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Qualea grandiflora Mart. e sua dependência química de alumínio: aspectos anatômicos, fisiológicos e moleculares

Qualea grandiflora Mart. and its chemical dependence on aluminum: anatomical, physiological, and molecular aspects

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Brasília-DF
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Tese apresentada ao Departamento de Botânica como parte do requisito para a obtenção do título de doutora em Botânica pela Universidade de Brasília.

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Ao meu pai, um botânico de coração,

À minha mãe, orgulhosa da filha pesquisadora,

À minha avó,

Aos meus professores.

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“A ciência é um esporte coletivo.”
(Walter Isaacson, em A decodificadora)

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RESUMO

O alumínio (Al) é um metal que em solos ácidos se torna tóxico para as plantas, e sua forma mais fitotóxica é o cátion Al^{3+}. No entanto, algumas plantas possuem tolerância/resistência ao Al, sendo que algumas acumulam consideráveis quantidades desse elemento. No Cerrado, várias espécies não apenas acumulam Al, mas precisam dele ao longo de seu desenvolvimento. As Vochysiaceae, uma das famílias mais importantes da flora do Cerrado, destacam-se nesse grupo, pois todas as suas espécies são acumuladoras de Al. *Qualea grandiflora* Mart. (Vochysiaceae), uma das espécies mais representativas do Cerrado, é hermafrodita, diplóide, com altas taxas de endogamia, baixa heterozigosidade e acumula grande quantidades de Al. Alguns estudos mostram que *Q. grandiflora* necessita de Al para o crescimento e desenvolvimento. Entretanto, ainda não se sabe como isso ocorre. Assim, essa tese busca entender como *Q. grandiflora* enfrenta as altas concentrações de Al durante a germinação e desenvolvimento inicial, além de realizar a busca por genes relacionados ao acúmulo/tolerância de Al no genoma desta espécie. Neste sentido, foi investigada a presença e distribuição de Al e outras substânciasde reserva das sementes desta planta. As sementes de *Q. grandiflora* acumularam 6,43 g de Al. Kg^{-1} de matéria seca, mesmo em solo com baixa disponibilidade deste elemento. Ademais, a análise histoquímica revelou que o Al estava localizado principalmente no embrião da semente, que também continha outros compostos de reserva, a saber, os lipídios e as proteínas. Esta tese propõe que os ácidos graxos, proteínas e Al são cruciais para a germinação e o crescimento inicial de plântulas de *Q. grandiflora*. Adicionalmente, o sequenciamento do genoma completo de *Q. grandiflora* foi realizado em plataforma Illumina, e devido à ausência de um genoma de referência, sua montagem foi do tipo *de novo*. O sequenciamento cobriu aproximadamente 81% do genoma total de *Q. grandiflora* que apresenta tamanho aproximado de 500 Mb. Cerca de 11 famílias gênicas que podem estar correlacionados com os mecanismos metabólicos do Al em *Q. grandiflora* foram identificadas. Cinco dessas famílias gênicas foram diferencialmente expressas em resposta ao Al no transcritoma de folhas de *Q. grandiflora*. Desta forma, essa tese traz dados de extrema importância para o entendimento da relação entre o Al e *Q. grandiflora*. Além de ser o primeiro projeto de sequenciamento genômico de *Q. grandiflora,* este trabalho faz uma correlação direta entre o Al e as substâncias de reserva (proteínas/lipídios) em sementes e plântulas *Q. grandiflora*. Tais resultados contribuirão para os estudos de conservação do Cerrado e, principalmente, de *Q. grandiflora.*

Palavras-chaves: *Qualea grandiflora*, alumínio, histoquímica, genoma, lipídios, proteína.

ABSTRACT

Aluminum (Al) is a metal that in acidic soils becomes toxic to plants, and its most phytotoxic form is the Al^{3+}. However, some plants have tolerance/resistance to Al, and some even accumulate considerable amounts of this element. In the Cerrado, several species not only accumulate Al, but need it throughout their development. The Vochysiaceae, one of the most important families of the Cerrado flora, and all its species are Al accumulators. *Qualea grandiflora* Mart (Vochysiaceae), one of the most representative species of the Cerrado, is hermaphroditic, diploid, with high inbreeding rates, low heterozygosity and accumulates large amounts of Al. Some studies have shown that *Q. grandiflora* needs Al for growth and development. However, this process is not yet known. Thus, this thesis seeks to understand how *Q. grandiflora* deals with Al from germination to the search for genes related to Al accumulation/tolerance in this species. In this sense, it was the presence and distribution of Al and other seed reserve components of this plant were investigated. The seeds of *Q. grandiflora* accumulated 6.43 g of Al. Kg^{-1} of dry matter, even in soil with low availability of this element. Furthermore, histochemical analysis revealed that Al was mainly located in the seed embryo, which also the storage place of other reserve compounds, like lipids and proteins. This thesis proposes that fatty acids, proteins, and Al were crucial for the germination and initial growth of *Q. grandiflora* seedlings. This sequencing was performed on an Illumina platform, and due to the absence of a reference genome, its assembly was *de novo*. The sequencing project covered approximately 81% of the total genome of *Q. grandiflora*, which is approximately 500 Mb in size. About 11 identified gene families may be correlated with Al metabolic mechanisms in *Q. grandiflora*. Five of these gene families were differentially expressed in response to Al in the transcriptome of *Q. grandiflora* leaves. Thus, this thesis brings extremely important data that will contribute to a better understanding of the relationship between Al and *Q. grandiflora*. Besides being the first genomic sequencing project of *Q. grandiflora*, this work makes a direct correlation between Al and reserve substances (proteins/lipids) during germination and seedlings of *Q. grandiflora*. These results will contribute to the conservation studies of the Cerrado and, mainly, of *Q. grandiflora*.

Keywords: *Qualea grandiflora*, aluminum, histochemistry, genome sequencing, lipids, protein.

INTRODUÇÃO GERAL

Qualea grandiflora Mart. é uma planta arbórea decídua, heliófita, que ocorre tanto em formações primárias como secundárias do Cerrado (Ferreira et al. 2001). Essa planta é popularmente conhecida como "pau-terra", "pau-terra-do-cerrado" e "pau-terra-da-folha-larga", pertence à família Vochysiaceae, e à ordem Myrtales (APG IV 2016). Morfologicamente, é caracterizada como uma árvore que pode alcançar aproximadamente 15 m de altura, com tronco tortuoso de casca escura, rugosa e pouco espessa (Silva-Júnior et al. 2005). As folhas são opostas, simples, pecioladas, inteiras, coriácea e perinervadas. As flores possuem rancemos terminais ou axilares, brácteas caducas, pedúnculos curtos e pétalas amarelas (Stafleu 1953). Os frutos são secos deiscente com frutificação entre os meses de junho e outubro e suas cápsulas são ovóides (Stafleu 1953). As sementes são aladas do tipo autogiro de 3,5 a 4,0 cm de comprimento, e apresentam síndrome de dispersão anemocórica (Stafleu 1953). A época de dispersão das sementes é na estação seca entre os meses de julho a setembro (Ferreira et al. 2001). A germinabilidade de *Q. grandiflora* é baixa e lenta, apresentando comportamento ortodoxo em relação à dessecação (Carvalho et al. 2006; Ribeiro 2010; Dousseau et al. 2013). Ou seja, são sementes que podem ser desidratadas a níveis baixos de umidade (5 a 7% de umidade) e armazenadas em ambientes com baixas temperaturas (Carvalho et al. 2006; Dousseau et al. 2013).

Além disso, *Q. grandiflora* está entre as dez espécies nativas mais importantes do Distrito Federal (Andrade et al. 2002) e possui ampla distribuição pelo Cerrado (Eiten 1972; Ratter et al. 2003). Adicionalmente, essa espécie não tem muitas restrições quanto ao nível de luminosidade, nutrientes e pH do solo (Haridasan 1982). Isso permite que *Q. grandiflora* seja utilizada em reflorestamento de áreas degradadas de preservação permanente (Dousseau et al. 2013). Ademais, essa espécie possui propriedades medicinais, tendo ação antibacterianas (Ayres et al. 2008), antiulcerosa (Hiruma-Lima et al. 2006), anticonvulsivo e antioxidante (Dousseau et al. 2013).

Q. grandiflora é uma espécie acumuladora de Al (Haridasan 2008; Andrade et al. 2011). Ela acumula Al tanto em solos ácidos como não ácidos sem prejuízos ao seu metabolismo. Por isso, trabalhos recentes investigam se *Q. grandiflora* tem dependência metabólica de Al (Silva 2012; 2017; Cury et al. 2019). Esses trabalhos relataram a existência de Al em plântulas cultivadas sem o elemento (Silva 2012; 2017; Cury et al. 2019). A hipótese sugerida, então, foi

que o Al era oriundo da semente. Sabe-se que as sementes de espécies do Cerrado acumulam grandes quantidades de Al (Haridasan 1987). Além disso, em plantas acumuladoras o Al endógeno parece desencadear os processos germinativos (Haridasan 1987; Rodrigues et al. 2019). Entretanto, pouco se sabe sobre o conteúdo de Al e seu padrão de distribuição nas sementes, e se há relação entre a germinação e o Al em espécies acumuladoras, principalmente em *Q. grandiflora*.

O Al é o metal mais abundante da crosta terrestre, compreendendo cerca de 8% da terra, e participa da estrutura de vários minerais primários como safiras, rubis, rochas ígneas e argilas (Skibniewska e Skibniewski 2019). Quando em solução no solo, os íons de Al sofrem processo de hidrólise e formam o cátion trivalente (Al^{3+}) (Haridasan 2008; Liu et al. 2014). O Al^{3+} é a forma mais tóxica para as plantas, provocando danos, principalmente no sistema radicular dos vegetais (Haridasan 2008; Liu et al. 2014). Em espécies sensíveis, o Al é responsável pela inibição do crescimento das raízes, tornando-as mais grossas, curtas e quebradiças (Ma 2007; Aggarwal et al. 2015). A nível celular, observa-se o rompimento da membrana plasmática das células, a inibição da atividade de muitas enzimas, como também afeta a replicação do DNA, a formação de microtúbulos e a disfunção das mitocôndrias (Ma 2007; Aggarwal et al. 2015).

Na América do Sul, os solos ácidos ocorrem em cerca de 57% das terras agricultáveis (Schmitt et al. 2016). No Brasil, o bioma cujo solo tem a maior acidez e concentração de Al é o Cerrado, principalmente, o Cerrado distrófico (Haridasan 2008). O Cerrado é o segundo maior bioma do Brasil, ocupando cerca de 2 milhões de km^2 do território nacional (Gomes et al. 2011). Em virtude da ameaça de extinção da riqueza florística e da quantidade de espécies endêmicas, o Cerrado é apontado como um dos *hotspots* para a conservação da biodiversidade mundial (Solórzano et al. 2012).

As plantas nativas do Cerrado desenvolveram mecanismos para lidar com o Al livre no solo (Al^{3+}). Essas plantas podem excluir e/ou absorver/inativar esse metal de modo que não há efeitos prejudiciais sobre o crescimento, reprodução e metabolismo dessas espécies (Haridasan 1987; 2008). Dentro do bioma Cerrado, as plantas que atraem mais atenção dos pesquisadores de todo o mundo são as plantas acumuladoras de Al. Estas plantas acumulam mais de 1 g de $Al.Kg^{-1}$ de matéria seca (MS) nas folhas (Chenery 1948). Nas plantas acumuladoras, o Al é absorvido pelas raízes, transportado para as partes aéreas da planta, onde é compartimentalizado e armazenado dentro das células, e, consequentemente, neutralizando os efeitos maléficos deste metal sobre o metabolismo celular, ou usado no metabolismo dessas plantas (Ma 2007; Muhammad et al. 2019; Pidjath et al. 2019).

Os mecanismos de acumulação de Al envolvem vários processos celulares e moleculares nas plantas que ainda são pouco conhecidos (Muhammad et al., 2019; Pidjath et al. 2019). O maior foco desses estudos está associado aos mecanismos de tolerância/resistência ao Al (Grevenstuk e Romano 2013; Pidjath et al. 2019). Plantas tolerantes são consideradas, exclusivamente, aquelas que utilizam mecanismos internos para desintoxicar o Al (Grevenstuk e Romano 2013; Kochian et al. 2015). Ainda não existe uma definição consistente para plantas acumuladoras que dependem do Al para crescer e se desenvolver, o que parece ser o caso de algumas plantas do Cerrado. Posto isso, sabe-se que para as plantas tolerantes mais estudadas, como arroz e *Camellia sinensis* (L.) KUNTZE, o Al induz uma série de expressões gênicas relacionadas a: modificação da parede celular (*STAR1*), exsudação de ácidos orgânicos (*ALMT* e *MATE*) e desintoxificação interna do Al (*NRAT1* e *ALS1*) (Kochian et al. 2015). Sabe-se ainda que nessas plantas os genes *STOP* e *ART* modulam a secreção de ácidos orgânicos, além de regular vários genes de tolerância ao Al (*ALS, NRAT* e *ALMT*) (Kochian et al. 2015; Daspute et. al. 2017).

Segundo Jansen et al. (2002) existem 45 famílias de espécies acumuladoras de Al. Dentre elas, a família Vochysiaceae se destaca por ser composta exclusivamente por espécies acumuladoras de Al (Medeiros e Haridasan 1985; Nogueira et al. 2019). Além disso, essa família é um componente importante nas formações vegetais neotropicais (Sajo e Rudall 2002). Por exemplo, *Q. grandiflora* é provavelmente a árvore mais frequente no Cerrado (Ratter e Dargie 1992; Sajo e Rudall 2002) e algumas espécies de *Vochysia*, *Callisthene* e *Salvertia* também são características dessa vegetação (Sajo e Rudall 2002).

Neste sentido, algumas preguntas nortearam este trabalho, como: em quais regiões da semente de *Q. grandiflora* o Al e as reservas estão localizadas? Quais são os compostos de reserva presentes na semente de *Q. grandiflora*? Qual a correlação entre as reservas nutricionais da semente e o Al? Além disso, buscando compreender a dependência metabólica de Al das plantas de *Q. grandiflora*, procedeu-se o sequenciamento do seu genoma para poder compreender os mecanismos de tolerância ao Al em nível molecular. Assim, em função disso algumas questões foram levantadas: qual o tamanho de genoma? Existe fundamentação no genoma de *Q. grandiflora* que suportam os estudos proteômicos e transcritômicos realizados por Silva (2017) e Cury et al. (2019, 2020)? Existem genes específicos responsáveis pelo processo de acúmulo de Al nessas plantas? Quais? Enfim, há inúmeras questões a serem respondidas sobre a função do Al em *Q. grandiflora*.

Para isso, esta tese foi dividida em três capítulos. O primeiro capítulo trata de uma revisão de literatura que discute os efeitos do Al nas plantas em geral, bem como os mecanismos de acúmulo, resistência e tolerância ao Al com ênfase em plantas nativas. Desta forma, intencionou-se compreender a importância do Al para as espécies do Cerrado, especialmente para as Vochysiaceae. Este capítulo foi publicado como artigo de revisão na revista "*Brazilian Journal of Botany*" (https://link.springer.com/article/10.1007%2Fs40415-021-00781-1). O segundo capítulo tem como objetivo determinar o padrão de distribuição de reservas e Al em sementes de *Q. grandiflora* e deteriminar a importância deste metal na germinação e desenvolvimento inicial de *Q. grandiflora*. O capítulo 2 foi formatado para ser submetido como artigo científico na revista "*Theoretical and Experimental Plant Physiology*", online ISSN: 2197-0025. Enquanto que o terceiro capítulo terá por base o sequenciamento do genoma de *Q. grandiflora* e a identificação de genes envolvidos na resposta ao Al. Esse capítulo foi formatado para ser submetido como artigo científico na revista "*The Plant Journal*", Online ISSN: 1365-313X.

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CHAPTER 1

Molecular and physiological aspects of plant responses to aluminum: what do we know about Cerrado plants?

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Abstract

Aluminum (Al) is a metal that in acid soils becomes toxic to plants, and its most phytotoxic form is Al^{3+}. Usually, Al hinders plant growth and development by impairing processes such as respiration, photosynthesis, and gene expression. Moreover, plants under Al stress upregulate oxidative stress metabolism to handle Al-toxicity. Nevertheless, some plants possess tolerance/resistance to Al. The mechanisms of Al tolerance/resistance involve its complexation, either internally or externally, with organic acids (citrate, malate, and oxalate). Also, some plants can accumulate more than 1 g of Al. Kg^{-1} of dry matter. In the Cerrado, several species not only accumulate Al, but need it throughout their development. The Vochysiaceae stand out as one of the most important families of Cerrado flora and all its species are Al-accumulators. At the molecular level, the gene families *ALMT* and *MATE* have been associated with Al tolerance/resistance in various plant species. Moreover, there have been several reports addressing the relationships between Cerrado plants and Al at the molecular, metabolic, and subcellular levels. Additionally, in Cerrado native species, molecular and physiological data revealed that Al upregulated several crucial processes, e.g., photosynthesis, respiration, genetic information processing, and cell wall synthesis. These studies have, therefore, supported the idea that Al is not a stress factor in these native plants. Nonetheless, there is much to unravel about the role of Al in Cerrado plants and the reasons they require it to grow and develop.

Keywords: Accumulation, mineral nutrition, molecular basis, Savanna Brazilian, Vochysiaceae.

1. Introduction

Aluminum (Al) comprises about 8% of the earth's crust, but usually it is not freely available in soils, with insoluble forms occurring as Al oxides and silicates (Kochian et al. 2005). The octahedral hexahydrate of Al $(H_2O)_6^{3+}$, commonly known as $A1^{3+}$, is soluble in soils with pH < 5 (Kochian et al. 2005). The solubilization process of Al^{3+}, its most phytotoxic form, is associated with soil acidification, which may be a result of acid rain, erosion, or anthropogenic action (Bojórquez-Quintal et al. 2017).

The presence of $A1^{3+}$ in soils is harmful to plants, fungi, and animals, including humans (Bojórquez-Quintal et al. 2017). In humans, Al may cause various diseases such as encephalopathy, dementia, Alzheimer's, and osteomalacia (Bojórquez-Quintal et al. 2017). In plants, Al affects several cellular and physiological processes that can impair growth and developmental processes (Kochian et al. 2005; Rahman et al. 2018). Hence, Al may interfere with cellular respiration, chemical/physical properties of the cell wall, photosynthesis, membrane permeability, genetic processing, and absorption of essential nutrients (Ca, Mg, K, P) (Rahman et al. 2018).

However, with the increased bioavailability of Al^{3+} in acidic environments, plants have developed strategies to deal with this element (Poschenrieder et al. 2019). Two mechanisms have been identified to cope with Al^{3+}. Firstly, an exclusion mechanism that prevents the Al^{3+} in the rhizosphere from entering the root system, which is called resistance. The second, termed tolerance, leads to the accumulation of Al by allowing its access to the inner root regions, and then, neutralizing and transporting it to various organs where it is stored in an inactive form (Kochian et al. 2015).

Cerrado plants have adapted to deal with Al without suffering its harmful effects on their metabolism, allowing them to grow and develop normally (Haridasan 2008). The Cerrado is the second largest Brazilian biome and occupies about 21% of the country (Souza and

Habermann 2012). Moreover, the Cerrado (known as Brazilian Savanna) is the largest and richest neotropical savanna as well as a biodiversity hotspot (Françoso et al. 2015). Furthermore, the Cerrado is a mosaic of phytophysiognomies mainly determined by its climate and edaphic conditions as well as the irregularity of wildfires (Haridasan 1987). The Cerrado may present dystrophic and mesotrophic soils. Dystrophic soils are acidic (pH <5), poor in nutrients and rich in exchangeable Al, while the mesotrophic soils are slightly acidic (pH ≥ 6), rich in calcium (Ca) and magnesium (Mg), and low in exchangeable Al (Haridasan 2008, Bressan et al. 2016; Souza et al. 2017). In addition, regardless of soil type, some Cerrado species accumulate large amounts of Al (Goodland 1971; Haridasan 1982; 1987; Haridasan and Araújo 1988).

The pioneering work of Goodland (1971) on Cerrado plants reported that several Vochysiaceae and Melastomataceae species could be classified as Al-accumulators. However, this author suggested that Al was toxic to these plants, and the scleromorphism (hyperplasia) of Cerrado plants was a result of an Al-related oligotrophism. Therefore, Goodland (1971) agreed with the idea of Arens (1963) who had postulated a direct correlation between aluminotoxic and scleromorphism in Cerrado plants. However, later, it was observed that the high concentrations of Al in native plants were not associated with low contents of essential nutrients in their leaves (Haridasan 1982; 1987). Thus, the Cerrado Al-accumulating species have adapted to high levels of Al, and this metal could not be considered toxic to them (Haridasan 2008).

The Vochysiaceae family comprises approximately 200 species, almost all tropical, and with most of its species confined to South and Central America (Negrelle et al. 2007). In Brazil, this family has the highest Importance Value Index (IVI) in several types of Cerrado vegetation (Machado 1985; Haridasan and Araújo 1988; Simon and Pennington 2012). Haridasan (1982) observed that *Qualea parviflora* Mart., *Q. grandiflora* Mart., *Q. multiflora* Mart., *Vochysia*

thyrsoidea Pohl. and *V. elliptica* Mart. stored large amounts of Al and did not show Al-related deleterious effects (Haridasan 1982; 1987). Thus, Cerrado Vochysiaceae species have evolved towards developing mechanisms to survive in poor and acid soils with high Al^{3+} saturation (Machado 1985). This fact has instigated our curiosity about the roles of this metal during the growth and development of native plants, and there are many questions that remain to be addressed, amongst which: do native plants really require Al to grow and develop? Could this metal be considered essential for these plants? Which molecular processes are responsive to Al in these plants? Thus, this review discusses the effects of Al on plants in general, as well as the mechanisms of Al accumulation, resistance, and tolerance. Moreover, we have attempted to answer the questions above, which involves the issue of how crucial Al is for Cerrado species, especially for the Vochysiaceae.

2. Aluminum-accumulating plants

Al-accumulating plants are those that store at least 1 g of Al $Kg^{-1}$of dry matter (DM) in their leaves (Chenery 1948). Additionally, in the hyperaccumulating species, the contents of Al in their leaves are significantly higher than 1 g of Al Kg^{-1} of DM (Metali et al. 2012). These plants are mainly distributed in tropical and temperate forests (Metali et al. 2012). For example, many species from temperate climates, such as some from the *Symplocos* genera can accumulate up to 24 g of Al Kg^{-1} DM in their organs (Schmitt et al. 2016). *Symplocos spicata* Roxb. is the species with the highest concentration of Al ever recorded with about 72 g of Al Kg^{-1} of DM in its leaves (Schmitt et al. 2016). In the tropical savanna (Cerrado), the arboreal species *Qualea parviflora* Mart. and *Vochysia thyrsoidea* Pohl. accumulate 10 and 17 g of Al Kg^{-1} of DM in their leaves, respectively (Table 1). In terms of Al accumulation, the following families should be highlighted in the Cerrado: Rubiaceae, Melastomataceae and Vochysiaceae (Haridasan 1982; Souza et al. 2015; Bressan et al. 2016; Souza et al. 2017). Moreover, the latter is one of the most important plant families of the Cerrado, and all its species are Al-

accumulating. The Vochysiaceae genera (*Vochysia*, *Qualea*, and *Salvertia*) are predominantly present in studies on the Cerrado flora, regardless of the soil type where they occur (Haridasan 1982; Machado 1985; Haridasan and Araújo 1988; Jansen et al. 2002).

Table 1: Determination of the amount of aluminum (Al Kg-1 of dry matter-DM) in different organs of the species of Vochysiaceae species.

Types of physiognomies	Species	Plant Organ	Al (g Kg-1 DM)	Source
Cerrado *sensu stricto*	*Qualea grandiflora*	leaf	3.9	Andrade et al. (2011)
Cerrado *sensu stricto*	*Q. multiflora*	seed	20.2	Haridasan (1987)
Cerrado *sensu stricto*	*Q. parviflora*	leaf	10	Bressan et al. (2016)
		seed	4.8	Haridasan (1987)
Cerrado *sensu stricto*	*Vochysia thyrsoidea*	leaf	16.6	Machado (1985)
		seed	36.5	Haridasan (1987)
Gallery Forest	*V. pyramidalis*	leaf	5.8	Andrade et al. (2011)
Cerrado *sensu stricto*	*V. tucanorum*	seed	40.5	Haridasan (1987)
Cerrado *sensu stricto*	*V. rufa*	seed	28.4	Haridasan (1987)
Semideciduous Forest	*Callisthene major*	leaf	6.5	Andrade et al. (2011)
Cerrado *sensu stricto*	*Salvertia convallariodora*	seed	28.4	Haridasan (1987)

Additionally, Al-accumulating species are classified as either mandatory/compulsory or optional (Haridasan 2008; Schmitt et al. 2016). Mandatory accumulators cannot normally grow and develop in the absence of Al (Haridasan 2008). For example, *Q. multiflora*, *Q. grandiflora*, *Q. parviflora* and *V. thyrsoidea* plants showed abnormal growth and chlorotic leaves when grown in the absence of Al (Andrade et al. 2011; Cury et al. 2020). In addition, the hemiparasite plant, *Psittacanthus robustus* (Mart.) Marloth, a mandatory Cerrado Al-accumulating species, infects only Al-accumulator hosts (Scalon et al. 2013). Meanwhile, optional Al-accumulating plants only accumulate when it is available in the soil or host, as observed in *Passovia ovata* (Pohl ex DC.) Kuijt (Scalon et al. 2013) and *Struthanthus polyanthus* Mart. (Souza et al.

2018b). These two hemiparasite species infect both Al-accumulating and non-accumulating plants (Scalon et al. 2013; Souza et al. 2018b). The presence of Al in the tissues of *P. ovata* and *S. polyanthus* depends on whether the host is an accumulator (Scalon et al. 2013; Souza et al. 2018b). Also, little is known about why these two categories of Al-accumulating plants coexist in the Cerrado; however, it has been proposed that there might be an evolutionary advantage for each aptitude related to some metabolic processes and associated with defense against herbivory and/or pathogens (Schmitt et al. 2016).

According to Ryan and Delhaize (2010), Al tolerance appears to be a common feature in endemic species of regions with acid soils, where the ability to handle Al^{3+} is a prerequisite for survival. For example, approximately 35% of Cerrado woody species accumulate Al in their leaves (Goodland 1971; Haridasan 1982). However, Al-accumulating Cerrado species are not restricted to a specific soil type (Haridasan and Araújo 1988). For example, *Q. multiflora* and *Q. grandiflora* may also be found in in either dystrophic or mesotrophic soils and accumulate Al in both soil types (Haridasan and Araújo 1988). In fact, according to Haridasan and Araújo (1988), *Q. grandiflora* leaves from trees in mesotrophic soil had higher concentrations of Al (7.2g of $Al.Kg^{-1}$) than those from trees in dystrophic soil (5.4 g of $Al.Kg^{-1}$). Moreover, the Al-accumulator *Callisthene fasciculata* Mart. is an indicator of Cerrado calcareous soils (Haridasan and Araújo 1988; Souza et al. 2019). Besides, this species is one of the most important species in the mesotrophic *Cerradão* (Cerrado phytophysiognomy), which occurs in several regions of Brazil (Haridasan 1982). Thus, the ability to accumulate Al is an intrinsic property of Vochysiaceae family (Jansen et al. 2002; Metali et al. 2012).

In addition, the levels of Al in soils do not interfere with the absorption of macro and micronutrients in Cerrado accumulating species (Haridasan and Araújo 1988). Furthermore, in Vochysiaceae the availability of Al^{3+} in the soil does not affect the capacity of its species to accumulate it (Machado 1985). Thus, based on these observations Al is not toxic to Cerrado

plants, and has beneficial effects on native species (Haridasan 1982; Machado 1985; Haridasan 2008). However, can it be considered an essential element for these plants? Further investigation is needed on this crucial issue. Nonetheless, for at least one Al-accumulating species, tea (*Camellia sinensis* L. Kuntze), this element has been regarded as essential for root growth (Sun et al. 2020). To the best of our knowledge, this is the first indication that Al might be an essential nutrient for some species. Even though, there remains much to discuss about this topic. For this we need to understand how Al affects plant metabolism, the effects of this metal on plant cells, the sites of Al-accumulation and, principally, which transcripts and/or proteins have their expression regulated by Al.

3. Aluminum and Plant Metabolism

To better understand Al response mechanisms, it is necessary to address its action sites in plants, as well as how plants physiologically react to it. Al is a metallic binder with a preference for oxygen donors and high affinity for carboxyl, hydroxyl, and phosphate groups (Poschenrieder et al. 2019). Moreover, Al can also bind to cell components such as the cell wall (pectin carboxyl groups) and chromatin, which contains abundant sugar-phosphates (Matsumoto 1991; Poschenrieder et al. 2019). This linkage can have serious consequences on oxidative stress metabolism, cell respiration, cell wall structure/functioning, cell membranes, cell division, and gene expression (Matsumoto 1991; Poschenrieder et al. 2019). In the following sections some Al-related physiological phenomena are described as well as how it affects plant metabolism. Also, we address the case of the native plants and their relationship with this metal.

3.1. Oxidative stress metabolism and Aluminum

Oxidative stress is a result of an imbalance between production and scavenging of reactive oxygen species (ROS) (Poljsak et al. 2013). ROS are strong oxidizing agents (i.e., O_2^-, H_2O_2, and OH^-) and harmful to cell structures. In a biological system, oxidative metabolism can lead

to an enhancement of ROS by either an increase in production or reduction in antioxidant agents (Munne-Bosch and Jubany-Mari 2001). ROS toxicity is not always correlated to the reactivity of a particular oxygen species but can be a factor of its half-life, allowing it to diffuse and reach longer distances within the cell and, therefore, amplifying the cellular damages (Dumanović et al. 2021). Thus, to prevent the harmful effects of ROS, plants trigger their defense mechanisms to prevent damage caused by ROS, which, usually, involve antioxidant enzymes (Mittler et al. 2004; Foyer and Noctor 2005). These mechanisms are mostly based on enzymes such as peroxidases and superoxide dismutases, whose activities result in the removal of ROS (Wang et al. 2018). For this reason, peroxidases and superoxide dismutases (SOD) have been considered the first line of defense against ROS (Giannakoula et al. 2010; Guo et a. 2018).

ROS overproduction appears to be a typical event in Al sensitive plants when exposed to Al. For instance, in Al sensitive wheat, an oxalate oxidase (Oxo) is upregulated in Al-exposed root tips (Delisle et al. 2001). This enzyme catalyzes the oxidation of calcium oxalate resulting in CO_2, Ca^{2+}, and H_2O_2 (Delisle et al. 2001). Furthermore, in this Al-sensitive wheat, there was a direct correspondence between Oxo transcript abundance, activity, and root growth inhibition (Delisle et al. 2001). Hence, the higher the expression of Oxo, the greater the inhibition of root growth in this plant (Delisle et al. 2001). The authors concluded that the increased expression of Oxo led to toxic levels of H_2O_2, which impaired root growth in these Al-sensitive plants. However, in plants, Al-induced ROS generation is not only a stress elicitor, but it is also crucial for a proper response to Al stress. ROS plays a role in the signaling and regulation of various oxidative stress defense systems, e.g., improving the production of antioxidant molecules, as well as inducing the upregulation of proteins and enzymes to keep up with enhanced demand for ROS removal (Ribeiro et al. 2012; Ranjan et al. 2021). For instance, in two rice cultivars, the exposure to Al triggered their antioxidant defense mechanisms, whose efficiency in dealing

with the Al stress was proportional to the inherent capacity of each cultivar (Ribeiro et al. 2012). Also, in rice seedlings (cultivar Pant-12), the presence of Al activated a defense system composed of at least six enzymes with antioxidant activities, among which SOD and monodehydroascorbate reductase (MDHAR) (Sharma and Dubey 2007).

As mentioned, Al-induced ROS production is correlated with the upregulation of antioxidant genes, which results in an enhancement of plant tolerance/resistance to Al (Guo et al. 2018). Nevertheless, not all plants can maintain a balanced Al-induced ROS production/scavenging only through the expression of antioxidant genes (Chandran et al. 2008; Giannakoula et al. 2010). The disparity between ROS production and scavenging leads to cell death and tissue necrosis (Simonovicova et al. 2004; Chandran et al. 2008). Chandran et al. (2008) studied diferential gene expression in Al-sensitive and -resistant lines of *Medicago truncatula* Gaertn. In this plant, Al induced similar transcript accumulation of ROS-generating enzymes in root tips of both Al-sensitive (S70) and resistant (T32) lines. However, the total number of upregulated ROS genes was lower in the resistant T32 root tips (Chandran et al. 2008). Moreover, a few additional Al resistance-associated genes such as a blue copper protein (BCB), two quinone-oxidoreductases, and a glutathione S-transferase (GST) had their expression significantly upregulated in the Al-resistant T32 line. Then, the combined expression of these genes was observed only in Al-resistant root tips of *M. truncatula* (T32), which could explain why this line had higher Al resistance (Chandran et al. 2008). Consequently, besides the antioxidant capacity of its own oxidative metabolism, the T32 line had an additional contribution of those Al-resistant genes to prevent the damaging effects of ROS accumulation (Chandran et al. 2008).

The upregulation of oxidative stress metabolism is part of a series of metabolic events to cope with Al toxicity in plants. However, the upregulation of this metabolic pathway was not observed by Cury et al. (2019, 2020) in roots of *Q. grandiflora* plants grown with Al (150 µM).

In fact, in this Cerrado species, many enzymes of the oxidative stress metabolism like SOD, MDHAR, disulfde isomerase (PDI), and GST had lower relative abundance compared with plants grown without Al supplementation (Cury et al. 2019). Additionally, the lack of Al also induced stress signs in *Q. grandiflora* plants such as chlorosis, lower biomass, and stuntedness (Cury et al. 2019). These data strengthen the idea that oxidative stress metabolism is not upregulated in *Q. grandiflora* plants by Al, because, as a mandatory accumulator, Al is not a stress elicitor in this species (Cury et al. 2019). Thus, this is the first time that molecular data are able to explain what had already been observed in the field (Haridasan 1982; Machado 1985; Haridasan and Araújo 1988; Haridasan 2008), which means, Al is required for the growth and development of *Q. grandiflora*. However, while this is an important first step it is still necessary to unravel how Al acts at the gene expression level.

In excessive quantities, Al might become toxic even to Al-accumulating plants. This hypothesis is consistent with what was observed by Bressan et al. (2020) with the Al-accumulating Cerrado species *Styrax camporum* Pohl. At lower Al quantities (0 and 740 µM), *S. camporum* had normal development; however, at 1480 µM, these plants showed signs of Al-toxicity (Bressan et al. 2020). Also, Rodrigues et al. (2019) studied the effects of various concentrations of Al (0, 200, 400, 600, 800 µM Al^{3+}) on *Eugenia dysenterica* (Mart.) DC, another Al-accumulating Cerrado species. Although no substantial changes in anatomical and physiological parameters were observed, at the highest concentrations (600–800 µM Al^{3+}), the activity of antioxidant enzymes was at their peak. Also, these high Al concentrations undermined the germinative vigor of *E. dysenterica* seeds (Rodrigues et al. 2019). The authors also suggested that, depending on the quantity, Al could compromise both seed vigor and seedling development, even in tolerant species. Besides, the significant increase in antioxidant activity can be a metabolic red flag, indicating that the Al levels could be reaching a toxicity threshold. Additionally, *E. dysenterica* root growth was stimulated when plants were grown

with 200 µM Al^{3+} (Rodrigues et al. 2019), which suggests that there might have an optimal amount of Al for accumulating plants.

3.2. Aluminum and cellular respiration

In plants, the depletion of mitochondrial respiration by Al stress results in ROS production (Panda et al. 2008). This event is commonly observed in most Al-sensitive plants (Mattiello et al. 2014; Wang et al. 2014). Nonetheless, in Al-resistant maize, the gene expression profile of Al-treated plants showed that TCA (tricarboxylic acid) cycle genes were upregulated in comparison with the sensitive cultivars (Mattiello et al. 2014). A similar phenomenon was observed in an Al-resistant rice cultivar— Nipponbare—which also showed an enhanced expression of the TCA cycle enzymes isocitrate dehydrogenase (IDH), aconitate hydratase (ACO), and succinyl-CoA ligase betachain (SUCL) (Wang et al. 2014). Additionally, the expression of TCA cycle enzymes may be correlated to an increase in production of organic acids (OAs) such as malate and citrate, which are frequently associated with Al tolerance/ resistance mechanisms (Mattiello et al. 2014).

Additionally, Wang et al. (2014) observed that some glycolysis genes were significantly upregulated in Al-resistant rice plants when exposed to Al. This fact could indicate an effort to maintain ATP production through glycolysis to handle Al^{3+} stress (Wang et al. 2014). Likewise, in Al-tolerant maize, the glycolytic pathway enzymes had their expression upregulated in comparison with the sensitive cultivar (Mattiello et al. 2014). However, in *Lotus corniculatus* L., key glycolytic enzymes like pyruvate kinase, enolase, and phosphoglycerate kinase were strongly downregulated in plants under Al stress (Navascués et al. 2012). The authors suggest that Al induced ROS accumulation and oxidative stress caused metabolic dysfunction in the mitochondria and other cellular compartments of *L. corniculatus*.

In the Al-accumulating species *C. sinensis*, a proteomic analysis compared the expression of proteins from various metabolic processes, e.g., photosynthesis, glycolysis, and TCA cycle

between Al-treated and non-treated plants (Xu et al. 2017). In *C. sinensis*, while Al upregulated glycolysis in roots, it significantly reduced glycolytic metabolism in leaves (Xu et al. 2017). Nevertheless, citrate synthase (CS), a TCA cycle enzyme, was upregulated in both root and leaf. It is believed that this increase in citrate synthesis would be needed to meet the demand for Al chelation and transportation (Xu et al. 2017). This fact is consistent with the higher contents of Al in *C. sinensis* leaves from plants grown with Al (Xu et al. 2017).

It also appears that Al tolerance/resistance is linked to a higher respiratory capacity. Plants capable of upregulating TCA cycle and/or glycolysis can deal much better with Al than those incapable of doing so (Mattiello et al. 2014; Wang et al. 2014; Xu et al. 2017). Therefore, it can be inferred that these metabolic pathways play a key role in plant responses to Al, therefore, they are excellent targets to improve Al resistance/tolerance in plants.

In *Q. grandiflora*, the TCA cycle was upregulated in roots from Al-supplemented plants (Cury et al. 2020). Furthermore, in this plant, Al induced greater relative abundance of various TCA cycle enzymes such as malate dehydrogenases (MDH1, and 2), isocitrate dehydrogenase (IDH1, 2, and 3), pyruvate dehydrogenase (PDH), succinyl-CoA synthetase 1 and 2 (Cury et al. 2020). In addition, five enzymes (ATP synthase subunit, NADH dehydrogenase ubiquinone Fe-S1/FeS2, V-type proton ATPase catalytic subunit A, and NADH dehydrogenase [ubiquinone] 1 subcomplex-subunit 2 alfa) of the oxidative phosphorylation process were upregulated in Al-treated *Q. grandiflora* plants (Cury et al. 2020).

3.3. Organic acids, phenolic compounds, and Aluminum

Aluminum induces the upregulation of genes involved in the efflux of organic acids (OAs) in response to the overproduction of ROS in higher plants (Chauhan et al. 2021). Organic acids, internally and/or externally, participate in mechanisms of Al tolerance/resistance (Barceló and Poschenrieder 2002; Grevenstuk and Romano 2013). Also, the efflux of OAs such as malate,

citrate and oxalate by roots is the usual response of many plant species to Al (Ma et al. 2001; Barceló and Poschenrieder 2002).

Furthermore, OA production is not only correlated to Al tolerance/resistance. The mechanism of Al accumulation in plants also encompasses the formation of Al-OAs complexes for transportation and storage as well (Singh et al. 2017; Xu et al. 2017). The OAs can neutralize free Al within plant cells, and, in accumulating plants, they also assist in its transportation from roots to shoots (Barceló and Poschenrieder 2002; Grevenstuk and Romano 2013; Singh et al. 2017). Furthermore, Shen and Ma (2001) investigated the distribution of Al in buckwheat (*Fagopyrum esculentum* Moench), an herbaceous species described as an Al-accumulator that can store a high quantity of Al (~1.68 g $Al.kg^{-1}$ of DM), mostly in its leaves. Despite the high Al content in this organ, buckwheat leaves had no signs of toxicity (Shen and Ma 2001). The internal Al content in buckwheat leaves was neutralized by oxalate, which formed a stable complex with Al (Ma et al. 1997; Ma et al. 1998; Ma 2001). This fact also shows that OA can be involved in Al detoxification as well as in its transportation and accumulation. Therefore, OAs could be crucial for the whole tolerance process of Al in plants.

There are three OA anions that usually complex with Al in the following order: citrate > oxalate > malate (Brunner and Sperisen 2013). These OAs are found in all plant cells because of mitochondrial respiration (Brunner and Sperisen 2013). Bressan et al. (2020) studied the effects of various concentrations of Al (0, 740, and 1480 µM) on mitochondria in root tip cells of *Styrax camporum*, a native optional Al-accumulating species. In this plant, the mitochondria were conspicuous in root cells from plants treated with lower concentrations of Al (0 and 740 µM), whilst at 1480 µM, these organelles were no longer seen. In addition, *S. camporum* seedlings grown at 740 µM of Al released more citric and oxalic acids compared with those exposed to 1480 µM (Bittencourt et al. 2020). Therefore, this phenomenon may be associated with a more active TCA cycle in *S. camporum* root tips treated with lower amounts of Al,

whose cells had abundant and conspicuous mitochondria (Bittencourt et al. 2020; Bressan et al. 2020). Furthermore, at 1480 µM of Al, the plants showed signs of Al-toxicity including lower growth of the root system and stunted plants, while plants supplemented with zero and 740 µM of Al had normal growth and development (Bressan et al. 2020).

The type and quantity of OAs produced in roots and leaves depends on the species as well as the availability of Al in the soil (Brunner and Sperisen 2013; Singh et al. 2017). In Al-accumulating Cerrado plants, the presence of citrate has been correlated with the transportation of Al from roots to shoots, likely via the xylem (Souza et al. 2019; Cury et al. 2020). *C. fasciculata* plants grown in soils with high concentrations of Al have greater concentrations of citrate and oxalate than those grown in soils with either low concentrations or no available Al (Souza et al. 2019). In addition, *Q. grandiflora* roots from plants supplemented with Al produced twice as much citrate than those not supplemented (Cury et al. 2020). Furthermore, two hemiparasites species found in the Cerrado, *P. ovata* and *S. polyanthus* (optional Al-accumulation), infecting *M. albicans* (mandatory accumulator of Al), had high levels of citrate (Souza et al. 2018a). Unlike the host plant, *M. albicans* had higher concentrations of oxalate (Souza et al. 2018a). Thus, besides being specific to each species, citrate, malate, and oxalate could be involved in the transportation of Al (xylem) from the roots to shoots in Cerrado species (Fig 1). However, the proteins involved in secreting/transporting Al-OA complexes across cell membranes in native species remain unknown.

Besides OAs, phenolic compounds may complex free Al as well. It has been noted that roots of *Vochysia tucanorum* and *Q. grandiflora* contain significant amounts of quinic acid (Souza et al. 2017; Cury et al. 2020), which is a precursor of some phenolic compounds such as chlorogenic acids, and possibly lignins (Wang et al. 2013c; Silva et al. 2019). Quinic acid was considered a potential marker of metabolic changes in *leaves* of *V. tucanorum* (Souza et al. 2017). Furthermore, quinic acid esters may act as antioxidants and the ability to deal with

oxidative stress is a key factor in metal plant accumulation process (Souza et al. 2018a). Therefore, in addition to using OAs, *V. tucanorum* and *Q. grandiflora* might also use phenolic compounds to handle Al in their organs.

3.4. Aluminum and photosynthesis

Carbon dioxide (CO_2) assimilation is reduced under Al stress in many plants including citrus (Chen et al. 2005; Jiang et al. 2009a; 2009b), *Quercus glauca* Thunb. (Akaya and Takenaka 2001), wheat (Moustakas et al. 1995), and maize (Lidon 1997). Nevertheless, the impacts of Al on Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) still needs clarification (Silva 2012). Some reports say that Rubisco activity did not change or, at most, undergoes only a slightly decrease in the presence of Al (Jiang et al. 2008, 2009a, b). However, the activity of Rubisco and other key enzymes of the Calvin cycle, e.g., NADP-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and phosphoribulokinase (PRK) are significantly enhanced in Al-treated leaves of *Citrus reshni* Hort. seedlings (Chen et al. 2005). Even though, in this plant a reduction in CO_2 assimilation was observed. Thus, this drop in CO_2 assimilation was not directly associated with lower activity of Rubisco/Calvin cycle enzymes (Chen et al. 2005). The likely cause of the Al-induced CO_2 assimilation decrease was an impairment of the capacity of electron transport due to a photoinhibition of Photosystem II, as well as lower chlorophyll content (Chen et al. 2005; Jiang et al. 2008). Hence, Al may severely hinder plant growth rate by diminishing photosynthetic efficiency in sensitive plants. Therefore, a functional photosynthetic system is crucial to dealing with Al toxicity.

Consistent with this statement, in other citrus species, the maintenance of photosynthesis as well as the expression of photosynthetic and energy-related proteins contributed to Al tolerance (Li et al. 2016). For instance, in *Citrus grandis*, Al lowered CO_2 assimilation (Li et al. 2016). Conversely, in *C. sinensis*, which has higher tolerance to Al, CO_2 assimilation was not affected by its presence (Li et al. 2016). Furthermore, Al induced a decrease of chlorophyll

contents in both *C. sinensis* e *C. grandis*; however, this drop in chlorophyll level was greater in the sensitive *C. grandis* leaves (Guo et al. 2018).

Concerning native plants, it has been reported that the lack of Al supplementation in *Q. grandiflora* might have altered the typical shape and structure of chloroplasts (Cury et al. 2020). Besides, *Q. grandiflora* chloroplasts accumulate Al (Andrade et al. 2011), which could be related to the fact that its absence harms chloroplast structure. Moreover, leaves from Al-treated *Q. grandiflora* Al had higher contents of photosynthetic pigments (chlorophyll *a* and *b*, and carotenes) than those grown without it (Cury et al. 2020). On the other hand, although *Qualea cordata* Spreng. grown in lower levels of acidity had an increased content of mineral elements in its leaves, it accumulated less biomass (Alvim et al. 2017). Additionally, the chlorophyll levels were not altered in low acidity conditions. However, these unusual growth conditions for a native species induced a significant 2-fold enhancement of carotenoid content in *Q. cordata* leaves, which may indicate a higher need for photoprotection in these plants (Alvim et al. 2017). Furthermore, *Q. cordata* plants that grew in deacidified soils with low Al^{3+} had significantly lower biomass in both roots and shoots. This fact might indicate a debilitated production of carbon skeletons, and consequently, less biomass yield. It appears that, like *Q. grandiflora*, *Q. cordata* may also require a certain level of Al for photosynthetic purposes.

Another aspect that must be taken into consideration is the fact that the roots from Al-treated *Q. grandiflora* plants had about 4- to 5-fold the amount of fructose and glucose than that those grown without it (Cury et al. 2020). It is likely that the better structured photosynthetic apparatus of Al-supplemented plants had resulted in a higher accumulation of carbohydrates in these roots, and therefore, higher biomass (Cury et al. 2020). However, a few crucial questions remain. Which photosynthesis-related processes require Al in *Q. grandiflora*? Why do *Q. grandiflora* chloroplasts store Al? Is Al required for chloroplast differentiation in this species? Is Al needed for photosynthesis in all Cerrado accumulating species? For certain,

there is much to learn about what roles Al may play in photosynthesis of mandatory accumulating plants.

4. Aluminum Effects on Plant Cells

4.1 Programed Cell Death

It appears that Al-induced ROS production mediates a cell death-like response, as seen in tobacco cells (Panda et al. 2008), barley (Simonovicova et al. 2004), yeast (Zheng et al. 2007), and wheat (Delisle et al. 2001). Molecular analyses showed that Al enhanced the expression of genes associated with cell death in the root tips of *M. truncatula* (Chandran et al. 2008). Initially, genes related to cell death, senescence, and cell wall degradation were upregulated in both Al-sensitive and tolerant *M. truncatula* lines (Chandran et al. 2008). However, after 48 h of Al treatment, these genes remained preferentially active in the S70 Al-sensitive line (Chandran et al. 2008).

At the subcellular level, several Al-binding sites have been identified, including the cell wall, plasma membrane, cytoskeleton, nucleus, chloroplast, and mitochondrion. For instance, Al triggers several mitochondrial abnormalities in sensitive nonchlorophyllic SL tobacco cell lines (Panda et al. 2008). When exposed to Al, SL tobacco cells had swollen mitochondria, with abnormal outer membrane architecture, among which membrane bursts, cristae breakage, and internal electron-dense material was detected (Panda et al. 2008). Mitochondrial swelling may indicate the opening of the Mitochondrial Permeability Transition (MPT) pores and Cytochrome c (Cyt. c) leakage, which, in plants and animals alike, are consistent with them undergoing programmed-cell-death (PCD) processes (Panda et al. 2008; Mashayekhi et al. 2014).

Additional abnormalities were seen in Al-treated SL tobacco cells that showed plasma membranes with unusual folding and plasmolysis (Panda et al. 2008). Furthermore, chromatin

condensation and fragmentation were also seen in these Al-treated cells, which are considered pre-apoptotic phenomena (Panda et al. 2008; Panda et al. 2009). Similar nuclear changes were observed in apoptotic BY-2 tobacco cells under UV-B light stress (Lytvyn et al. 2010). Therefore, all events described here are consistent with the idea that Al may induce a mitochondrial pathway PCD (Panda et al. 2008).

Additionally, in *Arabidopsis*, Al exposure induced the expression of *AOX1a* (alternative oxidase 1a) which is an Al-upregulated gene that maintains the electron flux and reduces the formation of mitochondrial ROS (Liu et al. 2014). AOX1a may also participate in the regulation of mitochondrial pathway PCD (Liu et al. 2014), and the expression of AOX1a triggers the expression of protective functional proteins that prevent the establishment of Al-induced PCD (Liu et al. 2014). In contrast, in the roots of *Q. grandiflora* there was no differential expression of proteins associated with programmed cell deaths in either treatment (Cury et al. 2020). It appears that an uncoordinated Al-induced PCD process is not common to Al-accumulating plants.

4.2 Aluminum and DNA Structure

Both endogenous and exogenous factors can block DNA replication and transcription. Additionally, appropriate DNA repair is crucial for chromatin integrity as well as for cell metabolism (Wood 1995; Bray and West 2005). It has been found that DNA damage is one of the effects of Al toxicity in yeast (Wang et al. 2013a). Wang et al. (2013a) identified several proteins that are targeted by Al in *Rhodotorula taiwanensis*, many of which are DNA repairing enzymes. For instance, Al diminished the abundance of the DNA N-glycosylase and apurinic/apyrimidinic lyase, which are responsible for removing oxidized ring-saturated pyrimidines from DNA (Meadows et al. 2003; Wang et al. 2013a). Further, in plants, the formation of DNA-Al complexes could result in a repression of the transcriptional process by altering chromatin structure due to a more rigid DNA double helix (Matsumoto 1991). In

Arabidopsis, it has also been proposed that Al may induce a Z- DNA conformation, which would tremendously impact gene expression (Nezames et al. 2012).

Several other chromosomal and nuclear abnormalities have been correlated with Al toxicity. In garlic roots (*Allium cepa* var. agrogarum), Al led to irregular nucleolar behavior during mitosis (Qin et al. 2010). Initially, the nucleoli of cells grown with Al were not disassembled as expected during the initial phases of mitosis. Subsequently, nucleolar reconstruction did not occur, and formation of sticky chromosomes was observed (Qin et al. 2010). Chromosome stickiness is an irreversible phenomenon that involves nuclear shrinkage and abnormal chromatin condensation and may lead to cell death (Rieger et al. 1976; Rayburn et al. 2002). It is noteworthy that this feature was also found in maize microsporocytes grown in the Brazilian Cerrado, which is notorious for its acid soils and high Al content (Caetano-Pereira et al. 1995). In addition, lagging chromosomes, chromosome fragmentation, and anaphase bridges were seen in cells of wheat root tips grown in soils with high levels of Al (Rayburn et al. 2002). Thus, Al toxicity can affect DNA structure and functionality as well as regular mitosis in plant cells.

In contrast, in Al-accumulating plants, Al may be necessary for genetic information processing. In a pioneering study on the essentiality of Al in *C. sinensis* plants, Sun et al. (2020) suggest that Al is required to maintain DNA integrity. In *C. sinensis* roots, Al was mainly found in the nuclei of meristematic root cells (Sun et al. 2020). Nevertheless, when the Al treatment was withdrawn, the nuclear Al^{3+} gradually moved to the cytosol. This movement caused damage to the DNA of *C. sinensis* root cells (Sun et al. 2020). Thus, it was concluded that the Al was crucial for cell division and root elongation in this species (Sun et al. 2020).

Likewise, Al might also favor DNA and chromatin structure, and genetic information processing in *Q. grandiflora*. A KEGG (Kyoto Encyclopedia of Genes and Genomes) analysis of the root proteome of this species showed that Al upregulated many cellular essential

processes, among which was genetic information processing (Cury et al. 2020). About 62% of the Al-upregulated proteins in *Q. grandiflora* are involved with genetic information processing and primary metabolism. Genetic information processing alone accounts for 30% of all the differentially expressed proteins in *Q. grandiflora* (Cury et al. 2020). In addition, Al also induced an increase in nucleotide and amino acid metabolism and ribosomal activity, which likely correlates with an intense processing of the genetic information in *Q. grandiflora* (Cury et al. 2020). The Al-treated *Q. grandiflora* plants grew faster, produced more biomass (cell division/elongation), and then, likely because they demand higher levels of gene expression, exhibited greater activity of nucleic and amino acid syntheses, and ribosomal activity as well. According to Cury et al. (2020), all these processes were upregulated by Al in this species. Thus, Al has no toxic effects on DNA/chromatin of *Q. grandiflora*.

4.3 Plasma membrane

Besides the peroxidation and breakdown of lipids, Al can also bind to proteins and fatty acids of the plasma membrane, which may alter its properties including membrane selective permeability. For instance, in sorghum and maize roots, Al induced modifications in fatty acid composition as well as lowered the double bond index (DBI) of root tip plasma membranes (Peixoto et al. 2001; Chaffai et al. 2005). The DBI is a measure of membrane fluidity. Then, the Al-induced-change of DBI suggests that the Al stress has significantly reshaped the permeability of membranes of an Al-sensitive sorghum cultivar (Peixoto et al. 2001). Furthermore, these changes in membrane lipids were the cause of Al-toxicity-associated effects that included an inhibition of root elongation as well as the swelling, bending, and darkening of root apices (Chaffai et al. 2005).

Moreover, membranes control the movement of molecules in and out of cells, as well as among its internal compartments. Thus, cellular membranes integrate the response mechanisms to Al in plants. There have also been reports that correlate the preservation of membrane H^+-

ATPases (vacuolar and mitochondrial) activities to plant Al resistance/tolerance mechanisms (Hamilton et al. 2001; Wang et al. 2014). ATPases play various roles in plant cells, among which as proton pumps, phloem sucrose loading/unloading, stomata opening, and stress responses (Wang et al. 2014). Thus, the maintenance of high levels of ATPases by tolerant/resistant cultivars are presumed to enhance Al^{3+}-related-stress responses. For example, under Al^{3+} stress, four H^+-ATPases (Accession no: LOC_Os01g49190, LOC_Os04g56160, LOC_Os06g43850, and LOC_Os02g03860) were upregulated in Al-tolerant rice cultivar (Wang et al. 2014). Conversely, in sensitive plants, such as Squash (*Cucurbita pepo* L. cv Tetsukabuto), in the plasma membrane of root cortex and epidermal cells underwent an Al-dependent H^+-ATPase inhibition, which was coupled with a zone-specific depolarization of plasma membrane surface potential (Ahn et al. 2001). Consequently, it negatively affected the membrane transportation kinetics, nutrient subcellular contents, and the absorption of important minerals such as NO^{3-}, Ca^{2+}, Na^+, and K^+ (Reid and Hayes 2003; White and Broadley 2003; Castillo et al. 2015).

4.4 Cell Wall

Concerning cell wall synthesis, Al stress can cause a reduction in the amount of Golgi proteins, which might affect the deposition of polysaccharides in the cell wall. In *R. taiwanensis,* Al diminished the abundance of some Golgi-associated proteins (Wang et al. 2013a). This fact may indicate that the Golgi apparatus is a target of Al toxicity, and therefore, it can modify how this organelle works in higher plants by disrupting enzymatic activities in this organelle (Matsumoto 2000; Wang et al. 2013a). This fact is relevant for plant cell growth and development. It has been shown that any distortion in cell wall synthesis or components may result in cell wall abnormalities (Pereira et al. 2006).

Furthermore, in roots of *Q. grandiflora* seedlings supplemented with Al, several proteins associated with cell wall synthesis had their abundance increased (Cury et al. 2020). For

instance, pectinesterases and *S*-adenosyl methionine synthetase (SAMS) were amongst the upregulated proteins in response to Al (Cury et al. 2020). Pectinesterases control the degree of pectin methylesterification, and therefore, they play a role in cell wall adhesion and rigidity (Pereira et al. 2006; 2007; Kohli et al. 2015). Concomitantly, SAMS is responsible for the formation of *S*-adenosyl methionine (SAM), which is the main methyl donor for transmethylation reactions (Moffatt and Weretilnyk 2001). SAM is used by SAM-dependent methyltransferases, which are crucial for the synthesis of several cell wall components, such as pectins and lignin (Moffatt and Weretilnyk 2001; Pereira et al. 2006; 2007).

It is noteworthy that, compared with non-treated plants, the relative abundance of quinate in roots of Al-treated *Q. grandiflora* plants was about 37-fold higher (Cury et al. 2020). This immense difference might have crucial metabolic implications. Quinate is a precursor of phenolic compounds, including lignin (Cesarino et al. 2012). Regarding abundance, lignin is second only to cellulose, and accounts for about 30% of organic carbon on earth (Cesarino et al. 2012). This polymer is commonly found in the primary and secondary cell walls of plant cells, and its deposition has major impacts on plant biomass. Proteomic, metabolomic, and growth analyses of *Q. grandiflora* roots are very coherent regarding the effects of Al on lignin synthesis (Cury et al. 2020). In addition, several enzymes involved in lignin synthesis were also upregulated by Al such as the caffeoyl shikimate esterase, laccase, four peroxidases, and a bifunctional 3-dehydroquinate dehydratase/shikimate dehydrogenase chloroplastic-like protein (Cury et al. 2020). Therefore, the existence of a positive correlation between lignin and Al in *Q. grandiflora* is highly likely.

Conversely, in another Al-accumulating species, *C. sinensis*, the binding of Al and phenolic acids in cell walls reduced lignin synthesis (Xu et al. 2017). In leaves and roots of *C. sinensis*, there was a downregulation of 4CL (4-coumarate CoA ligase), cinnamoyl-CoA reductase, cinnamyl alcohol dehydrogenase, peroxidase, and the expression of F5H (ferulate -

5-hydroxylase), which suggests a reduction of lignin synthesis in this plant (Xu et al. 2017). Hence, not all Al-accumulating plants behave the same in the presence of this element.

5. Aluminium accumulation sites in plants

Al can be accumulated in both vegetative (leaf, stem, and root) and reproductive organs (flower, fruit, and seed) in accumulating plants (Jansen et al. 2002; Grevenstuk and Romano 2013). For instance, when hydrangea (*Hydrangea macrophylla* Thunb.) is grown in acidic soils, the color of the sepals changes from red to blue because of the formation of an anthocyanin-Al complex in the vacuoles (Negishi et al. 2012). The vacuolar Al storage in hydrangea sepals can reach concentrations higher than 15 mM (Negishi et al. 2012).

It appears that, in Vochysiaceae, the amounts of Al in seeds may usually be higher than in the leaves (Table 1). For example, *V. thyrsoidea* had 17 and 36 g of Al Kg^{-1} of DM in leaves and seeds, respectively (Table 1). Hence, besides growth and development of this species, Al may also be crucial for seed germination and seedling establishment (Haridasan 1987).

In addition, the accumulation of Al in leaves of accumulating species has been thoroughly reported in the literature (Haridasan et al. 1987; Jansen et al. 2002; Grevenstuk and Romano 2013). The storage of Al in leaves of Vochysiaceae species is not dependent on the Cerrado physiognomy as seen in Table 1. It has been shown that *V. pyramidalis* stored 5.8 g of Al Kg^{-1} of DM in leaves when in gallery forest whose soils have approximately 1.06 $g.dm^{-3}$ of Al (Andrade et al. 2011). Likewise, *Q. grandiflora* accumulated 3.9 g of Al Kg^{-1} of DM in its leaves in Cerrado *sensu stricto* with 0.7 $g.dm^{-3}$ of Al in the soil (Andrade et al. 2011). Thus, the Al accumulation trait and the relationships between foliar elemental and Al concentrations may contribute to species habitat partitioning and ecosystem-level differences in biogeochemical cycles (Metali et al. 2015).

At the cellular and tissue levels, Al is commonly found in cell walls, vacuoles, as well as in the living cells of the phloem and xylem (Table 2) (Haridasan et al. 1987; Andrade et al.

2011; Bressan et al. 2016; Nogueira et al. 2019). The detection of Al in tissues and organs may vary according to the method used. The use of aluminon and hematoxylin are among the histochemical most used tests to determine the distribution of Al in plant tissues (Andrade et al. 2011).

Table 2 Histolocalization of aluminum in leaves of Vochysiaceae species as detected by Haridasan et al. (1987); Andrade et al. (2011); Bressan et al. (2016); Nogueira et al. (2019). (-) absence (+) presence (ud) Unidentified.

		Qualea				***Vochysia***			***Callisthene***
Plant Tissue	**Site**	*Q. grandiflora*	*Q. multiflora*	*Q. parviflora*	*V. thyrsoidea*	*V. pyramidalis*	*V. rufa*	*V. elliptica*	*C. major*
Epidermis	Cuticle	-	-	-	-	+	-	-	+
	Wall Cell	+	+	+	+	+	+	+	-
Parenchyma	Wall Cell	+	+	-	+	+	+	-	-
	Chloroplast	+	ud	+	ud	ud	ud	ud	+
Collenchyma	Wall Cell	-	-	-	-	-	-	+	-
Sclerenchyma	Wall Cell	-	-	-	-	-	-	-	-
Phloem	Sieve tube element	+	+	+	+	-	+	+	-
	Parenchyma	+	-	-	-	-	-	-	-
Xylem	Vessel element	-	-	-	-	-	-	-	-
	Parenchyma	+	-	-	-	+	-	+	+

Using aluminon, Haridasan et al. (1986) located Al in leaves of various Cerrado accumulating species, among which *Qualea grandiflora, Q. parviflora, Q. multiflora, Vochysia elliptica, V. thyrsoidea, V. rufa*. In these plants, Al was detected in the cell wall of the epidermis, spongy parenchyma, and collenchyma, and in all phloem elements of leaf veins (Haridasan et al. 1986). Nevertheless, Al was not observed in xylem vessel elements, libriform fibers, and leaf palisade parenchyma (Haridasan et al. 1986).

Diversely, the hematoxylin method showed a slightly different pattern of Al distribution in these Vochysiaceae species (Andrade et al. 2011). Additionally, Table 2 shows, in most plants, the presence of Al in the leaf parenchyma (spongy and palisade) as well as in the epidermal cell walls (Andrade et al. 2011). Besides, in *V. pyramidalis* and *C. major* Al was also found in the cuticle and cell wall of epidermal cells (Andrade et al. 2011). Regarding the presence of Al in the vascular tissues, it was detected in the sieve elements and parenchyma of phloem of *Q. grandiflora*; however, in *Q. multiflora, Q. parviflora, V. thyrsoidea, V. rufa,* and *V. elliptica,* Al was only seen in the sieve elements (Haridasan et al. 1987; Andrade et al. 2011; Bressan et al. 2016; Nogueira et al. 2019). Furthermore, this element was found in the xylary parenchyma of *Q. grandiflora*, *V. pyramidalis*, *V. elliptica* and *C. major* (Haridasan et al. 1987; Andrade et al. 2011; Bressan et al. 2016). Note that only hematoxylin could detect Al in the xylary parenchyma of some species (Andrade et al. 2011; Bressan et al. 2016). This small inconsistency between these histochemical tests might be associated with their sensitivity, and therefore, it could indicate that hematoxylin would be more sensitive for detecting Al in plant tissues (Andrade et al. 2011; Bressan et al. 2016). Besides this sensitivity issue, it also appears that Al distribution pattern in plant tissues may vary among the accumulating species as demonstrated in Table 2.

In addition, some Al storage sites are frequently reported in the Al-accumulating species. For example, cell walls are considered one of the most important sites of Al accumulation in

plants (Blamey 2001). It has been known that Al has a high affinity for cell wall components, such as cellulose, hemicelluloses, and pectins, which facilitates its immobilization in the cell wall (Yang et al 2011; Zhu et al 2015; Kopittke and Wang 2017). Table 2 shows the presence of Al in the cell wall of plant primary tissues of accumulating plants has been reported in *Q. grandiflora*, *Q. multiflora*, *V. thyrsoidea* (Haridasan et al. 1986), *V. pyramidalis* (Andrade et al. 2011), *Miconia albicans*, *M. rubiginosa* (Bressan et al. 2016). Additionally, in plants that tolerate but do not require Al in their metabolism, the cell wall works as a metal sequestration site, impeding the Al from coming into direct contact with metabolically active molecules/structures and essential biochemical processes (Grevenstuk and Romano 2013).

Besides the cell wall, Al was also observed in cuticles of epidermal cells of leaves of *V. pyramidalis* and *C. major*, as seen in Table 2 (Andrade et al. 2011). In general, all epidermis is covered by a cuticle of lipidic nature, and, although hydrophobic, it is permeable to ions and other polar compounds (Fernández et al. 2016). The presence of Al in the cuticle might indicate a mechanism of exclusion of this element, suggesting that the Al^{3+} were excluded from the epidermal cells of these species (Andrade et al. 2011). This fact is entirely plausible because the external periclinal cell walls have thread-like structures, named ectodesmata, which permit the absorption and excretion of substances through the outer wall of epidermal cells (Franke 1970). Thus, the mechanism of Al accumulation in *V. pyramidalis* and *C. major* might differ from other Al-accumulators, because besides accumulating, they can also exclude this element from their cells.

Aluminium can also be stored in chloroplast of Cerrado plants (Table 2). This was first noticed by Andrade et al. (2011) in leaves of *Q. grandiflora* and *C. major*. Subsequently, Al has also been detected in chloroplasts of the native herbaceous species *Borreria latifolia* (Aubl.) K.Schum and *Coccocypselum aureum* (Spreng.) Cham. & Schltdl. (Castro 2013). Furthermore, Al was also observed in chloroplasts of *Rudgea virburnoides* (Malta et al. 2016),

M. rubiginosa (Bressan et al. 2016), *E. dysenterica* (Rodrigues et al. 2019), and *Hancornia speciosa* Gomes (Rodrigues et al. 2017). Unfortunately, to this date, the reason for Al presence in this organelle has not been determined.

Concerning the transportation of Al to shoots, it likely takes place in the xylem via the transpiratory flow, and then, it is redistributed through the phloem to the various organs of the plant (Haridasan 2008; Schmitt et al. 2016). As mentioned, in *Q. grandiflora, Q. multiflora, Q. parviflora, V. thyrsoidea, V. rufa,* and *V. eliptica* the Al was detected in the radial parenchyma of the xylem, as well as in the sieve tube elements and companion cells of the phloem (Table 2) (Haridasan et al. 1986; Haridasan 1987). The transportation of Al throughout the plant is likely to occur as Al-OA complexes through the xylem (Haridasan 2008; Karak et al. 2015). Consistent with the idea of a xylem Al transportation, an analysis of the xylem sap of tea leaves by nuclear ^{27}Al magnetic resonance and ^{19}F NMR spectroscopy revealed an Al content of 0.29 mM in this plant organ (Morita et al. 2004). Besides, the root xylem sap of these plants formed Al-citrate complexes, which suggests that the Al was translocated throughout the plant complexed with this OA (Morita et al. 2004).

Additionally, the presence of Al has been documented in phloem cells of many accumulating plants, e.g., in tea plants, this metal was translocated via phloem (Hajiboland et al. 2015). Thus, it is not surprising that in *Arabidopsis* a phloem-related protein has been identified (ALS3) that is required for Al tolerance (Larsen et al. 2005). The *ALS3* gene has a phloem specific expression pattern in *Arabidopsis* and may be involved in the internal redistribution of Al (Larsen et al. 2005). Therefore, the transportation of Al via phloem would be essential to redistribute and store Al in various plant organs. In many tolerant plants, this redistribution mechanism would prevent an excessive accumulation of Al, and therefore, the damages that this element may inflict on plant metabolism (Larsen et al. 2005; Nezames et al.

2012). However, the mechanism by which Al is transported within the phloem has yet to be unravelled.

As previously discussed, the Al can be allocated to various plant organs and organelles. Further, the Al-accumulating species have different mechanisms of Al-neutralization and storage. Figure 1 depicts an outline of Al accumulation/transportation mechanisms. Thus, the Al is absorbed by the roots, complexed with either OAs or phenolic compounds, transported to the shoot through the xylem, and subsequently, redistributed via phloem (Watanabe and Osaki 2002; Kochian et al. 2005; Grevenstuk and Romano 2013). Finally, the Al can be sequestered in the cell walls, chloroplasts, and vacuoles (Kochian et al. 2005; Grevenstuk and Romano 2013).

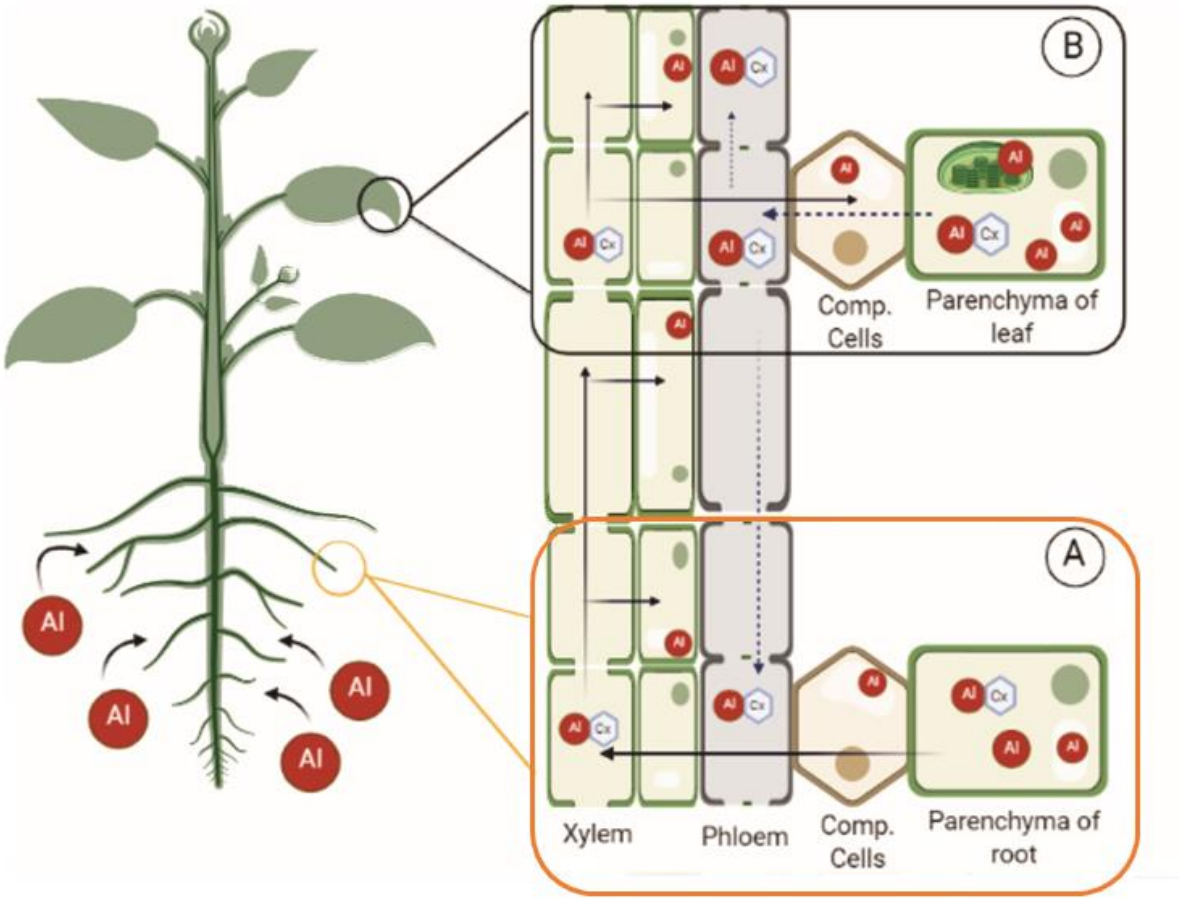

Figure 1. Scheme of Al accumulation and transportation in accumulating plants. A. The absorption of Al^{3+} from the soil solution occurs through the roots, in which the Al enters the root cells and might be stored in the vacuole, cell walls and transported to the shoot in a complexed form (Al:OA/phenolic compounds), via xylem. B. In leaves, the complexed Al (OAs/phenolic compounds) can be allocated to the chloroplast and/or the cell walls and vacuoles, and then redistributed through the phloem to the various organs of the plant. Legends: Al. Aluminum; Al-Cx. Complexed Al; Phloem: sieve element of the phloem; Comp Cells: companion cells

6. Molecular Basis of Aluminum tolerance/resistance.

In plants, several genes have been associated with the enhancement of Al tolerance/resistance by preventing the metabolic disruption related to its presence. One of the most frequent reactions to Al is the Al-activated exudation of OAs, mainly malate and citrate, to the rhizosphere, which plays a significant role in plant Al resistance. Furthermore, several genes have been identified, which are correlated to this phenomenon.

For instance, the *TaALMT1* (*Triticum aestivum* Al^{3+} Activated Malate Transporter), from wheat, was one of the very first Al^{-}resistant genes cloned from plants (Sasaki et al. 2004). The ALMT genes encode an anion transporter/channel and can transport organic and inorganic ions across the tonoplast and plasma membrane into cells (Ma et al. 2020). This gene family is linked to a large group of proteins with the potential to improve Al^{3+} resistance in plants. Delhaize et al. (2004) showed that transgenic barley plants expressing *TaALMT1* exudate malate, which led to higher Al resistance than those not expressing it (Delhaize et al. 2004). Therefore, the overexpression of genes involved in OAs synthesis may enhance Al resistance in plants (Delhaize et al. 2004).

Nevertheless, the efflux capacity of OAs to the rhizosphere constitutes a rate-limiting factor in plant Al resistance (Ryan and Delhaize 2001; Delhaize et al. 2004). Hence, OA production must be coupled with OA exudation. For instance, a citrate overproducing fungus, the *Aspergillus niger* mutant (*nuo* mutant), has a 30-fold increase in citrate accumulation; however, it releases 10-fold less citrate to the rhizosphere than the wild type (Prömper et al. 1993). Therefore, the *ALMT* gene family is crucial for OA efflux and effectively increases an OA based Al resistance. Besides, the ALMT transporter channels have been localized at the plasma membrane, which is critical for malate efflux from root apices. Malate in the rhizosphere can then chelate and immobilize the Al in the outer root layers and/or rhizosphere (Yamaguchi et al.2005).

In addition, another gene family, *MATE* (Multidrug and Toxic Compound Extrusion), is also involved with Al^{3+}-activated citrate release from roots. MATE genes can extrude a great variety of toxic compounds such as secondary metabolites, antibiotics, and various metal detoxification related substances (Li et al. 2002; Hvorup et al. 2003; Magalhaes et al. 2010). Moreover, in plants, several *MATE* genes have been associated with Al resistance like the *SbMATE*, from sorghum (Magalhaes et al. 2007; Wang et al. 2007), and *HvAACT1* from barley (Furukawa et al. 2007; Wang et al. 2007). Both, SbMATE and HvAACT1, have been localized at the plasma membrane of root epidermal cells (Furukawa et al. 2007; Magalhaes et al. 2007). In tobacco, the overexpression of *HvAACT1* increased root citrate secretion and improved Al resistance compared with non-transformed tobacco (Furukawa et al. 2007). The MATE protein family works as efflux carriers that depend on a transmembrane electrochemical gradient for the extrusion activity.

Another *MATE* family member, the *AtMATE* (*Arabidopsis thaliana* Multidrug and Toxic Compound Extrusion), a *SbMATE*/*HvAACT1* homolog, also encodes an Al-activated citrate transporter. Therefore, *Arabidopsis* roots, when exposed to Al, release citrate mediated by AtMATE (Liu et al. 2009). Although independently, AtMATE and AtALMT1 (Al-activated malate transporter) contribute to *Arabidopsis* Al tolerance, AtMATE contribution is not as significant as AtALMT1 (Liu et al. 2009).

Furthermore, several *MATE* genes associated with Al tolerance/resistance have been isolated and characterized from *Secale cereale (ScFRDL2)* (Yokosho et al. 2010), *Oryza sativa* (*SsFRDL4*) (Yokosho et al. 2011), and *Amaranthus hypochondriacus* (*AhMATE1*) (Fan et al. 2021). Additional Al tolerance genes have been identified in rice cultivars: *OsSTAR1* (related to cell wall modification), *OsNrat1*, *OsFRDL4* (a MATE member) and *OsALS1* (internal detoxification of Al), and genes related to ABC (ATP-binding cassette) transporters (Kochian et al. 2015).

Also, in *Arabidopsis*, an unforeseen mechanism of Al tolerance has been uncovered, which involves *AtATR* (ataxia telangiectasia-mutated and Rad3-related) gene homologs (Rounds and Larsen 2008; Nezames et al. 2012). ATR protein is responsible for detecting single-stranded breaks and replication fork blocks. Besides detecting DNA damages, ATR is also a cell cycle checkpoint and its activity results in the stoppage of root growth (Nezames et al. 2012). In response to Al, ATR activity leads to the extinction of the root apical meristem by inducing the complete differentiation of its quiescent center (Culligan et al. 2004; Nezames et al. 2012). Contrary to what should be expected, the lack of ATR activity enhances Al tolerance as verified in *atr* loss-of-function mutants (Rounds and Larsen 2008). These mutants do not recognize the ATR-DNA cell cycle checkpoints allowing the continuation of root growth by keeping intact the quiescent center (Rounds and Larsen 2008). In addition, *ALT2* (ALUMINUM TOLERANT 2) also responds to DNA damage similarly to *ATR* (Nezames et al. 2012). Consistently with *atr* mutants, *alt2* loss-of-function mutants also have higher Al tolerance and do not arrest root growth in the presence of Al (Nezames et al. 2012). These reports suggest that the cell cycle is a crucial target of Al toxicity.

Regarding Al-transportation-related genes, a functional analysis of the *ALS1* (ALUMINUM SENSITIVE 1) gene was conducted with *als1* mutants and GUS/GFP reporter genes (Larsen et al. 2007). This study revealed that this gene could be involved in intracellular movement of chelated Al throughout the plant (Larsen et al. 2007). In addition, the loss of *ALS1* function (*als1* mutant) had, in the presence of Al, a dramatic inhibition of root growth (Larsen et al. 2007). Also, GUS and ALS1:GFP analyses showed that *ALS1* was expressed in root tips and vascular tissues of the whole plant (Larsen et al. 2007). Therefore, it is likely that *ALS1* is part of an Al tolerance mechanism that facilitates its sequestration and redistribution (Larsen et al. 2007). In addition, studying *als3-1* (*Arabidopsis thaliana* Al-hypersensitive mutant) indicates that the gene *ALS3* is primarily expressed in the phloem and needed for Al

internal redistribution, whose expression could be complementary and/or overlapping with *ALS1* (Larsen et al. 2007; Nezames et al. 2012).

In rice, the genes *OsNrat1* (plasma membrane-localized Al transporter) and *OsALS1* (tonoplast-localized Al transporter) have similar expression patterns and localization (Zhang et al. 2019). The encoded proteins work cooperatively. Then, while Nrat1 is involved in Al uptake, the OsALS1 is responsible for Al sequestration in the vacuoles, which results in the detoxification of Al in rice roots (Zhang et al. 2019). Furthermore, in sepals of *H. macrophylla*, an Al-accumulating plant, it has been identified genes for vacuolar Al transporters (*VALT*) and plasma membrane Al transporter (*PALT1*), both members of the aquaporin family in hydrangea (Negishi et al. 2012).

With the advance of molecular investigations of Al accumulating plants, many other Al responsive genes have been identified and characterized such as *HbALMTs* in *Hevea brasiliensis* (Ma et al. 2020), *CsALMT1*, *CsNrat1*, *CsSTAR1/CsSTAR2*, *CsFRDL2*, *CsALS3* in *C. sinensis* (Zhao et al. 2018) and *Nrat1* (Nramp aluminum transporter 1) in rice (Xia et al. 2010). These genes play similar roles to those described above in response to Al.

However, for non-crop Al-accumulating plants, this type of information is scarce. There is one report addressing this matter through a transcriptome analysis of *Psychotria rubra* (Lour.) Poir (Rubiaceae), a species that grows in acid soil (Iguchi et al. 2019). In this plant several genes were found encoding ALMT and MATE members (Iguchi et al. 2019). However, this study did not compare the transcriptomes of *P. rubra* plants grown in acid with non-acid soils. It is known that these genes may be related to many physiological functions in plants, which includes Al detoxification (Magalhaes et al. 2007; 2010; Liu et al., 2009; Iguchi et al. 2019).

Nonetheless, no Al tolerance-associated gene/protein has been identified in *Q. grandiflora* roots, which supports the idea that this plant does not need to handle Al as a stress inducer. In fact, the results of Cury et al. (2019; 2020) point to an Al-requirement of this Cerrado plant.

Additionally, frequently, the expression pattern of genes in *Q. grandiflora* plants in the presence of Al does not match that of Al-tolerant/sensitive plants. For instance, an Al-induced upregulation of SAMS was observed in *Q. grandiflora* roots (Cury et al. 2020) contrasting to what has been observed in other plants such as in rice and rye under Al stress (Milla et al. 2002; Wang et al. 2013b). Moreover, while oxidative stress enzymes integrate a chain of response to Al and may contribute to Al tolerance (Chandran et al. 2008; Guo et al. 2018; Chauhan et al. 2021), these proteins were not upregulated in this Cerrado species (Cury et al. 2019; 2020).

7. Final Statements

Al toxicity has various deleterious effects on metabolism, growth, and development of various plants. Moreover, in plants, there is a strong correlation between Al-induced ROS production, oxidative metabolism upregulation, and OA production and exudation. This correlation determines whether the plant is sensitive, resistant, or tolerant. Many metabolic and developmental processes are targeted by Al toxicity including mitochondrial respiration, photosynthesis, genetic information processing, and cell wall structure and formation. Nevertheless, Cerrado plants do not respond to Al as most other plants. Additionally, in native species, Al promotes plant growth and development; however, it is still necessary to unravel the role of Al in the metabolism these plants. Therefore, only recently, the molecular basis of Al function in these plants has been investigated. Although still incipient, there have been recent advances in the understanding of Al in native plants. In Cerrado plants, this element favors photosynthesis, biomass production, and genetic information processing, cell wall and lignin syntheses, and organic acid metabolism. This review supports the idea that Al is not toxic to Cerrado accumulating species. On the contrary, some native species may need Al to grow and develop. It is imperative that the researchers may focus on the almost unexplored genetic resources of Cerrado.

Our group has recently sequenced the whole genome of *Q. grandiflora* (unpublished results). Its analysis has subsidized the previous studies on proteomics and metabolomics and can result in the discovery of novel genes in this plant species. Furthermore, under an increasing concern about climate change, it is crucial to broaden the research topics on how plants react to environmental hindrances, e.g., drought response, which has continuously been considered an essential research subject (Turner 2018).

Thus, we have also expanded our research spectrum by developing a propagation protocol for a Ni-accumulating plant, *Justicia lanstyakii* Rizz. (Acanthaceae), which has great potential as ornamental and for bioremediation purposes (Lima et al. 2022, in press).

Therefore, this constant knowledge build-up on molecular and metabolic processes and genes involved in plant stress tolerance is crucial for a better understanding and conservation of threatened biomes like the Cerrado.

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CHAPTER 2

Seed reserves and endogenous aluminum play crucial roles in the germination and seedling establishment of *Qualea grandiflora* Mart. (Vochysiaceae)

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Abstract

Qualea grandiflora Mart. is an Al-accumulating Cerrado species with a metabolic dependency on aluminum (Al). This study aimed to investigate the role of Al during germination and seedling establishment through the determination of the composition, anatomical distribution, and mobilization of Al and other seed reserves. Histochemical and energy dispersive X-ray spectroscopy (EDS) analyses were performed to detect Al, nutrients, and reserve compounds. Protein and lipid quantification were also performed. In soil with low exchangeable Al^{3+}, *Q. grandiflora* seeds accumulated about 6.43 g of $Al.kg^{-1}$ of DM and relatively high amounts of N, P, K, Ca, and Mg. Approximately 60% of the seed Al was translocated to seedling leaves, and exogenous Al had no effects on the germination of *Q. grandiflora*. Proteins and lipids were the main organic reserves of *Q. grandiflora* seeds. The histochemical analysis revealed that the bulk of Al in seeds was in the embryo, which was also the location of the other seed reserves. The most abundant fatty acid in *Q. grandiflora* seeds was lauric acid, which can serve as energy and carbon sources during germination and initial development, and this may be an Al-dependent process in *Q. grandiflora* seeds. Therefore, we propose that fatty acids, proteins, and Al were crucial for germination and the initial growth of *Q. grandiflora* seedlings.

Keywords: aluminum, Cerrado, fatty acids, germination, initial growth, proteins

1. Introduction

Aluminum (Al) is the third most abundant element in the earth's crust, and when in acid soils (pH <5.0), is present as a trivalent cation (Al^{3+}), which is highly phytotoxic (Haridasan 2008; Liu et al. 2014; Saha et al. 2019). Acid soils not only allow the formation of Al^{3+} but may also lead to the accumulation of other metal species such as Fe^{2+} and Mn^{2+}, which in high levels are toxic to plants as well (Awasthi et al. 2017). The formation of Al^{3+} can occur through phenomena like acid rain, erosion, and anthropogenic processes (Bojórquez-Quintal et al. 2017; Saha et al. 2019).

In plants, roots rapidly perceive the presence of Al^{3+}, whose toxicity can disrupt nutrient absorption, as well as induce cell wall and plasma membrane structural changes (Schmitt et al. 2016; Zhang et al. 2019). Furthermore, Al may also cause damage to the cytoskeleton, nucleus, and chromatin/DNA (Wang et al. 2013; Schmitt et al. 2016; Zhang et al. 2019). However, plants have adapted to cope with Al in acid soils. Al-excluding plants prevent its entrance into their tissues by arresting the Al in the outer regions of the roots (Schmitt et al. 2016; Zhang et al. 2019). However, plants have also been identified plants that tolerate the presence of Al in their system, and therefore, are designated as Al-tolerant. These plants can internally neutralize Al by complexing it with organic compounds and storing it in cellular compartments (Schmitt et al. 2016; Zhang et al. 2019). Moreover, some plants can accumulate more than 1 g of $Al.Kg^{-1}$ of dry matter (DM) in their tissues and because of this are termed Al-accumulators (Chenery 1948; Metali et al. 2015). In some native and tolerant species, the Al may interact with cellular structures/molecules without causing damage (Andrade et al. 2011; Metali et al. 2015; Zhang et al. 2019) and may even be required for normal plant growth and development (Silva 2017; Cury et al. 2019, 2020).

In the Brazilian savanna, known as Cerrado, the soils are mostly acid, with high levels of Al^{3+} and poor in nutrients (Haridasan 2008). Despite these natural edaphic conditions, native plants are adapted to these circumstances and show no signs of Al-toxicity (Haridasan 2008). For instance, *Qualea grandiflora* Mart. (Vochysiaceae), a woody native species capable of accumulating more than 3.9 g of Al. Kg^{-1} of DM in its leaves, is considered an Al-hyperaccumulator (Haridasan 2008; Andrade et al. 2011). In addition, transcriptomic and proteomic studies indicate that *Q. grandiflora* plants are metabolically dependent on Al (Silva 2017; Cury et al. 2019; 2020).

Since Al might be metabolically required and *Q. grandiflora* seeds accumulate large amounts of this metal, we have hypothesized that Al may also be crucial for germination and initial seedling growth. To test this hypothesis, we have evaluated the Al distribution pattern in seeds and whether it could have been translocated to leaves of 120-day-old plants. Additionally, we investigated which reserve compounds were present in *Q. grandiflora* seeds and quantified the contents of macronutrients in this plant organ. In addition, a chemical analysis of the soil near the parent plants was accomplished to determine the correlations between the contents of Al and nutrients in soils and seeds. Concomitantly, the effect of exogenous Al on germination was also examined.

2. Material and methods

2.1 Part 1 – Morpho-anatomical, histochemistry, chemical analyses, and SEM/EDS

2.1.1 Plant material

Seeds of *Qualea grandiflora* Mart. (Vochysiaceae) were collected in September 2018 in an area of Cerrado sensu stricto in the municipality of Água Fria de Goiás (14°59'09"S 47°47'02"W), Goiás state, Brazil. In addition, three soil samples (20 cm) were collected in the vicinity of the parent plants. The collected material was treated according to the usual herborization procedure (Mori et al., 1989) and incorporated into the collection of the Herbarium of the University of Brasília (UB), Voucher: 217284.

Eighty seeds of *Q. grandiflora* were germinated to investigate the distribution and relocation of Al in plants grown without Al supplementation. The seeds were disinfected in 70% (v/v) ethanol for 1 min and in a 2% (v/v) sodium hypochlorite (NaClO) solution for 20 min and washed in distilled water. Then, the seeds were placed in sterile Petri dishes on germitest paper with filter paper soaked in sterilized distilled water and incubated in a BOD (Biochemical Oxygen Demand-CE-300/350-F CIENLAB, Brazil) for 30 days. After the emergence of the cotyledons, the seedlings were transferred to containers with 500 mL of Agrofloc® vermiculite and grown at 25 °C (± 2), 16 h photoperiod, and light intensity of 80 $\mu mol.m^{-2}.s^{-1}$ (Cury et al. 2020). Subsequently, the seedlings were cultivated with $^1/_5$ MS (Murashige and Skoog 1962) nutrient solution (watered every three days) without Al supplementation for 90 days. At the end of the experiment, the seedlings had been cultivated for 120 days without Al supplementation.

2.1.2. Determination of Al and macronutrients in *Q. grandiflora* seeds and seedling leaves

The contents of Al, nitrogen (N), phosphorus (P), calcium (Ca), potassium (K), and magnesium (Mg) in *Q. grandiflora* seeds were quantified in five replicates (10 seeds each) that have been dried at 60º C for 72 h and ground in a mechanical mill. For Al, P,

C, K, and Mg quantification, seed samples (1 g each) were transferred to tubes containing 3:1 nitric-perchloric solution (v/v) and submitted to a digester block (Tedesco et al. 1995). To assess seed N content, the samples (1 g) were analyzed by combustion analysis (Oxenham et al., 1983). Subsequently, Al, P, Ca, K, Mg, and N were quantified in an atomic emission spectrometer with inductively coupled plasma (4200 MP-AES, Agilent Technology, USA) and expressed in $g.Kg^{-1}$ of dry matter (DM).

The amount of Al in leaves of *Q. grandiflora* plants was quantified (six replicates) by atomic emission spectrometry. The visual aspect of plants grown without Al was observed at the end of the experimental period.

2.1.3. Soil chemical analysis

Three soil samples of 300 g each were randomly collected in two areas (~16 m^2 each) near the parent plants. The soil collections were carried out at a depth of ~10 cm for soil chemical analysis, including soil pH (Embrapa 1997). The nutrient quantification in soil was performed using on a spectrophotometer, as described in Raij et al. (2001).

2.1.4. Morpho-anatomical and histochemical analyses

The description of external morphology (size, color, texture and tegument consistency, shape) and the analysis of the internal features (shape and embryo position, hypocotyl-root axis, and cotyledons) of seeds of *Q. grandiflora* were performed using a stereomicroscope. Morphological descriptions followed the nomenclature definitions contained in Stafleu (1953), Stearn (1983), and Barroso et al. (1999).

For anatomical characterization, seeds of *Q. grandiflora* were rehydrated for 1 h in distilled water, followed by serial ethanolic dehydration. Then, the samples were infiltrated and embedded in methacrylate Historesin (Leica, Heidelberg, Germany), with subsequent slow polymerization, according to Paiva et al. (2011). Subsequently, the embedded samples were sectioned transversely and longitudinally (~7 µm) on a semi-

automatic rotary microtome (RM, Leica Microsystems Inc., Deerfield, USA). Finally, the slides were stained with toluidine blue, mounted with glass varnish (Paiva et al. 2011), and photographed on a photomicroscope (BX-40 TRF, Olympus, Tokyo, Japan) equipped with a digital camera (Axion Cam HRc, Carl Zeiss Mikroskopie, Jena, Germany).

Cross-sections of embedded seed samples were submitted to histochemical tests, as follows: chrome azurol-S (CAS) (Kukachka and Miller 1980) and hematoxylin (Andrade et al. 2011) for Al detection. Bromophenol blue for proteins (Mazia et al. 1953), periodic acid-Schiff's reagent for neutral polysaccharides (O'Brien and McCully 1981), Lugol reagent for starch (Johansen 1940), Sudan red B for lipids (Brundrett et al. 1991 modified), phloroglucinol for lignin (Johansen 1940) and ferric chloride for total phenolic compounds (Johansen 1940). The control consisted of slides with non-tested sections mounted in water. All reagents and controls were prepared, as described in Ventrella et al. (2013). All histochemical tests were performed in triplicates. The slides were mounted with water and photographed using a photomicroscope (BX-40 TRF, Olympus, Tokyo, Japan) equipped with a digital camera (Axion Cam HRc, Carl Zeiss Mikroskopie, Jena, Germany).

2.1.5. X-ray dispersion microanalysis for Al detection (SEM/EDS)

Seed samples of *Q. grandiflora* were fixed in 0.5% (v/v) glutaraldehyde in 0.05 M Na-cacodylate buffer, dehydrated in an increasing series of acetone solutions and dried to the critical point (CPD 030, Bal-Tec, Balzers, Liechtenstein). The samples were placed on stubs and sputter-coated with gold and analyzed on a Scanning Electron Microscope coupled to the energy dispersive X-ray spectroscopy (EDS- JSM-7000F, Jeol, Tokyo, Japan) operated at 15 kV and Al (1.5 keV). The images were acquired at the Multi-User Microscopy and Microanalysis Laboratory (LMM) at the University of Brasilia (UnB).

Twelve points were randomly selected in *Q. grandiflora* seed samples: six in the tegument, and six in the embryo.

2.2. Part 2 – Protein and lipid quantification

2.2.1. Quantification of proteins and lipids in *Q. grandiflora* seeds

The quantification of lipids and proteins was performed using seeds collected in September 2020 in an area of Cerrado stricto sensu in the municipality of Luziânia (latitude "16°16'07.7" S, and longitude 47°58'54.9" W), Goiás, Brazil. Seeds were washed in running water for 10 min, disinfected in a 5% (v/v) sodium hypochlorite (NaClO) for 10 min, and washed in distilled water.

Subsequently, 40 seeds were germinated in wet germitest paper and grown without Al supplementation. The seeds were placed in petri dishes and incubated in a BOD (Biochemical Oxygen Demand-CE-300/350-F CIENLAB, Brazil), regularly watered for 42 days at 27 °C (± 0.5) and 12 h photoperiod (Dousseau et al. 2013). For the quantification of proteins and lipids, the seedlings were divided into hypocotyl-root and cotyledons, and the seed coat was removed.

For protein quantification, five seeds and 15 seedlings cultivated for 42 days were collected (five replicates with three seedlings each) and lyophilized for 72 h. Total protein was extracted and quantified by the method of Bradford (1976) using bovine serum albumin (BSA) as the standard. Measurements were carried out using a spectrophotometer microplate reader (SpectraMax 190, Molecular Devices) at a wavelength of 595 nm. Protein quantification was performed using SoftMax Pro 6.1 software (Molecular Devices).

For lipid quantification, five *Q. grandiflora* seeds and 42-day-old seedlings in five replicates (3 seedlings each) were lyophilized for 72 h and then ground in a mortar. Fatty

acids in dry biomass were converted to their methyl esters (FAMES) using a hydrochloric acid/methanol *in situ* method adapted from Laurens et al. (2012).

The samples were analyzed on a GC-MS device (Agilent Technologies 7820A GC system, 5975 series MSD; Agilent, USA) equipped with a standard INNOWAX column (30 m, 0.25 mm diameter, 0.25 µm film thickness, Agilent Technologies), and 1 µL of each sample was injected with a temperature of 250 °C and a split ratio of 30:1. Separation was achieved using a constant column helium gas flow of 1 $mL.min^{-1}$ with a temperature program starting at 70 °C and gradually increasing to 130 °C (at 20 $°C.min^{-1}$) and then 260 °C (10 $°C.min^{-1}$) before holding at 260 °C for 5 min. Electron impact ionization was performed at -70 eV, and mass spectra were acquired in scan mode. FAMEs peaks were identified by comparing retention times with authentic standards and mass spectra with the NIST mass spectral database using MSSearch software. Quantification was performed using Agilent ChemStation software, and standard curves were constructed using a certified mixture of FAMES (Grain FAME standard; Sigma-Aldrich, USA).

2.3. Part 3 – Seed germination

Eighty seeds were germinated (40 for each treatment) with (150 µM $AlCl_3$) or without Al supplementation. The germination percentages (GP) were calculated, as follows: GP = (number of seeds germinated/total number of seeds)*100 (Maguire, 1962). The germination rate index (GRI) was calculated with the formula: GRI = G1/N1 + G2/N2 +. . . + Gn/Nn; G1 is the number of germinated seeds on each day, and N is the number of the day of observation (Maguire, 1962). Furthermore, the rates of time, synchronization index, and average germination speed were measured, according to Ranal and Santana (2006) and Labouriau and Valadares (1976).

2.4. Statistical analysis

The data of seed germination means were compared by Student's T-test ($p < 0.05$) using the R 4.0.4 software (R Development Core Team 2021).

3. Results

3.1 Contents of Al and macronutrient in seeds and leaves of *Q. grandiflora* plants were not dependent on the respective amounts in soils

The nutrient contents of Cerrado soils near the parental *Q. grandiflora* plants were determined, and these soils contained 24.28 mg of $Al.dm^{-3}$, 0.87 mg of $N.dm^{-3}$, 1.38 mg of $P.dm^{-3}$, 160.31 mg of $K.\ dm^{-3}$, 230.00 mg of $Ca.dm^{-3}$, and 47.76 mg of $Mg.dm^{-3}$ of soil (Table 1). Furthermore, the soil pH was 6.0, it had an Al saturation considered moderately harmful (13.33%), it was poor in organic matter (25.75 $g.dm^{-3}$), with low base saturation (< 50%), and low cation exchange capacity (CEC; 4.70 $cmol_c.dm^{-3}$) (Table 1).

Table 1: Chemical analysis of the soil of the collection area Cerrado *sensu stricto*.

	Values	
Mineral soil contents	($cmol_c.dm^{-3}$)	($mg.dm^{-3}$)
Al^{3+}	0.27 ± 0.09	24.28 ± 8.09
N	-	0.87 ± 0.07
P	-	1.38 ± 0.06
K^{+}	0.41 ± 0.05	160.31 ± 19.55
Ca^{2+}	1.15 ± 0.15	230.00 ± 30.00
Mg^{2+}	0.36 ± 0.06	47.76 ± 7.29
Chemical properties	Values	
pH	6.05 ± 0.04	
Organic matter ($g.dm^{-3}$)	25.75 ± 4.40	
Base saturation (%)	42.33 ± 3.57	
Al saturation (%)	13.33 ± 4.86	
Cation exchange capacity (CEC) $cmol_c.dm^{-3}$	4.70 ± 0.29	

(±) standard error.

Note that *Q. grandiflora* seeds had about 6.43 g of $Al.Kg^{-1}$ of dry matter (DM), while leaves of young plants grown without Al-supplementation contained about 3.77 g $Al.Kg^{-1}$ (Table 2). In addition, the seeds also contained N (20.84 $g.Kg^{-1}$), K (12.05 $g.Kg^{-1}$), P (2.34 $g.\ Kg^{-1}$), Ca (2.66 $g.Kg^{-1}$), and Mg (1.57 $g.Kg^{-1}$) (Table 2).

Table 2: Quantification of Al and macronutrients in seeds and leaves from 120-day-old *Qualea grandiflora* Mart. seedlings grown with $^{1}/_{5}$ MS salts without Al supplementation ($g.Kg^{-1}$).

Sample	Al	N	P	K	Ca	Mg
Seed	6.43 ± 0.3	20.84 ± 0.3	2.34 ± 0.1	12.05 ± 0.5	2.66 ± 0.1	1.57 ± 0.1
Leaves	3.77 ± 0.4	-	-	-	-	-

(±) standard error.

3.2 Structure, pigment contents, histochemistry and Al in *Q. grandiflora* seeds

The seeds of *Q. grandiflora* were winged, bi-tegumented, exalbuminous, with an embryo that occupied the entire space of the seminal cavity (Fig. 1A- C). The tegument had eight to ten layers of cells, and externally, a thin lipid cuticle, and consisted of a single layer of cells compressed (Fig. 1D-E). The seeds had the remains of a nuclear endosperm with a narrow embryo that had two long and convoluted cotyledons (Fig. 1D). Additionally, the embryo axis was formed by dividing meristematic cells with a distinct protoderm, procambium, and ground meristem (Fig. 1C). The cotyledons were composed of a uniseriate protoderm, ground meristem, and procambium (Fig. 1F).

The presence of Al in the embryo axis and cotyledons resulted in purplish and blue stains when reacted with hematoxylin and CAS, respectively (Fig. 2A-C). Both reagents showed intense coloration for Al in the ground meristem of the cotyledons, and a weak stain in the protoderm (Fig. 2D-F). In addition, hematoxylin stained weakly for Al in the seed coat, which had no correspondence with CAS reaction (Fig. 2G-I). Furthermore, neither hematoxylin nor CAS detected Al in the vascular tissues of the cotyledons and embryo axis (Fig. 2J-K).

Regarding proteins in *Q. grandiflora* seeds, the bromophenol blue test produced an intense reaction for protein bodies in the embryo axis and cotyledons (Fig. 3A-B). Polysaccharides were detected in the cell walls with PAS (Fig. 3C-D), most likely because of their cellulose and pectin contents. Sudan red B, although not intense, detected the

presence of lipids in the protoderm. Moreover, lipid bodies were seen in the embryo axis and ground meristem of cotyledons (Fig. 3E). The ferric chloride test and phloroglucinol indicated the presence of phenolic compounds and lignin only in the tegument (Fig. 3F-G). Seeds of *Q. grandiflora* did not contain starch as a reserve substance.

The sites of Al accumulation in *Q. grandiflora* seeds observed by X-ray microanalysis were similar to those observed with hematoxylin tests, which detected Al in the cotyledons and tegument. The averages of the relative abundance of Al were 5.32% and 1.21% in the cotyledons and tegument, respectively (Fig. 4A-B).

3.3 Protein and lipid contents in seeds and seedlings of *Q. grandiflora*

The *Q. grandiflora* seeds were composed of about 7% protein and 37% total fatty acids (Table 3). Saturated fatty acids represented 94.3% of the total fatty acid composition, whilst monounsaturated and polyunsaturated fatty acids were 6.1% and 0.32%, respectively (Table 3). Furthermore, Table 4 shows the contents of different types of fatty acids in *Q. grandiflora* seeds and seedlings. In seeds, the saturated lauric acid (12:0) was the most abundant (81.4%), followed by myristic acid (14:0 – 8.02%), and the monounsaturated oleic acid (18:1 – 6.07%). Concerning seedlings, the most abundant fatty acids in the hypocotyl-root axis were the saturated palmitic acid (16:0 – 32.30%), the monounsaturated oleic acid (18:1 – 19.33%), and the polyunsaturated linoleic acid (18:2 – 24.69%) (Table 4). In contrast, in the cotyledons, the fatty acids with the highest relative abundance were the saturated lauric acid (12:0 – 79.19%) and myristic acid (14:0 – 7.77%) (Table 4).

Table 3: Total proteins and fatty acids (% of dry matter; ± standard error) and percentage of saturated, monounsaturated, and polyunsaturated fatty acids in the seeds and seedlings of *Qualea grandiflora* Mart. grown without Al.

		Fatty Acids			
Plant Organ	**Total Protein (%)**	**Total (%)**	**Saturated (%)**	**Monounsaturated (%)**	**Polyunsaturated (%)**
Seed	6.88 ± 0.72	36.92 ± 1.63	94.31 ± 0.92	6.10 ± 0.60	0.32 ± 0.04
42-day-old Hypocotyl-Root	0.05 ± 0.01	0.77 ± 0.03	27.53 ± 9.42	11.40 ± 3.41	20.88 ± 7.30
42-day-old Cotyledons	2.00 ± 0.52	20.35 ± 1.59	92.75 ± 1.47	6.48 ± 0.53	1.53 ± 0.31

Table 4: Fatty acid composition of seeds and 42-day-old seedlings of *Qualea grandiflora* Mart. grown without Al supplementation. Data expressed as percentage (± standard error) of total fatty acid. Fatty acids were quantified as their methyl-esters (FAMEs).

Fatty Acid	Seed (%)	Hypocotyl-Root Axis (%)	Cotyledons (%)
C12:0	81.40 ± 1.38	3.53 ± 1.19	79.19 ± 0.52
C14:0	8.02 ± 0.43	1.46 ± 0.15	7.77 ±0 .39
C16:0	3.60 ± 0.30	32.30 ± 0.58	4.26 ± 0.09
C18:0	0.54 ± 0.06	4.13 ± 0.04	1.52 ± 0.03
C18:1	6.07 ± 0.58	19.33 ± 0.71	5.71 ± 0.52
C18:2	0.24 ± 0.02	24.69 ± 0.26	0.83 ± 0.09
C18:3	0.13 ± 0.01	9.44 ± 0.25	0.70 ± 0.06
C20:0	-	0.86 ± 0.43	-
C20:2	-	0.19 ± 0.19	-
C22:0	-	3.17 ± 0.11	-
% of total fatty acid detected	100	99.0	99.98

3.4 Exogenous supplementation of Al had no effects on the germination of *Q. grandiflora* seeds

Table 5 depicts the effect of exogenous Al on the germination of *Q. grandiflora* seeds. Notice that there was no significant difference among any of the analyzed parameters (Table 5). Therefore, the results strongly suggest that the additional supplementation of Al might not be a factor during the germination of *Q. grandiflora* seeds.

Table 5: Effect of exogenous Al supplementation on the germination of *Qualea grandiflora* Mart. seeds (n=40).

Treatment	Germination index (Seeds per day)	Germination rate (%)	Average germination time (h)	Speed of germination (h)	Synchronization index (unitless)
No Al	1.54 ± 0.11	81.24 ± 4.73	261.8 ± 3.78	0.004 ± 0.0	0.05 ± 0.00
Al	1.77 ± 0.18	85.00 ± 4.08	259.3 ± 18.21	0.004 ± 0.1	0.14 ± 0.07
	N/S	N/S	N/S	N/S	N/S

N/S: non-significant

4. Discussion

Many Cerrado species, including *Q. grandiflora*, are known to accumulate large amounts of Al (Chenery 1948; Haridasan and Araújo 1988; Andrade et al. 2011; Souza et al. 2017; Rodrigues et al. 2019). Moreover, it has been shown that *Q. grandiflora* plants not only accumulate Al, but that this species has a high metabolic dependency on this element to grow and develop (Cury et al. 2019, 2020). However, little is known about the Al content and its distribution pattern in *Q. grandiflora* seeds. Here, we show evidence that endogenous Al may play a role during germination and seedling establishment.

4.1 Despite the low Al^{3+} availability in the surrounding soil at the location of the parental plants, *Q. grandiflora* seeds stored high amounts of this metal and other nutrients

The soils where *Q. grandiflora* seeds were collected can be categorized as slightly acidic (pH ≥ 6.0), dystrophic, with low CEC and base saturation (Embrapa 2006). Moreover, they were relatively poor in organic matter with considerable Ca, K, P, and Mg contents. Besides, it is interesting to point out that in soils with pH ≥ 6.0, most of the Al is in the insoluble form ($Al(OH)_3$), which dramatically decreases its toxicity in sensitive plants (Faquin 1994). Furthermore, some Al-accumulating Cerrado plants, including *Q. grandiflora*, may occur in both acid and calcareous eutrophic soils (Haridasan and Araújo 1988; Haridasan 2008; Pereira et al. 2018). Hence, it has been established that, independent of the edaphic conditions (acid/calcareous soils, etc.), these Al-accumulating Cerrado plants can acquire and store similar amounts of Al (Nogueira et al. 2019). Similarly, the *Q. grandiflora* seeds collected in this study accumulated high quantities of Al (6.43 g of $Al.Kg^{-1}$ of DM) in a soil with low levels of exchangeable Al^{3+}. Additionally, Andrade et al. (2011) verified that in several Vochysiaceae species, including *Q. grandiflora*, the internal concentration of Al^{3+} in these plants is not directly correlated to its soil availability. In general, the Al accumulation trait appears to be

intrinsic to the species and not greatly dependent on the edaphic conditions (Jansen et al. 2002; Metali et al. 2015). Furthermore, high amounts of Al have been detected in seeds of other native accumulating plants such as *Miconia albicans* (Sw.) Triana (Melastomataceae), *Palicourea rigida* H.B.K. (Rubiaceae), and *Qualea parviflora* Mart. (Vochysiaceae) (Haridasan et al. 1987). Consequently, these results are very consistent with the data showing that *Q. grandiflora* seeds could accumulate and store high quantities of Al.

In addition to Al, *Q. grandiflora* seeds also had high contents of Ca, K, P, N, and Mg relative to their corresponding soil levels. This fact may be crucial to *Q. grandiflora* germination and seedling establishment. All these minerals are macronutrients required in high quantities by plants during their germination, growth, and development (Pandey 2015). For instance, N is vital for plant growth, which is absorbed from the soil and used in the synthesis of essential molecules such as amino acids, nucleic acids, and chlorophyll. Thus, N is vital for DNA replication, transcription, protein synthesis, and photosynthesis (Masclaux-Daubresse et al. 2010).

Another mineral largely accumulated in *Q. grandiflora* seeds was the Al. Hence, it might not be circumstantial that *Q. grandiflora* seeds can accumulate Al and macronutrients, independently of their soil availability. The distribution pattern of Al in the seeds, primarily found in the cotyledons and embryo axis, supports the hypothesis that this metal might be stored in these seeds as a reserve component. This statement is supported by the evidence showing that, in *Q. grandiflora* seeds, the accumulation sites of Al and its reserve compounds (proteins and lipids) overlapped one another. Moreover, the germination analysis of *Q. grandiflora* seeds revealed that an exogenous supply of Al did not impact germination. Therefore, these data uphold the idea that the Al was stored by *Q. grandiflora* seeds in a similar fashion to that of reserve compounds.

Q. grandiflora seedlings were cultivated for 120 days without Al supplementation to determine whether it could be translocated from seeds to the growing plants. Accordingly, throughout this cultivation period, the only source of Al was the seed. Thus, it is worth noting that 3.77 g of $Al.Kg^{-1}$ of DM, approximately 60% of all Al in *Q. grandiflora* seeds, were carried to leaves of 120-day-old plants. Furthermore, during the germination and plant initial growth, it is likely that the translocation of Al from seeds to seedlings might reflect the pattern of other mineral nutrients as well as reserve substances. Leaves are responsible for major metabolic processes in plants, reinforcing the idea that Al transportation to *Q. grandiflora* leaves was not a casual phenomenon. Accordingly, there is a high level of coherency between this event and the data that shows a metabolic requirement of Al in these plants (Cury et al. 2019, 2020). Nonetheless, it has been noticed that, after 120 days of cultivation, leaves of *Q. grandiflora* plants started to turn yellow, a phenomenon that was associated with Al starvation (Cury et al. 2019, 2020).

4.2 Aluminum and reserves shared the same locations in seeds of *Q. grandiflora*

The distribution pattern of Al, reserve compounds, and other nutrients in the seeds of accumulating plants are still not well known. However, it is interesting to note that *Q. grandiflora* seeds have a high abundance of proteins and conspicuous lipid droplets, and like Al, these reserves were found in the embryo axis and cotyledons. According to Mayworm et al. (2011), the principal components of reserves in Vochysiaceae seeds are proteins (20%) and lipids (21.6%). The *Q. grandiflora* seed reserves were also proteins and lipids; however, their proportion was quite different (proteins - 7%; lipids - 37%). Furthermore, *Q. grandiflora* seeds were exalbuminous; thus, these reserve compounds were stored in the embryo, primarily in the cotyledons. Thus, it was remarkable that the cotyledons were the predominant accumulation site of Al, as well.

Therefore, proteins were an important reserve component in *Q. grandiflora* seeds. Proteins are macromolecules involved in fundamental processes in the cellular metabolism as enzymes, in structural roles, photosynthesis, DNA replication, as well as reserve substances (Showalter 1993; Herman and Larkins 1999; Wang et al. 2004; Alencar et al. 2012; Schuller et al. 2018; Zhao et al. 2018). Furthermore, Cury et al. (2020) reported a positive correlation between ribosomal activity, protein synthesis, and Al in the roots of *Q. grandiflora*. Proteins are macromolecules involved in fundamental cellular metabolic processes such as enzymes, structural roles, photosynthesis, genetic information processing, as well as reserve substances (Showalter 1993; Herman and Larkins 1999; Wang et al. 2004; Alencar et al. 2012; Schuller et al. 2018; Zhao et al. 2018). Besides, it was also demonstrated that Al induced the expression of proteins associated with primary metabolism involving carbohydrates metabolism, energy metabolism, and genetic information processing (Cury et al. 2019, 2020). Hence, it appears that in *Q. grandiflora*, all these processes require Al to work properly, not only for plant growth and development in general but also for germination and seedling establishment.

Concerning lipids in seeds, they are mobilized from reserve tissues during germination and initial seedling growth. For example, in oat, the scutellum absorbs either free fatty acids (FFAs) released from triacylglycerols (TAGs) or intact TAGs, which are promptly degraded to saturated and unsaturated FFAs (Leonova et al. 2010). These FFAs can be broken down, through the β-oxidation pathway and glyoxylate cycle, into sugars and other molecules to be used as energy and carbon backbone sources during germination and seedling development (Eccleston and Ohlrogge 1998; Eastmond et al. 2000; Muscolo et al. 2007; Leonova et al. 2010). It is noteworthy that Al induced the upregulation of eight enzymes of the glyoxylate cycle in *Q. grandiflora* roots (Cury et al.

2020). Hence, this fact may help explain the high levels of Al in seeds of this plant and support the idea of a crucial role of Al during germination and seedling establishment.

Although the association between seed reserves and endogenous Al during germination and seedling establishment in Al-accumulating species has not been demonstrated, it may be required for initial seedling growth in this species. As mentioned, there is a positive correlation between protein synthesis and Al in *Q. grandiflora* (Cury et al. 2020). *Q. grandiflora* requires Al to grow and develop properly. The question is: why would it not require during germination and seedling establishment? Thus, it is not out of the question that this element works as a reserve component for initial seedling growth. This hypothesis is further supported by the fact that in *Q. grandiflora,* an exogenous supply of Al did not interfere in germination and seedling growth.

4.3 Proteins and lipids were the main organic reserve compounds in *Q. grandiflora* seeds

Usually, seed reserve components are starch, lipids (TAGs), and storage proteins, and their relative abundance may vary among plant species (Baud et al. 2008). However, no starch was detected in *Q. grandiflora* seeds. In the case of *Q. grandiflora*, the reserves were composed of proteins and lipids, representing approximately 7 and 37% of seed DM.

4.3.1 *Q. grandiflora* seeds stored proteins as a reserve and used most of these compounds during germination and seedling establishment

Seed proteins can be categorized into three distinct groups: storage proteins, structural and metabolic proteins, and protective proteins (Shewry and Halford 2002). Moreover, in dry seeds, like those from *Q. grandiflora*, storage proteins are found in protein storage vacuoles (PSVs) in the endosperm/cotyledon, often surrounding the nuclei of embryo cells, and lipids in oil droplets at the cytoplasm periphery (Shewry and Halford 2002; Baud et al. 2008). In fact, this arrangement of reserves could be seen in *Q.*

grandiflora seeds. Note that the proteins were detected in more centralized structures and the lipidic bodies at the cytoplasm periphery of embryo cells (Fig. 3 B and E).

It has been shown that, in plant seeds, the predominant types of SSPs are albumins and globulins (Chagas and Santoro 1997; Dziuba et al. 2014; Verma and Bhatia 2019; van Wyk and Amonsou 2021). Storage proteins function as carbon, nitrogen, and sulfur sources for the initial development of seedlings (Krishnan and Coe 2001). Regarding *Q. grandiflora*, the 42-day-old seedlings had only about 30% of the original seed protein content, most of it in its cotyledons. This fact might indicate that, during seedling development, the reserve proteins might have been used at a higher rate than lipids during the initial growth. Hence, about 70% of the SSPs could have been broken down, and their moieties incorporated in molecules like chlorophylls, Coenzyme A (CoA), and vitamins. Although the 42-day-old seedlings were cultivated without Al and nutrient supplementation, they had green and fully expanded cotyledons. Consequently, photosynthetic pigments, e.g., chlorophyll *a* = 34.4 $\mu g.cm^{-2}$, *b* = 18.0 $\mu g.cm^{-2}$, and carotenes 9.04 $\mu g.cm^{-2}$ (data not shown) were synthesized, and required the use of carbon/nitrogen sources, likely, from SSP reserves.

4.3.2 Lipids may be the principal energy and carbon sources in *Q. grandiflora* seeds

The main reserve substances in *Q. grandiflora* seed were lipids, which constituted 37% of its DM. Also, as previously mentioned, lipids stored in seeds can be converted into sugars through the glyoxylate cycle and gluconeogenesis, and therefore, used as a source of energy (ATP) and carbon during germination and seedling establishment (Eccleston and Ohlrogge 1998; Eastmond et al. 2000; Muscolo et al. 2007; Gonçalves et al. 2016; Ma et al. 2016; Wu et al. 2020).

Additionally, 94% of lipids stored in seeds of *Q. grandiflora* were saturated, of which 84% was lauric acid (12:0). The proportion of fatty acids in seeds varies greatly

between plant species. For instance, while some Cuphea species (Thompson et al. 1990; Graham et al. 2016), *Astrocaryum vulgare* Mart., *Cocos nucifera* L., *Elaeis guineensis* Jacq., and *Q. grandiflora* contain lauric acid as the predominant seed fatty acid (Lalouckova et al. 2021), in alfalfa it was the polyunsaturated linoleic acid, 18:2 (Huang and Grunwald 1990). Lauric acid has been used against pathogenic agents such as viruses (flu, common cold, herpes, etc.) and bacteria like *Staphylococcus aureus* and *Clostridium* (Arora et al. 2011; Barker et al. 2019; Matsue et al. 2019). Besides, this fatty acid has raised concerns as a dietary product due to the risks of coronary artery diseases (Orsavova et al. 2015).

Regarding its biological function in seeds, it is likely that lauric acid could provide energy and carbon for biosynthetic processes during germination and growth. It has been suggested that, in plants, there might be a lauric-acid-derived signal to induce the expression of genes associated with the fatty acid β-oxidation/glyoxylate cycle, and gluconeogenesis (Eastmond and Graham 2001). This hypothesis is supported by the observation that in transgenic *Brassica napus* L. with increased production of lauric acid there was a significant increase in isocitrate lyase (ICL) activity, a unique enzyme of the glyoxylate cycle (Eccleston et al. 1996). Albeit there is no concrete evidence that this model (lauric acid/β-oxidation/glyoxylate cycle) is ubiquitous in plants, it has been established that, in some plants, there might be a correlation between this fatty acid and germination/seedling development (López-Villalobos et al. 2001).

Thus, tissues from germinating *C. nucifera* embryos accumulated significant lauric acid and other saturated and unsaturated fatty acids (López-Villalobos et al. 2001). In fact, fatty acid contents substantially increased in tissues from germinating *C. nucifera* embryos, and lauric acid comprised about 24-35% of all fatty acids in the haustorium. Concomitantly, the lipid content of the solid endosperm decreased (López-Villalobos et

al. 2001), indicating that there would have been a mobilization of lipids from the endosperm to embryo tissues. Moreover, coconut embryos used both sucrose and fatty acids, noticeably lauric acid, as energy and carbon sources (López-Villalobos et al. 2011). Even though in in vitro conditions, if added at day zero, the lauric acid inhibited germination, later adding this fatty acid improved the growth and development of *in vitro* cultivated coconut zygotic embryos and seedlings (López-Villalobos et al. 2011). Further, the use of ^{14}C-labelled lauric acid revealed its incorporation into longer chain fatty acids of complex lipids, mainly phospholipids (López-Villalobos et al. 2011).

Despite all information above, in *Q. grandiflora* seeds, the role of lauric acid (12:0) has yet to be determined. However, it was noticed that the FFAs were mobilized during the germination and seedling growth in this species. Compared with the respective amounts in seeds, the hypocotyl-root axis of 42-day-old seedlings presented an increase in the abundance of fatty acids with longer chains, more specifically, palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2), and linolenic acid (18:3). Conversely, the abundance of lauric acid was dramatically lower in this region of the seedling.

Interestingly, all fatty acids mentioned above are found in biological membranes (Reszczyńska and Hanaka 2020). Nonetheless, the fatty acid (saturated and unsaturated) composition of the membranes is variable throughout the plant. Thus, these membrane configuration differences between the hypocotyl-root axis and cotyledons are likely due to the tissue-specific demands of each region. Photosynthesizing organs, like leaves, have a different configuration of fatty acids than those without chloroplasts (Reszczyńska and Hanaka 2020). Moreover, as the cotyledons are the primary reserve organ, and thus, at the initial developmental stage, it was expected that the fatty acid composition would be closer to that of the seeds.

4.4 Polysaccharides and phenols in *Q. grandiflora* seeds could not be associated with responses to Al

Reserve polysaccharides, like starch, were not detected in the seeds of *Q. grandiflora*. Hence, polysaccharides may not have a significant role as reserve substances in these seeds. Instead, most polysaccharides were detected in the cell walls because of their pectic-cellulosic nature. In addition, the cell wall has been considered one of the main sites of Al toxicity response, where it can be immobilized (Horst et al. 2010; Aggarwal et al. 2015). Also, hemicellulose and pectins are regarded as the wall components that may interact with Al (Zhang et al. 2019). However, in *Q. grandiflora* seeds, there was no Al in the cell walls. Coherently, Andrade et al. (2011) did not find Al in the cell walls of *Q. grandiflora* leaves as well. Thus, the absence of Al in the cell wall indicates that its polysaccharides do not correlate with response to Al toxicity.

The seed coat of *Q. grandiflora* presented a large abundance of lignin and total phenolic compounds. Lignins are associated with cell wall stiffness and occur mainly in vascular and supporting tissues, such as xylem and sclerenchyma (Barros et al. 2015). In addition, several phenolic compounds may also be involved in the Al detoxification process (Barceló and Poschenrieder 2002). However, in *Q. grandiflora* seed, phenolic compounds and Al did not occur in the same seed sites. Thus, there is no reason to expect Al-detoxification by phenols in seeds of this species. In fact, the results support the idea that Al is accumulated in these seeds because it may be required for germination and the initial development of the plant (Cury et al. 2019, 2020).

5. Conclusion

This work is the first in-depth analysis of the role of Al in *Q. grandiflora* seeds, which was accumulated in large amounts compared to its levels in soil. Additionally, most of the Al was stored in the cotyledons together with proteins and lipids, which were the organic reserve components of these seeds. Besides, these seeds accumulated higher

mineral nutrients contents than those quantified in the soil. Also, the exogenous Al had no effects on the germination of *Q. grandiflora* seeds. Moreover, during the initial growth of *Q. grandiflora* plants, the Al was translocated from seeds to leaves. Furthermore, the fatty acid lauric acid might have been the most important source of energy and carbon during seedling development. Therefore, these events strengthened the hypothesis that the Al is a seed reserve component and may be critical for germination and plant establishment in this species.

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Figure captions

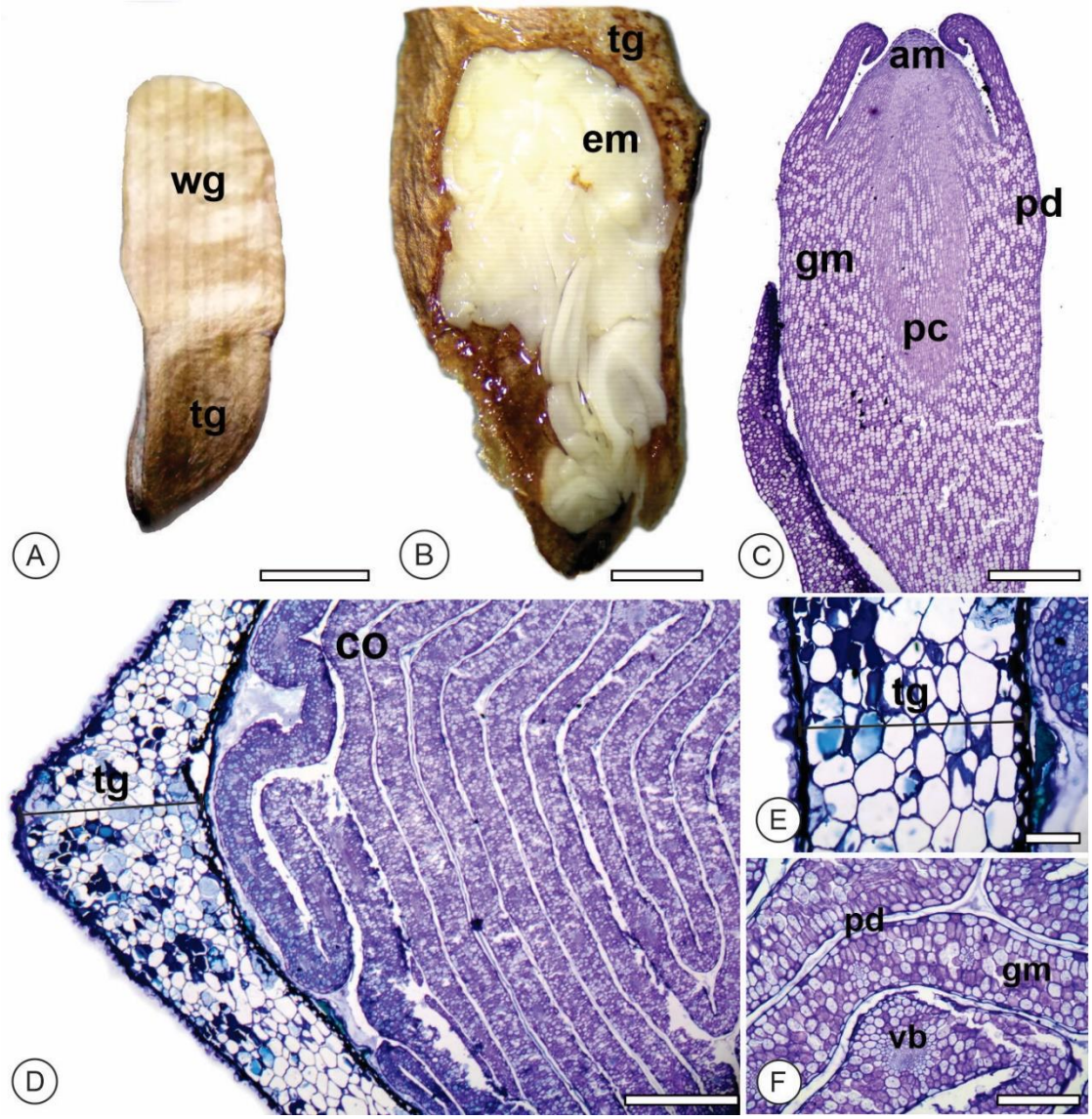

Fig. 1: Morphology and anatomy of *Qualea grandiflora* Mart. seeds stained with toluidine blue. A) General view of *Q. grandiflora* seeds. B) Longitudinal section of the seed showing the tegument and the embryo. C) Micrograph of the embryo axis in longitudinal section and meristematic tissues. D) Cross section of the seed showing the tegument and the convoluted cotyledon. E) Detail of the tegument. F) Detail of a cotyledon. (co) cotyledon; (em) embryo; (gm) ground meristem; (pd) protoderm; (tg) tegument; (pc) procambium; (am) apical meristem;(vb) vascular bundle; (wg) wing. Scale: A: 6mm; B: 5mm; C-D: 500µm; E: 50 µm; F: 100µm.

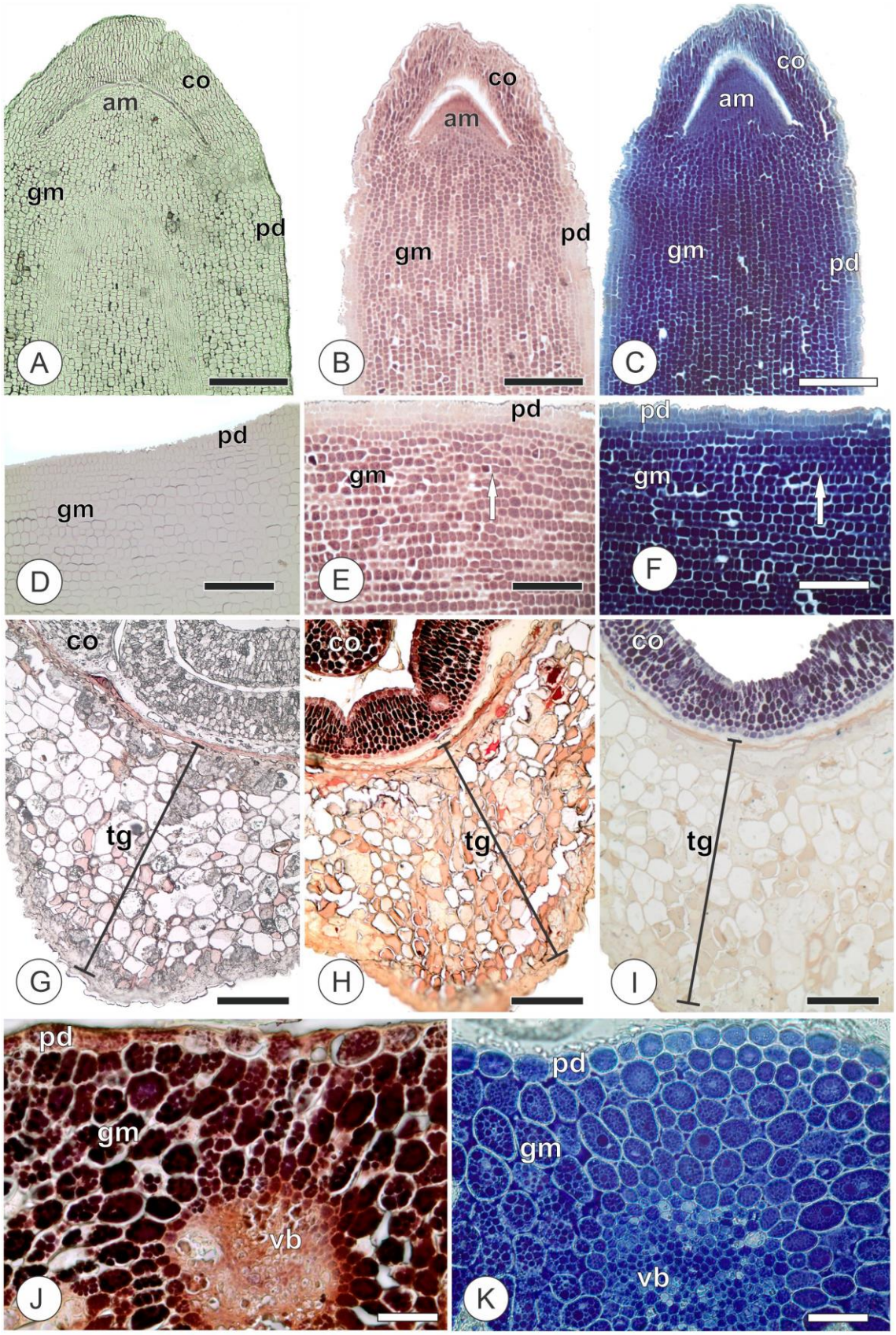

Fig. 2: Pattern of Al distribution in *Qualea grandiflora* Mart. seed. A) Control section. B) Longitudinal section of the embryo stained with hematoxylin for Al detection; C) Detection of Al with chrome azurol S (CAS) in longitudinal section of the embryo. D) Control showing a portion of the embryo. E) Detail of the embryo in longitudinal section stained with hematoxylin to detect Al, without staining in the cell wall (arrow) F) Reaction with CAS for Al detection in the embryo, without staining in the cell wall (arrow). G)

Cross-section control of the tegument and cotyledon. H) Seed tegument and cotyledon stained with hematoxylin. I) Reaction with CAS in the seed tegument and cotyledon. J) Detail of the cotyledon stained with hematoxylin in cross section. K) Cotyledon stained with CAS. (am) apical meristem; (co) cotyledon; (gm) ground meristem; (pc) procambium; (pd) protoderm; (tg) tegument; (vb) vascular bundle. Scale: A-C; G-I: 200 µm; D-F: 150 µm; J-K: 50 µm.

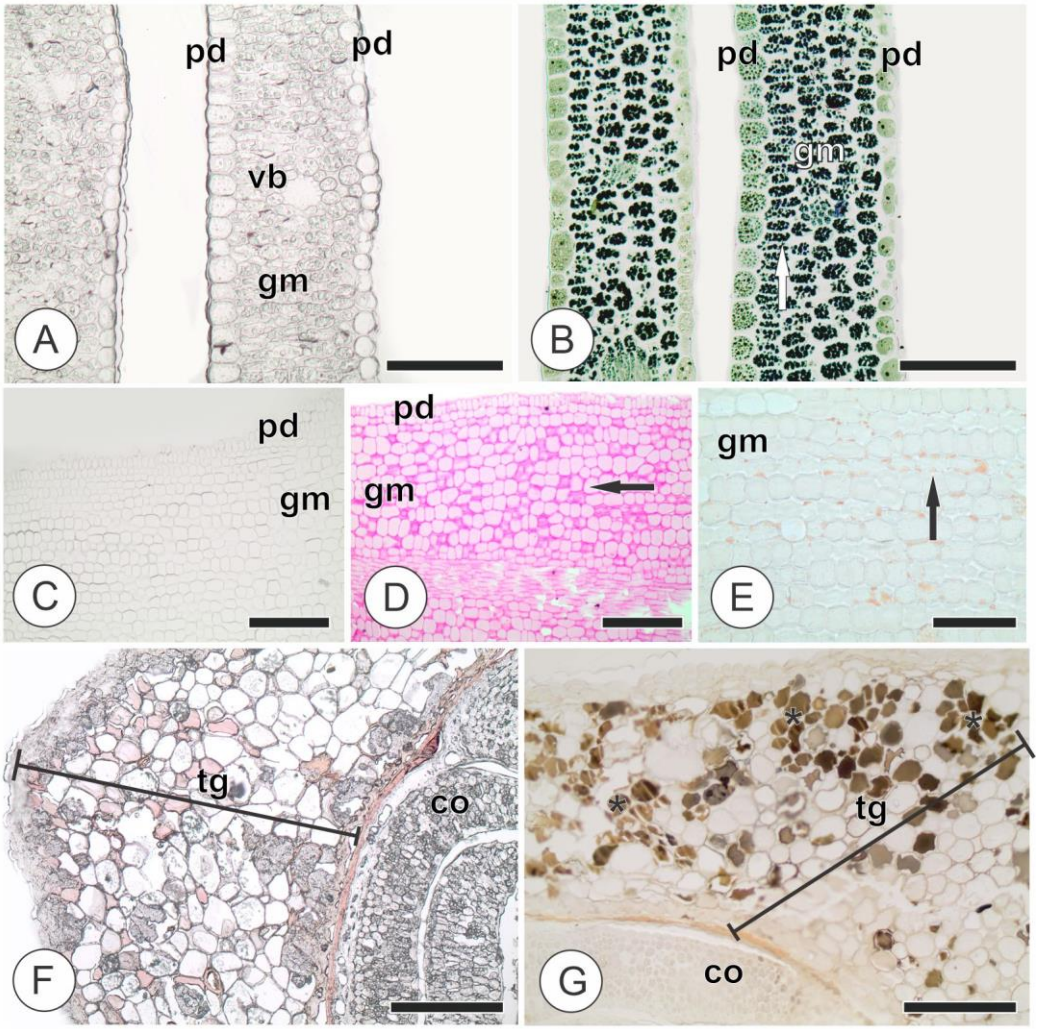

Fig. 3: Histochemical characterization of *Qualea grandiflora* Mart. seed. A) Control for the bromophenol blue reagent. B) Distribution of proteins (arrow) stained with bromophenol blue in the ground meristem of the cotyledon. C) Control in *Q. grandiflora* embryo. D) The embryo stained with Schiff's reagent to detect polysaccharide (arrow) in the embryo. E) Embryo stained with Sudan red B showing the presence of lipid (arrow) droplets in meristematic tissues of the embryo. F) Control for tegument and cotyledon. G) Tegument and cotyledon stained with ferric chloride to detect phenolic compounds (*). (co) cotyledon; (gm) ground meristem; (pd) protoderm; (tg) tegument; (vb) vascular bundle. Scale: A-D: 150 µm; E: 80 µm; F-G: 300 µm.

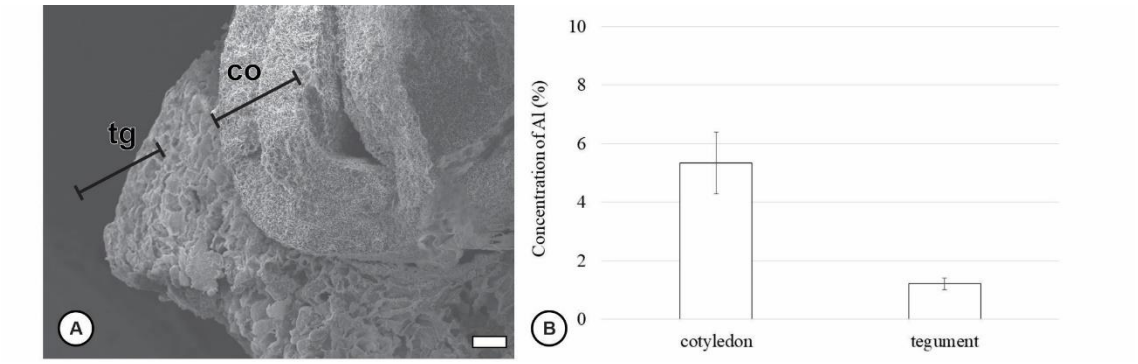

Fig. 4: Al detection in *Qualea grandiflora* Mart. seeds with SEM coupled to an X-ray probe. A) Cross-section of the seed with the six points marked for Al detection. B) Graph with the concentration of Al in the cotyledon (co) and tegument (tg). Scale: 50 µm.

CHAPTER 3

Whole-genome sequencing and de novo assembly reveale aluminum-regulated genes in the *Qualea grandiflora* Mart. (Vochysiaceae)

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Abstract

Qualea grandiflora Mart (Vochysiaceae) is an aluminum (Al)-accumulator species. Furthermore, *Q. grandiflora* is a hermaphroditic, diploid plant with high inbreeding rates and low heterozygosity. Some studies show that *Q. grandiflora* needs Al for its plant growth and development. Despite being widely distributed and one of the most important Cerrado species, *Q. grandiflora* does not have its genome sequenced. This sequencing is crucial to understand how *Q. grandiflora* deals with Al. Therefore, the aim of this work was to sequence, assembly, and annotate genes of this native species. The *Q. grandiflora* genome was sequenced using Illumina platform and assembled de novo, because of the lack of a reference genome. The paired-end sequencing of the *Q. grandiflora* genome generated approximately 57.7 Gb of data, with total coverage of 81% and excellent quality values. Moreover, 38,034 complete genes were annotated. In addition, the Gene Ontology (GO) analysis revealed 14,887 terms separated in Biological Process (7,754), Molecular Functions (3,686), and Cellular Components (3,447). Furthermore, it has been identified genes that could be associated with tolerance/accumulation of Al. About 11 gene families, e. g., *MATE* and *ALMT*, were identified that could be correlated to Al metabolic mechanisms in *Q. grandiflora*. Finally, the BlastKOALA metabolic pathways analysis disclosed several metabolic pathways/genes that have been shown to be regulated by Al. This paper will be the basis for many studies that will contribute to the understanding of the role of Al in this plant species.

Keywords: aluminum, Gene Ontology, genome-whole, native plant, Cerrado.

1. Introduction

Aluminum (Al) is the third most abundant element, the most abundant post-transition metal in the earth's crust (Bojórquez-Quinta et al., 2017; National Center for Biotechnology Information, 2022). Acid soils have greater Al trivalent availability (Al^{3+}), the most phytotoxic form of this metal (Liu et al., 2014). Al^{3+} can rapidly reach plant root cells and harm DNA structure and replication, hindering root growth and impairing essential signaling pathways of the stress response (*e.g.*, activation of Al tolerance gene expression) (Ma, 2007; Daspute et. al., 2017).

It is noteworthy that some plants have adapted to acid soil and high levels of Al^{3+} (Delhaize et al., 2012; Ma et al., 2020). Moreover, at physiological and anatomical levels, these adaptations have been associated with changes in cell wall properties as well as with processes of Al uptake, neutralization, and/or translocation from roots to shoots, which usually lead to the accumulation of Al in various organs (Kochian et al., 2015). Nonetheless, little is known about the molecular basis of Al tolerance and accumulation mechanisms (Ma et al., 2020). Despite this fact, in crop plants such as soybean, wheat, and rice, several genes have been identified and linked to Al tolerance and detoxification (Kochian et al., 2015; Zhang et al., 2019a). For instance, several Al-tolerant genes have frequently been correlated to the *MATE* (Al-activated citrate transporter) and *ALMT* (Al-activated malate transporter) families (Zhang et al., 2019a). Furthermore, a few transcription factors, like the proteins from the MYB, WRKY, NAC, and bZIP families, are the first to respond to environmental cues and can modulate the expression of genes necessary for responses to Al (Zhang et al., 2019a). However, regardless of numerous studies, the correlations between Al and these transcription factors in plant metabolism are still unknown. Moreover, studies are still incipient on Al-responsive genes in accumulating plants, especially related to native plants.

Qualea grandiflora Mart. (Vochysiaceae) is an Al-accumulating species, native to the Cerrado and widely distributed in this biome. In addition, *Q. grandiflora* is a hermaphrodite plant, diploid (2n=22), with high inbreeding and low heterozygosity rates (Yamagishi-Costa et al., 2018; Potascheff et al., 2019). Besides its ecological importance, this species has also raised pharmaceutical and medicinal interests, *e.g.*, *Q. grandiflora* bark extracts have antibacterial (Ayres et al., 2008) and antiulcer activities (Hiruma-Lima et al., 2006). Additionally, its leaf extracts have anticonvulsant, analgesic, and antioxidant properties (Dousseau et al., 2013), and the wood has been used for linings, toys, furniture, among other uses (Ferreira et al., 2001).

Furthermore, because of its capacity of accumulating and high tolerance to Al, *Q. grandiflora* has been the subject of various physiological, ecological, and, more recently, molecular studies. Hence, Andrade et al. (2011) observed that Al was accumulated in chloroplasts of *Q. grandiflora*, which did not affect their functionality. Moreover, the transcriptome of *Q. grandiflora* leaves grown with or without Al reinforced the idea that this species depends on Al for growth and development (Silva, 2017). The presence of Al increased the abundance of enzymes associated with cell wall biosynthesis, primary and secondary metabolism, phytohormones (brassinosteroids and salicylic acid), chloroplast biogenesis, plant defense response, kinase receptors, and Al transportation (Silva, 2017). Conversely, in *Q. grandiflora* plants, the Al downregulated the expression of transcripts related to stress-responsive hormones like ABA, ethylene, jasmonic acid, and proteins associated with biotic and abiotic stresses, amino acid degradation metabolism (Silva, 2017). We propose that fatty acids, proteins, and Al were crucial for germination and the initial growth of *Q. grandiflora* seedlings (Pers. Communication).

The great challenge in understanding the molecular processes responsive to Al in non-model plants is the almost absolute lack of reference genomes for these species.

Nevertheless, with the emergence of alternative sequencing platforms such as the next generation sequencing (NGS) Illumina, there has been an increase in throughput concomitantly with the respective lowering sequencing costs. Therefore, NGS techniques have provided useful resources for genomic studies of non-model plants (Unamba et al., 2015). Hirsch and Buell (2013) have discussed the obstacles to obtaining quality genome assembly from non-model species: 1. Gene and genome duplication (segmental, tandem, and complete genome); 2. Heterozygosity; 3. Ploidy level; and 4. Repetitive sequence composition. These factors might prevent a complete genome sequencing and assembly of non-model plants. However, different strategies and techniques have improved the quality of sequencing and data assembly (Unamba et al., 2015). For example, a good approach is sequencing several independent libraries with different insert sizes on different platforms with the subsequent combination of the data for assembly (Unamba et al., 2015). Thus, the compilation of these data leads to high genome coverage and, consequently, improves assembly quality (Unamba et al., 2015).

Thus, to understand the molecular basis on how *Q. grandiflora* deals with Al, this investigation has performed a *de novo* assembly of its genome to identify genes that are the best candidates for Al tolerance and accumulation in this species. Furthermore, the analysis of this genome aims to determine the gene sequences, including those involved in Al responsive processes, characterizing them according to their function. Therefore, to the best of our knowledge, the present study is the first sequencing of the *Q. grandiflora* genome, which is a crucial milestone towards unraveling the role of this metal in native plants.

2. Material and Methods

2.1 Plant material and DNA extraction

Qualea grandiflora Mart.seeds were collected in an area of Cerrado *stricto sensu* (14° 42' 19.31" S; 47° 40' 11.81" W) in the municipality of Água Fria de Goiás, Goiás-Brazil. Eighty seeds of *Q. grandiflora* were germinated without Al supplementation. The seeds were disinfected in 70% (v/v) ethanol for 1 min and in a 2% (v/v) sodium hypochlorite (NaClO) solution for 20 min and washed in distilled water. Then, the seeds were placed in sterile Petri dishes on germitest paper with filter paper soaked in sterilized distilled water and incubated in a BOD (Biochemical Oxygen Demand-CE-300/350-F CIENLAB, Brazil) for 30 days. After the emergence of the cotyledons, the seedlings were transferred to containers with 500 mL of Agrofloc® vermiculite and grown at 25 °C (± 2), 16 h photoperiod, and light intensity of 80 $\mu mol.m^{-2}.s^{-1}$ (Cury et al. 2020). Subsequently, the seedlings were cultivated with $^{1}/_{5}$ MS (Murashige and Skoog 1962) nutrient solution (watered every three days) without Al supplementation for 90 days. At the end of the experiment, the seedlings had been cultivated for 120 days without Al supplementation.

After germination, leaves from *Q. grandiflora* seedlings were frozen in liquid nitrogen and taken to the Plant Biotechnology Laboratory of the University of Brasília, UnB/DF, for total DNA extraction. The collected material was treated according to the usual herborization procedure (Mori et al., 1989) and incorporated into the collection of the Herbarium of the University of Brasília (UB), Voucher: 217284.

Total DNA extraction was accomplished using Qiagen DNeasy Plant Kit (Hilden, Germany). The concentration and quality of the DNA were verified on a NanoDrop 1000 (Thermo Fisher Scientific, Wilmington, USA), Bioanalyzer (2100, Agilent Technologies

Inc., Santa Clara, USA), Qubit from Life Technologies (Thermo Fisher Scientific, Wilmington, USA), and on 1% agarose gel (Figura S1).

2.2 Illumina sequencing

The sequencing was performed on Illumina HiSeq 2500 platform (San Diego, CA, USA), and four paired-end libraries were built with the TruSeq DNA Nano Low Throughput Library Prep Kit (Illumina, San Diego, CA, USA) with reads size 100-300 bp. In addition, quality checking of the readings was performed with FastQC (v0.11.3).

2.3 *De novo* genome assembly

Prior to assembly, Illumina reads were filtered and trimmed by the Trimmomatic software (Bolger et al., 2014). Next, K-mer analysis was performed in the libraries, using a K-mer of 89 as a parameter. Next, genome size estimation was performed with Genome Scope (Vurture et al., 2017) using Jellyfish v 2.0 (Marçais and Kingsford, 2011) to count and build the histogram of the K-mers. In addition, the genome was assembled using ABySS v2.0 software (Jackman et al., 2017) and DISCOVAR again (Love et al., 2016; https://software.broadinstitute.org/software/discovar). Finally, to assess the quality of the *de novo* genome assembly, the software used was QUAST (Gurevich et al., 2013) and BUSCO v3.0.2 (Simão et al., 2015).

2.4 Prediction and Functional Annotation of Genes

For gene prediction and annotation, it has been used the software Funannotate pipeline v1.8.1 (Palmer and Stajich, 2020), AUGUSTUS v2.5.5 (Stanke et al., 2006a), Snap (Korf, 2004), and GlimmerHMM (Cantarel et al., 2008). First, Funannotate cleaned up and sorted the sequences, and then, the RepeatModeler v2.0/RepeatMasker v4.0.1 software identified the repetitive elements (Stanke et al., 2006b). Also, Funannotate performed a training with the RNA-Seq data of *Q. grandiflora* (SRA:SRR5248188, SRA:SRR5248189, SRA:SRR5248190, SRA:SRR5248191, SRA:SRR5248192,

SRA:SRR5248193) and the sequence bank of Uniprot Myrtales proteins. AUGUSTUS was trained with alignments from the BUSCO dataset (Simão et al., 2015).

Protein annotations were assigned by similarity to the Pfam (Finn et al., 2014) and CAZy (Huang et al., 2018) domains using GlimmerHMM v3.0.472 (Cantarel et al., 2008); and MEROPS (Rawlings et al., 2014), eggNOG v4.5 (Huerta-Cepas et al., 2016) by BLAST and InterProScan v5.20-59.0 (Jones et al., 2014) using Funannotate standards. In addition, the categorization by Gene Ontology (GO) was performed using Blast2GO software (Conesa et al., 2005).

Furthermore, Blast2Go was used to identify gene sequences associated with the response to Al, combined with BLAST at the NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

2.5 Analysis of metabolic pathways

The analysis of metabolic pathways was performed by searching the KEGG database (Kyoto Encyclopedia of Genes and Genomes) using the BlastKOALA mapping platform (KEGG Orthology and Links Annotation) (Kanehisa et al., 2016) for functional annotation based on the following parameters: Plants taxonomic group, and family_eukaryotes database as reference data.

3. Results

3.1 Sequencing, assembly, and coverage

Whole-genome *Q. grandiflora* paired-end sequencing (100 bp-libraries) was accomplished using a HiSeq 2500 Illumina platform and generated about 57.7 Gb of data. In addition, there was an attempt to do a mate-pair sequencing, but the sequences had no sufficient quality to contribute with the whole-genome sequencing. The sequencing of paired-end libraries obtained an optimal quality score (Q>30) with the reliability of

approximately 89% to 92% and an accuracy of 99.9% (Table S1). The sequencing generated 922,309,828 reads, with 2% of the reads discarded data trimming (Table S1). Furthermore, the Illumina platform reading mapping showed average heterozygosity of 1.31%, with an error frequency of 0.78% (Table S2). This high heterozygosity may have been due to the DNA from two different *Q. grandiflora* individuals, although from the same parent plant. The sequencing assembly project covered approximately 81% of the total genome of *Q. grandiflora*, which is approximately 500 Mb in size (Table 1).

The Illumina dataset was assembled using two software (ABySS 2.0 and DISCOVAR again), however, the best results were obtained with the ABySS 2.0 and produced 406,048,254 bp of assembled sequences (Table 1). Furthermore, 277,998 scaffolds were assembled, with a maximum size of 600,552 bp. The size of N50 (shortest contig length at 50% of total genome size) was 2,499 bp, with an L50 of 35,899 and an overall 36.33% GC content in the assembled scaffolds. In addition, repeated sequences constituted about 50.17% (203,730,046 bp) of the genome sequenced (Table 1). Also, the BUSCO software was used to assess the completeness of the assembly by identifying conserved orthologs in the assembled sequences, as shown in Table 2. Therefore, the search found 1,440 groups in the Embryophyte ortholog bank (Table 2), of which 63.1% were "complete" genes. These “complete” genes (C) with more than one copy were designated as 'Duplicate' (D), which corresponded to 1.3% of the genes identified by BUSCO. Partially recovered genes were classified as "fragmented" (F), which was 13.1% (Table 2). Finally, non-recovered genes were classified as "missing" (M) and represented about 23.8% of the genes of the *Q. grandiflora* genome (Table 2).

Table 1: *Qualea grandiflora* Mart. genome assembly statistics.

Genome estimate size (Mb)	**500**
Total size (bp)	406,048,254
Nº of contigs	5,905,481
Nº of scaffolds	277,998
Maximum scaffold length (bp)	600,552
GC (%)	36.33
N50	2,499
N75	806
L50	35,899
L75	114,236
Masked repeats (bp)	203,730,046

Table 2: Summary of BUSCO evaluation of the gene prediction.

Parameter	**BUSCO groups**	**%**
Complete BUSCOs (C)	909	63.1
Complete and single-copy BUSCOs (S)	890	61.8
Complete and duplicated BUSCOs (D)	19	1.3
Fragmented BUSCOs (F)	188	13.1
Missing BUSCOs (M)	343	23.8
Total BUSCO groups searched	1,440	100.0

3.2 Annotation and prediction of *Qualea grandiflora* genome

The structural annotation of the genome of *Q. grandiflora* found 38,034 genes, of which 37,691 were mRNA and 343 tRNA (Table S3). The average gene length was 1,353.38 bp with 114,847 exons. The protein-coding gene sequences were determined based on the BUSCO software and predicted 58.4% complete, 2.6% duplicate, 17.0% fragmented, and 24.6% absent genes (Table S4).

Meanwhile, the predicted proteins were compared against the Interpro database using the Interproscan software for the genome functional annotation, and then BLAST searched against UniProt Clusters 90% (Uniref90) Viridiplantae protein database.

Furthermore, according to the Gene Ontology (GO) analysis, 7,754 gene sequences were placed in biological process (BP), 3,686 in Molecular Function (MF), and

3,447 in Cellular Component (CC) domains. The BP sequences were distributed in various categories among which: organic substance metabolic process (4.2%), primary metabolic process (4.0%), nitrogen compound metabolic process (3.7%), cellular metabolic process (4.0%), biosynthetic process (1.8%), gene expression (1.6%), and transport (1.3%) (Figure 1). Additionally, in the MF domain, the genes were distributed in several groups like protein binding (2.0%), catalytic activity acting on protein (2.3%), carbohydrate derivative binding (2.4%), oxidoreductase activity (2.6%), hydrolase activity (2.8%), small molecule binding (3.3%), transferase activity (3.5%), ion binding (5.0%), organic cyclic compound binding (5.6%), and 5.6% correlated with heterocyclic compound binding (Figure 2). Regarding the CC domain, the genes were mainly associated with intracellular anatomical structure (9.0%), organelle (7.1%), membrane (6.8%), cytoplasm (5.9%), intrinsic membrane component (4.3%), and cell periphery (1.8%) (Figure 3).

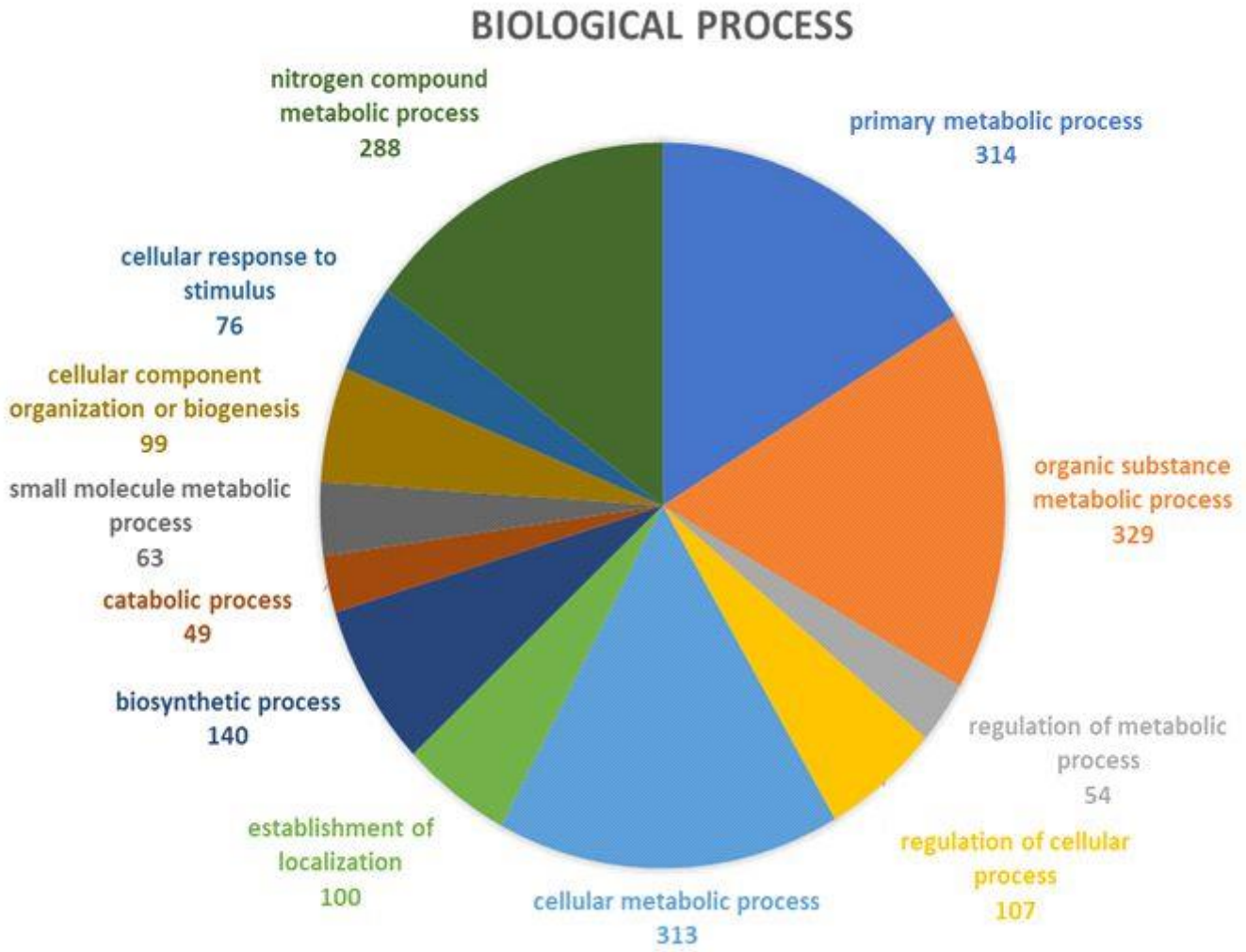

Figure 1: Number of *Qualea grandiflora* Mart. sequences associated with Biological Process categories as designated by GO analysis.

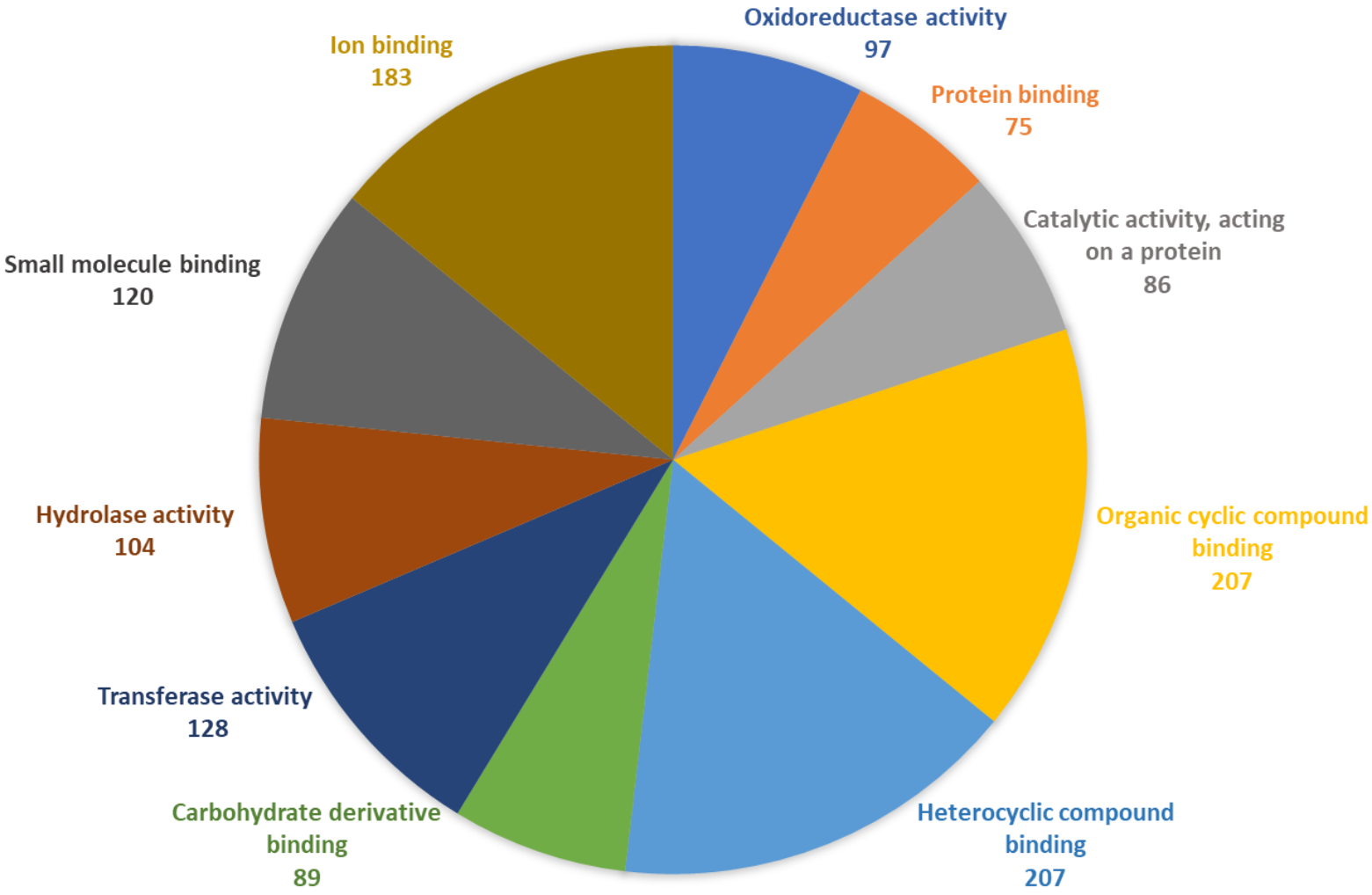

Figure 2: Molecular Function domain categories of *Qualea grandiflora* Mart. genes according to GO analysis (number of sequences).

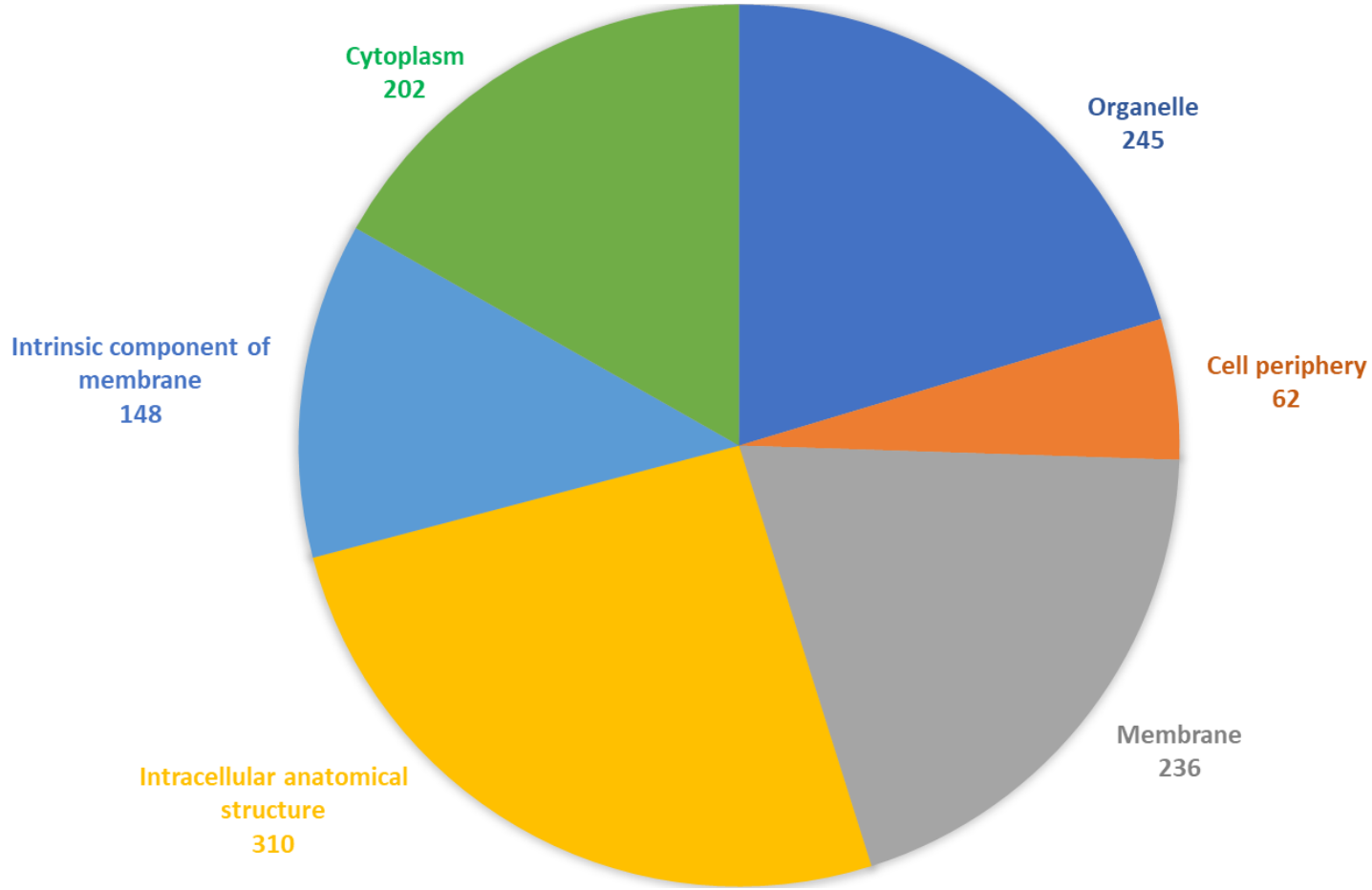

Figure 3: Cellular Component domain categories of *Qualea grandiflora* Mart. genes as determined by GO analysis (number of sequences).

3.3.Comparative analysis of *Qualea grandiflora* genome

The genome comparisons were performed between *Q. grandiflora* and five other Al-accumulating species: *Eucalyptus grandis* W. Hill ex Maiden., *Camellia sinensis* (L.) Kuntze, *Fagopyrum esculentum* Moench., *Citrus sinensis* L. Osbeck, as well as the native species of the cerrado *Caryocar brasiliense* Cambess. (Table 5). The genomic coverage of *Q. grandiflora* (81%) was higher than that of *Caryocar brasiliense* (45.6%), and *Eugenia dysenterica* (56.7%). Among the species analyzed, the number of reads of *Q. grandiflora* (922,309,828) sequencing was second only to *F. esculentum* (1,212,021,130). Additionally, the GC content of the *Q. grandiflora* genome (36.33%) was close to all other species and varied between 34 to 40%. The N50 scaffold varied widely among species, which in *Q. grandiflora* was 2,499 bp, 67,070 bp in *Camellia sinensis*, and 1,690,000 bp in *Citrus sinensis*. The number of predicted genes found in the genome of *Q. grandiflora* was the highest among the analyzed species, with the exception of *E. dysenterica*. For *C. brasiliense*, the number of genes was not informed.

Table 5: Comparison of *Qualea grandiflora* Mart. assembly and annotation with genome projects of Al-accumulating angiosperms.

Assembly	***Qualea grandiflora* Mart.**	***Eucalyptus grandis* W. Hill ex Maiden.**	***Camellia sinensis* L. Kuntze**	***Fagopyrum esculentum* Moench.**	***Citrus sinensis* L. Osbeck**	***Caryocar brasiliense* Cambess.**	***Eugenia dysenterica* DC.**
Reference	-	Myburg et al. (2014)	Wei et al. (2018)	Yasui et al. (2016)	Xu et al. (2013)	Nunes et al. (2019)	Ribeiro (2016)
Genome size (Mb)	~ 500	640	~ 2,980	1,200	367	464	442
Coverage (%)	81	> 94	93	98.3	87.3	45.7	56.7
Reads	922,309,828	3,446,208	1,325,000	Between 1,212,021,130 – 2,424,042,260	785,000,000	293,621,819	59,415,168
GC%	36.33	40.0	39.0	39.1	34.06	34.84	-
N50 scaffold (bp)	2,499	53,892,272	67,070	25,109	1,690,000	6,005	2,658
Number of predicted genes	38,034	36,376	33,932	35,000	29,445	-	60,171

3.4. Functional analysis of candidate genes from the *Qualea grandiflora* genome that might be differentially regulated in response to Al.

The sequence of genes associated with the response to Al found in the literature was compared with the *Q. grandiflora* genome to identify similar sequences, using Blast2GO and BLAST/NCBI (Table S4). Therefore, some *Q. grandiflora* genome sequences with greater similarity mean and lower e-value were selected. Moreover, a few Al-associated genes have been listed according to their Gene ID (Table 6). Therefore, genes associated with Al-resistance/tolerance, Al transportation, organic acids, plant growth regulators, and crucial metabolic pathways such as the methylation cycle and cell wall synthesis are good candidates for Al response in *Q. grandiflora*.

Table 6: Al-associated gene families found in the genome of *Qualea grandiflora* Mart.

Family Gene ID	Description	e-value	Similarity Means	PFAM	InterPro	GO	References
NRAT	Natural resistance-Al resistance transcription factor 1	6,50E-136	92.94	PF01566	IPR001046	GO_component: GO:0016020 - membrane [Evidence IEA]; GO_function: GO:0046873 - metal ion transmembrane transporter activity [Evidence IEA]; GO_process: GO:0009873	Kochian et al. (2015); Kopittke et al. (2017); Tao et al. (2018)
ALMT	Aluminium activated malate transporter	2,29E-124	82.75	PF11744	IPR020966	GO_process: GO:0015743 - malate transport [Evidence IEA]	Sasaki et al. (2004); Hoekenga et al. (2006); Piñeros et al. (2008a, b); Liu et al. (2009); Chen et al. (2011); Ligaba et al. (2012); Liang et al. (2013); Liu et al. (2017); Park et al. (2017); Zhang et al. (2017); Riaz et al. (2018); Gallo-Franco et al. (2020); Ma et al. (2020); Oliveira

							and Pinto-Maglio, (2020).
MATE	Multi-drug and toxic compound extrusion family	1,18E-36	82.36	PF01554	IPR002528	GO_component: GO:0016020 - membrane [Evidence IEA];GO_function: GO:0015297 - antiporter activity [Evidence IEA];GO_function: GO:0015238 - drug transmembrane transporter activity [Evidence IEA];GO_process: GO:0006855 - drug transmembrane transport [Evidence IEA]; GO_process: GO:0055085 - transmembrane transport [Evidence IEA]	Magalhães et al. (2007); Liu et al. (2009); Maron et al. (2010); Yokosho et al. (2010, 2011); Yang et al. (2017); Takanashi et al. (2017); Tanaka et al. (2017)
PME	Pectin methylesterase	1,95E-156	79.84	PF01095	IPR000070	GO_function: GO:0030599 - pectinesterase activity [Evidence IEA];GO_process: GO:0042545 - cell wall modification [Evidence IEA]	Gaffe et al. (1992); Schmohl et al. (2000); Eticha et al. (2005); Rangel et al. (2009); El-Moneim et al. (2014); Li et al. (2016); Zhang et al. (2019b); Zhu et al. (2019)

ME	Malic oxidoreductase	3,84E-169	96.17	PF00390; PF03949	IPR001891	GO_function: GO:0004471 - malate dehydrogenase (decarboxylating) (NAD+) activity [Evidence IEA];GO_function: GO:0051287 - NAD binding [Evidence IEA];GO_function: GO:0004470 - malic enzyme activity [Evidence IEA]; GO_process: GO:0055114 - oxidation-reduction process [Evidence IEA]	Copeland et al. (1989); Detarsio et al. (2003); Krill et al. (2010); Sun et al. (2019); Badia et al. (2020)
FDH	Formate dehydrogenase	1,44E-43	86.65	PF00389	IPR006139; IPR006140	GO_function: GO:0051287 - NAD binding [Evidence IEA];GO_function: GO:0016616 - oxidoreductase activity, acting on the CH-OH group of donors, NAD or NADP as acceptor [Evidence IEA];GO_function: GO:0008863 - formate dehydrogenase (NAD+) activity [Evidence IEA]; GO_process: GO:0055114 - oxidation-reduction process [Evidence IEA]	Hourton-Cabassa et al. (1998); Suzuki et al. (1998); David et al. (2010); Lou et al. (2016); Cury (2017); Cury et al. (2020)

SAHH	*S*-adenosyl-L-homocysteine hydrolase	2,42E-174	96.76	PF00670	IPR000043; IPR003726; IPR020082	GO_function: GO:0004013 - adenosylhomocysteinase activity [Evidence IEA]	Eticha et al. (2005); Yang et al. (2008); Krill et al. (2010); Zheng et al. (2014); Ezaki et al. (2016); Coelho et al. (2019)
SAMS	*S*-adenosylmethionine synthetase	0.0	98.67	PF00438; PF02772; PF02773	IPR002133; IPR022629; IPR022630	GO_function: GO:0005524 - ATP binding [Evidence IEA];GO_function: GO:0004478 - methionine adenosyltransferase activity [Evidence IEA]; GO_process: GO:0006556 - S-adenosylmethionine biosynthetic process [Evidence IEA]	Eticha et al. (2005); Yang et al. (2008); Krill et al. (2010); Zheng et al. (2014); Ezaki et al. (2016); Coelho et al. (2019)
GST	Glutathione *S*-transferase	6,94368E-57	89.46	PF02798; PF13409; PF13417	IPR004045; IPR010987	GO_function: GO:0005515 - protein binding [Evidence IEA]	Marrs (1996); Ezaki et al. (2004); Dmitriev et al. (2016); Silva (2017); Cury (2017); Cury et al. (2020)
ABC	ABC transporter	6,61E-153	97.29	PF00005; PF00664	IPR003439	GO_component: GO:0016021 - integral component of membrane [Evidence IEA]; GO_function: GO:0016887 - ATPase activity [Evidence	Larsen et al. (2007); Huang et al. (2009); Song et al. (2014); Tao et al. (2018); Huang et al. (2021)

						IEA];GO_function: GO:0042626 - ATPase activity, coupled to transmembrane movement of substances [Evidence IEA];GO_function: GO:0005524 - ATP binding [Evidence IEA]; GO_process: GO:0055085 - transmembrane transport [Evidence IEA]	
ASR	Abscisic acid (ABA), stress, and ripening	1,12E-13	94.62	PF02496	IPR003496		Carrari et al. (2004); Arenhart et al. (2013); Sade et al. (2016)

3.5 Analysis of metabolic pathways

According to the BlastKOALA platform, *Q. grandiflora* proteins were assigned to more than 300 different metabolic pathways. Furthermore, the results showed that the identified proteins, translated from the *Q. grandiflora* sequenced genome, were involved in pathways related to several metabolic processes like genetic information processing family (23%), carbohydrate metabolism (11%), signaling, and cellular process family (10%), and environmental information processing (8%) (Figure 4).

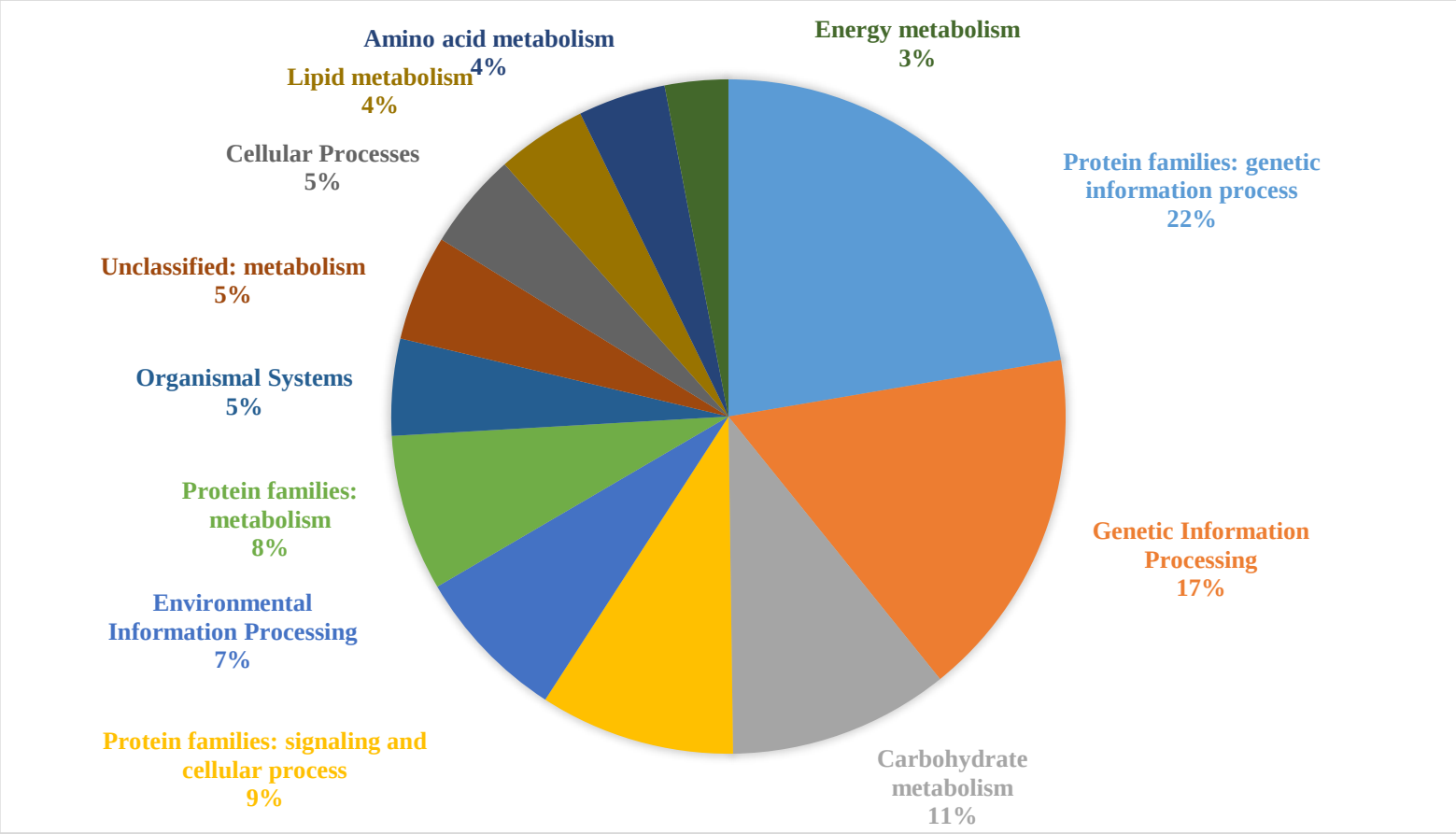

Figure 4: Main metabolic pathways assigned to *Qualea grandiflora* Mart. proteins.

4. Discussion

4.1. Assembly and GO analysis of *Qualea grandiflora* genome

The current study is the first whole-genome sequencing project of *Q. grandiflora*, an ecologically relevant species for the Cerrado. Considering the size and complexity of the genome and the absence of a reference genome, the sequencing of the *Q. grandiflora* genome was of good quality and coverage. In addition, the *de novo* assembly of the *Q. grandiflora* genome showed 63.1% complete genes, which is considered very reasonable for genome projects of non-model organisms (Song et al., 2016). In non-model plants, depending on the intrinsic difficulties of the species (*e.g.*, genome size and complexity, taxonomic position), a percentage > 50% of complete genes is considered acceptable (Seppey et al., 2019; Christiansen et al., 2021). Additionally, the comparison between *Q. grandiflora* genome sequencing/assembly and other Al-accumulating species (native and exotic) reveals a robust set of data. For example, *Q. grandiflora* sequence coverage (81%), which was considerably better than that of other native plants such as *C. brasiliense* (45.7%), and *E. dysenterica* (56.7%) (Ribeiro, 2016; Nunes et al., 2019).

The Gene Ontology (GO) Resource (http://geneontology.org/) provides useful knowledge through a computational model of biological systems that are supported by community inputs by its community (Harris et al., 2004; Hill et al., 2008). The purpose of this bioinformatics initiative is to categorize genes focusing on their products and functions through a predefined controlled vocabulary (Balakrishnan et al., 2013). Moreover, it is an essential computational tool for functional genomics of large-scale biological data (Reijnders and Waterhouse, 2021). The GO has three separate domains: Molecular Function (MF), Biological Process (BP), and Cellular Component (CC). In January 2022, the GO Resource contained 43,786 terms, of which 28,428 were categorized as BP, 11,175 MF, and 4,183 CC domains.

Additionally, there are 7,965,896 GO annotations and 1,566,018 annotated gene products. In *Q. grandiflora*, the GO analysis resulted in 14,887 terms (7,754 BP, 3,686 MF, and 3,447 CC) with 38,034 annotations.

The GO is a powerful tool for functional analysis of genomics that continuously grows with time because of constant input of data (Rhee et al., 2008). Thus, the rapid advances in molecular sequencing technologies have increased our understanding of molecular basis of the evolution of specific traits in almost all organisms (Primmer et al., 2013). Furthermore, the abundant information on gene products and functions due to GO (BP, MF, and CC) from model organisms can be used to derive orthologues in recently sequenced species (Primmer et al., 2013). Hence, the information on the *Q. grandiflora* genome acquired by its sequencing will contribute to our understanding of the process of evolution of adaptation of this species to Cerrado environmental conditions. Another important aspect of a GO analysis is the opportunity to investigate the functionality of genes of interest, as the focus of this computational analysis provides information on gene products and functions.

Moreover, Silva (2017) and Cury et al. (2019, 2020) investigated the differential expression of genes in *Q. grandiflora* leaves and root proteome in response to the presence of Al. It appears that the *Q. grandiflora* transcriptome and proteome have a high degree of consistency with genome data. Furthermore, differentially expressed transcripts and proteins in leaves and roots of *Q. grandiflora* were associated with several important metabolic processes responsive to Al, such as ionic binding, catalytic activity, and transport (Silva, 2017, Cury et al., 2019, 2020).

4.2 Genes associated with Al resistance/tolerance were identified in *Q. grandiflora* genome

Several genes associated with Al tolerance/resistance were identified in the *Q. grandiflora* genome. Genes related to transmembrane transport were identified in the genome

of *Q. grandiflora*, such as *ALMT* (Aluminum activated malate transporter), *MATE* (Multi-drug and toxic compound extrusion family), *ABC* (ABC transporter), and *NRAT* (Natural resistance-Al resistance transcription factor 1) (Magalhães et al., 2007; Huang et al., 2009; Kochian et al., 2015; Sasaki et al., 2004).

The *ALMT* gene is a malate transporter activated by Al^{3+} in roots that results in the exudation of malate into the rhizosphere, enabling the chelation of this cation externally. This phenomenon prevents Al damage in sensitive plants and is crucial for the Al resistance mechanism. This gene encodes transmembrane proteins that form anion channels, including transporting organic anions such as malate (Liu and Zhou, 2018). *ALMT* was first identified in the roots of a wheat genotype (*TaALMT1*) (Sasaki et al., 2004). In addition, this gene has been found in *Hevea brasilienses* L. (*HbALMT*) (Ma et al., 2020), *Camellia sinensis* (*CsALMT1*), on Al accumulating plant (Park et al., 2017), *Arabidopsis thaliana* (Hoekenga et al., 2006; Liu et al., 2009), alfalfa (Chen et al., 2011), and maize (Ligaba et al., 2012). To the best of our knowledge, this is the first record of the presence of *ALMT* in the genome of a Cerrado species.

Another gene associated with transmembrane transport found in the *Q. grandiflora* genome was *MATE* (DTX36_2). This gene belongs to the *MATE* family, and its expression is induced by the presence of Al^{3+} by the roots via citrate secretion (Magalhães et al., 2007; Kochian et al., 2015). This gene was first identified in sorghum (*SbMATE*. Magalhães et al., 2007) and since then found in barley (HvAACT1. Takanashi et al., 2017), *Arabidopsis* (Liu et al., 2009), corn (Maron et al., 2010), rice (Yokosho et al., 2011), and rye (Yokosho et al., 2010). Regarding the presence of *MATE* in the *Q. grandiflora* genome, its expression has been reported in response to Al in *Q. grandiflora* leaves (Silva, 2017). However, it is still not possible to know the role of this gene in *Q. grandiflora* leaves in response to Al.

Like the *MATE*, the *NRAT* gene is present in the genome of *Q. grandiflora* (*NRAMP2_2* - natural resistance-associated macrophage protein - Al transporter 2). In higher plants, Nramp proteins play important roles in transporting mineral elements from the soil to different plant organs and tissues (Lu et al., 2018). For instance, in Arabidopsis, the AtNramp1 protein is involved in transporting Mn^{2+} (Tyagi et al., 2020). AtNramp3 and AtNramp4 are located in the tonoplast and act in mobilizing Fe^{2+} from vacuolar stores to support the developing plant (Tyagi et al., 2020). Furthermore, in rice roots, the Al transporter 1 gene (*OsNrat1*) transports Al^{3+} from the cytoplasm to the vacuole of root cells (Xia et al., 2010; Kochian et al., 2015). It has been suggested that Nrat1 is necessary for the internal detoxification of Al through its sequestration in vacuoles (Xia et al., 2010). In *Q. grandiflora*, there is no record of the differentiated expression of this family of genes in response to Al (Cury et al., 2019, 2020).

Genes encoding ABC transporters (ATP-Binding Cassette) were also found in the genome of *Q. grandiflora*. Nine hundred and six isoforms of the *ABC* genes were found in *Q. grandiflora* (Table S4). Analysis of the transcriptome *Q. grandiflora* leaves revealed the presence of four differentially regulated *ABC* genes in response to Al (Silva, 2017). However, only one of these genes was upregulated in the presence of Al in *Q. grandiflora* leaves (Silva, 2017). ABC transporters hydrolyze ATP to energize various biological systems and can act in coordination with other genes, as occurs during Al detoxification in rice where and ABC transporter acts together with the Al transporter 1 (*OsNrat1*) gene (Kochian et al., 2015). The *ABC* gene family comprises eight subfamilies whose genes are associated with important physiological processes, including responses to environmental stress (Lane et al., 2016; Liu et al., 2021). Thus, it is not surprising that these ABC transporters act in Al detoxification in plants (Liu et al., 2021). Additionally, genes from this family are associated with Al tolerance, such as *Arabidopsis* (*ALS1* and *3* – Al-sensitive 1 and 3) (Larsen et al., 2007), barley (*HvABCB25*)

(Liu et al., 2021), soybean (Huang et al., 2021), and rice (*STAR1* and *2* – sensitive to aluminum rhizotoxicity, formerly known as *ALS1* and *3*) (Huang et al., 2009). However, the activation of the *ABC* genes does not seem to occur in *Q. grandiflora* in response to Al.

However, it is noteworthy that most genes associated with Al tolerance/resistance present in the genome of *Q. grandiflora* were not upregulated in the presence of Al (Silva, 2017, Cury et al., 2019, 2020). In fact, many were downregulated by Al^{3+} in leaves and roots of *Q. grandiflora* plants supplemented with Al (Silva, 2017, Cury et al., 2019, 2020). Furthermore, it is necessary to consider that even genes that are related to Al tolerance/resistance can be activated by other endogenous and exogenous factors (Piñeros et al., 2008b).

4.3 Genome identification of Al-upregulated genes in leaf transcriptome and root proteome of *Q. grandiflora*.

As expected, genes involved in ubiquitous processes such as *SAMS2_1* and *SAMS2_2* (*S*-adenosyl methionine synthetase 2) were found in the genome of *Q. grandiflora*. SAMS catalyzes the formation of SAM (*S*-adenosyl methionine), which is the source of methyl groups for most transmethylation reactions like the methylation of DNA, RNA, pectins, proteins, lipids, and the synthesis of ethylene and polyamines (Moffatt and Weretilnyk, 2001; He et al., 2019). Recently, it was demonstrated that the *SAMS* genes could confer Al tolerance in *Andropogo virginicus* L. (*AvSAMS1*) (Ezaki et al., 2016). Ezaki et al. (2016) suggested a correlation between *AvSAMS1* as the SAM source and a mediator that affects epigenetic stress regulation of Al response. It is important to mention that Al upregulated the *SAMS2* gene in the root proteome of *Q. grandiflora* (Cury et al., 2019, 2020). Concomitantly, the *SAHH2* (S-adenosylhomoysteine hydrolase 2; Table S4) and Adenosine Kinase (*ADK*; IPR001810) gene were also identified in the genome of *Q. grandiflora*. These genes are essential for maintaining SAM-dependent transmethylation reactions in plants (Moffatt et al., 2002; Pereira et al., 2006, 2007).

Additionally, a gene for pectin methylesterification (*PME29*) was identified in the genome of *Q. grandiflora*. The PME regulates the degree of pectin methylation in plant cell walls (Eticha et al., 2005; Pereira et al., 2006; El-Moneim et al., 2014). The cell wall is known as one of the most important sites of Al accumulation/action (Eticha et al., 2005; Kochian et al., 2015; Ma et al., 2020). Besides, the *Q. grandiflora* root proteome showed that PMEs were upregulated in the presence of Al, which may indicate that cell wall synthesis was favored in the presence of this metal (Cury et al., 2019, 2020).

The genes described here are related to the response of *Q. grandiflora* to Al. Thus, we observe that this work is just the beginning to understand the complex mechanisms involving Al in this plant. However, further analysis is required to functionally characterize not only the genes mentioned above but also to understand how the regulation of other genes is affected by the presence of this metal in *Q. grandiflora*.

4.4 Metabolic pathways of the *Q. grandiflora* genome.

It is noteworthy that the GO and BlastKOALA analyses of the *Q. grandiflora* genome reinforce what was found with transcriptome and proteome data (Silva, 2017; Cury et al., 2019, 2020).

The genome BlastKOALA analysis favored metabolic pathways that were very active in *Q. grandiflora* leaf transcriptome and root proteome analyses, such as genetic information processing, primary metabolism (carbohydrate, energy, and lipid metabolisms), environmental information processing, and signaling (Silva, 2017; Cury et al., 2019, 2020). Moreover, the genome sequencing of *Q. grandiflora* permits the identification of sequences of gene involved in pivotal metabolic routes that are directly responsive to Al and crucial for plant metabolism, *e. g.*, photosynthesis. For instance, six sequences related to Rubisco were identified in the genome of *Q. grandiflora* , mainly for its large subunit. Moreover, there is strong evidence that

this metabolic process requires Al to work properly in this species. Thus, Andrade et al. (2011) observed that *Q. grandiflora* chloroplasts accumulated Al and did not undergo any structural damage. Furthermore, Silva (2017) reported that Al was crucial for synthesizing photosynthetic pigments. Therefore, the sequenced genome of *Q. grandiflora* will contribute to understanding not only the involvement of Al in the photosynthetic process but also why Al is needed for the growth and development of this plant (Silva, 2017; Cury et al., 2019, 2020).

5. Conclusion

This is the first genome sequencing project of *Q. grandiflora*. 81% of total genome coverage was achieved with excellent quality values considering that there was no reference genome, and it involved a non-model species. In addition, 11 gene families were identified that would be involved in Al metabolic mechanisms in this species. These gene families are well known in the literature for their involvement in Al tolerance/resistance in many plants. Thus, this study provides a guide for gene selection to perform functional analysis, cloning, association analysis to understand the mechanism involving Al in *Q. grandiflora*.

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SUPPLEMENTARY INFORMATION

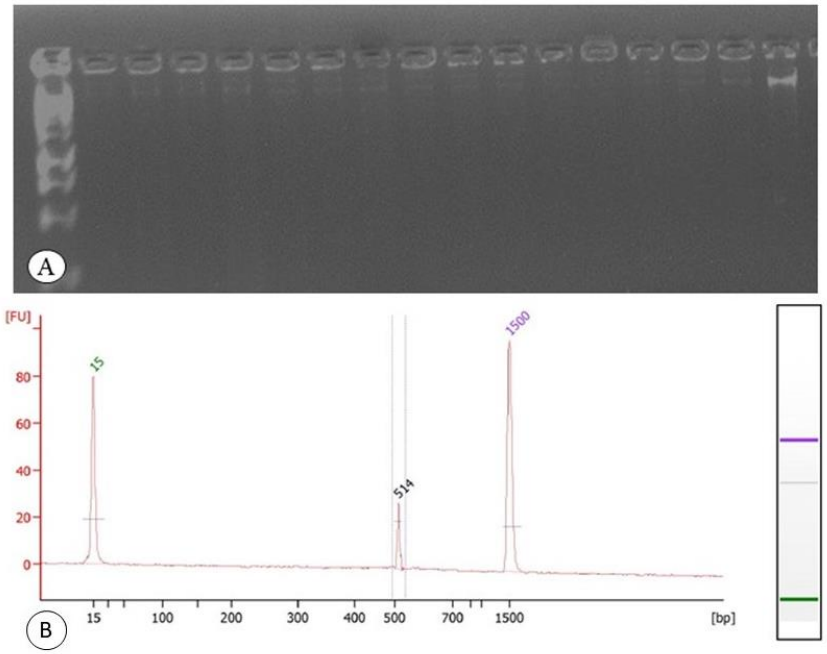

Figure S1: Quality and integrity of the total DNA extracted from *Qualea grandiflora* Mart. A. Agarose gel showing the integrity of *Q. grandiflora* DNA. B. Bioanalyzer data on *Q. grandiflora* DNA quality and integrity (by LACTAD).

Table S1: Summary of *Qualea grandiflora* Mart. genome DNA sequencing data.

Library insert size	Run	Reads	Gigabases	Coverage	Trimmed Reads	% Trimmed Reads	Gigabases Trimmed Reads	Coverage Trimmed Reads*	%Bases ≥ Q30
paired end	200825	106,747,006	10.78	21.56	102,244,660	95.78%	10.33	20.65	89.38
paired end	201002	277,906,232	28.07	56.14	267,869,548	96.39%	27.05	54.11	91.98
paired end	201210	161,070,374	16.27	32.54	160,308,418	99.53%	16.19	32.38	96.06
paired end	210615	376,586,216	38.04	76.07	375,794,586	99.79%	37.96	75.91	92.39
	Total	**922,309,828**	**93.15**	**186.31**	**906,217,212**		**91.53**	**183.06**	

Table S2: Avaliação da predição de acordo com o BUSCO.

K-mer value	Genome Haploid length(bp)	Genome Repear Length(bp)	Genome Unique Length (bp)	Genome size (Mb)	Heterozygosity rate (%)	Model Fit	Error rate (%)
21	496,325,302	146,236,230	350,089,072	500	1.31	96.81	0.78

Table S3: *Qualea grandiflora* Mart. final genome annotation statistics.

Parameter	BUSCO groups	
Complete BUSCOs (C)	840	58.4%
Complete and single-copy BUSCOs (S)	803	55.8%
Complete and duplicated BUSCOs (D)	37	2.6%
Fragmented BUSCOs (F)	245	17.0%
Missing BUSCOs (M)	355	24.6%
Total BUSCO groups searched	1440	

Table S4: Estimation of genome characteristics based on 21-mer statistics.

Number of genes	38,034
Average gene length (bp)	1,353.38
Number of exons	114,847
mRNA	37,691
tRNA	343

Table S5: Al-associated genes found in the genome of *Qualea grandiflora* Mart., using Blast2Go.

SeqName	Description	Length	Hits	e-value	sim mean (%)	NAME	GO ID	GO Names	Interpro Ids	GO ID	Interpro Name
QG_037386-T1	1358661UniRef90_A0A1S3U8U8 metal transporter Nramp2 isoform X1 n=17 Tax=Phaseoleae TaxID=163735 RepID=A0A1S3U8U8_VIGRR	225	5	6,50E-136	92.94	NRAMP2_2	P:GO:0006508; F:GO:0008233; C:GO:0019031	P:proteolysis; F:peptidase activity; C:viral envelope	IPR001046 (PRINTS); IPR001046 (PFAM); NON_CYTOPLASMIC_DOMAIN (PHOBIUS); NON_CYTOPLASMIC_DOMAIN (PHOBIUS); TRANSMEMBRANE (PHOBIUS); TRANSMEMBRANE (PHOBIUS); CYTOPLASMIC_DOMAIN (PHOBIUS); TRANSMEMBRANE (PHOBIUS); TRANSMEM	P:GO:0030001; F:GO:0046873; C:GO:0016020	P:metal ion transport; F:metal ion transmembrane transporter activity; C:membrane

									BRANE (PHOBIUS); TRANSMEMBRANE (PHOBIUS); CYTOPLASMIC_DOMAIN (PHOBIUS); CYTOPLASMIC_DOMAIN (PHOBIUS); NON_CYTOPLASMIC_DOMAIN (PHOBIUS)		
QG_025630-T1	1241465UniRef90_UPI0011D20388 aluminum-activated malate transporter 12-like n=1 Tax=Syzygium oleosum TaxID=219896 RepID=UPI0011D20388	250	5	2,29E-124	82.75	ALMT12_2			IPR020966 (PFAM); mobidb-lite (MOBIDB_LITE); NON_CYTOPLASMIC_DOMAIN (PHOBIUS); TRANSMEMBRANE (PHOBIUS); TRANSMEMBRANE (PHOBIUS); CYTOPLASMIC_DOMAIN (PHOBIUS);	P:GO:0015743	P:malate transport

									TRANSMEMBRANE (PHOBIUS); NON_CYTOPLASMIC_DOMAIN (PHOBIUS); CYTOPLASMIC_DOMAIN (PHOBIUS); TRANSMEMBRANE (PHOBIUS); TRANSMEMBRANE (PHOBIUS); CYTOPLASMIC_DOMAIN (PHOBIUS); TRANSMEMBRANE (PHOBIUS); NON_CYTOPLASMIC_DOMAIN (PHOBIUS); CYTOPLASMIC_DOMAIN (PHOBIUS)		
QG_025630-T1	1241465UniRef90_UPI0011D20388 aluminum-activated malate transporter 12-like	250	5	2,29E-124	82.75	ALMT12_2			IPR020966 (PFAM); mobidb-lite (MOBIDB_LITE);	P:GO:0015743	P:malate transport

	n=1 Tax=Syzygium oleosum TaxID=219896 RepID=UPI0011D20388								NON_CYTOPLASMIC_DOMAIN (PHOBIUS); TRANSMEMBRANE (PHOBIUS); TRANSMEMBRANE (PHOBIUS); CYTOPLASMIC_DOMAIN (PHOBIUS); TRANSMEMBRANE (PHOBIUS); NON_CYTOPLASMIC_DOMAIN (PHOBIUS); CYTOPLASMIC_DOMAIN (PHOBIUS); TRANSMEMBRANE (PHOBIUS); TRANSMEMBRANE (PHOBIUS); CYTOPLASMIC_DOMAIN (PHOBIUS); TRANSMEM		

									BRANE (PHOBIUS); NON_CYTOPLASMIC_DOMAIN (PHOBIUS); CYTOPLASMIC_DOMAIN (PHOBIUS)		
QG_015524-T1	4760474UniRef90_A0A7J0G6B6 MATE efflux family protein n=1 Tax=Actinidia rufa TaxID=165716 RepID=A0A7J0G6B6_9ERIC	100	5	1,18E-36	82.36	DTX36_2	P:GO:0006278; P:GO:0090305; F:GO:0003964; F:GO:0004518; F:GO:0004519; F:GO:0016740; F:GO:0016779; F:GO:0016787	P:RNA-dependent DNA biosynthetic process; P:nucleic acid phosphodiester bond hydrolysis; F:RNA-directed DNA polymerase activity; F:nuclease activity; F:endonuclease activity; F:transferase activity; F:nucleotidyltransferase activity; F:hydrolase activity	IPR002528 (PFAM); CYTOPLASMIC_DOMAIN (PHOBIUS); TRANSMEMBRANE (PHOBIUS); CYTOPLASMIC_DOMAIN (PHOBIUS); NON_CYTOPLASMIC_DOMAIN (PHOBIUS); TRANSMEMBRANE (PHOBIUS); TRANSMEMBRANE (PHOBIUS); NON_CYTOPLASMIC_DOMAIN (PHOBIUS)	P:GO:0006855; P:GO:0055085; F:GO:0015297; F:GO:0042910; C:GO:0016020	P:xenobiotic transmembrane transport; P:transmembrane transport; F:antiporter activity; F:xenobiotic transmembrane transporter activity; C:membrane

QG_019816-T1	2675630UniRef90_UPI0011D218B7 probable pectinesterase 29 n=1 Tax=Syzygium oleosum TaxID=219896 RepID=UPI0011D218B7	349	5	1,95E-156	79.84	PME29	P:GO:0006508; F:GO:0004190; F:GO:0008233; F:GO:0016787	P:proteolysis; F:aspartic-type endopeptidase activity; F:peptidase activity; F:hydrolase activity	IPR012334 (G3DSA:2.160.20.GENE3D); IPR000070 (PFAM); IPR033131 (PROSITE_PATTERNS); SIGNAL_PEPTIDE_C_REGION (PHOBIUS); NON_CYTOPLASMIC_DOMAIN (PHOBIUS); SIGNAL_PEPTIDE_N_REGION (PHOBIUS); SIGNAL_PEPTIDE (PHOBIUS); SIGNAL_PEPTIDE_H_REGION (PHOBIUS); SignalP-TM (SIGNALP_EUK); IPR011050 (SUPERFAMILY)	P:GO:0042545; F:GO:0030599	P:cell wall modification; F:pectinesterase activity
QG_025316-T1	932917UniRef90_A0A6P8CWJ3 Malic enzyme	263	5	3,84E-169	96.17	NADPME4	P:GO:0006811; P:GO:000681	P:ion transport; P:potassium	IPR001891 (PRINTS); IPR012301	P:GO:0055114; F:GO:00	P:obsolete oxidatio

	n=5 Tax=Punica granatum TaxID=22663 RepID=A0A6P8CWJ3_PUNGR						3; C:GO:0016020; C:GO:0016021	ion transport; C:membrane; C:integral component of membrane	(SMART); IPR012302 (SMART); IPR012302 (PFAM); IPR001891 (PIRSF); G3DSA:3.40.50.720 (GENE3D); IPR012301 (PFAM); IPR037062 (G3DSA:3.40.50.GENE3D); IPR015884 (PROSITE_PATTERNS); IPR036291 (SUPERFAMILY); SSF53223 (SUPERFAMILY)	04470; F:GO:0004471; F:GO:0051287	n-reduction process; F:malic enzyme activity; F:malate dehydrogenase (decarboxylating) (NAD+) activity; F:NAD binding
QG_008681-T1	2348016UniRef90_A0A6A2ZCL4 Formate dehydrogenase, mitochondrial n=3 Tax=malvids TaxID=91836 RepID=A0A6A2ZCL4_HIBSY	384	5	0.0	93.13	FDH1	P:GO:0006779; P:GO:0006783; P:GO:0006937; P:GO:0006942; F:GO:0004729; F:GO:0016491;	P:porphyrin-containing compound biosynthetic process; P:heme biosynthetic process; P:regulation of muscle contraction; P:regulation	G3DSA:3.40.50.720 (GENE3D); IPR006139 (PFAM); G3DSA:3.40.50.720 (GENE3D); IPR006140 (PFAM); IPR029753 (PROSITE_P		

							C:GO:0005737; C:GO:0005861	of striated muscle contraction; F:oxygen-dependent protoporphyrinogen oxidase activity; F:oxidoreductase activity; C:cytoplasm; C:troponin complex	ATTERNS); IPR029753 (PROSITE_PATTERNS); IPR029752 (PROSITE_PATTERNS); SIGNAL_PEPTIDE_H_REGION (PHOBIUS); SIGNAL_PEPTIDE (PHOBIUS); SIGNAL_PEPTIDE_N_REGION (PHOBIUS); NON_CYTOPLASMIC_DOMAIN (PHOBIUS); SIGNAL_PEPTIDE_C_REGION (PHOBIUS); IPR033689 (HAMAP); IPR033689 (CDD); SignalP-TM (SIGNALP_GRAM_POSITIVE); SignalP-noTM (SIGNALP_G		

									RAM_NEGATIVE); IPR036291 (SUPERFAMILY); SSF52283 (SUPERFAMILY)		
QG_042365-T1	1628703UniRef90_A0A446MS47 Adenosylhomocysteinase n=4 Tax=Triticum turgidum subsp. durum TaxID=4567 RepID=A0A446MS47_TRITD	271	5	2,42E-174	96.76	SAHH2	P:GO:0005975; P:GO:0006541; P:GO:1901135; P:GO:1901137; F:GO:0004360; F:GO:0008483; F:GO:0016740; F:GO:0097367; C:GO:0005737	P:carbohydrate metabolic process; P:glutamine metabolic process; P:carbohydrate derivative metabolic process; P:carbohydrate derivative biosynthetic process; F:glutamine-fructose-6-phosphate transaminase (isomerizing) activity; F:transaminase activity; F:transferase activity; F:carbohydr	IPR015878 (SMART); IPR000043 (SMART); G3DSA:3.40.50.1480 (GENE3D); IPR015878 (PFAM); G3DSA:3.40.50.720 (GENE3D); IPR000043 (PFAM); IPR020082 (PROSITE_PATTERNS); IPR036291 (SUPERFAMILY); SSF52283 (SUPERFAMILY)	F:GO:0004013	F:adenosylhomocysteinase activity

								ate derivative binding; C:cytoplasm			
QG_044625-T1	5471100UniRef90_UPI000D18A6C5 glutathione S-transferase U24-like n=1 Tax=Populus trichocarpa TaxID=3694 RepID=UPI000D18A6C5	132	5	6,94E-57	89.46	GST3			G3DSA:1.20.1050.10 (GENE3D); IPR004045 (PFAM); G3DSA:3.40.30.10 (GENE3D); SFLDG00358 (SFLD); SFLDG01152 (SFLD); SIGNAL_PEPTIDE_C_REGION (PHOBIUS); SIGNAL_PEPTIDE (PHOBIUS); SIGNAL_PEPTIDE_N_REGION (PHOBIUS); NON_CYTOPLASMIC_DOMAIN (PHOBIUS); SIGNAL_PEPTIDE_H_REGION (PHOBIUS); IPR004045	F:GO:0005515	F:protein binding

									(PROSITE_PROFILES); IPR010987 (PROSITE_PROFILES); cd03058 (CDD); IPR036249 (SUPERFAMILY)		
QG_021256-T1	1877868UniRef90_A0A059B5H9 S-adenosylmethionine synthase n=2 Tax=Myrtales TaxID=41944 RepID=A0A059B5H9_EUCGR	393	5	0.0	98.67	SAMS2_1	P:GO:0006811; P:GO:0006821; P:GO:0007165; P:GO:0007214; P:GO:0007268; P:GO:0009791; P:GO:0030534; P:GO:0034220; P:GO:0042391; P:GO:0050877; P:GO:0051932; P:GO:0060078; P:GO:0071420;	P:ion transport; P:chloride transport; P:signal transduction; P:gamma-aminobutyric acid signaling pathway; P:chemical synaptic transmission; P:post-embryonic development; P:adult behavior; P:ion transmembrane transport; P:regulation of membrane potential;	IPR022629 (PFAM); IPR022630 (PFAM); IPR002133 (TIGRFAM); G3DSA:3.30.300.10 (GENE3D); IPR022628 (PFAM); IPR002133 (PIRSF); G3DSA:3.30.300.10 (GENE3D); G3DSA:3.30.300.10 (GENE3D); IPR022631 (PROSITE_PATTERNS); IPR022631 (PROSITE_PATTERNS); IPR002133		

							P:GO:1902476; P:GO:1904862; F:GO:0004888; F:GO:0004890; F:GO:0005216; F:GO:0005230; F:GO:0005237; F:GO:0005254; F:GO:0005515; F:GO:0008503; F:GO:0016917; F:GO:0022851; F:GO:0030594; F:GO:1904315; C:GO:0005737; C:GO:0005886; C:GO:0005887; C:GO:0016020;	P:nervous system process; P:synaptic transmission, GABAergic; P:regulation of postsynaptic membrane potential; P:cellular response to histamine; P:chloride transmembrane transport; P:inhibitory synapse assembly; F:transmembrane signaling receptor activity; F:GABA-A receptor activity; F:ion channel activity; F:extracellular ligand-gated ion channel	(HAMAP); IPR022636 (SUPERFAMILY); IPR022636 (SUPERFAMILY); IPR022636 (SUPERFAMILY)		

							C:GO:0016021; C:GO:0030054; C:GO:0030424; C:GO:0030425; C:GO:0030659; C:GO:0031410; C:GO:0032590; C:GO:0034707; C:GO:0042995; C:GO:0043005; C:GO:0045202; C:GO:0045211; C:GO:0060077; C:GO:0098794; C:GO:0098978; C:GO:0098982; C:GO:0099060; C:GO:1902710;	activity; F:inhibitory extracellular ligand-gated ion channel activity; F:chloride channel activity; F:protein binding; F:benzodiazepine receptor activity; F:GABA receptor activity; F:GABA-gated chloride ion channel activity; F:neurotransmitter receptor activity; F:transmitter-gated ion channel activity involved in regulation of postsynaptic membrane potential;			

							C:GO:1902711	C:cytoplasm; C:plasma membrane; C:integral component of plasma membrane; C:membrane; C:integral component of membrane; C:cell junction; C:axon; C:dendrite; C:cytoplasmic vesicle membrane; C:cytoplasmic vesicle; C:dendrite membrane; C:chloride channel complex; C:cell projection; C:neuron projection; C:synapse; C:postsynaptic membrane; C:inhibitory synapse;			

								C:postsynapse; C:glutamatergic synapse; C:GABA-ergic synapse; C:integral component of postsynaptic specialization membrane; C:GABA receptor complex; C:GABA-A receptor complex			
QG_025835-T1	1660114UniRef90_A0A6J1D9S1 S-adenosylmethionine synthase n=6 Tax=rosids TaxID=71275 RepID=A0A6J1D9S1_MOMCH	393	5	0.0	98.58	SAMS2_2	P:GO:0016032	P:viral process	IPR002133 (PIRSF); G3DSA:3.30.300.10 (GENE3D); IPR022629 (PFAM); G3DSA:3.30.300.10 (GENE3D); IPR022630 (PFAM); IPR002133 (TIGRFAM); G3DSA:3.30.300.10 (GENE3D);		

									IPR022628 (PFAM); IPR022631 (PROSITE_PATTERNS); IPR022631 (PROSITE_PATTERNS); IPR002133 (HAMAP); IPR022636 (SUPERFAMILY); IPR022636 (SUPERFAMILY); IPR022636 (SUPERFAMILY)		
QG_017384-T1	5917447UniRef90_A0A0B2SPC8 Abscisic stress-ripening protein 2 n=1 Tax=Glycine soja TaxID=3848 RepID=A0A0B2SPC8_GLYSO	60	5	1,12E-13	94.62				IPR003496 (PFAM); mobidb-lite (MOBIDB_LITE); mobidb-lite (MOBIDB_LITE)	no GO terms	no GO terms
QG_030266-T1	756187UniRef90_A0A059BX08 ABC transporter domain-containing protein n=4 Tax=Myrtoideae TaxID=1699513	233	5	1,44E-107	85.3	ABCG26_1			G3DSA:3.40.50.300 (GENE3D); IPR003439 (PFAM); mobidb-lite (MOBIDB_LITE); IPR027417	F:GO:0005524; F:GO:0016887	F:ATP binding; F:ATP hydrolysis activity

	RepID=A0A059BX08_EUCGR								(SUPERFAMILY)		
QG_035002-T1	3427326UniRef90_A0A059APW1 ABC transporter domain-containing protein n=4 Tax=Myrtoideae TaxID=1699513 RepID=A0A059APW1_EUCGR	239	5	5,17E-115	82.12	ABCI11_1	F:GO:0005198	F:structural molecule activity	IPR003439 (PFAM); G3DSA:3.40.50.300 (GENE3D); SIGNAL_PEPTIDE_C_REGION (PHOBIUS); NON_CYTOPLASMIC_DOMAIN (PHOBIUS); SIGNAL_PEPTIDE_N_REGION (PHOBIUS); SIGNAL_PEPTIDE_H_REGION (PHOBIUS); SIGNAL_PEPTIDE (PHOBIUS); IPR003439 (PROSITE_PROFILES); cd03225 (CDD); IPR027417 (SUPERFAMILY)	F:GO:0005524; F:GO:0016887	F:ATP binding; F:ATP hydrolysis activity
QG_021827-T1	998459UniRef90_A0A059BVF2	329	5	4,16E-159	81.66	ABCG10_1			IPR003593 (SMART);	F:GO:0005524;	F:ATP binding;

	ABC transporter domain-containing protein n=5 Tax=Myrtoideae TaxID=1699513 RepID=A0A059BVF2_EUCGR								G3DSA:3.40.50.300 (GENE3D); IPR003439 (PFAM); IPR017871 (PROSITE_PATTERNS); IPR003439 (PROSITE_PROFILES); cd03213 (CDD); IPR027417 (SUPERFAMILY)	F:GO:0016887	F:ATP hydrolysis activity
QG_023879-T1	145371UniRef90_UPI00192E9188 ABC transporter B family member 13-like n=7 Tax=Myrtoideae TaxID=1699513 RepID=UPI00192E9188	305	5	7,22E-168	89.42	ABCB13_3	P:GO:0000272; P:GO:0005975; P:GO:0006814; P:GO:0008152; P:GO:0008643; P:GO:0009401; P:GO:0016310; P:GO:0030245; P:GO:0033611; P:GO:0055085;	P:polysaccharide catabolic process; P:carbohydrate metabolic process; P:sodium ion transport; P:metabolic process; P:carbohydrate transport; P:phosphoenolpyruvate-dependent sugar phosphotran	IPR003593 (SMART); IPR003439 (PFAM); G3DSA:3.40.50.300 (GENE3D); IPR036640 (G3DSA:1.20.1560.GENE3D); IPR017871 (PROSITE_PATTERNS); NON_CYTOPLASMIC_DOMAIN (PHOBIUS); SIGNAL_PEPTIDE_H_REG	F:GO:0005524; F:GO:0016887; C:GO:0016021	F:ATP binding; F:ATP hydrolysis activity; C:integral component of membrane

							F:GO:0000166; F:GO:0000287; F:GO:0003824; F:GO:0004553; F:GO:0008810; F:GO:0008949; F:GO:0015293; F:GO:0016301; F:GO:0016740; F:GO:0016787; F:GO:0016798; F:GO:0016829; F:GO:0016831; F:GO:0022857; F:GO:0030976; F:GO:0042802; F:GO:0043531; F:GO:0046872;	sferase system; P:phosphorylation; P:cellulose catabolic process; P:oxalate catabolic process; P:transmembrane transport; F:nucleotide binding; F:magnesium ion binding; F:catalytic activity; F:hydrolase activity, hydrolyzing O-glycosyl compounds; F:cellulase activity; F:oxalyl-CoA decarboxylase activity; F:symporter activity; F:kinase activity; F:transferase	ION (PHOBIUS); SIGNAL_PEPTIDE_C_REGION (PHOBIUS); SIGNAL_PEPTIDE (PHOBIUS); SIGNAL_PEPTIDE_N_REGION (PHOBIUS); IPR003439 (PROSITE_PROFILES); cd03249 (CDD); SignalP-noTM (SIGNALP_EUK); IPR027417 (SUPERFAMILY)		

							C:GO:0005886; C:GO:0016020; C:GO:0016021	activity; F:hydrolase activity; F:hydrolase activity, acting on glycosyl bonds; F:lyase activity; F:carboxy-lyase activity; F:transmembrane transporter activity; F:thiamine pyrophosphate binding; F:identical protein binding; F:ADP binding; F:metal ion binding; C:plasma membrane; C:membrane; C:integral component of membrane			
QG_041699-T1	657490UniRef90_A0A7J8YMI4	329	5	4,01E-151	86.15	ABCG36			G3DSA:3.40.50.300	F:GO:0005524;	F:ATP binding;

	ABC transporter domain-containing protein n=2 Tax=Gossypium TaxID=3633 RepID=A0A7J8YMI4_GOSAI								(GENE3D); IPR029481 (PFAM); IPR003439 (PFAM); mobidb-lite (MOBIDB_LITE); IPR027417 (SUPERFAMILY); IPR027417 (SUPERFAMILY)	F:GO:0016887	F:ATP hydrolysis activity
QG_005544-T1	3141675UniRef90_UPI0011E54386 ABC transporter I family member 1 isoform X1 n=3 Tax=Rhodamnia argentea TaxID=178133 RepID=UPI0011E54386	231	5	6,61E-153	97.29	ABCI1			IPR003593 (SMART); IPR005895 (TIGRFAM); IPR003439 (PFAM); G3DSA:3.40.50.300 (GENE3D); IPR003439 (PROSITE_PROFILES); IPR005895 (PROSITE_PROFILES); IPR027417 (SUPERFAMILY)	P:GO:0017004; F:GO:0005215; F:GO:0005524; F:GO:0016887	P:cytochrome complex assembly; F:transporter activity; F:ATP binding; F:ATP hydrolysis activity
QG_008049-T1	2890936UniRef90_A0A7J9EVW7 ABC transporter domain-	263	5	2,25E-130	86.09	ABCG9_1			IPR003593 (SMART); IPR003439 (PFAM);	F:GO:0005524; F:GO:0016887	F:ATP binding; F:ATP hydrolys

	containing protein n=2 Tax=Gossypium TaxID=3633 RepID=A0A7J9EVW7_9ROSI								G3DSA:3.40.50.300 (GENE3D); IPR017871 (PROSITE_PATTERNS); IPR003439 (PROSITE_PROFILES); cd03213 (CDD); IPR027417 (SUPERFAMILY)		is activity
QG_010187-T1	810295UniRef90_A0A059BFG7 ABC transporter domain-containing protein n=4 Tax=Myrtoideae TaxID=1699513 RepID=A0A059BFG7_EUCGR	344	5	3,21E-172	83.52	ABCG21_1	P:GO:0006260; P:GO:0006275; P:GO:0006351; P:GO:0006355; P:GO:0016032; P:GO:0039693; F:GO:0000166; F:GO:0003677; F:GO:0003700; C:GO:0042025	P:DNA replication; P:regulation of DNA replication; P:transcription, DNA-templated; P:regulation of transcription, DNA-templated; P:viral process; P:viral DNA genome replication; F:nucleotide binding; F:DNA binding;	IPR003593 (SMART); IPR003439 (PFAM); G3DSA:3.40.50.300 (GENE3D); mobidb-lite (MOBIDB_LITE); IPR017871 (PROSITE_PATTERNS); IPR003439 (PROSITE_PROFILES); IPR027417 (SUPERFAMILY)	F:GO:0005524; F:GO:0016887	F:ATP binding; F:ATP hydrolysis activity

								F:DNA-binding transcription factor activity; C:host cell nucleus			
QG_010339-T1	239633UniRef90_UPI0011E51C27 ABC transporter C family member 3-like isoform X3 n=2 Tax=Rhodamnia argentea TaxID=178133 RepID=UPI0011E51C27	240	5	5,37E-94	73.6				IPR025314 (PFAM); G3DSA:3.40.50.300 (GENE3D); IPR003439 (PFAM); IPR027417 (SUPERFAMILY)	F:GO:0005524; F:GO:0016887	F:ATP binding; F:ATP hydrolysis activity
QG_018690-T1	2597368UniRef90_UPI0011E51842 putative multidrug resistance protein n=1 Tax=Rhodamnia argentea TaxID=178133 RepID=UPI0011E51842	133	5	5,71E-54	82.88		P:GO:0000160; P:GO:0007165; P:GO:0016310; F:GO:0000155; F:GO:0005198; F:GO:0016772; C:GO:0016020; C:GO:0016021; C:GO:0019028	P:phosphorelay signal transduction system; P:signal transduction; P:phosphorylation; F:phosphorelay sensor kinase activity; F:structural molecule activity; F:transferase activity, transferring	IPR003593 (SMART); IPR003439 (PFAM); G3DSA:3.40.50.300 (GENE3D); IPR027417 (SUPERFAMILY)	F:GO:0005524; F:GO:0016887	F:ATP binding; F:ATP hydrolysis activity

								phosphorus-containing groups; C:membrane; C:integral component of membrane; C:viral capsid			
QG_021358-T1	2273112UniRef90_A0A2R6QB08 ABC transporter B family member protein n=1 Tax=Actinidia chinensis var. chinensis TaxID=1590841 RepID=A0A2R6QB08_ACTCC	221	5	1,23E-75	74.6	ABCB21_1	P:GO:0001525; P:GO:0001952; P:GO:0002693; P:GO:0006469; P:GO:0007010; P:GO:0007155; P:GO:0007229; P:GO:0007267; P:GO:0030334; P:GO:0030336; P:GO:0034116; P:GO:0043066; P:GO:0043113;	P:angiogenesis; P:regulation of cell-matrix adhesion; P:positive regulation of cellular extravasation; P:negative regulation of protein kinase activity; P:cytoskeleton organization; P:cell adhesion; P:integrin-mediated signaling pathway; P:cell-cell signaling;	IPR003593 (SMART); IPR003439 (PFAM); G3DSA:3.40.50.300 (GENE3D); IPR003439 (PROSITE_PROFILES); IPR027417 (SUPERFAMILY)	F:GO:0005524; F:GO:0016887	F:ATP binding; F:ATP hydrolysis activity

							P:GO:0043547; P:GO:0045576; P:GO:0046549; P:GO:0046777; P:GO:0048041; P:GO:0048147; P:GO:0050731; P:GO:0050771; P:GO:0050852; P:GO:0050860; P:GO:0050870; P:GO:0051281; P:GO:0051894; P:GO:0061099; P:GO:0070571; P:GO:0098609; P:GO:2000298; F:GO:0005096;	P:regulation of cell migration; P:negative regulation of cell migration; P:positive regulation of heterotypic cell-cell adhesion; P:negative regulation of apoptotic process; P:receptor clustering; P:positive regulation of GTPase activity; P:mast cell activation; P:retinal cone cell development; P:protein autophosphorylation; P:focal adhesion assembly; P:negative regulation of fibroblast			

							F:GO:0005178; F:GO:0005515; F:GO:0019899; F:GO:0019901; F:GO:0034235; C:GO:0005783; C:GO:0005829; C:GO:0005886; C:GO:0009897; C:GO:0009986; C:GO:0016020; C:GO:0016324; C:GO:0030425; C:GO:0030426; C:GO:0030673; C:GO:0031225; C:GO:0031362; C:GO:0032590;	proliferation; P:positive regulation of peptidyl-tyrosine phosphorylation; P:negative regulation of axonogenesis; P:T cell receptor signaling pathway; P:negative regulation of T cell receptor signaling pathway; P:positive regulation of T cell activation; P:positive regulation of release of sequestered calcium ion into cytosol; P:positive regulation of focal adhesion assembly; P:negative			

							C:GO:0032809; C:GO:0045121; C:GO:0046658	regulation of protein tyrosine kinase activity; P:negative regulation of neuron projection regeneration; P:cell-cell adhesion; P:regulation of Rho-dependent protein serine/threonine kinase activity; F:GTPase activator activity; F:integrin binding; F:protein binding; F:enzyme binding; F:protein kinase binding; F:GPI anchor binding; C:endoplasmic reticulum;			

								C:cytosol; C:plasma membrane; C:external side of plasma membrane; C:cell surface; C:membrane ; C:apical plasma membrane; C:dendrite; C:growth cone; C:axolemma ; C:anchored component of membrane; C:anchored component of external side of plasma membrane; C:dendrite membrane; C:neuronal cell body membrane; C:membrane raft; C:anchored component			

								of plasma membrane			
QG_022693-T1	1632928UniRef90_UPI000B8C9B00 ABC transporter D family member 1-like n=1 Tax=Carica papaya TaxID=3649 RepID=UPI000B8C9B00	306	5	1,85E-174	90.58	ABCD1_2	P:GO:0019062; P:GO:0019064; P:GO:0019065; P:GO:0039654; P:GO:0039663; P:GO:0046718; P:GO:0046761; P:GO:0075509; P:GO:0075512; F:GO:0046789; C:GO:0016020; C:GO:0016021; C:GO:0019031; C:GO:0020002; C:GO:0033644; C:GO:0055036	P:virion attachment to host cell; P:fusion of virus membrane with host plasma membrane; P:receptor-mediated endocytosis of virus by host cell; P:fusion of virus membrane with host endosome membrane; P:membrane fusion involved in viral entry into host cell; P:viral entry into host cell; P:viral budding from plasma membrane; P:endocytosis involved in	IPR003593 (SMART); G3DSA:3.40.50.300 (GENE3D); IPR003439 (PFAM); IPR017871 (PROSITE_PATTERNS); IPR003439 (PROSITE_PROFILES); cd03223 (CDD); IPR027417 (SUPERFAMILY)	F:GO:0005524; F:GO:0016887	F:ATP binding; F:ATP hydrolysis activity

								viral entry into host cell; P:clathrin-dependent endocytosis of virus by host cell; F:host cell surface receptor binding; C:membrane; C:integral component of membrane; C:viral envelope; C:host cell plasma membrane; C:host cell membrane; C:virion membrane			
QG_023779-T1	2273112UniRef90_A0A2R6QB08 ABC transporter B family member protein n=1 Tax=Actinidia chinensis var. chinensis TaxID=1590841	298	5	5,40E-168	89.85	ABCB21_2			IPR003593 (SMART); IPR036640 (G3DSA:1.20.1560.GENE3D); G3DSA:3.40.50.300 (GENE3D); IPR003439	F:GO:0005524; F:GO:0016887; C:GO:0016021	F:ATP binding; F:ATP hydrolysis activity; C:integral component of

	RepID=A0A2R6QB08_ACTCC								(PFAM); IPR017871 (PROSITE_PATTERNS); SIGNAL_PEPTIDE_C_REGION (PHOBIUS); SIGNAL_PEPTIDE (PHOBIUS); NON_CYTOPLASMIC_DOMAIN (PHOBIUS); SIGNAL_PEPTIDE_N_REGION (PHOBIUS); SIGNAL_PEPTIDE_H_REGION (PHOBIUS); IPR003439 (PROSITE_PROFILES); cd03249 (CDD); SignalP-TM (SIGNALP_GRAM_POSITIVE); IPR027417 (SUPERFAMILY)		membrane

QG_026497-T1	850628UniRef90_UPI00192EDA8E ABC transporter G family member 25 n=3 Tax=Myrtoideae TaxID=1699513 RepID=UPI00192EDA8E	369	5	1,15E-151	80.24	ABCG25_2	P:GO:0006351; F:GO:0003677; F:GO:0003899; F:GO:0016740; F:GO:0016779	P:transcription, DNA-templated; F:DNA binding; F:DNA-directed 5'-3' RNA polymerase activity; F:transferase activity; F:nucleotidyltransferase activity	IPR003593 (SMART); G3DSA:3.40.50.300 (GENE3D); IPR003439 (PFAM); mobidb-lite (MOBIDB_LITE); mobidb-lite (MOBIDB_LITE); TRANSMEMBRANE (PHOBIUS); CYTOPLASMIC_DOMAIN (PHOBIUS); NON_CYTOPLASMIC_DOMAIN (PHOBIUS); IPR003439 (PROSITE_PROFILES); cd03213 (CDD); IPR027417 (SUPERFAMILY)	F:GO:0005524; F:GO:0016887	F:ATP binding; F:ATP hydrolysis activity
QG_028414-T1	4628443UniRef90_A0A7J6V1E6 Lipid A export ATP-	202	5	1,34E-77	91.77	ABCB4_1	P:GO:0006260; P:GO:0039686;	P:DNA replication; P:bidirectional double-	IPR003439 (PFAM); G3DSA:3.40.50.300	F:GO:0005524; F:GO:0016887;	F:ATP binding; F:ATP hydrolys

	binding/permease protein MsbA n=1 Tax=Thalictrum thalictroides TaxID=46969 RepID=A0A7J6V1E6_THATH						F:GO:0003677	stranded viral DNA replication; F:DNA binding	(GENE3D); IPR036640 (G3DSA:1.20.1560.GENE3D); IPR027417 (SUPERFAMILY)	C:GO:0016021	is activity; C:integral component of membrane
QG_029056-T1	684353UniRef90_A0A5J5AL86 ABC transporter domain-containing protein n=1 Tax=Nyssa sinensis TaxID=561372 RepID=A0A5J5AL86_9ASTE	191	5	5,38E-83	88.37	STR2_2			G3DSA:3.40.50.300 (GENE3D); IPR003439 (PFAM); IPR027417 (SUPERFAMILY)	F:GO:0005524; F:GO:0016887	F:ATP binding; F:ATP hydrolysis activity
QG_031010-T1	834154UniRef90_UPI0011E55AE4 ABC transporter D family member 2, chloroplastic isoform X4 n=1 Tax=Rhodamnia argentea TaxID=178133 RepID=UPI0011E55AE4	311	5	2,87E-145	77.69		P:GO:0006278; P:GO:0015074; P:GO:0090305; F:GO:0003676; F:GO:0003964; F:GO:0004518; F:GO:0004519; F:GO:0016740; F:GO:0016779;	P:RNA-dependent DNA biosynthetic process; P:DNA integration; P:nucleic acid phosphodiester bond hydrolysis; F:nucleic acid binding; F:RNA-directed DNA polymerase	IPR003593 (SMART); IPR003439 (PFAM); IPR017871 (PROSITE_PATTERNS); IPR003439 (PROSITE_PROFILES); cd03223 (CDD); IPR027417 (SUPERFAMILY)	F:GO:0005524; F:GO:0016887	F:ATP binding; F:ATP hydrolysis activity

							F:GO:0016787	activity; F:nuclease activity; F:endonuclease activity; F:transferase activity; F:nucleotidyltransferase activity; F:hydrolase activity			
QG_031173-T1	396085UniRef90_UPI0011D22B42 receptor-like kinase TMK4 n=1 Tax=Syzygium oleosum TaxID=219896 RepID=UPI0011D22B42	163	5	2,36E-23	86.64				IPR032675 (G3DSA:3.80.10.GENE3D); IPR001611 (PFAM); IPR003439 (PFAM); G3DSA:3.40.50.300 (GENE3D); IPR027417 (SUPERFAMILY); SSF52058 (SUPERFAMILY)	F:GO:0005515; F:GO:0005524; F:GO:0016887	F:protein binding; F:ATP binding; F:ATP hydrolysis activity
QG_033207-T1	1231273UniRef90_A0A067F8U5 ABC transporter domain-containing protein n=2 Tax=Citrus sinensis TaxID=2711	241	5	3,39E-156	96.6	ABCF5_2			IPR003439 (PFAM); IPR032781 (PFAM); G3DSA:3.40.50.300 (GENE3D); G3DSA:3.40.5	F:GO:0005524; F:GO:0016887	F:ATP binding; F:ATP hydrolysis activity

	RepID=A0A067F8U5_CITSI								0.300 (GENE3D); IPR027417 (SUPERFAMILY)		
QG_034170-T1	2084871UniRef90_A0A7J9IQU6 ABC transporter domain-containing protein n=1 Tax=Gossypium armourianum TaxID=34283 RepID=A0A7J9IQU6_9ROSI	163	5	1,44E-71	85.35	ABCD1_3	P:GO:0006779; P:GO:0006783; F:GO:0004325; F:GO:0016829; C:GO:0005739; C:GO:0005743; C:GO:0016020	P:porphyrin-containing compound biosynthetic process; P:heme biosynthetic process; F:ferrochelatase activity; F:lyase activity; C:mitochondrion; C:mitochondrial inner membrane; C:membrane	G3DSA:3.40.50.300 (GENE3D); IPR003439 (PFAM); IPR027417 (SUPERFAMILY)	F:GO:0005524; F:GO:0016887	F:ATP binding; F:ATP hydrolysis activity
QG_038380-T1	1006067UniRef90_A0A6A2YHV5 ATP-binding cassette sub-family E member 1 n=8 Tax=Malvoideae TaxID=214907 RepID=A0A6A2YHV5_HIBSY	159	5	7,71E-81	77.25	ABCE2_4	C:GO:0005730	C:nucleolus	IPR003593 (SMART); G3DSA:3.40.50.300 (GENE3D); IPR003439 (PFAM); IPR027417 (SUPERFAMILY)	F:GO:0005524; F:GO:0016887	F:ATP binding; F:ATP hydrolysis activity
QG_042756-T1	2450845UniRef90_W9SAL7 ABC transporter C	193	5	9,73E-98	89.76	ABCC3_5			IPR003439 (PFAM); G3DSA:3.40.5	F:GO:0005524;	F:ATP binding; F:ATP

	family member 3 n=1 Tax=Morus notabilis TaxID=981085 RepID=W9SAL7_9ROSA								0.300 (GENE3D); IPR027417 (SUPERFAMILY)	F:GO:0016887	hydrolysis activity
QG_000790-T1	3847299UniRef90_A0A7J6WVY1 Lipid A export ATP-binding/permease protein MsbA n=1 Tax=Thalictrum thalictroides TaxID=46969 RepID=A0A7J6WVY1_THATH	198	5	1,58E-87	82.66	ABCB12			IPR036640 (G3DSA:1.20.1560.GENE3D); IPR003439 (PFAM); G3DSA:3.40.50.300 (GENE3D); IPR027417 (SUPERFAMILY)	F:GO:0005524; F:GO:0016887; C:GO:0016021	F:ATP binding; F:ATP hydrolysis activity; C:integral component of membrane
QG_002259-T1	143534UniRef90_UPI0011D2706C ABC transporter C family member 10-like n=1 Tax=Syzygium oleosum TaxID=219896 RepID=UPI0011D2706C	113	5	3,19E-54	93.33	ABCC10_1	P:GO:0001934; P:GO:0006468; P:GO:0007165; P:GO:0007166; P:GO:0007167; P:GO:0007169; P:GO:0007399; P:GO:0007422; P:GO:0007507;	P:positive regulation of protein phosphorylation; P:protein phosphorylation; P:signal transduction; P:cell surface receptor signaling pathway; P:enzyme linked receptor protein	G3DSA:3.40.50.300 (GENE3D); IPR003439 (PFAM); IPR027417 (SUPERFAMILY)	F:GO:0005524; F:GO:0016887	F:ATP binding; F:ATP hydrolysis activity

							P:GO:0007528; P:GO:0008045; P:GO:0008284; P:GO:0009058; P:GO:0009103; P:GO:0009243; P:GO:0010628; P:GO:0014065; P:GO:0016310; P:GO:0018108; P:GO:0019305; P:GO:0030182; P:GO:0030307; P:GO:0032886; P:GO:0033088; P:GO:0033674; P:GO:0035556; P:GO:0042060;	signaling pathway; P:transmembrane receptor protein tyrosine kinase signaling pathway; P:nervous system development; P:peripheral nervous system development; P:heart development; P:neuromuscular junction development; P:motor neuron axon guidance; P:positive regulation of cell population proliferation; P:biosynthetic process; P:lipopolysaccharide biosynthetic			

							P:GO:0042552; P:GO:0043406; P:GO:0043410; P:GO:0043547; P:GO:0045226; P:GO:0045727; P:GO:0045765; P:GO:0045785; P:GO:0045943; P:GO:0046777; P:GO:0048709; P:GO:0050679; P:GO:0070372; P:GO:0071363; P:GO:0071364; P:GO:0090314; F:GO:0000166; F:GO:0000287;	process; P:O antigen biosynthetic process; P:positive regulation of gene expression; P:phosphatidylinositol 3-kinase signaling; P:phosphorylation; P:peptidyl-tyrosine phosphorylation; P:dTDP-rhamnose biosynthetic process; P:neuron differentiation; P:positive regulation of cell growth; P:regulation of microtubule-based process; P:negative regulation of immature T cell			

							F:GO:0001042; F:GO:0004672; F:GO:0004713; F:GO:0004714; F:GO:0004888; F:GO:0005515; F:GO:0005524; F:GO:0008022; F:GO:0008879; F:GO:0016301; F:GO:0016740; F:GO:0016779; F:GO:0019838; F:GO:0019903; F:GO:0042802; F:GO:0043125; F:GO:0046872; F:GO:0046982;	proliferation in thymus; P:positive regulation of kinase activity; P:intracellular signal transduction; P:wound healing; P:myelination; P:positive regulation of MAP kinase activity; P:positive regulation of MAPK cascade; P:positive regulation of GTPase activity; P:extracellular polysaccharide biosynthetic process; P:positive regulation of translation; P:regulation of angiogenesis			

							C:GO:0005634; C:GO:0005737; C:GO:0005768; C:GO:0005769; C:GO:0005829; C:GO:0005886; C:GO:0005887; C:GO:0009925; C:GO:0010008; C:GO:0016020; C:GO:0016021; C:GO:0016323; C:GO:0016324; C:GO:0031410; C:GO:0038143; C:GO:0043209; C:GO:0043235; C:GO:0048471	; P:positive regulation of cell adhesion; P:positive regulation of transcription by RNA polymerase I; P:protein autophosphorylation; P:oligodendrocyte differentiation; P:positive regulation of epithelial cell proliferation; P:regulation of ERK1 and ERK2 cascade; P:cellular response to growth factor stimulus; P:cellular response to epidermal growth factor stimulus; P:positive			

								regulation of protein targeting to membrane; F:nucleotide binding; F:magnesium ion binding; F:RNA polymerase I core binding; F:protein kinase activity; F:protein tyrosine kinase activity; F:transmembrane receptor protein tyrosine kinase activity; F:transmembrane signaling receptor activity; F:protein binding; F:ATP binding; F:protein C-			

								terminus binding; F:glucose-1-phosphate thymidylyltransferase activity; F:kinase activity; F:transferase activity; F:nucleotidyltransferase activity; F:growth factor binding; F:protein phosphatase binding; F:identical protein binding; F:ErbB-3 class receptor binding; F:metal ion binding; F:protein heterodimerization activity; C:nucleus; C:cytoplasm;			

								C:endosome; C:early endosome; C:cytosol; C:plasma membrane; C:integral component of plasma membrane; C:basal plasma membrane; C:endosome membrane; C:membrane ; C:integral component of membrane; C:basolateral plasma membrane; C:apical plasma membrane; C:cytoplasmic vesicle; C:ERBB3:ERBB2 complex; C:myelin sheath; C:receptor complex; C:perinuclea			

								r region of cytoplasm			
QG_002581-T1	741822UniRef90_A0A059AAN2 ABC transporter domain-containing protein n=7 Tax=Myrtoideae TaxID=1699513 RepID=A0A059AAN2_EUCGR	154	5	1,30E-84	92.73	ABCG15_1			IPR003439 (PFAM); G3DSA:3.40.50.300 (GENE3D); IPR027417 (SUPERFAMILY)	F:GO:0005524; F:GO:0016887	F:ATP binding; F:ATP hydrolysis activity
QG_005231-T1	2450845UniRef90_W9SAL7 ABC transporter C family member 3 n=1 Tax=Morus notabilis TaxID=981085 RepID=W9SAL7_9ROSA	188	5	6,65E-90	88.93	ABCC3_1	P:GO:0002052; P:GO:0006357; P:GO:0014019; P:GO:0016360; P:GO:0022008; P:GO:0030536; P:GO:0046672; P:GO:0097150; P:GO:2000035; F:GO:0000977; F:GO:0009981; C:GO:0005634	P:positive regulation of neuroblast proliferation; P:regulation of transcription by RNA polymerase II; P:neuroblast development; P:sensory organ precursor cell fate determination; P:neurogenesis; P:larval feeding behavior; P:positive regulation of	G3DSA:3.40.50.300 (GENE3D); IPR003439 (PFAM); IPR027417 (SUPERFAMILY)	F:GO:0005524; F:GO:0016887	F:ATP binding; F:ATP hydrolysis activity

								compound eye retinal cell programmed cell death; P:neuronal stem cell population maintenance; P:regulation of stem cell division; F:RNA polymerase II transcription regulatory region sequence-specific DNA binding; F:DNA-binding transcription factor activity, RNA polymerase II-specific; C:nucleus			
QG_012828-T1	1953550UniRef90_A0A059ASR2 ABC transporter domain-	147	5	8,85E-84	92.79	ABCG1_3			IPR003439 (PFAM); G3DSA:3.40.50.300	F:GO:0005524; F:GO:0016887	F:ATP binding; F:ATP hydrolys

	containing protein n=2 Tax=Eucalyptus grandis TaxID=71139 RepID=A0A059ASR2_EUCGR								(GENE3D); IPR027417 (SUPERFAMILY)		is activity

CONSIDERAÇÕES FINAIS

Esta tese apóia a ideia de que o Al não é tóxico para as espécies acumuladoras do Cerrado, como *Q. grandiflora*. A semente de *Q. grandiflora* acumulou 6,43 g de $Al.Kg^{-1}$ de MS, em solo com baixa concentração de Al. Em laboratório, essas sementes foram germinadas sem e com Al, observou-se que o Al não interferiu no processo de germinação. Além disso, o Al presente na semente foi mobilizado para as plântulas de *Q. grandiflora* independente do tratamento. Aproximadamente 60% do Al da semente foi translocado para as folhas das plântulas crescidas em 120 dias de vida.

As sementes de *Q. grandiflora* apresentaram proteínas e lipídios como principais substâncias de reserva. Além disso, o Al e as proteínas foram detectados nos mesmos sítios (embrião) nas sementes e nas plântulas de *Q. grandiflora*. O ácido graxo mais abundante nas sementes de *Q. grandiflora* foi o ácido láurico (C12:0), que pode servir como fonte de energia e carbono durante a germinação e desenvolvimento inicial, podendo ser um processo dependente de Al em sementes de *Q. grandiflora*. Assim, o Al parece estar relacionado as substâncias de reserva, proteínas e lipídios, em *Q. grandiflora*, o Al é endógeno em plantas de *Q. grandiflora*.

Neste sentido, realizou-se o sequenciamento e montagem do genoma de *Q. grandiflora* numa tentativa de identificar genes responsivos ao Al. O sequenciamento apresentou boa qualidade. O projeto de montagem cobriu aproximadamente 81% do genoma total de *Q. grandiflora* que apresenta tamanho aproximado de 500 Mb. Onze genes foram identificados como candidatos ao Al. Dentre eles, cinco dessas famílias gênicas foram expressas em resposta ao Al no transcriptoma de *Q. grandiflora*. As famílias gênicas, *MATE, ABC, NRAMP, GST e FDH* estavam presentes tanto no genoma quanto no transcriptoma de *Q. grandiflora*, indicando que são possíveis genes relacionados ao acúmulo de Al nesta espécie. Não obstante, os genes relacionados aos transportadores de ácidos orgânicos, síntese da parede celular, transportadores de membrana relacionados ao Al, também foram identificados no genoma de *Q. grandiflora*. Assim, este estudo fornece um guia para entender o mecanismo envolvendo Al em *Q. grandiflora*.

Desta forma, essa tese traz informações novas e importantes sobre a relação do Al em plantas de *Q. Grandiflora*, pois é o primeiro trabalho a relacionar e identificar Al no

mesmo sítio que as proteínas em sementes e plântulas desta espécie. Além disso, essa tese apresenta o primeiro projeto de sequenciamento genômico de *Q. grandiflora.* Assim, como são estudos pioneiros ainda há muitas perguntas a serem respondidas e experimentos a serem realizados para sanar as lacunas existentes. Fornecemos, aqui, um caminho para novos trabalhos nessa linha de pesquisa.