Universidade de Brasília – UnB
Instituto de Biologia
Departamento de Botânica

Análises de transcritoma e de metaboloma revelam que *Qualea grandiflora* Mart. possui um metabolismo Alumínio-dependente

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Brasília-DF
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Antoine de Saint-Exupéry

Como é precioso o teu amor, ó Deus!

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Salmos 36:7

LISTA DE ABREVIAÇÕES

ABA - Ácido abiscísico
ABP - Proteína de ligação a auxina
ADP - adenosina difosfato
AMDIS - Software automatizado de descoberta e identificação espectral de massa
ATP - Adenosina tri fosfato
BIN - Principais categorias funcionais
BR - Brassinoesteroide
BWA - Alinhador de Burrows-Wheeler
CDF - Formato de documento em computador
cDNA - DNA complementar
CE-MS - Espectrometria de massa por electroforese capilar
CT - Controle
cpSecA - Subunidade de translocase de proteína de tilacoide
CTAB - Brometo de Cetil Trimethyl de Amônio
DEG - Diferença de expressão gênica
DNA - Ácido desoxirribonucleico
EST - Etiqueta de sequência expressa
ET - Etileno
GA - Ácido giberélico
GABA - Ácido Gama-Aminobutírico
GC-MS - Espectrometria de massa por cromatografia gasosa
GMD - Base de dados do *Golm Metabolome*
GO - Ontologia gênica
GSH - Glutationa
GTP - Guanosina trifosfato
IBGE - Instituto Brasileiro de Geografia Estatística
Imaging MS - Imagem química por espectrometria de massas
IP6 - Hexafosfato
JA - Ácido jasmônico
KEGG - Kyoto Enciclopédia de Genes e Genomas
KO - Ortologia do KEEG
KOG - Clusters de grupos ortólogos eucarióticos de proteínas
LaCTAD - Laboratório Central de Tecnologias de Alto Desempenho
LC-MS - Espectrometria de massa por cromatografia líquida
LecRKs - Receptoras de quinases de lectina
M&S - Murashige e Skoog
m/z - Razão massa sobre a carga
MeV - Microscópio eletrônico de varredura
MS - Espectrometria de massas
MSTFA - N-metil-N- (trimetilsilil) trifluoroacetamida
NADH - Nicotinamida adenina dinucleotídeo
NCBI - Centro Nacional de Informação Biotecnológica
NGS - Sequenciamento de nova geração
NIST - Instituto Nacional de Padrões e Tecnologia
NMR - Espectroscopia de ressonância magnética nuclear
NR - Proteína não redundante
OA - Ácido orgânico
ORF - Janela aberta de leitura

PC - Fitoquelatina
PCR - Reação em cadeia de polimerase
pH - Potencial Hidrogeniônico
Pi - Fosfato inorgânico
PIB - Proteínas translocadoras de metais
PME - Pectina metil esterase
RLKs - Receptor quinase
RNA - Ácido ribonucleico
RNAm - RNA mensageiro
ROS - Espécies Reativas ao Oxigênio
RPKM - Transcrição por milhão de reads mapeadas
SA - Ácido salicílico
SBP - Proteína de ligação ao selênio
SOD - Superóxido dismutase
SOLid - Sequenciamento por ligadura e detecção de oligonucleotídeos
SRA - Arquivo de Leitura de Sequência
TCA - Ciclo do ácido tricarboxílico
UV - Ultravioleta

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1. Análises de transcritoma e de metaboloma revelam que *Qualea grandiflora* Mart. possui um metabolismo Alumínio-dependente

1.1. Resumo: A toxicidade do alumínio (Al) é, atualmente, um dos principais fatores responsáveis por perdas na produção agrícola. Em plantas do Cerrado, devido ao alto teor de Al e acidez do solo, vários mecanismos são acionados para lidar com esse metal. Investigar os efeitos do Al no metabolismo vegetal, principalmente em nível de regulação gênica e produtos dos genes envolvidos, é essencial para se entender os processos fisiológicos associados a esse metal, e abrir a possibilidade de identificar genes que poderão resultar em novos produtos biotecnológicos visando a obtenção de cultivares mais resistentes às condições edáficas do Cerrado. É importante destacar que muitas espécies do Cerrado não só acumulam Al, mas o requerem para seu crescimento e desenvolvimento. Uma dessas plantas é *Qualea grandiflora*, uma das oito espécies mais importantes na composição florística do Cerrado que acumula entre 3 e 5 g de $Al.kg^{-1}$ em matéria seca. Estudos proteômicos em folhas dessa espécie mostraram que várias proteínas são diferencialmente expressas em resposta à presença ou ausência de Al. No presente estudo, foram avaliados os mecanismos moleculares, via análise transcritômica de folhas, e os fluxos metabólicos, via análise cromatográfica gasosa de raízes e folhas, buscando elucidar os mecanismos envolvidos no metabolismo do Al em *Q. grandiflora*. Os resultados aqui descritos serão utilizados para estudos de caracterização gênica, fundamentais para entender melhor a função fisiológica do Al em plantas acumuladoras.

Palavras-chave: tolerância ao Al, expressão gênica diferencial, transcritoma, metaboloma, Cerrado.

2. Transcriptomic and metabolomic analyzes reveal that *Qualea grandiflora* Mart. has aluminum-dependent metabolism

2.1. Abstract: The toxicity of aluminum (Al) is currently one of the main factors responsible for losses in agricultural production. In Cerrado plants, several metabolic mechanisms are triggered to deal Al due to the high metal content and acid soils. Investigating the effects of Al on plant metabolism, especially at the level of gene regulation and products of the genes involved, is essential to understand the physiological processes associated with this metal, and to open the possibility of identifying genes that may result in new biotechnological products aimed at cultivars that are more resistant to soil conditions in the Cerrado. It is important to note that many Cerrado species not only accumulate Al but require it for their growth and development. One of these plants is *Qualea grandiflora*, one of the eight most important species in the Cerrado floristic composition that accumulates between 3 and 5 g of Al.kg-1 in dry matter. Leaves proteomic studies of this species have shown several proteins differentially expressed in response to the presence or absence of Al. In the present study, the molecular mechanisms, via transcriptic analysis of leaves, and metabolic fluxes through gas chromatographic analysis of roots and shoots, seeking to elucidate the mechanisms involved in metabolism of Al in *Q. grandiflora* were performed. The results described here will be used for gene characterization studies, fundamental to better understand the physiological function of Al in accumulating plants.

Keywords: Al tolerance, differential gene expression, transcriptome, metabolome, Cerrado.

3. INTRODUÇÃO GERAL

O Cerrado *sensu stricto* brasileiro, com formações florestais savânicas e campestres, ocupa aproximadamente 22% do território nacional, com uma área de, aproximadamente, 200 milhões de hectares distribuídos entre a sua área "core" (nuclear de domínio), Região Centro-Oeste e as áreas disjuntas nas regiões Sul, Sudeste, Norte e Nordeste (Alvin e Araújo, 1952; Rizzini, 1979 e Câmara, 1993, Vasconcelos et al., 2010).

Como é o segundo maior bioma nacional (Ratter et al., 1997), o Cerrado é classificado como um complexo vegetacional devido a variação de suas fitofisionomias que vão desde paisagens adensadas até as mais abertas (Coutinho, 1978). Estudos sobre a flora desse bioma ressaltam a riqueza deste em biodiversidade, com grande endemismo de espécies vegetais (Castro et al. 1999). A distribuição da vegetação e as adaptações dessas plantas são determinadas por condições climáticas (temperatura e sazonalidade de chuvas), por condições edáficas (relevo e tipo de solo) e pelos efeitos do fogo (Scariot et al., 2005).

No que se refere ao solo deste bioma, em grande parte, a acidez é um fator relevante (Oliveira et al., 2005). São, em geral, ricos em alumínio (Al) e pobres em nutrientes (Kochian, 1995; Souza et al, 2007). Essas características estão diretamente relacionadas com a baixa resposta à fertilização e baixa produtividade de biomassa vegetal. Adicionalmente, o alto teor Al é considerado um dos fatores mais importantes nas questões de produtividade agrícola desse bioma (Kochian, 1995).

O íon trivalente Al^{3+}, encontrado em solos ácidos, é considerado o mais danoso para as espécies cultivadas e sensíveis ao Al (Kochian, 1995). Quando plantadas nesses tipos de solos, as plantas sensíveis apresentam um pobre desenvolvimento radicular, o que acentua as deficiências nutricionais, acarretando em maior susceptibilidade à seca, pois essas plantas não são capazes de alcançar as camadas mais profundas do solo (Kinraide e Parker, 1990; Rao et al., 2016; Schmitt et al., 2016).

Como já mencionado, as relações entre a acidez do solo e a solubilidade do Al, assim como os efeitos desse metal sobre as plantas já são conhecidos há décadas (Kochian, 1995). Apesar disso, o entendimento das vias metabólicas associadas à sensibilidade, resistência e tolerância ao Al em plantas, ainda é limitado. Os termos "resistência" e "tolerância" ao Al são frequentemente usados como sinônimos, contudo, como se referem a estresses abióticos, é importante estabelecer que o termo "resistência" refere-se a mecanismos que impedem a entrada de Al na planta; já o termo "tolerância" está associado a mecanismos que desintoxicam ou sequestram o Al internamente (Delhaize et al., 2007). Além disso, alguns autores relatam que algumas espécies, comumente classificadas como tolerantes, podem acumular Al, e passam a ser denominadas acumuladoras ou hiperacumuladoras.

Esse terceiro grupo de plantas é capaz de imobilizar esse metal em compartimentos celulares e também transportá-lo das raízes até as partes aéreas (Haridasan et al., 2008[1]).

O Al prejudica o crescimento e o desenvolvimento de plantas sensíveis. Algumas espécies entretanto, como aludido anteriormente, dispõem de mecanismos que as capacitam tolerar os efeitos tóxicos deste íon. Essas plantas tolerantes e acumuladoras de Al têm sido identificadas, como *Miconia fallax* DC., *Vochysia thyrsoidea* Pohl, *Vochysia rufa* Mart., *Vochysia haenkiana* (Spreng.) Mart., *Vochysia elliptica* Mart., *Qualea dichotoma* (Mart.) Warm. e *Qualea multiflora* Mart. (Haridasan, 1982, 2008[1]; Delhaize et al., 2012). Efeitos fisiológicos externos, visualizados nessas espécies crescidas em solos ricos em Al, têm demonstrado a possível ocorrência de mecanismos associados ao melhor desenvolvimento vegetal como um todo.

Há tempos se conhece plantas acumuladoras ou hiperacumuladoras de Al, especificamente plantas nativas do Cerrado, e esse metal parece ser importante para o crescimento e desenvolvimento destas espécies (Haridasan et al., 2008[2]), cujo teor de Al, nos diferentes órgãos, atinge valores acima de $1 g.kg^{-1}$ de matéria seca (Chenery, 1948; Schmitt et al., 2016). Dentro das hiperacumuladoras podem haver, contudo, diferentes formas de se lidar com o Al, sugerindo que essas espécies possam ter evoluído com mecanismos variáveis para tolerar esse metal, o que não se verifica em plantas sensíveis (Jansen et al., 2002). Apesar do Cerrado conter muitas espécies acumuladoras de Al, estudos sobre mecanismos metabólicos, bioquímicos e de regulação gênica são escassos. Assim, análises envolvendo espécies do Cerrado, acumuladoras de Al, podem auxiliar na identificação de genes com funções-chave, ou mesmo diferente daquelas existentes nos mecanismos já estudados em outras espécies (Silva, 2012).

Nesse bioma, três famílias se destacam por possuírem um maior número de espécies de plantas acumuladoras: Melastomataceae, Rubiaceae e Vochysiaceae. Essas famílias têm espécies que acumulam de 3 a 14 g de $Al.Kg^{-1}$ de matéria seca em órgãos vegetativos e reprodutivos (Haridasan, 1982). No presente estudo, a espécie nativa *Qualea grandiflora* Mart. (Vochysiaceae) foi selecionada por se tratar de uma planta acumuladora de Al. Esta espécie está entre as principais lenhosas do Cerrado, possuindo ampla distribuição pelo bioma, sendo encontrada tanto em ambientes abertos como em formações florestais (Eiten, 1972). *Q. grandiflora* é capaz de armazenar grandes quantidades de Al em partes aéreas, mesmo quando em solos neutros e alcalinos (Araújo, 1984). Este trabalho se propõe a realizar análises de resposta transcritômica e metabólica dessa espécie na presença e ausência de Al para estudar os efeitos moleculares e fisiológicos desse metal em seu organismo.

4. REVISÃO BIBLIOGRÁFICA

4.1. O alumínio (Al) nos solos.

O Al é o metal mais abundante no solo, uma vez que a maior parte dos minerais primários e secundários das rochas, formados pela ação do intemperismo, são aluminosilicatos que, quando decompostos pela água carregada de gás carbônico, liberam o Al na forma trocável e altamente solúvel (Al^{3+}). Trata-se de um elemento anfótero, ou seja, que pode atuar como cátion em meio ácido e como ânion em meio básico. No solo, o pH é o principal fator que controla a disponibilidade desse metal (Malavolta, 1980). A solubilidade desse elemento aumenta em pH abaixo de 5,5 e acima de 7,5. Essas condições induzem a toxicidade iônica com o excesso de Al, e às vezes o manganês (Mn), além de realçar restrições nutricionais devido à carência de Ca^{+2} e Mg^{+2}. Aliados a todos esses fatores, as condições descritas acima também levam à baixa disponibilidade de fósforo (P) para as plantas (Chen et al., 2012).

Adicionalmente, a lixiviação retira elementos químicos do solo, em especial cálcio (Ca) e magnésio (Mg) através da água de percolação, que substitui as bases por hidrogênio (H) e Al, intensificando a acidificação. Somado a isto, os teores de nitrogênio (N) e potássio (K), nestes tipos de solos tendem a ser deficientes devido ao alto intemperismo e aos baixos teores de matéria orgânica. (Malavolta, 1980; Fageria, 1988; Foy, 1988; Fernandes, 2006). Assim, à medida que os solos se acidificam, íons Al^{3+} passam a ocupar as posições de troca catiônica, em superfícies eletronegativas dos coloides, em substituição aos cátions removidos pela lixiviação (Rengel e Zhang, 2003).

A maior parte dos solos do Cerrado é ácida, com valores de pH que permitem a ocorrência do íon trivalente Al^{3+} tanto na solução do solo, como em sítios de troca. Nessas condições, o Al é disponibilizado e se torna tóxico para plantas sensíveis (Kochian, 1995; Haridasan, 2008[1]). Em geral, essas plantas têm seu desenvolvimento comprometido, primariamente por prejudicar o funcionamento normal das raízes, inibindo o seu crescimento e bloqueando os mecanismos de aquisição e transporte de água e nutrientes (Vitorello et al., 2005; Tabaldi, 2008).

4.2. Efeitos bioquímicos e fisiológicos do Al em plantas

Há um conjunto de fatores que modulam as respostas ao Al nos vegetais, e isso pode variar de acordo com a espécie, genótipo de planta, tipo e horizonte do solo, pH do solo, concentração e espécie de Al, estrutura e aeração do solo e clima (Vitorello et al., 2005).

Em geral, as plantas sensíveis ao serem afetadas pelo cátion tóxico Al^{3+}, com frequência

apresentam sinais de deficiência nutricional de P, K, Ca, Mg e Mo, uma vez que ele interfere no processo de absorção, translocação e transporte de muitos nutrientes. (Sivaguru e Horst, 1998; Delhaize e Ryan,1995; Rao et al., 2016). Isso ocorre porque o Al^{3+} é mais fortemente atraído pelas partículas do solo com cargas negativas do que importantes elementos minerais de menor valência, como o Na^{+}, K^{+}, Ca^{2+} e Mg^{2+}. Com isto, o Al^{3+} permanece no solo enquanto os outros cátions tendem a serem lixiviados. Em função disso, os solos tropicais tendem a ter altos teores de Al^{3+} e baixos de Ca^{2+}, Mg^{2+}, Na^{+} e K^{+} nas formas trocáveis (Bohnen, 2000). Além disso, altas concentrações de Al também interferem no metabolismo do N, elemento essencial na síntese de aminoácidos e ácidos nucleicos. (Mendoza-Soto et al., 2015; Rao et al., 2016). Em culturas hidropônicas tratadas com Al, Freitas et al. (2006) reportaram redução significativa da absorção dos macronutrientes P, Mg, Ca e K em genótipos de arroz. O impacto negativo deste elemento sobre as principais culturas e, em especial, sobre as que produzem grãos é evidente e amplamente discutido na literatura científica (Chenery, 1948; Kinraide, 1991; Kochian, 1995; Panda et al., 2009; Mendoza-Soto et al., 2015).

São vários os sintomas causados pelo Al^{3+} em plantas sensíveis e a deficiência dos nutrinetes essenciais ou situações que afetem a disponibilidade destes para as plantas podem induzir distúrbios no metabolismo, ocasionando anormalidades no desenvolvimento vegetal e, consequente, decréscimo dos teores de moléculas estruturais, como proteínas e ácidos nucleicos. Em particular, a absorção do elemento P, de extrema importância para os vegetais, presente no DNA e RNA, é diretamente prejudicada, pois este se torna menos solúvel por se ligar ao Al formando precipitados de aluminofosfatos (Chenery, 1948; Kinraide, 1991; Kochian, 1995; Mendoza-Soto et al., 2015). A precipitação de P é enfatizada por Diniz e Calbo (1990) em tomate, no qual observou-se maiores sinais de deficiência deste elemento em cultivares que o receberam via solução nutritiva com alto teor de Al, em comparação com aqueles que receberam nutrientes por pulverização, pois a espécie apresentou maiores teores de P em matéria seca.

Em nível celular, ambas as regiões (apoplástica e simplástica) podem estar envolvidas na toxicidade ao Al^{3+} (Massot et al., 2002). É válido, entretanto, considerar que esse metal tem rápido acesso ao apoplasto e que interações com a parede celular e a membrana plasmática precedem qualquer transporte para dentro do simplasto. Por concomitância, qualquer interação é potencialmente nociva pela complexação do Al^{3+} com elementos envolvidos em processos metabólicos, como, por exemplo, enzimas, tubulinas, ATP e DNA (Delhaize e Ryan, 1995).

O cátion trivalente Al^{3+} possui múltiplos sítios de ação nos revestimentos e delimitações celulares. Neste sentido, no espaço apoplástico, o Al se ligaria à parede celular e à camada externa da membrana plasmática unindo-se a grupos carboxílicos e ao material péctico da lamela média aumentando sua rigidez (Horst, 1999; Li et al., 2016). Esta união acarretaria em efeitos nas

propriedades físicas que resultariam na diminuição da extensibilidade da parede celular e da permeabilidade da membrana, afetando o crescimento normal das plantas. Possivelmente, ocorre uma interação entre o Al e as cadeias de ácido poligalacturônico das paredes das células jovens e formam compostos pécticos diferentes pelo deslocamento do cálcio, que acarreta em perda da plasticidade e, consequentemente, diminuição do volume celular (Horst, 1999; Li et al., 2016).

Além de interações nas membranas e paredes celulares, o Al altera permeabilidade celular e modifica as proteínas de transporte, assim como no citoesqueleto e núcleo, este último, com redução da replicação do DNA (Meharg, 1993; Li et al., 2016). Wallace e Anderson (1984) reportaram que mudas de Trigo (*Triticum aestivum* L.) expostas a 0,19 mM de Al, durante 4 h, apresentaram uma inibição de 32% na incorporação subsequente de 3H-timidina no DNA, e que esse processo foi aumentando com a duração da exposição ao Al. Este trabalho sugere que o Al tenha dois efeitos sobre cultivares susceptíveis: uma rápida inibição do alongamento da raiz seguido de uma inibição da síntese do DNA.

Estudos anteriores também reportam que o Al provoca a interferência da atividade celular na região meristemática da raiz induzindo o aparecimento de um número elevado de células com dois núcleos, o que sugere uma interrupção da citocinese. (Malavolta, 1980; Horst, 1999; Li et al., 2016).

Além disso, o Al^{3+} pode formar complexos de baixo peso molecular com vários ligantes tais como grupos carboxilato, sulfato e fosfato (Rossiello e Netto, 2006). Essa espécie de Al também pode se ligar a grupos mais complexos de ácidos orgânicos como citrato, malato e oxalato e também com macromoléculas a interferir no metabolismo de açúcares, proteínas fosforiladas, nucleotídeos e ácidos nucléicos (Minocha et al. 1992; Kochian, 1995; Ma et al., 2001).

O Al^{3+} também influencia na mudança da homeostase celular do Ca^{2+} e também compete por canais desse íon. A inibição da absorção celular de Ca^{2+} pelo Al^{3+} afeta vários processos celulares, tais como a mitose, a citocinese, o crescimento polar, as correntes citoplasmáticas e a sinalização celular (Huang et al., 1992; Zhang e Rengel, 1999; Qian et al., 2016) e pode interagir e inibir a enzima fosfolipase C, associada ao transporte do Ca (Jones e Kochian, 1995; Ma et al., 2007).

O metabolismo energético também é afetado, uma vez que o Al^{3+} é capaz de formar complexos com ATP e inibir as ATPases e outras fosfatases da membrana plasmática. Isso dificulta ou impede a utilização da energia contida nas ligações do ATP (Calbo e Cambraia, 1980; Cambraia, 1989; Kochian et al., 2004).

Em decorrência desses fatores físico-químicos e celulares afetados pelo Al, o crescimento inicial e o desenvolvimento do sistema radicular de plantas sensíveis são rapidamente comprometidos. Esse metal, quando em contato com as raízes promove uma rápida paralisação de crescimento, tornando-as atrofiadas em função da injúria ou mesmo morte dos tecidos na região meristemática.

Especificamente, a parte distal da zona de transição no ápice das raízes, onde as células estão na fase de alongamento e diferenciação, tem sido identificada como o sítio da ação tóxica primária do Al (Sivaguru e Horst, 1998; Sade et al., 2016). Consequentemente, as raízes podem apresentar sinais de engrossamento, degeneração da epiderme, tortuosidade, ramificações secundárias e escurecimento nas pontas, em parte pela oxidação de compostos fenólicos (Peixoto et al., 2007). Miguel et al., 2008 observaram alguns desses sintomas em *Brachiaria ruziziensis* cv. em solução nutritiva com concentrações crescentes de Al (0, 15, 30, 45 e 60 ppm). As raízes das plantas estudadas apresentavam-se amareladas, grossas, quebradiças e com volume reduzido. Esses sintomas se acentuavam quanto maior o teor desse íon na solução (Martins et al., 2011).

Assim, altas concentrações de Al solúvel no solo, além de serem tóxicas para a maioria das plantas por afetar o desenvolvimento normal das raízes, também resultam em deficiência na aquisição e transporte de água e nutrientes essenciais. Como resultado, além de impedir o crescimento da planta, quando aliados a períodos de menor disponibilidade de água, o Al pode reduzir, de forma drástica, a produtividade dos vegetais em solos ácidos (Delhaize e Ryan 1995; Echart e Cavalli-Molina, 2001; Fernandes, 2006; Sade et al., 2016).

O efeito do Al^{3+} no crescimento da parte aérea é apresentado como uma consequência dos danos causados por este metal no sistema radicular, especialmente em decorrência das deficiências de absorção de água e nutrientes provocadas (Matsumoto, 2000; Vitorello et al., 2005). A parte aérea mal desenvolvida se caracteriza pela deficiência de fósforo que retarda o crescimento global da planta, o que pode levar a ocorrência de folhas pequenas, amareladas e/ou escuras - devido a interferência na biossíntese de clorofila -, com maturidade precoce, além do encurtamento dos nós e colapso do ápice e pecíolo. (Boris, 2016; Sade et al., 2016).

Dessa forma, vários efeitos de toxicidade em plantas sensiveis são descritos na literatura, sendo que os principais envolvem diretamente o cátion Al^{3+} (Hartwig et al., 2007; Sade et al., 2016). Em trigo (*Triticum aestivum* L.) e outros cereais, Beckmann (1954) observou o amarelecimento das folhas e a redução do crescimento da planta, fenômeno que denominou de “crestamento”. Diversos registros, relacionados a rápida ação tóxica do Al foram observados em soja (*Glycine max* L.) (Balestrasse et al., 2006), ervilha (*Pisum sativum* L.) (Li et al., 2016), arroz (*Oryza sativa* L.) (Yang et al., 2015), trigo (*Triticum aestivum* L.) (Ma et al., 2004; Yu et al., 2016), milho (*Zea mays* L.) (Li et al., 2000; Yang et al.,2015), feijão (*Phaseolus vulgaris* L.) (Massot et al., 1999, 2002) e sorgo (*Sorghum bicolor* L. Moench) (Andrade Júnior et al., 2005). Nessas espécies, verificou-se uma rápida e exacerbada diminuição e/ou inibição do crescimento radicular diretamente associada a vários fenômenos intra e extracelulares (Cakmak e Horst, 1991; Yamamoto et al., 2002; Sade et al., 2016).

4.3. Mecanismos de tolerância ao Al

A tolerância ao Al é variável e as espécies de plantas diferem significativamente quanto ao grau dessa tolerância (Rampim e Lana., 2013). Há uma variedade de mecanismos os quais indicam diferentes funções (genes) ou mudanças de funções (alelos) selecionados ao longo da evolução e divergência das espécies. Desta forma, muitos mecanismos têm sido propostos por conferir às plantas a condição de inativar a entrada do Al ou armazená-lo nas raízes e folhas em formas não tóxicas e utilizáveis pelo seu metabolismo. (Ma et al., 2001, 2004; Fernandes, 2006; Schimitt et al., 2016).

Existem mecanismos externos e internos de tolerância. No mecanismo externo, as plantas não permitem a entrada de Al ou agem no sentido de expulsá-lo depois de absorvido. A complexação desse metal ocorre com ácidos orgânicos na rizosfera, pois estes compostos são exsudados pelo sistema radicular. Essas alterações na rizosfera provem da capacidade da planta de adequar-se às condições físico-químicas adversas, o que minimiza os problemas causados pelo Al em solos ácidos (Freitas et al., 2006; Nolla et al., 2007; Hartwig et al., 2007).

Os mecanismos de tolerância interno são aqueles em que o Al entra no simplasto e a tolerância ocorre pela formação de quelatos no citosol, compartimentalização no vacúolo, ligação do Al à proteínas e atuação de enzimas tolerantes ao Al. Nesse mecanismo, conhecido como mecanismo interno ou de reparo, não há barreiras à entrada desse elemento na célula e a sua ação fitotóxica é neutralizada internamente (Ma et al., 2001).

A seleção de plantas que suportem elevados níveis de Al vem sendo considerada uma das melhores alternativas para o aumento da produtividade em solos ácidos e com altas concentrações desse cátion Al^{3+}. Assim, práticas envolvendo diferentes métodos de crescimentos de plantas na presença de Al estão sendo empregadas tanto em cultura de campo como em soluções nutritivas. (Sanchez-Chacón et al., 2001; Echart e Cavallimolina, 2001). Essa estratégia é considerada indispensável em programas de melhoramento genético por visar a identificação de plantas mais produtivas que apresentem maior adaptabilidade em condições de estresse. Assim, a seleção de cultivares mais adaptadas às condições descritas, com consequente aumento de produtividade, tem sido observada em várias culturas, como a soja (Duressa et al., 2010; 2011), o trigo (Ryan et al., 1995 *in* Ryan et al., 2001) e o arroz (Mendonça et al., 2005).

Outras espécies, além de não apresentarem sensibilidade ao Al, acumulam esse metal nas partes vegetativas, como verificado em *Miconia fallax* DC. (Melastomataceae), *Vochysia thyrsoidea* Pohl (Vochysiaceae) e *Palicourea rigida* Kunth. (Rubiaceae), três espécies lenhosas do Cerrado (Haridasan, 1982; Haridasan, 2008[2]). Nos últimos anos, tem se procurado entender como muitas espécies consentem elevados níveis de Al solúvel no solo (Haridasan, 1988). Conjectura-se que essas espécies

possuem mecanismos internos ainda não totalmente esclarecidos de desintoxicação desse metal (MA et al., 2001; Ryan et al., 2011; Schimitt et al., 2016). Mais recentemente, no entanto, alguns autores têm proposto que para algumas espécies, este elemento além de não ter nenhum efeito tóxico, favorece o crescimento e desenvolvimento dessas plantas (Haridasan et al., 2008[1,2]; Silva, 2012). Portanto, falar de mecanismos de desintoxicação talvez não seja apropriado nesses casos.

Nessas plantas, a capacidade de absorção de nutrientes essenciais para o crescimento e reprodução não é prejudicada por altas concentrações de Al no solo (Haridasan et al., 2008[2]; Schimitt et al., 2016). Em *Miconia albicans* (Sw.) Triana, uma espécie arbustiva acumuladora de Al, notam-se exemplares que crescem naturalmente em latossolos ácidos com elevados teores de Al, da região do Cerrado do Brasil central. Em uma experiência de cultura em vaso, plantas dessa espécie foram cultivadas em três condições: solo distrófico fortemente ácido, solo ácido fértil e solo cálcario. Observou-se que as mudas cresceram melhor no solo ácido fértil do que no solo distrófico. Concomitantemente, essas plantas não conseguiram crescer no solo calcário, produzindo apenas um único par de folhas amareladas, necróticas, com baixa concentração de Al após o surgimento de um primeiro par de folhas verdes. Plantas com folhas cloróticas, transplantadas do solo calcário para os solos ácidos, mostraram recuperação completa das folhas e um simultâneo aumento da concentração de Al nesses órgãos (Haridasan, 2008[3]). As plantas com folhas cloróticas que cresceram no solo calcário também mostraram recuperação completa quando partes de seus sistemas radiculares foram cultivadas em cloreto de aluminio ($AlCl_3$) em uma concentração contendo 10 mg de $Al.L^{-1}$. Isso mostra que o Al provavelmente desempenhe algum papel específico no metabolismo desta espécie (Haridasan, 2008[3]).

Em um outro estudo, objetivou-se quantificar nutrientes minerais em oito espécies pertencentes a três famílias acumuladoras de Al que estocam em suas folhas quantidades consideráveis (4,31 a 14,12 $mg.kg^{-1}$) desse metal em matéria seca de um latossolo vermelho escuro fortemente ácido com baixo teor de nutrientes catiônicos disponíveis. As espécies selecionadas foram *Miconia ferruginata* (DC.) Cogn., *Miconia pohliana* Cogn. (Melastomataceae), *Palicourea rigida* H. B. K. (Rubiaceae), *Qualea grandiflora* Mart., *Qualea multiflora* Mart., *Qualea parviflora* Mart. (Vochysiaceae), *Vochysia elliptica* (Spr.) Mart., e *Vochysia thyrsoidea* Pohl (Vochysiaceae). Nesse estudo, notou-se que altos níveis de Al nas folhas dessas plantas não estavam associados com baixos níveis foliares de Ca, Mg, K, Fe, Mn, Z ou P, em comparação com as outras espécies que não acumulam Al. Apesar do baixo pH e da baixa saturação da base do solo, as espécies acumuladoras de Al parecem ter um mecanismo eficiente para absorver todos os cátions do solo (Haridasan, 1982).

Como mencionado, o Cerrado tem muitos exemplares de acumuladoras e essas plantas absorvem e acumulam grandes quantidades de Al nas folhas e em outros órgãos, como sementes e

frutos. Isso foi observado também em *Faramea cyanea* Müll. Arg. (Rubiaceae), *Miconia pohliana* Cogn (Melastomataceae) e *Qualea dichotoma* (Mart.) (Vochysiaceae) (Haridasan, 2008[1]). Medeiros (1983) sugere que o Al é importante no metabolismo dessas espécies. Em adição, *Qualea grandiflora* Mart., *Callisthene fasciculata* Mart., e outras Vochysiaceae acumulam Al nas folhas mesmo quando presentes em condições de Cerradão mesotrófico, que caracteriza-se por apresentar solos com pH acima de 6 e possuírem altos níveis de Ca e Mg, além de baixa saturação de Al (Araújo 1984).

4.4. Transcritoma

O transcritoma é toda a coleção de RNA presente em uma célula. Esses podem ser utilizados para diferentes tipos de análises, como estágios de desenvolvimento, condições de crescimento, tecidos, e outros critérios (Wang et al., 2009). Entender o transcritoma de uma planta é essencial para a interpretação de elementos funcionais do genoma. Dentre as técnicas de análise, a hibridização consiste em incubar cDNA marcados com microarranjos gênicos (Wang et al., 2009) e outras técnicas são baseadas no sequenciamento direto do cDNA. Alguns dos métodos que utilizam-se do sequenciamento de cDNAs são o sequenciamento pelo método de Sanger de cDNA em bibliotecas de EST (Boguski e Tolstoshev et al., 1994), a Análise Serial da Expressão do Gene (SAGE) (Velculescu et al., 1995), e sequenciamento de nova geração (NGS) por meio do RNA-Seq (Mortazavi et al., 2008; Wang et al., 2009).

A demanda por tecnologias revolucionárias para a obtenção de informaçoes genômicas rápidas, baratas e precisas tornou-se proeminente e esse desafio impulsionou a origem dos equipamentos para NGS (Egan et al., 2012). Os métodos de NGS permitem que se sequenciem milhões de bases de uma vez a uma fração de custo relativamente mais baixo quando comparado a outras plataformas. A produção de dados em larga escala e o baixo custo, na ordem de milhões de leituras de sequências de DNA em uma única corrida são as principais vantagens sobre os métodos convencionais e está mudando o cenário da genética, possibilitando expandir o leque de abordagens científicas na pesquisa básica aplicada e clínica para além da determinação da ordem das bases (Egan et al., 2012; Strickler et al. 2012).

Além disso, o NGS desenvolve-se em paralelo com a disponibilidade de um vasto conjunto de dados *on line* e publicações científicas, colocando a capacidade de sequenciamento de um grande centro genômico nas mãos de pesquisadores individuais permitindo abordar uma variedade de análises, não possíveis anteriormente (Shendure e Ji, 2008; Egan et al., 2012). Dentre as tecnologias de nova geração, destacam-se as plataformas SOLiD da Applied Biosystems, Ion Torrent e HiSeq 2000/2500 da Illumina (Mardis, 2008; Metzker, 2010).

O RNA-seq da plataforma Illumina é um método de analise do NGS que sequencia o transcritoma (todos os transcritos de RNA) e pode mostrar as sequências expressas em tecidos específicos em um momento específico e está rapidamente substituindo outros métodos de estudo da expressão gênica, como os microarrays (Egan et al., 2012). É prático para plantas não modelos, principalmente porque não é necessário um genoma de referência, e pode identificar e quantificar transcritos raros, além de fornecer informações a respeito de *splicing* alternativo e variações de sequência de genes (Metzker, 2010; Egan et al., 2012).

Diante da explosão de dados genômicos obtidos pelo RNAseq, torna-se cada vez mais necessária a análise de informações comparativas em genes homólogos e subprodutos gênicos (Egan et al., 2012). Algumas informações são bem definidas e utilizadas em várias bases de dados como o GI (General Identifier) e o Taxonomy ID atribuídos pelo NCBI. Eles são peças chave para os processos de integração (Kitano et al., 2005).

Vários esforços são feitos por grandes grupos de pesquisa visando a padronização da estrutura das bases de dados. O primeiro passo foi a criação de uma padronização para a representação dos dados de interações proteicas, o próximo passo é a aplicação dessa representação na montagem de vias, com a propagação de informação para o maior número de entradas possível, com um processo de curadoria manual supervisionada pelo próprio usuário. O desafio de integrar bases de dados de modo a automatizar, acelerar e facilitar a análise de dados é essencial para futuras análises em larga escala (Kitano et al., 2005; Matsuoka et al., 2010).

O transcritoma de plantas em exposição a metais pode ser uma análise-chave para elucidar mecanismos moleculares envolvidos em respostas de estresse e tolerância. Em *Silene dioica* utilizou-se a tecnologia de sequenciamento Illumina (MiSeq) para sequenciar, realizar a montagem *De novo* e anotar os transcritomas dos indivíduos tolerantes ao Cu (cobre). As sequências de transcritoma montadas podem ser acessadas no NCBI com os seguintes números de acesso: GFCG00000000 (para os indivíduos masculinos); GFCH00000000 (para os indivíduos femininos). O sequenciamento do RNAm normalizado foi feito em raízes, caules, botões florais e flores para cada sexo. Os programas *Trinity* e *Detonate* foram usados para a montagem *De novo* (Cegan et al., 2017).

A regulação transcricional em resposta ao tratamento com Cd (cádmio) foi investigada tanto em raízes como em folhas de *Arabidopsis*, utilizando o microarray de todo o genoma contendo pelo menos 24.576 conjuntos de sondas independentes. As plantas de *Arabidopsis* foram tratadas hidroponicamente com concentrações baixas (5 μM) ou altas (50 μM) de Cd durante 2, 6 e 30 horas. Uma das principais respostas observadas nas raízes foi a indução de genes envolvidos na assimilação de enxofre-redução e metabolismo da glutationa (GSH). Os resultados sugerem que, para lidar com o Cd, as plantas ativam a via de assimilação de enxofre aumentando a transcrição de genes relacionados

para proporcionar uma maior produção de GSH para a biossíntese de fitoquelatina (PC) (Herbette et al., 2006).

Alguns estudos têm reportado à identificação de genes que respondam ao Al, como por exemplo, em *Anthoxanhum sp.*, em que identificaram genes envolvidos na exsudação de ácidos orgânicos (*TaALMT1*, para malato e *ZmMATE* para citrato), além de genes envolvidos na modificação da parede celular (*OsSTAR1*), e desintoxicação interna ao Al (*OsNRAT1*) (Gould et al., 2014, 2015). Analogicamente, outros estudos com soja identificaram um total de 117 regiões de multidrogas e extrusão de compostos (MATE) e suas funções potenciais foram propostas por análise filogenética. Os transportadores de efluxo multidrogas que extrudam compostos orgânicos, transportam uma ampla gama de substratos, como ácidos orgânicos, hormônios vegetais e metabólitos secundários. Os elementos cis e os padrões de expressão de oito genes MATE de soja relacionados à destoxificação e translocação de Al foram analisados e GmMATE75 foi identificado como um gene candidato para a tolerância a esse metal nessa espécie (Liu et al., 2016). Além disso, *Setaria viridis* (L.) foi utilizada como uma planta modelo para sobre-expressar um gene MATE recentemente identificado em *Brachypodium distachyon* Pal. (BdMATE), estreitamente relacionado à SbMATE, para ensaios de tolerância ao Al. Plantas transgênicas de *S. viridis* sobre-expressando um BdMATE apresentaram um fenótipo de tolerância ao Al melhorado, caracterizado pelo crescimento de raízes e exclusão de Al do ápice radicular em plantas transgênicas, como confirmado pelo ensaio de hematoxilina. Além disso, as plantas transgênicas apresentaram maior exudação de citrato na rizosfera, sugerindo que a melhoria na tolerância ao Al nestas plantas pode estar relacionada com a quelação do metal ao ácido orgânico. Estes os resultados sugerem que o gene BdMATE pode ser usado para transformar culturas de plantas C4 economicamente importantes, melhorando a tolerância destas ao Al.

Em chá (*Camellia sinensis* L. O. Kuntze), o transcritoma em resposta ao Al foi sequenciado e montado com base na análise De novo. Foi identificado um grande número de transportadores, fatores de transcrição, citocromo P450, ubiquitina ligase, biossíntese de ácidos orgânicos, proteínas de choque térmico diferencialmente expressos em resposta ao Al, que provavelmente foram candidatos ideais envolvidos na tolerância ou acumulação desse metal. Além disso, alguns dos genes candidatos Al-responsivos relacionados ao sequestro de Al, modificação da parede celular e excreção de ácido orgânico foram bem elucidados como já foi encontrado em Arabidopsis, arroz e trigo sarraceno (Li et al., 2017).

Já em trigo mourisco, os estudos reportaram genes Al-responsivos envolvidos na defesa da toxicidade da parede celular e estresse oxidativo. Os dados de RNA-seq dessa espécie também revelaram que é pouco provável que o metabolismo dos ácidos orgânicos seja induzido por Al (Zhu et al., 2015).

Portanto, caracterizar em larga escala a expressão diferencial de genes envolvidos na tolerância ao Al em *Q. grandiflora* poderá elucidar mecanismos de regulação gênica até então não descritos anteriormente.

4.5. Metaboloma

O interesse renovado na pesquisa metabólica nas últimas duas décadas inspirou uma explosão de desenvolvimentos tecnológicos para estudar o metabolismo de uma grande variedade de organismos. Na vanguarda da inovação metodológica está uma abordagem chamada de metaboloma (Zamboni et al., 2015). O termo "metaboloma" tem aparecido em paralelo ao genoma, transcritoma e proteoma, visando denominar, identificar e quantificar um conjunto de todas as moléculas, largamente indefinidas, ou seja, todos os metabólitos (primários e secundários) de uma célula, tecido, órgão ou organismo em uma determinada condição biológica (Fiehn, 2002; Bino, et al., 2004; Zamboni et al., 2015).

Metaboloma significa o conjunto de reações químicas que continuamente estão ocorrendo em uma célula. A identificação de enzimas específicas que catalisam essas reações, estabelecendo o que se denominam rotas metabólicas é um processo chave. Os compostos químicos formados, degradados ou transformados são chamados de metabólitos. Os compostos químicos produzidos pelos vegetais podem ser divididos em dois grandes grupos: os primários, que são moléculas essenciais a todos os seres vivos - nesse grupo, estão incluídos os lipídios, nucleotídeos, protídeos e glicídios, com funções vitais bem definidas e - os secundários, que geralmente apresentam estrutura complexa, marcantes atividades biológicas e, diferentemente daqueles do metabolismo primário, somente são encontrados em determinados grupos de plantas. Muitos desses metabólitos vegetais podem atuar na defesa da planta contra estresses bióticos e abióticos, agindo como toxinas e contra a herbivoria. Por outro lado, alguns metabólitos secundários atuam de maneiras opostas, atraindo insetos, pássaros, morcegos e até mesmo ratos, responsáveis pela polinização e dispersão de sementes de muitas plantas, além de terem aplicações importantes como medicamentos, aromatizantes, antibióticos, toxinas e matéria prima para indústria farmacêutica (Gottlieb e Borin, 2001) e biotecnologia.

A análise metabolômica permite descrever quais são os metabólitos presentes em uma planta e quais as concentrações destes de acordo com o ambiente em que esta se encontra (ou seja, a influência dos fatores bióticos e abióticos na biossíntese de metabolitos da espécie) para obter informações sobre os processos metabólicos ativos (Caprini, 2007).

Estratégias por espectrometria de massas (MS) vêm sendo cada vez mais empregadas em metabolômica, e têm sido comumente muitos utilizadas (Bedair e Sumner, 2008; Lei et al., 2011). Isso

porque as técnicas de metabolômica baseadas em MS oferecem excelente combinação de sensibilidade e seletividade, e são, portanto, uma plataforma indispensável em biologia (Lei et al., 2011). Além disso, por desenvolver equipamentos altamente sensíveis, seletivos e com alta precisão, as técnicas analíticas empregadas em metabolômica têm se diversificado consideravelmente, podendo-se citar desde análises por cromatografia líquida de ultra-alta pressão a análises diretas (Abdelnur et al., 2008) e imagem química por espectrometria de massas (Imaging MS) (Yang et al., 2009; Li et al., 2008; Abdelnur, 2010, 2011).

Resumidamente, a MS é uma técnica que detecta a razão massa sobre a carga (m/z) de íons provenientes de uma fonte de ionização. Esta fonte gera íons na fase gasosa, a partir de moléculas neutras ou de moléculas carregadas (Abdelnur, 2011). Com o decorrer dos anos, a espectrometria de massas vem obtendo grandes avanços nos campos instrumentais e de aplicação. Por exemplo, a Cromatografia Gasosa (GC) é uma técnica de separação que possui alta sensibilidade e através desta é possível diferenciar isômeros além de ser muito eficiente na separação e isolamento dos compostos (Abdelnur, 2011).

A aplicação da metabolômica, utilizando GC, tem sido empregada para analisar as respostas de plantas a estresses abióticos diversos. Alguns estudos reportam respostas a estresse salino, como em *Arabidopsis* e *Thellungiella*, em que se avaliou uma gama mais ampla de metabólitos. Kim et al. (2007) investigou a resposta metabólica no nível celular ao usar células cultivadas de *Arabidopsis* T87. Os resultados sugerem que o ciclo de metilação para o fornecimento de grupos metil, da via fenilpropanóide para a biossíntese de lisina e glicina betaína são sinergicamente induzidos como resposta de curto prazo ao estresse salino. Os resultados também sugerem a co-indução de glicólise e metabolismo de sacarose, bem como co-redução do ciclo de metilação como respostas a longo prazo para o stress ocasionado por sal (Obata e Fernie, 2012).

Outros trabalhos reportam a resposta de plantas na presença de metais. Jahangir et al. (2008) analisaram os efeitos de Cu (cobre), Fe (ferro) e Mn (manganês) sobre os níveis de metabólitos de *Brassica sp.*, que é uma planta acumuladora de metais. Os glucosinolatos e ácidos hidroxicinâmicos conjugados com malato, bem como os metabólitos primários - carboidratos e aminoácidos - foram considerados os metabolitos diferencialmente regulados. Além disso, estudos com plantas de *Arabidopsis* tratadas com Cd, exibiram um aumento dos níveis de alanina, β-alanina, prolina, serina, putrescina, sacarose e outros metabolitos com propriedades de solubilidade compatíveis, nomeadamente GABA, rafinose e trealose (Sun et al., 2010). O teor de vários outros metabolitos, incluindo lactato, frutose, uracila e alanina, aumentados pela exposição ao Cr (cromo) em *Arabidopsis* sugerem a modulação da via de degradação da sacarose envolvendo as três principais vias de fermentação que operam como um mecanismo de resgate quando a respiração é inibida (Dubey et al.,

2010).

Apesar de alguns estudos reportarem as variações metabólicas de resposta ao Al em plantas, mais estudos são necessários para se obter uma melhor compreensão dos mecanismos subjacentes a estas. É evidente que a metabolômica possui grande impacto no futuro, principalmente, no entendimento global de um sistema biológico. As aplicações da metabolômica têm se expandido amplamente, assim como as técnicas genômicas, proteômicas e transcritômicas fizeram nos últimos anos. A utilização dessas técnicas nesse trabalho tem impulsionado a análise de vias metabólicas de *Q. grandiflora* em resposta ao Al. Espera-se assim, esclarecer os mecanismos de tolerância ao Al, até então não reportado em plantas acumuladoras.

4.6. Filogenia das plantas acumuladoras de Al

De acordo com Jansen et al. (2002), grande parte das espécies de plantas acumuladoras pertence ao grupo das Rosídeas e Asterídeas (Figura 1). Ao todo, são conhecidas 45 famílias com espécies que acumulam Al; das quais 32% das espécies pertencem à família Rubiaceae (Jansen et al., 2002). Além de Rubiaceae, as famílias Anisophyllaceae, Celastraceae, Cornaceae, Diapensaceae, Geissolomataceae, Grossulariaceae, Melastomataceae, Pentaphylaceae, Polygonaceae, Proteaceae, Symplocaceae, Theaceae e Vochysiaceae também possuem um considerável número de espécies acumuladoras de Al. Evolutivamente, essa característica se originou em diferentes épocas durante o processo de evolução vegetal (Jansen et al., 2002).

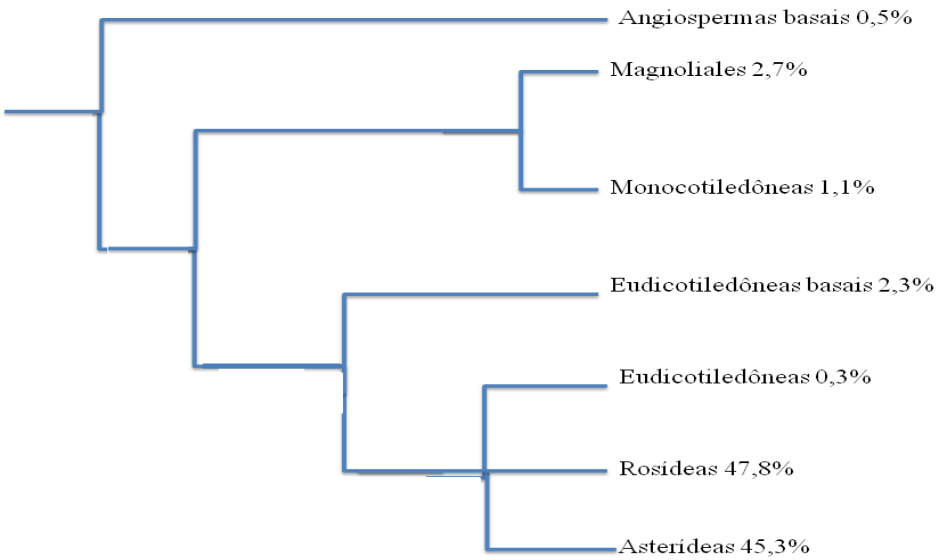

Figura 1- Filogenia das angiospermas: pocentagem de plantas acumuladoras de Al nos grupos (Adaptado de Jansen et al., 2002).

4.7. *Qualea grandiflora* Mart. (Vochysiaceae)

A família Vochysiaceae é uma das espécies acumuladoras de Al mais importantes do Cerrado (Haridasan e Araújo, 1987) e Goodland (1971) aponta *Q. grandiflora* como uma das espécies mais abundantes desse bioma.

Qualea grandiflora (Figura 2) é popularmente conhecida como "pau-terra", "pau-terra-do-campo", "pau-terra-do-cerrado", "ariavá", entre outros (Lorenzi, 2000). Trata-se de uma espécie arbórea xerofítica, heliófita e seletiva, nativa do Cerrado brasileiro, que apresenta interesse comercial, como ornamental e medicinal, podendo ainda ser empregada em reflorestamentos heterogêneos, destinados à recomposição e restauração de áreas degradadas de preservação permanente (Lorenzi, 2000).

Esta espécie pode atingir 15m de altura, com tronco de 30cm a 40cm de diâmetro. É uma árvore, em geral, bem tortuosa, protegida por uma casca grossa. As folhas são bem delicadas, glabras e brilhantes. Os frutos, ovados-oblongos são cápsulas que abrigam sementes aladas cujo formato facilita a dispersão pelo vento. As flores são amarelas e vistosas (Lorenzi, 1992; 2000).

As sementes de *Qualea grandiflora* são ortodoxas e têm uma forte dessecação no final da maturação na planta-mãe, sendo capazes de manter seu potencial de germinação por longos períodos de armazenamento em estado seco. Em geral, a semente é alada e possui embrião axial com cotilédones enrolados. A germinação é epígea fanerocotiledonar, com baixa taxa de mortalidade, o que facilita sua germinabilidade em ensaios de crescimento *in vitro* (Ferreira et al., 2001; Silva, 2012). Geralmente, o embrião expõe sua raiz por volta de sete dias (Silva, 2012).

Essa espécie possui proriedades químicas que potencializam sua comercialização. Por meio de seus frutos verdes é realizada a extração de corantes (Almeida et al., 1998). Além disso, o caule e as folhas desta espécie, por serem ricos em polifenois, como taninos e triterpenos, inibem o ataque de patógenos herbívoros e são popularmente usados em chás contra úlceras, inflamações, dores abdominais, diarréia e na desinfecção de ferimentos externos (Lorenzi, 1992). O extrato bruto de sua casca é antibacteriano (Alves et al., 2000) e o extrato hidroalcoólico da casca tem ação antiulcerosa (Hiruma-Lima et al., 2006). De suas folhas extraem-se o extrato hidroalcoólico, um analgésico com potencial anticonvulsivo (Gaspi et al., 2006) e o extrato de etanol, que possui efeitos antioxidantes (Sousa et al., 2007) e antibacterianos (Ayres et al., 2008).

Ao estudar aspectos nutricionais de um Cerrado e Cerradão no Distrito Federal, Ribeiro (1983) concluiu que *Q. grandiflora* está entre as oito espécies mais importantes na composição florística do bioma dessa região. Araújo (1984) reforçou esse conceito e destaca que essa espécie está entre as oito mais importantes de um Cerradão distrófico no Distrito Federal. Além do Distrito Federal, *Q.*

grandiflora ocorre em regiões de Cerrado da Amazônia, São Paulo, Minas Gerais, Goiás e Mato Grosso do Sul, tanto em solos distróficos como mesotróficos (Araújo e Haridasan, 1988). Adicionalmente, *Q. grandiflora* é uma espécie pioneira, decídua, ocorrendo tanto em formações vegetais primárias como secundárias (Lorenzi, 1992).

Além dessa espécie se destacar pela abundância nas diferentes fisionomias do Cerrado, estudos indicam que *Q. grandiflora* pode acumular de 3,91 g a 5,16 g de $Al.Kg^{-1}$ de matéria seca, principalmente nas partes aéreas (Andrade et al., 2007; Haridasan, 1982). Em virtude disso, *Q. grandiflora* foi selecionada para estudos moleculares transcricionais e metabólicos envolvendo os processos fisiológicos relacionados à função do Al em plantas nativas.

Figura 2 - *Qualea grandiflora* Mart. (Vochysiaceae). A- Vista geral da árvore adulta; B- detalhes dos ramos; C- fruto fechado; D- fruto aberto; E- flor (Adaptado de Haridasan et al., 2008[1]).

5. JUSTIFICATIVA

O Al é o metal mais abundante e o terceiro elemento de maior constituição dos solos e, sua toxicidade, é muitas vezes o principal fator limitante em culturas de plantas, na sua maioria sensíveis a esse metal. No Cerrado, muitas espécies nativas são acumuladoras de Al e crescem em condições de acidez no solo, possuindo adaptações resultantes da pressão seletiva sofrida ao longo do processo evolutivo. Além disso, essas espécies parecem necessitar de Al para crescer e se desenvolver normalmente. No entanto, os mecanismos moleculares e metabólicos envolvidos nesse processo ainda são pouco conhecidos. Assim, este estudo visa a identificação de transcritos e metabólitos abundantemente diferentes em plantas de *Q. grandiflora*, cultivadas na presença e ausência de Al. Essa proposta, portanto, abre a possibilidade da identificação de genes com potencial biotecnológico, tanto para programas de conservação destas plantas, quanto para o melhoramento genético de plantas sensíveis cultivadas, abrindo a oportunidade de se realizar práticas agrícolas com menor impacto ambiental e aumento da produtividade de forma sustentável.

6. HIPÓTESES

- O Al não é um fator de estresse para *Q. grandiflora*;
- Essa espécie possui metabolismo Al-dependente;
- O Al é um nutriente importante para o seu metabolismo;
- As análises de transcritoma e metaboloma são cruciais na elucidação da função metabólica do Al em plantas.

7. OBJETIVO GERAL

Realizar a análise do transcritoma e metaboloma de plantas de *Qualea grandiflora* Mart. (Vochysiaceae) cultivadas na presença e ausência de Al com o propósito de identificar genes e produtos gênicos relacionados ao metabolismo desse metal nesta espécie.

7.1. Objetivos específicos:

- Sequenciar o transcritoma de plantas de *Q. grandiflora* visando determinar o padrão de expressão gênica em plântulas de *Q. grandiflora* crescidas na presença e ausência de Al;
- Identificar as sequências nucleotídicas dos transcritos de *Q. grandiflora* associadas a processos

metabólicos de resposta ao Al;

- Estabelecer uma relação entre a concentração desses metabólitos e a ocorrência dos transcritos das enzimas e proteínas associadas a processos metabólicos de resposta ao Al;

- Depositar as sequências dos dados brutos envolvidos na tolerância em bancos de dados *in silico*;

- Investigar e comparar o perfil metabólico comparativo de plantas de *Q. grandiflora* crescidas na presença e ausência de Al por meio da técnica GC-MS de forma a delinear rotas associadas ao Al;

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CAPÍTULO I - Transcriptome Analysis to Investigate the Metabolic Role of Aluminum in *Qualea grandiflora* Mart. using RNAseq

Transcriptome Analysis to Investigate the Metabolic Role of Aluminum in *Qualea grandiflora* Mart. using RNAseq

Abstract. About 40% of the agricultural land of the world is acid with high levels of aluminium (Al). In acid soils, there is accumulation of Al^{3+}, which is the most toxic form of Al for plants. There are few reports about Cerrado Al-accumulating species and studies at the molecular level on their mechanisms to deal with this metal are even rarer. *Qualea grandiflora* is a high Al-tolerant and accumulating species and requires it for proper growth and development. In previous studies, the root and shoot length, biomass and photosynthetic pigment contents of Al-treated plants were shown to be greater than those of non-treated plants. Moreover, a histochemical analysis also detected Al in leaves, stems and roots and showed the path of Al throughout the plant. In the present study, leaves from *Q. grandiflora* plants grown with or without Al were analysed to observe the mRNA in response to the presence of this metal. Therefore, the expression pattern of Al-responsive genes was investigated in this plant species. Therefore, a *De novo* assembly of the transcripts followed by sequence clustering yielded 130,704 non-redundant reads, from which 580 transcripts were differentially expressed between the two treatments. Moreover, among the differentially expressed transcripts, 265 were downregulated and 315 upregulated. The MapMan metabolism enrichment analysis revealed among the differentially expressed some genes encoded cell wall enzymes, as well as involved in brassinosteroid and salicylic acid functions, including cell expansion, antioxidant activity and defence regulation. Additionally, receptor-like kinases, proteins against pathogens, transporters associated with Al-translocation were upregulated. Furthermore, a few candidate genes likely related to Al sequestration, cell wall modification have also been identified. Moreover, the lack of Al-induced the transcription of stress-related genes mainly associated with jasmonate, abscisic acid and ethylene synthesis as well as signalling molecules, programmed cell death and amino acid metabolism activity. Therefore, the transcriptome analysis of *Q. grandiflora* of leaves revealed a gene network responsive to Al that will provide valuable information on role of this metal in *Qualea* metabolism.

1. INTRODUCTION

Acid soils are relatively common throughout the globe and comprises about 3.95 billion ha worldwide. This kind of soil represents around 50% of world's agricultural lands (Von Uexküll and Mutert, 1995; Kochian et al., 2015). In general, acid soils are the result of a combination of many factors, involving climate conditions such as high temperature and rainfall intensity. These conditions favor a rapid soil weathering, which results in physical and chemical changes and facilitates the leaching of exchangeable bases, soil erosion, elevated soil acidity and higher Al levels. Thus, most tropical soils are acid with high contents of Al (Martin, 1988), especially Al^{3+}, which is highly phytotoxic to many plants (Kochian, 1995; Delhaize and Ryan, 1995).

Al phytotoxicity in plants is a well-documented phenomenon (Delhaize and Ryan, 1995; Horst et al., 1999; Marienfeld et al., 2000). In many countries, with naturally acid soils, Al toxicity is a major agricultural problem, and it is intensively studied in crop plant (LeNoble et al., 1996). In addition, it is generally known that sensitive plants grown in acid soils with high Al contents have reduced root systems and exhibit a variety of nutrient deficiency signs, as well as a depleted crop yield (Delhaize and Ryan, 1995).

Nevertheless, it has been found that some plant species do not resent the presence of Al. Additionally, there is considerable variability in Al tolerance among plants and this fact has been useful to breed and develop Al-tolerant cultivars. Al tolerance is a very complex phenomenon that involves various mechanisms at cellular, tissue, organ levels (Yang et al., 2011; Panda et al. 2013). Al response in tolerant plants involves different pathways, which results in different gene expression profiles. Some plant species or cultivars have developed a variety of strategies to tolerate or cope with Al toxicity. Plants Al tolerance phenotypes revealing several plant lines from different species and families. For instance, in the Cerrado, several genera of Vochysiaceae such as *Vochysia*, *Qualea*, *Callisthene* have high Al-accumulating species. Plants from other families have also Al tolerant species such as *Miconia* spp (Melastomataceae) and *Palicourea* spp. (Rubiaceae). Besides, some crop plants have cultivars genotypes that also tolerate/resist high amounts of Al, e.g., maize, soybean, sorghum, barley and tea (Delhaize and Ryan, 1995; Kochian, 1995; Ma et al., 2001; Matsumoto, 2000; Osawa and Matsumoto, 2001; Haridasan, 2008).

Numerous studies have been performed to identify genes that contribute to Al tolerance. Most of these studies involve crop plants such as rice bean (*Vigna umbellata*) (Fan et al., 2014), soybean (*Glycine max* L.) (Wang et al., 2016) common buckwheat (*Fagopyrum esculentum*) (Wang, 2015; Yokosho et al., 2015) and buckwheat (*Fagopyrum esculentum* Moench.) (Xu et al., 2017).

Moreover, tea plant (*Camellia sinensis* L.O. Kuntze) and hortensia (*Hydrangea macrophylla*)

are capable of accumulating Al at high levels without exhibiting signs of toxicity (Matsumoto et al. 1976; Ma et al. 1997; Naumann and Horst 2003). These studies have contributed to understand several aspects of Al tolerance and resistance in plants. However, only a limited number of genes have been identified as part of mechanism associated with Al tolerance/resistance, which is not yet sufficient to characterize the molecular and metabolic processes that may be involved in Al-coping mechanisms.

Nowadays, a lot of attention has been paid to the molecular basis of plant metal tolerance. Gene expression may change in response to the exposure time and type metal. The next-generation sequencing (NGS) technologies has progressively revolutionized genomic studies and has been employed for studying both model and non-model organisms (Wang et al., 2010; Oshlack et al., 2010). This technique has allowed an evaluation of global gene expression changes in response to various stimulus, which includes Al, and in this specific case, led to the identification of a broader number of genes modulated by this ion (Maron et al., 2008; Guo et al., 2007; Xu et al., 2017). Guo and colleagues (2007) studied in wheat putative Al-associated genes, and identified 28 differentially expressed genes, including genes for Al-activated malate transporter-1, ent-kaurenoic acid oxidase-1, β-glucosidase, lectin and histidine kinase. Additionally, in *Anthoxanthum odoratum* L. candidate genes for Al tolerance were also found through transcriptome analysis. In this species, genes involved in organic acid exudation (TaAL MT1, ZenMATE), cell wall modification (OsSTAR1), and internal Al detoxification (OsN RAT1) were upregulated after exposure to Al (Gould, et al., 2015). Moreover, candidate genes for Al resistance were reported in buckwheat and 589 upregulated genes were identified (Xu et al. 2017). Also, 255 genes were downregulated under Al exposure. About these, 30 transporter genes and 27 transcription factor (TF) were induced by Al. The authors concluded that TFs play critical role in transcriptional regulation of Al resistance genes in buckwheat. In addition, they suggested a possible role for gene duplication in the species Al-resistance (Xu et al., 2017).

As mentioned, genes from several metabolic processes respond to Al. Kumari et al. (2008) reported that the exposure to Al induces ribosomal protein genes, peptidases and phosphatases. Additionally, Maron et al. (2008) compared gene expression from two maize genotypes that differed in Al tolerance capacity and found that several genes involved in processes such as cell wall remodeling, response to oxidative stress and Pi starvation were differentially regulated. Furthermore, an Al-responsive transcriptome of two buckwheat species, *Fagopyrum esculentum* L. and *Fagopyrum tataricum* L., were investigated and revealed that the expression of genes involved in cell wall defense, toxicity and oxidative stresses were preferentially induced by Al (Yokosho et al., 2016; Zhu H. et al., 2015).

For non-model organisms, as in the present research, RNA sequencing followed by *De novo* assembly and clustering is necessary to generate a reference transcriptome. Alternatively, genome

sequences and expressed sequence tag (EST) of related species can be used as references for sequence analysis (Yang et al., 2016). This is crucial, because it opens the possibility for plant whose genomes are still not sequenced to be studied using a high throughput RNA-seq approach. This becomes even more important for Cerrado plants, which have a huge variety of very interesting phenotypes and very little molecular data available.

Some Al-accumulating plants benefit from Al presence. In fact, they need Al to properly grow and develop. *Q. grandiflora* plants grown without Al had their growth and development impaired and showed signs of chlorosis in leaves (Silva, 2012). Hence, the Al metabolic role in these native accumulating plants remains widely unknown, mainly at molecular level. Nevertheless, Silva (2012) initiated studies on physiological, histochemical and proteomics aspects of Al metabolism in *Q. grandiflora*. It was observed that root and shoot growth was stimulated rather than inhibited by Al (Figure 1). In leaves, the presence of Al was crucial to chlorophyll a, b and carotene syntheses. Furthermore, histochemical analysis detected Al presence in all plant organs. Moreover, proteome analysis of leaves revealed the presence of antioxidant proteins as well as ATPsynthases, RubisCO, proteins associated with light harvesting and electrons transportation were upregulated by Al. Definitely, the leaves are key-organs to study the effects of Al on plant metabolism (Silva, 2012). Thus, the results shown here support the idea that Al may be absorbed by the roots and transported to leaves, where it can be accumulated and used to important metabolic roles (Silva, 2012).

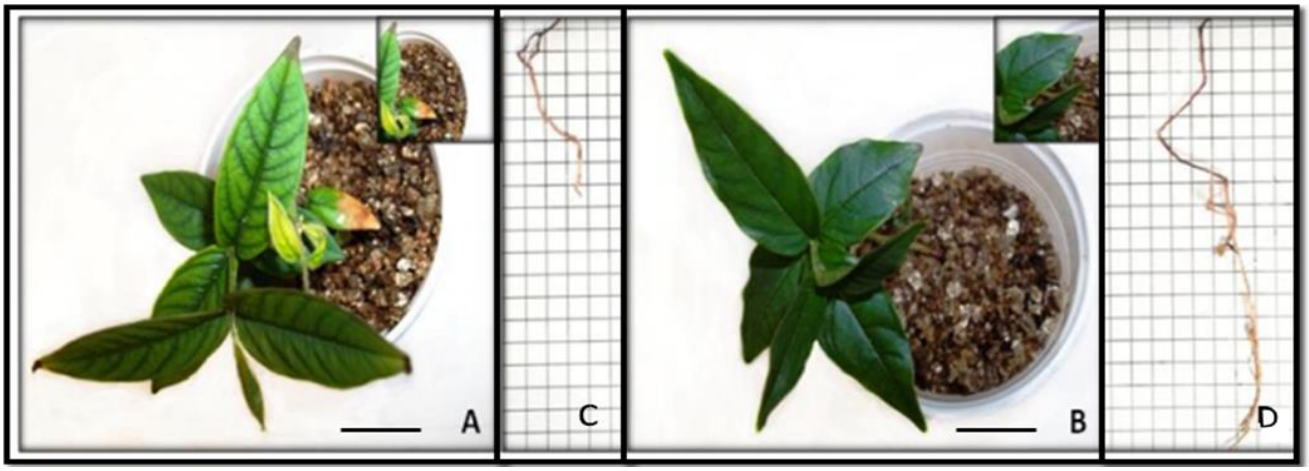

Figure 1. *Qualea grandiflora* seedlings at the 120th day of cultivation. A) Upper view of a *Q. grandiflora* seedling grown without Al. Detail of shoot - A and root - C) Upper view of a *Q. grandiflora* seedling grown with Al. Detail of shoot - B and root - D) Lateral view of the *Q. grandiflora* seedlings grown with Al and without Al. Note the difference between the treatments in terms of size and chlorosis. Scale bars = 1 cm.

The present work provides useful insights about changes in plants of *Qualea grandiflora* Mart. (Vochysiaceae) submitted to two distinct growth conditions, with and without Al. In the current study,

transcriptome analysis was to investigate the expression dynamics in *Qualea* leaves in response to Al. This analysis identified genes of several metabolic pathways, whose expressions were modified in response to the presence/absence of Al. The present work has identified several potential gene candidates associated with Al tolerance and accumulation in *Q. grandiflora*. The results obtained in this study will extend the knowledge of the genetic basis of *Q. grandiflora* response to Al accumulating at the transcriptional and metabolomic level.

2. MATERIAL AND METHODS

2.1. Plant material and growth conditions

Seeds of *Q. grandiflora* were collected in the vicinity of the University of Brasília, Brazilian Institute of Geography and Statistics (IBGE), and Fazenda Água Limpa (FAL). Subsequently, the seeds were soaked in bleach (2% active chlorine) for 30 min, and 70% ethanol for 1 min. Then, the seeds were washed three times in sterile double-distilled water, placed in Petri dishes and put to geminate for 30 days in wet germitest paper at 30 °C, 70% relative humidity, and 16 h photoperiod. Due to the slow growth of *Qualea* plants a total of 40 seedlings, at similar developmental stage, were transferred to plastic bags with sterile vermiculite and grown for eight months until the third pair leaves were well developed. The conditions during this phase were 25 °C, 70% relative humidity, and 16 h photoperiod. Throughout this period *Qualea* plants were treated with $^1/_5$ MS (Murashige and Skoog, 1962) nutrient solution (no sugars/vitamins), pH 4.5-4.8 with either aluminum (200 µM $AlCl_3$) or without Al (20 plants per treatment).

2.2. Sample Collection, RNA-seq Library Preparation and Sequencing

Five leaves from the 2nd and 3rd nodes of each one of six leaves samples (three samples per treatment) from 8-month-old *Q. grandiflora* plants cultivated with and without Al were collected at 3 PM and immediately frozen in liquid nitrogen and stored at - 80 °C (Silva, 2012). The total RNA was extracted using a modified CTAB (Cetyl Trimethyl Ammonium Bromide) method (Codeiro et al., 2010). The RNA integrity was firstly evaluated in denaturing agarose gel. The quantity and quality of the RNA were determined by NanoDrop 1000 spectrophotometer (Thermo Scientific, Wilmington, USA) and a 2100 Bioanalyzer RNA Nanochip (Agilent Technologies GmbH, Berlin, Germany) (Figure S1), respectively.

For sequencing RNA-seq libraries were prepared from collected tissues of three biological replicates of each treatment using a custom high-throughput method for the Illumina RNA-seq library (Kumar et al., 2012). These RNA-seq libraries were sequenced at the University of Campinas, High-Performance Technologies Central Laboratory in Life Sciences (LaCTAD) facility on a single lane were sequenced in HiSeq 2500 platform (Illumina), with length reads of 100-bp paired-end format. Six cDNA libraries were pooled by lane. The raw sequence data obtained have been deposited at the NCBI in the Short Read Archive (SRA) database under the accession number: PRJNA358394. (Sequencing data available at http://www.lactad.unicamp.br/DATA/FernandoTorres/150820).

2.3. *De Novo* Assembly and Sequence Clustering

The raw reads were cleaned by trimming the adapter sequences, low quality fragments were removed using Trimmomatic [10.1093/bioinformatics/btu170] observing the criteria of Phred Quality Score (Q) > 5 at 5' and 3' extremities; and an average of Q > 15 in a sliding window of 4 nucleotides (Lohse et al., 2012).

De novo assembly of the clean reads from all six samples was performed using Trinity paired-end model to transcripts (Grabherr et al., 2011). Trinity focuses more on splice isoforms. It first forms contigs that represent the significant parts of individual isoforms. Then, it clusters the contigs into individual groups such that contigs from isoforms likely to be from the same gene are grouped together. Lastly, each group of contigs is processed separately. Furthermore, Trinity is based on paired-end reads and attempts to reconstruct transcripts for splice isoforms in each group Trinity built a K-mer dictionary from all clean reads based on frequency and considered each K-mer as an initial contig. Each initial contig (in descending frequency order) was extended by selecting the most frequent K-mer in the dictionary with K-1 overlaps with the current contig end, until neither direction could be extended further. Subsequently, contigs were pooled if they shared at least one K-1-mer and there were reads across the junction sites. A de Brujin graph was constructed for each contig pool. Finally, each de Brujin graph was compacted and linear sequences representing each alternative splicing form and/or high similar transcripts were produced (Grabherr et al., 2011).

From the assembled transcripts, coding sequence prediction was performed using Transdecoder. To annotate the assembled transcripts, BLASTx searches (E-value <1e-5) were performed against the following protein databases: NCBI non-redundant protein (NR) database, Swiss-Prot, Kyoto Encyclopedia of Genes and Genomes (KEGG), and Clusters of eukaryotic Orthologous Groups of proteins (KOG). All the unigenes were translated into potential proteins according to ORF prediction by Getorf (http://emboss.sourceforge.net/apps/cvs/emboss/apps/getorf). Manual annotation of

hypothetical genes from transcriptome was performed using InterProScan. Gene annotations were used to characterize the transcriptome in *Q. grandiflora* and perform a differential gene expression analysis between the treatments.

The reads were mapped to the assembled transcripts using BWA (Burrows-Wheeler Aligner) software in paired-read mapping mode with parameters minimum seed length (-k):1, clipping penalty (-l): 25, maximum edit distance (-n) 0.04, maximum number of gap extensions (-e): 15, disallow an indel within INT bp towards the ends (-i): 10 (Li and Durbin, 2009), and quantified using bed tools. Differentially expressed transcripts were identified using DESeq2 (Love et al., 2014) package in a R environment. Candidates were selected according the criteria of adjusted p-value (q-value) < 0.01 and fold change between treatments >= 1 or =< -1. The values are depicted as Base Mean Expression among samples of the same treatment, and the difference in expression is shown as log2FoldChange, which is equivalent to log2 (Base Mean Expression in Al / Base Mean Expression in Control).

The transcripts abundance was normalized by the reads per kilobase of transcript per million mapped reads (RPKM) value using the RSEM (RNASeq by Expectation Maximization) package (Li B and Dewey, 2011). And those transcripts with RPKM value equal or larger than 0.1 were defined as expressed.

2.4. Differential expression analysis and GO enrichment analysis

To annotate the assembled sequences with gene ontology (GO) terms describing biological processes, molecular functions, and cellular components, the BLASTp results were imported into Blast2GO version 4.1 [Conesa et al., (2005); Conesa and Go'tz, (2008)], which is a software package that retrieves GO terms and allows gene functions to be determined and compared by enrichment and refinement. BLASTP and InterProScan search results were loaded into Blast2GO and the Blast2GO function Annotation > Perform Annotation Step menu was used to perform GO annotation. MySQL databases (b2g) from Blast2Go.com were downloaded and installed on a local server to enable faster processing. Blast2GO results were saved in plain text format (Additional file 2) and in Blast2GO format and added to the bitbucket in a subdirectory named Blast2GO. These GO terms were assigned to query sequences, and produced a broad overview of groups of genes catalogued in the transcriptome, biological processes, molecular functions, and cellular components of *Q. grandiflora*.

2.5. Pathway analysis

Gene expression data were analyzed in terms of metabolic functionalities using the Mercator pipeline (Lohse *et al.*, 2014) to ascribe potential gene function and MapMan BINs (Usadel *et al.*, 2005). Fisher's exact test, adjusted with Bonferroni correction for multiple testing, was employed for identification of MapMan BIN categories that were considered significantly enriched (adjusted *P*-value <0,05). Functional annotations of the differential unigenes were performed to search against the NR, Swiss-Prot, GO (Gene Ontology) and KOG database.

3. RESULTS

3.1. Physiological responses of *Q. grandiflora* in Al presence

Qualea grandiflora plants require Al to grow and develop. Visually, Al-treated and non-treated plants showed substantial morphological differences. It was observed that around 240 days of cultivation more than 80% of untreated plants had signs of chlorosis and necrosis at leaf apices and margins (Figure 2-A). Also, the untreated plants were smaller than those that were supplemented with Al (Figure 2 A-B). Differently, plants grown with Al were significantly healthier, no signs of chlorosis in leaves and a well-developed root system. Plants grew more uniformly throughout the experimental period without any sign of toxicity (Figure 2-B).

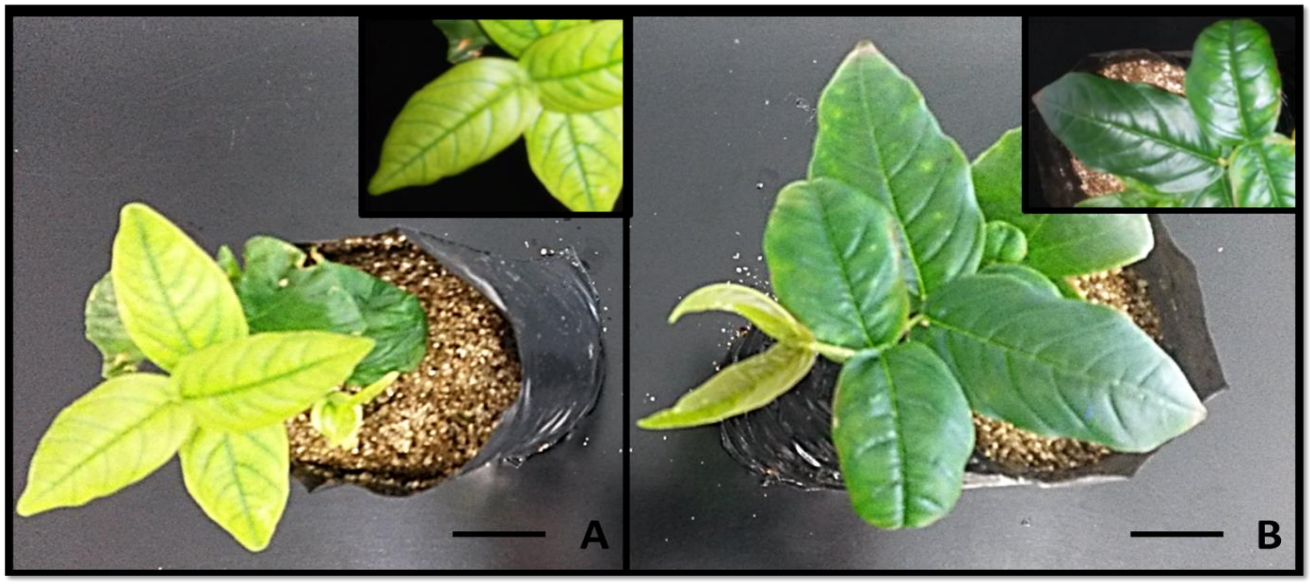

Figure 2. Plants of *Q. grandiflora* after 240th day of growth. A-B Overview of leaves grown in the absence and presence of Al. A- Grown in the absence of Al (Al-untreated). B- Grown in the presence of Al (Al-treated). Scale bars = 1 cm.

3.2. RNA-Seq analyses

It is important to state that the whole genome sequence of *Q. grandiflora* has not yet been accomplished. Therefore, the transcriptome analysis based on actual genomic data from Qualea was not feasible for this study. Complementarily, the cytogenetic analysis showed 22 chromosomes (11 pairs) e confirms that this species is diploid. Consequently, a *De novo* assembly was the best approach to investigate the expression changes associated with Al in this plant.

A high throughput RNA-seq (Illumina) approach was performed to understand the regulation of Al-responsive genes at the transcript level. Thus, leaves from the 2nd and 3rd nodes of Al-treated and non-treated plants were collected, and a total of six cDNA libraries prepared. Consequently, the cDNA sequencing yielded about 350 million clean reads in all six samples (Table 1). The sequencing result for all samples presented reliable quality, with 89,52-90,13% of base call higher than Q30 (Table 1). Hence, the clean reads constituted a total of ~16 GB of sequenced data. In addition, the actual number of paired-ends generated 51,024,474; 41,879,446; and 29,802,642 reads from Al-treated samples Al1, Al2 and Al4, respectively. Sequencing of C2, C3 and C4 samples from non-treated plants produced 41,987,304; 86,347,228 and 94,268,006 reads, respectively (Table 1). The overall rate of assembled reads was on average 87% of all samples (Table 1).

Table 1. Mining and quality of RNA-seq data. The reads and respective percentages of Q≥30 bases of clean reads from six *Q. grandiflora* leaf samples of Al-treated plants (AL1, AL2 and AL4) and Al-untreated plants (C2, C3 and C4).

Sample	Lane	Reads	Yield (Mases)	% of >= Q_{30} Bases (PF)	Overall rate of assembled reads *Q. grandiflora* (%)
C 2	1	41.987.304	4,199	89,93	86.61% (36,365,203)
C 3	1	86.347.228	8,635	89,99	87.84% (75,847,405)
C 4	1	94.268.006	9,427	89,81	85,01% (80,137,231)
Al 1	1	51.024.474	5,102	89,98	88,68% (45,248,503)
Al 2	1	41.879.446	4,188	89,52	87,19% (36,514,688)
Al 4	1	29.802.642	2,980	90,13	85.36% (25,439,535)

3.3. Global representation of differential gene expression of *Q. grandiflora*

Several criteria were used to determine the desirable sequence assembly, such as the number of reads, total transcriptome length, average contig length, N50, and annotation by UniProt, GO, KO and KEEG Orthology database. The differential gene expression analyses in *Qualea* leaves from Al-

treated, and non-treated samples were performed using scripts from the DESeq2 package. Furthermore, it was generated a total of 130,704 raw transcripts of *Q. grandiflora* from both treatments. Subsequently, the filter baseMean > 100, padj < 0.01 was applied, as well as the absolute value of log2FoldChange > 1 resulting in a total of 580 transcripts differentially expressed, from which 315 were upregulated (higher expression in Al-supplemented plants) and 265 downregulated (lower expression in Al-treated plants) (Table 2). Moreover, five genes were annotated as uncharacterized (Supplementary Table S2). Subsequently, these uncharacterized genes were functionally re-annotated based on the domain signature by InterProScan (Supplementary Table S3).

Table 2. Summary of transcripts from *Q. grandiflora* sequencing, assembly and annotation.

Al responsive transcripts by RNAseq expression pattern	**Number**
Total number of transcripts	130,704
Total number of transcripts significant	580
Transcripts significant upregulated*	315
Transcripts significant downregulated*	265

Representation of difference of expressed genes (DEGs) between the two treatments in response to Al in *Q. grandiflora* was based on expression levels (log2FC). The transcripts were presented graphically as volcano scatter plots in Figure 3, and as heat maps with hierarchical cluster analysis of expression patterns in Al-treated and non-treated plants (Figure S2). Expression varied considerably between the treatments, with six heat map cluster groups for DEGs was observed in *Q. grandiflora* (Figure S2). Different gene expression modulation patterns were seen in Al-treated and -nontreated plants, most likely reflecting a differential response to either presence or absence of Al. Table 3 represent main DEGs between treatments showing possible key-candidates in Al-response process. All DEGs relative to each treatment that were identified are listed in Table S1.

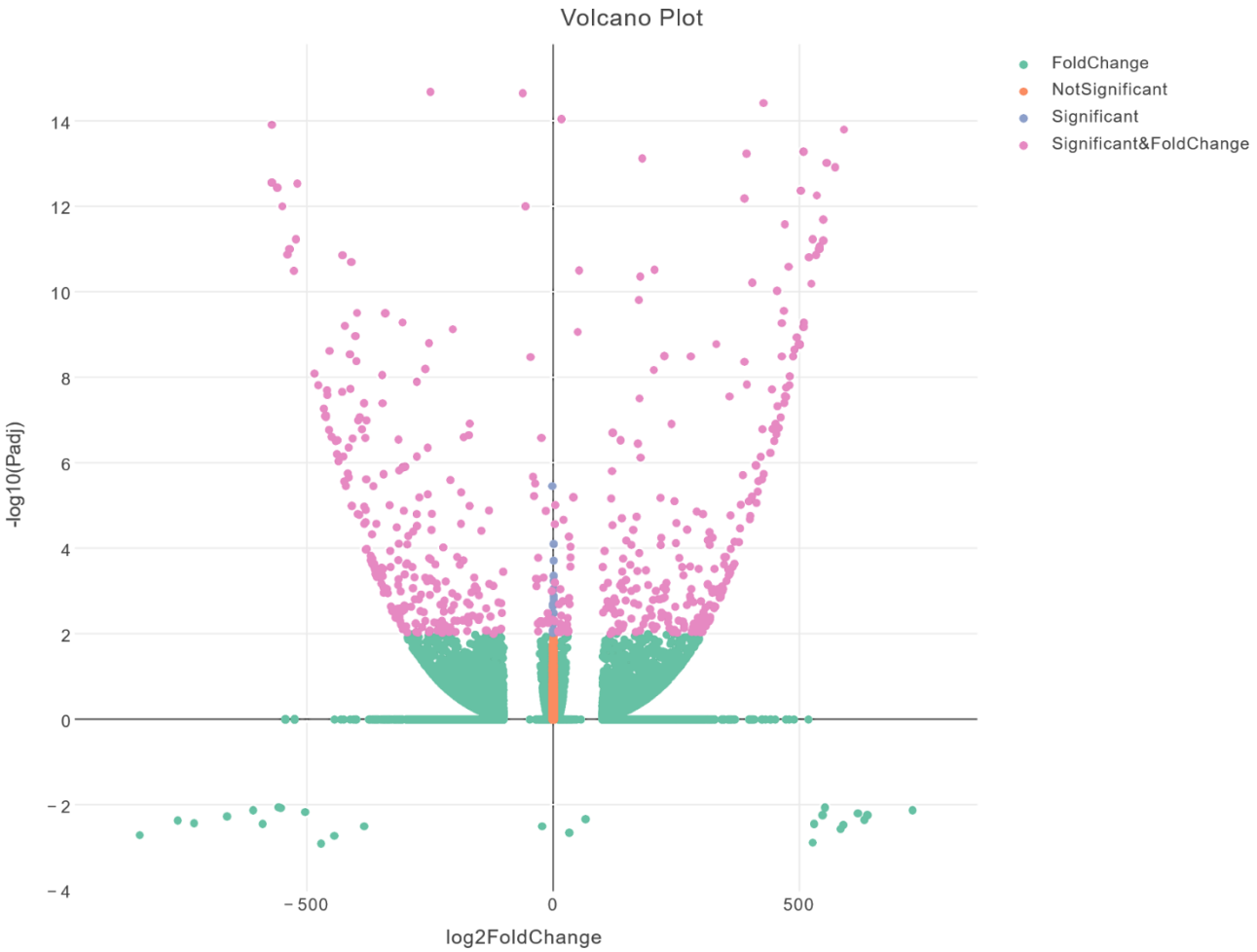

Figure 3. Volcano scatter plots for differentially expressed genes (DEGs) in *Q. grandiflora*. Genes significant&foldchange that were highly modulated in response to Al, in relation to Al-untreated, farther to the left and right sides, with highly significant changes appearing above on the plot in pink. Genes that presented significant HR values, but not exceeding the limit of +1 or -1 are in blue. In green are the genes that had FC> +1 or <-1 but did not present Padj <0.01, i.e. not pass the established limit. Genes non-significant wich presented values equal to 1 are represented in orange.

Table 3. Differentially expressed genes in *Q. grandiflora* leaves in response to Al using RNA-Seq data.

ID (*Q. grandiflora* TR)	Description	Base Mean	Log2Fold Change	*P-adj*
TR25696|c2_g1_i1|g.61553	ARHGDI, RHOGDI; Rho GDP-Dissociation Inhibitor	338.772	-120.864	0.000753
TR26970|c1_g1_i1|g.76196	ATP-Binding Cassette, Subfamily B (MDR/TAP), Member 1	162.529	10.029	0.008459
TR32793|c1_g3_i1|g.165085	Cellulose Synthase A	990.304	540.753	0. 874E-12
TR31544|c0_g13_i10|g.143010	Cellulose Synthase A	175.464	343.123	0.000778
TR33427|c0_g2_i1|g.177392	DNA Repair Protein RAD7	201.834	-104.804	0.00192
TR32498|c0_g4_i2|g.159812	Gpmb; Probable Phosphoglycerate Mutase	220.505	147.471	0.001657
TR16450|c0_g1_i3|g.9199	Jasmonate O-Methyltransferase	540.489	286.014	0.005732
TR17561|c0_g2_i1|g.11044	LRR Receptor-Like Serine/Threonine-Protein Kinase	496.677	314.419	0.000718
TR21549|c0_g3_i1|g.26155	Pectate Lyase	209.383	-188.836	0.000242
TR23901|c0_g1_i1|g.43344	Pectinesterase	143.829	-362.541	0.00023
TR25481|c1_g1_i15|g.59418	Pola; DNA Polymeras e I	129.601	299.724	0.009117
TR20746|c0_g2_i2|g.21902	Rhamnogalacturonan Endolyase	186.374	-414.661	0. 22E-6
TR13675|c0_g2_i1|g.6209	Ribosomal RNA Methyltransferase Nop2	130.379	-35.092	0.000509
TR33771|c0_g2_i12|g.184272	Small Nuclear Ribonucleoprotein Component	113.787	-312.817	0. 77E-4

TR32855|c3_g2_i20|g.166375	Stromal Membrane-Associated Protein	152.837	300.312	0.00172
TR34300|c6_g10_i14|g.194391	Tryptophan Synthase Alpha Chain	138.195	-206.406	0.002805
TR30352|c0_g2_i4|g.123278	Xyloglucan:Xyloglucosyl Transferase	291.909	-34.035	0.000763

3.4. GO enrichment analysis

The abundance of DEGs was analysed according to GO classifications (Supplementary Data Table S1–S3, Figure S3). Analysis of Fisher's exact test in *Q. grandiflora* showed enrichment of transcripts levels to hormone regulation, defense response, RNA polymerase regulatory, RNA binding, xylan activity, cellulose biosynthetic process, cellulose synthase activity, chloroplast biogenesis, photosystem repair, regulation of phenylpropanoids, lignin biosynthetic process, cytosol and plant-type primary biogenesis (Figure S3).

The abundant Al-related transcripts were classified into several Biological processes (Figure 4). The differential abundant transcripts were predominantly related to cellular process, metabolic process, single-organism process, response to stimulus, biological regulation, regulation of biological process, development process, multicellular organismal process, cellular component organization of biogenesis, reproductive process, signalling, positive and negative regulation of biological process and immune system.

Moreover, the Al-related pathways may be located in the following cellular regions: organelle, membrane, organelle region, macromolecular complex, membrane-enclosed lumen, extracellular region, and cell junction, plasma membrane, intracellular, nuclear lumen, chloroplast, bound to membrane organelle, cytosol, mitochondrion, Golgi apparatus, extracellular region, vacuole and plasmodesma (Figure 5-A-B).

To determine which molecular functions were more responsive to Al, the transcripts of Q. grandiflora were annotated and attributed to three main metabolic pathways: binding, catalytic activity and transporter activity (Figure 6-A). These were associated with eight metabolic subsets: protein binding, hydrolase activity, transporter activity, metal ion binding, kinase activity, phosphotransferase activity - alcohol group as acceptor, DNA binding, ATP binding (Figure 6-B).

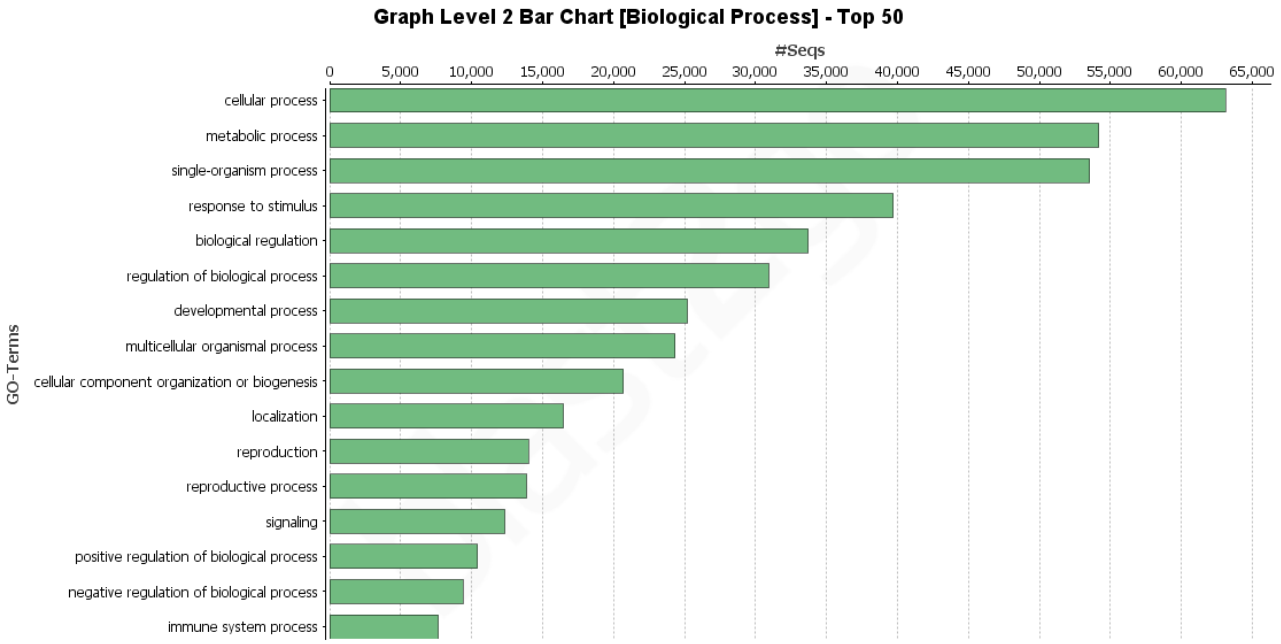

Figure 4. Distribution of differentially expressed transcripts in *Q. grandiflora* in response to Al, according to the biological processes in which they were classified. The numbers represent the occurrence of each GO term.

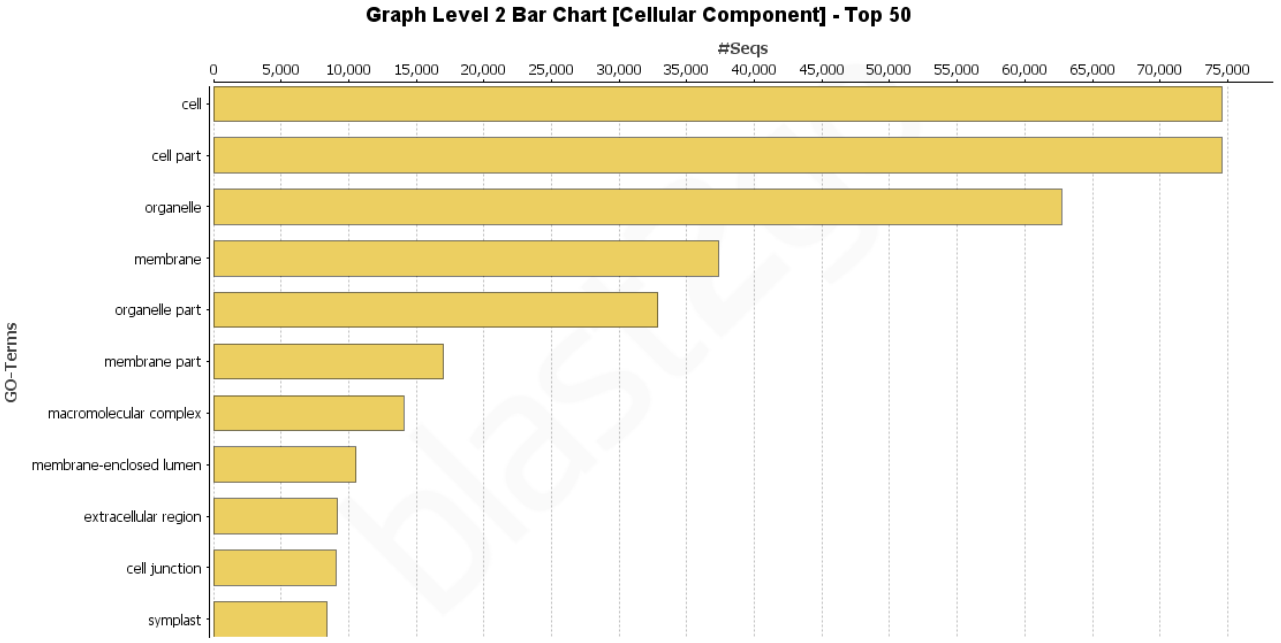

Figure 5. Distribution of differentially expressed transcripts in *Q. grandiflora* in response to Al, according to the cellular component. The numbers represent the occurrence of each GO term.

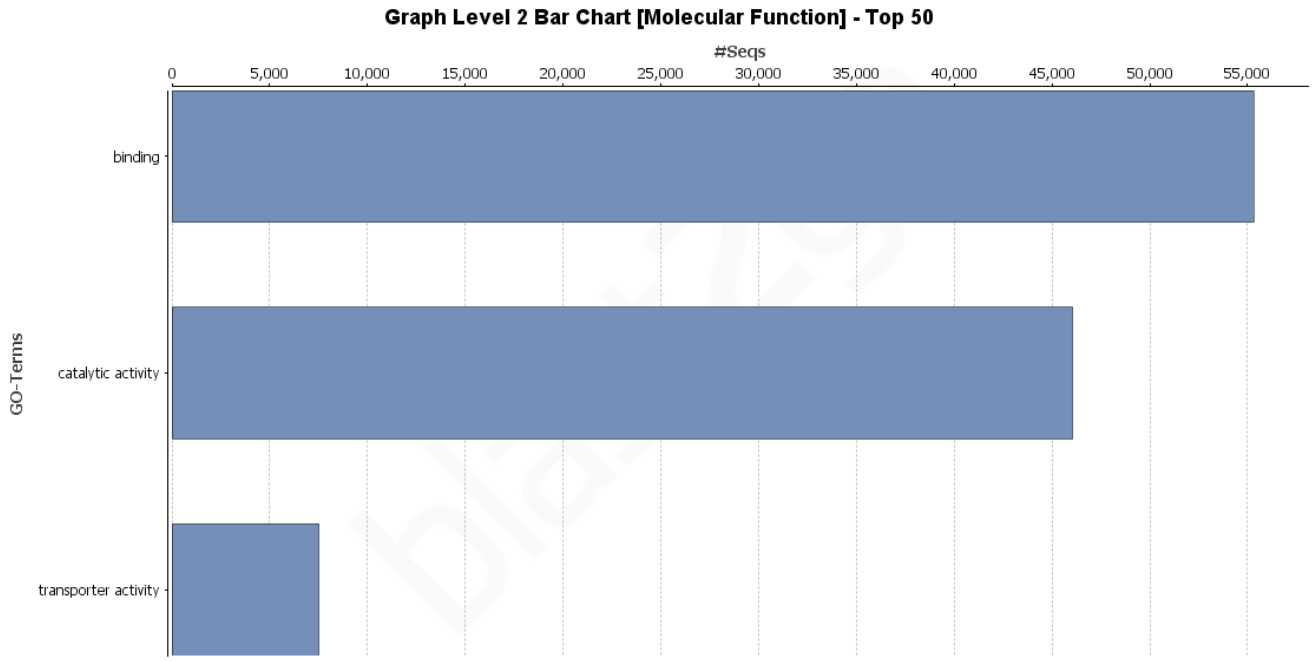

Figure 6. Distribution of differentially expressed transcripts in *Q. grandiflora* in response to Al, according to the molecular functions. The numbers represent the occurrence of each GO term.

3.5. Mercator analysis

Genes responsive to Al in *Q. grandiflora* and considered upregulated had a positive value of Log2 Fold Change, and those downregulated had a negative value of Log2 Fold Change (Table S1). Therefore, the transcript abundance analysis of differentially expressed genes revealed that RNA sequences may be associated with 34 metabolic categories (Figure 7). Most DEGs in *Qualea* leaves were involved in carbohydrate metabolism (0,77%) cell wall (1,07%), lipid metabolism (1,61%), amino acid metabolism (1,19%), secondary metabolism (1,40%), hormone metabolism (1,53%), stress (4,52%), misc enzymes (3,51%), RNA (7,96%), DNA (1,95%), protein metabolism (12,22%), signalling (6,25%), cell organization (2,81%), development (2,01%) and substance transport (4,31%). Nevertheless, there was a considerable part of genes that were not assigned (35%).

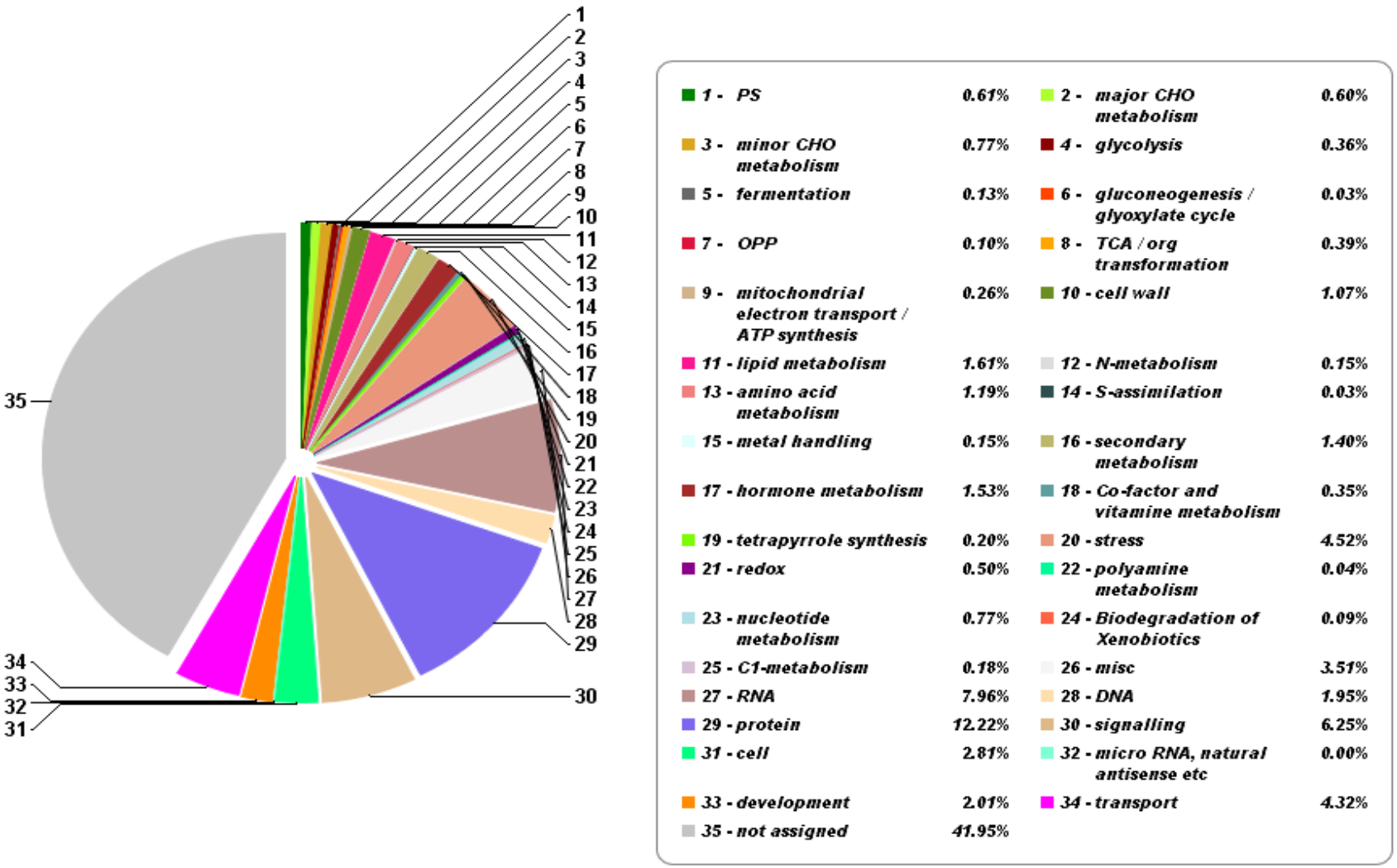

Figure 7 - Functional distribution of Al-responsive genes in *Q. grandiflora* leaves as determined by Mercator. Fisher's exact test, adjusted with Bonferroni was employed for identification of MapMan BIN categories that were considered significantly enriched (adjusted P-value <0·05). PS: Photosynthesis, CHO: Carbohydrate.

Analysis of DEGs using Mercator web application provided an assign MapMan "BINs" (major functional categories), that corresponded to arbitrary protein input sequences through a representation of gene expression across selected metabolic pathways, which was based on a manually curated binning of the reference database entries.

Among the BINs checked there were several lectin receptor kinases (LecRKs), as well as cell upregulated wall enzymes. Additionally, crucial components of genetic information processing mechanisms such as nucleobases and transcription factors had higher abundance in Al-treated *Q. grandiflora*. Genes associated with primary and secondary metabolism and some plant growth regulators were also found to be upregulated in Al-treated *Qualea* plants. BINs encoding transporters cell wall-anchored, transmembrane proteins of plasma and chloroplast and mitochondrial membranes were positively responsive to Al (Table 4S).

3.6. Mapman

Analysis of DEGs in response to Al in *Q. grandiflora* using MapMan provided a representation of gene expression across selected metabolic pathways (Figure 8). The changes of expression were displayed via a false colour code: genes whose expression did not change were coloured white, and the significant increase (upregulation) or decrease (downregulation) in expression was shown as intense blue or red colour, respectively.

Genes were identified based on homology to genome banks and allocated to MapMan BINs. Significant upregulation of genes involved in carbohydrate metabolism (BIN2), glycolysis (BIN4), fermentation (BIN5), cell wall (BIN10), lipid metabolism (BIN11), metal handling (BIN15), secondary metabolism (BIN16), RNA (BIN27), DNA (BIN28), protein (BIN29) and signalling (BIN30) were observed in Al-treated leaf samples of *Qualea*. Differently, in these plants, it was observed a downregulation of genes related to amino acid metabolism (BIN13), hormone metabolism (BIN17), stress biotic (BIN20), nucleotide metabolism (BIN23), misc enzymes (BIN26) and development unspecified (BIN33) (Figure 8-A, Table S4).

The current build organised all genes into 35 ‘BINS’ above (Table S4), which was split into about 23 subcategories termed as ‘subBINs’ (Figure 8-B and Table S4). Among these subBINs, the most upregulated Al-responsive genes were related to

hormone metabolism of BR and SA, synthesis of cell wall components, DNA synthesis, RNA transcription and regulation, transcription factors, protein targeting and vesicle transport. Moreover, signalling of phosphoinositides and signalling receptor kinases, as well as fermentation aldehyde dehydrogenase, metal handling by selenium-biding, major and minor CHO metabolism and phenols metabolism were also found to be positively regulated by Al. Conversely, genes associated with protein modification, hormone metabolism (ABA, ET, GA, JA), biotic stress proteins and abiotic stress, misc enzymes (cytochrome P450 and oxidases) as well as amino acid metabolism of degradation were downregulated in Al-treated *Qualea* plants.

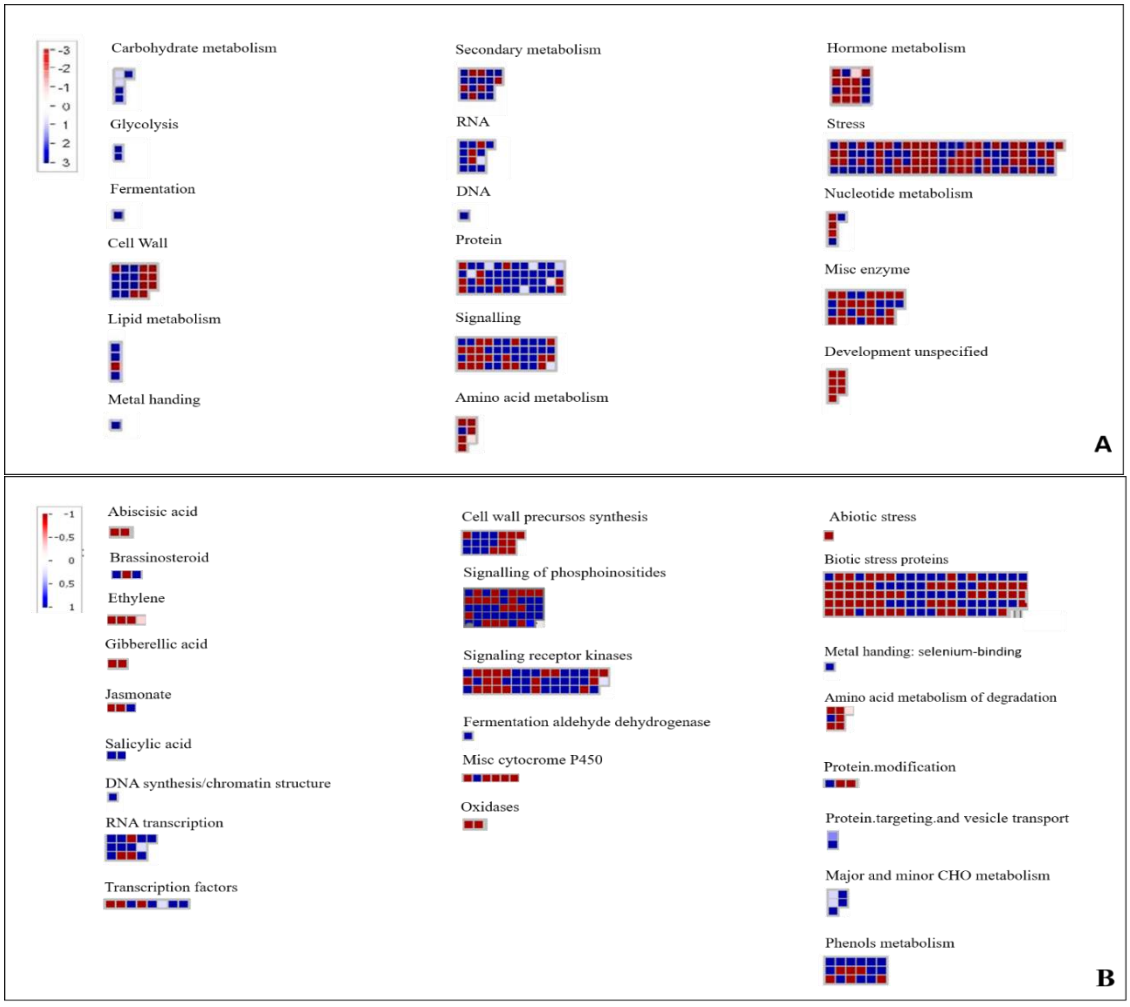

Figure 8. MapMan-derived chart showing the expression profiles of differentially expressed genes (DEGs) in *Q. grandiflora*. Differential expression patterns were determined based on log2FCs of mRNA transcripts from leaves from both treatments. A- Metabolism overview represented by functional categories - BIN codes. B- Metabolic pathways represented by functional subcategories – subBINs codes. Each dot represents the presence of a paralogue gene that encodes a particular enzyme within a metabolic pathway. Compounds with significant differences are shown in colour representations ($p < 0.05$).
Blue colour = upregulated gene; White colour = not significant; red colour = downregulated gene.

As mentioned, it was observed Al-stimulated phytohormone correlated genes in *Q. grandiflora* leaves: BR (brassinosteroid) and SA (salicylic acid). Moreover, genetic information processing was positively influenced by Al, and, proteins involved in RNA transcription (C2C2(Zn) GATA transcription factor family), DNA synthesis [pentatricopeptide repeat-containing protein(PPR)], pyrophosphatase and adenylate kinase proteins were upregulated. Also, transcription factors genes were increased by Al, which was directly related to gene expression as well (Figure 8-B). On the other hand, metabolic pathways associated with the phytohormones ABA, ET, GA, JA were downregulated in Al-supplemented plants.

Concerning to cell wall biosynthesis pathways, MapMan analysis and GO enrichment were also highly consistent. Therefore, cell wall associated genes encoding a phosphoglycerate/bisphosphoglycerate mutase, pectinesterases, a cellulose synthase, as well as precursors of fructose-6-P and glucose-6-P were upregulated.

Furthermore, there was an induction of signalling proteins such as signalling receptor kinases in Al-treated *Q. grandiflora* leaves as well (Figure 6-B, 8-B). It has been observed that these upregulated signalling pathways involved receptor kinases containing leucine rich repeats.

Moreover, the oxidation pathway was upregulated, shown by an increased expression of aldehyde dehydrogenase (NAD) protein family (Figure 8-B), whose members 3-chloroallyl aldehyde dehydrogenase and coniferyl-aldehyde dehydrogenase were actived by Al.

In contrast, the pathways containing genes that encode enzymes like misc cytochrome P450 and oxidases were downregulated by Al (Figure 8-B). Furthermore, the amino acid pathways that were downregulated by Al encoded methionine homocysteine S-methyltransferase and tryptophan synthase. Also, it was identified an upregulated gene encoding a putative metal handling (selenium-binding protein, SBP), and two genes that encode chloroplast targeted proteins known as Sec-dependent protein sorting (cpSecA) and superoxide dismutases (SODs).

Furthermore, Al-upregulated genes associated with primary metabolism were also involved in starch synthesis, e.g. starch synthase, starch limit dextrinase/pullulanase and family raffinose synthases putative (e.g. galactinol synthase). The secondary metabolism pathways upregulated by Al were related to phenols and dihydroflavonols biosynthesis and isoprenoids mevalonate biosynthesis, as well as components of lignin biosynthetic

process, and essential photosystem accessory pigments such as carotenoids (Watanabe et al., 2013).

4. DISCUSSION

Studies on plant adaptation to acid soils have provided some of the most concrete examples of how fast plant evolution takes place (Macnair, 1987; Linhart and Grant, 1996; Wright et al., 2013). Information on the genetic basis of edaphic tolerance continues to be based on molecular researches involving domesticated plants like *Arabidopsis thaliana*, sorghum (*Sorghum bicolor* (L.) Moench) and soybean [*Glycine max* (*L*.) Merr and provide a useful comparative resource (Zhang et al., 2016; Chen et al., 2017; Mangeon et al., 2017; Zhou et al., 2017). Furthermore, RNA sequencing from native plants also is a valuable tool to search for and identify genes that are potentially associated with plant adaptations to Al exposure, examining, a priori, candidates for investigating the genetic basis of plant adaptation to the environment (Metali et al., 2011).

4.1. Physiological responses

Physiological studies on Al-adaptation in *Q. grandiflora* growing in Cerrado soils were one of the earliest to document native Al accumulating plants (Haridasan, 2008). Previous studies report an enhancement of root and shoot length and biomass of *Q. grandiflora* plants supplemented with Al. Moreover, Al-treated *Qualea* plants had better visual aspects with no signs of senescence (Silva, 2012). Conversely, non-Al-treated plants were chlorotic with clear signs of stress (Silva, 2012). Consequently, this description is very consistent with the morphological aspects of 240-day-old plants used in this investigation.

Therefore, this research was focused on investigating the transcript response to Al to contribute to the understanding of the molecular basis of Al function in this native plant. Additionally, the identification of genes that may be potentially involved in mediating metabolic responses associated with Al will shed some light on the mechanisms of tolerance and accumulation. Hence, a comparative transcriptome analysis of *Q. grandiflora* grown with and without Al might reveal valuable information on gene regulation as well as on metabolic processes associated with this metal.

4.2. Al and phytohormones response

Regarding hormonal changes in *Q. grandiflora* plants, it was observed changes in five categories: BR (brassinosteroid) and SA (salicylic acid) were upregulated, and ABA (abscisic acid), ET (ethylene), GA (gibberellic acid), JA (jasmonic acid) downregulated. In *Q. grandiflora* two upregulated genes of BR metabolism (synthesis/degradation) and two genes related to SA metabolism (synthesis/degradation) were stimulated by Al. The role of BR and SA in plants has been intensively studied in the last few years. *Arabidopsis* mutant analysis demonstrated that the ability to synthesise, perceive and respond to BR is essential to normal plant growth and development (Clouse et al., 1996). Many studies have demonstrated that BR alone or interacting with other plant hormones is involved in cell elongation and seed germination (Haubrick and Assmann 2006). SA is is an important signal molecule in plant defense and the signaling is mediated by at least two mechanisms, one requiring the NON-EXPRESSOR OF PR1 (NPR1) gene and a second that is independent of NPR1. SA is involved in resistance against a variety of biotic and abiotic stresses through morphological, physiological and biochemical mechanisms as observed in *Cicer arietinum* L. (War et al., 2011). However, SA role goes beyond defence and affects several physiological phenomena crucial for plant growth and development such as chloroplast structure and RuBisCO activity, protection against oxidative stress, and cellular respiration (Vicente and Plasencia 2011). The regulatory process leading to efflux of organic acids in Cassia tora L. showed the possible involvement of salicylic acid (SA) in regulating Al-induced citrate release. Increased citrate efflux due to the SA treatment was associated with decreased inhibition of root growth and Al content in root tips, suggesting that exogenous SA could confer Al tolerance by increasing citrate efflux in this plant. However, both citrate synthase activities and citrate accumulation remained unaffected. These results indicate that SA-promotion of Al-induced citrate efflux is not correlated with increase in citrate production but are indirectly pathways involved in Al-tolerant mechanism (Yang et al., 2003). In contrast, little is known about how BR and SA may be connected to Al and which physiological and development responses are crucial *Q. grandiflora* metabolism. Therefore, more investigation is needed to understand the role of these growth regulators in this plant in response to Al.

Concerning to the downregulated phytohormones due to Al presence, two genes involved in the synthesis/degradation and alcohol dehydrogenase activity of ABA were observed in *Q. grandiflora*, ABA synthesis-degradation short chain alcohol

dehydrogenmase (Aba2) and ABA synthesis-degradation 8-hydroxylase that catalyzes the conversion of xanthoxin to abscisic aldehyde and catalyzes the first step in the oxidative degradation of ABA, respectively (González-Guzmán et al., 2002; Krochko et al., 1998). It is known that ABA is a major phytohormone and plays an essential role in a varied of stress-related processes such as heavy metal, drought, heat, high salinity, low temperature, and radiation stress (Vishwakarma et al., 2017). Moreover, ABA also plays a role in seed dormancy, and stoma closure (Vishwakarma et al., 2017).

Additionally, two JA and one ET encoding genes were downregulated in Al-treated Q. grandiflora. For JA the 12-oxophytodienoate reductase and llene oxidase synthase, which is required for jasmonate biosynthesis (Liechti, 2005). The latter gene encodes a member of the cytochrome p450 CYP74 family that functions as an allene oxide synthase. This enzyme catalyses the transformation of hydroperoxides in JA biosynthetic and acts as a vital signalling compound in a diverse plant stress responses and development (Zarate et al., 2007). For ET the aminocyclopropane-1-carboxylic acid synthase (ACC synthase, ACS), an enzyme that catalyzes the synthesis of 1-Aminocyclopropane-1-carboxylic acid (ACC). This enzyme is a precursor for ethylene, from S-Adenosyl methionine (Zhang et al., 2004). JA-ET pathway in *Arabidopsis* is activated in response to pathogens, as well as in tissue-damaged by herbivores and wounding (Kessler and Baldwin, 2002; Glazebrook, 2005). Finally, two genes of gibberellin-regulated family protein were found to have decreased abundance in Al-treated *Qualea* plants. JA signalling does not work alone while mediating defence responses in plants. It regularly functions in various crosstalk network with other phytohormone signalling pathways such as GA (Dar et al., 2015).

Similarly, four genes that encode a member of the dehydration responsive element binding DREB subfamily A-5 of ERF/AP2 transcription factor family were downregulated in *Q. grandiflora* in response to Al. This gene family is involved in ET perception in plant response to salinity and other stresses (Tao et al., 2015). Recently, an inhibitory plant growth mechanism associated with ET was proposed, in which several ET-inducible proteins (including NtTCTP, NEIP2 in tobacco, AtSAUR76/77/78, and AtARGOS em Arabidopsis) were involved in a negative feedback mechanism that is regulated by this phytohormone (Tao et al., 2015).

Therefore, we propose that Al may be an important component of signalling and phytohormone response in several metabolic processes in *Q. grandiflora*. Moreover, the lack of Al can impair morphogenetic response in this species. Consequently, further

investigation of the hormonal response, as well as their quantification will be essential to elucidate this important topic for plant growth and development.

4.3. Aspects of primary metabolism responsive to Al in *Q. grandiflora*

4.3.1. Al effects on carbohydrate metabolism of *Q. grandiflora*

The genes upregulated in *Q. grandiflora* is of phosphoglycerate/bisphosphoglycerate mutase protein that is associated with catalytic activity in chloroplasts (Hermans et al., 2010). Additionally, previous studies reported this protein family had a critical role in the stomatal movement, as well as in vegetative growth, and pollen production in *Arabidopsis thaliana* (Zhao and Assmann, 2011). Stomatal movements require massive changes in guard cell osmotic balance, and both stomatal opening and closure have been shown to be energy-requiring processes (Zhao and Assmann, 2011). Glycolysis, oxidisation of glucose to pyruvate is a central metabolic pathway and yields a net gain of 2 ATP, 2 NADH. Moreover, the 2,3-biphosphoglycerate-independent phosphoglycerate mutase (iPGAM) is a key enzyme in glycolysis. This enzyme catalyses the reversible interconversion of 3-phosphoglycerate to 2-phosphoglycerate. In Arabidopsis, the lack of iPGAM activity impaired plant growth mutants and pollen production (Zhao and Assmann, 2011). To grow, plants require a considerable amount of energy, and, therefore, energy supplier metabolic pathways should be working properly. Consequently, we firmly believe that; it is not without reason that genes related to glycolytic activity were upregulated in *Q. grandiflora*. To support this statement, in *Avena sativa* the increase in glucose metabolism in response to gibberellic acid (GA) stimulus promoted plant growth and enhanced synthesis of cell wall material (Montague and Ikuma, 1978).

Plant cell wall synthesis is highly dependent upon carbohydrate metabolism. Plant cell wall is a complex matrix of polysaccharides that provides support and strength for plant cell, and it is essential for plant survival (Scurfield and Wardrop 1962). The cell wall, not only participates in the structure and support of the plant, but also has crucial roles in cell growth and differentiation, intercellular communication, water movement and defence (Cosgrove, 2005; Ochoa-Villarreal et al. 2012). Additionally, plant cells synthesise wall polysaccharides and assemble them into a strong fibrous network, which directly affects wall expansion during cell growth (Cosgrove, 2005).

The main biologically active components of the cell wall are cellulose, pectins and hemicelluloses (Scurfield and Wardrop 1962) and in *Q. grandiflora* genes encodes pectinesterases, enzymes involved pectin production, was upregulated.

Pectins are a major component of plant cell wall and the most complex polysaccharides among organisms, which play several critical functions in plant growth and development (Willats et al., 2001; Vincken et al., 2003; Kohli et al., 2015). Moreover, studies have shown that pectins are the primary targets of attack by invading microbes and their breakdown works as potent elicitors in plant-defence responses (Kohli et al., 2015). These wall polysaccharides can form Ca cross-linked gels by pectinesterase activity, which results in higher wall stiffness and cell adhesion. The de-esterification (Ca-mediated cross-linking) is a controlled and reversible process, essential for cell expansion and plant growth (Iwai et al., 2002).

Pectinesterases are the first enzymes to act on pectin (Kohli et al., 2015). It was observed that activity pectin methyl esterase (PME) and other pectinesterase increase during separation of cells from pea root caps, and it is correlated with an increase of acidic pectin and a decrease in cell wall pH (Stephenson and Hawes, 1994). This study was performed using an antisense transgenic plant transformed with a pectin methyl esterase gene (*rcpme1*) obtained by screening a root cDNA library (Wen et al., 1999). Analysis of this plant showed that *rcpme1* expression is required for the maintenance of extracellular pH, elongation of the cells within the root tip and for cell wall degradation leading to border cell separation.

As previously mentioned, cell wall plays several roles in plants, which comprise resistance and protection against biotic or abiotic stresses, without preventing the entrance of nutrients, gases as well as not blocking intercellular signalling (Scurfield and Wardrop 1962). It is noteworthy that a group of genes in *Q. grandiflora*, encoding a pectinesterase and cellulose synthase proteins, were upregulated by Al, which may indicate that cell wall synthesis is favoured in the presence of this metal (Figure 9).

4.3.2. Morphological and transcriptional data indicated that Al-induced promotes the growth and development of *Q. grandiflora*

Al-treated *Q. grandiflora* plants did not have any signs of senescence. Differently, those plants not supplemented with Al were chlorotic and visually smaller. Moreover, a previous study quantified the growth differences between treated and non-treated *Qualea*

plants (Silva, 2012). All measured parameters (biomass, size, and photosynthetic pigments) showed that Al-treated plants had significantly grown and developed better (Silva, 2012). Moreover, leaf proteomic analysis revealed that higher relative abundance of RuBisCo in plants grown with Al, which may indicate higher photosynthetic activity can be correlated with Al presence (Silva, 2012).

The transcriptome data appears to support these findings. Many transcripts linked to photosynthesis and respiration such as protein targeting chloroplast involved in chloroplast biogenesis and the regulation of photosynthesis and glycolysis targeted phosphoglycerate mutase family protein, involved in glycolysis, which may lead to an increase of primary metabolites and ATP formation were positively responsive to Al (Figure 9). Additionally, transcripts and metabolites (Chapter 2) have been identified in Al-treated *Q. grandiflora* such as genes encoding for starch synthase, as well as for dextrinase/pullulanase (BIN 2 on Table S4) family, and putative raffinose synthases (BIN 3 on Table S4). These enzymes participate of sugars metabolic pathways of glucose, fructose, raffinose, and galactose and glycolate (Sengupta et al., 2015). These are plastidial enzymes and crucial for the synthesis of amylopectin, starch hydrolysis and raffinose family oligosaccharide accumulation (Pattanagul et al., 2001). Hence, these genes can also be associated with physiological processes that include seed growth, embryogenesis as well as resistance to biotic and abiotic stresses, and protection against oxidative damages in plants (Nishizawa et al., 2008).

Primary metabolites in plants are directly involved in growth, development, and reproduction of the organism. Primary metabolites are typically formed in energy metabolism. High photosynthetic rates can improve plant survival by offering the individual competitive advantages (Dodd, 2005). This fact is consistent with the idea that Al promotes growth and development of *Q. grandiflora* (Figure 1 and 2). Thus, the Al may confer important adaptive advantages in primary metabolism favouring innumerable metabolic pathways such as enhancing the photosynthesis of *Q. grandiflora*. Moreover, these positively regulated primary metabolism involves proteins associated with starch synthesis such as starch synthase. As mentioned this plastidial enzyme participates in the synthesis of an important component of starch (amylopectin), and strengthens the hypothesis that the mechanisms of energy production/reserve in *Q. grandiflora*, somehow, need Al to work properly.

4.3.3. Al affected amino acid metabolism in *Q. grandiflora* plants

As mentioned, the lack of Al in the nutritional solution induced the expression of several transcripts related amino acid metabolism in *Q. grandiflora*. Amino acids have various functions in plants. Besides being crucial for protein biosynthesis, they also represent the building blocks of several other biosynthesis pathways, and play pivotal roles in signalling processes as well as in plant stress response. Amino acids can also contribute to the energy pool of plant cells under certain physiological conditions, e.g. carbon starvation (Hildebrandt et al., 2015).

In this topic, we discuss the biological role of the following amino acids: tryptophan, alanine, aspartate, valine, leucine, isoleucine, threonine and pyroglutamate. Moreover, the current knowledge on amino acid degradation pathways and their regulation in the context of possible physiological roles in Al-non-treated *Q. grandiflora* plants. We observed that amino acid downregulated pathways contain genes that encode enzymes such as homocysteine S-methyltransferase and tryptophan synthase, which are involved in the biosynthesis of methionine and tryptophan, respectively (Ganu et al., 2015). Methionine partakes in several metabolic processes such as synthesis of S-adenosylmethionine, (SAM) the major methyl donor for transmethylation reactions (Moffatt and Weretilnyk, 2001). Furthermore, SAM is the precursor for the synthesis of polyamines, ethylene, nicotianamine (Moffatt and Weretilnyk, 2001). Polyamines and ethylene are plant-related stress hormones (Gill and Tuteja, 2010), and nicotianamine is a known metal-chelating compound ubiquitously present in higher plants (Takahashi et al., 2003). Tryptophan biosynthesis in plants is not constitutive and can be induced by different stress (Hsiao et al., 2007). Therefore, these upregulated genes in *Qualea* non-treated plants might be associated with stress response, which is very consistent with the morphological aspect of these plants.

Moreover, Hildebrandt et al. (2015) state that amino acid metabolism may also provide an energetic connection allowing plants to survive under prolonged stress conditions. During senescence, nutrients are reallocated from the source (leaves) to sink tissues such as in developing fruits and seeds, and, therefore, protein and amino acid degradation may take place (Watanabe et al., 2013). Also, during carbon starvation or even in the regular plant life cycle, proteins can be degraded, and the resulting amino acids may produce the energy required to support the needs of certain organs (Hildebrandt et al., 2015). This data strongly indicates that amino acids can be catabolized and involved

in stress response. As previously shown, *Q. grandiflora* plants not supplemented with Al were chlorotic, and thereby, it may have their photosynthetic capacity compromised. Hence, they may need to search for other energy sources to cope with the metabolic needs, and amino acids may be an option.

4.4. Secondary metabolism

Secondary metabolites are organic compounds produced from the modification of primary metabolite. Secondary metabolites do not play a role in growth, development, and reproduction as primary metabolites do. However, they may confer adaptive advantages to plants. Secondary metabolites are typically formed at the end or near the stationary phase of growth. Many of the known secondary metabolites have a role in ecological function, including defence mechanisms, by serving as antibiotics and by producing pigments (http://www.boundless.com/microbiology/textbooks).

It is noticeable that in *Q. grandiflora*, Al induced the upregulation of a considerable number of genes involved in the synthesis of secondary metabolites (phenols, isoprenoids mevalonate, terpenoids and carotenoids - BIN 16 on Table S4). Some of these compounds were identified by CG/MS as flavonols, isoflavonols, dihydroflavonols, isoprenoids mevalonate, phenylpropanols and carotenoids, essential components of lignin and tannin biosynthetic processes, photosystem accessory pigments and response to metals (topic detailed in Chapter 2). The presence of these transcripts and metabolites may shed some light on how *Q. grandiflora* has adapted to Cerrado environment, especially to its edaphic conditions.

Some reports have revealed that phenols, isoprenoids, terpenoids and carotenoids might be involved in several physiological processes, e.g., plant growth and development, as well as in response to environmental changes (Fornazier et al., 2003). These compounds also protect plants from damage caused by UV and visible light and are essential to the integrity of the photosynthetic apparatus (Tanaka et al., 2008).

At the molecular level, the response to Al in *Q. grandiflora* kept similarities to what has been described in other accumulating plants, such as *Camellia sinensis* L. Kuntze and *Hydrangea macrophylla* Thunb. Ser. (Chen et al., 2015). In these species, the number of transcripts related to the biosynthesis of secondary metabolites involved in environmental adaptation in the of presence Al was increased (Houde and Diallo, 2008; Chen, 2015). The transcriptome data from Al-treated *Qualea* indicates that the total

number of Al-responsive transcripts was within the same range observed in other Al-accumulating plants such as *Fagopyrum esculentum* (Yokosho et al., 2014) and *Hydrangea macrophylla* (Chen et al., 2015).

Flavonoids and terpenoids are widely distributed secondary metabolites with different metabolic functions in plants. Various phenylpropanoids, including flavonoids, isoprenoids and terpenoids possess excellent antioxidant activity and estrogenic, protection against UV, antiviral, antibacterial, and anticancer activities (Maía et al., 2012; Tholl, 2015).

Based on what was seen in *Q. grandiflora* supplemented with Al, the high expression of transcripts involved in secondary metabolites production could play many essential functions, protection against pathogens, detoxification, electron carrier, light absorption, structural functions, and plant propagation (Dinelli et al., 2006; Samanta et al., 2011). Moreover, within the plant, these substances can have an essential protective role, e.g., UV-irradiation protection or attacks by insects, fungi or bacteria (Figure 9).

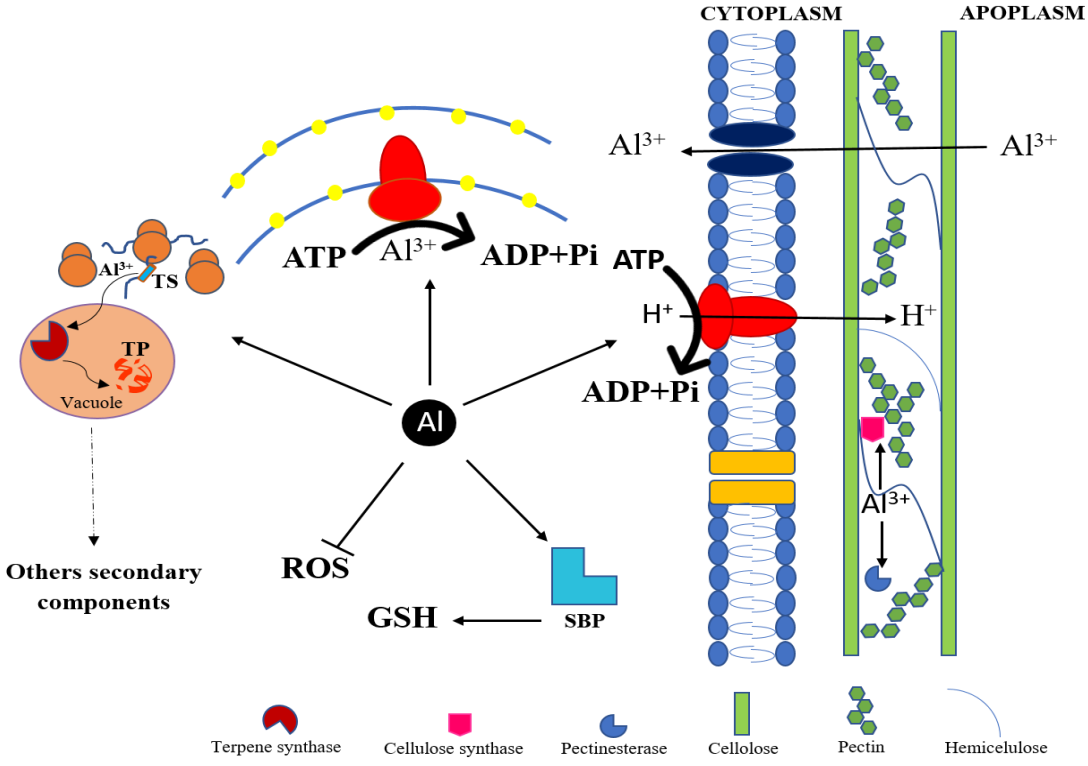

Figure 9. Diagram of Al and its association with cell wall synthesis and other cellular processes (Xu et al., 2017 with modifications). Al induced the expression of genes encoding cellulose synthase and pectinesterase. Moreover, the biosynthesis of terpene and others secondary metabolites (terpene synthase), and ATP formation in Al-treated *Q. grandiflora* leaves. Also, a putative Al role in preventing reactive oxygen species (ROS) production. Arrow and bar in black indicate up and downregulated changes, respectively. GSH: glutathione, ROS: reactive oxygen species, TP: Terpene, TS: Targeting signal.

4.5. Metal handling proteins, superoxide dismutases and targeting chloroplast proteins in response to Al in *Q. grandiflora*

Genes upregulated that encode a putative metal handling protein - selenium-binding protein 1 (SBP1) - proposed in this paper as Al-binding -, two targeting chloroplast proteins - Sec-dependent protein sorting (cpSecA) and superoxide dismutases (SODs) may play crucial roles in leaf cells of Al-treated *Q. grandiflora*. SBP1 is a known a cadmium and selenium binding and mediates the response to stress requiring glutathione (GSH), increasing the tolerance to, e.g., cadmium, selenate, hydrogen peroxide excess and sulphate assimilation (Dutilleul et al., 2008). The cpSecA proteins act as ATPases subunits of the chloroplast translocation machinery beyond acting a metal ion binding function (Liu et al., 2010). SODs are composed of enzymes that catalyse the conversion of superoxide radical (O2•-) to oxygen (O2) and hydrogen peroxide and are considered the first defence mechanism (Fukai and Fukai, 2011).

The putative selenium-binding protein (SBP1) decreases sensitivity to stress requiring glutathione (GSH) for metal tolerance. Moreover, it participates in cadmium and selenium response, hydrogen peroxide detoxification as well as in sulphate assimilation in *Arabidopsis* (Dutilleul et al., 2008). In *Arabidopsis* cadmium-treated seedlings, the SBP1 expression level was increased in roots. In shoots, SBP1 transcripts accumulated later due to higher Cd quantity (Dutilleul et al., 2008). Likewise, in *Saccharomyces cerevisiae*, an elevated SBP1 expression led to an increased Cd tolerance in *ycf1* Cd-hypersensitive mutant (Dutilleul et al., 2008). Therefore, SBP1 can bind Cd. These data highlight the importance of maintaining the adequate SBP protein level and suggest that, during Cd exposure, SBP accumulation efficiently helps to detoxify and enhance Cd tolerance through direct binding (Dutilleul et al., 2008).

These results suggest the SBP proteins in *Q. grandiflora* plays a major role in the maintenance of Al entrance into cells by endowing membranes with the capacity to serve as an initial carrier of Al. Moreover, it also may activate glutathione to avoid oxidative stress into the cytoplasm. It is consistent that the *Q. grandiflora* SBP protein can work as a potential new player in Al-accumulation plant cells (Figure 10).

The chloroplast-associated proteins are included in the endosymbiont-derived Sec-dependent protein sorting (cpSecA) pathway and work as a P-P-bond-hydrolysis-driven protein transmembrane transporter with ATP binding activity. These are an ATPase subunits of the chloroplast Sec translocation machinery, which plays an essential

role in biogenesis in this organelle (Liu et al., 2010). Another function is to import proteins into the thylakoid lumen and consequently regulate the photosynthesis reaction. The absence these proteins triggers a retrograde signal that eventually leads to a reprogramming of chloroplast and mitochondrial gene expression (Liu et al., 2010). Two loss-of-function mutants of cpSecA, the ATPase subunit of the chloroplast Sec translocation machinery, were analysed in Arabidopsis (Liu et al., 2010). Real-time PCR and microarray analyses revealed that the suppressed transcription of these subunits induced alteration of chloroplast protein translocation machinery and mitochondrial-encoded respiratory complexes (Liu et al., 2010). The homozygous mutants were albino, and the mutations were lethal under autotrophic conditions. The seedlings remained dwarf and infertile and only survived with an exogenous carbon supply (Liu et al., 2010). Furthermore, these results above confirm the importance of these proteins to photosynthetic apparatus of *Q. grandiflora*, and these are more produced in Al presence. Previous studies of the ultrastructural analysis in *Q. grandiflora* reported Al absence led to a progressive disintegration of chloroplasts (Melo, 2016).

Our findings also indicate that cpSecA, in Al-treated *Q. grandiflora*, can contribute to chloroplast sub-organelle structure and function. It also suggests that Al is likely to prevent the damage to chloroplast structure, by balancing the cellular redox status and maintaining the vitality of the plant in photosynthesis and respiration process (Liu et al., 2010). Probably this protein may also be involved in Al accumulation by acting as an initial carrier of metal ions into cells in *Q. grandiflora*, which can improve the photosynthetic activity of *Qualea* leaves (Figure 10). Consequently, the upregulated genes encoding chloroplast targeting proteins such as the ATPase subunit chloroplast sec might play an essential role in chloroplast biogenesis and photosynthesis regulation, which could be an Al-dependent process in this plant.

For superoxide dismutases (SODs) activity need a metal co-factor properly work (Gupta et al., 1993). SODs can be classified into four categories: iron SOD (FeSOD), which needs iron to work, copper-zinc SOD (CuZnSOD), which requires copper and zinc together, manganese SOD (MnSOD), which has manganese as redox active metal, and nickel SOD (NiSOD) that uses nickel co-factor (Alvarez et al., 2004). All these SOD types are present in prokaryotes, nevertheless, in eukaryotes, FeSOD is found in chloroplasts, MnSOD is typically located in mitochondria and peroxisomes (Alvarez et al., 2004). CuZnSOD is usually the most abundant SOD and present in chloroplasts, cytosol and extracellular space as well (Alvarez et al., 2004). Previous studies have

suggested the involvement of SOD in protection against oxidative stress during a broad spectrum of biotic and abiotic stresses in fenugreek, mainly in ultraviolet (UV) radiation exposure (Fukai and Fukai, 2011; Gupta and Mandal., 2016). Moreover, Ghanati et al. (2005) reported that Al-induced increased the activities of antioxidant enzymes - SODs - , resulting in increased membrane integrity and delayed lignification and ageing in tea plants. In *Q. grandiflora*, Al-induced SOD production may play a major role in preventing DNA damage and conferring protection against mechanical injuries to the photosynthetic apparatus. Further, it might confer UV irradiation protection by inducing antioxidant enzymes in Al-treated plants, which possibly will result in a better oxidative stress management in *Q. grandiflora* throughout its process of growth and development (Figure 10).

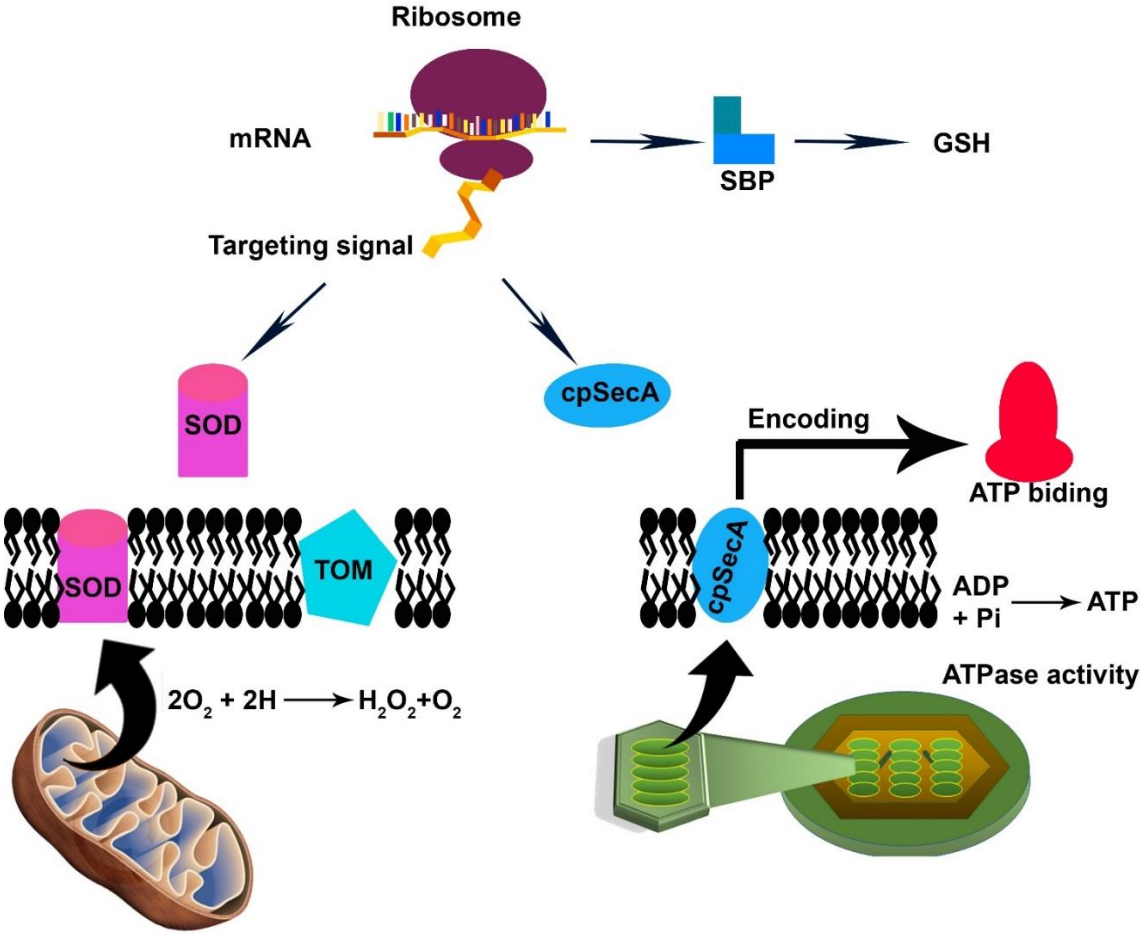

Figure 10. Diagram representing the proposed roles of Al in peroxidation on the mitochondrial membrane, and in cpSecA ATPase activation on thylakoid membranes, GSH induced by SBP protein in *Q. grandiflora* leaves Al-treated. GSH: glutathione, SOD: superoxide-dismutase, cpSecA: Sec-dependent protein sorting, TOM: translocase of the outer membrane.

4.6. Genetic information processing was affected by Al in *Q. grandiflora*

It is noteworthy that transcriptome analysis indicated that about 12% of the differentially abundant expressed genes were associated with DNA, RNA, nucleotide and amino acid metabolism. That might not be a coincidence that the processes upregulated by Al in *Q. grandiflora* cells involves routes of DNA and RNA synthesis, transcriptional regulation. Some of the upregulated genes were directly associated with transcriptional regulation, nucleotide metabolism and pyrophosphatase and adenylate kinase. The transcriptional regulation proteins [C2C2(Zn) GATA transcription factor family] are a group of DNA binding proteins broadly distributed in eukaryotes. These proteins control the rate of gene transcription by helping or hindering RNA polymerase to bind to the DNA strand and have been implicated in light-dependent and nitrate-dependent control of transcription (Jacob and Monod, 1961; Reyes et al., 2004). The nucleotide metabolism associated proteins [Pentatricopeptide repeat proteins (PPR)] are a large family of RNA-binding proteins that mediate several aspects of gene expression in organelles (plastids and mitochondria) but also in the nucleus (Mana, 2015). These proteins facilitate processing, editing, stability, splicing and translation of RNAs (Mana, 2015). Finally, the proteins -pyrophosphatase and adenylate kinase - are related to phytochrome control and enzyme control of chloroplasts (Butler and Bennett, 1969).

RNA is present in all living organisms. The role of RNA resides on protein synthesis in the cytosol, as well as in chloroplasts and mitochondria. Protein synthesis also involves different types of RNA such as mRNA, rRNA. RNA also plays a role in the regulation of gene expression, e.g., in RNA interference/gene silencing in both unicellular and multicellular eukaryotes (Baulcombe, 2004; Molnár et al., 2007; Huang et al., 2011; Norden-Krichmar et al., 2011).

Previous reports show how protein synthesis rate, and plant growth rate could be increased by maximising protein synthesis rate per unit RNA in organisms (Raven, 2013). The most obvious way of relating RNA content to the Growth Rate Hypothesis (GRH) is through RNA per unit protein (e.g., Matzek and Vitousek, 2009). If the ribosomes are operating at their maximum peptide elongation rates (Karpinets et al., 2006), there is a positive correlation between RNA:protein and growth rate. Moreover, experimental and theoretical data indicated that the requirement of ribosomes for protein synthesis is proportional to the RNA:protein ratio (Karpinets et al., 2006).

Furthermore, others studies demonstrate that the RNA:DNA ratio may also be

used as an indicator of growth rate. Hence, a fast growth means that protein synthesis is upregulated to attendant the individual needs during the growth process and requires more rRNA, mRNA, and tRNA per unit DNA (Mizuta et al., 2003; Reef et al., 2010, 2012).

An additional support that correlates RNA concentration with organism growth is biomass, which can be expressed by dry and fresh weight, or volume (e.g., Mizuta et al., 2003). Nicklisch and Steinberg (2009) examined the RNA, DNA, and protein concentration of five species of eukaryotic microalgae whose growth rates were submitted to various light intensities and concentrations of P, N and Si. All five showed a significant positive correlation between RNA:DNA ratio with growth rate; and most had a positive relationship between RNA:protein and growth rate (Nicklisch and Steinberg, 2009). Therefore, the data support the idea that higher levels of RNA can result in an increased growth rate.

In vascular plants, *Pinus contorta* and *P. muricata* submitted to an ideal nutrient solution had three times the RNA:protein ratio and biomass in leaves than did plants grown with a low-nutrient solution (Matzek and Vitousek, 2009). Furthermore, the plant relative growth rate of plants treated with nutrient abundant solution plants was almost twice as that observed in plants grown in low-nutrient (Matzek and Vitousek, 2009).

Higher contents of protein:RNA values were also found for shoot linear extension rate of two species of the mangrove tree, which reflected the faster growth of the plant (Reef et al., 2010). Thus, there was a positive correlation between linear extension rate of the shoot and the RNA:DNA ratio (Reef et al., 2010).

These studies support the growth analysis data of Al-treated and untreated *Q. grandiflora* (Silva, 2012). It was established that Al is required for the growth and development of this plant. Consequently, a higher level of genetic information processing should be expected for Al-treated plants, which was exactly what happened. Transcriptome data showed that Al-treated *Qualea* plants had an upregulation of genes involved in all steps of genetic information processing. Similarly, a proteomic analysis of *Q. grandiflora* roots was very coherent with transcriptome analysis performed in leaves (Cury, 2017). Therefore, molecular data confirm that *Q. grandiflora* requires Al for growth and development.

4.7. Signalling and signalling receptors Kinases in response to Al

Signalling was another mechanism upregulated by Al in *Q. grandiflora.* Al

induced the expression of genes encoding receptor kinases leucine rich repeat, receptor kinases DUF 26 and signalling receptor kinases misc protein family, which is believed to be involved in environmental adaptation and pathogen. The predicted sequences of these proteins carry both a receptor-like serine motif and a threonine-protein kinase domain.

Signalling receptor kinases containing leucine rich repeats in specific organs originated from the shoot apical meristem. These unigenes are involved in shoot growth (Torii et al., 2004). Moreover, all these proteins may play a role in cell surface recognition of a pathogen ligand and subsequent activation of an intracellular defence response in *Arabidopsis thaliana* (Chinchilla et al., 2006). Consistently, these pathways involve the auxin-binding protein ABP19, which can be associated with biotic stress responses (Ohmiya et al., 1998). These enzymes are also involved in leucine biosynthesis and biotin catabolic process in response to heat or high-light stress (Garrido et al., 2005).

Another investigation indicates that a growing number of these receptor-like kinases (RLKs) are implicated in the regulation of a wide range of developmental and defence-related processes (Torii, 2004). Also, the structural features of the RLKs suggest that these proteins might act as receptors for extracellular signals. Some of these proteins were found have perception systems, including for brassinolide, a plant hormone involved in stem elongation and cell division, which was observed in *Brassica napus* L. (Grove et al., 1979; Kinoshita et al., 2005). Characterization of this transcript should facilitate the understanding of the resistance machinery in *Q. grandiflora* in Al-presence.

5. CONCLUSIONS AND FUTURE DIRECTIONS

In conclusion, this was the first study into the dynamics of Al expression in *Q. grandiflora*. A comprehensive transcriptome was characterised based on leaves of *Q. grandiflora* plants grown with and without Al by the Illumina sequencing technology. This study expands the understanding of the regulatory network involved in Al adaptation in this species. The Al-associated transcripts identified in *Q. grandiflora* included previously reported Al-responsive transcripts found in other species as well as some novel differentially regulated transcripts induced by Al.

The results provide evidence for early defence and stress responses from JA/ET/ABA/GA signalling events was downregulated and SA/BR involved in shoot growth was upregulated by Al.

Moreover, the genes encode for cell wall synthesis proteins such as cellulose

synthase, pectinesterase and phosphoglycerate/bisphosphoglycerate mutase protein were upregulated.

The identification of transcripts related to metabolism of carbohydrate (starch synthase and raffinose synthase), lipid (metabolism phenylpropanoids family protein) and amino acids (amino acid metabolism synthesis family protein) by RNAseq analysis will become a new source for molecular studies of positive pathways regulations in Al-accumulating plants.

Genes upregulated that encodes a putative metal handling proteins (SBP), two targeting chloroplast proteins known endosymbiont-derived Sec-dependent protein sorting (cpSecA) and superoxide-dismutase (SOD) protein groups can disclose some light on the role of Al into cells of *Q. grandiflora.* Our study also revealed the better understanding the mechanism by which plants recognise and uptake Al, which plays an essential role in chloroplast biogenesis as well as in the regulation of light-harvesting photosynthesis promoting growth and avoid UV damages in *Q. grandiflora.*

Some of the upregulated genes were directly associated with transcriptional regulation, nucleotide metabolism and pyrophosphatase and adenylate kinase. Moreover, genes encoding signalling and signalling receptor kinases leucine rich repeat, receptor kinases DUF 26 and signalling receptor kinases misc protein family were upregulated in Al-response.

This article provides good evidence that *Q. grandiflora* transcripts can function as a potential new player in Al tolerance/accumulation to engineer plants studies aiming capable of cleaning Al-polluted soils through phytoremediation techniques or preventing nutritional disease by restricting the introduction of heavy metals into the food chain.

Ultimately the molecular functions of many newly identified Al-responsive DEGs remain unknown. However, the results shown here provide a significant resource for gene discovery and transcript quantification in Al metabolism. Transgenic assay, complementation assay, and subcellular localisation could be employed to elucidate their possible contribution on Al role in native plants.

6. SUPPLEMENTARY INFORMATION

Supplementary data are available at pdf document – attached – and consist of the following. Figure S1: RNA extraction of leaves of *Q. grandiflora* in two treatments: without Al (C2, C3, C4) and with Al (Al1, Al2, Al4). Visualization by 1% agarose gel stained with ethidium bromide. All samples showed ratio A260/280 between 1.8 to 2.0 and RIN score above 8.0 measured by Bioanalyzer. C – Control (Aluminum-untreated) sample and Al – Aluminum-treated sample. Figure S2: Histogram of *Q. grandiflora* samples from two treatments: without Al (control) and with Al. RPKM averaged values were represented based on the colour scale shown at the top. The expression map was generated using MeV s/w (http://www.tm4.org/mev/). Table S1: Differentially expressed genes in *Q. grandiflora* leaves in response to Al using RNA-Seq data. Table S2: Differentially expressed genes of uncharacterized protein in *Q. grandiflora* using RNA-Seq data. Table S3: Differentially expressed genes of uncharacterized protein functionally re-annotated in *Q. grandiflora* based on the domain signature by InterProScan using RNA-Seq data. Table S4: Functional re-annotation of differentially expressed genes between treatments of *Q. grandiflora* using MapMan.

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CAPÍTULO II - GC-MS-Based Metabolic Analysis Helps to Unravelling the Role of Aluminum (Al) in *Qualea grandiflora* Mart. (Vochysiaceae)

GC-MS-Based Metabolic Analysis Helps to Unravelling the Role of Aluminum (Al) in *Qualea grandiflora* Mart. (Vochysiaceae).

Abstract. *Qualea grandiflora* Mart. is a native Cerrado species that depends on aluminium (Al) to grow and develop properly. In this study, the metabolic profile of roots and leaves grown with or without Al was investigated. Using gas chromatography/mass spectrometry (GC/MS), we automatically quantified 13 and 23 distinct compounds from Al-treated and untreated *Q. grandiflora* leaf and root extracts, respectively. A comparison between the two treatments showed that *Q. grandiflora* possesses distinct and Al responsive metabolic profiles. The presence or the absence of Al caused changes in the contents and types of sugars, amino acids and organic/inorganic acids. Vanted analysis demonstrated that Al resulted in biochemical changes in several metabolic pathways including carbohydrate metabolism, photosynthesis, amino acid metabolism and oxidative phosphorylation-related pathways. These results show that the use of metabolite profiling is a tool that can significantly extend and enhance the power of functional genomics approaches and will contribute to elucidating the metabolic role of Al in this native species. Furthermore, an integrated analysis of the effects of Al on *Q. grandiflora* metabolism will be performed at metabolites and transcripts levels. The results presented here will contribute to elucidating the metabolic role of Al in this native species.

1. INTRODUCTION

Aluminum (Al) is one of the most abundant metal in soils. Since many plant species are sensitive to micromolar concentrations of Al, its phytotoxicity must be taken into consideration for agricultural purposes (Delhayze and Ryan, 1995). Al toxicity is a major constraint for crop production in acid soils worldwide. One of the reasons for that is the fact that in acid soils (pH < 5), the Al3+ is solubilized and then enters the roots and impairs plant development (Panda et al., 2009). Furthermore, in sensitive plants, Al can also induce several morphological, physiological and biochemical damages, which includes lower growth rate, photosynthesis depletion, imbalance of carbohydrate metabolism, stresses related to mineral nutrition, water deficit, and oxidative stress (Barceló and Poschenrieder, 2002).

Nevertheless, plants have also evolved several endogenous mechanisms to cope with Al toxicity. These mechanisms involve signal transduction proteins, antioxidant compounds, activation of transport systems and biosynthesis of chelating compounds (Lopez-Bucio et al., 2000; Ma, 2000; Li and Ma, 2000; Ryan et al., 2001; Watanabe and Osaki, 2002). It is crucial to investigate the mechanisms of Al tolerance, as well as the identification of cellular and biochemical targets that encompass plant physiological responses to it. However, a systemic understanding of metal tolerance and accumulating mechanisms of plant physiological responses are complex and challenging.

Recently, the arrival of "omics" approaches have been widely used in modern biology and have facilitated a massive characterization of molecular mechanisms of living systems at different levels. Therefore, it can provide a systemic view of cell biochemical architecture as well as functional biology networks. Also, in a post-genomic era, the elucidation of gene function is one the focus in molecular biology research. "Plant functional genomics couples the generation of transgenic and mutant plants with the multi-parallel analysis of gene products such as mRNA and proteins". However, these methods do not supply direct information about how a change in mRNA or protein is correlated with changes in biological function (Fiehn, 2000).

Metabolomics is a new genomics approach that aims at measuring all or a subset of metabolites in the cell. Moreover, this systematic identification and quantification can provide information on complex mechanisms by measuring the amounts of cellular products derived from various biochemical pathways. With the use of technologies such as GC-MS (gas chromatography-mass spectrometry), a variable number of metabolites

can now be quantified by using minimal amounts of biological samples, which allows obtaining a global analysis of metabolic systems. GC-MS has also opened the door for discoveries that link cellular pathways to biological phenomena and deepen our understanding at molecular, cell biology and physiology levels. Besides GC-MS, there are other analytical platforms such as liquid chromatography (LC)-MS, capillary electrophoresis (CE)-MS and nuclear magnetic resonance spectroscopy (NMR), which are commonly used in plant metabolomics research. However, to date, GC-MS is the most widely used technique for studying plant metabolomics, especially for facilitating the identification and quantification of metabolites involved in the major pathways of the primary metabolism such as photosynthesis, organic acids, amino acids, polyamines, sugars and sugar alcohols (Schauer, 2006).

Increasing evidence has revealed that metabolomics studies are playing important roles in the post-genomic era, and it has been able to characterise the physiological responses to various metals in plants better. For instance, a metabolite profiling was done to determine radish response to cadmium (Cd) and lead (Pb) stresses using GC-MS and LC-MS (Wang et al., 2015). The results demonstrated that the exposure of radish to Pb stress induced profound biochemical changes, which included carbohydrate metabolism. As a result, there was an increase of glucose and fructose, energy metabolism (an increase of citrate and decrease of malate) and amino acid metabolism (decrease of serine, glycine, alanine and isoleucine) (Wang et al., 2015). Concerning Cd there was a significant variation in energy production, amino acid metabolism and oxidative phosphorylation-related pathways took place in this plant, which resulted in a decrease of compounds associated with these routes such as fructose, glucose, citrate, alanine and threonine (Wang et al., 2015). Also, a metabolite profiling was carried out in *Arctium lappa* L. (Asteraceae) using copper (Cu) as the stress eliciting factor (Jung et al., 2015). The changes in metabolite levels due to Cu included increased amounts of phenols and decreased quantities of several primary metabolites such as glucose (Jung et al., 2015). It was also revealed enhanced levels of unsaturated fatty acids and diminished amounts of sterols in Cu-treated plants. These results showed that Cu stress leads to the activation of the phenylpropanoid pathway and growth inhibition (Jung et al., 2015). Moreover, GC/MS metabolic analysis of Cerrado native Al-accumulating plants was performed to discriminate compounds associated with Al and determine which pathways are affected by the presence and absence of this metal. For instance, in in leaves and roots of Al-treated *Qualea dichotoma* (Mart.) Warm. was detected a greater abundance of myo-

inositol, quinic acid and sucrose, which may indicate that Al was crucial in metabolic routes that are related to these compounds (Moreira, 2016).

It has been reported that Cerrado plants take up Al during growth (Chenery, 1955). *Qualea grandiflora* Mart. (Vochysiaceae) is one of the most important Al-accumulating species of this biome. It occurs in savannas areas of Amazon forest, as well as in Cerrado of Sao Paulo, Minas Gerais, Goiás and Mato Grosso do Sul. *Qualea grandiflora* is known to be an Al-hyperaccumulating plant, and its growth is enhanced by this metal (Haridasan 20081; Silva, 2012). On a dry-weight basis, this species can accumulate up 3 to 5 g of $Al.Kg^{-1}$ in different organs (Haridasan,1982; Haridasan et al., 1986). Moreover, Al probably promotes photosynthetic activity (noticed by an increase of RubisCO abundance) as well as an increase in biomass accumulation (Silva, 2012). Moreover, it induced the activities of antioxidant proteins such as peroxiredoxin (Silva, 2012).

Also, this study identified exclusively expressed proteins in roots and leaves in both treatments (Silva, 2012). Besides, recently, using a more efficient method, 2520 proteins were identified in roots of *Q. grandiflora* in response to Al. Among which, there were 410 were differentially abundant proteins in *Qualea* roots (274 positively regulated and 136 negatively regulated) (Cury, 2017). Also, this study reported that Al had played active roles in signalling and regulating processes associated with glyoxylate metabolism, oxidative phosphorylation, fatty acid degradation, genetic information processing, and metabolism of amino acids (Cury, 2017).

Furthermore, an integrated analysis of the effects of Al was performed on the levels of gene transcripts from our previous transcriptome work in leaves of *Q. grandiflora*. Six cDNA libraries were constructed from leaves of *Q. grandiflora* plants supplemented or not with Al (Chapter 1). Therefore, a set of differentially expressed transcripts involved in several metabolic routes such as secondary metabolism, photosynthesis, signaling-related pathways and carbohydrate metabolism was detected. Moreover, there have been identified exclusively Al-responsive transcripts, many of which were found to target metal transport systems and transcription factors. These results would facilitate a deeper understanding of the mechanisms of Al accumulation/tolerance in plants.

Accordingly, a metabolite profiling analysis of roots and leaves from 8-month-old Q. grandiflora plants grown for with or without Al supplementation was performed. The aim of this investigation was to determine metabolic changes associated with Al

metabolism in this species. Hence, a broad range of Al-responsive metabolites was quantified, as well as the changes in several metabolic pathways such as carbohydrate metabolism, energy metabolism, amino acid metabolism and oxidative-related pathways. We describe here the use of metabolite profiling as a tool for a comparative display of gene function that has the potential to provide deeper insight into complex regulatory processes of *Q. grandiflora* in response to Al. Furthermore, this work aims to deliver a metabolic and molecular framework for a better understanding of mechanisms of Al physiological role in this species. The results will support functional gene studies that may facilitate crop plants genetic manipulation towards obtaining tolerant plants in acid soils.

2. MATERIAL AND METHODS

2.1. Plant material

The growth conditions followed the method described in Chapter 1. *Q. grandiflora* seeds were immersed in 70% alcohol for 1 min and 2% commercial bleach (2.5 active chlorine) for 30 min. Then the seeds were washed three times in sterile double-distilled water, placed in Petri dishes on wet germitest and incubated at 30 °C, 70% relative humidity and photoperiod of 18 h. Thirty days after germination, the seedlings were transferred to plastic bags containing sterile vermiculite. The plants were watered two times a week with $^1/_5$ MS (Murashige and Skoog, 1962) nutrient solution, no sugars, no vitamins, with or without Al. The Al was provided by the addition of 150 mM of $AlCl_3$ in the nutrient solution. The pH of the nutrient solution to both treatments was adjusted and maintained to 4.5- 4.8 in order to maintain the Al solubilized. The seedlings were grown in a growth chamber at 25 °C, 70% relative humidity, 18 h photoperiod, for 8 months.

2.2. *Q. grandiflora* metabolite extraction

Metabolites were extracted as described in the “Lisec Method” with some modifications (Lisec et al. 2006). *Q. grandiflora* roots and leaves were lyophilized and then frozen in liquid nitrogen, and then manually ground in a mortar. Subsequently, 10 mg of each sample was transferred into a 2-mL centrifuge tube, and subsequently, mixed

with 1.4 mL of 100%-methanol (Sigma-Aldrich) + 60 µL of Ribitol solution (0.2 mg.mL^{-1}) (Sigma-Aldrich), vortexed for about 10 s.

After, the samples were incubated at 70 ^{o}C for 10 min in a water bath. Additionally, the samples were vortexed, and centrifuged for 10 min at 10.000 g and 4 °C. The supernatant was decanted and 750 µL of it was collected into a 2-mL screw-top tube, and then added 375 µL of cold chloroform and 750 µL of cold deionized water and centrifuged again for 10 min. Afterwards, amounts of 200 µL from the aqueous layer were transferred into a plastic vial for vacuum-dry for 2h at room temperature. In addition, blank samples (water) were also prepared and pooled. The blank samples were analyzed in each analytic run along with the treatment samples (Lisec et al. 2006). The dried samples were resuspended and derivatized using a two-step procedure before injection into GC-MS for analysis. First, 40 µL of the methoxyamine hydrochloride (Sigma-Aldrich) mixture dissolved (20 mg·mL^{-1}) in 100%-pyridine were added to the vial, vortexed for 10 s and shook in dry-block for 2 h and vortex900 rpm at 37 ^{o}C. Then, this procedure was followed by trimethylsilylation with the addition of 70 µL of N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) (SigmaAldrich) to each tube, and subsequently shook in dry-block for 30 min at 37 ^{o}C. Finally, the derived samples were cooled down to room temperature before injection.

2.3. Gas chromatography-mass spectrometry (GC-MS) analysis

GC-MS analysis was performed on a 7890A-5975C GC-MS system (Agilent Technologies, Santa Clara, CA, USA) equipped with an HP-5MS capillary column (30 m × 0.25 mm × 0.25 µm) (Agilent J &W Scientific, Folsom, CA). All the samples and replicates were continuously injected as one batch, in random order to discriminate the technical from biological variations. Each 1 µL aliquot of the derivatized sample solution was injected in split mode into the GC column at 230 °C by a TriPlus auto sampler at a constant flow rate of helium of 1.5 mL.min^{-1}. The temperature program started at 70 °C for 5 min, followed by an increase of 5 °C.min^{-1} up to 330 °C and maintained at this temperature for 10 min. The mass spectrometry parameters were performed using mass range recorded from m/z 70 to m/z 600 and the filament bias current was -70 V.

2.4. Metabolite identification

Unprocessed MS files were firstly converted into Net CDF format by Agilent MSD Station. The metabolite identification in the raw metabolite profiles were based on Automated Mass Spectral Deconvolution and Identification Software (AMDIS) [National Institute of Standards and Technology (NIST), Golm Metabolome Database (GMD)]. Before statistical analyses, all peak areas were normalized to the internal standard (ribitol).

2.5. Statistical analysis of metabolic pathway construction

For the metabolic profile data, the T test was applied comparing values to the values obtained the VANTED 2.5 program itself. The software used was BioStat 5.3.

3. RESULTS

3.1. Metabolic changes in response to Al in *Q. grandiflora*

Q. grandiflora plants grown with or without Al were investigated with the purpose to determine the effects og this metal on their metabolism. It is noteworthy that these plants were morphologically distinct, reflecting the presence or absence of Al in the nutritional solution (Figure 1). Plants grown with Al were visually healthier than those that did not receive it. Thereby, 8-month-old plants treated with Al had greener leaves and larger roots and shoots. Conversely, plants grown without Al had unhealthy appearance, showing signs of chlorosis and stunted aspect compared with those supplemented with Al.

Concerning the GC-MS analysis of *Q. grandiflora* tissue samples, both treatments showed a strong signal, large peak capacity, and reproducible retention time, which indicates the reliability of the dataset. Moreover, chromatographic differences were observed between the treatments encompassing several compounds as a result of the growth conditions (Figures 2 and 3). Also, a total of 36 metabolites were identified (13 in roots and 23 in leaves). The compounds detected in each treatment are shown in Tables 1 and 2.

Figure 1. Plants of *Q. grandiflora* at eight months of age. Note the visual differences between plants on each treatment. Plants treated with Al were healthier than those that did not receive this metal. On the left, non-treated *Qualea grandiflora* plant (no Al), and on the right, Al-treated plant. Scale bar = 1cm.

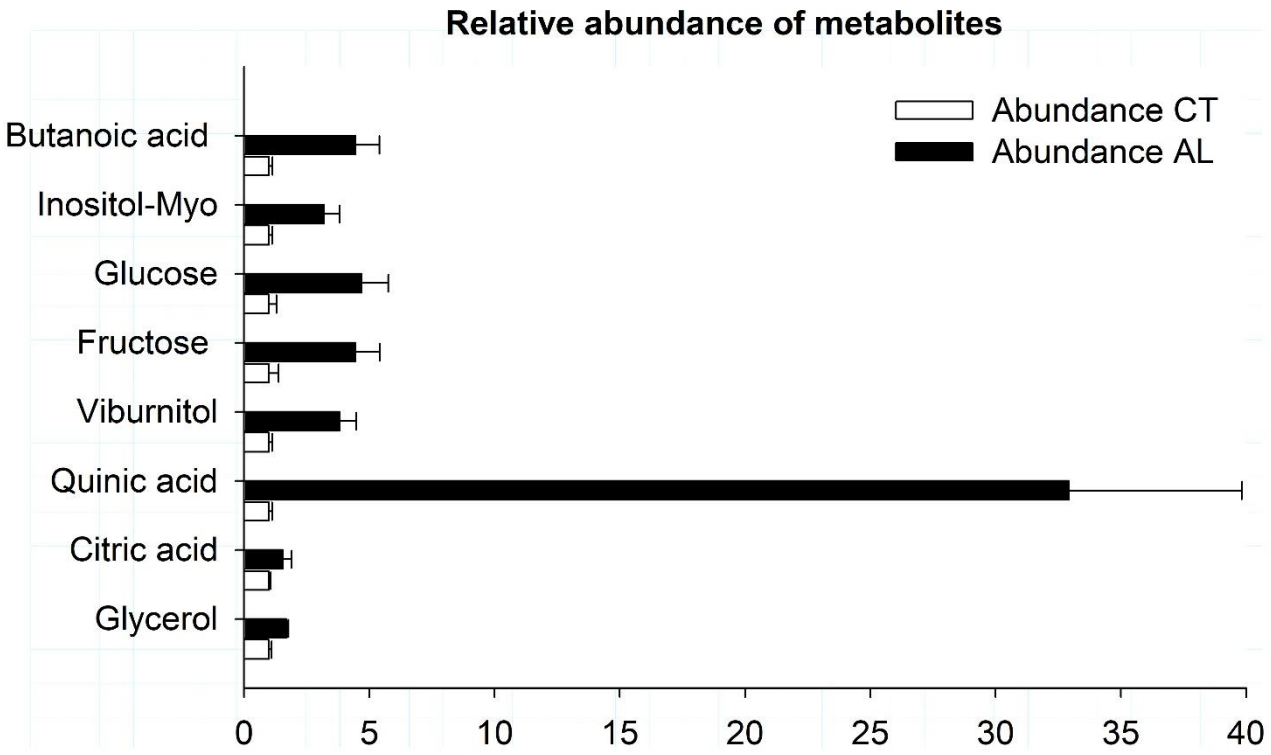

Figure 2 - Relative abundance of significative metabolites in *Q. grandiflora* roots of both treatments. Lezgend: Non-treated – white bar; Al-grown plants – black bar, with standard error bar.

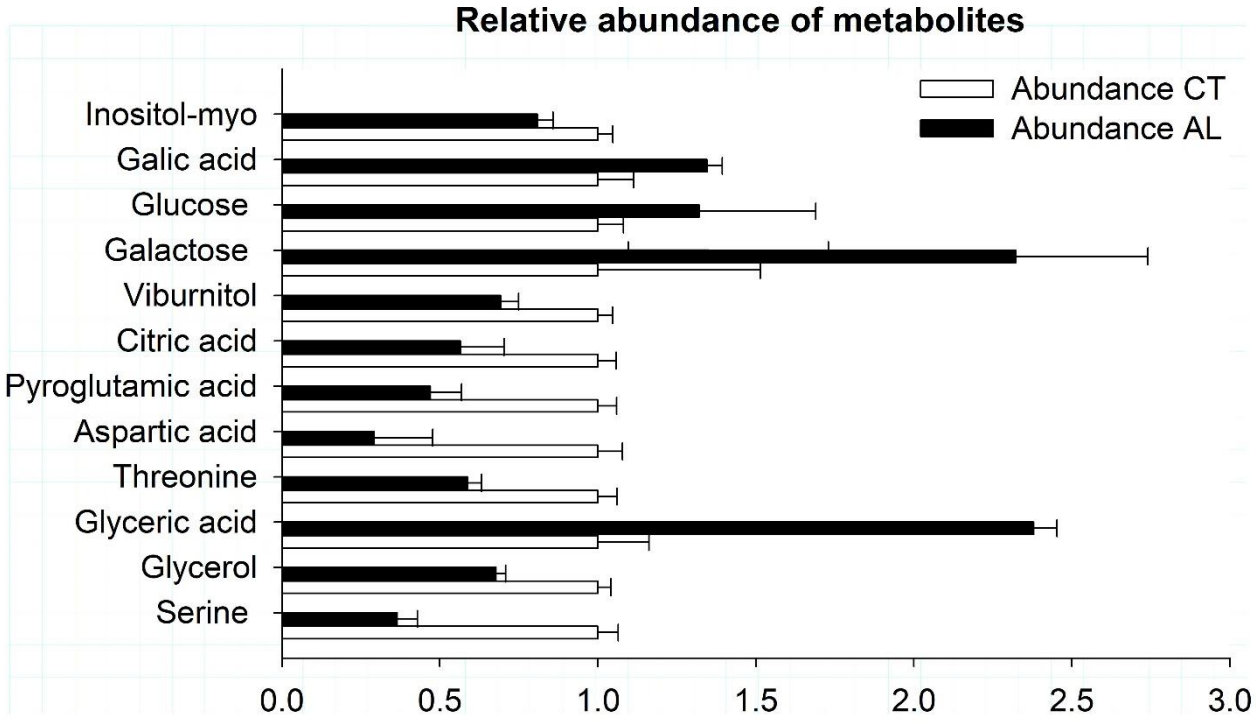

Figure 3 - Relative abundance of metabolites in *Q. grandiflora* leaves of both treatments. Legend: Non-treated – white bar; Al-grown plants – black bar, with standard error bar.

3.2. Metabolites in roots of *Q. grandiflora* plants in both treatment

Five classes of compounds have been found in roots of *Q. grandiflora* plants: organic acids, inorganic acids, amino acids, alcoholic sugars, and reducing sugars (Table 1). Figure 2 depicts the relative abundance of several compounds that presented higher content in roots from plants treated with Al. However, among the 13 different compounds detected in roots, eight were significantly different between the two treatments (Table 1). Hence, the citric acid, quinate, glycerol, inositol- myo, viburnitol, fructose, glucose ($p < 0.05$) and phosphoric acid ($p < 0.1$) showed significantly higher amounts than those from non-treated plants (Table 1 and Figure 2). Note that, in roots, all compounds with significant differences between treatments, the highest amounts were always found in samples from Al-treated plants, and the differences varied from 1.73-fold (citrate) to about 36.5-fold (quinate) (Table 1). Moreover, the butanoic acid, asparagine, glutamate, and glutamine-DL were exclusively detected in roots samples (Table 1)

3.3. Metabolites in leaves of *Q. grandiflora* grown with and without Al.

Similar to roots, the same classes of compounds were present in *Q. grandiflora* leaf samples. Nevertheless, the number of compounds present in leaves was higher than in roots (Table 2). It has been identified 23 compounds in leaves distributed as following: seven organic acids, two inorganic acids, five amino acids, six alcoholic sugars, and three reducing sugars (Table 1). Among the compounds detected in leaves, twelve had significant differences between the treatments. Figure 3 and Table 2 show all compounds with their respective amounts detected in *Q. grandiflora* leaves of both treatments. Therefore, the following compounds were found to have significantly higher abundance in samples from plants treated with Al: gallate (1.34 fold), glycerate (2.37 fold), galactose (2.32 fold) and glucose (1.32 fold). Conversely, in leaf samples from the non-treated plants there were eight compounds with significantly higher quantities than those seen in Al-treated plants: citrate (0.56 fold), aspartate (0.29 fold), pyroglutamate (0.46 fold), serine (0.36 fold), threonine (5.66 fold), glycerol (0.67 fold), Myo-inositol (0.8 fold) and Viburnitol (0.69 fold) (Table 2).

Furthermore, in leaves, there were 14 compounds solely detected in this organ

(Table 2). Among which, five organic acids (dehydroascorbic acid dimer, gallate, glycerate, malate, and oxalate), one inorganic acid (hydroxylamine), four amino acids (alanine, pyroglutamate, serine, threonine), three alcoholic sugars (galactinol, glucopyranose, sorbitol), and one reducing sugar (galactose). Furthermore, gallate, glycerate, pyroglutamate, serine, threonine, glucose and galactose presented significant differences between the treatments (Table 2).

Table 1. Relative abundance of metabolites in roots of Al-treated (AL) and non-treated: control (CT) of *Q. grandiflora* plants.

Metabolic Compounds	m/z	Retention Time (min)	*Folds* (AL/CT)
Organic Acids			
Aspartic acid	160	20475233	1.38
Butanoic acid*	174	23170567	4.44
Citric acid*	273	29925783	1.79
Quinic acid*	345	30908466	36.50
Inorganic Acids			
Phosphoric acid**	299	16544518	7.56
Amino Acids			
Asparagine	159	24697933	0.45
Glutamate	246	25394234	1.16
Glutamine-DL	156	28740950	1.17
Sugar alcohols			
Glycerol*	205	16639967	1.73
Inositol-Myo*	217	35299633	3.47
Viburnitol*	217	31133066	4.06
Reducing Sugars			
Fructose *	217	31222918	4.92
Glucose*	319	31593533	4.96

Means followed by (*) sign are significantly different at $p < 0.05$; and those followed by (**) are significantly different at $p<0.1$

Table 2. Relative abundance of metabolites in leaves of Al-treated (AL) and non-treated: control (CT) *Q. grandiflora* plants

Metabolic Compounds	m/z	Retention Time (min)	*Folds* (AL/CT)
Organic Acids			
Citric acid**	273	29914366	0.56
Dehydroascorbic Acid Dimer	173	30442200	0.57
Gallic acid**	281	32570400	1.34
Glyceric acid*	189	18217684	2.37
Malic acid	233	22328083	1.06
Oxalic acid	147	11170484	1.01
Quinic acid	255	30908283	0.81
Inorganic Acids			
Hydroxylamine	249	12658533	1.03
Phosphoric acid	299	16533100	1.09
Amino Acids			
Alanine	188	18790449	0.95
Aspartate*	160	20475033	0.29
Pyroglutamate*	156	22940166	0.46
Serine*	219	15932266	0.36
Threonine*	219	17021633	5.66
Sugar alcohols			
Galactinol	204	49438782	1.85
Glycerol*	205	16634167	0.67
Glucopyranose	217	28864300	1.50
Myo-inositol	217	35293835	0.81
Sorbitol	319	32345798	0.80
Viburnitol*	230	31127266	0.69
Reducing Sugars			
Fructose*	217	31217117	1.42
Galactose*	205	31593349	2.32
Glucose*	160	31728117	1.32

Means followed by (*) sign are significantly different at $p < 0.05$; and those followed by (**) are significantly different at $p < 0.1$

3.4. Pathway mapping and the metabolite-to-metabolite network visualization.

As mentioned, in roots, all compounds with statistically significant differences had higher contents in plants that received Al and in leaf samples (Tables 1 and 2). Therefore, a metabolic network containing Al-responsive metabolites as well as those more abundant in the absence of Al were mapped based on Vanted software. This analysis revealed six and ten metabolic pathways related to Al-treated and non-treated *Qualea* plants, respectively (Figures 4 and 5). Therefore, it can be noticed that glucose, galactose, fructose, sucrose, and organic acids syntheses were upregulated in roots and/or leaves from plants treated with Al. The absence of Al in leaves may have resulted in an increase

of photorespiration products, since serine involved in this metabolism were evident (Wingler et al., 2000) (Figures 4 and 5). Figures 4 and 5 depict biosynthetic networks assigned for roots and leaves, which can be divided into two groups. One group consisted of compounds mainly involved in carbohydrate metabolism that include fructose, galactose and glucose biosynthesis (amino sugar metabolism and biosynthesis of secondary metabolites). The second was composed of several acid compounds, which included some amino acids (alanine, aspartate, pyroglutamate, serine and threonine), organic acids (citrate and malate), as well as inorganic acids (phosforic acid).

Figura 4 - Metabolic map of primary pathways in roots samples from *Q. grandiflora* plants grown with (black bar) or without (white bar) Al. Compounds with significant differences are marked with *, which are: citrate, quinate, glycerol, inositol-myo, viburnitol, fructose and glucose ($p < 0.05$), phosphoric acid ($p<0,1$).

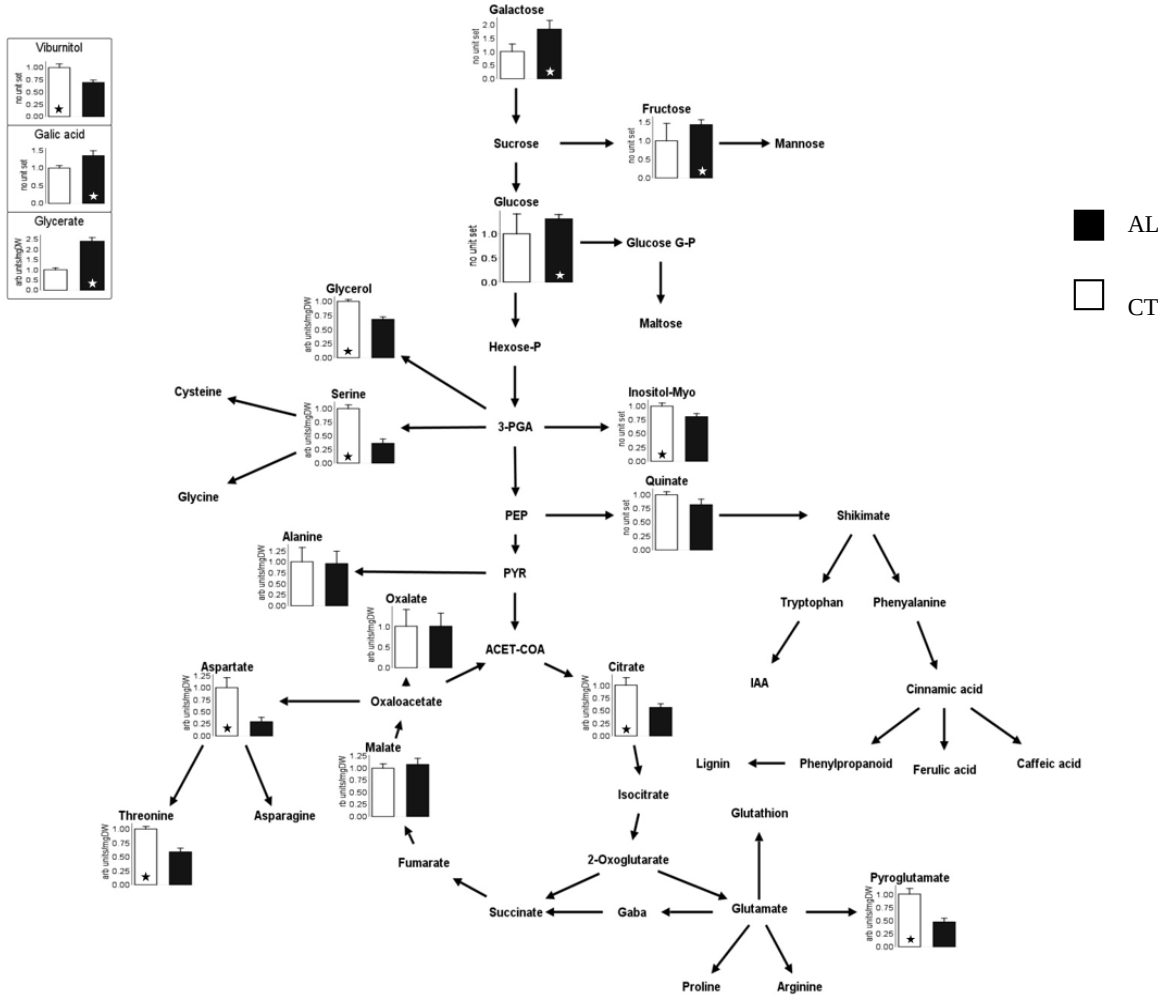

Figure 5. Metabolic map of primary pathways in leaves samples from *Q. grandiflora* plants grown with (black bar) or without (white bar) Al. Compounds with significant differences are are marked with *, which are: glycate, aspartate, pyroglutamate, serine, threonine, glycerol, viburnitol, galactose and glucose ($p<0,05$); citrate and gallate ($p<0,1$).

4. DISCUSSION

The capacity of accumulating Al in plants required the evolution of intricate mechanisms to respond and adapt to acid soil with high levels of Al. Therefore, some plants were naturally selected based on their capacity of accumulating large amounts of this metal (> 1 g of $Al.kg^{-1}$ of dry matter). Besides some species went further and incorporated Al into their metabolism in such way that they need it grow and develop properly. *Q. grandiflora* fits into this class of plants. This Cerrado Al-accumulating plant can uptake and accumulate Al in both roots and shoots, and require it during its development (Silva, 2012). Moreover, the lack of Al induces severe defects at the at morphological and physiological levels (Haridasan, 20081; Silva 2012), which also

reflects on its proteomic profile (Silva 2012, Cury 2017).

However, to the best of our knowledge, there are no reports on metabolite changes in *Q. grandiflora* plants growing in either presence or absence of Al. This kind of work is regarded as an important research field in the post-genomic analysis, especially to investigate plant physiological responses to various stimuli (Arbona et al., 2013; Brunetti et al., 2013). The present study reports a comprehensive analysis of metabolic changes in *Q. grandiflora* growing in two different conditions (with and without Al) using a GC-MS-based metabolomics approach. The data indicated that either the presence or the absence of Al resulted in considerable metabolite alterations, mainly in sugars, amino acids, and organic acids contents.

4.1. Metabolic responses of *Q. grandiflora* to Al

The results showed that, in roots and leaves from Al-treated in *Q. grandiflora*, there were compounds whose syntheses were significantly upregulated. A considerable part of such substances is intrinsically connected to primary metabolic processes. For instance, the sugars and most of the organic acids are products of the primary metabolism. In fact, these compounds with increased abundance in Al-supplemented plants may be directly correlated with primary metabolic processes such as cellular respiration and photosynthesis. This fact may support higher growth rate and biomass in Al-treated *Q. grandiflora* plants (Silva, 2012).

Conversely, in Al-sensitive plants, Al exposure negatively affects photosynthesis and respiration as seen in soybean, maize and barley (Cakmak and horst, 1991; Basu et al., 1994; Boscolo et al., 2003. Guo et al., 2004). However, in *Q. grandiflora*, the lack of Al was effective to induce growth inhibition and chlorosis (Silva, 2012), which may result in an altered level of carbohydrate production, as well as other primary products. These facts confirmed what was observed in other native Al-accumulating plants as *Vochysia thyrsoidea*, *Miconia albicans*, *malabathricum* L. and *Camellia sinensis* (L.) Kuntze (Haridasan, 1988, 2008[2]; Ghanati, et al., 2005). Therefore, in these plants, the lack of Al induced lower biomass accumulation, decreased growth rate and chlorosis in leaves (Haridasan, 1988, 2008[2]; Jansen, 2002). The results strongly support an Al role in the metabolism of *Q. grandiflora*, as well as in other Al accumulating plants.

4.1.1. Organic and inorganic acids

It has been established that Al supplementation is beneficial for the growth and development of *Q. grandiflora* (Haridasan, 2008[1,2]; Silva, 2012). This fact indicates that *Qualea* might be better equipped for photosynthesis and respiration, which can lead to the accumulation of essential cell compounds that are required for the maintenance of cell metabolism. On the other hand, the synthesis and accumulation of certain substances may indicate a stressful situation. For example, in dealing with Al, some plants tend to accumulate organic acids in roots and rhizosphere as seen in buckwheat (*Fagopyrum esculentum*), *Hydrangea macrophylla* and *Melastoma malabathricum* (Ma et al., 2001).

Moreover, organic acids play a role in plants in providing redox equilibrium, involved in ionic gradients on membranes, as well as in pH adjustment in the extracellular medium. These compounds are synthesised in plants as a result of the incomplete oxidation of photosynthetic products and compose carbon pools accumulated from different transient conversion times of carbon compounds in metabolic pathways (Igamberdiev and Eprintsev, 2016). Nonetheless, the accumulation of organic acids in leaves together with higher content of sugars may also indicate proper junctions of the photosynthetic apparatus as well as cellular respiration and redox equilibrium (Jansen et al., 2002; Watanabe and Osaki, 2002; Wang et al., 2015; Igamberdiev and Eprintsev, 2016).

Independent of Al supplementation, it was observed that *Q. grandiflora* showed roots and leaves presented differences in accumulation of organic acids (Tables 1 and 2). Therefore, except for citrate, the other organic acids accumulated in this plant following an organ specific fashion. Roots showed higher amounts of citrate and quinate, while leaves had greater contents of gallate and glycerate. Although citrate occurred in both organs, it accumulated differently. While roots of Al-treated samples had higher abundance of citrate, in leaves, the largest amounts were seen in samples from Al-non-treated samples.

Few questions remain: firstly, “is the higher amounts of citrate (1.79-fold) and quinic acid (36.5-fold) in roots of Al-supplemented plants associated with a stress condition or other metabolic processes?”. Secondly, “what is the metabolic meaning of the elevated citrate contents in leaf samples from plants that did not receive Al supplementation?”. Finally, “why do leaves of Al-treated plants have high levels of gallic and glyceric acids?

Firstly, Al-chelation by low molecular weight organic acids is a common and effective mechanism to deal with this metal in many plant species (Delhaize et al., 2001; Yang et al., 2013). It has been shown that some Al-accumulating species detoxify internal Al^{3+} by forming Al-organic acid complexes, mainly Al-citrate (Ma and Hiradate, 2000; Ma et al., 2001; Fan et al., 2016) and Al-quinate (Wenzl et al., 2001; Negishi et al., 2012). Hence, in tolerant plants, these organic acids can chelate and efficiently reduce the activity of Al^{3+} in the cytosol, preventing the formation of harmful complexes between Al and cellular components. It is known that this metal may be stored in specific organelles such as chloroplasts and vacuole (Figure 6). For instance, in *Melastoma* organic acids are thought to be stored in the vacuole (Watanabe and Osaki, 2002). Nonetheless, Al is not a stress eliciting factor in *Q. grandiflora*. Studies with woody plants provide hints where the Al may be stored in leaf tissues, as well as in subcellular compartments, like chloroplasts or possibly vacuoles. Hence, the chloroplasts of *Qualea grandiflora* (Silva, 2012) and *Callisthene major* Mart. have been suggested as a compartment for Al sequestration (De Andrade et al., 2011).

Consequently, in *Q. grandiflora* roots grown with Al the amounts of citrate (1.79-fold higher than in non-treated plants) quinate (36.5-fold higher than in non-treated plants), glycerate (2,37- fold↑) and gallate (1.34-fold↑) in leaves are not likely involved in Al-related stress. Therefore, organic acids are usually participating in metabolic mechanisms that deal with Al in several physiological processes. There is evidence that the transportation of Al^{3+} is made through Al-organic acid complexes, as in *Hydrangea* and *Camellia si*nensis L., where the Al-citrate complex has a ratio of 1:1 (Ma et al.,1997; Watanabe et al., 1998). Furthermore, in *Melastoma malaba*thricum L. (Melastomataceae) e *Fagopyrum esculentum* Moench. (Polygonaceae), the transport of Al (Al:citrate - 1:1) is likely through xylem (Watanabe & Osaki, 2002; Ma et al., 2001). Moreover, Haridasan (2008[2]) also suggests that in Cerrado species Al might be transported to aerial parts via xylem. Thus, we hypothesise that the high levels of citrate and quinate in roots and glycerate and gallate in leaves of *Q. grandiflora* Al-treated (Figure 6) could be an indication of its involvement in Al transportation from roots to shoots.

It is important to emphasise that *Q. grandiflora* plants that received Al did not have signs of stress. Moreover, these observations are consistent with previous reports that showed the importance of Al for the growth and development of this species (Haridasan,1986, 2008[2]; Silva, 2012; Melo, 2016; Schmitt et al., 2016). In fact, *Qualea* plants not supplemented with Al were those that resented its absence. Moreover, these

plants presented a leaf citrate concentration 0.56-fold higher than in Al-treated leaves. Thus, what roles citrate may play in leaves of Al-non-treated plants? One possible explanation is that the amount of citrate in leaves of non-supplemented *Q. grandiflora* could be associated with an impairing of the enzyme that performs the conversion of organic acids in respiration routes (TCA cycle). Several studies with TCA mutants have shown that any harm on organic acid interconversions have a dramatic effect, not only on TCA cycle but also on organic acid distribution (Atwell, 1999). Thereby, the availability of these compounds is crucial for many cellular processes such as photosynthesis, plant biomass accumulation, root growth, photorespiration, nitrogen assimilation, amino acid metabolism, and stomatal function (Atwell, 1999).

The hypothesis raised here is that the higher citrate content in leaves of Al-non-supplemeted plants occurred due to the enzymatic interruptions resulting of stress due to Al absence that affected the krebs cycle. As consequence, without Al, *Q. grandiflora* may not be able to carry out the conversion of citrate to isocitrate and 2-oxoglutarate. Another possibility would be the use of citrate in other pathways involve in plant stress response as a compensatory mechanism (Atwell, 1999).

Another reason could be the fact that the presence of certain organic acids can confer some adaptative advantage in *Q. grandiflora*. For instance, the larger amounts of quinate in Al-treated roots and gallate in Al-treated leaves may be involved in the biosynthesis of tannin. These compounds are formed by prodelphinidin units in the route of chinic acid (Dewick and Haslam 1969; Bruneton, 1991). Tannins are polyphenols and important molecules that play a role in plant defence mechanisms. They prevent herbivore attack by decreasing leaf palatability and digestibility, and are involved in the production of toxic compounds, which also protect against pathogenic microorganisms (Ferrell, 2006). Moreover, high levels of polyphenols can also influence the phytohormonal network, that shape the core of plant defence organization through antimicrobial activity (Scala et al., 2013).

Santos et al. (2002) found that tannins are an important factor in plant defence system, responsible for internal antiradical and antimicrobial effects. Other authors observed a high degree of tannin polymerization in *Stryphnodendron* genus, which is associated with a controlling factor of insects, fungi and bacteria (Bruneton, 1991, Ishida et al., 2009). Xu et al. (2016) compared two cultivars of tea and observed that the shikimic pathway - tannin route - was enhanced in roots by Al with higher levels of quinic acid. Moura et al. (2012) observed amYn association between the presence of tannins with

antibacterial activity in leaves of *Q. grandiflora*. High amounts of gallic and glyceric acids in Al-treated *Q. grandiflora* leaves may also correlate with tannins. Gallic acid and glyceric acid are organic acids found in a variety of plants and well known as powerful antioxidants. Gallic acid is also found as part of tannins, which are antimicrobials plant polyphenols (Haddock, 1982). Consequently, in *Q. grandiflora*, the presence of precursors of tannin synthesis could be related to some Al-dependent evolutionary advantage correlated to the biome climate, soil and water regime peculiarity. Conversely, there was an increase of citric acid in *Q. grandiflora* leaves of Al-non-treated plants (Table 2). As these plants are not receiving an extra amount of Al, it appears that the higher levels of this organic acid are not related to Al translocation in this plant, as it was previously proposed. The hypothesis is that this organic acid would to compensate the poor activity of ATP synthases trying to increase the TCA cycle unsuccessfully (Chapter 1). The high levels of citrate produced in the absence of Al could be generated to increase the respiratory activity of *Q. grandiflora* cells to compensate the lower amount of available ATP synthase. Another hypothesis would be the use of this by-product of the TCA cycle in non-cyclic flux what would decrease ATP production.

The tricarboxylic acid (TCA) cycle is a crucial component of respiratory metabolism in plant organs (Araújo et al., 2012). In tomato. all major TCA cycle genes have been cloned recently, which allows the generation of transgenic plants to study the role of each TCA associated gene (Araújo et al., 2012). These studies have provided a good picture of the controls of this important metabolic pathway (Araújo et al., 2012). The unusual distribution of this enzymes and increase of some products might be consistent with specific non-cyclic flux (Araújo et al., 2012).

4.1.2. Sugar Alcohol

It is interesting that there was a significantly higher abundance of glycerol, myo-inositol, and viburnitol in root samples from *Qualea* Al-treated plants (Tables 1 and 2). Additionally, these sugar alcohols contents varied approximately from 1.73- to 4.06-fold higher in Al-treated roots. Sugar alcohols are also significant photosynthetic products and have been considered as osmoprotectants, which means, they help to maintain cell osmotic balance (Yancey, 2005). Besides, these compounds can also work as a reservoir of energy for the plant (Yancey, 2005). Moreover, polyols also have an antioxidant capacity (Williamson et al., 2002).

Myo-inositol, for example, is a sugar-like carbohydrate produced by most plants and is necessary for phosphate storage, cell wall biosynthesis, production of stress related molecules, cell-to-cell communication, storage and transport of plant hormones (Loewus, 1990, 2000; Styer, 2000). This component is also involved in the storage of phosphate in seeds. Phosphate is a vital plant nutrient. Usually, plants store essential nutrients into seeds to support the initial growth of seedlings. In seeds, inorganic phosphate is stored as inositol hexaphosphate (IP6). Also, during cell wall biosynthesis the inositol-derived sugars provide components for other vital pathways involved in plant storage, transport, and development (Loewus, 1990, 2000; Styer, 2000). Myo-inositol is an essential component of a signalling pathway, more specifically, in the so-called Pi signalling pathway and probabily correlated with Al-supplementation in *Q. grandiflora* (Loewus, 2000). This pathway has been shown to partake in a variety of plant responses including the ability of roots to grow downward in response to gravity, and pressure changes in leaf pores that control wilting (Styer, 2000). Moreover, myo-inositol has been hypothesised to play a central role in the control of the plant hormone auxin (Loewus, 1990; Kowalczyk, 1991).

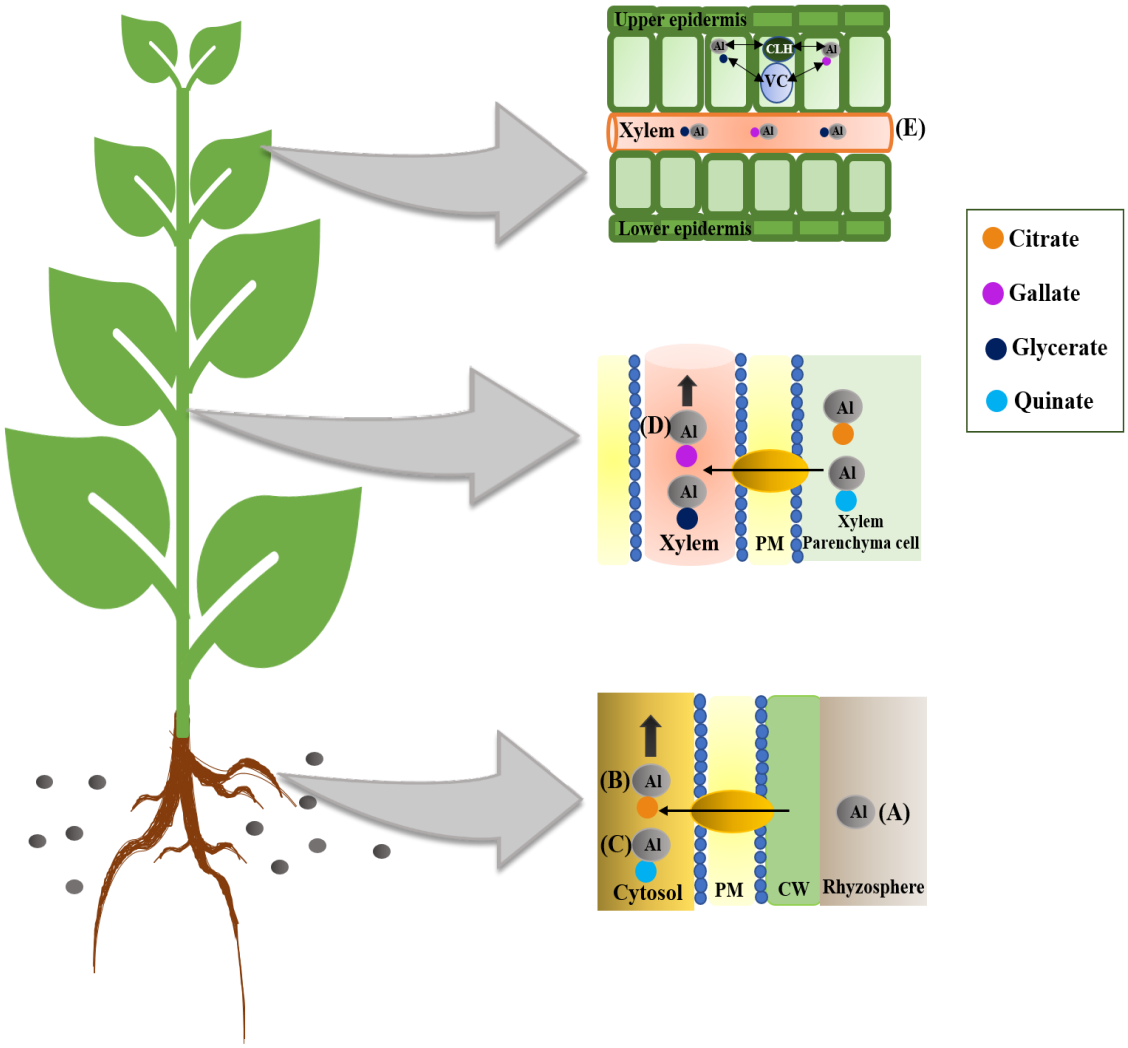

Figure 6- Diagrammatic representation of the organic acids transport in the growth of *Q. grandiflora*. (A) The Al is complexed by the roots (B) by Al-citrate complex is absorbed to the cation exchange sites of the roots. (C) Absorbed Al forms a complex with quinine and (D) is transported to the shoots in the xylem. (E) In the leaves, Al exchanges its citrate ligand for an gallate-ligand, glycerate-ligand or becomes free. CHL: chloroplast, CW: cell wall, PM: plasm membrane, VC: vacuole.

Sugar alcohols link up with auxins forming hormone conjugates that are temporarily inactive (Bandurski, 1979). These inactive conjugates allow for safe storage and transport auxins and may regulate the availability of active auxins for physiological responses (Loewus and Loewus, 1983; Loewus, 1990). Corn enzymes capable of conjugating myo-inositol to auxin have been isolated, as well as an enzyme that may be involved in the release of auxin from its inositol conjugate (Loewus, 1990; Kowalczyk and Bandurski, 1991; Munnik, 1998; Perera, 1999; Styer, 2000).

In *Q. grandiflora*, it is not inconsistent to consider that sugar alcohols may play similar roles as described above. The supply of Al may enhance photosynthesis in *Qualea* and consequently, the polyols products of this reaction may be efficiently utilised as

osmoprotectants, and can also act as antioxidants.

Furthermore, it is known that other sugars from photosynthesis can promote the grown of *Q. grandiflora* - as described above -. The smaller molecules such as ions and monosaccharides have an enormous effect on the osmotic balance of plants (Hill et al., 2003). It is reasonable to think that due to the higher growth rate of Al-treated plants (Haridasan, 20082; Silva, 2012), they can efficiently uptake larger amounts of minerals than those not supplemented with Al. It is noteworthy that *Q. grandiflora* plants received the same amount of minerals in both treatments, the only exception was the presence of Al supplementation. The presence of the Al, by itself, may constitute a factor that may be a required additional effort to mantainig the osmotic homeostasis in root cells.

4.1.3. Sugars

The assertion that Al is a crucial for growth and development in *Q. grandiflora* by influencing a myriad of metabolic and physiological processes is not unreasonable (see chapter 1). The concept that Al works as a cellular regulator of metabolism is new. Al is an essential element for *Q. grandiflora* has yet to be demonstrated.

In both leaves and roots, Al-treated *Q. grandiflora* had increased sugars contents compared with those not supplemented, particularly fructose and glucose. This fact suggests that photoassimilates were produced in higher rate in *Q. grandiflora* supplemented with Al. (Figures 4 and 5). It appears that these results are directly related to the presence of Al in the nutritional solution, which may have a positive effect on photosynthesis and respiration. This idea is supported by the fact that plants supplemented with Al grow better than those that are not (Haridasan 2008; Silva, 2012). Moreover, Kieffer et al. (2009) reported that the photoassimilates were stored in the form of hexoses and complex sugars in *Populus*, a tolerant species, under cadmium exposure, which could associate the tolerance mechanism with the better rate of growth and development in this species.

It has been suggested that in *Q. grandiflora* the Al induces CO_2 uptake by leaves, electron transport, and photophosphorylation by chloroplasts. Moreover, Al may increase ATPase activity in mitochondria (discussed in chapter 1). Nevertheless, a CO_2 assimilation by RubiCO as well as a mitochondrial activity analyses should be performed to confirm the transcriptomic and metabolome results.

In addition to their roles in carbon and energy metabolism as well as in polymer

biosynthesis, sugars may play hormone-like functions as primary messengers in signal transduction. The pivotal role of sugars as signalling molecules is well illustrated by a variety of sugar sensing and signaling mechanisms discovered in free-living microorganisms such as bacteria and yeast (Rolland et al., 2002).

Moreover, high sugar accumulation during early seedling development may reflect undesirable growth conditions at crucial developmental stages (Lopez-Molina et al., 2001), and result in a reversible developmental arrest that acts as a protection mechanism. Sugar-dependent seedling phenotypes have been used extensively for the selection of sugar signalling mutants in *Arabidopsis*, including both sugar-insensitive and sugar-hypersensitive mutants (Rolland, 2002, 2006).

As a new concept, it can be hypothesised that the synergistic interaction between sugars (or sugar-like compounds) and phenolic compounds form part of an integrated redox system, quenching ROS and contributing to enhance stress tolerance, especially in tissues or organelles with high soluble sugar concentrations (Hajiboland et al., 2010). Sugars also play critical roles in adjustments or protection of cell constituents in *Populus* (Kieffer et al., 2009). Keunen et al. (2013) reported that plant sugars are crucial players in the oxidative challenge. This fact ensures optimal synthesis and use of carbon and energy resources and allows for the adaptation of carbon metabolism to changing environmental conditions and to the availability of other nutrients.

Sugar sensing and signalling also are involved in the regulation of leaf senescence that coincides with a decline in chlorophyll content and photosynthetic activity (Jiang et al., 1993; Bleeker and Patterson, 1997; Quirino et al., 2000). The recent demonstration also suggests a role for sugar signalling in senescence and defence in *Arabidopsis* (Yoshida et al., 2002). Moreover, sugar-dependent sensing and signalling mechanisms are involved in the control of growth and development during the entire plant life cycle (Hasaneen, 2009). Accordingly, the higher sugar content in *Q. grandiflora* is likely related to higher biomass production, due to a photosynthetic stimulus promoted by Al supplementation. This fact might indicate that Al is a good candidate to become an essential mineral for *Qualea*.

4.1.4. Amino Acids

Q. grandiflora leaves from plants not supplemented with Al accumulated significantly higher contents of aspartate, pyroglutamate, serine and threonine. It has been

shown that, in some plants, an enhancement in amino acid contents take place due to either synthesis or protein degradation and could be associated with response mechanisms to abiotic stress (Arbona et al. 2013).

Abiotic stresses, such as low temperature and high salinity conditions, can induce serine accumulation in plants (Ros et al., 2014). Serine, a protein amino acid, is associated with plant adaptation to abiotic stresses, such as heat in *Agrostis stolonifera*, a perennial grass (Yang et al., 2014). Besides, serine synthesis may also respond to salinity stress, which directly affects photosynthesis, respiration, and nitrogen assimilation (Ros et al., 2014). Additionally, in *Gelidium coulteri* was observed that under salinity stress was correlated with protein degradation and resulted in high levels of serine and threonine (Wingler, 2000; Hagemann et al., 2016). In addition, salinity indirectly affected the photosynthate levels and enhanced photorespiration rate (Wingler, 2000; Hagemann et al., 2016). Concerning *Q. grandiflora*, it was observed a high amount of serine in leaves from plants not supplemented with Al. This result reinforces the idea that there may have been degradation of proteins due to Al-absence. Furthermore, the photorespiration in plants grown without Al could be higher, what regarding productivity, reduces the net photosynthesis rate that could harm the growth of this plants.

Another amino acid found in significant amounts in Al-non-treated Q. grandiflora leaves was threonine.

Threonine accumulation has also been correlated to abiotic stresses, which could affect the synthesis and activity of the antioxidant enzymes, ion transport regulation, modulation of stomatal opening and sequestration of toxic metals (e.g. cadmium ions) in the cytosol or vacuole (Rai, 2002; Catala et al., 2007; Kieffer et al., 2008). In *Phaseolus vulgaris* under biotic stress was observed a high level of threonine (Zimmerlin et al., 1994), as well as in *Arabidopsis thaliana* under abiotic stress (Pires, 2012). Moreover, it has been observed that in coffee plants submitted to water stress, there was a significant increase in the content of AAs (Silva et al., 2005). In summary, amino acids residues may be stress signals.

As mentioned before, protein degradation under stress conditions have been correlated to this increase in amino acid content (Catala et al., 2007), a phenomenon that also could also be taking place in *Q. grandiflora* due to the stress caused by the lack of Al. Therefore, the contributions of amino acid catabolism to the energy status requirements of developing *Q. grandiflora* appears critical compared with plants that receive Al supplementation causing limits to growth conditions.

4.2. Metabolite networks of *Q. grandiflora* in response to Al

Al-induced metabolic responses of *Q. grandiflora* roots and leaves showed differences, but also complimentary alterations mainly in organic acids, sugars, amino acids. The metabolic profiles demonstrated that exposure of *Q. grandiflora* to Al affected profoundly several processes. Concomitantly, these plants resented the lack of this metal as well.

These data corroborate the results described in chapter 1. The metabolome and transcriptome analisys may provide important information on molecular and metabolic pathways associated with Al.

These findings may provide useful information for the understanding of molecular mechanisms of the metabolic role of Al in native plants

4.2.1. A proposed model of integration of RNAseq and metabolic pathways in response to Al in *Q. grandiflora*

With the purpose of integrating genes and metabolic pathways involved in the Al metabolism in this native species, as well as to make functional links between annotated/assigned function (MapMan, GO, and KEEG) and respective pathways are shown in Figure 7.

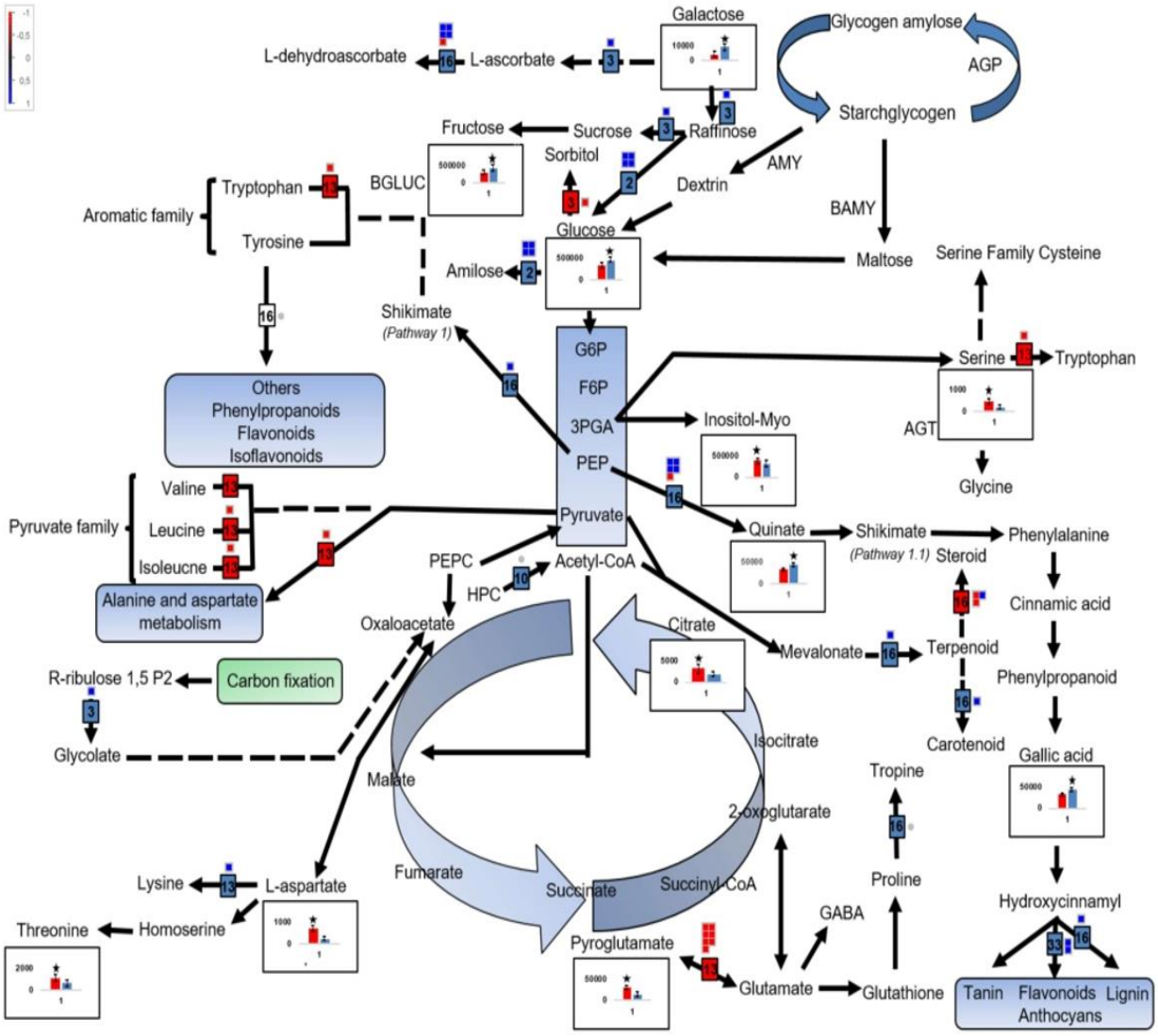

Figure 7. Metabolite and transcript pathway network analysis of *Q. grandiflora* responses to Al. Gene-to-metabolite and metabolite-to-gene network fluctuations in leaves in response to Al. Metabolite fold changes are represented in bar graphs, and significant changes based on t-test statistics are indicated by a star ($P<0.05$). The blue and red colours represent most abundance and less abundance of components respectively. Transcripts are represented as arrows connecting metabolites. Transcript fold-changes (1.5-fold threshold; $P<0.05$) based on results are represented by boxes. Blue illustrates an increase in transcript abundance in response to Al, while red represents a decrease ($P< 0.05$ and fold change >1.5 or <−1.5). Capital lettering beside the boxes indicates gene name abbreviations (see Table 1). Black arrows without boxes represent transcripts detected in RNAseq, but not significantly affected by Al. Solid arrows represent single-step reactions, while hashed arrows represent multi-step reactions. AGP: alpha-glucanphosphorylase, AGT: Alanine-glyoxylate transaminase, BAMY: Beta-amylase, BGLUC*:* Beta-glucosidase, CoA: Coenzyme A, GABA: gamma-aminobutyric acid PEP: Phosphoenolpyruvate.

The integrated metabolome and transcriptome analysis reveal the major processes that were responsive to Al. Metabolite changes between treatments matched the physiological/morphological signs of *Qualea* plants and appeared to be consistent with MapMan transcript annotations (discussed in detail in chapter 1) (Figure 7). Upregulated BINs related to the primary metabolism and secondary in Al-treated *Q. grandiflora* and the respective metabolic profile of these plants revealed an enhanced sugar metabolism and ribulose 1,5 2P activity. Moreover, in the shikimate pathways related production of secondary compounds of structural composition, signalling and defence [Figure 7 and Figure 8-B (chapter 1)]. Moreover, a large number of BINs (chapter 1) and compounds associated with the secondary metabolism such as those involved in the biosynthesis of flavonols, isoflavonols and phenylpropanoids were also consistent.

Downregulated BINs in *Q. grandiflora* and the metabolic profile of Al-untreated leaves revealed pathways likely involved in amino acid metabolism and protein modification [Figure 6 and Figure 8-B (chapter 1)]. This fact indicates that the lack of Al induced signalling specific of stress related processes by amino acid catabolism in these plants.

5. CONCLUSIONS

The data presented here is the first report on Al-responsive metabolites in *Q. grandiflora*. The results provide a new perspective on the Al responses of *Q. grandiflora*, which can be summarised as follows:

(1) Organic acids were more abundant in roots from Al-treated plants and showed significantly higher levels of citric acid and quinate. Leaves had higher contents of gallate and glycerate in Al-supplemented samples suggesting the metabolic response to Al follows an organ specific fashion;

(2) Other compounds, including phosphoric acid, had obvious differences in *Q. grandiflora* roots plant treated with Al;

(3) The abundance of amino acids in *Q. grandiflora* leaves was enhanced by the absence of Al, which reflected on aspartate, pyroglutamate, serine and threonine contents;

(4) Higher sugar alcohol contents and reducing sugars such as fructose, glucose and galactose were found in *Q. grandiflora* roots and leaves of Al-treated plants;

(5) Combined transcriptome and metabolome showed correlations between pathways of primary and secondary metabolism related to Al.

The present results suggest that Al is a fundamental element for the growth of *Q. grandiflora* and the metabolism of organic acids, inorganic acids, amino acids, sugar alcohols and sugars were differently affected by the presence or absence of Al.

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7. CONCLUSÃO FINAL E PERSPECTIVAS

Os resultados obtidos neste estudo fornecem uma nova perspectiva sobre as respostas de Al em uma planta acumuladora nativa, *Q. grandiflora*, e as conclusões deste trabalho podem ser resumidas a seguir:

- Este é o primeiro estudo a identificar genes em folhas e quantificar metabólitos em raízes e folhas de *Q. grandiflora*, utilizando uma abordagem transcritômica e metabolômica, respectivamente;

- No período experimental de oito meses, notou-se alterações morfológicas nas raízes e folhas de *Q. grandiflora*, onde as plantas cultivadas sem Al apresentaram sinais de estresse ao se desenvolverem menos sem a suplementação do metal;

- Os resultados sugerem que o Al é um elemento químico importante para *Q. grandiflora* e pode ser considerado um nutriente essencial para o seu crescimento e desenvolvimento;

- As diferentes condições de crescimento em meio nutritivo às quais as plântulas de *Q. grandiflora* foram submetidas, influenciaram nos parâmetros transcritômicos e metabolômicos avaliados.

- As análises transcritômicas e metabolômicas evidenciaram que as alterações induzidas pelo Al em *Q. grandiflora* não caracterizaram uma resposta de estresse;

- Os resultados obtidos neste trabalho e suas próximas etapas, abrem perspectivas para a continuidade e criação de novas linhas de pesquisa em plantas acumuladoras de Al a nível molecular. Estas linhas se basearão principalmente no emprego de ferramentas de validação dos dados in silico, com foco em análise da expressão gênica em tempo real e transgenia, além do estudo biofisico, análise da estrututa secundária e dinâmica de proteinas, visando a identificação de marcadores-alvo relacionados à tolerância ao Al.

SUPPLEMENTARY INFORMATION

Title: Transcriptome Analysis to Investigate the Metabolic Role of Aluminum in *Qualea grandiflora* Mart.

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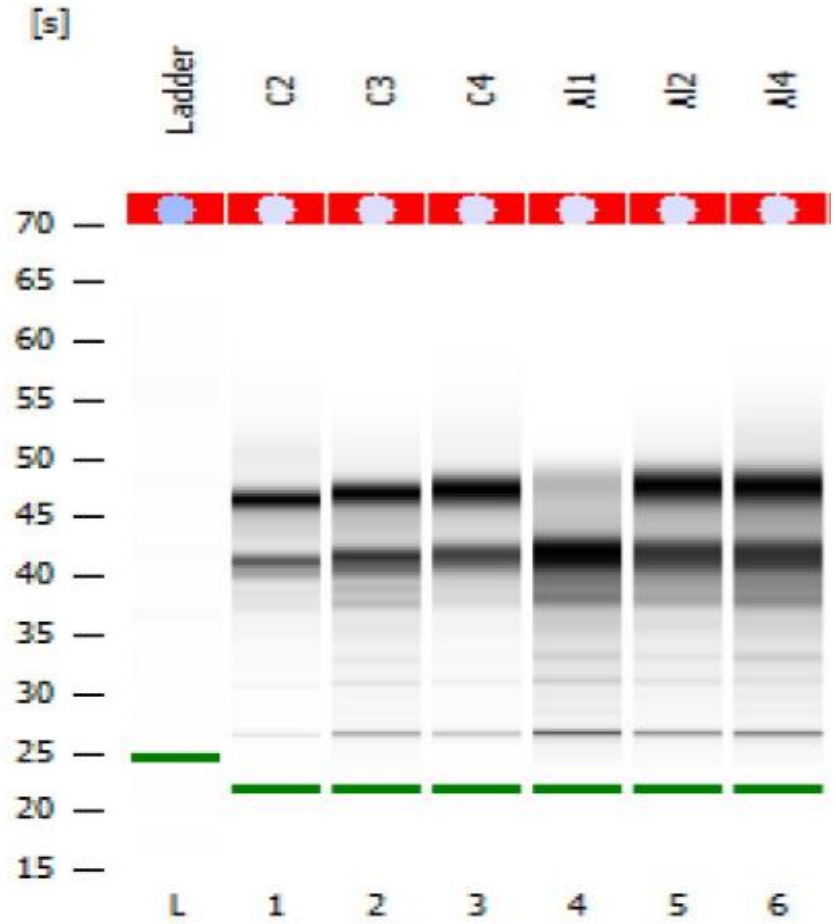
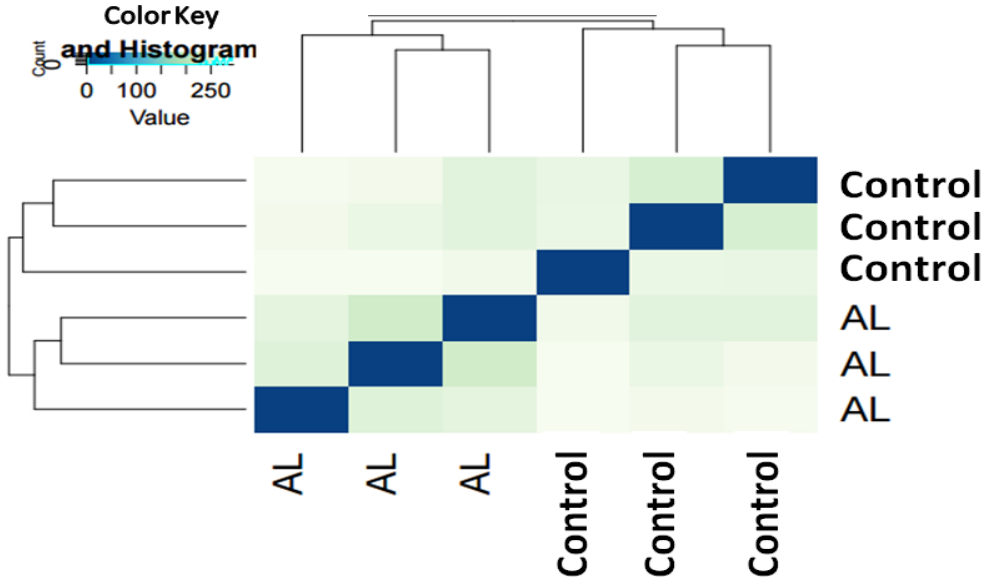
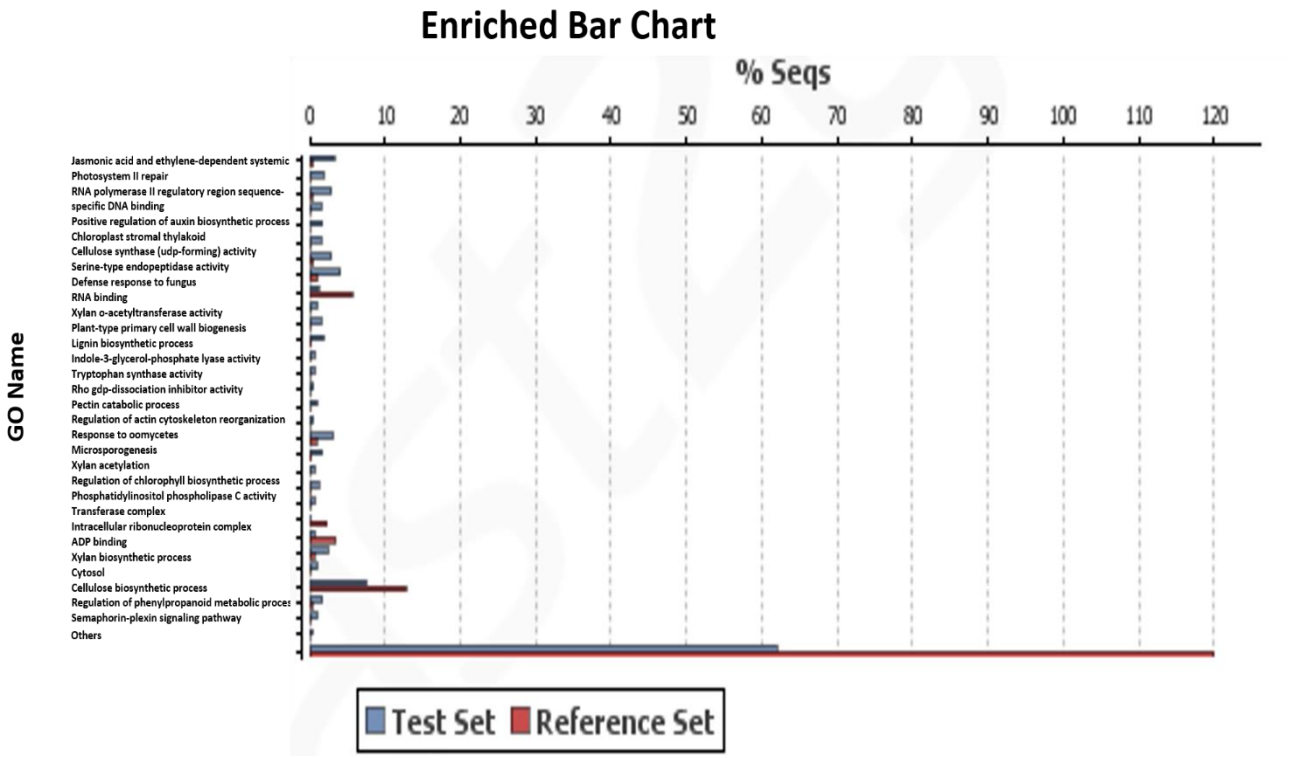


Supplementary Figure 1: RNA extraction of leaves of *Q. grandiflora* in two treatments: without Al (C2, C3, C4) and with Al (Al1, Al2, Al4). Visualization by 1% agarose gel stained with ethidium bromide. All samples showed ratio A260/280 between 1.8 to 2.0 and RIN score above 8.0 measured by Bioanalyzer. C – Control (Aluminum-untreated) sample and Al – Aluminum-treated sample.



Supplementary Figure 2: Histogram of *Q. grandiflora* samples from two treatments: without Al (control) and with Al. RPKM averaged values were represented based on the color scale shown at the top. The expression map was generated using MeV s/w (http://www.tm4.org/mev/). De novo assembly was carried out by the Trinity method (Grabherr et al., 2011), which resulted in a color key and histogram that were obtained by clustering using RPKM (Li and Godzik, 2006). It is clear that either the presence or absence of Al induced changes expression profiles of *Qualea* plant. This conclusion was based on the fact that the distance between the expression profiles were smaller within the treatments than between them.



Supplementary Figure 3: GO-terms (GO-Slim) differentially distributed in *Q. grandiflora* (Fisher's exact test, $P < 0.05$). For analisys, the enriched GO terms were mapped to a GO Slim annotation.

Supplementary Table 1. Differentially expressed genes in *Q. grandiflora* leaves in response to Al using RNA-Seq data.

ID (*Q. grandiflora* TR)	Description	Base Mean	Log2Fold Change	*P-adj*	*P-value*
TR10108|c0_g1_i1|g.3959	frmA, ADH5, adhC; S-(hydroxymethyl)glutathione dehydrogenase / alcohol dehydrogenase	118.157	-255.306	0.000586128976463665	8,5E-04
TR10373|c0_g2_i1|g.4107	\N	231.338	-366.995	0.000230909459537084	3,4E-03
TR10570|c0_g3_i1|g.4251	\N	363.823	453.452	0.000000214424674312562	4,2E-06
TR10646|c0_g1_i1|g.4286	\N	623.789	303.532	0.00891162436604222	2,8E-06
TR11133|c0_g1_i1|g.4553	HSPA1_8; heat shock 70kDa protein 1/8	140.519	397.371	0.00000799253998640821	1,7E-05
TR11912|c0_g1_i2|g.5012	\N	215.433	424.345	0.00000244390175142925	8,16E-05
TR12902|c0_g1_i1|g.5675	IRAK4; interleukin-1 receptor-associated kinase 4 [EC:2.7.11.1]	282.932	-360.024	0.000404027752620581	3,19E-05
TR13675|c0_g2_i1|g.6209	NOP2; ribosomal RNA methyltransferase Nop2 [EC:2.1.1.-]	130.379	-35.092	0.000509210024975566	4,34E-03
TR14641|c0_g1_i1|g.7089	\N	358.428	-395.487	0.000001018397043648	0,001373
TR14678|c0_g1_i2|g.7128	IRAK4; interleukin-1 receptor-associated kinase 4 [EC:2.7.11.1]	650.546	-347.997	0.000404556517478482	9,10E-08
TR14683|c0_g1_i1|g.7137	RGP, UTM; reversibly glycosylated polypeptide / UDP-arabinopyranose mutase [EC:2.4.1.-5.4.99.30]	953.467	-23.679	0.000000262291024366322	6,07E-06
TR14683|c0_g1_i1|g.7138	\N	953.467	-23.679	0.000000262291024366322	0,008765
TR15288|c0_g1_i1|g.7764	CYP26A; cytochrome P450, family 26, subfamily A	432.994	-309.119	0.00101403145061102	7,13E-06
TR16133|c0_g5_i1|g.8836	\N	579.227	480.635	0.000000009533798976529 2	1,08E-05
TR16450|c0_g1_i3|g.9199	E2.1.1.141; jasmonate O-methyltransferase [EC:2.1.1.141]	540.489	286.014	0.00573211704787919	1,31E-05

TR16528|c0_g3_i1|g.9287	\N	553.599	164.373	0.00190997419576299	0,000659
TR16787|c0_g2_i1|g.9662	\N	941.117	202.933	0.0016109550207439	0,003446
TR16796|c0_g1_i1|g.9681	\N	798.288	-259.015	0.0049487798135259	1,66E-15
TR16809|c0_g1_i1|g.9702	\N	403.273	-269.011	0.00292430376219502	0,000187
TR16864|c0_g1_i2|g.9775	RPS2; disease resistance protein RPS2	536.869	487.162	0.0000000032722134220986 2	0,00904
TR16864|c0_g1_i3|g.9776	RPS2; disease resistance protein RPS2	116.715	590.768	0.0000000000001610867822233358	2,19E-08
TR17001|c0_g1_i2|g.10010	\N	263.763	381.214	0.00000960081370769553	6,32E-49
TR17185|c0_g1_i1|g.10346	\N	265.552	-301.681	0.00216061318095566	1,10E-68
TR17203|c1_g1_i1|g.10378	\N	239.482	-259.567	0.0000000063902491506565 3	0,004096
TR17203|c1_g1_i2|g.10379	\N	140.033	-186.738	0.0000267180878047012	0,005569
TR17294|c0_g1_i3|g.10509	RPS2; disease resistance protein RPS2	675.061	444.599	0.0000001935979531579 42	0,00179
TR17300|c0_g1_i1|g.10525	ACS; 1-aminocyclopropane-1-carboxylate synthase [EC:4.4.1.14]	229.393	-311.129	0.00285259042468242	3,12E-06
TR17561|c0_g2_i1|g.11044	FLS2; LRR receptor-like serine/threonine-protein kinase FLS2 [EC:2.7.11.1]	496.677	314.419	0.000718967560506654	1,98E-20
TR17816|c0_g2_i1|g.11536	PIP; aquaporin PIP	217.836	-235.749	0.000666116020003248	1,65E-13
TR18151|c0_g2_i2|g.12311	\N	713.744	219.824	0.00411967899173926	0,000386
TR18342|c0_g1_i2|g.12870	\N	121.433	-274.033	0.00927502325656353	9,53E-06
TR18533|c0_g1_i1|g.13375	EREBP; EREBP-like fator	697.331	-194.229	0.000159477134287186	1,34E-

					09
TR18671|c0_g1_i1|g.13767	\N	367.313	-408.352	0.0000101875113857631	1,58E-05
TR18671|c0_g1_i1|g.13768	\N	367.313	-408.352	0.0000101875113857631	0,001744
TR18671|c0_g1_i1|g.13769	\N	367.313	-408.352	0.0000101875113857631	3,80E-06
TR18671|c0_g1_i1|g.13770	\N	367.313	-408.352	0.0000101875113857631	0,001403
TR19035|c0_g1_i2|g.14876	HSPA1_8; heat shock 70kDa protein 1/8	200.228	301.429	0.00700551143316178	3,61E-06
TR19189|c0_g1_i1|g.15355	CYP1A1; cytochrome P450, family 1, subfamily A, polypeptide 1 [EC:1.14.14.1]	522.938	297.598	0.00433420267306293	5,19E-10
TR19263|c0_g1_i1|g.15549	\N	203.342	-173.946	0.00183778120999769	2,05E-19
TR19576|c0_g1_i2|g.16590	\N	157.223	3.491	0.000627004208698461	0,000897
TR19623|c0_g2_i1|g.16829	\N	591.776	508.777	0.0000000006605517243394 35	0,00885
TR19643|c0_g1_i1|g.16877	\N	559.056	-30.532	0.000165006057706992	6,90E-11
TR19650|c0_g1_i2|g.16896	CYP75A; flavonoid 3',5'-hydroxylase [EC:1.14.13.88]	445.237	272.249	0.0000365818524363358	0,008696
TR19650|c0_g1_i2|g.16897	\N	445.237	272.249	0.0000365818524363358	1,04E-24
TR19744|c0_g1_i4|g.17277	\N	371.519	205.171	0.00779538567527221	0,000107
TR19795|c1_g1_i1|g.17492	cdd, CDA; cytidine deaminase [EC:3.5.4.5]	273.525	-171.225	0.00548750665250789	9,53E-06
TR19903|c1_g2_i1|g.17931	\N	441.902	458.371	0.000000153815760680628	1,34E-09
TR20176|c0_g1_i2|g.18995	\N	475.211	-220.109	0.00166128343564817	1,58E-05

TR20453|c0_g3_i1|g.20244	TR1; tropinone reductase I [EC:1.1.1.206]	147.018	290.001	0.00579342681002613	0,001744
TR20479|c0_g3_i7|g.20424	\N	136.025	30.552	0.00822683435554161	3,80E-06
TR20633|c0_g2_i1|g.21312	\N	447.503	-336.684	0.000896303930404586	0,001403
TR20731|c0_g2_i2|g.21818	ABA2; xanthoxin dehydrogenase [EC:1.1.1.288]	318.867	-272.237	0.00155653364264727	3,61E-06
TR20746|c0_g2_i2|g.21902	RGL4, rhiE; rhamnogalacturonan endolyase [EC:4.2.2.23]	186.374	-414.661	0.00000220226973920177	5,19E-10
TR20879|c2_g1_i2|g.22674	\N	445.146	-428.079	0.000000221059965954534	2,05E-19
TR20879|c2_g1_i5|g.22680	\N	355.604	-36.725	0.00000303589034162244	0,000897
TR21030|c1_g1_i2|g.23308	\N	259.454	-378.113	0.000103066068684508	0,00885
TR21112|c0_g2_i1|g.23765	E1.2.1.3; aldehyde dehydrogenase (NAD+) [EC:1.2.1.3]	104.768	296.894	0.00700551143316178	6,90E-11
TR21169|c1_g5_i7|g.24049	\N	186.853	-357.555	0.000398193125437499	0,008696
TR21169|c1_g5_i7|g.24050	\N	186.853	-357.555	0.000398193125437499	1,04E-24
TR21299|c2_g1_i1|g.24823	E1.10.3.3; L-ascorbate oxidase [EC:1.10.3.3]	645.997	174.411	0.0000000001554101676418 78	0,000107
TR21299|c2_g1_i1|g.24825	\N	645.997	174.411	0.0000000001554101676418 78	4,20E-05
TR21299|c2_g1_i5|g.24827	E1.10.3.3; L-ascorbate oxidase [EC:1.10.3.3]	578.303	181.367	0.0000000000000758218261348226	0,000386
TR21299|c2_g1_i7|g.24834	E1.10.3.3; L-ascorbate oxidase [EC:1.10.3.3]	193.709	177.007	0.0000000004439438565727731	9,53E-06
TR21299|c2_g1_i8|g.24836	E1.10.3.3; L-ascorbate oxidase [EC:1.10.3.3]	439.379	172.293	0.000000358819426968147	1,34E-09
TR21332|c1_g1_i1|g.25023	\N	357.094	555.849	0.0000000000009564588410716 46	1,58E-05

TR21549|c0_g3_i1|g.26155	pel; pectate lyase [EC:4.2.2.2]	209.383	-188.836	0.000242767614391446	0,001744
TR21549|c0_g3_i2|g.26157	pel; pectate lyase [EC:4.2.2.2]	155.494	-181.407	0.000000252007355202594	3,80E-06
TR21606|c0_g2_i10|g.26512	FAM50, XAP5; protein FAM50	832.378	-217.527	0.000595303197368738	0,001403
TR21609|c0_g2_i1|g.26527	\N	227.394	427.773	0.00000183379974590387	3,61E-06
TR21636|c0_g1_i1|g.26786	\N	139.349	124.332	0.00146768154468998	5,19E-10
TR21662|c1_g7_i1|g.26971	\N	194.151	332.122	0.00106356075444609	2,05E-19
TR21697|c0_g2_i1|g.27211	E3.4.23.25; saccharopepsin [EC:3.4.23.25]	203.343	183.441	0.00593295986231455	0,000897
TR21775|c0_g1_i1|g.27638	\N	294.279	441.655	0.000000587878806857194	0,00885
TR21775|c1_g2_i11|g.27644	\N	108.231	358.133	0.000406829250874179	6,90E-11
TR21775|c1_g2_i25|g.27655	\N	672.721	310.056	0.00657158052002931	0,008696
TR21775|c1_g2_i26|g.27656	\N	944.373	352.066	0.000580499723149547	1,04E-24
TR21830|c0_g3_i1|g.28071	\N	106.276	-559.727	0.0000000000000368490046699974	0,000107
TR21939|c0_g1_i3|g.28762	\N	145.238	-265.433	0.006951054350259	4,20E-05
TR21939|c0_g1_i6|g.28766	\N	758.158	-345.545	0.000448683468729846	
TR22188|c0_g7_i2|g.30597	\N	618.326	309.568	0.00241213059651198	
TR22238|c0_g1_i2|g.30902	FLS2; LRR receptor-like serine/threonine-protein kinase FLS2 [EC:2.7.11.1]	580.644	-238.443	0.000237754135362889	
TR22512|c0_g3_i2|g.32498	\N	122.754	-169.201	0.000000122333636463737	3,19E-05
TR22512|c0_g3_i2|g.32499	\N	122.754	-169.201	0.000000122333636463737	4,34E-

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TR22633|c0_g1_i3|g.33398	: No such data.	454.699	-23.733	0.00503758566581327	0,001373
TR22668|c0_g1_i2|g.33665	\N	116.053	-204.223	0.00111963987400012	9,10E-08
TR22668|c0_g1_i3|g.33667	\N	663.408	-301.016	0.00000123256905286037	6,07E-06
TR22668|c0_g1_i9|g.33672	\N	529.802	-346.626	0.000000008930270726109 77	0,008765
TR22678|c0_g9_i1|g.33733	FUCA; alpha-L-fucosidase [EC:3.2.1.51]	338.229	21.747	0.00796010114688777	7,13E-06
TR22897|c0_g2_i1|g.35245	\N	384.802	469.604	0.000000040155517852462 5	1,08E-05
TR22897|c0_g2_i2|g.35252	\N	527.549	489.842	0.000000002234973277490 29	1,31E-05
TR23153|c0_g4_i1|g.37149	IRAK4; interleukin-1 receptor-associated kinase 4 [EC:2.7.11.1]	142.747	-328.892	0.00228853117275598	0,000659
TR23178|c0_g4_i1|g.37263	XRN2, RAT1; 5'-3' exoribonuclease 2 [EC:3.1.13.-]	542.051	500.076	0.000000001701439456881 79	0,003446
TR23178|c0_g4_i1|g.37264	DICER1, DCR1; endoribonuclease Dicer [EC:3.1.26.-]	542.051	500.076	0.000000001701439456881 79	1,66E-15
TR23178|c0_g4_i1|g.37265	E5.4.99.8; cycloartenol synthase [EC:5.4.99.8]	542.051	500.076	0.000000001701439456881 79	0,000187
TR23178|c0_g4_i1|g.37267	\N	542.051	500.076	0.000000001701439456881 79	0,00904
TR23178|c0_g4_i1|g.37268	DICER1, DCR1; endoribonuclease Dicer [EC:3.1.26.-]	542.051	500.076	0.000000001701439456881 79	2,19E-08
TR23266|c1_g1_i1|g.37962	IRAK4; interleukin-1 receptor-associated kinase 4 [EC:2.7.11.1]	252.902	-249.214	0.000000000000002110602 05252258	6,32E-49
TR23266|c1_g1_i2|g.37963	IRAK4; interleukin-1 receptor-associated kinase 4 [EC:2.7.11.1]	173.664	-293.607	0.0000513230527081832	1,10E-68
TR23266|c1_g1_i4|g.37966	IRAK4; interleukin-1 receptor-associated kinase 4 [EC:2.7.11.1]	158.701	-312.239	0.0000015144745933553 8	0,004096

TR23266|c1_g1_i5|g.37968	IRAK4; interleukin-1 receptor-associated kinase 4 [EC:2.7.11.1]	175.046	-302.756	0.0000132710394024847	0,005569
TR23266|c1_g1_i6|g.37969	IRAK4; interleukin-1 receptor-associated kinase 4 [EC:2.7.11.1]	129.041	-254.768	0.000004424429087440 97	0,00179
TR23294|c0_g1_i2|g.38147	\N	600.378	-251.172	0.000170034146148775	3,12E-06
TR23363|c1_g1_i2|g.38755	\N	260.767	-359.998	0.000391230489911572	1,98E-20
TR23444|c0_g3_i1|g.39433	\N	168.557	302.898	0.00531199795784024	1,65E-13
TR23540|c0_g1_i1|g.40394	OPR; 12-oxophytodienoic acid reductase [EC:1.3.1.42]	670.139	-313.737	0.000002837825571554 91	0,000386
TR23688|c1_g4_i1|g.41631	K06889; uncharacterized protein	742.629	240.165	0.000001231783147727 79	9,53E-06
TR23688|c1_g7_i1|g.41633	K06889; uncharacterized protein	110.292	256.269	0.000167637765188468	1,34E-09
TR23765|c0_g3_i4|g.42216	RPS2; disease resistance protein RPS2	338.527	-313.763	0.000188697582387698	1,58E-05
TR23821|c2_g1_i2|g.42645	BAK1; brassinosteroid insensitive 1-associated receptor kinase 1 [EC:2.7.10.1 2.7.11.1]	256.975	278.133	0.00130847158772739	0,001744
TR23868|c1_g19_i2|g.43084	MOB1, Mats; MOB kinase activator 1	431.245	-380.357	0.000244847545184186	3,80E-06
TR23884|c0_g5_i2|g.43220	\N	177.334	-259.245	0.000485241957845911	0,001403
TR23901|c0_g1_i1|g.43344	E3.1.1.11; pectinesterase [EC:3.1.1.11]	143.829	-362.541	0.000230909459537084	3,61E-06
TR23901|c0_g1_i2|g.43345	E3.1.1.11; pectinesterase [EC:3.1.1.11]	192.595	-396.276	0.000158309443281141	5,19E-10
TR23901|c0_g1_i3|g.43346	E3.1.1.11; pectinesterase [EC:3.1.1.11]	210.708	-344.832	0.000302319614661218	2,05E-19
TR23926|c0_g1_i1|g.43637	\N	977.771	-251.699	0.000000001600373990736 3	0,000897
TR23932|c2_g11_i1|g.4368	CALM; calmodulin	278.156	-357.605	0.000467119489781625	0,00885

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TR23950|c0_g1_i3|g.43776	E2.6.1.83; LL-diaminopimelate aminotransferase [EC:2.6.1.83]	398.561	104.825	0.000115676434924939	6,90E-11
TR24026|c0_g1_i3|g.44436	IRAK4; interleukin-1 receptor-associated kinase 4 [EC:2.7.11.1]	513.672	-224.671	0.00882660463345376	0,008696
TR24026|c0_g1_i3|g.44437	\N	513.672	-224.671	0.00882660463345376	1,04E-24
TR24026|c0_g1_i3|g.44438	\N	513.672	-224.671	0.00882660463345376	0,000107
TR24179|c0_g1_i20|g.45995	\N	177.066	271.864	0.0021113645056728	4,20E-05
TR24294|c2_g4_i2|g.47180	IRAK4; interleukin-1 receptor-associated kinase 4 [EC:2.7.11.1]	241.021	168.013	0.00895418320991184	4,72E-19
TR24364|c0_g1_i7|g.47724	\N	392.569	-136.135	0.00828468593348326	7,53E-11
TR24389|c0_g1_i1|g.48014	GST, gst; glutathione S-transferase [EC:2.5.1.18]	386.757	295.664	0.000302319614661218	0,000125
TR24476|c1_g1_i1|g.48705	\N	338.031	169.485	0.0000182468997254096	9,59E-05
TR24476|c1_g1_i1|g.48706	\N	338.031	169.485	0.0000182468997254096	0,000814
TR24476|c1_g1_i2|g.48707	\N	275.622	219.571	0.0000566924238034035	1,33E-08
TR24476|c1_g1_i3|g.48709	\N	196.416	19.135	0.00171559756768349	1,32E-07
TR24476|c1_g1_i3|g.48710	\N	196.416	19.135	0.00171559756768349	0,005168
TR24521|c1_g1_i5|g.49159	\N	296.196	-345.844	0.000301543781450837	0,000799
TR24607|c0_g1_i3|g.50001	SCPL-I; serine carboxypeptidase-like clade I [EC:3.4.16.-]	114.022	224.006	0.00081910601258775	0,00231
TR24688|c0_g3_i5|g.50884	\N	503.828	-335.187	0.00111828877026714	1,41E-07

TR24688|c0_g3_i6|g.50885	\N	465.023	-435.565	0.000009232488671402 08	3,80E-06
TR24717|c0_g1_i4|g.51138	VAC8; vacuolar protein 8	486.749	208.876	0.000987219922163304	0,009623
TR24717|c0_g1_i4|g.51140	\N	486.749	208.876	0.000987219922163304	0,008765
TR25072|c1_g1_i12|g.54636	\N	195.866	-31.029	0.00569520221633303	2,56E-14
TR25072|c1_g1_i12|g.54637	E2.1.1.76; flavonol 3-O-methyltransferase [EC:2.1.1.76]	195.866	-31.029	0.00569520221633303	2,40E-09
TR25072|c1_g1_i8|g.54628	\N	204.509	-340.053	0.000857091824730419	1,30E-16
TR25072|c1_g1_i8|g.54630	E2.1.1.76; flavonol 3-O-methyltransferase [EC:2.1.1.76]	204.509	-340.053	0.000857091824730419	0,00904
TR25129|c0_g1_i2|g.55238	\N	391.131	-149.959	0.00125380963022267	0,000376
TR25147|c0_g1_i4|g.55467	map; methionyl aminopeptidase [EC:3.4.11.18]	155.587	116.211	0.00997950371074647	0,009849
TR25229|c0_g2_i1|g.56466	K07497; putative transposase	515.693	404.341	0.000000000060952535143 6813	0,008765
TR25229|c0_g2_i1|g.56467	K07497; putative transposase	515.693	404.341	0.000000000060952535143 6813	2,56E-14
TR25229|c0_g2_i2|g.56468	K07497; putative transposase	644.133	502.805	0.000000000000428630284 63125	2,40E-09
TR25229|c0_g2_i2|g.56469	K07497; putative transposase	644.133	502.805	0.000000000000428630284 63125	1,30E-16
TR25381|c3_g1_i3|g.58210	IRAK4; interleukin-1 receptor-associated kinase 4 [EC:2.7.11.1]	404.555	-203.433	0.000000000748645428249 161	0,00904
TR25440|c3_g6_i3|g.58928	\N	434.803	52.588	0.000000000031673140561 3019	0,000376
TR25454|c5_g1_i1|g.59062	\N	143.298	368.329	0.000228596343025488	0,009849
TR25470|c3_g1_i1|g.59232	HSPA1_8; heat shock 70kDa protein 1/8	279.407	-379.692	0.000002443901751429 25	5,14E-

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TR25470|c3_g8_i1|g.59241	HSPA1_8; heat shock 70kDa protein 1/8	815.553	329.374	0.00230977650892351	0,000128
TR25470|c3_g9_i4|g.59249	HSPA1_8; heat shock 70kDa protein 1/8	148.273	368.674	0.0000700450049316083	7,05E-06
TR25481|c1_g1_i14|g.59417	DPO1, polA; DNA polymerase I [EC:2.7.7.7]	831.856	33.209	0.00200495324478373	1,74E-06
TR25481|c1_g1_i15|g.59418	DPO1, polA; DNA polymerase I [EC:2.7.7.7]	129.601	299.724	0.0091171703580109	0,000375
TR25496|c1_g2_i1|g.59500	\N	797.075	291.098	0.00836286571420311	1,65E-13
TR25607|c1_g3_i2|g.60730	\N	456.124	187.694	0.00435460741686427	5,75E-60
TR25680|c1_g1_i6|g.61406	E2.4.1.218; hydroquinone glucosyltransferase [EC:2.4.1.218]	203.086	224.756	0.00355630335187434	0,001543
TR25696|c2_g1_i1|g.61553	ARHGDI, RHOGDI; Rho GDP-dissociation inhibitor	338.772	-120.864	0.000753297122476949	0,000487
TR25696|c2_g1_i1|g.61554	ARHGDI, RHOGDI; Rho GDP-dissociation inhibitor	338.772	-120.864	0.000753297122476949	
TR25696|c2_g1_i3|g.61556	ARHGDI, RHOGDI; Rho GDP-dissociation inhibitor	326.299	-10.262	0.0055224912439108	
TR25770|c0_g3_i2|g.62168	\N	243.349	32.152	0.00143119881869192	
TR25770|c0_g3_i8|g.62170	\N	609.433	35.081	0.0000909606403501319	
TR25794|c1_g2_i9|g.62452	\N	159.753	-110.272	0.00183848801476469	
TR25794|c1_g2_i9|g.62453	\N	159.753	-110.272	0.00183848801476469	
TR25958|c0_g1_i1|g.64135	K14165; atypical dual specificity phosphatase [EC:3.1.3.16 3.1.3.48]	376.226	139.322	0.00064165627959406	3,19E-05
TR25970|c0_g1_i10|g.64288	\N	664.025	-228.859	0.0060284111090882	4,34E-06
TR25970|c0_g1_i10|g.64289	\N	664.025	-228.859	0.0060284111090882	0,001373
TR25992|c0_g7_i3|g.64620	SRD5A3; 3-oxo-5-alpha-steroid 4-	318.095	248.012	0.00232193029721257	9,10E-156

	dehydrogenase 3 [EC:1.3.1.22 1.3.1.94]				08
TR26025|c2_g3_i2|g.64887	FLS2; LRR receptor-like serine/threonine-protein kinase FLS2 [EC:2.7.11.1]	130.788	-19.534	0.000477813346779515	6,07E-06
TR26025|c3_g2_i12|g.64900	E2.7.1.-	478.161	-296.062	0.000223598368293421	0,008765
TR26025|c3_g2_i13|g.64901	E2.7.1.-	583.476	-275.981	0.0000299176383001979	7,13E-06
TR26025|c3_g2_i13|g.64902	IRAK4; interleukin-1 receptor-associated kinase 4 [EC:2.7.11.1]	583.476	-275.981	0.0000299176383001979	1,08E-05
TR26025|c3_g2_i4|g.64892	E2.7.1.-	288.609	-281.023	0.000474135980169793	1,31E-05
TR26221|c0_g2_i2|g.66894	\N	619.994	-245.211	0.000565004209758113	0,000659
TR26261|c0_g1_i1|g.67341	\N	927.845	278.463	0.00026595089513735	0,003446
TR26309|c4_g4_i2|g.67906	ANXA7_11; annexin A7/11	835.537	-173.872	0.00856034442879052	1,66E-15
TR26497|c0_g1_i1|g.69955	\N	365.712	360.396	0.0000169865843552568	0,000187
TR26564|c1_g1_i14|g.70823	EEF1A; elongation factor 1-alpha	868.156	-148.354	0.00396016406229538	0,00904
TR26578|c3_g1_i2|g.71012	\N	971.143	356.544	0.000430229042851604	2,19E-08
TR26581|c0_g1_i5|g.71040	F6H1; feruloyl-CoA ortho-hydroxylase [EC:1.14.11.-]	273.444	-365.091	0.00000349305258514788	6,32E-49
TR26645|c0_g2_i1|g.72109	\N	159.907	445.772	0.00000016249245490748	1,10E-68
TR26645|c0_g2_i2|g.72111	\N	141.164	41.022	0.00000645770051342008	0,004096
TR26645|c0_g2_i2|g.72112	\N	141.164	41.022	0.00000645770051342008	0,005569
TR26645|c0_g2_i6|g.72119	\N	115.699	364.649	0.000270189065812953	0,00179

TR26645|c0_g2_i8|g.72126	\N	155.873	348.106	0.000159391273675815	3,12E-06
TR26645|c0_g2_i8|g.72127	\N	155.873	348.106	0.000159391273675815	1,98E-20
TR26651|c1_g2_i7|g.72226	E1.11.1.7; peroxidase [EC:1.11.1.7]	845.779	-304.763	0.007740071203806	1,65E-13
TR26679|c1_g2_i1|g.72468	\N	141.828	-308.114	0.00282993812217941	0,000386
TR26697|c0_g4_i3|g.72662	GST, gst; glutathione S-transferase [EC:2.5.1.18]	282.375	280.739	0.000893863185576531	9,53E-06
TR26736|c0_g1_i9|g.73140	\N	231.618	314.224	0.0000647217457636189	1,34E-09
TR26795|c2_g7_i1|g.73839	\N	393.259	0.859256	0.000436398735368363	1,58E-05
TR26795|c2_g7_i1|g.73840	LRRK2; leucine-rich repeat kinase 2 [EC:2.7.11.1]	393.259	0.859256	0.000436398735368363	0,001744
TR26822|c2_g1_i2|g.74101	HSPA1_8; heat shock 70kDa protein 1/8	445.446	-314.678	0.00409853326706653	3,80E-06
TR26826|c0_g1_i2|g.74135	tehA; tellurite resistance protein	682.023	-228.343	0.00207057671741244	0,001403
TR26862|c1_g4_i11|g.74673	\N	588.594	-460.929	0.0000008560296926884 74	3,61E-06
TR26862|c1_g4_i13|g.74678	\N	520.803	-449.398	0.000002486638979781 15	5,19E-10
TR26862|c1_g4_i13|g.74679	\N	520.803	-449.398	0.000002486638979781 15	2,05E-19
TR26862|c1_g4_i2|g.74652	HSPA1_8; heat shock 70kDa protein 1/8	179.193	-571.176	0.000000000000279869620631752	0,000897
TR26862|c1_g4_i2|g.74653	\N	179.193	-571.176	0.000000000000279869620631752	0,00885
TR26862|c1_g4_i2|g.74654	\N	179.193	-571.176	0.000000000000279869620631752	6,90E-11
TR26862|c1_g4_i3|g.74655	HSPA1_8; heat shock 70kDa protein 1/8	137.374	-526.075	0.000000000032121474614	0,00869

				771	6
TR26862|c1_g4_i3|g.74656	\N	137.374	-526.075	0.0000000000321214746147 71	1,04E-24
TR26862|c1_g4_i3|g.74657	\N	137.374	-526.075	0.0000000000321214746147 71	0,000107
TR26862|c1_g4_i5|g.74664	HSPA1_8; heat shock 70kDa protein 1/8	209.823	-326.515	0.00292860138277739	4,20E-05
TR26862|c1_g4_i8|g.74670	\N	199.418	-367.974	0.000191857536992241	4,72E-19
TR26862|c1_g4_i9|g.74671	\N	401.513	-420.587	0.00000349305258514788	7,53E-11
TR26970|c1_g1_i1|g.76196	ABCB1; ATP-binding cassette, subfamily B (MDR/TAP), member 1 [EC:3.6.3.44]	162.529	10.029	0.00845938300176919	2,56E-14
TR27062|c0_g1_i1|g.77426	\N	229.042	281.694	0.00884997169460722	2,40E-09
TR27062|c0_g1_i1|g.77427	\N	229.042	281.694	0.00884997169460722	1,30E-16
TR27062|c0_g1_i1|g.77428	\N	229.042	281.694	0.00884997169460722	0,00904
TR27169|c0_g1_i1|g.78932	BCS1; mitochondrial chaperone BCS1	396.451	-0.926182	0.0049487798135259	0,000376
TR27178|c0_g1_i1|g.78995	\N	199.123	331.296	0.000000001691466468059 39	0,00984 9
TR27242|c0_g1_i1|g.79739	crtZ; beta-carotene 3-hydroxylase [EC:1.14.13.129]	565.152	358.045	0.000000027977120925013 1	5,14E-06
TR27296|c0_g3_i4|g.80311	\N	234.349	-301.751	0.00274383182033973	0,000128
TR27330|c0_g4_i8|g.80704	\N	639.364	264.071	0.00514357879040099	7,05E-06
TR27383|c0_g1_i6|g.81341	\N	112.261	-222.533	0.0000950630446533352	1,74E-06
TR27412|c0_g1_i8|g.81685	\N	154.638	-15.044	0.0000132710394024847	0,000375

TR27412|c0_g1_i8|g.81686	\N	154.638	-15.044	0.0000132710394024847	1,65E-13
TR27514|c1_g1_i1|g.82939	\N	144.801	340.347	0.00138142981365887	5,75E-60
TR27514|c1_g1_i2|g.82941	\N	181.486	399.527	0.0000208347449096808	0,001543
TR27514|c2_g1_i1|g.82943	RP-L28, MRPL28, rpmB; large subunit ribosomal protein L28	278.831	413.005	0.00000869095856424849	0,000487
TR27514|c2_g1_i2|g.82944	RP-L28, MRPL28, rpmB; large subunit ribosomal protein L28	827.605	573.026	0.000000000000122558324464268	
TR27514|c2_g1_i3|g.82945	RP-L28, MRPL28, rpmB; large subunit ribosomal protein L28	933.545	548.937	0.0000000000063400497408621	
TR27559|c1_g5_i5|g.83558	\N	210.755	464.311	0.00000000327221342209862	
TR27580|c0_g3_i1|g.83816	HSPA1_8; heat shock 70kDa protein 1/8	914.902	-235.347	0.00556810079145285	
TR27715|c1_g4_i2|g.85367	\N	398.334	230.188	0.00064165627959406	
TR27829|c6_g1_i3|g.86692	EPHX2; soluble epoxide hydrolase / lipid-phosphate phosphatase [EC:3.3.2.10 3.1.3.76]	157.802	-153.876	0.0041319940060561	
TR27834|c0_g1_i3|g.86753	DPYS, dht, hydA; dihydropyrimidinase [EC:3.5.2.2]	738.179	-409.771	0.000000000020004105071555	3,19E-05
TR27834|c0_g1_i9|g.86769	DPYS, dht, hydA; dihydropyrimidinase [EC:3.5.2.2]	384.118	-2.822	0.00459424801466385	4,34E-06
TR27964|c3_g12_i1|g.88665	\N	197.123	305.938	0.00334946057891803	0,001373
TR27967|c1_g1_i1|g.88686	HSPA1_8; heat shock 70kDa protein 1/8	160.689	-319.702	0.00411759678114782	9,10E-08
TR28080|c1_g1_i2|g.90139	\N	366.228	-340.529	0.000000000317666562128693	6,07E-06
TR28080|c1_g1_i2|g.90140	\N	366.228	-340.529	0.000000000317666562128693	0,008765
TR28280|c1_g7_i4|g.92956	: No such data.	220.637	360.868	0.00010305145838668	7,13E-06

TR28280|c1_g7_i7|g.92958	: No such data.	106.945	316.677	0.00505945714955292	1,08E-05
TR28282|c0_g1_i1|g.92976	\N	492.967	-0.851568	0.00579342681002613	1,31E-05
TR28282|c0_g1_i1|g.92977	\N	492.967	-0.851568	0.00579342681002613	0,000659
TR28288|c0_g1_i7|g.93064	\N	118.877	140.445	0.00034976893041274	0,003446
TR28347|c0_g1_i11|g.93824	MRPL46; large subunit ribosomal protein L46	199.427	246.423	0.00801688665678371	1,66E-15
TR28347|c0_g1_i2|g.93816	MRPL46; large subunit ribosomal protein L46	161.961	-129.121	0.000677822802861601	0,000187
TR28347|c0_g1_i4|g.93819	MRPL46; large subunit ribosomal protein L46	973.271	388.596	0.0000000043407842731921	0,00904
TR28385|c1_g1_i7|g.94238	\N	361.061	205.831	0.0000000000306528553088391	2,19E-08
TR28513|c1_g4_i14|g.95962	K07095; uncharacterized protein	133.369	-116.284	0.00834386956764275	6,32E-49
TR28513|c1_g4_i3|g.95956	K07095; uncharacterized protein	799.098	-208.116	0.0000025407204526563	1,10E-68
TR28535|c0_g1_i3|g.96264	adk, AK; adenylate kinase [EC:2.7.4.3]	327.307	162.628	0.0000371447163717107	0,004096
TR28535|c0_g1_i3|g.96265	adk, AK; adenylate kinase [EC:2.7.4.3]	327.307	162.628	0.0000371447163717107	0,005569
TR28602|c0_g2_i1|g.97213	RPS2; disease resistance protein RPS2	861.807	345.048	0.000887803521892917	0,00179
TR28602|c0_g2_i1|g.97214	RPS2; disease resistance protein RPS2	861.807	345.048	0.000887803521892917	3,12E-06
TR28602|c0_g2_i1|g.97218	\N	861.807	345.048	0.000887803521892917	1,98E-20
TR28602|c0_g2_i3|g.97225	RPS2; disease resistance protein RPS2	103.492	322.606	0.000918039861892101	1,65E-13
TR28602|c0_g2_i3|g.97226	RPS2; disease resistance protein RPS2	103.492	322.606	0.000918039861892101	0,000386

TR28602|c0_g2_i3|g.97227	\N	103.492	322.606	0.000918039861892101	9,53E-06
TR28602|c0_g2_i4|g.97232	RPS2; disease resistance protein RPS2	128.304	27.797	0.00605526792578742	1,34E-09
TR28602|c0_g2_i4|g.97233	RPS2; disease resistance protein RPS2	128.304	27.797	0.00605526792578742	1,58E-05
TR28602|c0_g2_i4|g.97237	\N	128.304	27.797	0.00605526792578742	0,001744
TR28621|c7_g7_i1|g.97527	HSPA1_8; heat shock 70kDa protein 1/8	215.491	290.231	0.00895418320991184	3,80E-06
TR28659|c0_g5_i10|g.98023	\N	359.641	392.904	0.0000000000000583494194182017	0,001403
TR28680|c0_g1_i3|g.98331	PLCD; phosphatidylinositol phospholipase C, delta [EC:3.1.4.11]	117.807	284.196	0.00713554507765617	3,61E-06
TR28680|c0_g1_i3|g.98332	PLCD; phosphatidylinositol phospholipase C, delta [EC:3.1.4.11]	117.807	284.196	0.00713554507765617	5,19E-10
TR28706|c0_g6_i3|g.98809	E2.1.1.76; flavonol 3-O-methyltransferase [EC:2.1.1.76]	204.328	-271.175	0.00000645770051342008	2,05E-19
TR28743|c5_g4_i1|g.99544	HSPA1_8; heat shock 70kDa protein 1/8	163.214	-393.282	0.0000164708035500145	0,000897
TR28877|c0_g1_i1|g.101282	SLC15A3_4, PHT; solute carrier family 15 (peptide/histidine transporter), member 3/4	823.124	175.861	0.000000031062401111959	0,00885
TR28897|c2_g1_i7|g.101578	RP-S5, MRPS5, rpsE; small subunit ribosomal protein S5	420.306	193.019	0.00072072448916451	6,90E-11
TR29155|c0_g4_i6|g.105415	\N	826.435	119.649	0.00000155619953181109	0,008696
TR29184|c0_g2_i6|g.105847	\N	396.161	233.244	0.00376234578571883	1,04E-24
TR29215|c1_g3_i1|g.106350	RPM1, RPS3; disease resistance protein RPM1	121.811	279.317	0.00152317317776235	0,000107
TR29240|c0_g6_i4|g.106689	\N	614.169	-305.543	0.0000000005187140820 68427	4,20E-05
TR29263|c6_g7_i1|g.10692	HSPA1_8; heat shock 70kDa protein 1/8	180.834	-30.532	0.00871980082748988	4,72E-

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TR29272|c1_g1_i3|g.107019	\N	113.531	323.678	0.00109434781762559	7,53E-11
TR29319|c2_g2_i1|g.107612	E5.4.99.7, LSS, ERG7; lanosterol synthase [EC:5.4.99.7]	170.279	-369.838	0.000149363613791801	0,000125
TR29385|c0_g1_i3|g.108606	\N	823.094	-344.519	0.00000183379974590387	9,59E-05
TR29400|c0_g1_i17|g.108790	CTH; cystathionine gamma-lyase [EC:4.4.1.1]	465.702	279.986	0.0000000032722134220986 2	0,000814
TR29400|c0_g1_i8|g.108772	CTH; cystathionine gamma-lyase [EC:4.4.1.1]	573.304	169.167	0.000862648347291429	1,33E-08
TR29400|c0_g1_i8|g.108773	CTH; cystathionine gamma-lyase [EC:4.4.1.1]	573.304	169.167	0.000862648347291429	1,32E-07
TR29448|c8_g3_i1|g.109559	\N	217.975	-314.065	0.000722581851624003	0,005168
TR29507|c0_g1_i2|g.110369	\N	191.481	-129.612	0.0000130728880511395	0,000799
TR29507|c0_g1_i7|g.110372	SFRS2; splicing factor, arginine/serine-rich 2	458.892	14.593	0.00578017603851415	0,00231
TR29524|c1_g2_i11|g.110579	K07119; uncharacterized protein	132.319	-267.748	0.00119833858391131	1,41E-07
TR29524|c1_g2_i21|g.110585	K07119; uncharacterized protein	780.503	-235.308	0.000887803521892917	3,80E-06
TR29585|c0_g1_i1|g.111462	AOS; hydroperoxide dehydratase [EC:4.2.1.92]	138.101	-246.461	0.0000371447163717107	0,009623
TR29585|c0_g1_i1|g.111464	\N	138.101	-246.461	0.0000371447163717107	0,008765
TR29590|c0_g3_i1|g.111576	\N	157.104	-249.629	0.000506441407972995	2,56E-14
TR29592|c0_g1_i9|g.111672	lpxB; lipid-A-disaccharide synthase [EC:2.4.1.182]	829.792	132.528	0.00867305127225192	2,40E-09
TR29615|c0_g1_i2|g.111857	ABCF2; ATP-binding cassette, subfamily F, member 2	197.945	-11.276	0.00325461172579187	1,30E-16

TR29625|c0_g1_i9|g.112030	\N	305.833	-304.711	0.00686278409317965	0,00904
TR29673|c2_g1_i5|g.112666	E2.4.1.82; raffinose synthase [EC:2.4.1.82]	309.803	136.775	0.000000299647736955389	0,000376
TR29724|c1_g2_i7|g.113390	\N	178.023	-383.053	0.0000105809980558692	0,009849
TR29743|c1_g2_i1|g.113733	\N	106.227	335.316	0.000889998271639765	9,59E-05
TR29755|c0_g2_i1|g.113858	\N	435.157	-521.817	0.0000000000059438714926125 1	0,000814
TR29755|c3_g3_i2|g.113869	inlA; internalin A	160.555	-367.304	0.0000470547837790469	1,33E-08
TR29909|c3_g3_i1|g.116093	HSPA1_8; heat shock 70kDa protein 1/8	146.011	-549.629	0.0000000000010025867384 0145	1,32E-07
TR29967|c0_g1_i16|g.117016	NPEPPS; puromycin-sensitive aminopeptidase [EC:3.4.11.-]	969.477	228.798	0.000918015253444718	0,005168
TR29967|c0_g1_i16|g.117017	\N	969.477	228.798	0.000918015253444718	0,000799
TR29967|c0_g1_i16|g.117018	NPEPPS; puromycin-sensitive aminopeptidase [EC:3.4.11.-]	969.477	228.798	0.000918015253444718	0,00231
TR30067|c0_g4_i1|g.118723	\N	132.511	-399.227	0.0000000041646567409243 7	1,41E-07
TR30082|c2_g2_i9|g.118872	ABC.ATM; mitochondrial ABC transporter ATM	330.172	-309.785	0.00512456153297445	3,80E-06
TR30133|c1_g8_i10|g.119565	\N	380.282	-422.752	0.0000000006217510479160 06	0,009623
TR30133|c1_g8_i13|g.119570	\N	356.749	-378.514	0.000000102423637642515	0,008765
TR30214|c0_g1_i4|g.120776	\N	113.699	136.001	0.000737153908776892	2,56E-14
TR30243|c0_g2_i8|g.121314	RPS2; disease resistance protein RPS2	472.125	-366.738	0.000174227705216879	2,40E-09
TR30276|c0_g1_i6|g.12178	\N	247.231	107.906	0.00253375608647352	1,30E-

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TR30322|c0_g1_i1|g.122736	\N	369.904	-416.481	0.00000177546055648599	0,00904
TR30343|c2_g1_i1|g.123143	cfa; cyclopropane-fatty-acyl-phospholipid synthase [EC:2.1.1.79]	205.197	201.583	0.00635674553604179	0,000125
TR30343|c2_g1_i1|g.123144	cfa; cyclopropane-fatty-acyl-phospholipid synthase [EC:2.1.1.79]	205.197	201.583	0.00635674553604179	9,59E-05
TR30352|c0_g2_i4|g.123278	E2.4.1.207; xyloglucan:xyloglucosyl transferase [EC:2.4.1.207]	291.909	-34.035	0.000763570879860974	0,000814
TR30423|c1_g3_i7|g.124358	RFWD2, COP1; E3 ubiquitin-protein ligase RFWD2 [EC:2.3.2.27]	613.701	0.807188	0.000574596904440582	1,33E-08
TR30523|c3_g3_i1|g.125844	\N	157.192	254.806	0.00488478116497287	1,32E-07
TR30542|c0_g3_i8|g.126149	CCR; cinnamoyl-CoA reductase [EC:1.2.1.44]	109.478	196.849	0.00263493876242983	0,005168
TR30542|c0_g3_i8|g.126150	CCR; cinnamoyl-CoA reductase [EC:1.2.1.44]	109.478	196.849	0.00263493876242983	0,000799
TR30542|c0_g4_i1|g.126143	CCR; cinnamoyl-CoA reductase [EC:1.2.1.44]	430.991	-285.361	0.000273097866600562	0,00231
TR30889|c0_g1_i1|g.131445	bglB; beta-glucosidase [EC:3.2.1.21]	604.625	16.317	0.00371225088606821	1,41E-07
TR30939|c1_g2_i2|g.132415	SLC50A, SWEET; solute carrier family 50 (sugar transporter)	307.323	-154.203	0.000899823227170804	3,80E-06
TR31013|c0_g3_i7|g.133440	ABCB1; ATP-binding cassette, subfamily B (MDR/TAP), member 1 [EC:3.6.3.44]	227.865	-347.838	0.000284261378447516	0,009623
TR31013|c0_g3_i7|g.133441	ABCB1; ATP-binding cassette, subfamily B (MDR/TAP), member 1 [EC:3.6.3.44]	227.865	-347.838	0.000284261378447516	0,008765
TR31040|c0_g3_i13|g.134146	E1.14.-.-	165.003	-245.672	0.0000156153239724181	2,56E-14
TR31040|c0_g3_i6|g.134140	E1.14.-.-	202.609	-21.084	0.00570673777477169	2,40E-09
TR31053|c0_g6_i1|g.134348	SORD, gutB; L-iditol 2-dehydrogenase [EC:1.1.1.14]	250.654	-249.898	0.00828468593348326	1,30E-16

TR31096|c0_g3_i5|g.134971	\N	493.197	441.005	0.000000591084518912195	0,00904
TR31116|c1_g9_i10|g.135382	\N	204.598	-361.241	0.000317780757671408	0,000376
TR31154|c2_g1_i1|g.136152	\N	118.084	-172.138	0.00395279855990468	0,009849
TR31205|c0_g6_i5|g.137141	\N	123.603	-225.322	0.00494661393159938	9,59E-05
TR31220|c1_g2_i1|g.137368	ftsH, hflB; cell division protease FtsH [EC:3.4.24.-]	211.612	233.814	0.00415927439377126	0,000814
TR31228|c2_g2_i1|g.137475	clpP, CLPP; ATP-dependent Clp protease, protease subunit [EC:3.4.21.92]	851.753	101.955	0.000831695586522477	1,33E-08
TR31228|c2_g2_i6|g.137481	clpP, CLPP; ATP-dependent Clp protease, protease subunit [EC:3.4.21.92]	351.397	0.849462	0.000192587964159912	1,32E-07
TR31228|c2_g2_i7|g.137483	clpP, CLPP; ATP-dependent Clp protease, protease subunit [EC:3.4.21.92]	513.413	0.839825	0.00556810079145285	0,005168
TR31311|c2_g1_i1|g.138775	EHMT; euchromatic histone-lysine N-methyltransferase [EC:2.1.1.43]	717.662	473.373	0.000000017511030443009	0,000799
TR31312|c5_g4_i2|g.138783	RPS2; disease resistance protein RPS2	411.847	285.068	0.00181662215139185	0,00231
TR31374|c2_g1_i2|g.139956	\N	147.135	314.824	0.00511584287033724	1,41E-07
TR31382|c0_g4_i1|g.140052	K07497; putative transposase	435.558	249.453	0.0000753372437091071	3,80E-06
TR31382|c0_g4_i2|g.140053	K07497; putative transposase	701.906	246.371	0.00000792917272275729	0,009623
TR31382|c0_g4_i3|g.140054	K07497; putative transposase	534.587	317.657	0.0000420000270467533	0,008765
TR31382|c0_g4_i3|g.140055	\N	534.587	317.657	0.0000420000270467533	2,56E-14
TR31382|c0_g4_i4|g.140056	K07497; putative transposase	746.395	262.076	0.000273097866600562	2,40E-09
TR31382|c0_g4_i5|g.14005	K07497; putative transposase	495.913	304.432	0.0000158309443281141	1,30E-

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TR31382|c0_g4_i5|g.140058	\N	495.913	304.432	0.0000158309443281141	2,56E-14
TR31394|c0_g3_i2|g.140211	\N	128.703	548.924	0.0000000000020583291049512 2	2,40E-09
TR31440|c0_g1_i1|g.141103	\N	265.194	126.894	0.00176375583768674	1,30E-16
TR31440|c0_g1_i1|g.141104	\N	265.194	126.894	0.00176375583768674	0,00904
TR31544|c0_g13_i10|g.143010	CESA; cellulose synthase A [EC:2.4.1.12]	175.464	343.123	0.000778570286315278	0,000376
TR31544|c0_g13_i10|g.143011	CESA; cellulose synthase A [EC:2.4.1.12]	175.464	343.123	0.000778570286315278	0,009849
TR31544|c0_g13_i5|g.143002	CESA; cellulose synthase A [EC:2.4.1.12]	144.041	304.103	0.00661160960048127	5,14E-06
TR31544|c0_g13_i5|g.143003	CESA; cellulose synthase A [EC:2.4.1.12]	144.041	304.103	0.00661160960048127	0,000128
TR31544|c0_g13_i6|g.143004	CESA; cellulose synthase A [EC:2.4.1.12]	101.422	309.511	0.00494515249184955	7,05E-06
TR31544|c0_g13_i6|g.143005	CESA; cellulose synthase A [EC:2.4.1.12]	101.422	309.511	0.00494515249184955	1,74E-06
TR31544|c0_g13_i9|g.143007	CESA; cellulose synthase A [EC:2.4.1.12]	187.835	356.461	0.000316754125781747	0,000375
TR31544|c0_g13_i9|g.143008	CESA; cellulose synthase A [EC:2.4.1.12]	187.835	356.461	0.000316754125781747	1,65E-13
TR31544|c0_g13_i9|g.143009	CESA; cellulose synthase A [EC:2.4.1.12]	187.835	356.461	0.000316754125781747	5,75E-60
TR31561|c0_g5_i2|g.143261	SELENBP1; selenium-binding protein 1	153.537	393.225	0.0000001476355881431 87	0,001543
TR31566|c0_g1_i4|g.143355	\N	186.601	-0.967664	0.00833458639494051	0,000487
TR31566|c0_g1_i4|g.143356	\N	186.601	-0.967664	0.00833458639494051	

TR31570|c0_g1_i11|g.143413	GSN; gelsolin	608.867	176.428	0.00836223666696665	
TR31592|c3_g1_i5|g.143880	\N	601.905	-104.907	0.00754871744374189	
TR31599|c1_g2_i12|g.144032	PRORP; proteinaceous RNAse P [EC:3.1.26.5]	882.765	-126.638	0.00393150411373079	
TR31599|c1_g2_i12|g.144037	\N	882.765	-126.638	0.00393150411373079	
TR31618|c0_g2_i3|g.144317	\N	747.347	2.592	0.00494661393159938	
TR31618|c0_g2_i3|g.144318	\N	747.347	2.592	0.00494661393159938	3,19E-05
TR31641|c0_g3_i1|g.144662	CYP1A1; cytochrome P450, family 1, subfamily A, polypeptide 1 [EC:1.14.14.1]	140.279	-150.677	0.00473188499358318	4,34E-06
TR31650|c1_g3_i1|g.144776	\N	197.086	27.966	0.00891162436604222	0,001373
TR31650|c1_g3_i1|g.144777	\N	197.086	27.966	0.00891162436604222	9,10E-08
TR31738|c1_g1_i2|g.146392	\N	130.816	3.232	0.0000269926045535531	6,07E-06
TR31740|c0_g1_i3|g.146426	\N	159.529	400.505	0.0000175131480047655	0,008765
TR31796|c1_g4_i9|g.147357	NPEPPS; puromycin-sensitive aminopeptidase [EC:3.4.11.-]	151.263	0.987256	0.00149102411649453	7,13E-06
TR31819|c1_g1_i2|g.147775	\N	542.155	-224.542	0.00939537434102578	1,08E-05
TR31847|c2_g5_i3|g.148290	HSPA1_8; heat shock 70kDa protein 1/8	759.209	-221.592	0.00303511324909899	1,31E-05
TR31849|c0_g2_i1|g.148301	\N	137.518	301.983	0.00867305127225192	0,000659
TR31878|c1_g6_i1|g.148768	\N	980.133	325.492	0.0022355258703583	0,003446
TR31922|c0_g2_i2|g.14952	E1.10.3.3; L-ascorbate oxidase [EC:1.10.3.3]	469.939	-222.442	0.00845938300176919	1,66E-

6					15
TR31923|c1_g5_i3|g.149552	ATPeV0D, ATP6D; V-type H+-transporting ATPase subunit d	598.163	535.868	0.000000000000560611905890082	0,000187
TR31923|c1_g6_i5|g.149548	FLS2; LRR receptor-like serine/threonine-protein kinase FLS2 [EC:2.7.11.1]	213.299	340.768	0.00125380963022267	0,00904
TR31937|c1_g1_i2|g.149760	\N	425.497	-313.282	0.00415927439377126	2,19E-08
TR31937|c1_g1_i2|g.149761	\N	425.497	-313.282	0.00415927439377126	6,32E-49
TR31937|c1_g1_i2|g.149762	\N	425.497	-313.282	0.00415927439377126	1,10E-68
TR31978|c1_g1_i1|g.150657	E1.14.13.93; (+)-abscisic acid 8'-hydroxylase [EC:1.14.13.93]	118.946	-251.549	0.00895418320991184	0,004096
TR31978|c1_g1_i1|g.150658	\N	118.946	-251.549	0.00895418320991184	0,005569
TR32042|c1_g1_i1|g.151839	\N	610.107	-249.748	0.00122388177033315	0,00179
TR32042|c1_g1_i2|g.151841	\N	903.023	-219.139	0.00622978970684524	3,12E-06
TR32071|c0_g1_i3|g.152369	\N	676.498	-0.7413	0.00531141943447102	1,98E-20
TR32071|c0_g1_i6|g.152381	\N	981.376	-0.760515	0.00568225122992551	1,65E-13
TR32071|c0_g1_i6|g.152382	\N	981.376	-0.760515	0.00568225122992551	0,000386
TR32081|c0_g2_i1|g.152540	PTCD1; pentatricopeptide repeat domain-containing protein 1	456.241	-158.164	0.000782404018216876	9,53E-06
TR32081|c0_g2_i1|g.152541	\N	456.241	-158.164	0.000782404018216876	1,34E-09
TR32081|c0_g2_i1|g.152543	\N	456.241	-158.164	0.000782404018216876	1,58E-05
TR32085|c0_g1_i1|g.152599	EREBP; EREBP-like factor	590.149	111.315	0.000641427928322333	0,001744

TR32085|c0_g1_i1|g.152600	\N	590.149	111.315	0.000641427928322333	3,80E-06
TR32175|c0_g1_i14|g.153925	: No such data.	303.869	121.386	0.000002002347871836 17	0,001403
TR32175|c0_g1_i17|g.153930	: No such data.	106.632	175.012	0.000129237154496786	3,61E-06
TR32175|c0_g1_i9|g.153914	: No such data.	136.216	139.654	0.000019842089256296 9	5,19E-10
TR32175|c0_g1_i9|g.153915	\N	136.216	139.654	0.000019842089256296 9	2,05E-19
TR32215|c1_g2_i1|g.154556	\N	394.899	421.775	0.000007272275139412 78	0,000897
TR32235|c0_g3_i1|g.154879	\N	700.335	-280.402	0.00155055197524578	0,00885
TR32278|c2_g1_i3|g.155466	FLS2; LRR receptor-like serine/threonine-protein kinase FLS2 [EC:2.7.11.1]	403.482	318.059	0.000083003393380137 8	6,90E-11
TR32278|c2_g1_i3|g.155467	ATPeV0D, ATP6D; V-type H+-transporting ATPase subunit d	403.482	318.059	0.000083003393380137 8	0,008696
TR32312|c1_g2_i7|g.156273	: No such data.	778.445	-13.024	0.0048853179307707	1,04E-24
TR32312|c1_g2_i7|g.156274	: No such data.	778.445	-13.024	0.0048853179307707	0,000107
TR32319|c2_g1_i1|g.156496	\N	128.137	-284.132	0.000853226949628565	4,20E-05
TR32319|c2_g1_i1|g.156497	\N	128.137	-284.132	0.000853226949628565	4,72E-19
TR32319|c2_g1_i1|g.156498	\N	128.137	-284.132	0.000853226949628565	7,53E-11
TR32319|c2_g1_i4|g.156513	\N	118.003	-278.495	0.00571969944666464	0,000125
TR32319|c2_g1_i4|g.156514	\N	118.003	-278.495	0.00571969944666464	9,59E-05
TR32319|c2_g1_i4|g.15651	\N	118.003	-278.495	0.00571969944666464	0,00081

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TR32319|c2_g1_i4|g.156516	\N	118.003	-278.495	0.00571969944666464	1,33E-08
TR32319|c2_g1_i4|g.156517	\N	118.003	-278.495	0.00571969944666464	1,32E-07
TR32385|c0_g1_i2|g.157615	\N	636.474	-182.756	0.00488570133399866	0,005168
TR32385|c0_g1_i6|g.157619	\N	107.837	470.852	0.0000000000262833067362409	0,000799
TR32385|c0_g1_i7|g.157620	\N	100.073	519.718	0.000000000015510356464 9495	0,00231
TR32413|c3_g7_i1|g.158088	HSPA1_8; heat shock 70kDa protein 1/8	515.104	-358.569	0.0000267180878047012	1,41E-07
TR32413|c3_g7_i2|g.158089	HSPA1_8; heat shock 70kDa protein 1/8	487.498	-330.359	0.000114749573241459	3,80E-06
TR32452|c1_g11_i1|g.158913	\N	730.916	-135.279	0.00252311664369906	0,009623
TR32498|c0_g4_i2|g.159812	gpmB; probable phosphoglycerate mutase [EC:5.4.2.12]	220.505	147.471	0.00165749149908149	0,008765
TR32498|c0_g4_i3|g.159813	gpmB; probable phosphoglycerate mutase [EC:5.4.2.12]	212.046	136.181	0.00317694036917807	2,56E-14
TR32554|c0_g2_i4|g.160857	XRN2, RAT1; 5'-3' exoribonuclease 2 [EC:3.1.13.-]	263.447	403.902	0.00000614979399328159	2,40E-09
TR32554|c0_g3_i1|g.160852	DICER1, DCR1; endoribonuclease Dicer [EC:3.1.26.-]	643.034	540.327	0.0000000000101138948236 93	1,30E-16
TR32554|c0_g3_i1|g.160853	\N	643.034	540.327	0.0000000000101138948236 93	0,00904
TR32564|c0_g6_i5|g.161036	\N	208.208	411.798	0.00000114104744436498	0,000376
TR32564|c0_g6_i5|g.161037	\N	208.208	411.798	0.00000114104744436498	0,009849
TR32564|c0_g6_i5|g.161038	\N	208.208	411.798	0.00000114104744436498	9,59E-05

TR32564|c0_g6_i6|g.161039	\N	211.985	412.181	0.0000011709828263639	0,000814
TR32564|c0_g6_i6|g.161040	\N	211.985	412.181	0.0000011709828263639	1,33E-08
TR32581|c1_g11_i1|g.161305	HSPA1_8; heat shock 70kDa protein 1/8	124.011	416.995	0.00000269835614289468	1,32E-07
TR32581|c1_g3_i1|g.161296	HSPA1_8; heat shock 70kDa protein 1/8	584.347	-283.737	0.0000405528008373355	0,005168
TR32581|c1_g3_i3|g.161298	HSPA1_8; heat shock 70kDa protein 1/8	720.509	-458.651	0.000000020200391078318 1	0,000799
TR32581|c1_g3_i5|g.161300	HSPA1_8; heat shock 70kDa protein 1/8	587.975	-411.213	0.000000018801728453969 1	0,00231
TR32581|c1_g3_i6|g.161301	HSPA1_8; heat shock 70kDa protein 1/8	798.568	-2.994	0.000987219922163304	1,41E-07
TR32591|c1_g2_i1|g.161427	HSPA1_8; heat shock 70kDa protein 1/8	141.745	327.089	0.00250348830130213	3,80E-06
TR32615|c1_g10_i1|g.161778	\N	126.463	-316.277	0.00276798352688485	0,009623
TR32615|c1_g5_i4|g.161772	\N	172.203	415.728	0.00000472194333688034	0,008765
TR32615|c1_g5_i5|g.161774	\N	257.116	-379.941	0.0000126590762605252	2,56E-14
TR32615|c2_g4_i5|g.161791	inlA; internalin A	409.039	-38.898	0.00000593060460838273	2,40E-09
TR32615|c2_g4_i6|g.161792	inlA; internalin A	574.165	-458.076	0.000000026093351464076 1	1,30E-16
TR32633|c0_g8_i1|g.162160	\N	184.371	-295.852	0.00916761323764697	0,00904
TR32638|c0_g8_i2|g.162264	\N	162.614	-319.288	0.00417621442903093	2,56E-14
TR32638|c0_g8_i3|g.162271	\N	530.382	170.992	0.000338930960506273	2,40E-09
TR32638|c0_g8_i4|g.16227	\N	284.336	-308.326	0.00610508862748722	1,30E-

5					16
TR32659|c0_g4_i1|g.162616	: No such data.	699.602	-346.154	0.000000040822659278347	0,00904
TR32681|c0_g1_i11|g.163085	\N	757.258	121.419	0.00905767407917999	0,000376
TR32701|c0_g1_i1|g.163366	E6.4.1.4B; 3-methylcrotonyl-CoA carboxylase beta subunit [EC:6.4.1.4]	964.399	-0.812445	0.00224890213434762	0,00984 9
TR32730|c0_g2_i1|g.163889	\N	267.935	14.525	0.00089747794371668	5,14E-06
TR32737|c0_g2_i2|g.164110	\N	303.331	236.424	0.00732583797218122	0,00012 8
TR32737|c0_g3_i1|g.164108	\N	859.638	262.249	0.00512421980723083	7,05E-06
TR32753|c1_g4_i1|g.164318	E4.2.3.111; (-)-alpha-terpineol synthase [EC:4.2.3.111]	289.743	243.476	0.00902228961621212	1,74E-06
TR32755|c0_g4_i6|g.164351	TPS1; valencene/7-epi-alpha-selinene synthase [EC:4.2.3.73 4.2.3.86]	883.889	-331.614	0.00000981720257804314	0,00037 5
TR32793|c1_g3_i1|g.165085	CESA; cellulose synthase A [EC:2.4.1.12]	990.304	540.753	0.0000000000874217589329487	1,65E-13
TR32813|c2_g1_i3|g.165437	\N	159.423	455.636	0.0000000478934113414132	5,75E-60
TR32845|c1_g4_i1|g.166191	E2.4.1.207; xyloglucan:xyloglucosyl transferase [EC:2.4.1.207]	604.646	266.072	0.00610508862748722	0,00154 3
TR32855|c3_g2_i20|g.166375	SMAP; stromal membrane-associated protein	152.837	300.312	0.00172053207374666	0,00048 7
TR32856|c0_g2_i2|g.166377	\N	303.018	-100.726	0.000353170775456242	
TR32856|c0_g2_i2|g.166378	\N	303.018	-100.726	0.000353170775456242	
TR32921|c0_g3_i11|g.167514	SLC15A3_4, PHT; solute carrier family 15 (peptide/histidine transporter), member 3/4	119.393	-168.957	0.00207057671741244	
TR32972|c3_g8_i1|g.168487	\N	492.134	326.998	0.00225068015960729	

TR32975|c0_g6_i13|g.168583	SULTR2; sulfate transporter 2, low-affinity	407.887	13.679	0.0091171703580109	
TR32975|c0_g6_i13|g.168584	\N	407.887	13.679	0.0091171703580109	
TR32983|c1_g3_i2|g.168740	\N	788.185	316.206	0.00494661393159938	3,19E-05
TR33072|c1_g1_i1|g.170381	\N	194.382	20.753	0.0000213335987188992	4,34E-06
TR33079|c0_g1_i1|g.170525	PIGQ, GPI1; phosphatidylinositol glycan, class Q	414.146	217.338	0.00155055197524578	0,001373
TR33116|c1_g5_i1|g.171377	idi, IDI; isopentenyl-diphosphate delta-isomerase [EC:5.3.3.2]	131.752	454.575	0.000000000095140946156 6365	9,10E-08
TR33120|c0_g1_i3|g.171478	\N	422.787	-329.661	0.000273097866600562	6,07E-06
TR33141|c0_g1_i1|g.171799	\N	196.523	-315.481	0.00256090595987187	0,008765
TR33158|c1_g2_i8|g.172121	secA; preprotein translocase subunit SecA	439.136	0.834832	0.00828468593348326	7,13E-06
TR33158|c1_g2_i8|g.172123	\N	439.136	0.834832	0.00828468593348326	1,08E-05
TR33171|c4_g2_i1|g.172387	HSPA1_8; heat shock 70kDa protein 1/8	686.936	-193.752	0.00135663651095327	1,31E-05
TR33171|c4_g2_i4|g.172390	HSPA1_8; heat shock 70kDa protein 1/8	527.944	-276.394	0.000000012816654480727 5	0,000659
TR33175|c0_g4_i3|g.172470	SLC25A39_40; solute carrier family 25, member 39/40	211.646	4.098	0.00000964941789874202	0,003446
TR33199|c0_g2_i4|g.172809	\N	581.331	-287.741	0.00232193029721257	1,66E-15
TR33199|c0_g3_i2|g.172817	\N	150.214	-348.219	0.000669763413982325	0,000187
TR33213|c1_g1_i3|g.172992	\N	105.246	508.805	0.000000000000052833739 9166665	0,00904
TR33215|c0_g1_i4|g.17302	\N	267.801	-305.483	0.00000128940939423348	2,19E-

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TR33215|c1_g1_i2|g.173028	\N	336.159	-295.622	0.0000810349877005222	6,32E-49
TR33235|c1_g2_i1|g.173641	\N	594.365	264.752	0.000428343420291237	1,10E-68
TR33235|c1_g4_i8|g.173660	secY; preprotein translocase subunit SecY	162.112	120.024	0.00845938300176919	0,004096
TR33244|c2_g1_i2|g.173776	E3.1.4.46, glpQ, ugpQ; glycerophosphoryl diester phosphodiesterase [EC:3.1.4.46]	163.334	226.036	0.00000000315993020789637	0,005569
TR33265|c2_g1_i10|g.174343	\N	208.501	327.757	0.000302319614661218	0,00179
TR33297|c0_g2_i1|g.174911	RPS2; disease resistance protein RPS2	418.333	473.308	0.0000000288726242673392	3,12E-06
TR33304|c2_g3_i4|g.175091	\N	553.123	218.121	0.00000661222723249007	1,98E-20
TR33308|c2_g2_i12|g.175166	HSPA1_8; heat shock 70kDa protein 1/8	383.913	317.934	0.00208898894581281	1,65E-13
TR33308|c2_g2_i12|g.175167	HSPA1_8; heat shock 70kDa protein 1/8	383.913	317.934	0.00208898894581281	0,000386
TR33308|c2_g2_i4|g.175155	HSPA1_8; heat shock 70kDa protein 1/8	121.344	379.695	0.0000341956818462699	9,53E-06
TR33309|c0_g1_i1|g.175172	\N	185.956	-362.931	0.000269751026522934	1,34E-09
TR33309|c2_g1_i1|g.175173	HSPA1_8; heat shock 70kDa protein 1/8	618.003	-454.777	0.0000017087114151323 5	1,58E-05
TR33309|c2_g1_i2|g.175175	HSPA1_8; heat shock 70kDa protein 1/8	442.602	-438.654	0.0000062829659916613 5	0,001744
TR33309|c4_g1_i3|g.175179	\N	710.805	-414.838	0.0000044134104472909 1	3,80E-06
TR33309|c5_g7_i1|g.175189	HSPA1_8; heat shock 70kDa protein 1/8	725.368	-519.155	0.0000000000029426734 8658207	0,001403
TR33318|c1_g4_i1|g.175298	HSPA1_8; heat shock 70kDa protein 1/8	252.584	-358.683	0.000418782435419095	3,61E-06

TR33331|c1_g2_i3|g.175664	RPM1, RPS3; disease resistance protein RPM1	428.871	324.773	0.000894017939331004	5,19E-10
TR33331|c1_g2_i5|g.175666	RPM1, RPS3; disease resistance protein RPM1	512.412	35.044	0.000161659600599264	2,05E-19
TR33381|c1_g1_i6|g.176535	GST, gst; glutathione S-transferase [EC:2.5.1.18]	923.947	-165.455	0.0010731770164519	0,000897
TR33403|c2_g1_i2|g.176881	ETR, ERS; ethylene receptor [EC:2.7.13.-]	398.658	-106.741	0.00791485350844621	0,00885
TR33403|c2_g1_i4|g.176885	ETR, ERS; ethylene receptor [EC:2.7.13.-]	209.303	-0.983169	0.00589985452681995	6,90E-11
TR33418|c0_g6_i3|g.177175	\N	390.484	-208.184	0.00704591886238095	0,008696
TR33420|c0_g6_i1|g.177229	\N	427.328	218.466	0.0000841838051455304	1,04E-24
TR33427|c0_g2_i1|g.177392	RAD7; DNA repair protein RAD7	201.834	-104.804	0.00192073359410508	0,000107
TR33438|c2_g3_i1|g.177637	\N	449.556	425.183	0.00000016524982446266	4,20E-05
TR33449|c3_g1_i2|g.177769	RPS2; disease resistance protein RPS2	760.158	-159.621	0.000480038703883705	4,72E-19
TR33452|c0_g3_i1|g.177780	CAF16; CCR4-NOT complex subunit CAF16	366.992	291.708	0.0000138377869032036	7,53E-11
TR33455|c1_g1_i10|g.177881	\N	883.295	-441.041	0.000000310472722959372	0,000125
TR33455|c1_g1_i11|g.177882	\N	631.861	-476.545	0.0000000154438935051316	9,59E-05
TR33455|c1_g1_i12|g.177883	\N	667.282	-462.858	0.00000008115642722945	0,000814
TR33455|c1_g1_i2|g.177875	\N	258.311	-343.639	0.00109206100604014	1,33E-08
TR33455|c1_g1_i3|g.177876	\N	722.686	-41.001	0.0000021042905021635 1	1,32E-07
TR33455|c1_g1_i7|g.17787	\N	772.338	-484.308	0.000000008230250335839	0,00516

8				81	8
TR33533|c2_g5_i5|g.179467	IRAK4; interleukin-1 receptor-associated kinase 4 [EC:2.7.11.1]	526.751	468.847	0.0000000002782656868766 25	0,00079 9
TR33650|c0_g4_i2|g.181954	E2.3.1.133, HCT; shikimate O-hydroxycinnamoyltransferase [EC:2.3.1.133]	133.059	300.003	0.00785404493984448	0,00231
TR33656|c2_g1_i4|g.182017	\N	159.863	-182.115	0.00474680307164421	1,41E-07
TR33656|c2_g1_i4|g.182019	\N	159.863	-182.115	0.00474680307164421	3,80E-06
TR33676|c0_g8_i1|g.182307	RPM1, RPS3; disease resistance protein RPM1	847.021	220.171	0.00318121396427107	0,00962 3
TR33706|c3_g19_i4|g.182917	AFG3; AFG3 family protein [EC:3.4.24.-]	981.329	226.623	0.00139638429098556	0,00876 5
TR33710|c1_g4_i1|g.182935	\N	429.063	-423.735	0.00000273396886415238	2,56E-14
TR33714|c0_g1_i6|g.182999	UBE2O; ubiquitin-conjugating enzyme E2 O [EC:2.3.2.24]	725.975	0.994585	0.00895418320991184	2,40E-09
TR33714|c0_g1_i6|g.183000	\N	725.975	0.994585	0.00895418320991184	1,30E-16
TR33762|c1_g1_i10|g.184100	PLCD; phosphatidylinositol phospholipase C, delta [EC:3.1.4.11]	716.476	142.523	0.0060982034743051	0,00904
TR33762|c1_g1_i10|g.184101	PLCD; phosphatidylinositol phospholipase C, delta [EC:3.1.4.11]	716.476	142.523	0.0060982034743051	0,00037 6
TR33762|c1_g1_i11|g.184104	PLCD; phosphatidylinositol phospholipase C, delta [EC:3.1.4.11]	339.045	158.731	0.000990555963391977	0,00984 9
TR33762|c1_g1_i4|g.184078	PLCD; phosphatidylinositol phospholipase C, delta [EC:3.1.4.11]	833.077	213.925	0.00153684191434096	9,59E-05
TR33762|c1_g1_i7|g.184087	PLCD; phosphatidylinositol phospholipase C, delta [EC:3.1.4.11]	260.428	156.689	0.000242767614391446	0,00081 4
TR33762|c1_g1_i8|g.184091	PLCD; phosphatidylinositol phospholipase C, delta [EC:3.1.4.11]	269.236	148.073	0.000548679564513871	1,33E-08
TR33762|c1_g1_i8|g.184092	PLCD; phosphatidylinositol phospholipase C, delta [EC:3.1.4.11]	269.236	148.073	0.000548679564513871	1,32E-07

TR33771|c0_g2_i12|g.184272	EFTUD2; 116 kDa U5 small nuclear ribonucleoprotein component	113.787	-312.817	0.0000777806666296793	0,005168
TR33771|c0_g2_i13|g.184274	STX16; syntaxin 16	577.379	-425.558	0.000000714998607505638	0,000799
TR33776|c4_g1_i1|g.184332	\N	283.408	347.438	0.00023897257784565	0,00231
TR33849|c1_g7_i1|g.185806	E2.4.1.21, glgA; starch synthase [EC:2.4.1.21]	444.833	0.941883	0.00829204466928939	1,41E-07
TR33849|c1_g7_i1|g.185807	E2.4.1.21, glgA; starch synthase [EC:2.4.1.21]	444.833	0.941883	0.00829204466928939	3,80E-06
TR33849|c1_g7_i2|g.185809	E2.4.1.21, glgA; starch synthase [EC:2.4.1.21]	378.515	10.653	0.00893551629661289	0,009623
TR33849|c1_g7_i2|g.185811	E2.4.1.21, glgA; starch synthase [EC:2.4.1.21]	378.515	10.653	0.00893551629661289	0,008765
TR33851|c0_g14_i1|g.185839	\N	685.021	-193.954	0.00493817985316607	2,56E-14
TR33909|c2_g2_i3|g.186717	\N	705.551	-186.227	0.00000491219360257437	2,40E-09
TR33923|c1_g4_i1|g.187053	PRCP; lysosomal Pro-X carboxypeptidase [EC:3.4.16.2]	228.254	293.972	0.00415927439377126	1,30E-16
TR33940|c1_g1_i2|g.187348	\N	470.025	498.107	0.0000000017203047510382 6	0,00904
TR33940|c1_g1_i3|g.187349	\N	378.397	462.267	0.000000087058903510985 2	2,56E-14
TR33940|c1_g1_i4|g.187350	\N	353.084	449.233	0.000000310335481991735	2,40E-09
TR33940|c3_g1_i1|g.187355	\N	328.775	470.923	0.000000027890123463167 2	1,30E-16
TR33940|c3_g1_i4|g.187358	RP-L28, MRPL28, rpmB; large subunit ribosomal protein L28	544.596	509.561	0.000000000529579678324 879	0,00904
TR33940|c3_g1_i5|g.187359	\N	363.483	451.768	0.000000123178314772779	0,000376
TR33946|c4_g12_i4|g.1874	\N	462.983	314.069	0.000682959279659194	0,00984

21					9
TR33970|c2_g3_i3|g.187901	\N	203.303	338.145	0.00143119881869192	5,14E-06
TR34001|c1_g3_i8|g.188523	\N	769.077	250.455	0.0000256465516169682	0,000128
TR34036|c3_g6_i2|g.189247	\N	146.123	-297.925	0.00666204376036632	7,05E-06
TR34049|c2_g4_i4|g.189430	\N	838.379	323.703	0.0000562513518398272	1,74E-06
TR34049|c2_g4_i4|g.189431	\N	838.379	323.703	0.0000562513518398272	0,000375
TR34049|c2_g4_i9|g.189436	\N	105.832	31.801	0.0000528769910662572	1,65E-13
TR34049|c2_g4_i9|g.189437	\N	105.832	31.801	0.0000528769910662572	5,75E-60
TR34055|c0_g1_i17|g.189501	E3.2.1.41; pullulanase [EC:3.2.1.41]	847.711	148.567	0.0000657321799238414	0,001543
TR34060|c7_g1_i1|g.189571	\N	379.642	-350.031	0.000348742713255863	0,000487
TR34074|c1_g1_i9|g.189901	: No such data.	189.455	464.527	0.0000000005408601861020179	
TR34074|c2_g3_i1|g.189938	: No such data.	691.873	494.768	0.0000000011712761373075 2	
TR34132|c1_g1_i1|g.191169	\N	280.787	527.334	0.0000000000059438714926 1251	
TR34132|c1_g1_i3|g.191171	HSPA1_8; heat shock 70kDa protein 1/8	323.323	478.251	0.0000000000260024452295176	
TR34138|c4_g6_i2|g.191278	IRAK4; interleukin-1 receptor-associated kinase 4 [EC:2.7.11.1]	330.094	-412.118	0.0000000029143491685994 6	
TR34138|c4_g6_i6|g.191282	IRAK4; interleukin-1 receptor-associated kinase 4 [EC:2.7.11.1]	365.032	-401.737	0.0000000010798135447727 8	
TR34138|c4_g7_i1|g.191279	IRAK4; interleukin-1 receptor-associated kinase 4 [EC:2.7.11.1]	356.112	427.082	0.0000000000000038838332 8046605	3,19E-05

TR34213|c2_g3_i11|g.192794	\N	149.838	142.124	0.000908291135063031	4,34E-06
TR34213|c2_g3_i12|g.192795	\N	238.829	117.964	0.00000685326366567333	0,001373
TR34224|c1_g1_i2|g.192973	XRN2, RAT1; 5'-3' exoribonuclease 2 [EC:3.1.13.-]	282.708	-300.837	0.000406829250874179	9,10E-08
TR34224|c1_g1_i2|g.192974	DICER1, DCR1; endoribonuclease Dicer [EC:3.1.26.-]	282.708	-300.837	0.000406829250874179	6,07E-06
TR34245|c6_g4_i1|g.193299	E2.7.1.-	112.702	-26.248	0.00503758566581327	0,008765
TR34253|c1_g3_i3|g.193543	\N	188.408	-120.452	0.00996871927986428	7,13E-06
TR34263|c1_g2_i4|g.193705	\N	219.845	319.135	0.00404640379649076	1,08E-05
TR34275|c5_g4_i7|g.193878	E3.2.1.24; alpha-mannosidase [EC:3.2.1.24]	830.879	-163.062	0.00372361756975457	1,31E-05
TR34275|c5_g7_i12|g.193890	E3.2.1.24; alpha-mannosidase [EC:3.2.1.24]	149.227	-169.003	0.0000102049969788078	0,000659
TR34291|c1_g1_i3|g.194200	\N	293.624	200.006	0.00372361756975457	0,003446
TR34291|c1_g1_i3|g.194201	SMG1; PI-3-kinase-related kinase SMG-1	293.624	200.006	0.00372361756975457	1,66E-15
TR34291|c1_g1_i3|g.194202	\N	293.624	200.006	0.00372361756975457	0,000187
TR34300|c6_g10_i14|g.194391	trpA; tryptophan synthase alpha chain [EC:4.2.1.20]	138.195	-206.406	0.00280504988731629	0,00904
TR34300|c6_g1_i1|g.194370	trpA; tryptophan synthase alpha chain [EC:4.2.1.20]	712.424	-181.661	0.000190592917045299	2,19E-08
TR34300|c6_g6_i1|g.194371	trpA; tryptophan synthase alpha chain [EC:4.2.1.20]	828.623	-387.755	0.00000016524982446266	6,32E-49
TR34300|c6_g7_i1|g.194374	trpA; tryptophan synthase alpha chain [EC:4.2.1.20]	585.075	-306.349	0.00297856005594892	1,10E-68
TR34311|c8_g11_i6|g.1945	RPS2; disease resistance protein RPS2	179.211	-298.044	0.000238350277314859	0,00409

76					6
TR34311|c8_g11_i9|g.194578	RPS2; disease resistance protein RPS2	462.168	-398.084	0.000000000313083480617551	0,005569
TR34311|c8_g8_i3|g.194569	\N	151.576	-31.688	0.000528545349956929	0,00179
TR34311|c8_g8_i5|g.194573	\N	768.138	-380.965	0.000000262291024366322	3,12E-06
TR34358|c8_g4_i1|g.195334	RPS2; disease resistance protein RPS2	799.191	-570.949	0.0000000000000125133349205787	1,98E-20
TR34358|c9_g5_i3|g.195345	\N	113.989	-222.751	0.00212578782943131	1,65E-13
TR34358|c9_g5_i3|g.195346	RHM; UDP-glucose 4,6-dehydratase [EC:4.2.1.76]	113.989	-222.751	0.00212578782943131	0,000386
TR34393|c1_g8_i2|g.195622	\N	802.594	-406.783	0.000002707950863711 54	9,53E-06
TR34423|c7_g1_i4|g.195764	\N	118.632	305.316	0.00312007361441307	1,34E-09
TR39738|c0_g1_i1|g.197018	SLC15A3_4, PHT; solute carrier family 15 (peptide/histidine transporter), member 3/4	604.899	30.451	0.00863972740422505	1,58E-05
TR41716|c0_g1_i1|g.197481	\N	496.052	533.932	0.000000000013557636658003	0,001744
TR5218|c0_g1_i1|g.1870	\N	499.461	-234.584	0.00828468593348326	3,80E-06
TR55359|c0_g1_i1|g.200059	RPS2; disease resistance protein RPS2	104.135	344.177	0.000987219922163304	0,001403
TR7026|c0_g1_i1|g.2528	\N	243.841	-369.912	0.000189720199797678	3,61E-06
TR7131|c0_g2_i1|g.2587	\N	104.556	318.821	0.00453483650011329	5,19E-10
TR8915|c0_g1_i1|g.3332	\N	461.548	479.944	0.000000015472640195444	2,05E-181

				2	19
TR9727|c0_g1_i1|g.3760	\N	131.247	30.555	0.00887877963968719	0,000897

Supplementary Table 2. Differentially expressed genes of uncharacterized protein in *Q. grandiflora* using RNA-Seq data.

ID (*Q. grandiflora* TR)	Description	Base Mean	Log2Fold Change	*P-adj*	*P-value*
TR23688|c1_g4_i1|g.41631	Uncharacterized protein	742.629	240.165	0.000000123178314772779	9,53E-06
TR23688|c1_g7_i1|g.41633	Uncharacterized protein	110.292	256.269	0.000167637765188468	1,34E-09
TR28513|c1_g4_i14|g.95962	Uncharacterized protein	133.369	-116.284	0.00834386956764275	6,32E-09
TR28513|c1_g4_i3|g.95956	Uncharacterized protein	799.098	-208.116	0.0000025407204526563	1,10E-08
TR29524|c1_g2_i11|g.110579	Uncharacterized protein	132.319	-267.748	0.00119833858391131	1,41E-07
TR29524|c1_g2_i21|g.110585	Uncharacterized protein	780.503	-235.308	0.000887803521892917	3,80E-06

Supplementary Table 3. Differentially expressed genes of uncharacterized protein functionally re-annotated in *Q. grandiflora* based on the domain signature by InterProScan using RNA-Seq data.

ID (*Q. grandiflora* TR)	Domain - InterProScan annotation	GO: biological process	GO: molecular function	GO: cellular component
TR23688|c1_g4_i1|g.41631	IPR029058 - Alpha/Beta hydrolase	Involved in surface recognition	Organic substance catabolic process	-
TR23688|c1_g7_i1|g.41633	IPR029058 - Alpha/Beta hydrolase fold	-	-	-
TR28513|c1_g4_i14|g.95962	IPR029052 - Metallo-dependent phosphatase-like	Retrograde transport, endosome to Golgi	-	Retromer complex
TR29524|c1_g2_i11|g.110579	IPR013149 - Alcohol dehydrogenase, C-terminal	Oxidation-reduction process	-	-
TR29524|c1_g2_i21|g.110585	IPR013149 - Alcohol dehydrogenase, C-terminal	Oxidation-reduction process	-	-

Supplementary Table 4. Functional re-annotation of differentially expressed genes between treatments of *Q. grandiflora* using MapMan.

BIN	GO: Biological Process	Interproscan Annotation	GO: Molecular Function	GO: Cellular Component	Elements	P-value
2	Major Cho Metabolism				5	0.99
2.1	Major Cho Metabolism.Synthesis				4	0.99
2.1.2	Major Cho Metabolism.Synthesis.Starch				4	0.9E-2
2.1.2.2	Major Cho Metabolism.Synthesis.Starch.Starch Synthase	IPR011835, IPR013534, PR001296	Amylopectin Biosynthetic Process	Chloroplast	4	0,8E-2
2.2	Major Cho Metabolism.Degradation				1	0.92
2.2.2	Major Cho Metabolism.Degradation.Starch				1	0.92
2.2.2.9	Major Cho Metabolism.Degradation.Starch.Limit Dextrinase/ Pullulanase	IPR011839, IPR017853, IPR013781, IPR006047	Limit Dextrinase Activity, Pullulanase Activity, Alpha-Amylase Activity;	Chloroplast	1	0.92
3	Minor Cho Metabolism				2	0.92
3.1	Minor Cho Metabolism.Raffinose Family				1	4E=2
3.1.2.2	Minor Cho Metabolism.Raffinose Family.Raffinose Synthases.Putative	IPR017853, IPR008811			1	7E-2
3.3	Minor Cho Metabolism.Sugar Alcohols	IPR011032, IPR013154, IPR002328, IPR013149, IPR002085	Oxidation Reduction	Cell Surface	1	0.89
3.99	Minor Cho Metabolism.Sugar Alcohols	IPR006357	Phosphoglycolate Phosphatase Activity	Chloroplast	1	0,89

4	Glycolysis				2	0.89
4.3	Glycolysis.Unclear/Dually Targeted				2	0.89
4.3.12	Glycolysis.Unclear/Dually Targeted.Phosphoglycerate Mutase	IPR013078, IPR001345	Catalytic Activity	Chloroplast	2	0.89
5	Fermentation				1	0.89
5.10	Fermentation.Aldehyde Dehydrogenase	IPR016161, IPR015590, IPR016160	3-Chloroallyl Aldehyde Dehydrogenase Activity, Coniferyl-Aldehyde Dehydrogenase Activity, Aldehyde Dehydrogenase (NAD) Activity		1	0.89
10	Cell Wall				19	0.89
10.1	Cell Wall.Precursor Synthesis				1	0.89
10.1.10	Cell Wall.Precursor Synthesis.Rhm	IPR001509, IPR016040	Synthesis Of Cell Wall Precursors/Biosynthesis Of Rhamnose		1	0.89
10.2	Cell Wall.Cellulose Synthesis				11	2,11E-02
10.2.1	Cell Wall.Cellulose Synthesis.Cellulose Synthase	IPR005150	Cell Wall Biogenesis	Plasma Membrane, Endoplasmic Reticulum	11	2,11E-02
10.5	Cell Wall.Cell Wall Proteins				1	0.98
10.5.5	Cell Wall.Cell Wall Proteins.Rgp	IPR004901	Glycogen Glycosyltransferase Activity	Plasma Membrane	1	0.98

10.6	Cell Wall.Degradation				3	0.85
10.6.3	Cell Wall.Degradation.Pectate Lyases And Polygalacturonases	IPR010325, IPR013784, IPR008979	Cell Wall Degradation/Pectase Activity Liase	Endomembrane System	3	0.85
10.8	Cell Wall.Pectin*Esterases				3	0.35
10.8.1	Cell Wall.Pectin*Esterases.Pme	IPR006633, IPR018040, IPR011050, IPR006501, IPR012334	Cell Wall Modification	Plasma Membrane, Cell Wall	3	1,77E-02
10.8.2	Cell Wall.Pectin*Esterases.Pme	IPR004963	Carboxylesterase Activity	Endomembrane System, Cell Wall	3	1,77E-02
10.8.3	Cell Wall.Pectin*Esterases.Pme, Secondary Metabolism.Flavonoids.Dihydroflavonols	IPR002213	Quercetin 3-O-Glucosyltransferase Activity, UDP-Glycosyltransferase Activity, Transferase Activity, Transferring Glycosyl Groups			
10.8.3.3	Cell Wall.Pectin*Esterases.Pme, Secondary Metabolism.Flavonoids.Dihydroflavonols	IPR001128	Flavonoid 3' Hydroxylase Activity			
10.8.99	Cell Wall.Pectin*Esterases.Pme	IPR018040	Cell Wall Modification/Invertase/Pectin Methylesterase/Copper Ion Binding		1	1,77E-02
11	Lipid Metabolism				4	0.99
11.3	Lipid Metabolism.Phospholipid Synthesis				2	0.89
11.3.7	Lipid Metabolism.Phospholipid	IPR002937	Cyclopropane-	Endomembrane	2	0.89

	Synthesis.Cyclopropane-Fatty-Acyl-Phospholipid Synthase		Fatty-Acyl-Phospholipid Activity	System		
11.8	Lipid Metabolism.'Exotics'(Steroids, Squalene Etc)				1	0.85
11.8.6	Lipid Metabolism.'Exotics' (Steroids, Squalene Etc).Cycloartenol Synthase	IPR002365	Synthesis And Degradation Of Steroids	Vacuole	1	1,60E-01
11.9	Lipid Metabolism.Lipid Degradation				1	0.89
11.9.3	Lipid Metabolism.Lipid Degradation.Lysophospholipases				1	0.89
11.9.3.3	Lipid Metabolism.Lipid Degradation.Lysophospholipases.Glycerophosphodiester Phosphodiesterase	IPR017946	Metabolic Process Of Glycerol	Vacuole	1	0.89
11.9.4.9	Lipid Metabolism.Lipid Degradation.Lysophospholipases.Glycerophosphodiester Phosphodiesterase		Fatty Acid Beta-Oxidation	Cell Wall, Peroxisome, Nucleolus	1	0,89
11.4.9.13	Lipid Metabolism.Lipid Degradation.Lysophospholipases.Glycerophosphodiester Phosphodiesterase	IPR004262	Oxidoreductase Activity/Fatty-Acyl-Coa Reductase	Endoplasmic Reticulum	4	0.89
11.9.4.14	Lipid Metabolism.Lipid Degradation.Lysophospholipases.Glycerophosphodiester Phosphodiesterase	IPR018376	Fatty Acid Catabolic Process - Seed Germination	Peroxisome	1	0.89
11.10.3	Lipid Metabolism. Glycolipid Synthesis	IPR001509	Phosphate Starvation/Sulfolipid Biosynthetic Process/Glycolipid Biosynthetic Process/Zinc Ion Binding	Chloroplast	1	0.89

11.10.4	Lipid Metabolism. Glycolipid Synthesis	IPR001296	Sulfolipid Biosynthesis	Chloroplast, Chloroplast Envelope		0.89
13	Amino Acid Metabolism				7	0.85
13.1	Amino Acid Metabolism.Synthesis				6	0.85
13.1.3	Amino Acid Metabolism.Synthesis.Aspartate Family				2	0.99
13.1.3.5	Amino Acid Metabolism.Synthesis.Aspartate Family.Lysine				1	0.99
13.1.3.5.3	Amino Acid Metabolism.Synthesis.Aspartate Family.Lysine.Ll-Diaminopimelic Acid Aminotransferase	IPR019942, IPR015424, IPR004839, IPR015421, IPR015422	Biosynthetic Process Of Lysine Via Diaminopimelate, Systemic Acquired Resistance, Salicylic Acid Mediated Signaling Pathway, COPPER BIDING	Chloroplast, Chloroplast Stroma	1	0.99
13.1.3.4	Amino Acid Metabolism.Synthesis.Aspartate Family.Methionine	IPR013216	Methyltransferase Activity	Chloroplast, Plastoglobule	1	0.99
13.1.3.4.12	Amino Acid Metabolism.Synthesis.Aspartate Family.Methionine.Homocysteine S-Methyltransferase	IPR013216	Methyltransferase Activity	Chloroplast, Plastoglobulum	1	0.99
13.1.6	Amino Acid Metabolism.Synthesis.Aspartate Family.Lysine				4	6,78E-02
13.1.6.5	Amino Acid Metabolism.Synthesis.Aromatic Aa.Tryptophan				4	6,78E-02
13.1.6.5.5	Amino Acid Metabolism.Synthesis.Aromatic Aa.Tryptophan.Tryptophan Synthase	IPR018204, IPR013785, IPR011060,	Tryptophan Biosynthesis	Chloroplast	4	6,78E-02

		IPR002028				
13.2	Amino Acid Metabolism.Degradation				1	0.99
13.2.4	Amino Acid Metabolism.Degradation.Branched Chain Group	IPR011762	Leucine Degradation In Mitochondria/Biotin Carboxylase Activity, Cobalt Ion Binding, Methylcrotonoyl-Coa Carboxylase Activity, Zinc Ion Binding	Mitochondrion, Mitochondrial Matrix	1	0.99
13.2.4.4	Amino Acid Metabolism.Degradation.Branched Chain Group.Leucine	IPR011762	Biotin Carboxylase Activity, Cobalt Ion Binding, Methylcrotonoyl-Coa Carboxylase Activity, Zinc Ion Binding; INVOLVED IN: Leucine Catabolic Process	Mitochondrion, Mitochondrial Matrix	1	0.99
13.2.6.6	Amino Acid Metabolism.Degradation.Aromatic Aa.Tryptophan	IPR001753	Catalytic Activity - Embryo Development Ending In Seed Dormancy	Plasma Membrane,Peroxisome	5	0,99
13.2.7	Amino Acid Metabolism.Degradation.Branched Chain Group.Leucine	IPR015424	Pyridoxal Phosphate Binding/Carboxy-Lyase Activity		1	0,89
13.99	Amino Acid Metabolism. Glutamate Metabolism	IPR002912	Amino Acid	Cytosol	8	0,89

			Binding/Response To Cytokinin Stimulus			
15	Metal Handling		Selenium Binding ,Cadmium Biding		1	0.85
16	Secondary Metabolism				17	0.89
16.1	Secondary Metabolism.Isoprenoids				5	0.92
16.1.2	Secondary Metabolism.Isoprenoids.Mevalonate Pathway				1	0.85
16.1.2.7	Secondary Metabolism.Isoprenoids.Mevalonate Pathway.Isopentenyl Pyrophosphate:Dimethyllallyl Pyrophosphate Isomerase	IPR015797	Isopentenyl-Diphosphate Delta-Isomerase Activity, Chlorophyll Biosynthetic Process	Cytosol, Mitochondrion, Chloroplast	1	0.85
16.1.4	Secondary Metabolism.Isoprenoids.Carotenoids				1	0,21
16.1.4.6	Secondary Metabolism.Isoprenoids.Carotenoids.Carotenoid Beta Ring Hydroxylase	IPR006694	Carotene Beta-Ring Hydroxylase Activity,TERPENE METABOLISM / Xanthophyll Biosynthesis Process, Metabolic Process Of Carotene	Endoplasmic Reticulum, Chloroplast	1	0.85
16.1.5	Secondary Metabolism.Isoprenoids.Terpenoids	IPR002365, IPR008930, IPR018333, IPR001330	Terpene Synthase, Metal Binding Domain	Vacuole	3	0,26
16.2	Secondary Metabolism.Phenylpropanoids	IPR003480,	Biosynthetic Process Of Chlorophyll,	Cytosol, Mitochondria, Chloroplast	4	0,99

			Transferase Activity, Transferring Acyl Groups Other Than Amino-Acyl Groups			
16.2.1	Secondary Metabolism.Phenylpropanoids	IPR002213	Quercetin Activity 3-O-Glucosyltransferase, UDP-Glycosyltransferase Activity / Lignin Biosynthesis		2	0,29
16.2.1.9	Secondary Metabolism.Phenylpropanoids.Lignin Biosynthesis.Comt	IPR011991, IPR012967, IPR001077, IPR016461	Myricetin 3'-O-Methyltransferase Activity, Quercetin 3-O-Methyltransferase Activity, Caffeate O-Methyltransferase Activity	Cytosol, Nucleus, Plasma Membrane, Cytoplasm	2	0.88
16.4	Secondary Metabolism.N Misc		Transfer Activity		2	0.89
16.4.1	Secondary Metabolism.N Misc.Alkaloid-Like	IPR020904, IPR016040, IPR002347, IPR002198, IPR016166, IPR012951, IPR006094	Arginine And Proline Degradation/Oxidoreductase Activity		2	0.89
16.8	Secondary Metabolism.Flavonoids				2	0.89
16.8.3	Secondary Metabolism.Flavonoids.Dihydroflavonols		Quercetin 3-O-Glucosyltransferas		2	0,33

			e Activity, UDP-Glycosyltransferase Activity			
16.8.3.3	Secondary Metabolism.Flavonoids.Dihydroflavonols.Flavonoid 3''-Monooxygenase	IPR001128, IPR002401, IPR017972	Flavonoid 3' Hydroxylase Activity		1	0,45
16.10	Secondary Metabolism.Simple Phenols	IPR011707, IPR017761, IPR011706, IPR008972, IPR002355, IPR001117	Ascorbate Metabolism/ Secondary Cell Wall Biogenesis/Flavonoid Biosynthetic Process- Lignin/ Response To Copper Ion	Endomembrane System, Apoplast	5	0.89
17	Hormone Metabolism				14	0.59
17.1	Hormone Metabolism.Abscisic Acid				2	0.85
17.1.1	Hormone Metabolism.Abscisic Acid.Synthesis-Degradation				2	0.85
17.1.1.1	Hormone Metabolism.Abscisic Acid.Synthesis-Degradation.Synthesis				1	0.89
17.1.1.1.11	Hormone Metabolism.Abscisic Acid.Synthesis-Degradation.Synthesis.Short Chain Alcohol Dehydrogenmase (Aba2)				1	0.89
17.1.1.2	Hormone Metabolism.Abscisic Acid.Synthesis-Degradation.Degradation				1	0.89
17.1.1.2.1	Hormone Metabolism.Abscisic Acid.Synthesis-Degradation.Degradation.8-Hydroxylase	IPR001128	Oxygen Binding, (+)-Abscisic Acid 8'-Hydroxylase Activity	Endomembrane System	1	0.89
17.3	Hormone Metabolism.Brassinosteroid				3	0.99
17.3.1	Hormone Metabolism.Brassinosteroid.Synthesis-				2	0.89

	Degradation					
17.3.1.1	Hormone Metabolism.Brassinosteroid.Synthesis-Degradation.Brs	IPR001104	Oxidoreductase Activity	Endoplasmic Reticulum	1	0.89
17.3.1.1.1	Hormone Metabolism.Brassinosteroid.Synthesis-Degradation.Brs.Det2	IPR020904	Oxidoreductase Activity, Binding, Catalytic Activity		1	0.89
17.3.1.2	Hormone Metabolism.Brassinosteroid.Synthesis-Degradation.Sterols				1	0.85
17.3.1.2.99	Hormone Metabolism.Brassinosteroid.Synthesis-Degradation.Sterols.Other	IPR002365	Cycloartenol Synthase Activity	Vacuole	1	0.85
17.3.2	Hormone Metabolism.Brassinosteroid.Signal Transduction				1	0.89
17.3.2.99	Hormone Metabolism.Brassinosteroid.Signal Transduction.Other	IPR002365	Leu-Rich Receptor Serine/Threonine Protein Kinase	Vacuole	1	0.89
17.5	Hormone Metabolism.Ethylene				4	0.88
17.5.1	Hormone Metabolism.Ethylene.Synthesis-Degradation				1	0.87
17.5.1.1	Hormone Metabolism.Ethylene.Synthesis-Degradation.1-Aminocyclopropane-1-Carboxylate Synthase	IPR001176			1	0.87
17.5.2	Hormone Metabolism.Ethylene.Signal Transduction	IPR009082	Ethylene Binding, Protein Histidine Kinase Activity, Receptor Activity, Glycogen Synthase Kinase 3 Activity	Endomembrane System, Endoplasmic Reticulum Membrane	3	0.89
17.6	Hormone Metabolism.Gibberelin				2	8,54E-02
17.6.1	Hormone Metabolism.Gibberelin.Synthesis-	IPR005123	Oxidoreductase		1	8,54E-

	Degradation		Activity			02
17.6.3	Hormone Metabolism.Gibberelin.Induced-Regulated-Responsive-Activated	IPR003854		Endomembrane System	1	8,00E-02
17.7	Hormone Metabolism.Jasmonate				3	8,00E-02
17.7.1	Hormone Metabolism.Jasmonate.Synthesis-Degradation				3	0.89
17.7.1.3	Hormone Metabolism.Jasmonate.Synthesis-Degradation.Allene Oxidase Synthase	IPR001128	Hydro-Lyase Activity, Allene Oxide Synthase Activity, Oxygen Binding	In 7 Components	1	0.89
17.7.1.5	Hormone Metabolism.Jasmonate.Synthesis-Degradation.12-Oxo-Pda-Reductase	IPR001155	12-Oxophytodienoate Reductase Activity	Peroxisome	1	0.87
17.7.1.10	Hormone Metabolism.Jasmonate.Synthesis-Degradation.Jasmonate-O-Methyltransferase	IPR005299	Methyltransferase Activity		1	0.89
17.8	Hormone Metabolism.Salicylic Acid				1	0.89
17.8.1	Hormone Metabolism.Salicylic Acid.Synthesis-Degradation	IPR005299	UDP-Glycosyltransferase Activity, Transferase Activity/Methilation	Nucleous	1	0.89
17.8.1.1	Hormone Metabolism.Salicylic Acid.Synthesis-Degradation.Synthesis				1	0.89
17.8.1.1.5	Hormone Metabolism.Salicylic Acid.Synthesis-Degradation.Synthesis	IPR002213	Transferase Activity, Transferring Hexosyl Groups, UDP-	Cytosol	6	0,89

			Glycosyltransferase Activity			
17.8.1.1.7	Hormone Metabolism.Salicylic Acid.Synthesis-Degradation.Synthesis.Methyl-Sa Methylesterase	IPR005299	Jasmonic Acid Carboxyl Methyltransferase		1	0.89
20	Stress				67	0.47
20.1	Stress.Biotic				64	0.59
20.1.2	Stress Biotic Receptors	IPR003593	Transmembrane Receptor Activity, Nucleoside-Triphosphatase Activity, Nucleotide Binding, ATP Binding	Intrinsic To Membrane	15	2,57E-03
20.1.4	Stress.Biotic.Kinases				3	0.44
20.1.7	Stress.Biotic.Pr-Proteins				47	0.59
20.2	Stress.Abiotic				3	0.88
20.2.1	Stress.Abiotic.Heat				1	0.89
20.2.3	Stress.Abiotic.Drought/Salt				1	0.99
20.2.99	Stress.Abiotic.Unspecified		Manganese Ion Binding, Nutrient Reservoir Activity	Apoplast, Cell Wall	1	0.85
23	Nucleotide Metabolism				5	0.89
23.2	Nucleotide Metabolism.Degradation	IPR006263, IPR016192, IPR002125, IPR016193, IPR013171	Deoxycytidylate Deaminase Activity/Zinc Binding Domain		3	0.85
23.2.1	Nucleotide Metabolism.Degradation.Pyrimidine				2	0.85

23.2.1.4	Nucleotide Metabolism.Degradation.Pyrimidine.Dihydropyrimidinase	IPR011778, IPR006680, IPR011059	Uracil Metabolism		2	0.85
23.4	Nucleotide Metabolism.Phosphotransfer And Pyrophosphatases				2	9E-3
23.4.1	Nucleotide Metabolism.Phosphotransfer And Pyrophosphatases.Adenylate Kinase	IPR000850	Nucleobase, Nucleoside, Nucleotide Kinase Activity, Nucleotide Kinase Activity, ATP Binding	Mitochondrion	2	0.89
26	Misc				30	5E-2
26.1	Misc.Misc2				1	0.89
26.2	Misc.Udp Glucosyl And Glucoronyl Transferases	IPR002213	Quercetin 3-O-Glucosyltransferase Activity, UDP-Glycosyltransferase Activity, Transferase Activity, Transferring Glycosyl Groups		1	0.89
26.3	Misc.Gluco-, Galacto- And Mannosidases	IPR001360	Cation Binding, Hydrolase Activity, Hydrolyzing O-Glycosyl Compounds, Catalytic Activity	Endomembrane System	3	0.89
26.3.3	Misc.Gluco-, Galacto- And Mannosidases.Alpha-Mannosidase	IPR011013		Endomembrane System	2	0.89
26.6	Misc.O-Methyl Transferases				1	0.89

26.7	Misc.Oxidases - Copper, Flavone Etc	IPR011032	Oxidoreductase Activity, Binding, Zinc Ion Binding, Catalytic Activity		2	7E-3
26.8	Misc.Nitrilases, *Nitrile Lyases, Berberine Bridge Enzymes, Reticuline Oxidases, Troponine Reductases	IPR016166	Oxidoreductase Activity, Binding, Catalytic Activity		2	0.89
26.10	Misc.Cytochrome P450	IPR001128	Electron Carrier Activity, Monooxygenase Activity, Iron Ion Binding, Oxygen Binding, Heme Binding		6	4E-2
26.11	Misc.Alcohol Dehydrogenases	IPR011032	Oxidoreductase Activity, Zinc Ion Binding		4	0.91
26.11.1	Misc.Alcohol Dehydrogenases.Cinnamyl Alcohol Dehydrogenase	IPR001509	Alcohol Dehydrogenase (NAD) Activity	Plama Membrane	3	0.99
26.14	Misc.Oxygenases				1	5E-2
26.16	Misc.Myrosinases-Lectin-Jacalin				1	0.89
26.22	Misc.Short Chain Dehydrogenase/Reductase (Sdr)				1	0.89
26.27	Misc.Calcineurin-Like Phosphoesterase Family Protein				2	0.89
26.28	Misc.Gdsl-Motif Lipase	IPR013831	Hydrolase Activity, Hydrolase Activity, Acting On Ester Bonds, Carboxylesterase Activity		1	0.89

26.9	Misc.Glutathione S Transferases				4	0.99
27	RNA				13	0.89
27.1	RNA.Processing				1	0.99
27.1.1	RNA.Processing.Splicing				1	0.99
27.2	RNA.Transcription				4	5E-2
27.3	RNA.Regulation Of Transcription				8	9E-2
27.3.3	RNA.Regulation Of Transcription.Ap2/Erebp, Apetala2/Ethylene-Responsive Element Binding Protein Family				2	0.96
27.3.6	RNA.Regulation Of Transcription.Bhlh,Basic Helix-Loop-Helix Family				1	0.89
27.3.9	RNA.Regulation Of Transcription.C2c2(Zn) Gata Transcription Factor Family				1	0.89
27.3.67	RNA.Regulation Of Transcription.Putative Transcription Regulator				1	0.99
27.3.99	RNA.Regulation Of Transcription.Unclassified				2	0.89
28	DNA		Nucleobase, Nucleoside, Nucleotide Kinase Activity, ATP Binding	Mitochondria	1	0.85
28.1	DNA.Synthesis/Chromatin Structure	IPR002625	DNA Synthesis/Chromatin Structure	Chloroplast	1	0.85
29	Protein				47	1,13E-02
29.1	Protein.Aa Activation				1	0.88
29.1.7	Protein.Aa Activation.Alanine-TRNA Ligase	IPR012947	Ligase Activity, Forming Aminoacyl-TRNA And Related Compounds,	Cytoplasm	1	0.88

			Alanine-TRNA Ligase Activity, Nucleotide Binding, ATP Binding, Nucleic Acid Binding			
29.2	Protein.Synthesis				3	0.85
29.2.1	Protein.Synthesis.Ribosomal Protein				1	0.89
29.2.1.1	Protein.Synthesis.Ribosomal Protein.Prokaryotic				1	0.89
29.2.1.1.1	Protein.Synthesis.Ribosomal Protein.Prokaryotic.Chloroplast				1	0.89
29.2.1.1.1.1	Protein.Synthesis.Ribosomal Protein.Prokaryotic.Chloroplast.30s Subunit				1	0.89
29.2.1.1.1.1.5	Protein.Synthesis.Ribosomal Protein.Prokaryotic.Chloroplast.30s Subunit.S5				1	0.89
29.2.4	Protein.Synthesis.Elongation				2	5,00E-02
29.3	Protein.Targeting				4	0.89
29.3.3	Protein.Targeting.Chloroplast	IPR005692, IPR007378		Chloroplast Inter Membrane	2	0.99
29.3.4	Protein.Targeting.Secretory Pathway				2	0,89
29.3.4.99	Protein.Targeting.Secretory Pathway.Unspecified				2	0,89
29.4	Protein.Postranslational Modification	IPR020417			3	0.89
29.5	Protein Degradation				36	2,16E-04
29.5.1	Protein.Degradation.Subtilases	IPR003137	Serine-Type Endopeptidase Activity	Endomembrane System	5	5,00E-02
29.5.5	Protein Degradation Serine Proease	IPR015724			16	2,16E-04
29.5.7	Protein.Degradation.Metalloprotease	IPR005936	ATP-Dependent Peptidase	Mitochondrion, Chloroplast Thylakoid	3	0.89

			Activity, Atpase Activity; INVOLVED IN: Proteolysis, Protein Catabolic Process;	Membrane		
29.5.9	Protein.Degradation.Aaa Type				2	0.92
29.5.11	Protein.Degradation.Ubiquitin				4	0.99
29.5.11.3	Protein.Degradation.Ubiquitin.E2	IPR016135			1	0.99
29.5.11.4.3	Protein.Degradation.Ubiquitin.E3.Scf				2	0.92
29.5.11.4	Protein.Degradation.Ubiquitin.E3				3	0.99
29.5.11.4.2	Protein.Degradation.Ubiquitin.E3.Ring				1	0.89
29.5.11.4.3.2	Protein.Degradation.Ubiquitin.E3.Scf.Fbox	IPR001810			2	0.92
30.2.25	Signalling.Receptor Kinases.Wall Associated Kinase				4	0.94
30	Signalling				41	5,02E-02
30.2	Signalling.Receptor Kinases				26	5,02E-02
30.2.2	Signalling.Receptor Kinases.Leucine Rich Repeat Ii	IPR002290			1	5,00E-02
30.2.8	Signalling.Receptor Kinases.Leucine Rich Repeat Viii				14	5,00E-02
30.2.8.2	Signalling.Receptor Kinases.Leucine Rich Repeat Viii.Viii-2	IPR017441	Protein Serine/Threonine Kinase Activity, Kinase Activity, ATP Binding	Endomembrane System	14	5,90E-02
30.2.10	Signalling.Receptor Kinases.Leucine Rich Repeat	IPR017441			1	0.85

	X					
30.2.11	Signalling.Receptor Kinases.Leucine Rich Repeat Xi				1	0.85
30.2.12	Signalling.Receptor Kinases.Leucine Rich Repeat Xii				1	0.85
30.2.13	Signalling.Receptor Kinases.Leucine Rich Repeat Xiii				1	0.85
30.2.17	Signalling.Receptor Kinases.Duf 26				4	0.59
30.2.99	Signalling.Receptor Kinases.Misc				2	0.89
30.4	Signalling.Phosphinositides				9	0.85
30.4.4	Signalling.Phosphinositides.Phosphoinositide Phospholipase C	IPR015359	Phospholipase C Activity, Phosphoinositide Phospholipase C Activity, Phosphoric Diester Hydrolase Activity		9	0.85
30.5	Signalling.G-Proteins				4	0.99
30.11	Signalling.Light				2	0.89
3.1	Minor Cho Metabolism.Raffinose Family				1	0.94
31.1.1	Cell.Organisation.Cytoskeleton				1	0.89
31.1.1.1	Cell.Organisation.Cytoskeleton.Actin				1	0.89
31.1.1.1.3	Cell.Organisation.Cytoskeleton.Actin.Actin Binding				1	0.89
3.1.2	Minor Cho Metabolism.Raffinose Family.Raffinose Synthases				1	0.94
3.1.2.2	Minor Cho Metabolism.Raffinose Family.Raffinose Synthases.Putative				1	0.94
31	Cell				10	0.99
31.1	Cell.Organisation	IPR006843	Calcium-	Thylakoid, Chloroplast	10	0.99

			Dependent Phospholipid Binding, Calcium Ion Binding	Thylakoid Membrane, Chloroplast, Plastoglobule		
33	Development				7	0.35
33.99	Development.Unspecified	IPR011046	DNA Binding Transcription Factor Activity/Regulation Of Transcription	Vacuolar Membrane, Nucleous	7	1.47E-2
34	Transport				13	0.87
34.6	Transport.Sulphate				1	0.99
34.9	Transport.Metabolite Transporters At The Mitochondrial Membrane				2	0.89
34.13	Transport.Peptides And Oligopeptides				2	0.99
34.15	Transport.Potassium				1	0.89
34.16	Transport.Abc Transporters And Multidrug Resistance Systems				5	0.85
34.19	Transport.Major Intrinsic Proteins				1	0.89
34.19.1	Transport.Major Intrinsic Proteins.Pip				1	0.89
34.99	Transport.Misc	IPR002528	Atpase Activity, Coupled To Transmembrane Movement Of Ions, MATE Efflux Protein Activity - Antiporter Activity.		1	0.92
35	Not Assigned				291	0.97
35.1	Not Assigned.No Ontology				11	0.89
35.1.5	Not Assigned.No Ontology.Pentatricopeptide				1	0.89

	(Ppr) Repeat-Containing Protein					
35.2	Not Assigned.Unknown				280	0.99