGED
A0A444YL53	2	951,73	Rhodanese domain-containing protein		81831	40921	39770	74500	43593	31683	54174	49925	0,832440413	0,1178	UNCHANGED
A0A444X1J3	3	949,58	Alpha-galactosidase	carbohydrate metabolic process	7096	10817	10057	12982	12260	7860	9323	11034	0,432489975	-0,2431	UNCHANGED
A0A445BLZ1	3	933,53	ATP-dependent Clp protease proteolytic subunit	proteolysis	40421	52004	46878	29897	32290	33223	46434	31803	0,013837329	0,5460	UNCHANGED
A0A445D0V9	6	932,43	Uncharacterized protein		39713	35747	39422	27201	30105	30631	38294	29312	0,005693329	0,3856	UNCHANGED
A0A444XXH8	5	930,10	Xylose isomerase	D-xylose metabolic process	6790	5658	7224	13565	8486	17291	6557	13114	0,064827343	-1,0000	UNCHANGED
A0A445B002	5	923,61	PHB domain-containing protein		45363	48842	50525	35608	49968	51984	48243	45853	0,679550726	0,0733	UNCHANGED

A0A444X2Q0	2	920,39	Eukaryotic translation initiation factor 3 subunit F	formation of cytoplasmic translation initiation complex	22357	21858	23704	24093	19544	24634	22640	22757	0,948457053	-0,0075	UNCHANGED
A0A445DBF2	5	909,65	UDP-glucose 6-dehydrogenase	UDP-glucuronate biosynthetic process	74598	71239	90696	96580	94380	76636	78844	89199	0,300412318	-0,1780	UNCHANGED
A0A445CZP0	6	892,41	HATPase_c domain-containing protein		20823	27805	28263	18229	27864	24395	25630	23496	0,595485303	0,1254	UNCHANGED
A0A445ENE7	3	877,50	Xylose isomerase	D-xylose metabolic process	3901	4597	10150	6996	4796	5909	6216	5900	0,886569691	0,0752	UNCHANGED
A0A445APA9	4	858,11	Peptidase_S9 domain-containing protein	proteolysis	24814	21068	17716	25992	24154	21938	21199	24028	0,297096673	-0,1807	UNCHANGED
A0A445A4W1	5	847,26	Acetohydroxy-acid reductoisomerase	isoleucine biosynthetic process; valine biosynthetic process	8879	25587	33475	8118	18232	44480	22647	23610	0,944679437	-0,0601	UNCHANGED
A0A444ZF59	2	846,44	Uncharacterized protein	intracellular protein transport	16437	12660	12342	17463	16161	16721	13813	16781	0,095826785	-0,2809	UNCHANGED
A0A445EEN9	4	830,12	AAA domain-containing protein	protein catabolic process	5190	8431	14256	3927	12496	20667	9292	12363	0,607168049	-0,4120	UNCHANGED
A0A445CIH8	9	827,95	Uncharacterized protein		38170	56612	74626	33922	58965	79467	56469	57451	0,956338492	-0,0249	UNCHANGED
A0A444YFW7	5	827,56	Coproporphyrinogen oxidase	protoporphyrinogen IX biosynthetic process	26867	27121	49364	16606	26060	35491	34451	26052	0,414672136	0,4031	UNCHANGED
A0A445CFC7	2	826,77	Uncharacterized protein	carbohydrate metabolic process	9066	13991	14975	9323	16235	19553	12677	15037	0,53981101	-0,2463	UNCHANGED
A0A445EHL2	2	825,66	Glycosyltransferase		12154	35087	41573	32394	25939	20936	29605	26423	0,755021492	0,1640	UNCHANGED
A0A445A266	6	810,46	Beta-galactosidase	carbohydrate metabolic process	32274	49137	39104	28780	32608	31706	40172	31031	0,143433886	0,3725	UNCHANGED
A0A445ACD1	2	797,07	Cation_ATPase_N domain-containing protein		31053	20524	12944	20189	33314	24714	21507	26072	0,521807299	-0,2777	UNCHANGED
A0A444ZTC6	4	796,86	Uncharacterized protein	translation	7287	10705	8781	4966	12026	8540	8924	8511	0,863924333	0,0685	UNCHANGED
A0A445EPQ0	2	793,90	Pectinesterase	cell wall modification; pectin catabolic process	17310	12346	12530	14391	16031	19461	14062	16628	0,309668717	-0,2418	UNCHANGED
A0A445AFM7	3	785,64	S-(hydroxymethyl)glutathione dehydrogenase	ethanol oxidation	20029	26545	27603	13919	22143	32353	24726	22805	0,758490752	0,1167	UNCHANGED
A0A444XV99	4	785,48	D-3-phosphoglycerate dehydrogenase	L-serine biosynthetic process	45814	44243	37154	46632	42873	42367	42404	43958	0,630138207	-0,0519	UNCHANGED
A0A444WV29	3	784,32	Thioredoxin reductase	removal of superoxide radicals	20035	25633	35278	15370	21759	23699	26982	20276	0,259920721	0,4122	UNCHANGED
A0A445D3V5	11	769,85	Pyruvate kinase		30690	27456	25890	23548	26095	28915	28012	26186	0,433157314	0,0972	UNCHANGED
A0A445BGN5	3	768,52	Malate dehydrogenase [NADP], chloroplastic	malate metabolic process	8652	27033	33210	5569	17218	32549	22965	18445	0,695607251	0,3162	UNCHANGED
A0A445DJW8	2	763,01	Uncharacterized protein	isoleucine biosynthetic process; valine biosynthetic process	9462	23940	31391	5835	13918	46997	21597	22250	0,965401999	-0,0430	UNCHANGED
A0A445DC67	2	760,29	Uncharacterized protein	proteolysis	81401	87761	54348	111311	106293	109979	74503	109194	0,028545669	-0,5515	UNCHANGED
A0A445ALI9	4	755,45	Glucose-6-phosphate 1-dehydrogenase	glucose metabolic process; pentose-phosphate shunt, oxidative branch	15936	16940	18305	17779	21662	22788	17060	20743	0,091488746	-0,2820	UNCHANGED
A0A445BEC7	2	751,58	Coatomer subunit delta	protein transport; retrograde vesicle-mediated transport, Golgi to endoplasmic reticulum	36031	34686	15237	24853	36718	25292	28651	28954	0,970709067	-0,0152	UNCHANGED

A0A445AEC3	4	748,28	Uncharacterized protein	methylation; organic substance metabolic process	17214	31273	25175	13178	13379	14953	24554	13837	0,059523583	0,8275	UNCHANGED
A0A445DHF2	8	745,87	Pyruvate kinase		17812	21063	22974	14126	18082	21719	20617	17976	0,377074202	0,1978	UNCHANGED
A0A445CA05	5	745,33	Delta-aminolevulinic acid dehydratase	chlorophyll biosynthetic process; protoporphyrinogen IX biosynthetic process	24303	23062	20723	14924	18192	21541	22696	18219	0,109190913	0,3170	UNCHANGED
A0A445E402	4	744,35	Lipoxygenase	fatty acid biosynthetic process; oxylipin biosynthetic process	9096	19264	17609	3725	9159	10708	15323	7864	0,120813355	0,9624	UNCHANGED
D8KXZ2	2	739,80	3-oxoacyl-[acyl-carrier-protein] reductase	fatty acid biosynthetic process	43580	44149	45379	42093	57603	32326	44369	44007	0,963189155	0,0118	UNCHANGED
A0A444XEA4	3	732,07	PKS_ER domain-containing protein		4085	18661	17957	16661	18173	21324	13568	18719	0,355926736	-0,4644	UNCHANGED
A0A445E3Z0	2	711,36	Uncharacterized protein	carbohydrate metabolic process	11895	14622	23382	8354	17667	41548	16633	22523	0,603940103	-0,4373	UNCHANGED
A0A445BFZ5	2	708,29	Cytochrome b5 heme-binding domain-containing protein	response to oxygen-containing compound	29163	23360	26486	28265	26102	20465	26336	24944	0,65261581	0,0784	UNCHANGED
A0A445BWP6	2	702,87	Epimerase domain-containing protein		12686	11269	9403	22821	13315	14512	11119	16883	0,139996716	-0,6025	UNCHANGED
A0A445DXY2	4	690,39	Uncharacterized protein		15455	18697	23863	19311	23461	26351	19338	23041	0,310104323	-0,2528	UNCHANGED
A0A445AAF6	5	686,86	Lactoylglutathione lyase		32174	47904	42507	26388	36863	45312	40862	36188	0,549437789	0,1753	UNCHANGED
A0A444ZS36	2	684,19	Lipoxygenase	fatty acid biosynthetic process; oxylipin biosynthetic process	15432	15334	15476	14042	14521	11568	15414	13377	0,090203631	0,2045	UNCHANGED
A0A445B5D2	2	676,21	Glutamine amidotransferase type-1 domain-containing protein	de novo pyrimidine nucleobase biosynthetic process; glutamine metabolic process	19774	25193	21374	-	8621	22494	22113	15557	-	-	-
A0A445A437	2	674,79	CN hydrolase domain-containing protein	nitrogen compound metabolic process	19869	18723	20081	22530	28009	20252	19558	23597	0,159422406	-0,2709	UNCHANGED
A0A445E1I0	2	674,58	PKS_ER domain-containing protein		30208	32554	31455	31368	38876	33166	31405	34470	0,264354145	0,1343	UNCHANGED
A0A445A2Q1	2	672,52	Uncharacterized protein		20105	10503	13385	12697	14109	20307	14664	15704	0,791624563	-0,0988	UNCHANGED
A0A444X2S7	2	670,79	MPN domain-containing protein		27428	25051	14133	28137	22516	23150	22204	24601	0,619727123	-0,1479	UNCHANGED
A0A444ZB77	2	669,37	D-3-phosphoglycerate dehydrogenase	L-serine biosynthetic process	25705	30193	26089	20584	23164	26999	27329	23582	0,186491104	0,2127	UNCHANGED
A0A444ZW85	3	667,60	Cysteine synthase	cysteine biosynthetic process from serine	33167	39562	30098	38944	29331	29289	34276	32521	0,701073338	0,0758	UNCHANGED
A0A445E7D6	2	655,93	Adenosine kinase	AMP salvage; phosphorylation; purine ribonucleoside salvage	25868	23553	29595	34961	28474	24502	26339	29312	0,445784326	-0,1543	UNCHANGED
A0A445BNG1	2	636,11	Carboxypeptidase	proteolysis	52141	75578	90024	63773	40610	50957	72581	51780	0,182481018	0,4872	UNCHANGED
A0A444YPW2	2	617,52	Pyridoxal kinase	pyridoxal 5'-phosphate salvage	17683	11779	14904	16213	11907	14762	14789	14294	0,827170609	0,0491	UNCHANGED
A0A445DJ2	4	610,57	Uncharacterized protein		13268	17299	17161	15092	23989	20365	15909	19815	0,249457593	-0,3167	UNCHANGED
A0A445DW96	5	609,15	Aldehyd domain-containing protein		182632	122338	96079	148260	111167	118111	133683	125846	0,793695461	0,0872	UNCHANGED
A0A445DG07	5	600,50	Serine hydroxymethyltransferase	glycine biosynthetic process from serine; tetrahydrofolate interconversion	23838	20358	19560	15530	24876	14868	21252	18424	0,463086697	0,2060	UNCHANGED
A0A445E4X9	2	591,74	Epimerase domain-containing protein		10502	13509	11633	16144	10158	13078	11881	13127	0,555549991	-0,1438	UNCHANGED

A0A445CME4	2	585,86	Methenyltetrahydrofolate cyclohydrolase	one-carbon metabolic process	5331	10554	13665	6050	9806	12299	9850	9385	0,885676686	0,0697	UNCHANGED
A0A445BDW2	2	574,79	Formyltetrahydrofolate synthetase	tetrahydrofolate interconversion	13274	18354	18361	12736	17281	26461	16663	18826	0,647144479	-0,1761	UNCHANGED
A0A444X8G9	9	557,66	Alpha-1,4 glucan phosphorylase	carbohydrate metabolic process	40399	32333	34927	43043	48318	46186	35886	45849	0,024393354	-0,3534	UNCHANGED
A0A445CMX4	4	557,10	Prolyl-tRNA synthetase	prolyl-tRNA aminoacylation	6795	9863	12116	6282	12066	13354	9591	10567	0,732870646	-0,1398	UNCHANGED
A0A444XW20	4	543,09	Aldo_ket_red domain-containing protein		9722	14342	11976	10807	14761	12694	12014	12754	0,694972471	-0,0863	UNCHANGED
A0A444XC48	3	527,43	Uncharacterized protein		29804	27112	22869	23323	27747	21282	26595	24117	0,422794765	0,1411	UNCHANGED
A0A445A3U6	2	522,94	Uncharacterized protein		22240	24480	21022	19452	29988	19340	22580	22926	0,929485701	-0,0219	UNCHANGED
A0A444Y8A4	3	521,08	Uncharacterized protein		23027	19961	20398	26164	26860	19403	21129	24142	0,305003878	-0,1924	UNCHANGED
A0A444YS36	4	511,22	Alpha-mannosidase	mannose metabolic process	16671	18307	19702	25226	26184	26605	18227	26005	0,001292573	-0,5127	UNCHANGED
A0A445DIN3	3	504,99	Uncharacterized protein	carbohydrate metabolic process	7036	9245	6727	4597	11118	9068	7669	8261	0,790406582	-0,1072	UNCHANGED
A0A445E0Z9	5	502,34	Pyruvate kinase		18241	23054	20439	12493	16714	17706	20578	15638	0,080113559	0,3961	UNCHANGED
A0A445AI36	3	502,34	Carboxypeptidase	proteolysis	22806	25053	20118	40270	33606	24282	22659	32719	0,106754607	-0,5301	UNCHANGED
A0A445E495	5	499,05	Uncharacterized protein	D-xylene metabolic process; UDP-D-xylene biosynthetic process	21620	17962	25377	28279	31910	25772	21653	28654	0,065809332	-0,4041	UNCHANGED
A0A445D9P1	2	496,90	Uncharacterized protein	protein folding	12453	12874	8155	21175	20232	9834	11161	17080	0,206848804	-0,6139	UNCHANGED
A0A444WVZ4	3	493,35	Fn3_like domain-containing protein	xylan catabolic process	28231	32334	38134	34757	39846	43103	32900	39236	0,167421053	-0,2541	UNCHANGED
A0A445AJL2	3	476,29	UMP pyrophosphorylase	UMP salvage; uracil salvage	8359	10951	15275	5761	7570	10970	11529	8100	0,246900251	0,5092	UNCHANGED
A0A444X9N8	2	473,10	Cytochrome c domain-containing protein		4039	7625	10249	3782	5490	13348	7304	7540	0,948880201	-0,0458	UNCHANGED
A0A445EPG6	2	466,70	Aldehd domain-containing protein		27406	29113	28722	23327	28969	26239	28413	26178	0,260947088	0,1182	UNCHANGED
A0A445BG52	5	460,09	Tripeptidyl-peptidase II	proteolysis	31302	24976	19858	40817	38623	29560	25379	36333	0,083581454	-0,5177	UNCHANGED
A0A444XYS5	2	445,87	Uncharacterized protein	mitochondrial ADP transmembrane transport; mitochondrial ATP transmembrane transport	42860	46955	49949	41754	65895	65706	46588	57785	0,247415687	-0,3107	UNCHANGED
A0A445C1V2	3	442,49	Uncharacterized protein		23327	21622	24365	13497	22126	14665	23105	16763	0,087649207	0,4629	UNCHANGED
A0A445AM64	3	433,69	Uncharacterized protein		28564	21168	13463	14194	11471	16382	21065	14015	0,198985967	0,5878	UNCHANGED
A0A445B273	2	432,88	CN hydrolase domain-containing protein	nitrogen compound metabolic process	23158	25196	21279	24916	26643	29017	23211	26859	0,090274661	-0,2106	UNCHANGED
A0A444ZBJ3	2	431,82	Aldo_ket_red domain-containing protein		11107	14543	15281	8500	14737	16018	13644	13085	0,843552427	0,0603	UNCHANGED
A0A445E4I3	2	428,30	Uncharacterized protein		-	20739	22830	-	21843	20393	21784	21118	-	-	
A0A444ZBZ1	2	423,26	Uncharacterized protein		9598	9439	8808	14809	10247	10905	9282	11987	0,134301606	-0,3690	UNCHANGED
A0A445A532	2	399,55	Amine oxidase	amine metabolic process; response to chemical	26872	21514	22233	28474	25827	26098	23539	26800	0,157541796	-0,1871	UNCHANGED
A0A445DQT9	3	379,32	Mg-protoporphyrin IX chelatase	chlorophyll biosynthetic process; photosynthesis	107810	112771	85802	102838	116246	111089	102128	110058	0,435560345	-0,1079	UNCHANGED
A0A445DAA8	4	367,11	Transket_pyr domain-containing protein	tricarboxylic acid cycle	24957	19818	12942	31619	33758	23726	19239	29701	0,086623705	-0,6265	UNCHANGED

A0A444XSB2	3	364,17	Carboxypeptidase	proteolysis	23255	20574	24246	26761	20225	23363	22692	23450	0,74591640 2	- 0,0474	UNCHANGED
A0A445ALL5	2	353,07	Alpha-mannosidase	mannose metabolic process	7896	19637	26326	20562	23102	34201	17953	25955	0,30594643 1	- 0,5318	UNCHANGED
A0A445DQU5	2	349,52	Mg-protoporphyrin IX chelatase	chlorophyll biosynthetic process; photosynthesis	926	3737	3919	2702	3177	434	2860	2104	0,58835154 5	0,4428	UNCHANGED
A0A445EU63	2	347,64	Uncharacterized protein		21164	41248	33150	19969	28431	29657	31854	26019	0,42534095 7	0,2919	UNCHANGED
A0A445AN05	3	322,19	Alpha-1,4 glucan phosphorylase	carbohydrate metabolic process	18282	23064	21772	14778	17786	19770	21039	17445	0,15224283 7	0,2703	UNCHANGED
A0A445A708	2	291,85	Carboxypeptidase	proteolysis	9045	7720	6212	6090	6069	12631	7659	8263	0,80838078 3	- 0,1095	UNCHANGED
A0A444X3L0	2	281,40	Clathrin heavy chain	intracellular protein transport; vesicle-mediated transport	20135	14389	12178	15857	23331	18071	15567	19086	0,33927049 2	- 0,2940	UNCHANGED
A0A445A3P8	2	279,01	Phosphoenolpyruvate carboxylase	carbon fixation; tricarboxylic acid cycle	11301	8221	11210	9266	12582	11884	10244	11244	0,52254705 7	- 0,1344	UNCHANGED