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Amine oxidases in the oxidative pathway of polyamines in *Medicago truncatula* grown under different nitrogen sources

presentado por

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GRADO EN INGENIERÍA AGROALIMENTARIA Y DEL MEDIO RURAL
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1 Abstract

Crop productivity relies heavily on fertilization. However, the augmented use of fertilizers by industrial farming results in an over-accumulation of nutrients in the soil, which induces nutritional stresses. The utilization of nitrate, ammonium, and urea as nitrogen sources leads to an elevated intracellular accumulation of ammonium, which makes indispensable to understand and elucidate the mechanism by which plants face ammonium toxicity. Early studies on plant metabolism of polyamines pointed to their involvement in responses to different environmental stresses. However, the precise mechanisms by which polyamines control plant responses to stress stimuli have not been elucidated yet. Previous results of our laboratory suggested the importance of the "urea cycle" on the tolerance of *Medicago truncatula* seedlings to ammonium, in which polyamine catabolism might play a key role in such tolerance, but there are still important gaps to fulfill. The excess of ammonium originated either from ammonium nutrition or from polyamine metabolism may be suggested to act as a feedback inhibitor of amine oxidases since polyamines, mainly putrescine, were accumulated while the content of γ-aminobutyric acid decreased. In the present study, an intra- and interspecific phylogenetic analyses of amine oxidases are performed in order to characterize them. Moreover, the differential effect of distinct nitrogen nutrition and doses on diamine oxidases and polyamine oxidases activities involved in the catabolic processes of polyamines in connection to the "urea cycle" was assayed. In general terms, the results obtained in this study showed higher diamine oxidase activities in *Medicago truncatula* plant shoots than that observed in roots. In addition, plants supplied with low dose of ammonium exhibited significantly higher diamine oxidase activity in shoots in comparison with nitrate- and urea-fed plants, whereas diamine oxidase activity was significantly higher in roots of ammonium-fed plants at high dose. Since diamine oxidase activity has been shown to be increased under ammonium conditions in *Medicago truncatula* tissues, this might suggest the importance of polyamine catabolism in the tolerance against high ammonium conditions. Although alternative mechanisms underlying ammonium stress/tolerance response are proposed in this report, future research should be performed to elucidate the mechanisms underlying plant ammonium tolerance responses.

2 Abbreviations

ADC: Arginine decarboxylase
AIH: Agmatine iminohydrolase
ALMT: Aluminum-activated malate transporter
AMT: Ammonium transporter
Arg: Arginine
Asn: Asparagine
Cad: Cadaverine
CaM: Carbon-terminal calmodulin
CAPK: Cytosol-associated protein kinase
CuAO: Copper-containing amine oxidase
DAO: Diamine oxidase
dcSAM: decarboxylated S-adenosylmethionine
FAD: Flavin adenine dinucleotide
GABA: γ-aminobutyric acid
GAD: Glutamate decarboxylase
Gln: Glutamine
GOGAT: Glutamate synthase
GS: Glutamine synthetase
H_2O_2: Hydrogen peroxide
N: Nitrogen
NAGK: N-acetyl-L-glutamate kinase
NH_4^+: Ammonium
NO_3^-: Nitrate
OAT: Ornithine-δ-aminotransferase
ODC: Ornithine decarboxylase
Orn: Ornithine
PA: Polyamine
PAO: Polyamine oxidase
Pro: Proline
Put: Putrescine
P5CS: Delta 1-pyrroline-5-carboxylate synthetase
ROS: Reactive oxygen species
SAM: S-adenosylmethionine
SAMDC: S-adenosylmethionine decarboxylase
Spd: Spermidine
SPDS: Spermidine synthase
Spm: Spermine
SPMS: Spermine synthase

3 Introduction

Crop productivity relies heavily on fertilization. The availability of nitrogen in soils affects plant growth, and limits the productivity in agricultural ecosystems due to its involvement in key functions in plants. Nitrogen fertilizers for crops typically contain nitrate and ammonium salts, and urea. The augmented use of fertilizers by industrial farming, results in an over-accumulation of nutrients in the soil leading to environmental issues such as eutrophication. In this context, it is interesting to understand how plants utilize nutrients, and also the mechanism by which plants deal with nutritional stresses such as nitrogen over-accumulation in soils.

3.1 Nitrogen nutrition

Nitrogen (N), one of the main nutrients, is essential for the optimal growth and development of plants since it is the constituent of many plant cell components and biomolecules. Plant are able to uptake nitrogen in distinct forms such as nitrate (NO_3^-), ammonium (NH_4^+), urea ($CO(NH_2)_2$), molecular nitrogen (N_2) in symbiosis with nitrogen-fixing bacteria and/or amino acids under particular conditions of soil composition (Buchanan, 2015). Atmospheric N cannot be directly used by plants but legumes can form symbiotic relationships with soil bacteria, mainly from the Rhizobium family, and consequently utilize this N_2. Specifically, *Medicago truncatula* is found in symbiosis with *Sinorhizobium sp.*, which results in the formation of N-fixing nodules on the roots of the host plant (Wais *et al.,* 2000). However, the biological fixation of atmospheric N, as a way of converting atmospheric N into another more accessible N form, is neither sufficient for maintaining the yield of crop production required to supply the present human population needs nor the expected needs of human population in the future. Therefore, the use of N fertilizers containing nitrate, ammonium and/or urea, is required by agriculture (Sutton *et al.,* 2011).

Although its absorption and assimilation implies higher energy consumption, nitrate turns as the most commonly uptaken N source by roots (Britto and Kronzucker, 2002). Nitrate is assimilated to ammonium in a two steps process catalyzed by the consecutively action of the nitrate reductase and nitrite reductase enzymes. Urea is the most used fertilizer and its uptake by plants can be both in the form of ammonium resulting from the action of soil ureases and/or directly by transporters (Witte, 2011; Yang *et al*., 2015; Pinton *et al.*, 2016). Whatever the source of N is, only ammonium can be incorporated into amino acids (Lea and Miflin, 2011). Should be mentioned that at low concentrations, ammonium is the N source typically preferred by plants, however, it becomes toxic above certain threshold (Britto and Kronzucker, 2002). The ammonium tolerance/toxicity depends on plant species and, indeed, on crop varieties (Esteban *et al.*, 2016b). Thus, ammonium exclusive nutrition can lead to physiological and morphological disorders affecting the plant normal growth and development (Li *et al.,* 2014; Bittsánszky *et al.,* 2015; Esteban *et al*., 2016b).

The utilization of either, nitrate, ammonium, or urea as N sources leads to an elevated intracellular accumulation of ammonium, which makes indispensable to understand and elucidate the mechanism by which plants face ammonium toxicity. Ammonium nutrition has been studied along different species and, although some tolerance mechanisms have been proposed, the way in which plants cope with ammonium toxicity has not been clarified yet (Britto and Kronzucker, 2002; Bittsánszky *et al.,* 2015; Esteban *et al*., 2016b).

3.2 Ammonium nutrition: uptake, assimilation and transport

For plant economy, ammonium may be thought as a favorable N nutrient. The assimilation of ammonium takes place at a lower energy cost in comparison to nitrate; since it can be assimilated directly once it is uptaken by roots. However, the presence of ammonium as the only N source is paradoxically toxic for most plants, since high levels of ammonium dissipate the required transmembrane proton gradients for essential processes for plant development such as photosynthesis and respiration (Gerendás *et al.,* 1998; Cruz *et al.,* 2011; Li *et al.,* 2014; Esteban *et al.,* 2016a). The excess of ammonium also causes changes in ion balance by the inhibition of cations (K^+, Mg^{2+} or Ca^{2+}) uptake, intracellular alkalinization and extracellular acidification, interference with photosynthetic activity, altered expression/activity of ammonium assimilating enzymes, and high energy cost to maintain low levels of cytosolic NH_4^+ content, as reviewed by Esteban *et al.,* 2016b.

Ammonium uptake by roots is mediated by transporters, and once inside the plant cell it is distributed to intracellular compartments also via different classes of transporters. The transport of ammonium across the plasma membrane has been proposed to be through ammonium transporters (ATMs), non-specific cation channels (Coskun *et al.,* 2013), and aquaporins (Bertl and Kaldenhoff, 2007; Coskun *et al.,* 2013; Kirscht *et al.,* 2016). Moreover, the transport of ammonium via ATMs takes place at low ammonium concentrations, whereas it transport at high ammonium concentrations via non-specific cation channels and aquaporins appears to be the cause of toxicity (Pantoja, 2012; Bittsánszky *et al.,* 2015). Plant cells tend to assimilate the ammonium close to the site where it is absorbed or generated, and store any excess in their vacuoles. An accurate balance among the uptake, production, and assimilation of ammonium has to be also maintained (Bittsánszky *et al.,* 2015).

The ammonium derived from either the transformation of other N sources or directly from the ammonium uptake is firstly assimilated into amino acids. The incorporation of such ammonium in the carbon chains is mainly produced by the sequential actions of glutamine synthetase (GS) and glutamate synthase (GOGAT) enzymes, the so called GS-GOGAT cycle. However, when ammonium is supplied as the sole source of nitrogen, alternative pathways are induced, as the reversible deamination of glutamate to 2-oxoglutarate catalyzed by the glutamate dehydrogenase (Skopelitis *et al.,* 2006; Sarasketa *et al.,* 2014).

Nitrogen compounds synthesized in the roots, are transport along the plant mainly in the form of amides and ureids (Marschner, 2012). These organic compounds transfer the N from the source organs to the sink tissues, and acts as transient storage during periods of high N availability (Hawkesford *et al.,* 2012). Temperate legumes, as *Medicago truncatula*, transport N in the form of amides as the amino acids asparagine (Asn) and glutamine (Gln) (Lea and Azevedo, 2007). Moreover, studies with non-nodulated soybean and pea plants showed that under high ammonium concentration, the accumulation of lower carbon-to-nitrogen ratio amino acids such as Asn or arginine (Arg) increased (Ueda *et al.,* 2008; Ariz *et al.,* 2013).

Arginine is the amino acid with the lowest carbon-to-nitrogen ratio. It is considered a N store molecule, as well as a signal molecule. Arginine is produced within the "urea cycle" in plants (Zhou *et al.,* 2000), which serves as a distribution and repackaging hub for inorganic carbon and nitrogen, and it contributes significantly to the metabolic response against ammonium toxicity. Thus, the "urea cycle" may be a regulatory control point in nitrogen metabolism (Esteban *et al.,* 2016b). Moreover, the "urea cycle" is related with polyamine (PA) and γ-aminobutyric acid (GABA) metabolisms.

3.3 Mechanisms of tolerance

3.3.1 The "urea cycle"

The "urea cycle" activation as a metabolic response to high nitrogen availability, as described by Allen *et al.,* 2011 in diatoms, can reduce an excess of ammonium intracellular content. Nevertheless, the "urea cycle" is not considered complete in plants, since the urea cannot be synthetized directly from ammonium. Consequently, the internal ammonium content would not be reduced during stress. In contrast, plants can synthesize urea from glutamine (Zhou *et al.,* 2000) (Figure 1).

In the "urea cycle", arginine can be further degraded to urea and ornithine (Orn), or it can act as substrate to form agmatine (Zhou *et al.,* 2000). However, arginine can be synthesized from Orn in a metabolic process catalyzed by the N-acetyl-l-glutamate kinase (NAGK) (Chellamuthu *et al.,* 2014). The Orn, as well as the agmatine, are precursors for the biosynthesis of putrescine (Put), which is the first PA synthetized. Put acts as the precursor of PAs of higher molecular weight, mainly spermidine (Spd) and spermine (Spm). Putrescine and Spd can be oxidized to 4-aminobutanal (which spontaneously cycles to Δ-pyrroline and it is further converted to GABA), ammonium and H_2O_2 through the enzymatic activity of amine oxidases (Zhou *et al.,* 2000). Therefore, PA metabolism can be a sink as well as a source of ammonium (Minocha *et al.,* 2014). Moreover, PA catabolism is a major node of nitrogen and carbon recycling, with the concomitant production of hydrogen peroxide (H_2O_2) (Moschou *et al*., 2012). However, the importance of this pathway in plants is still unclear.

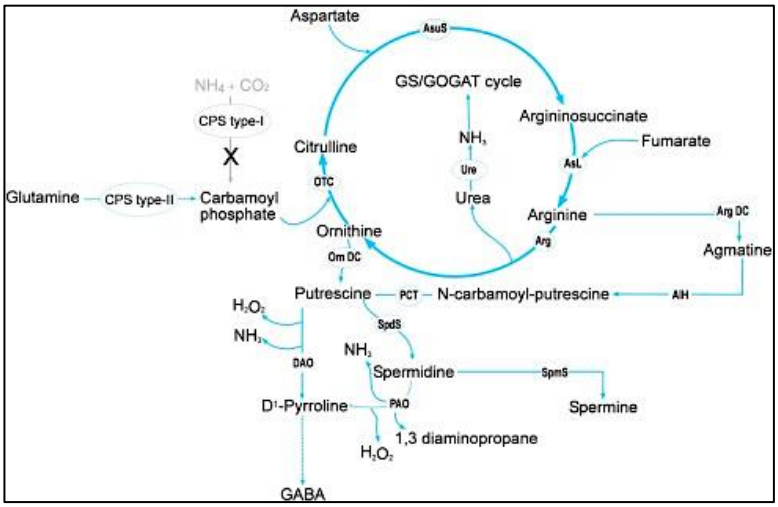

Figure 1: The "urea cycle" in plants and PA synthesis. *In the "urea cycle" in plants, the carbamoyl phosphate is synthetized in plants from glutamine instead of from ammonium. The arginine, as well as the ornithine, can act as precursors in PA synthesis. Ammonium and hydrogen peroxide molecules resulted from oxidases catalysis are given rise in inter-conversions of PAs. The enzymes are represented inside a circle as: agmatine imidohydrolase (AIH); argininosuccinate lyase (AsL); arginase (ARG); arginine decarboxylase (Arg DC); argininosuccinate synthase (AsuS); diamine oxidase (DAO); ornithine decarboxylase (Orn DC); transcarbamylase (OTC); polyamine oxidase (PAO); putrescine carbamoyl transferase (PCT); spermidine synthase (SpdS); spermine synthase (SpmS); urease (Ure). Illustration from* Esteban *et al.,* 2016b.

3.3.2 Polyamine metabolism

Polyamines are small ubiquitous aliphatic cations with low molecular weight present mainly in cell apoplast. The major PAs are putrescine, spermidine and spermine. They are involved in several physiological processes such as cell proliferation, differentiation and defense responses (Tiburcio *et al.,* 2014); which makes the synthesis of PAs essential to cell viability.

The biosynthesis of PAs (Figure 2) starts from Arg to produce Put. Arg is converted to the diamine Put through both pathways via agmatine, which is only present in plants and bacteria, and via Orn. The synthesis of Put from agmatine is catalyzed by three sequential enzymes: arginine decarboxylase (ADC), agmatine iminohydrolase (AIH) and *N*-carbamoylputrescine amidohydrolase (CPA). The synthesis of Put via Orn is catalyzed by the enzymes arginase (ARG) and ornithine decarboxylase (ODC). Once Put is synthesized, it is converted to Spd when an aminopropyl group from decarboxylated S-adenosylmethionine (dcSAM), which is formed from the decarboxylation of S-adenosylmethionine (SAM) catalyzed by SAM decarboxylase (SAMDC), is transferred in a reaction catalyzed by spermidine synthase (SPDS). Spermidine, which is a triamine, is further converted to a tetraamine spermine in a reaction catalyzed by spermine synthase (SPMS) where one more aminopropyl group is added (Alcázar *et al.,* 2010).

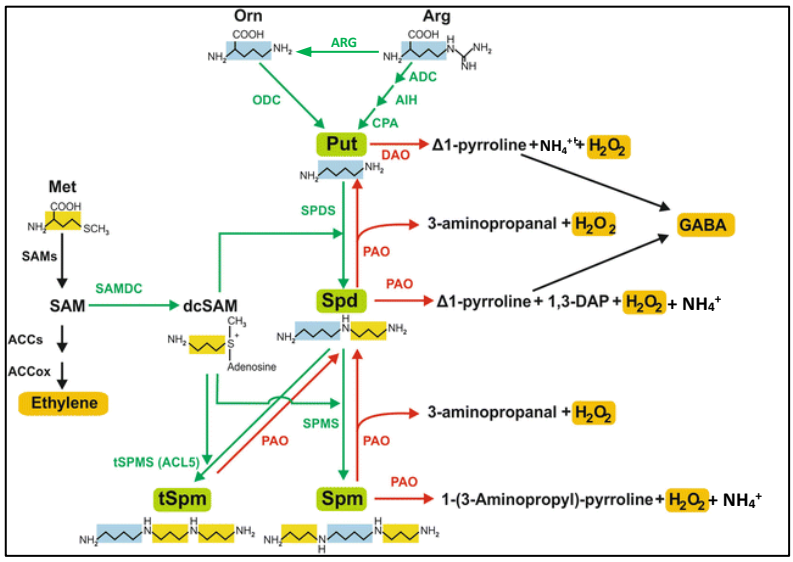

Figure 2: Polyamines metabolism and interaction with other metabolic routes. *Green arrows represent PA biosynthetic pathways, while red arrows represent PA catabolic pathways.*

As well as PA biosynthesis, PA catabolism is an important process to maintain PA homeostasis within plant cells. PA oxidation is catalyzed by a heterogeneous class of enzymes including diamine oxidases (DAO, EC 1.4.3.6) and the polyamine oxidases (PAO, EC 1.5.3.3) (Cona *et al.,* 2006a; Angelini *et al.,* 2010; Tavladoraki *et al.,* 2012). During PA catabolism where the PAs are deaminated by the DAO and PAO enzymes, there is a simultaneous generation of H_2O_2. This H_2O_2 has both effects the generation of oxidative stress and the activation of anti-oxidative defense responses as the lignification of plant cell wall (Gupta *et al.,* 2016).

Polyamines accumulation correlated with an enhanced tolerance to environmental stresses (Marco *et al.,* 2015). When plants are exposed to one or more abiotic stresses, several genes involved in the biosynthesis pathway of PAs have been reported to be upregulated, being the Put the first PA which is accumulated inside plant cells (Alcázar *et al.,* 2006a).

The metabolic pathway of PAs is interconnected with other metabolic routes in which signaling molecules and metabolites involved in plant stress response are synthesized. PA and ethylene biosynthesis share a common precursor, SAM, which undergo antagonistic effects during leaf and flower senescence, and fruit ripening (Pandey *et al.,* 2000). The H_2O_2 generated during PA oxidation by DAO and PAO enzymes is involved in both biotic and abiotic stress signaling, as well as in the stomatal closure induced by ABA (Cona *et al.,* 2006). Another important product from Put and Spd terminal catabolism is the GABA, which is a non-protein amino acid that rapidly accumulates in plant tissues when they are exposed to biotic and abiotic stresses, and regulates plant growth. According to Ramesh *et al.,* 2015, GABA regulates negatively the anion flux through plant aluminum-activated malate transporter (ALMT) proteins present in root apical cells, which is activated by anions. This novel signaling pathway of GABA-mediated regulation of ALMT proteins is able to translate this metabolite stress concentration changes into plant

physiological outputs. Finally, PA catabolism is closely related to proline (Pro) accumulation in response to salt stress (Aziz *et al.,* 1998). Proline levels increased in response to abiotic stress, which may be related to the accumulation of PA under these conditions since both share a common precursor, the Orn (Filippou *et al.,* 2013). It has been reported that Pro is synthesized from Orn during supra-optimal nitrogen conditions and seedling development (Roosens *et al.,* 1998; Armengaud *et al.,* 2004; Dar *et al.,* 2016). Therefore, PA metabolism is interconnected with several hormonal and metabolic pathways involved in plant development, nitrogen assimilation, respiratory metabolism and stress response. Early research of plant PAs outlines their involvement in abiotic stress tolerance. However, the precise molecular mechanisms by which PAs modulate plant stress responses to abiotic stress stimuli are largely unknown although PA signaling is recently showed to interact with different routes and intricate hormonal cross-talks (Alcázar *et al.,* 2010).

3.3.3 Polyamine catabolism: amine oxidases

Polyamine homeostasis is essential to plant cell viability. During PA catabolism and/or interconversion, H_2O_2 is generated; this reactive oxygen species (ROS) acts up or downstream of various signaling cascades and its accumulation in a given time is the determinant whether ROS are a part of the prime event of the cascade, usually at low levels, or a harmful event, at high levels (Foyer and Noctor, 2005; Miller *et al.,* 2010). Amine oxidases located in the apoplast have been reported as a key source of reactive oxygen species such as H_2O_2, and nitric oxide (NO) regulating differentiation events, as well as participating in signaling of plant both local and systemic defense responses.

Polyamines are catabolized through the activity of both types of amine oxidases DAOs and PAOs. The first one shows a strong preference for diamines Put and Cad, while the latter oxidize only higher PAs such as Spd and Spm. DAOs, which are present at high levels in dicots especially pea, chickpea, lentil and soybean seedlings, are copper-containing proteins which tend to form homodimers whose each sub unit contains a single copper ion and a 2,4,5-trihydroxyphenylalanine quinone cofactor. From this point, these enzymes will be called either diamine oxidases or copper-containing amine oxidases (CuAO). CuAOs catalyze the oxidation of Put to 4-aminobutanal with the concomitant production of NH_4^+ and H_2O_2, being the resulting aldehyde further converted to GABA (Figure 2). On the other hand, PAOs are monomeric, flavin adenine dinucleotide (FAD) dependent amine oxidases where FAD cofactor is bounded non-covantly (Kusano *et al.,* 2015). They are highly expressed in monocots. Nowadays, there is a current consensus which indicates that plants have two types of PAOs, those involved in PA terminal catabolism and those involved in back-conversion of PAs. Through terminal catabolism, PAOs convert Spd into 4-aminobutanal, with the concomitant production of 1,3-diaminopropane, NH_4^+ and H_2O_2; or Spm to N-(3-aminopropyl)-4-aminobutanal, NH_4^+ and H_2O_2 (Šebela *et al.,* 2001). The back-conversion reaction converts Spm into Spd, and Spd into Put, with the concomitant production of 3-aminopropanal and H_2O_2 (Kusano *et al.,* 2008) (Figure 2).

3.3.4 Involvement of polyamine metabolism in abiotic stress response

The involvement of PA metabolism in stress tolerance responses has been reported. Some PA functions in stress tolerance have been related with the production of GABA and H_2O_2, since 4-aminobutanal produced during the Put and Spd terminal oxidation catalyzed by DAOs and PAOs, respectively, can be further converted to GABA (Zarei *et al.,* 2015b), which enter into the Krebs cycle constituting a link between nitrogen and carbon metabolism (Moschou *et al.,* 2012). GABA is also synthesized from glutamate through the action of glutamate decarboxylase (GAD) (Snedden *et al.,* 1995; Snedden *et al.,* 1996). Under salt stress, Put catabolism via DAO contributes to Pro accumulation (Aziz *et al.,* 1998); moreover, under salinity conditions DAO and PAO activities have been observed to increase in tomato leaves, soybean roots, and shoots and roots of rice (Xing *et al.,* 2007; Quinet *et al.,* 2010; Legocka *et al.,* 2017).

The participation of PAs in the response to abiotic stresses is the topic of interest in the present study since the supply of ammonium as the sole source of nitrogen causes plant abiotic stress. High concentrations of exogenous ammonium have been reported to have several effects in carbon and nitrogen metabolism, which can be alleviated by the application of high light intensity that allows a higher carbon and energy availability, higher root respiration and lower the endogenous cell content of ammonium (Ariz *at al.,* 2011; Ariz *et al.,* 2013). Under high ammonium concentration, pea plants show an increase of the Arg content that is decreased and apparently transformed into Put under high light intensity conditions, while Spd and Spm contents remain constant increasing the ratio Put/(Spd+Spm) (Ariz *et al.,* 2013). Although PAs are stress-associated molecules linked to the "urea cycle" in plants, their contribution to the response to ammonium nutrition remains still unknown.

Previous studies performed by our laboratory showed the lack of stress symptoms when the legume *Medicago truncatula* was grown under low ammonium concentration since the correct growth and development of the plant was not affected (Esteban *et al.*, 2016a). The same study showed that the application of high concentration of nitrogen did not have important effects on plant growth and development since *Medicago truncatula* seemed not to be affected by being cultured under these conditions. It suggest that this legume is able to efficiently adapt its metabolism to different nitrogen sources availability, as well as to different nitrogen source concentrations applied (Royo, 2017).

Under axenic culture conditions, a comparative study of the differential effect on the "urea cycle" and PA synthetic pathway of ammonium and urea as the sole source of N against nitrate-based nutrition was performed by our laboratory. Its results highlighted the accumulation of Arg and PA, mainly Put, and the decrease of GABA content when high dose of ammonium was applied (Cerdan, 2017). It has been also proposed the activation of GABA shunt under ammonium nutrition to face with the excess of ammonium inside the plant cells (Allen *et al.,* 2011; Ariz *et al.,* 2013; Bittsánszky *et al.,* 2015). Moreover, the possible block of the oxidative metabolism of PAs by the effect of

ammonium as a product of the amine oxidases catalytic steps might be occurring (Cerdan, 2017). Esteban *et al.,* 2016b speculated that the excess of ammonium originated either from ammonium nutrition or from PA metabolism can act as a feedback inhibitor of PA deamination, further impairing PA metabolism.

Therefore, the importance of "urea cycle" on the tolerance of *Medicago truncatula* seedlings to ammonium has been proposed, in which polyamines catabolism might play a key role in such tolerance, but there are still important gaps in our knowledge. The present study seeks to elucidate whether the high intracellular ammonium content of *Medicago truncatula* plants grown under ammonium nutrition as the sole nitrogen source, in comparison to those nitrate- and urea- fed plants, may be blocking the terminal catabolism and the back-conversion of PAs.

4 Aim

The general purpose of this work was to study in deep the **amine oxidases in plants, especially in the legume *Medicago truncatula*.** In previous studies carried out by our group, a higher importance of "urea cycle" on the tolerance of *Medicago truncatula* seedlings to ammonium has been proposed. Polyamine catabolism might play a key role in such tolerance, but there are still important gaps in our knowledge. Therefore, the central aim was carried out through two major and consecutive goals (see details below).

1. A phylogenetic analysis of plant amine oxidases proteins to predict *Medicago truncatula* amine oxidases cellular location, substrate preference and polyamine catabolism pathway they are involved in.

The identification of *Medicago truncatula* amine oxidases and their orthologous proteins in other relevant species was conducted prior to the performance of the analysis. Both intra- and inter-specific phylogenetic analysis of amine oxidases using the identified sequences were carried out (Section 6.1 and 7.1).

2. A study of the differential effect of distinct nitrogen nutrition and doses on diamine oxidases and polyamine oxidases activities involved in the catabolic process of polyamines in connection to the urea cycle.

Medicago truncatula plants were grown on nitrate, ammonium or urea as a sole nitrogen source in axenic culture. This kind of axenic culture is of great interest because it allows the modeling of the plants response, without contamination with interfering bacteria and/or fungus which can be avoided. Thereby, an attempt to measure the diamine oxidases and polyamine oxidases activities spectrophotometrically on plant extracts was performed and, the role of such activities on ammonium- or urea-induced "stress" will be discussed (Section 6.2 and 7.2).

5 Material and Methods

5.1 Phylogenetic analysis

In order to fulfill the first specific goal of the present study, a phylogenetic analysis of the amine oxidases proteins was performed using bioinformatics tools (Jill Harrison and Langdale, 2006; Cvrčková, 2016). In particular, both intra- and interspecific study of *M. truncatula* and plant amine oxidases were carried out as described below.

5.1.1 *Medicago truncatula* amine oxidases

First of all, a research of all amine oxidases of the species *Medicago truncatula* was performed against Uniprot (http://www.uniprot.org) database. Secondly, the identified amine oxidases of *M. truncatula* were clustered into two groups as follow: diamine oxidases (DAO) and polyamine oxidases (PAO). In Table 1, a relation of all the *Medicago truncatula* proteins is accordingly depicted. Finally, a file with the FASTA sequences and gene locus was created to make easier the forthcoming analysis (see Supplementary Table 1 and 2).

Table 1:* Medicago truncatula *amine oxidases. *A list of the* Medicago truncatula *(**A**) diamine oxidases and (**B**) polyamine oxidase identified; and the correspondence between the gen identifier and the Uniprot ID of each protein is showed. The gen identifier of* M. truncatula *proteins was assigned as cited in Jill Harrison and Langdale, 2006. Thus, two initial letters corresponding to the species name followed by the type of amine oxidase and an arbitrary number were used as gen code.*

A) Diamine oxidases	
Gen identifier	Uniprot ID
MtDAO1	A0A072TRW3
MtDAO2	A0A072TRL3
MtDAO3	A0A072TRL9
MtDAO4	A0A072TRW9
MtDAO5	A0A072TSH3
MtDAO6	A0A072TTH8
MtDAO7	G7JYY1
MtDAO8	A0A072UDP7
MtDAO9	G7J4S8
MtDAO10	G7J7B0
MtDAO11	G7ID64
MtDAO12	G7ID65
MtDAO13	A0A072URB1
MtDAO14	A0A072UT22
MtDAO15	A0A072V0B7
MtDAO16	A0A072UUP2
MtDAO17	A0A072V290

B) Polyamine oxidases	
Gen identifier	Uniprot ID
MtPAO1	A2Q567
MtPAO2	A0A072UVV1
MtPAO3	G7K2Z6
MtPAO4	G7KD02
MtPAO5	A0A072UNG5
MtPAO6	A0A072UW35
MtPAO7	G7J7X8
MtPAO8	G7J7Y0
MtPAO9	G7J7X9
MtPAO10	G7IDT4
MtPAO11	G7IM95
MtPAO12	A0A072TCJ7
MtPAO13	G7J0U8

5.1.2 Identification of orthologous amine oxidase proteins

With the purpose of performing an interspecific phylogenetic analysis of *Medicago truncatula* both amine oxidases, DAOs and PAOs, orthologous proteins from several plants species were retrieved through *BLAST*p search (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The criterion followed to choose the orthologous amino acid sequences was an identity higher than 96%; and the e-score, the closer the e-score is to 0, the higher degree of homology between two sequences. This BLAST search was performed for each amine oxidase of *Medicago truncatula*. Moreover, in order to be able to characterized the proteins of interest, amine oxidases of both model plants *Arabidopsis thaliana* and *Oryza sativa*, which have been already characterized, were added (Jill Harrison and Langdale, 2006; Cvrčková, 2016).

Table 2: Selected orthologous amine oxidases. *A list of the identified and selected (**A**) diamine oxidases and (**B**) polyamine oxidase orthologous proteins of several species; and the correspondence between the gen identifier and the Uniprot ID of each protein is showed. The gen identifier was cited as previously assigned in literature or, in the case that no terminology was found, a new gen identifier was assigned (Jill Harrison and Langdale, 2006). Thus, two initial letters corresponding to the species name followed by the type of amine oxidase and an arbitrary number were used as gen code.*

A) Diamine oxidases	
Gen identifier	Uniprot ID
AtDAO1	Q8H1H9
AtDAO2	Q9M2B9
AtDAO3	F4IAX1
AtDAO4	P0DO00
AtDAO5	F4IAX0
AtDAO6	O23349
CaDAO	O65749
GmDAO1	K7LI85
GmDAO2	K7LI86
LcDAO	P49252
MdDAO1	A0A096ZNR5
MdDAO2	A0A096ZNU5
NtDAO	A0A075EZT1
OsDAO1	A3AUU3
OsDAO2	Q7XWW0
PsDAO	Q43077
SlDAO	K4CNU3
TpDAO	A0A2K3NT42
TrDAO	AQQ81871
ZmDAO	A0A096SGQ3

B) Polyamine oxidases	
Gen identifier	Uniprot ID
AtPAO1	Q9FNA2
AtPAO2	Q9SKX5
AtPAO3	Q9LYT1
AtPAO4	Q8H191
AtPAO5	Q9SU79
GsPAO1	A0A0B2QXT4
GsPAO2	KHN38359.1
HvPAO1	Q93WM8
HvPAO2	Q93WC0
MdPAO	A0A1C8M5I8
OsPAO1	Q5NAI7
OsPAO2	Q0DUC7
OsPAO3	Q7X809
OsPAO4	Q7XR46
OsPAO5	Q0J954
OsPAO6	Q0J291
OsPAO7	Q0J290
SmPAO	A6U6Y8
TpPAO1	A0A2K3P3K3
TpPAO2	A0A2K3JRK6
TrPAO	A0A286QIQ2
ZmPAO	O64411

Species used in the study: *Arabidopsis thaliana* (At), *Cicer arietinum* (Ca), *Glycine max* (Gm), *Glycine soja* (Gs), *Hordeum vulgare* (Hv), *Lens culinaris* (Lc), *Malus domestica* (Md), *Nicotiana tabacum* (Nt), *Oryza sativa* (Os), *Pisum sativum* (Ps), *Sinorhizobium medicae* (Sm), *Solanum lycopersicum* (Sl), *Trifolium pratense* (Tp), *Trifolium repens* (Tr) *and Zea mays* (Zm).

In this case, also Uniprot database was utilized to obtain the FASTA sequences of orthologous proteins. In Table 2, a relation of all the orthologous proteins is accordingly depicted. Additionally, the FASTA sequences and gene locus were presented in Supplementary Table 3 and 4.

5.1.3 Phylogenetic analyses

The next step in the completion of *Medicago truncatula* amine oxidases phylogenetic analysis was to carry out a multiple sequence alignment. The same procedure was followed in both intra- and inter-specific phylogenetic analyses, taking into account that the interspecific phylogenetic study of both DAO and PAO were independently performed. MAFFT 7 alignment software (https://mafft.cbrc.jp/alignment/software/) was selected to perform the multiple sequence alignment. In particular, the MAFFT 7 E-INS-I algorithm was utilized (Katoh *et al.,* 2017). Then, once the amino acid sequences were aligned, CD-HIT tool was applied in order to remove 100% redundant sequences. Finally, the phylogenetic tree corresponding to each phylogenetic analysis was obtained through the Neighbor Joining method and the statistical Bootstrap test with 1000 replications.

5.2 Plant material and culture conditions

In the present study, seedlings of *Medicago truncatula* were used to achieve the second specific objective previously cited in section 4: to study the differential effect of distinct N nutrition and doses on diamine oxidase and polyamine oxidase activities involved in the catabolic process of PAs in connection the urea cycle.

5.2.1 *Medicago truncatula*

The model plant *Medicago truncatula* (Gaertn.) belongs to the Fabaceae family. The choice of *Medicago truncatula* as a model plant is given by its short life cycle, its high yield of seed production and because its genome has been already sequenced (Tang *et al.,* 2014).

5.2.2 Culture conditions

Seeds of *Medicago truncatula* ecotype Jemalong A17 were scarified (Figure 3A) with 95% sulfuric acid during 8 minutes, and washed 3-4 times with mQ water. Further, seeds were sterilized with 50% (v/v) sodium hypochlorite solution for 5 minutes under a laminar flow cabinet, and consecutively washed with milliQ sterile water until the pH was approximately 7. In order to synchronize germination, seeds were kept overnight in sterile water at 4ºC in darkness. Then, they were germinated on Petri dishes containing 0.4% (w/v) plant agar during 4 days at 14°C also in darkness (Figure 3B). After seed germination (Figure 3C), the sprouts were transferred to glass jars under sterile conditions (Figure 3D) which contained 100 ml of culture media.

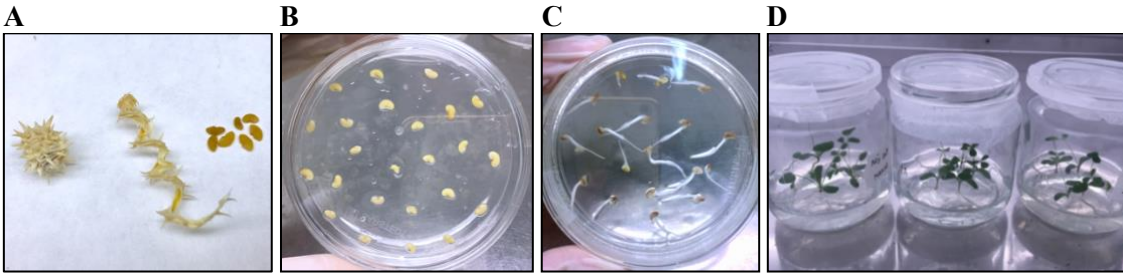

Figure 3:* Medicago truncatula *growth steps in axenic conditions. *(**A**) Fresh, (**B**) sterilized, and (**C**) germinated* Medicago truncatula *seeds; and (**D**) 15 days-old seedlings.*

The Fahraeus nutrient solution was used as culture media and its composition is showed in Table 3.

Table 3: Fahraeus nutrient solution for* Medicago truncatula *growth under axenic conditions. *Macro- and micronutrients composition and concentrations of Fahraeus medium. PhytagelTM was added to have a final concentration of 5.5% (w/v) agar.*

Macronutrients	Concentration (mM)	Micronutrients	Concentration (µM)
$CaCl_2$	0,9	$MnCl_2$	0,8
$MgSO_4$	0,5	$CuSO_4$	0,6
KH_2PO_4	0,7	$ZnCl_2$	0,7
Na_2HPO_4	0,8	H_3BO_3	1,6
Ferric citrate	0,02	Na_2MoO_4	0,5

For the study of the differential effect of nitrate, ammonium and urea nutrition, either calcium nitrate ($Ca(NO_3)_2$), ammonium sulfate ($(NH_4)_2SO_4$), or urea ($CO(NH_2)_2$) were supplied as the sole source of N. Moreover, two different situations were considered depending on the supplied nitrogen concentration: 1 mM (low dose) and 25 mM (high dose) of N, as can be seen in Table 4. In addition, to compensate the Ca^{2+} applied on the nitrate medium, ammonium and urea containing solution were also supplied with the same Ca^{2+} concentration as $CaSO_4$. An exhaustive control of the pH was performed during the preparation of the culture media for the purpose of achieving a pH value around 6.5.

Table 4: Nitrogen sources utilized to grow* Medicago truncatula *under axenic conditions. *Calcium sulfate was applied to ammonium and urea media in order to balance the amount of Ca ion compared with the other nitrogen media.*

Nitrogen source	Concentration (mM)	$CaSO_4$ (mM)
Nitrate	1	-
$Ca(NO_3)_2$	25	-
Ammonium	1	0.5
$(NH_4)_2SO_4$	25	12.5
Urea	1	0.5
$CO(NH_2)_2$	25	12.5

Medicago truncatula seedlings were grown during 15 days in a growth chamber under controlled environmental conditions with a day/night temperature of 24.5/22°C, 80% of relative humidity, day/night photoperiod of 16/8 hours and 70 $\mu mol^2 s^{-2}$ of photosynthetically active radiation. After the growth period, root and shoot were separately harvested, frozen in liquid nitrogen, and samples were stored at -80 °C until use.

5.3 Diamine and polyamine oxidases activity assay

The catalytic activity of *Medicago truncatula* diamine oxidase (DAO; EC 1.4.3.6) and polyamine oxidase (PAO; EC 1.5.3.3) activities were measured following the protocol described in Wisniewski *et al.,* 2000. *Medicago truncatula* frozen tissues (300 mg) were homogenized in a chilled mortar with 100 mM sodium phosphate pH 6.5 buffer, the extracts were centrifuged for 20 min at 12000 *g* at 4ºC, and the recovered supernatants were used for the analytical measurements. The reaction mixture contained 100 µl of plant extract and 100 µl of the following reaction buffer composed by 100 mM sodium phosphate pH 6.5, 0.1 mg/ml 3,3´,5,5´-tetramethylbenzidine, 0.3 mU horseradish peroxide and 20 mM of the correspondent polyamine. Specifically, putrescine was used to test DAO activity, while spermidine and spermine were used in PAO activity assay. The reaction was incubated at room temperature for 10 min, and then 50 µl of 1 M H_2SO_4 was added to stop the reaction. The spectrophotometric assay was performed in a 96-wells plate and the absorbance at a wavelength of 450 nm was read in a microplate reader (SpectraMax 340pc, Molecular Devices). To calculate the specific activity, either *o*-phenanthroline (Wisniewski *et al.,* 2000) or guazatine (Cona *et al.,* 2006) were used as inhibitors of DAO and PAO, respectively. Thus, the absorbance obtained when samples were incubated with the specific inhibitor (2 mM final) for 15 min prior to add the reaction buffer was subtracted from the sample absorbance.

5.4 Statistical analysis

Statistical analyses were performed with IBM Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp. The data obtained in this study were analyzed statistically using the mean as a measure of central tendency and the standard error (S.E.) as a measure of dispersion. All data were tested for normality (Kolmogorov-Smirnov test) and homogeneity of variances (Levene test). Differences among treatments were evaluated with one-way ANOVA and post-hoc Student-Newman-Keuls or non-parametric T3-Dunnet test. Finally, the resulting *p* values were considered to be statistically significant at $\alpha = 0.05$.

6 Results

6.1 Phylogenetic study of plant amino oxidases enzymes

Plant amino oxidases are a large family of proteins which catalyze different reactions on the catabolism of polyamines. As the metabolic pathway they are involved is related to several stress situations, the phylogenetic study of this proteins is performed thereafter.

6.1.1 Intraspecific phylogenetic analysis of *Medicago truncatula* amine oxidases

After the research of *M. truncatula* amino oxidases, 30 different proteins were identified which were grouped according to the prediction of the presence or absence of a copper ion bound (Table 1). Thus, 17 out of those proteins belong to the diamine oxidase family (DAO; CuAO), whereas 13 were identified as polyamine oxidase (PAO). Then, the sequence of all those 30 amino oxidases were used to perform the intraspecific phylogenetic analysis. The resulting phylogenetic tree (Figure 4) clearly differentiates two clades corresponding with both types of *Medicago truncatula* amine oxidases. As expected, this clustering matches with the classification showed earlier in Table 1. So, while Clade I grouped the 13 PAO proteins, Clade II grouped the 17 diamine oxidases.

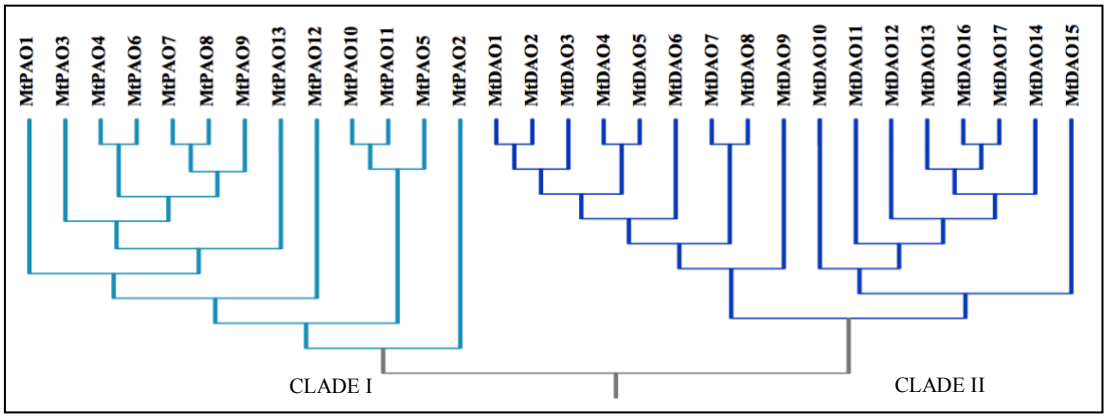

Figure 4: Phylogenetic tree of the intraspecific study of* Medicago truncatula *amine oxidases. *A phylogenetic analysis of 30 amine oxidases sequences of* M. truncatula *were performed using MAFFT 7 E-INS-I; the phylogenetic tree was obtained through the Neighbor Joining method and the statistical Bootstrap test with 1000 replications. Bootstrap values were higher than 90 for all the branches of the tree (not shown). Clade I (light blue) corresponded to* Medicago truncatula *PAOs, while in Clade II (dark blue) the DAOs were clustered.*

6.1.2 Interspecific phylogenetic analysis of *Medicago truncatula* amine oxidases

Once *Medicago truncatula* amine oxidases were clearly grouped into two types of proteins, an interspecific phylogenetic analysis was performed for both amine oxidases, respectively. In those analyses, *M. truncatula* proteins and their identified orthologous (Table 2) were included in each phylogenetic study.

The resulting phylogenetic tree of diamine oxidases interspecific analysis showed three different clusters (Figure 5). Thus, the DAOs analyzed were grouped on Clade I that was mainly composed by *Medicago truncatula* DAOs; Clade II and Clade III.

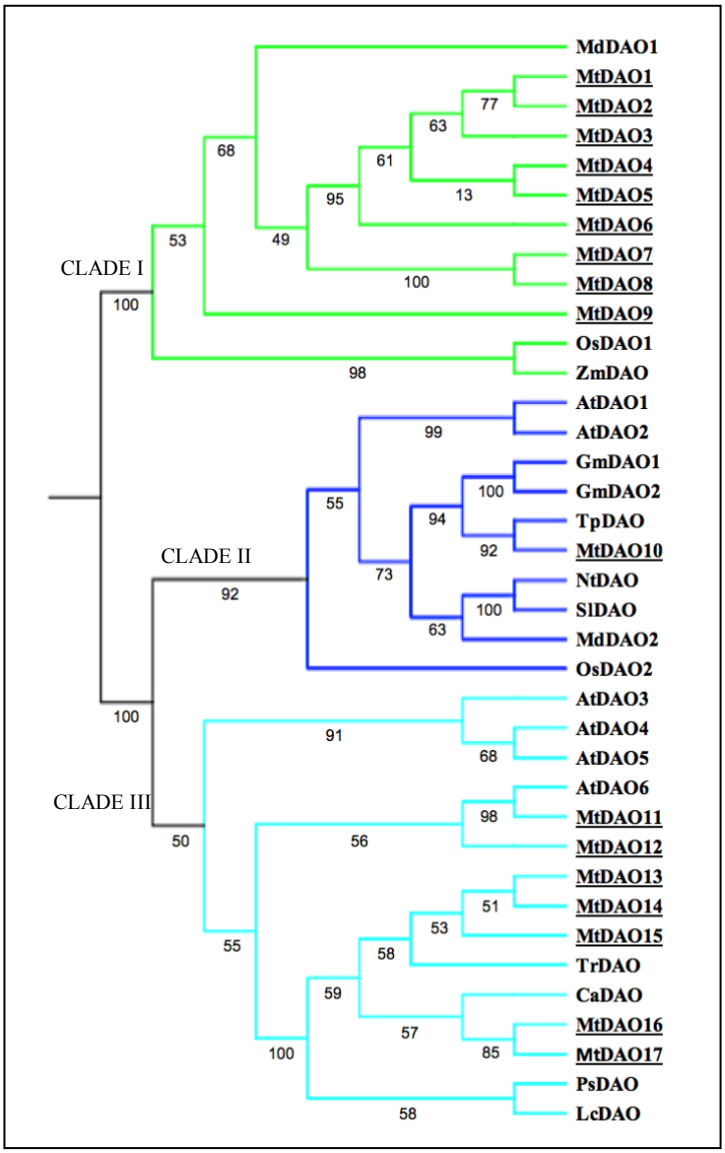

Figure 5: Phylogenetic tree of the interspecific study of diamine oxidases. *A phylogenetic analysis of plant diamine oxidases sequences including the 17 previously identified from* M. truncatula *were performed using MAFFT 7 E-INS-I. The phylogenetic tree was obtained through the Neighbor Joining method and the statistical Bootstrap test with 1000 replications. Bootstrap values were indicated at the nodes meaning the percentage of times the same branch was observed in the same place when the phylogenetic reconstruction was repeated. Green branches represented Clade I, dark blue corresponded to Clade II, and light blue to Clade III.*

More specifically, Clade I was formed by nine *M. truncatula* proteins (from MtDAO1 to MtDAO9) along with one of *Malus domestica*, one of *Oryza sativa* and another of *Zea mays* (MdDAO1, OsDAO1 and ZmDAO). Moreover, the clustering showed that Clade II and Clade III proteins were more similar, but higher species heterogeneity was observed on Clade II. The protein MtDAO10 was the only *M. truncatula* DAO found in this Clade II; in which additionally AtDAO1, AtDAO2, GmDAO1, GmDAO2, TpDAO, NtDAO, SlDAO, MdDAO2 and OsDAO2 were represented. Finally, in Clade III we found seven *M. truncatula* CuAOs (from MtCuAO11 to MtCuAO17), three *A. thaliana* DAOs (from AtDAO3 to AtDAO5) represented as an outgroup, and DAOs from four species more were clustered such as *Trifolium repens*, *Cicer arietinum*, *Lens culinaris* and *Pisum sativum* (TrDAO, CaDAO, PsDAO and LcDAO).

The interspecific phylogenetic analysis of PAOs resulted in a phylogenetic tree that also showed three different clusters (Figure 6). In this case, the *M. truncatula* protreins were more widespread among the different Clades. Thus, the Clade I was only formed by six proteins: MtPAO1, MtPAO2, AtPAO5, GsPAO1, OsPAO1 and TpPAO1. Clade II, the largest one, was composed by seven *Medicago truncatula* polyamine oxidases (from MtPAO3 to MtPAO9) in addition to AtPAO2, AtPAO3, AtPAO4, GsPAO2, MdPAO, OsPAO3, OsPAO4, OsPAO5, SmPAO, TpPAO2 and TrPAO. Finally, in Clade III four *Medicago truncatula* PAOs (from MtPAO10 to MtPAO13) were grouped along with AtPAO1, HvPAO1, HvPAO2, OsPAO2, OsPAO6, OsPAO7, and ZmPAO.

It should be mentioned that among the PAO homologous sequences used, it has been included a non-plant protein from *Sinorhizobium medicae* (SmPAO). Its inclusion on the analysis was because of the symbiotic relation that this bacterial species can form with the different *Medicago sp.*

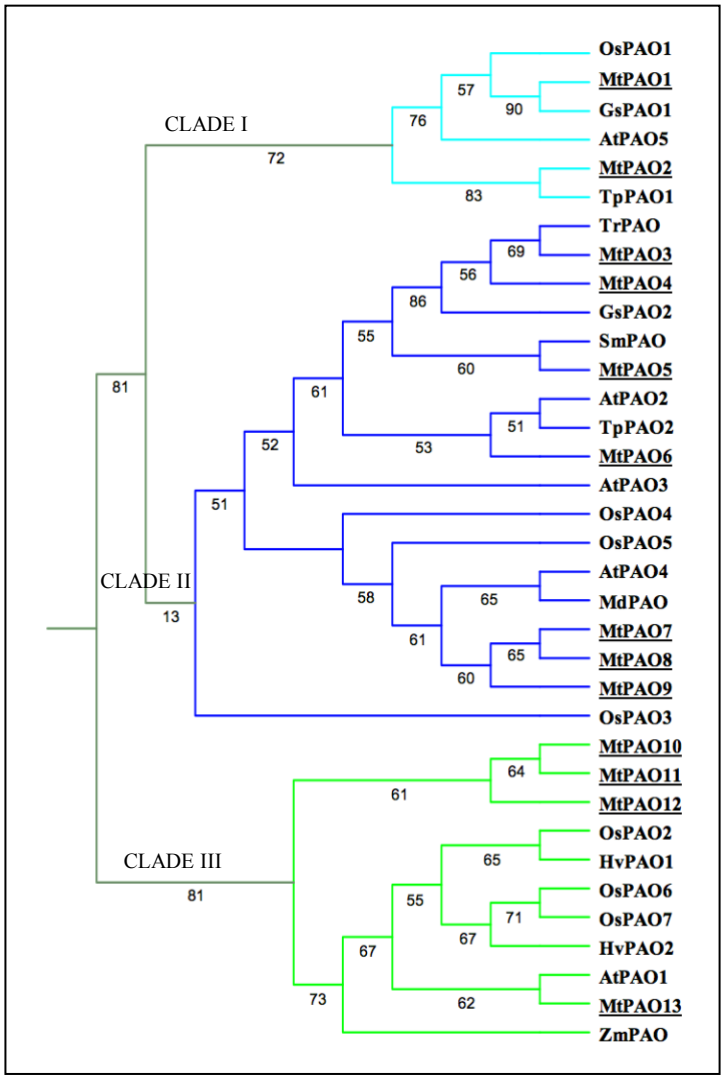

Figure 6: Phylogenetic tree of the interspecific study of polyamine oxidases. *A phylogenetic analysis of plant polyamine oxidases sequences including the 13 previously identified from* M. truncatula *were performed using MAFFT 7 E-INS-I. The phylogenetic tree was obtained through the Neighbor Joining method and the statistical Bootstrap test with 1000 replications. Bootstrap values were indicated at the nodes meaning the percentage of times the same branch was observed in the same place when the phylogenetic reconstruction was repeated. Light blue branches represented Clade I, dark blue corresponded to Clade II, and green to Clade III.*

The present phylogenetic study enables us to make a characterization of *Medicago truncatula* amine oxidases by means of comparing both *Medicago truncatula* DAOs and PAOs to those belonging to the same cluster and have been already characterized. In the following Table 5, the characteristics of interest of the orthologous diamine oxidases and polyamine oxidases, respectively, were summarized.

Table 5: Characteristics of the orthologous proteins utilized in the interspecific analysis of amine oxidases. *The research of the identified orthologous (A) diamine oxidases and (B) polyamine oxidases characteristics included the cellular location, substrate preference, function, and the polyamine catabolism pathway they are involve in.*

Proteins	Cellular location	Substrate preference	Function	TC/ BC*	References
A) Amine oxidases					
CLADE I					
MdDAO1	Apoplast	Put	-	TC	Zarei et al. (2015a)
ZmDAO	Peroxisome	Put	-	TC	Qi et al. (2014)
CLADE II					
At DAO1	Apoplast	Put, Spd	PA- and ABA-ediated NO production	TC	Møller and McPherson, 1998;
MdDAO2	Peroxisome	Put, Cad	-	TC	Zarei et al., 2015a
CLADE III					
At DAO3	Peroxisome	Put, Spd	ABA-induced stomatal closure	TC	Planas-Portell et al., 2013
AtDAO6	Apoplast	Put, Spd	-	TC	Wimalasekera et al., 2011; Planas-Portell et al., 2013
CaDAO	Apoplast	Put	Defense response	TC	Rea et al., 2002
LcDAO	Apoplast	Put, Spd, Spm	-	TC	Medda et al., 1996; Tavladoraki et al., 2012
PsDAO	Apoplast	Put, Spd, Spm	-	TC	Tipping and McPherson, 1995; Moschou et al., 2012
B) Polyamine oxidases					
CLADE I					
AtPAO5	Cytosol	Spm	PA homeostasis, plant growth, stress response	BC	Kim et al., 2014; Zarza et al., 2017
GsPAO1	-	Spm	-	BC	Qi et al., 2014
OsPAO1	Cytosol	Spm	Plant growth	BC	Liu et al., 2014a,c
TpPAO1	-	Spm	-	-	Ištvánek et al., 2014; 2017

Table 5: Continuation.

Proteins	Cellular location	Substrate preference	Function	TC/ BC*	References
CLADE II					
AtPAO2	Peroxisome	Spm, Spd	Stress response	BC	Moschou et al., 2008b; Takahashi et al., 2010
AtPAO3	Peroxisome	Spd, Spm	Stress response. Pollen tip growth	BC	Moschou et al., 2008b; Takahashi et al., 2010
AtPAO4	Peroxisome	Spm	Senescence	BC	Moschou et al., 2008b; Kamada-Nobusada et al., 2008; Takahashi et al., 2010
CLADE II					
GsPAO2	-	Spm, Spd	-	BC	Qi et al., 2014
OsPAO3	Peroxisome	Spd, Spm	-	BC	Ono et al., 2012
OsPAO4	Peroxisome	Spm	-	BC	Ono et al., 2012
OsPAO5	Peroxisome	Spm	-	BC	Ono et al., 2012
TpPAO2	-	Spd, Spm,	-	-	Ištvánek et al., 2014; 2017
CLADE III					
AtPAO1	Cytoplasm	Spm	Stress response	BC/ TC	Takahashi et al., 2010; Sagor et al., 2016
HvPAO1	Apoplast	-	-	TC	Cervelli et al., 2001; Cona etal., 2006a
HvPAO2	Vacuole	Spm, Spd	-	TC	Cervelli et al., 2001; Cona et al., 2006a
OsPAO6	Apoplast	-	-	-	Liu et al., 2014b
OsPAO7	Apoplast	Spm, Spd	-	TC	Liu et al., 2014b
ZmPAO	Apoplast	Spm, Spd	Cell wall differentiation	TC	Cona et al., 2006a

* Terminal catabolism of PAs (TC) or back-conversion pathway (BC)

6.2 Effect of nitrogen-source on *Medicago truncatula* amine oxidases activity

Since the metabolic pathway in which plant amine oxidases are involved, is related to several stress situations, the enzymatic activity of DAOs and PAOs was measured as described in section 5. Previous results obtained by our laboratory suggested that the oxidative catabolism of PAs might be blocked in ammonium-fed plants due to the high dose of NH_4^+ supplied either as a result of a general stress originated by NH_4^+, or as a inhibition since NH_4^+ is a final product of several reactions in the urea cycle and PA metabolism. Thereby, in this section the results for *Medicago truncatula* amine oxidases activity are shown.

Medicago truncatula plants were grown under axenic conditions in differentially nitrogen-supplemented media: nitrate, ammonium and urea; and the specific activities were determined by spectrophotometry in both, shoots and roots (Figures 7 and 8). In general terms, the DAO activity was higher in *M. truncatula* shoots than in roots. Here, seedlings grown under low dose of ammonium presented a significantly higher DAO activity in shoots (Figure 7A), while no significant differences among treatments were observed at high N doses in this plant tissue (Figure 7B).

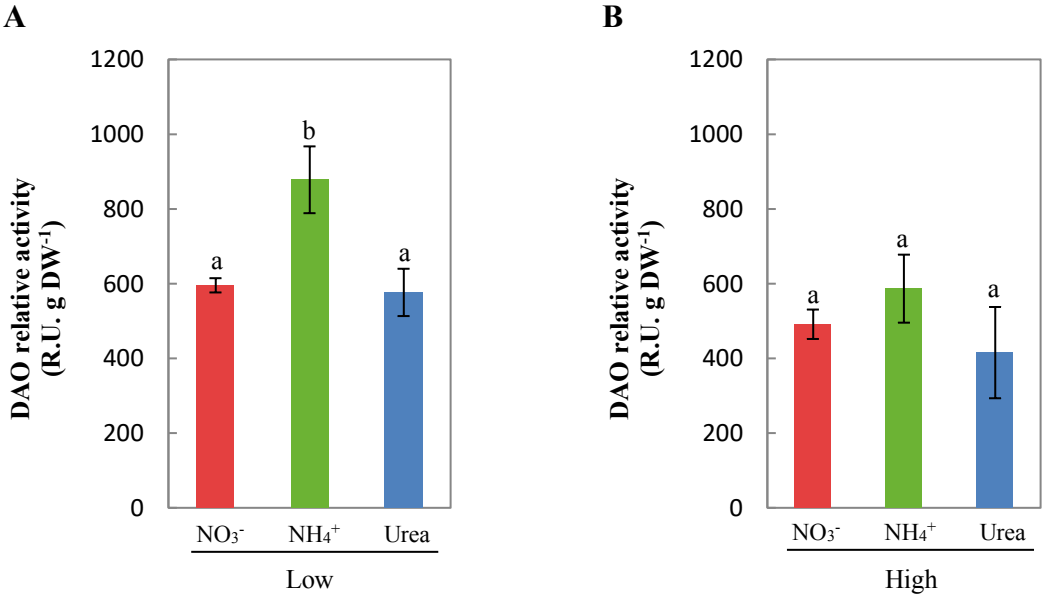

Figure 7: DAO relative activity of* Medicago truncatula *shoots. *The differential effect of the distinct nitrogen sources on DAO activity was assayed in shoot extracts of* Medicago truncatula *seedlings grown under axenic conditions at* ***(A)*** *low and* ***(B)*** *high doses of N supplied as NO_3^-, NH_4^+, and urea. The values are the mean ± S.E. (n = 3-5). Different letters denote statistically significant differences at α = 0.05 using the Student-Newman-Keuls test.*

Concerning to DAO activity in *Medicago truncatula* roots, although higher activity was found on ammonium-fed seedlings grown at low dose, it did not represent a significant difference (Figure 8A). However, when N was supplied at high dose, ammonium-fed seedlings showed a significant higher DAO activity in roots in comparison with both, nitrate- and urea-fed seedlings (Figure 8B).

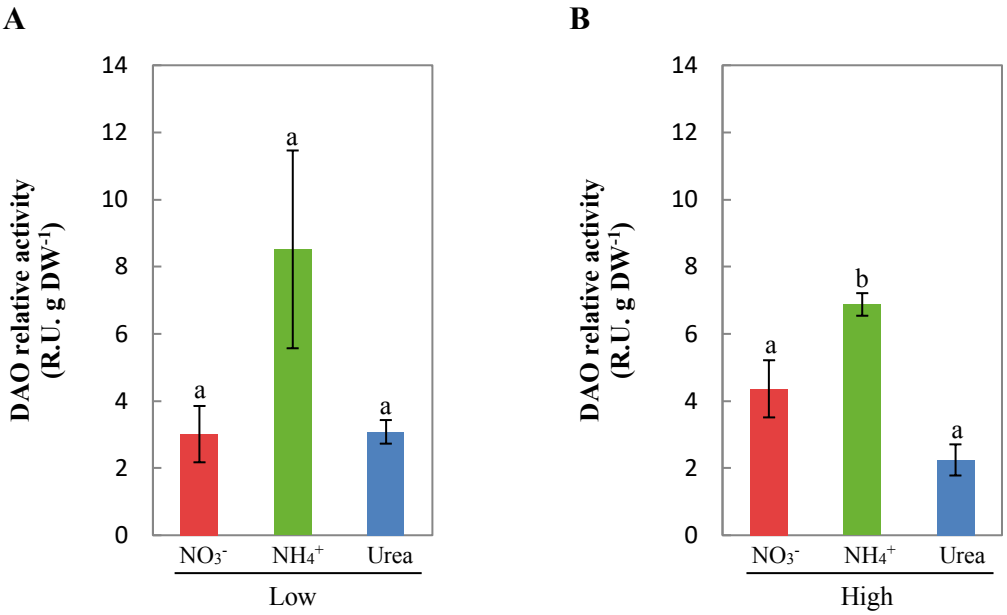

Figure 8: DAO relative activity of* Medicago truncatula *roots. *The differential effect of the distinct nitrogen sources on DAO activity was assayed in root extracts of* Medicago truncatula *seedlings grown under axenic conditions at* ***(A)*** *low and* ***(B)*** *high doses of N supplied as NO_3^-, NH_4^+, and urea. The values are the mean ± S.E. (n = 3-5). Different letters denote statistically significant differences at α = 0.05 using the Student-Newman-Keuls test*

Finally, attempts to measure PAO activity on both roots and shoots of *Medicago truncatula* seedlings were assayed. However, we failed to obtain results of PAO activity on both tissues. We tried to measure the activity in some different conditions but any of them worked for us, and therefore the PAO activity was not detected under our experimental conditions.

7 Discussion

Ammonium sensitivity of plants is a worldwide problem, constraining crop production. Prolonged application of ammonium as the sole nitrogen source may result in physiological and morphological disorders that lead to toxicity and decreased plant growth (Esteban *et al.,* 2016a). However, the threshold in which ammonium is toxic depends on various factors such as plant species, even varieties (Domínguez- Valdivia *et al.,* 2008; Cruz *et al.,* 2011; Li *et al.,* 2011; Sarasketa *et al.,* 2014). *Medicago truncatula* is usually used for the study of atmospheric nitrogen fixation, but it is also utilized for the study of nitrogen nutrition. Several studies with *Spinacia oleracea*, *Pisum sativum* and *Medicago truncatula* have reported that the different sources of nitrogen can affect the plant growth and development in different ways, especially at phenotypic level (Domínguez- Valdivia *et al.,* 2008; Ariz *et al.,* 2011; Esteban *et al.,* 2016a). The model legume *M. truncatula* was chosen to perform the present study due to its relative tolerance to ammonium nutrition and its ability to be cultured under axenic conditions. By means of this technique, it was possible to prevent the growth of bacteria and other microorganisms that can interfere in plant metabolism, as well as to avoid nodulation.

7.1 *Medicago truncatula* amine oxidases phylogenetic analyses

Diamine oxidases, which are present at high levels in dicots especially pea, chickpea, lentil and soybean seedlings, catalyze the terminal oxidation of Put to 4-aminobutanal with the concomitant production of NH_4^+ and H_2O_2, being the resulting aldehyde further converted to GABA. In contrast, polyamine oxidases are highly expressed in monocots. Nowadays, there is a current consensus which indicates that plants have two types of PAOs, those involved in PA terminal catabolism and those involved in back-conversion of PAs. Through terminal catabolism, PAOs convert Spd into 4-aminobutanal, with the concomitant production of 1,3-diaminopropane, NH_4^+ and H_2O_2; or Spm to N-(3-aminopropyl)-4-aminobutanal, NH_4^+ and H_2O_2 (Šebela *et al.,* 2001). The back-conversion reaction converts Spm into Spd, and Spd into Put, with the concomitant production of 3-aminopropanal and H_2O_2 (Kusano *et al.,* 2008).

Phylogenetic analyses have been performed in order to characterize *Medicago truncatula* amine oxidases. The intraspecific phylogenetic analysis has allowed to identify between both DAOs and PAOs that *Medicago truncatula* possesses, while the interspecific phylogenetic analysis has allowed to suggest the cellular location, substrate preference, function and their involvement in the terminal catabolism or back-conversion of PAs (Table 5), since *Medicago truncatula* amine oxidases were clustered with their previously characterized orthologous proteins of other plant species. Through the utilization of MAFFT 7 (E-INS-i) software alignment (Katoh *et al.*, 2017), *Medicago truncatula* amine oxidases were placed into the context of existing knowledge for already characterized orthologous proteins. Amine oxidases of both model species *Oryza sativa* and *Arabidopsis thaliana,* which should be included in a multiple sequences alignment (Cvrčková, 2016), and the closest orthologous amine oxidases to those of *Medicago*

truncatula were retrieved from BLAST tool. In general, bootstrap values are higher than 50%, even being up to 90-100% in some cases. This means that the resulting phylogenetic trees, obtained by the Neighbor Joining method, are stable (Jill Harrison and Langdale, 2006).

After performing the intraspecific phylogenetic analysis, *Medicago truncatula* amine oxidases were clearly clustered into two different clades (Figure 4). In Clade I, 13 polyamine oxidases were grouped, while in Clade II there were clustered 17 diamine oxidases.

Despite plant DAOs have been less studied and characterized than plant PAOs, a little characterization of *Medicago truncatula* DAOs has been carried out. The resulting phylogenetic tree showed the existence of three different clades in which plant DAOs are grouped. As it can be seen in Figure 5, nine *Medicago truncatula* proteins (from MtDAO1 to MtDAO9) were clustered in Clade I along with MdDAO1, OsDAO1 and ZmDAO. MdDAO1 is located in the apoplast (Zarei *et al.,* 2015a), while ZmDAO has been reported to be in the peroxisomes. Both MdDAO1 and ZmDAO show a substrate preference for Put (Zarei *et al.,* 2015a), and they are involved in the terminal catabolism of PAs oxidizing Put. MtDAO10 was clustered in Clade II in addition to AtDAO1, AtDAO2, GmDAO1, GmDAO2, MdDAO2, NtDAO, OsDAO2, SlDAO and TpDAO. AtDAO1 and MdDAO2 are located within the apoplast (Møller and McPherson, 1998) and peroxisomes (Zarei *et al.,* 2015a), respectively. Both diamine oxidases are involved in PA terminal catabolism. AtDAO1, which has been related to PA- and ABA-mediated NO production, shows a substrate preference for Put and Spd (Møller and McPherson, 1998). In contrast, MdDAO2 shows a substrate preference for Put and Cad (Zarei *et al.,* 2015a). Finally, in Clade III there were seven *Medicago truncatula* diamine oxidases (from MtDAO11 to MtDAO17) along with AtDAO3, AtDAO4, AtDAO5, AtDAO6, CaDAO, LcDAO, PsDAO and TrDAO. In this clade, some diamine oxidases have been reported to be in the apoplast such as AtDAO6 which is related to PA- and ABA-mediated NO production (Wimalasekera *et al.*, 2011; Planas-Portell *et al.*, 2013), CaDAO which is related to defense response, (Rea *et al.*, 2002), LcDAO (Medda *et al.,* 1996*;* Tavladoraki *et al.,* 2012) and PsDAO (Tipping and McPherson, 1995*;* Moschou *et al.,* 2012); whereas AtDAO3 is located in the peroxisomes (Planas-Portell *et al.,* 2013). All of them are involved in the terminal catabolism of PAs. CaDAO shows a substrate preference for Put; AtDAO3 and AtDAO6 can oxidize Put and Spd; and LcDAO and PsDAO oxidize Put, Spd and Spm. Although DAOs can also oxidized Spd and Spm, they show a lower affinity than Put, being the activity of LcDAO with Put, Spd and Spm at a ratio of 100:42:20; whereas those of PsDAO are at ratio of 100:35:0.3 (Šebela *et al.,* 2001). In general, plant diamine oxidases are located within the apoplast and/or peroxisomes. As it can be seen in Figure 5, Clade II and III shared the same branch of the phylogenetic tree, whereas Clade I was clearly separated at the first node. This suggests Clade II and III may share more common characteristics than those shared with Clade I.

The resulting phylogenetic tree obtained from the interspecific phylogenetic analysis of *Medicago truncatula* polyamine oxidases also showed three different clades in which plant PAOs can be clustered (Tavladoraki *et al.,* 2016; Takahashi *et al.,* 2018). Clade I corresponds to those plant PAOs generally localized in the cytosol and involved in PA homeostasis, stress response and plant growth, whose preferred substrate is Spm, and participate in PA back-conversion enzymatic reactions (Ištvánek *et al.,* 2014; Kim *et al.,* 2014; Qi *et al.,* 2014; Liu *et al.,* 2014a,c; Zarza *et al.,* 2017; Ištvánek *et al.,* 2017). This may suggest that *Medicago truncatula* MtPAO1 and MtPAO2 grouped in the present cluster, could share the same characteristics as OsPAO1, AtPAO5, GsPAO1 and TpPAO1. Regarding to Clade II, AtPAO2, AtPAO3, AtPAO4, OsPAO3, OsPAO4 and OsPAO5 are localized within the peroxisomes and they are involved in the back-conversion of PAs (Moschou *et al.,* 2008b; Takahashi *et al.,* 2010; Ono *et al.,* 2012; Sagor *et al.,* 2016). Moreover, these *Arabidopsis thaliana* amine oxidases are related with stress response and senescence. GsPAO2, from soybean (*Glycine max*), is also present in this clade and it is involved in the back-conversion of PAs (Qi *et al.,* 2014). All polyamine oxidases present in Clade II show preference for Spd and Spm as substrates. There are seven *Medicago truncatula* PAOs (from MtPAO3 to MtPAO9) clustered in Clade II; two of them have been already studied by Young *et al.,* 2011, who found that MtPAO7 is localized in the peroxisomes in accordance to the rest of already characterized PAOs present in this clade. Clades I and II shared the same branch of the phylogenetic tree (Figure 6), which may suggest they were more similar between them in comparison to Clade III, since the latter belonged to a tree branch clearly separated at the first node. Plant PAOs clustered in Clade III are located in the apoplast as OsPAO6, OsPAO7, HvPAO1 (Liu *et al.,* 2014b) and ZmPAO (Cona *et al.,* 2006a), with some exceptions such as AtPAO1 and HvPAO2 which are located in the cytosol (Tavladoraki *et al.,* 2006; Takahashi *et al.,* 2010; Sagor *et al.,* 2016) and vacuole (Cervelli *et al.,* 2001; Cona *et al.,* 2006a), respectively. These amine oxidases have been reported to be involved in PA terminal catabolism, excepting AtPAO1 that participates in the back-conversion of PAs (Tavladoraki *et al.,* 2006). HvPAO2, OsPAO7 and ZmPAO are able to oxidase Spd as well as Spm; however, AtPAO1 shows a substrate preference for Spm. AtPAO1 participates in stress response, whereas ZmPAO participates in cell wall differentiation (Cona *et al.,* 2006a).

Taking all together, *Medicago truncatula* amine oxidase predicted proteins will be characterized based on their shared characteristics with the orthologous proteins clustered in the same clade. On the one hand, *Medicago truncatula* has its 17 DAOs grouped in three different clusters. In Clade I there were nine DAOs (from MtDAO1 to MtDAO9), which may be located in the apoplast and/or peroxisomes. They may show a substrate preference for Put, and may catalyze the terminal oxidation of this PA. In Clade II, MtDAO10 was the only *Medicago truncatula* diamine oxidase clustered that may be located in the apoplast and/or peroxisomes. It may show a substrate preference for Put, Spd and/or Cad, and may catalyze the terminal oxidation of these PAs. In Clade III, there were grouped seven *Medicago truncatula* amine oxidases (from MtDAO11 to MtDAO17), which may be located in the apoplast. They might show a substrate

preference for Put, although they could oxidize with lower affinity Spd and Spm. On the other hand, *Medicago truncatula* has its 13 PAOs also clustered in three different clades. MtPAO1 and MtPAO2, from Clade I, may be located in the cytosol. They may show a substrate preference for Spm, and might catalyse the back-conversion of this PA into Spd. In Clade II, there were six *Medicago truncatula* polyamine oxidases (from MtPAO3 to MtPAO9), which may be located in the peroxisomes. They may show a substrate preference for Spd and Spm, and be involved in the back-conversion of these PAs oxidizing Spm to Spd, and Spd to Put. Finally, in Clade III there were clustered four *Medicago truncatula* polyamine oxidases (from MtPAO10 to MtPAO13), which may be localized mainly in the apoplast. They may show a substrate preference for Spd and Spm, and may catalyse the terminal oxidation of these PAs. However, although *Medicago truncatula* amine oxidases will be expected to share the characteristics of the clade they are clustered in, in order to elucidate the exact location of *Medicago truncatula* DAOs and PAOs, respectively, and the expression profiles of amine oxidases genes under ammonium nutrition, a future gene expression analysis of amine oxidases genes involved in PA catabolism will be required.

7.2 *Medicago truncatula* diamine oxidases activity under ammonium nutrition

During the NH_4^+ stress response episodes, there could be two possible reasons for PA accumulation within plant cells, (i) it could be attributed to an increased PA biosynthesis and/or (ii) to a decreased PA catabolism. However, in general, the accumulation of PAs in plants grown under stress conditions has largely been attributed to an increase of *de novo* synthesis of free PAs since biosynthetic genes have been found to be up-regulated under different abiotic stress conditions. Sometimes, there exists a disparity between the expected genes expression profiles and the content of PAs, which has been suggested to result from possible changes in PA catabolism depending on plant species (Marco *et al.,* 2015). Anyway, an understanding of the PA biosynthetic genes expression patterns of *Medicago truncatula* under ammonium stress conditions is important in order to understand the regulation of PA levels; as well as the elucidation of the effect of ammonium stress on the catalytic activity of the enzymes involved in PA degradation since the exact mechanisms involved still remain unelucidated.

Plant amine oxidases are responsible for PA catabolism. In the present study both DAO and PAO relative activities were analysed applying the protocol described in Wisniewski *et al.,* 2000. DAOs are involved in the terminal catabolism of PAs, especially the diamines Put and Cad; whereas PAOs are involved in PA terminal catabolism as well as in the back-conversion of PAs, especially Spd and Spm (Šebela *et al.,* 2001). Previous results have evidenced an increase in the products of the oxidative pathway of PA synthesis, which is downstream of the urea cycle as PAs remarkedly accumulate in legumes like pea (Ariz *et al.,* 2013) or *Medicago truncatula* (Cerdan, 2017) as a result of NH_4^+ stress. The initial hypothesis of the present study was the fact that ammonium accumulation in plants might be blocking the interconversion and catabolism of PAs by its effect on both

DAO and PAO activities within the urea cycle of plants, as NH_4^+ is a final product from these reactions. Previous results of our laboratory showed (i) an over-accumulation of Put, (ii) a decrease of GABA content, (iii) a decrease of Orn content, and (iv) an over-accumulation of Arg in *Medicago truncatula* ammonium-fed seedlings with respect to nitrate-fed seedlings. Moreover, *M. truncatula* seedlings grown in axenic medium with ammonium as the sole source of nitrogen at high dose, showed a significantly higher accumulation of ammonium in both plant tissues shoots and roots. In general terms, the results obtained in this study, on one hand, showed a higher DAO activities in *Medicago truncatula* plant shoots than that observed in roots. On the other hand, plants supplied with low dose of ammonium exhibited significantly higher DAO activity in shoots in comparison with nitrate- and urea-fed plants, while DAO activity was significantly higher in roots of ammonium-fed plants at high dose in comparison to nitrate- and urea-fed plants.

Medicago truncatula ammonium-fed plants at low dose showed significant higher DAO activity in shoots, whereas root tissues did not show significant differences in comparison to nitrate- and urea- fed plants. In contrast, *Medicago truncatula* plants grown under high ammonium conditions did not show significant differences in shoots, while DAO activity in roots was significantly higher. The previous results, cited above, obtained by our laboratory showed a decrease of the content of GABA in *Medicago truncatula* plants grown under ammonium nutrition in comparison to nitrate- and urea- fed plants, except for shoot tissues of *Medicago truncatula* plants grown under ammonium nutrition at low dose. Under high ammonium nutrition, *Medicago truncatula* plant shoots, where DAO activity did not show significant differences, showed a significant increase of the contents in ammonium, Arg, Put and Spd; whereas the content of GABA decreased significantly in comparison to low ammonium-fed plants. However, in roots, where DAO activity showed to be significantly higher, there was a significant increase in the content of ammonium, Arg and Put, while the content of Orn decreased significantly, and the content of GABA did not show significant differences in comparison to ammonium-fed plants at low dose. Put oxidation by plant DAOs has been reported to contribute up to 22% of GABA content in roots of lupine seedlings grown under salt stress (Legocka *et al.,* 2017; Yang *et al.,* 2018), which suggested the importance of DAO activity as stress tolerance mechanism since GABA accumulation is related to enhanced stress tolerance (Ramesh *et al.,* 2015). Increased DAO activity corresponded to higher Put and/or Cad content in olive, barley and soybean (Asthir *et al.,* 2002; Gomez-Jimenez *et al.,* 2010; Quinet *et al.,* 2010). These data fit with the results obtained in root tissues under high ammonium nutrition, where DAO activity was showed to be significantly higher in comparison to nitrate- and urea-fed plants while Put was accumulated concomitantly.

An up-regulation of genes involved in the biosynthesis of PAs have been considered crucial for plants to be tolerant against abiotic stresses; which corresponds with the observation of Marco *et al.,* 2015 about the PA accumulation correlation with an enhanced tolerance to environmental stresses, being Put the first PA accumulated (Gupta

et al., 2016). *Arabidopsis thaliana* plants grown under different types of abiotic stresses showed different induction patterns of PA biosynthetic genes expression. Under drought conditions, ADC, SPDS and SPDM expressions were induced (Alcázar *et al.,* 2006a; Alcázar *et al.,* 2010), where Put levels increased while the content of Spd and Spm did not increase above the basal levels. ADC and SPMS gene expressions were induced under salt stress, which resulted in an increase of Put and Spm contents (Urano *et al.,* 2003; Alcázar *et al.,* 2010). Also, under cold ADC and SAMDC genes were induced, where the content of Put increased in contrast to Spd and Spm contents, which remained constant or even decreased. The lack of correlation between the induction of SAMDC and the decrease of Spm level has been suggested to be due to an increase of Spm catabolism (Cuevas *et al.,* 2008; Alcázar *et al.,* 2010). In *Arabidopsis thaliana* ODC transgenic plants, since this species does not possess its own ODC gene according to Hanfrey *et al.,* 2001, Majumdar *et al.,* 2013 demonstrated that an increase up to 12-fold of Put from Orn did not have any significant impact in Put production from Arg by ADC; even ADC was not inhibited under both short-term inducible and constitutive conditions by a 40-fold increase in cellular Put. This suggest that Put metabolism via ADC may not be inhibited by neither the induction of ODC gene nor the accumulation of Put. This is in accordance with the previous results obtained by our laboratory where 25 mM ammonium-fed plant shoots presented an increase in the content of Put and Spd; whereas *Medicago truncatula* roots showed an elevated increase in Put content while Spd level remained constant; and in both plant tissues Spm contents did not show any significance difference. Until now, there has not been performed yet any assay related with the expression of biosynthetic genes of PAs under ammonium stress conditions. Taking into account that *Medicago truncatula* showed different tolerance responses to ammonium stress from those showed under salt stress, it makes interesting to analyse the expression of ADC, ODC, SPDS, SPMS and SAMDC biosynthetic genes of PA synthesis pathway under ammonium nutrition.

PA export to the apoplast where the majority of amine oxidases involved in PA terminal catabolism are present, their oxidation by DAOs and PAOs, and H_2O_2 production activating ROS-induced Ca^{2+} influx across the plasma membrane is a common response of plants to abiotic stresses, hormonal regulation, growth and development, and even program cell death scenarios (Moschou *et al.,* 2012; Tavaldoraki *et al.,* 2012). Plants grown under ammonium nutrition as the sole source of nitrogen show, as *Medicago truncatula* showed in previous results of our laboratory, a diminution of essential cations such as Mg^{+}, K^{+} and Ca^{2+} in comparison to nitrate-fed plants (Britto *et al.,* 2001; Britto *et al.,* 2002). From this point of view, PA overproduction under ammonium stress has been reported by Fernández-Crespo *et al.,* 2012 to contribute to the maintenance of cellular ionic balance, which correlates with the results obtained previously where *Medicago truncatula* plants fed by high dose of ammonium accumulated higher PA contents. Although sensing and regulatory mechanisms of ammonium in plant cells are not fully elucidated yet, there has been proposed by Bai *et al.,* 2014 the existence of a tonoplast-localized receptor-like kinase encoded by cytosol-associated protein kinase (CAPK) genes, which regulates root hair tip growth by maintaining cytoplasmic Ca^{2+}

gradients through the influx of ammonium into the vacuole via CAPK and/or CAPK-induced aquaporins, since an excess of ammonium in the cytosol seems to inhibit the influx of Ca^{2+}. As CAPK1 in *Arabidopsis thaliana* is almost expressed in all plant tissues its function as ammonium tolerance mechanism could be extended not only to root hair cells, but also to the rest of plant tissues (Liu and Von Wirén, 2017). Moreover, apoplastic H_2O_2 generated by PA catabolism has been reported by Pottosin *et al.,* 2014 to induce Ca^{2+} influx, which suggests that the increase activity of DAOs in *Medicago truncatula* roots under high ammonium nutrition could be a tolerance mechanism in order to reestablish the Ca^{2+} intracellular content despite the total content of Ca^{2+} of ammonium-fed plants is largely reduced in comparison to nitrate-fed plants.

On the other hand, stress-induced PA accumulation in response to abiotic stress such as salinity, drought, chilling, and hypoxia is widely reported (Alcázar *et al.,* 2010; Gill and Tuteja, 2010); all these stresses have a common denominator, which is the increase of cytosolic free Ca^{2+}. In this sense, PA-induced activation of plasma membrane Ca^{2+}-ATPase may be an important component of adaptive mechanism to restore the basal cytosolic Ca^{2+} level (Bose *et al.,* 2011). Besides, cytosolic pH could affect the activity of membrane transporters; especially, cytosolic alkalization leads to the inhibition of K^+ and Ca^{2+} influx, and the activation of both cations efflux observed by Alcázar *et al.,* 2010. Under high ammonium nutrition, *Medicago truncatula* plants showed a significant increase in Put content in shoots and roots, while Spd increased significantly in shoots, and Spm remained unchangeable. High level of Put has been shown to be able to induce the plasma membrane H^+-ATPase activity which results in alkalization of the cytosol (Alcázar *et al.,* 2010). This suggest that the cytosol of *Medicago truncatula* cells may be alkalized under ammonium nutrition at high concentration, which along with a low cytosolic Ca^{2+} content could inhibit the activity of the enzyme involved in the synthesis of GABA from glutamate, the GAD; this could be a reason for the decrease of GABA content in *Medicago truncatula* under high ammonium nutrition showed in previous results of our laboratory.

GAD is localized in the cytosol, its optimum pH is slightly acidic 5.8, and it is related with intracellular pH regulation since GAD activity consumes H^+. However, the presence of GAD carbon-terminal calmodulin (CaM)-binding domain enables in vitro GAD activity at neutral pH when the intracellular Ca^{2+} content is elevated, as under abiotic stress conditions (Ueno *et al.,* 2000; Shelp *et al.,* 2017). Even, GAD activity at pH 5.8 is 3 to 9 times higher than activity at pH 7.3 in the presence of saturating Ca^{2+} and CaM (Snedden *et al.,* 1995; Snedden *et al.,* 1996). This obervation makes a possible difference between the distinct tolerance response of *Medicago truncatula* under both salt and ammonium stress conditions. During salt stress, the transport of Na^{2+} through the tonoplast and/or the efflux through the cell plasma membrane requires a Na^{2+}/H^+ antiporter (Jan *et al.,* 2013). The accumulation of cytosolic Ca^{2+} and the acidification of the cytosol because of the vacuolar Na^{2+} compartmentalization, may activate GAD activity with the concomitant synthesis of GABA (Shelp *et al.,* 1999). Therefore, it could be suggested that the intracellular alkalization induced by ammonium uptake in the plant

cell, as well as in the vacuole can be driven by aquaporins (Esteban *et al.,* 2016a), and the lack of accumulation of Ca^{2+}, accentuated in root tissues in *Medicago truncatula* cells would block GAD activity and thereby the synthesis of GABA. Hence, it is possible to have a decrease of the GABA content because of the inhibition of GAD activity instead of the inhibition of DAO and PAO activities. Furthermore, if GAD was actually inhibited under high ammonium nutrition, the portion of remaining GABA found in *Medicago truncatula* plants under these abiotic stress conditions could be suggested to result from terminal catabolism of PAs, which may confer tolerance against ammonium stress through the activity of the plant amine oxidases DAOs and PAOs, since DAO activity showed to be significantly higher in the present study. Taking all this together, it would be interesting to discern whether GAD activity is totally-, partially- or non-inhibited under ammonium stress conditions in *Medicago truncatula*.

Orn has been postulated to be a key metabolite in PA biosynthesis (Majumdar *et al.,* 2013); moreover, Orn is not only precursor of the diamine Put, but also of the osmolite Pro, which suggests that the levels of both metabolites could be related. At this point, there has been observed discrepancies between different authors about whether the content of Put and Pro increase in a parallel way under abiotic stress, or the contents of both metabolites show a negative correlation. In previous results of our laboratory, there has been observed an accumulation of Pro in *Medicago truncatula* in high ammonium-fed plants, especially in shoots, while the increase of Put level was lower than that accumulated in roots. This could be related to the significant higher activity of *Medicago truncatula* DAOs obtained in the present study in plant shoots compared to roots. An increase of DAO activity means a concomitant increase of H_2O_2 production, and the possible increase of H_2O_2-induced Pro. 15 to 20% of Pro accumulation in *Glycine max* leaves under salt stress derived from PA catabolism while the application of a DAO inhibitor resulted in a decrease of Pro in the same percentage (Su and Bai, 2008; Legocka *et al.,* 2017). Conversely, as Put and Pro have Orn as a common precursor, it could be suggested that Pro may be synthesized from Orn via OAT instead from glutamate via P5CS. In response to osmotic stress, *Medicago truncatula* Pro synthesis from Glu is strongly activated since MtP5C5 transcript is seven-fold accumulated; while OAT transcript, four-fold. Despite Pro accumulation in shoots was correlated with both MtP5C5 and MtOAT transcripts, Pro accumulation in roots was only related with MtOAT alone (Armengaud *et al.,* 2004). At a first sight, but still inconclusive, the notably higher Pro accumulation in *Medicago truncatula* shoots under high ammonium nutrition could correspond with the activation of both MtP5C5 and MtOAT, being the MtOAT the only responsible of the little increase of Pro accumulation in roots. However, since *Medicago truncatula* tolerance response to salt stress differs from that of ammonium stress, it could be interesting to perform a transcriptional analysis of MtP5C5 and MtOAT under ammonium stress to elucidate whether they are up-regulated under these conditions.

Finally, previous results of our laboratory showed an over-accumulation of Arg in *Medicago truncatula* seendlings grown under high ammonium dose in comparison to nitrate- and urea-fed seedlings. Arginine can be synthesized from Orn within chloroplasts

(Slocum, 2005), which suggests the possible relation between the increase of Arg content in ammonium-fed *Medicago truncatula* plants and the parallel decrease of Orn content of these plants. Ammonium is rapidly assimilated via GS-GOGAT cycle when GS catalyzes the ATP-synthesis of Gln from ammonium and glutamate, once ammonium has entered plant cells. It has been proposed a central role for Gln, reflecting the plant cell nitrogen status (Xu *et al.,* 2012). In a recent study, it has been identified a protein-mediated Gln sensing mechanism in higher plants, PII, which co-ordinates the assimilation of ammonium with cellular energy in plant chloroplasts as well as its nitrogen status (Chellamuthu *et al.,* 2014). NAGK is a main enzyme involved in the synthesis of Arg, which is inhibited by a feedback mechanism when the content of Arg increase. However, NAGK regulation can be mediated by PII signal transduction protein which has been firstly described in cyanobacteria (Heinrich *et al.,* 2004), and in *Arabidopsis thaliana* (Burillo *et al.,* 2004).

NAGK, is feedback-inhibited by its downstream metabolic product Arg and hence NAGK is a crucial regulator of nitrogen metabolism via Arg. When Gln binds to the carbon-terminal domain in PII makes this to change conformationally allowing the interaction between PII and NAGK with the concomitant activation of NAGK antagonizing the inhibitory effect of Arg; and therefore, promoting Arg synthesis (Heinrich *et al.,* 2004; Slocum, 2005; Bourrellier *et al.,* 2009). This could be an explanation for the accumulation of Arg under ammonium stress conditions in *Medicago truncatula*, whose Gln content also increased under these conditions. The carbon-terminal domain is widely present in higher plants excepting for Brassicaceae. Moreover, PII proteins from *Oryza sativa*, an ammonium tolerant species, shows a high requirement of Gln to interact with NAGK, while the ammonium sensitive model *Arabidopsis thaliana* PII showed to behave independent from Gln; which suggests a possible role of PII proteins in regulating the amino acid flow through Arg/Orn pathway and, in addition, PA metabolism (Chellamuthu *et al.,* 2014). Taking all together, the possible over-production of Arg from Orn because of Gln-PII-NAGK interaction could be inducing PA biosynthesis via ADC in order to avoid the biosynthesis of Put from Orn, since the arginase and posterior urease activity results in a concomitant production of ammonium, which indeed could increase plant ammonium toxicity.

Medicago truncatula plants show to be ammonium tolerant since they do not show any acute symptoms when grown under high ammonium nutrition. To elucidate the tolerance mechanisms that *Medicago truncatula* possesses against ammonium stress, further research should be performed. PA metabolism has been widely related to plant abiotic stress tolerance. Previous results of our lab showed a large accumulation of Put in ammonium-fed plants, as well as Arg and Pro content increased; however, the content of GABA, which is a plant stress tolerance-related metabolite (Ramesh *et al.,* 2015), and Orn decreased. The inhibition of DAOs and PAOs, which are the enzymes in charge of PA catabolism, by ammonium was the initial hypothesis. However, our results show that DAO activity showed a significant higher activity in ammonium-fed plants shoots at low dose, and in roots at high dose. So, it may suggest the importance of PA catabolism as

stress tolerance mechanism against ammonium stress since it seems to be a source of GABA under these conditions as GAD activity could be inhibited by the insufficient content of cytosolic Ca^{2+} to be active in an alkalinized cytosol by Put-induced H^{+}-ATPase. Orn content decrease could result from the synthesis H_2O_2-induced Pro, MtOAT induction under ammonium stress conditions, and/or because of the Arg overproduction from Orn due to the possible interaction between Gln-PII-NAGK, which could induced PA biosynthesis via ADC to avoid ammonium production by arginase and urease activities. Once DAO activity has been shown to be increased under ammonium stress conditions in *Medicago truncatula*, tissues the following assays should be performed to elucidate the mechanisms underlying plant ammonium tolerance responses: (i) PAO activity assay, (ii) gene expression of DAOs and PAOs involved in PA catabolism, (iii) PA biosynthetic genes expression of *Medicago truncatula* ADC, ODC, SPDS, SPMS and SAMDC, (iv) GAD activity assay, (v) transcriptional analysis of MtP5C5 and MtOAT, and (vi) Gln-PII-NAGK analysis.

8 Conclusion

In summary, after performing the intraspecific phylogenetic analysis, *Medicago truncatula* 17 diamine oxidases and 13 polyamine were clearly differentiated. The interspecific phylogenetic analyses of both DAOs and PAOs, respectively, enabled us to make a characterization of *Medicago truncatula* amine oxidases by means of comparing both kind of proteins to those belonging to the same cluster and have been already characterized. While *Medicago truncatula* diamine oxidases are suggested to be involved in the terminal catabolism of polyamines, mainly putrescine; *Medicago truncatula* polyamine oxidases, which mainly oxidize spermidine and spermine, have been suggested to be involved in either the terminal catabolism of polyamines or the polyamine back-conversion. Moreover, *Medicago truncatula* amine oxidases have been proposed to be located in the apoplast and/or peroxisomes. Regarding to *Medicago truncatula* diamine oxidase activity assay, in general terms, the results obtained in this study showed higher diamine oxidase activities in *Medicago truncatula* plant shoots than that observed in roots. In addition, plants supplied with low dose of ammonium exhibited significantly higher diamine oxidase activity in shoots in comparison with nitrate- and urea-fed plants, whereas diamine oxidase activity was significantly higher in roots of ammonium-fed plants at high dose. Since diamine oxidase activity has been shown to be increased under ammonium conditions in *Medicago truncatula* tissues, this may suggest the importance of polyamine catabolism in the tolerance against high ammonium conditions. Although alternative mechanisms underlying ammonium stress/tolerance response are proposed in this report, future research should be performed to elucidate the mechanisms underlying plant ammonium tolerance responses.

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Supplementary data: amine oxidases identification

Table 1: **Medicago truncatula** ***identified diamine oxidases.*** *The locus, length of amino acid sequences, and FASTA sequences were obtained when* M. truncatula *proteins were researched against Uniprot database.*

Diamine oxidase	Uniprot Entry	Locus	Length	FASTA
MtDAO1	A0A072TRW3 http://www.uniprot.org/uniprot/A0A072TRW3	MTR_8g069505	774	>A0A072TRW3 MATVEEKTTPICCSLQNKNKTAASATSPNVPQQKQQLPFISAVDSRLDPPPKSASSKELKDRKETISFDIGITVMAKAQTCHPLDPLSAAEISVAVATVRAAGATPEVRDGIRFIEVGLVEPEKQVVALADAYFPPFQPSLLPRIKGGPVIPSKLPPRKARLVVYNKKSNETSIWIVELTEVHATTRGGHHRGKVITSTVVPDVQPPMDAVEYAECEAVVKDFPPFREAMKKRGIEDMDLVMVDPWCAGYHSEGDAPSRRLAKPLFFCRTESDCPMENGYARPVEGIHVLVDMQNMVVLEFEDRKLVPLPQADPLRNYTSGETRGGVDRSDVKPLQIIQPDGPSFRVNGNFIQWQKWNFRIGFTPREGLVIYSVAYIDGSRGRRPVAHRLSFVEMVVPYGDPNDPHYRKNAFDAGEDGLGKNAHSLKKGCDCLGYIKYFDAHFTNFYGGVETIENCVCMHEEDHGMLWKHQDWRTGLAEVRRSRRLTVSFICTVANYEYGFYWHFYQDGKIEAEVKLTGILSLGALQQGETRKYGTTIAPGLYAPVHQHFFVARMDMAVDCKPGEAFNQVVEVNVKVEEPGKNNVHNNAFYAEEKLLKSELEAMRDCDPLSARHWIVRNTRSVNRTGHLTGYKLVPGSNCLPLAGSEAKFLRRAAFLKHNLWVTPYARDELHPGGEFPNQNPRVGEGLATWVKQNRPLEEADIVLWYVFGVTHIPRLEDWPVMPVEHIGFMLMPHGFFNCSPAVDVPPSPGDLDDKENGMPAKHSQNGLIAKL
MtDAO2	A0A072TRL3 http://www.uniprot.org/uniprot/A0A072TRL3	MTR_8g069505	761	>A0A072TRL3 MATVEEKTTPICCSLQNKNKTAASATSPNVPQQKQQLPFISAVDSRLDPPPKSASSKGITVMAKAQTCHPLDPLSAAEISVAVATVRAAGATPEVRDGIRFIEVGLVEPEKQVVALADAYFPPFQPSLLPRIKGGPVIPSKLPPRKARLVVYNKKSNETSIWIVELTEVHATTRGGHHRGKVITSTVVPDVQPPMDAVEYAECEAVVKDFPPFREAMKKRGIEDMDLVMVDPWCAGYHSEGDAPSRRLAKPLFFCRTESDCPMENGYARPVEGIHVLVDMQNMVVLEFEDRKLVPLPQADPLRNYTSGETRGGVDRSDVKPLQIIQPDGPSFRVNGNFIQWQKWNFRIGFTPREGLVIYSVAYIDGSRGRRPVAHRLSFVEMVVPYGDPNDPHYRKNAFDAGEDGLGKNAHSLKKGCDCLGYIKYFDAHFTNFYGGVETIENCVCMHEEDHGMLWKHQDWRTGLAEVRRSRRLTVSFICTVANYEYGFYWHFYQDGKIEAEVKLTGILSLGALQQGETRKYGTTIAPGLYAPVHQHFFVARMDMAVDCKPGEAFNQVVEVNVKVEEPGKNNVHNNAFYAEEKLLKSELEAMRDCDPLSARHWIVRNTRSVNRTGHLTGYKLVPGSNCLPLAGSEAKFLRRAAFLKHNLWVTPYARDELHPGGEFPNQNPRVGEGLATWVKQNRPLEEADIVLWYVFGVTHIPRLEDWPVMPVEHIGFMLMPHGFFNCSPAVDVPPSPGDLDDKENGMPAKHSQNGLIAKL
MtDAO3	A0A072TRL9 http://www.uniprot.org/uniprot/A0A072TRL9	MTR_8g069505	619	>A0A072TRL9 MATVEEKTTPICCSLQNKNKTAASATSPNVPQQKQQLPFISAVDSRLDPPPKSASSKELKDRKETISFDIGITVMAKAQTCHPLDPLSAAEISVAVATVRAAGATPEVRDGIRFIEVGLVEPEKQVVALADAYFPPFQPSLLPRIKGGPVIPSKLPPRKARLVVYNKKSNETSIWIVELTEVHATTRGGHHRGKVITSTVVPDVQPPMDAVEYAECEAVVKDFPPFREAMKKRGIEDMDLVMVDPWCAGYHSEGDAPSRRLAKPLFFCRTESDCPMENGYARPVEGIHVLVDMQNMVVLEFEDRKLVPLPQADPLRNYTSGETRGGVDRSDVKPLQIIQPDGPSFRVNGNFIQWQKWNFRIGFTPREGLVIYSVAYIDGSRGRRPVAHRLSFVEMVVPYGDPNDPHYRKNAFDAGEDGLGKNAHSLKKGCDCLGYIKYFDAHFTNFYGGVETIENCVCMHEEDHGMLWKHQDWRTGLAEVRRSRRLTVSFICTVANYEYGFYWHFYQDGKIEAEVKLTGILSLGALQQGETRKYGTTIAPGLYAPVHQHFFVARMDMAVDCKPGEAFNQVVEVNVKVEEPGKNNVHNNAFYAEEKLLKSELEAMRDCDPLSARHWIKL
MtDAO4	A0A072TRW9 http://www.uniprot.org/uniprot/A0A072TRW9	MTR_8g069505	629	>A0A072TRW9 MATVEEKTTPICCSLQNKNKTAASATSPNVPQQKQQLPFISAVDSRLDPPPKSASSKGITVMAKAQTCHPLDPLSAAEISVAVATVRAAGATPEVRDGIRFIEVGLVEPEKQVVALADAYFPPFQPSLLPRIKGGPVIPSKLPPRKARLVVYNKKSNETSIWIVELTEVHATTRGGHHRGKVITSTVVPDVQPPMDAVEYAECEAVVKDFPPFREAMKKRGIEDMDLVMVDPWCAGYHSEGDAPSRRLAKPLFFCRTESDCPMENGYARPVEGIHVLVDMQNMVVLEFEDRKLVPLPQADPLRNYTSGETRGGVDRSDVKPLQIIQPDGPSFRVNGNFIQWQKWNFRIGFTPREGLVIYSVAYIDGSRGRRPVAHRLSFVEMVVPYGDPNDPHYRKNAFDAGEDGLGKNAHSLKKGCDCLGYIKYFDAHFTNFYGGVETIENCVCMHEEDHGMLWKHQDWRTGLAEVRRSRRLTVSFICTVANYEYGFYWHFYQDGKIEAEVKLTGILSLGALQQGETRKYGTTIAPGLYAPVHQHFFVARMDMAVDCKPGEAFNQVVEVNVKVEEPGKNNVHNNAFYAEEKLLKSELEAMRDCDPLSARHWIVSYYSFIFIPKKGKKNHQRLIIFPI
MtDAO5	A0A072TSH3 http://www.uniprot.org/uniprot/A0A072TSH3	MTR_8g069505	642	>A0A072TSH3 MATVEEKTTPICCSLQNKNKTAASATSPNVPQQKQQLPFISAVDSRLDPPPKSASSKELKDRKETISFDIGITVMAKAQTCHPLDPLSAAEISVAVATVRAAGATPEVRDGIRFIEVGLVEPEKQVVALADAYFPPFQPSLLPRIKGGPVIPSKLPPRKARLVVYNKKSNETSIWIVELTEVHATTRGGHHRGKVITSTVVPDVQPPMDAVEYAECEAVVKDFPPFREAMKKRGIEDMDLVMVDPWCAGYHSEGDAPSRRLAKPLFFCRTESDCPMENGYARPVEGIHVLVDMQNMVVLEFEDRKLVPLPQADPLRNYTSGETRGGVDRSDVKPLQIIQPDGPSFRVNGNFIQWQKWNFRIGFTPREGLVIYSVAYIDGSRGRRPVAHRLSFVEMVVPYGDPNDPHYRKNAFDAGEDGLGKNAHSLKKGCDCLGYIKYFDAHFTNFYGGVETIENCVCMHEEDHGMLWKHQDWRTGLAEVRRSRRLTVSFICTVANYEYGFYWHFYQDGKIEAEVKLTGILSLGALQQGETRKYGTTIAPGLYAPVHQHFFVARMDMAVDCKPGEAFNQVVEVNVKVEEPGKNNVHNNAFYAEEKLLKSELEAMRDCDPLSARHWIVSYYSFIFIPKKGKKNHQRLIIFPI
MtDAO6	A0A072TTH8 http://www.uniprot.org/uniprot/A0A072TTH8	MTR_8g069505	724	>A0A072TTH8 MKFFLLSELKDRKETISFDIGITVMAKAQTCHPLDPLSAAEISVAVATVRAAGATPEVRDGIRFIEVGLVEPEKQVVALADAYFFPPFQPSLLPRIKGGPVIPSKLPPRKARLVVYNKKSNETSIWIVELTEVHATTRGGHHRGKVITSTVVPDVQPPMDAVEYAECEAVVKDFPPFREAMKKRGIEDMDLVMVDPWCAGYHSEGDAPSRRLAKPLFFCRTESDCPMENGYARPVEGIHVLVDMQNMVVLEFEDRKLVPLPQADPLRNYTSGETRGGVDRSDVKPLQIIQPDGPSFRVNGNFIQWQKWNFRIGFTPREGLVIYSVAYIDGSRGRRPVAHRLSFVEMVVPYGDPNDPHYRKNAFDAGEDGLGKNAHSLKKGCDCLGYIKYFDAHFTNFYGGVETIENCVCMHEEDHGMLWKHQDWRTGLAEVRRSRRLTVSFICTVANYEYGFYWHFYQDGKIEAEVKLTGILSLGALQQGETRKYGTTIAPGLYAPVHQHFFVARMDMAVDCKPGEAFNQVVEVNVKVEEPGKNNVHNNAFYAEEKLLKSELEAMRDCDPLSARHWIVSYYSFIFIPKKGKKNHQRLIIFPI
MtDAO7	G7JYY1 http://www.uniprot.org/uniprot/G7JYY1	MTR_5g033170	737	>G7JYY1 MATAQEKTTPDPSQDPTRNTDSFPPKTTPTTKGIPVMMRAQTCHPLDPLSAAEISVAVATVRAAGATPEVRDSMRFVEVVLVEPVKQVVALADAYFFPPFQPSLLPRTKGGGPLIPTKLPTRKARLVVYNKKSNETSVWIVELREVHAATRGGPHRGKVISSQVVPNVQPPMDAMEYAECEAVVKDFPPFREAMKRRGIEDMDLVMVDAWCVGYHSEADAPNRRLAKPLIFCRSESDCPMENGYARPVEGIVVLVDMQNMVVLEFEDRKLIPLPTDPLRNYTSGETRGGVDRSDVKPLQIIQPEGPSFRVNGHFIQWQKWNFRIGFTPREGLVIYSVAYIDGSRGRRPVAHRLSFVEMVVPYGDPNDPHYRKNAFDAGEDGLGKNAHSLKKGCDCLGYIKYFDAHFTNFTGGVETIENCVCLHEEDHGILWKHQDWRTGLAEVRRSRRLTVSFICTVANYEYGFFWHFYQDGKIEAEVKLTGILSLGALQPGETRKYGTTIAPGLYAPVHQHFFVARMDMAVDCKPGEAFNQVVEVDVKVEDPGKNNVHNNAFYAEERLLKSELEAMRDCNPLSARHWIVRNTRTVNRTGQLTGYRLVPGSNCLPLAGSEAKFLRRAAFLKHNLWVTPYVHNEMHPGGEFPNQNPRVGDGLATWVKQNRSLEEADIVLWYVFGVTHIPRLEDWPVMPVERIGFMLMPHGFFNCSPAVDVPPSAGDLDDKENGMPAKHPQNEIIAKL
MtDAO8	A0A072UDP7 http://www.uniprot.org/uniprot/A0A072UDP7	MTR_5g033170	714	>A0A072UDP7 MATAQEKTTPDPSQDPTRNTDSFPPKTTPTTKGIPVMMRAQTCHPLDPLSAAEISVAVATVRAAGATPEVRDSMRFVEVVLVEPVKQVVALADAYFFPPFQPSLLPRTKGGGPLIPTKLPTRKARLVVYNKKSNETSVWIVELREVHAATRGGPHRGKVISSQVVPNVQPPMDAMEYAECEAVVKDFPPFREAMKRRGIEDMDLVMVDAWCVGYHSEADAPNRRLAKPLIFCRSESDCPMENGYARPVEGIVVLVDMQNMVVLEFEDRKLIPLPTDPLRNYTSGETRGGVDRSDVKPLQIIQPEGPSFRVNGHFIQWQKWNFRIGFTPREGLVIYSVAYIDGSRGRRPVAHRLSFVEMVVPYGDPNDPHYRKNAFDAGEDGLGKNAHSLKKGCDCLGYIKYFDAHFTNFTGGVETIENCVCLHEEDHGILWKHQDWRTGLAEVRRSRRLTVSFICTVANYEYGFFWHFYQDGKIEAEVKLTGILSLGALQPGETRKYGTTIAPGLYAPVHQHFFVARMDMAVDCKPGEAFNQVVEVDVKVEDPGKNNVHNNAFYAEERLLKSELEAMRDCNPLSARHWIVRNTRTVNRTGQLTGYRLVPGSNCLPLAGSEAKFLRRAAFLKHNLWVTPYVHNEMHPGGEFPNQNPRVGDGLATWVKQNRSLEEADIVLWYVFGVTHIPRLEDWPVMPVERIGFMLMVFNPTLTYFHSFCANIK

Table 1: Continuation.

Diamine oxidase	Uniprot Entry	Locus	Length	FASTA
MtDAO9	G7J4S8 http://www.uniprot.org/uniprot/G7J4S8	MTR_3g077080	769	>G7J4S8 MASASQKTTSSPSSSCCTPLDSSRLAAVSAAPAVAQSDHVALIRPLDSLPEPSSTNAPT SKGITSMTRPQSSHPLDPLSAAEISVAVATVRAAGATPELRDSMRFIEVVLLEPDKHVV ALADAYFFPPFQPSLLPRSKGGPVIPTKLPPRCARLVVYNKKSNETTLWIVELSQVHAV TRGGHHRGKVISSNVVPDVQPPMDAEEYAECEAVVKSFPPFIEAMKKRGIEDMDLVMV DPWCVGYHSEADAPGRRLAKPLLFCRSESDCPMENGYARPVEGIYVLVDMQNMVVIE FEDRKLVPLPPVDPLRNYTPGESRGGSDRSDVKPLQIIQPEGPSFRVNGYYVEWQKWN FRIGFTPKEGLIIYSVAYVDGSRGRRPVAHRLSFVEMVVPYGDPTEPHYRKNAFDAGED GLGKNAHSLKKGCDCLGYIKYFDAHFTNFTGGVETIENCVCLHEEDHGILWKHQDWRT GLAEVRRSRRLSVSFVCTVANYEYAFFWHFYQDGKIEAEVKLTGILSLGALMPGEVRK YGTMIAPGLYAPVHQHFFVARMDMSVDSRPGEALNQVVEVNMKVEEPGEKNHNNAF YAEETLRSELEAMRDCNPLTARJHWIVRNTRTGNRTGQLTGYKLVPGSNCLPLAGSEA KFLRRAAFLKHNVWVTAYSRDEMFPGGEFPNQNPRVGEGLATWIKQNRSLEETNIVLW YVFGITHVPRLEDWPVMPVEHIGFMLMPHGFFNCSPAVDVPPNSCEVESKDSDIKDNG ASKPIQGGIASKL
MtDAO10	G7J7B0 http://www.uniprot.org/uniprot/G7J7B0	MTR_3g080500	731	>G7J7B0 MDARNLLKFLVFSFGIALVILATWFHLPSSFNKEALDCNIFSGWCTSKNRFQSSNPIHKP SFSTRRQQPNHESDEPRHPLDPLTIQEFNKVRAILSTHPLFKSSNSVTLNSIVLEEPDKEL VLKWKNGQPLLPRKASVVALDKRVTHTLTVDLSTEITNHETRPGSSGYPTMTLEEMV AVLDVPLKSGEFNSTIRKRGVNLADLACLPVASGWYGTPVEENRRLIKVQCYSSKGTV NFYMKPIEGLTVLVDMDKREVVSITDNGLNIPVANGIDTDYRYSVQKLNGELNLINPISL EQPKGPSFTVDGHLVKWANWEFHLKPDPRAGTIISQAKVRDPDTLEMRNVIYKGFTSEL FVPYMDPTDGWYFKTYMDAGEYGFGLQAMPLDPLNDCPRNAYYMDGVFTSADGTPY VQPNMICIFESYAGDIAWRHAECPITDIKVTIEVRPKVTLVVRMAAAVANYDYIMDWEF QTDGLIRSKVGLSGILMVKGTTYDHMNQVPDQEYLYGTLLSENIGVIHDHYVTYYLDM DIDGSDNSFVKVNIKKQETSPGESPRKSYLKAVRKVAKTEKDAQIKLQLYNPSEFHMVN PSKKTRVGNPVGYKLVPGATAASLLDHDDPPQKRAAFTNNQIWVTPYNKSEEWAGGL LVYQSQGDDTLQVWSDRDRPIENKDIVLWYTVGFHHVPCQEDYPIMPTVSSSFDLKPV NFFERNPILRMPPNFQDDLPVCKAQDSA
MtDAO11	G7ID64 http://www.uniprot.org/uniprot/G7ID64	MTR_1g104550	658	>G7ID64 MIISSRFGKATTMIAQCLVLAFFLKFSFINSYSHPLDPLSPTEINKTRQIVQQSYLGAIPNI TYHFVDVEEPNKNNVLKWLSSSTTKQKPSIPRQAKVVVRAKGETHELVVDLTKGLIVSD KIYKGHGYPPFTFIELFKASKLPLTYPKFKESIAKRGLNLSEISCVPFTIGWYGEKITRRAL KVSCFYRDESVNIWARPVEGFTLLVDVDLIKIIMYNDRYRVPMPKAEGTNFQSSSKESKI FATCNISNGFTIKGNEVKWGNWIFHVGFNARAGMIISTASIFDDKKQKYRRVMVRGHV SETFVPYMDPTLEWYFRTFMDVGEFGFGRSADSLQPKVDCPGNAVYMDGFMVGPNG EVQQVPRAICMFERNSGNVAWRHMEINNPTKLIRDGEADITLVVRMIATVGNYDYILD WEFLKSGSIKVGVALTGVLEMKAVPYTHKNQIKERVFGTLVAENTIANYHDHLVTYYL DLDIDDNANSFINAKLQKVKASGFGTPRKSYWTVNKEAVKREAEARITLGLEPNELLIV NPNKMTKLGNQVGYRLISGQPVSSLLDDDDYPQRRASYTKYQVWVTPYNKSERWAGG FYADRSRGDDGLAVWSQRNREIENTDIVLWHTVGIHHVPYQEDFPVMPTVQGGFELRP ANFFESNPLL
MtDAO12	G7ID65 http://www.uniprot.org/uniprot/G7ID65	MTR_1g104590	675	>G7ID65 MKMKLKLIFSFLLMLLCSSIECSHIPHPLDPLTPSEINLVRNIILKSVQTKHYNLTFHYVG LQEPDKPLIQSWLSSNTKTKTLLPPPRQAFVIVRFQKQSLEIIVDFSTRSIISTKLYKGQGY PILTFGEQTASQLPFTYEPFKHSLNKRNINISNVLCAAFTVGWFGEEKSKRTVKVKCYY KNGSANLYARPLEGVAAVVDLDEMKIVGYSDRHVIPVPKAEGTEYRASKMKPPFGPM LKGIAVSQHDGPGFTIQGHSVSWANWVFHIGFDIQVGPIISLASIYDLENQKYREVLYKG LISEVFVPYQDPSEEWYYTTYFDCGEYGFGGQTMSSLQPFTDCPANAVFLDAYYSASDG TPVKISNAFCIFEKYAGDIMWRHTEIAIPNEVITEVRSDVSLVVRSVSTVGNYDYVIDWE FKPSGSIKLGVGLTGILGIKAGTYTNTDQIKEDIHGTLLADNTIGIYHDHFFTYYLDLDIDG EANSFVKTNLETVRVKDQTIPRKSYWTVVKETARTEADARVNIGIKPSELLVVNPSKKT KQGNQIGYRLLPGPIVHPLLLTDDYPQIRGAFTNYNVWVTPYNKTEKWAGGLYVDHSR GDDTLAVWSLRDRKIENKDIVLWHTMGFHHVPSQEDFPVMPTLSGGFELRPTNFFESN PVLKTKSPKAVHWPNCTFQH
MtDAO13	A0A072URB1 http://www.uniprot.org/uniprot/A0A072URB1	MTR_4g117660	675	>A0A072URB1 MAFSTTMKLVLFSALTLLSFQSVFSVTPLHFQHPLDPLTKEEFLTVQTLVHNKYPTSKN TVSFHYIGLDDPEKDAILKWETLKPSVITIPRKAFIIAIINGQNHEILIDLRLKRIVYDNVYK GNGFPTLTVDEQAIAIELPKKYPPFIASVQKRGLNLSEVVCSSFTMGWFGEKENRRTVR VDCFMKESTVNIYVRPITGITMVADLSLMKIVEYHDRDVEAVPTAENTDYRVSKQSPPF GPKQHSLASYQPQGPGFQINGHSVSWANWKFHIGFDVRAGIVISLASIYDLEKHKSRRV LYKGYISELFVPYQDPTEEFYFKTFFDSGEFGFGLSTVSLIPNRDCPPHAQFIDTYIHSAD GTPSLLKNAICVFEQYGNIMWRHTETGIPNEFVEESRTEVNLIVRTVVTVGNYDNVLDW EFKASGSIKPAIALSGILEIKGTNIKHKDEIKEDLHGKLVSANSIGIYHDHFYMYYLDFDID GVQNSFEKTSLKTVKITDGSSKRKSYWTTETQIAKTESDAKITLGLAPGELAIVNPNKKTI VGNDVGYRLIPAIPAHPLLTEDDYPQIRGAFTNYNVWVTPYNRTEKWAGGLYVDHSRG EDTLAVWTKKNRDIVNKDIVMWHVVGIHHVPAQEDFPIMPLLSTSFELRPTNFFERNPV LKTLSPRDVAWPGCSK
MtDAO14	A0A072UT22 http://www.uniprot.org/uniprot/A0A072UT22	MTR_4g117670	591	>A0A072UT22 MTSHSSKSGHATKANCPVSSPSLDGLWAGPVVSDNVYKGNGFPTLSVDEQSIAIELPKK YPPFIASVKKRGLNLSEIVCSTFSMGWFGEKESRRTVRIDCFMKESTVNIYVRPISGLTIV VDLGRMKIVEYHDREIETVPTAENNEYQVSKQNPPFGPKQHSLASHQPQGPGFQINGHS VSWANWKFHIGFDVRAGVIISLASTYDLEKHKSRRVLYKGYISELFVPYQDPSDEFYFK TFFDAGEFEFGLSTVSLIPNPDCPPNAEFIDTYTHSAEGAPIPLKNAVCIFEQYNSIMWRH IETGISDEFIEESRTEVNLIVRSVVTVGNYDNIIDWEFKASGSIKPSIALSGILEIKATNIKHK DEIKEDLHGTLVSENSIGVYHDHFYIYYLDLDIDGTHNSFEKTSLKTVKITDGSSKRKSY WHETEQTVKTESDAKITIGLAPGELAFVNPNKKTVVGNEVGYRLIPAIPAHPLLTEDDYPQI RGAFTNYNVWVAPYNRTEKWAGGLYVDHSRGDDTLAVWTKKNRDIVNKDIVMWHVV GIHHVPAQEDFPIMPLLSTGFELRPTNFFERNPVLKTLSPRDVAWPGCPK
MtDAO15	A0A072V0B7 http://www.uniprot.org/uniprot/A0A072V0B7	MTR_4g098910	149	>A0A072V0B7 MASTTIKFALFLTLTLLFLQVVSCTNIGLDDPEKDDILKWVSSKPSVITIPRKAFVIAIINN VESHEIVIDLRLKSIVSDNIHKGNGFPTLSVDEQTVAVGLPLKYPPFIASMKKRGLNLSEI VEYHDRVIEAVPTDENTEFRASDKKF
MtDAO16	A0A072UUP2 http://www.uniprot.org/uniprot/A0A072UUP2	MTR_4g045887	675	>A0A072UUP2 MASTTPMKLALFSILTLLYFQAVISLTPLHEHPLDPLTKEEFLTVQTLVHNKYHTSKNKV AFHYIGLDDPDKDYISKYEHEKVPIVIHRKSFVIAIINGQSHEIVIDIRLKTIVSDNVHKGYG FPMLSLEEQGLVTKLPLKYPPFIASLKKRGLNLSEVVCAPFSMGWFGEEESKRTVRVD CFMKENTVNFFVRPISGLTMVADLGLMKIVEYHDREVEVVPTAENTEYKASKQRPPFG PKQHSLASHQPQGPGFLINGHSVSWANWKFHIGFDVRAGIISLASIYDLEKHKSRRVLY KGYISELFVPYQDPTEEFYFKTFFDAGEYGFGRSTVSLIPNRDCPPHSHFIDTYIHSVDGT PSLLKNAICVFEQYGSIMWRHTETSIPNEFIEESRTEVNLIVRTVVTVGNYDNVLDWEFK TSGSIKPSIALSGILEIKATNIKHNDEIKGELYGKLVSANSIGIYHDHFYIYYLDFDIDGAEN SFEKTNLKTVRITDGSSKRKSYWTTETQTAKTESDAKITIGSAPAELVVINPNKKTAIGNH IIGYRLIPAIPAHSLLTEDDYPQIRGAFTNYNVWVTPYNRTEKWAGGLYVDHSRGDDTL AVWTKQNRDIVNKDIVMWHVVGIHHVPAQEDFPIMPLLSTSFELRPTNFFERNPVLKTL SPGDVVWHGCPKSN
MtDAO17	A0A072V290 http://www.uniprot.org/uniprot/A0A072V290	MTR_4g117610	668	>A0A072V290 MKLALFSVLTLLYFQAVVSVKPPLRQHPLDPLTNEEFLTVQTLVHNKYPTSKNKVAFH YIGLDDPAKDYIAIYERSLIPVVIRRKSFVLAIINGQSHEIVIDIRLKTIDSDNVHKGYGFPIL SVEEQGVAIELPLKYPAFIASVKKRGLNLSEVVCSTFSMGWFGEEESKRTVRVDCFMK ENSVNIVVRPISGLITVVDLGLQKIVEYHDREIETVPTAENTEYQVSKQSPPFGPNQHSLT SHQPQGPGFQIKGHSVSWANWKFHIGFDVRAGIVISLASIYDLEKHIYRRVLYKGYISEL FVPYQDPTEEFYFKTFFDSGEFGFGLSTVSLIPNRDCPPHAQFIDTYIHSDDGTPSILKNAI CVFEQYDSIMWRHTETGIPDEFIEESRTEVNLIVRTVVTVGNYDNVLDWEFKTSGSIKPSI ALSGILEIKGTNIKHTNEIKGELYGKLVSANSIGVYHDHFYMYYLDFDIDGPENSFEKTSL KTVRITDGSSKRKSYWTTETQTAKTESDAKITIGPTPAELVVINPNKKTAIGNHIGYRLIP AIPAHPLLTEDDYPQIRGAFTNYNVWITPYNRTEKWAGGLYVDHSRGDDTLAVWTKQN RNIVNKDIVMWHVVGIHHVPAQEDFPIMPLLSTSFELRPTNFFERNPVLKTLSPKDVPW PGCSN

Table 2: **Medicago truncatula** ***identified polyamine oxidases.*** *The locus, length of amino acid sequences, and FASTA sequences were obtained when* M. truncatula *proteins were researched against Uniprot database.*

Polyamine oxidase	Uniprot Entry	Locus	Length	FASTA
MtPAO1	A2Q567 http://www.uniprot.org/uniprot/A2Q567	MTR_2g039160	546	>A2Q567 MVVKKPKIVIIGAGMAGLTAANKLYTSTASKDLFELIVVEGGTRIGGRINTSEFGGDKIE MGATWHHGIGNSPIHKIAQQJHSLHSDQPWECMDGNNSNDESLTTISEGGFNLQPSIVDP VSKLFKVLMEYSQGKLTKETAKGEEVLSVYVNMAVKAASSNFASKKNLSIGSFLRQQLD AYFESLKDEEEEVKGVGDWNKKLLEEAVFAMYENTERTYTSAGDLECLDYEAESFYR MFPGEEITIAKGYLSIIEYIASVLPPGLIQLGKKVVKKIEWQSQKKSYDDNCFRPVKLHFCD GSIMYADHVIVTVSLGILKASISHHDDDDDKGMLFSPNLPSFKVEAISRLGFGVVNKLF MQLSTQKTTNLDDENSEGLFPFLQMVFHSPQNETKDKKIPWWMRKTATLFPIYNNSSV LLSWFAGEEALALESLKDEEIINGVTSTVSSFLPQNEVKFDKVLKSQWGTDPLFLGSYSY VQVGSSGEDLDTMAEPLPMMKDNSNFSYPLQILFAGEATHRTHYSTTHGAYFSGLREA NRLLQHYHCVGIFNN
MtPAO2	A0A072UVV1 http://www.uniprot.org/uniprot/A0A072UVV1	MTR_3g054420	283	>A0A072UVV1 MLLMGEHFGCLEEVLTIVSMLSVPSVFFRPKDRAEESDAARERFFVPESDHLTLYNVY QQWKQHDYRVKGLRKAREVMSQLLDILMVHTHVSQSSGNDYDILAENVGNRLFFAG EATSRQYPATMHGAFMSGLREASRIYQLNPVQQPCPKKSLSKNIAHNNGILVNLFKRPN LEIGNFAFICDPLQDDSQSKAIMKFTFGGIDESYKEIYTILSLEQMDQMQEINGGDENRLL YLTKNLGLKLMGLSALLIAGNDVIASVAASRNVDQRTERKIPHNPKKK
MtPAO3	G7K2Z6 http://www.uniprot.org/uniprot/G7K2Z6	MTR_5g087920	136	>G7K2Z6 MSQLLIHTHARLLTKLIKFEPKLLDWKEAAIADIRVGVENKIILHFKNVFWPNEDFLEVV AEISNGCSYFLNLHKAAGHSVLVYMPVGRQAKDIEKMSDEAAANFAFKQLKKILPDAS SPVTIIVLNLFNSFFTMG
MtPAO4	G7KD02 http://www.uniprot.org/uniprot/G7KD02	MTR_5g090300	488	>G7KD02 MESRIKTNPNSRKGLCYVNVDQQPRRSPSVIVIGGGMAGIAAARALHDASFQVVLLESR DRIGGRIHTDYSFGFPVDLGASWLHGVCNENPLAPLIGRLGLPLYRTCEDNSVLYDHDL ESYALFDMEGNQVPQELVTEVGKTFEMILQETDNVRQEFSEDMSILRALSIVFERKPEL RLEGLSHKVLQWYLCRMEGWFAADSDSISLKCWDQEELLPGGHGLMVRGYLPVIHTL AKGLDIRLGHRATKIVRGYNGVKVTTENGKTFVADAAIIAVPLGVLKANVIKFEPKLPD WKEAAIADIGVGVENKILHFKSVFWPNVEFLGVVAETSYGCSYFLNLHKAAGHPVLVY MPAGRLAKDIEKMSDEAAADFAFTQLKKILPDASSPIQYLVSRWGTDINSLGSYSFDAV GKPHGLYERLRVPVDNLFFAGEATSVLYTGSVHGAYSTGTMAAEDCRMRVLERYGEL DIFQPELEEGSVIPLLISRI
MtPAO5	A0A072UNG5 http://www.uniprot.org/uniprot/A0A072UNG5	MTR_4g094295	470	>A0A072UNG5 MTHTISLPSTNPSIFFPSVPKPFKFNPKCSSQLTPTPSSLPKTGVIIIGAGLAGLAAATHLN SQNIPFVLLESSNAVGGRVRTDIVDGFLLDRGFQIFITAYPEAQKLLNYQSLNLQKFYSG AKIFYNGQFHTVADPLRHFFDSAKSLTNPIGSVFDKLIGTTRIGVLTKSDEQILTAEEIPT IDLLKKLGFSDSIIGRFFRPFFGGIFFDPELETTSRLFDFIFKCLALGENTLPAKGISAIPEQ LAARLPSDSILLNTKAVSVDFDDSNPPRVRLQNGDVLSGELGVIVAVEEPALVKLLPGK KNPVFKKPVRSTVCLYFTANPDEIPVRDPVLFLNGSGQGIVNNMFFVTNVAPSYAPPNK ALISVSLIGLFEDVSEDELVLKVIQELSGWFGDKMVKEWKHLRTYRIEYAQPNQCPPTN LKKNPTIEQGLYLCGDYLTSATFDGALVSGRRAAESLLNDRAFFG
MtPAO6	A0A072UW35 http://www.uniprot.org/uniprot/A0A072UW35	MTR_3g449620	490	>A0A072UW35 MESGNKSNAKLTRALCYGNVSSVQERSPSVIVIGGGMAGIAAARALQDASFQVVLLES RERLGGRIHTDYSFGFPVDLGASWLHGVCKENPLASVIGRLGLPLYRTSGDNSVLYDH DLESYALYDMDGNQVPQELVTKVGEVFETILLETNKVRSEFNEDMSIHRGLSIVFERNP ELRLEGLAHKVLQWYLCRMEGWFAADSDTISLNGWDQEVLLPGGHGLMVRGYLPVIN TLAKGLDIRLGHRVTKVVRRYNGVKVTVESGKTFIADAAIVAVPLGVLKAKRISFEPKL PDWKEAAIAELGVGLENKIILHFENVFWPNVEFLGVVADTSYGCSYFLNLHKAARHPVL VVMPSGRLAKDIEKMSDEAAADFAFVQLKKILPDASSPIQYLVSRWGSDINSLGSYSYD QVGKPHELYERLRVPVDNLFFAGEATSMSYPGSVHGAFSTGMMAAEDCRMRVLERY GELDLFQPVMGEMASLTIPLQISRM
MtPAO7	G7J7X8 http://www.uniprot.org/uniprot/G7J4S9	MTR_3g064370	492	>G7J7X8 MKKRGIEDMDLVMVDPWCVGYHSEADAPGRRLAKPLLFCRSESDCPMENGYARPVE GIYVLVDMQNMVVIEFEDRKLVPLPPVDPLRNYTPGESRGGSDRSDVKPLQIPQPEGPSF RVNGYYVEWQKWNFRIGFTPKEGLIIYSVAYVDGSRGRRPVAHRLSFVEMVVPYGDPT EPHYRKNAFDAGEDGLGKNAHSLKKGCDCLGYIKYFDAHFTNFTGQVETIENCVCLHE EDHGILWKHQDWRTGLAEVRRSRRLSVSFVCTVANYEYAFFWHFYQDGKIEAEVKLT GILSLGALMPGEVRKYGYGTMIAPGLYAPVHQHFFVARMDMSVDSRPGEALNQVVEVNM KVEEPGEKNIHNNAFYAEETLLRSELEAMRDCNPLTARHWIVRNTRTGNRTGQLTGYK LVPGSNCLPLAGSEAKFLRRAAFLKHNVWVTAYSRDEMFPGGEFPNQNPRVGEGLAT WIKQNRSLEETNIVLWYVFGITHVPRLEDWPVMPVEHIGFMLMPHGFFNCSPAVDVPP NSCEVESKDSDIKDNGASKPIQGGIASKL
MtPAO8	G7J7Y0 http://www.uniprot.org/uniprot/G7J7Y0	MTR_3g064370	415	MGASWLHGVCNENPLAPLIRCLGLTLYRTSGDDSVLYDHDLESCMLFDIDGHQVPQQT VIEVGETFKRILEETGKVRDEHPEDISVSEAISIVLDRHPQLRQQGLSHEVLQWYICRMEA WFAADADMISLKTWDQEHVLSGGHGLMVQGYKPVINALAKDIDIRLNHRVTKISSGYN KVMVTLEDGRNFVADAAIIVPIGILKANLIEFEPRLPDWKVSAISDLGVGNENKIALKFD KVFWPDVELMGVVAPTSYACGYFLNLHKATGNPVLVYMAAGRFAYDLEKLSDESAA NFVMLQLKKMFPDACEPVQYLVSHWGTDPNSLGCYSYDLVGKSMDVYDKLRAPLGNI FFGGEAMSLDNQGSVHGAYSAGVMAAENCQRYLWEKQGNLESLSQVSARHETLGTNF PLQISRI
MtPAO9	G7J7X9 http://www.uniprot.org/uniprot/G7J7X9	MTR_3g064370	390	>G7J7X9 MDPKLFFSNNFLDGTITSLIDSQQRPAPSVIVVGAGISGIAAARILHDASFKVTLLESRDR LGGRIHTDYSFGCPVDMGASWLHGVCNENPLAPLIRCLGLTLYRTSGDDSVLYDHDLE SCMLFDIDGHQVPQQTVIEVGETFKRILEETGKVRDEHPEDISVSEAISIVLDRHPQLRQQ GLSHEVLQWYICRMEAWFAADADMISLKTWDQEHVLSGGHGLMVQGYKPVINALAKD IDIRLNHRVTKISSGYNKVMVTLEDGRNFVADAAIITVPIGILKANLIEFEPRLPDWKVSAI SDLGVGNENKIALKFDKVFWPDVELMGVVAPTSYACGYFLNLHKATGNPVLVYMAAG RFAYDLEKLSDESAANFVMLQLKKMFPDACEPVS
MtPAO10	G7IDT4 http://www.uniprot.org/uniprot/G7IDT4	MTR_1g087400	80	>G7IDT4 MELIVVVNGVDLVGLAAARQTFWFGYKVVVLEGRYRPGRRVYIEKIGNKAVLARQLSI PLHKVRVNCPLYKSSGEKLIKQ
MtPAO11	G7IM95 http://www.uniprot.org/uniprot/G7IM95	MTR_1g087430	135	>G7IM95 MKESKLYSYDEDLMDGELYEKNLVGNLNKNHLDPWRGSVRVWLTKGQIREFFPEMM EGIVVVIGVVLVGFSAERQLLSFDCKVVVLEGRNHPGGRVYTQRMGSEAVLAQQLSIPL HKVRINFSLYKLNGEPVDK
MtPAO12	A0A072TCJ7 http://www.uniprot.org/uniprot/A0A072TCJ7	MTR_2042s0010	355	>A0A072TCJ7 MPGEVGGQTVDFGGQWVGASQNLLRQQAIELDVPMYRQYTSGNSLVNIDGKVHAYSS TPKLPFLSTLELGLTLHRWKRDMQTLPVGAPWLAENALEWDAMSIEVWLDKHVDTTA ARDFARLVTSAITCTESLHVSYLFFLECLRQGGGLEAMMAVEGGAQQDKFLGGAWQIP KRMAERLDGCIVLNAAVSAIEQNANGVRVTCAQGAFEASYAIVTAPPPLASRIRFVPPL PVKRAGLLQRMQMGAVIKMHVAYPTPFWRRHGLNGSVASVGHHLGFVFDQSPEDESI GVLVGLIEGNHALELSGLSQEARREHIVADLVHYFGDEAAAPLDYADYDWTTDEWALG GYASHMPPG
MtPAO13	G7J0U8 http://www.uniprot.org/uniprot/G7J0U8	MTR_3g033000	507	>G7J0U8 MDSTNRSSVIIIGAGISGISAAKVLSENGVEDIVMLEASDRIGGRIRKECFGGVSVELGAG WIAGVGGREANPVWELAVQHNLKTCFSDYSNARFNIYDQSGKLIPSGIADDSYKKAVES AIQKLRDEEVEEDDEEANDDGKVTKPSLTPKTPVELAIDFILHDFEMAEVEPISTYVDFG EREFLVADERGYDHLLYKMAEGFLFTSEGRILDDRLKLNKVVRELQHSRNGVTVITEDG CVYEANYVILSVSIGVLQSDLLAFNPPLPRWKLEAIEKCDVMVYTKIFLKFPYRFWPCG PEKEFFMYAHEQRGYYTFWQHMDNAYPGSNILVVTLTNGESKRVEAQTNEETLREAM AVLRDMFGPNIPDAIDILVPCWWNNRFQRGSYSNFPIISNGKVFYNIKAPVGRIFFTGEH TSERFNGYVHGGYLAGIDTGKALVEEIRKENERESESQTLLLEPLLALTGSLTKSKPETV SNIHKCDIPTQLYLSGKLGIPEAIL

Table 3: Orthologous identified diamine oxidases. *The locus, length of amino acid sequences, and FASTA sequences were obtained when orthologous proteins were researched against Uniprot database.*

Diamine oxidase	Uniprot Entry	Locus	Lenght	FASTA
AtDAO1	Q8H1H9 http://www.uniprot.org/uniprot/Q8H1H9	At1g62810	712	>Q8H1H9 MAEPSFARLFLLFFSFLLIFATYSWVFGPDSGFLFGTRVRKTLGSNRQVHVDHSLEKPH HPLDPLTVREINRVRTILSNHDPGFGSGSATIHSMALDEPEKSRVVQWKKGNKLLSRRA AVVAYWGGQTHEITVDLDSGRVVSDVINRTSGYPILTLNDVFAASQVPLKSLEFNRSIEA RGVKFSDLACTIPFAGWFGSEEEGRRVIRVQCFTLQGTTNYFMRPLEGLYVTVDLDKLE VIKHDKGPIPIPKASGTEYRFGVQNKPVHMDRINPISMEQPDGPSFRVEDGHLVKWAN WVFHVKADQRAGMIISQATVRDSETGEPRSVMYKGFPSELFVPYMDPEEGWYYKGYM DAGELGLGPTAMPLVPLNDCPRNSYYIDGVFASPDGKPIVQPNMICLFERYAGDISWRH SEILFANADIRESRPKVTLVARMATSVGNYDYIFDWEFQTDGLIRVTVAASGMLMVKG TPYDNVDDLGDREDDAGPLISENVIGVVHDHFITFHLDMDIDGPMNNSLVKVHLEKQRV PTGKSPRKSYLKVKKYIAKTEKDAQIKLSLYDPYEFHIVNPNRKSRVGNPAGYRIVPGG NAASLLDHDDPPQIRGAFTNNQWVTPYNRSEQYAGGVLIYQSQGDDTLQVWSDRDRSI ENKDIVLWYTLGFHHVPCQEDYPVMPTVAASFELKPANFFESNPILGSAPFFEKDLPVC RPFASS
AtDAO2	Q9M2B9 http://www.uniprot.org/uniprot/Q9M2B9	At3g43670	687	>Q9M2B9 MVELSFSQLLVLLLSLLFLFTTLASSSKTPRFKYSLEKPHHPLDPLTTPEIKRVQTILSGHD PGFGSGSTIIHAMALDEPDKQRVIRWKKGDRLPPRRAEILAMSNGESHVLTVDLKSGRV VSDLVNPTFGYPILTMKDIIAVSQVPYKSVEFNRSIEARGIPFSGLICTIPFAGWYGPDEE GRRVIKIQCFSKQDTVNFYMRPIEGLYLTVDMDKLEIIKIVDNGPVPVPKSTGTEYRYGF LNETVYMDRVNPMSMEQPDGPSFQVEDGYLVKWANWKFHIKPDQRAGMIISQATVRD SKTGEARSVMYKGFASELFVPNMDPGEGWYSKAYMDAGEFGLGPSSMPLVPLNDCPR NAYYIDGFFASPEGIPILQPNMICLFERYAGDTSWRHSEILFGVDIRESRAKVTLVARM ACSVGNYDYIFDWEFQMDGVIRVTVAASGMLMVKGTAYENVEDLGEKEDDSGPLISEN VIGVVHDHFISFHLDMDIDGSANNSFVKVHLEKQRLPPGESRRKSYLKVKKYVAKTEKD AQIKMSLYDPYEFHLVNPNRLSRLGNPAGYKLVPGGNAASLLDHDDPPQMRGAFTNNQ IWVTRYNRSEQWAGGLLMYQSRGEDTLQVWSDRDRSIENKDIVLWYTLGFHHVPCQE DFPVMPTIASSFELKPVNFFESNPVLGISPFFEKDLPVC
AtDAO3	F4IAX1 http://www.uniprot.org/uniprot/F4IAX1	At1g31710	681	>F4IAX1 MAPLHFTILILFSFVIVVSSSSFTPPRHPFDPLTETELKLVRTIINKSYPVGPNHKFTFQVV GLNEPNKSLVLSWYSSPNHTIKPPPRQAFVIARDNGKTREIVLDFSSSRAIVSDKIHVGNG YPMLSNDEQEASTELVVKFKPFIDSVAKRGLNVSEIVFTTSTIGWYGETKAEAERVIRLM PFYLDGTVNMYLRPIEGMTIIVNLDEMKVSEFKDRSVVTMPIANGTEYRISKLNPPFGPT LHNAVLLQPDGPGFKVDGHIVRWANWEFHISFDVRAGIVISLASLFDTDVNKYRQVLYK GHLSEMFIPYMDPSDDWYFITYLDCGDFGCGQCAVSLQPYTDCPAGAVFMDGIFAGQD GTPAKIPKVMCIFEKYAGDIMWRHTEAEIPNLETEVRPDVSLVARVITTVGNYDYIVDY EFKPSGSIKMGVGLTGVLEVKPVEYIHTSEIKLGEDHGITVADNTVGVNHDHFVTFRLLH LDIDGTENSFVRNELVTRSPKSVNTPRKTYWTTKPKTAKTEAEARVKLGLKAEELVVV NPNRKTKHGNEVGYRLLHGSAAGPLLAQDDFPQIRAAFTNYNVWITPYNRSEVWAGGL YADRSQGDDTLAVWSQRNRKIEKEDIVMWYTVGFHHVPSQEDYPTMPTLSGGFELRPT NFFERNPVLKTKPVKVTTARKCTPKND
AtDAO4	P0DO00 http://www.uniprot.org/uniprot/P0DO00	At1g31672	662	>P0DO00 MSQLLLFTILVFSSVFVIGSLSFIPPPHPFDPLTEIELNLVRNIINERYPIGLEHRFTFQYVG LNEPDKSLVLSWYSSQYHNVKSPPRQAFVIARDHGKTREIVVDFASQAIVSEKIHVGNG YPMLTIDEQQAATSELVLKFKPFRDSIRRRGLNVSEVVVTTSTMGWFGEAKPERLIKKRP FYLNGSVNTYLRPIEGMTIIVNLDQMKVTKFRDRFTSPLPNAKGTEFRISKLKPPFGPSL QNAVLFQSEGPGFKIDGHTNRWANWEFHMSFDVRAGLVISLASIFDMDVNKYRQVLY KGHLSEIFVPYMDPSEDWYFRTFFDCGEFGCGQYAVSLEPYTDCPGNAAFMDGVFASQ DGTPIKITNVMCIFEKYAGDIMWRHTEIEIPGLKVRPDVSLVVRMVTTVGNYDYIVDYE FKPSGSIKIGVGLTGVLEVKPVEYVNTSEIKEDDIHGITIVADNTIGVNHDHFVTYRLLDI DGTDNSFVRSELVTKRTPKSVNTPRKSYWTIKRLKAEELLVVNPSRKTKHGNEVGYRL LHGPASEGPLLAQDDYPQIRAAFTNYNVWITPYNNTEVWASGLYADRSQGDDTLAVWS QRNRKIEKTDVMWYTVGFHHVPCQEDFPTMPTLFGGFELRPTNFFEQNPDLKTKPIKL NTTPTCTARND
AtDAO5	F4IAX0 http://www.uniprot.org/uniprot/F4IAX0	At1g31690	677	>F4IAX0 MAQVHLTIFIFSSIFVISSSSFIPPPHPFDPLTETELKLVRNIINKSYPIGHNHKFTFQVVGL NEPEKSLVLSWHSSPDRNVKPPPRQAFVIARDKGMSREIVIDFSTRAIVSNKIHVGNGNP MLTIDEQQAATAVVQKYKPFCDSIIKRGLNLSEVVVTSSTMGWFGETKTKRFIRTIPFYL NGSVNTYLRPIEGMTIIVNLDQMKVTGFKDRFTGPMPKANGREYRISKLKPPFGPSLRS AVVFQPDGPGFKIDGHVVRWANWEFHMSFDVRAGLVISLASIFDMDMNRYRQVLYKG HLSEMFVPYMDPNDDWYFISYLDCGEFGCGQYAVSLEPYTDCPPNAAFMDGIFPGQDG TPTKISNVMCIFEKYAGDIMWRHTEAEVPGLKITEVRPDVSLVARMVTTVGNYDYIIEY EFKPSGSIKMGVGLTGVLEVKPVEYVHTSEIKEDDIYGTIVADNTVGVNHDHFVTFRLDL DIDGTENSFVRTELVTKRTPKSVNTPRKSYWTTKRNTAKTEADARVKLGLRAEELVVVN PTKKTKHGNEVGYRLLPGPASSPLLVQDDYPQIRAAFTNYNVWITPYNKSEVWASGLY ADRSQGDDTLAVWSQRDREIENKDIVMWYTVGFHHVPCQEDFPTMPTMFGGFELRPT NFFEQNPVLKAKPFNLTTIPKCTTKNE
AtDAO6	O23349 http://www.uniprot.org/uniprot/O23349	At4g14940	650	>O23349 MNTSILAILFLIQCVFTLGLHFHPLDPLTPQEINKTSFIVKKSHLGNLKDLTFHYLDLEEPN KSHVLQWLSPNPSKKPPPPRRRSFVVVRAGGQTYELIIDLTTSKIASSRIYTGHGFPSFTF HLFKASKLPLTYPPFKKSILDRSLNISENSCIPFTVGWYGETTTRREHLKASCFYRDGSVN VFTRPIEGITVTIDVDSMQVIKVSLDRFRKPIPDKEGNDFRTKHRPFPFFCNVSDTGFKILG NRVKWANWKFHVGFTARAGVTISTASVVLDPRTKRFRRVMYRGHVSEITFVPYMDPTYE WYYRTFMDIGEFGFGRSAVNLQPLIDCPQNAAFLDGHVAGPDGTAQKMTNVMCVFEK NGYGASFRHTEINVPGQVTSGEAEISLVVRMVATLGNYDYIVDWEFKKNGAIRVGVDL TGVLEVKATSYTSNDQITENVYGTLVAKNTIAVNHDHYLTYYLDLDVDGNGNSLVKAKL KTVRVTEVNKTSSRRKSYWTTVVKETAKTEADGRVRLGSDPVELLIVNPNKKTKIGNTVG YRLIPEHLQATSLLTDDDYPELRAGYTKVPVWVTAYDRSERWAGGFYSDRSRGDDGLA VWSSRNREIENKDIVMWYNVGFHHIPYQEDFPVMPTLHGGFTLRPSNFFDNDPLIG
CaDAO	O65749 http://www.uniprot.org/uniprot/O65749	LOC101491799	670	>O65749 MASTTIKLSFFEAITLIFLQAVTPLNLQHPLDPLTKEEFSIVQTVVLHKVPTSKNSVAFHYI GLDDPIKDDVLKYETQPTLITIPRKSFVVAINSSQNHEIVIDLRLKTIVSDNIHKGYGFPILS ADEQGVAIKLPLKYPPFIASVNKRGLNLSEIVCSTFSMGWFGEEKNSRTVRVDCFMKEN TVNIYVRPISGITIVVDLQLMKIVEYNDRGIEAVPTAEKTDYRFSHQNPPFGPKLNSLVSH QPQGSGAHINGYSVSWANWRFHIGFDARAGIVISLASIYDLEKHKSRRVLYKGYISELFV PYQDPTEEFYFKTFFDSGEFGFGLSTVSLIPNRDCPSHAQFIDTYIHSADGSPILLKNAICV FEQYGNIMWRHTETGIPNEFPEESRTEVNLIVRTVVTVGNYDNVLDWEFKTSGSIKPAIA LSGILEIKGANIKHKDEIKEDQYGTLVSANSIGIYHDHFYMYYLDFDVDGVNNSFEKTSL KTVRITDGSSKRKSYWTTETQTAKTESDAKITIGLSPAELVVVNPNKKTAVGNDVGYRLI PAIPAHPLLTEDDYPQIRGAFTNYNVWVTPYNRTEKWAGGLVYDHSRGDDTLAVWTQK NRDIENKDIVLWHVVGHHIVPAQEDFPIMPLLSTSFELRPTNFFERNPVLNTLSPRDVNW PGCSN
GmDAO1	K7LI85 http://www.uniprot.org/uniprot/K7LI85	GLYMA_10G090600	734	>K7LI85 MEAKNLWRFLVLSIGVVLVLLVTWLPNKEALDCNLYSGWCTSKNRFYSSAHPNNPIKK PSFSTYHHHKNQHHESEIPQHPLDPLTIQEFNKVRTILLNYPLFKSSSSYTLNSVVLEEPD KKLVLKWKKGDLPLPRKASVVAYVKGDSHVLTVDLETGQVVSQEAITWSVSGYPTMTL EDMVGVLEVPLKSTEFNRSITKRGVNLADLACLPISSGWYGTQVEENTRLIKVQCYSKE GTVNFYMKPIEGVTALVDMNKKEVLSISDNGQNIPVANGINTDYRYSIQKLNGGELRML NPISLEQPKGPSFTINGHLVKWANWEFHLRPDPRAGIIISQAKVRDPDTSKLRSVMYKGF TSELFVPYMDPTQDWVFKTYMDAGEYGFGLQAMPLDPLNDCPKNAYYMDGVFASSD GTPVLQPNMICIFESYAGDIAWRHAFCPTIDLKVTEVRPKVTLVVRMAAAVANYDYIVD WEFQTDGLIRAKVGLSGILMVKGTTYENMDQVPNQEYLYGTLLSENIIGVHHDHFITYYL DMDVDGSDNSFVEVNIKKQETSPGESPRKSYLKAVKKVAKTEKDAQIRLQLYDPSEFHV VNPLKKTRIGNPVGYKLVPGATAASLLDPEDPPQKRAAFTNNQIWVTPYNKSEQWAGG LFAYQSKGDDTLQVWSNRDRPIENKDIVLWYTIGFHHIPCQEDYPVMPTVSSSFDLKPA NFFERNPILGVPPNFEDDLPVCKARDSA

Table 3: Continuation.

Diamine oxidase	Uniprot Entry	Locus	Lenght	FASTA
GmDAO2	K7LI86 http://www.uniprot.org/uniprot/K7LI86	GLYMA_10G090600	664	>K7LI86 MEAKNLWRFLVLSIGVVLVLLVTWLPNKEALDCNLYSGWCTSKNRFYSSAHPNNPIKK PSFSTYIHHHKNQHHESEIPQHPLDPLTIQEFNKVRTILLNYPLFKSSSSSYTLNSVVLEEPD KKLVLKWKKGDLPLRKASVVAYVKGDSHVLTVDLETGQVVSQEAITWSVSGYPTMTL EDMVGVLEVPLKSTEFNRSITKRGVNLADLACLPISSGWYGTQVEENTRLIKVQCYSKE GTVNFYMKPIEGVTALVDMNKKEVLSISDNGQNIPVANGINTDYRYSIQKLNGGEELRML NPISLEQPKGPSFTINGHLVKWANWEFHLRPDPRAGIISQAKVRDPDTSKLRSVMYKGF TSELFVPYMDPTQDWYFKTYMDAGEYGFGLQAMPLDPLNDCPKNAYYMDGVFAASD GTPVTLQPNMICIFESYAGDLAWRHAECPITDLKVTEVRPKVTLVVRMAAAVANYDYIVD WEFQTDGLIRAKVGLSGILMVKGTTYDNMQQVPNQKYLYGTLSENIIGVHHWHFTTYYL DMDVDGSDNSFVEVNIKKQETSPGESPRKSYLKAVKKVVAKTEKDAQIRLQLYDPSEFHV VNPLKKTRIGNPVGYKLVPGATAASLLDPEDPPQKRAAFTNNQIWVTPYNKSEQWAGG LFAYQSKGDDTLQVWSNRLLIF >K7LI86 MEAKNLWRFLVLSIGVVLVLLVTWLPNKEALDCNLYSGWCTSKNRFYSSAHPNNPIKK PSFSTYIHHHKNQHHESEIPQHPLDPLTIQEFNKVRTILLNYPLFKSSSSSYTLNSVVLEEPD KKLVLKWKKGDLPLRKASVVAYVKGDSHVLTVDLETGQVVSQEAITWSVSGYPTMTL EDMVGVLEVPLKSTEFNRSITKRGVNLADLACLPISSGWYGTQVEENTRLIKVQCYSKE GTVNFYMKPIEGVTALVDMNKKEVLSISDNGQNIPVANGINTDYRYSIQKLNGGEELRML NPISLEQPKGPSFTINGHLVKWANWEFHLRPDPRAGIISQAKVRDPDTSKLRSVMYKGF TSELFVPYMDPTQDWYFKTYMDAGEYGFGLQAMPLDPLNDCPKNAYYMDGVFAASD GTPVTLQPNMICIFESYAGDLAWRHAECPITDLKVTEVRPKVTLVVRMAAAVANYDYIVD WEFQTDGLIRAKVGLSGILMVKGTTYDNMQQVPNQKYLYGTLSENIIGVHHWHFTTYYL DMDVDGSDNSFVEVNIKKQETSPGESPRKSYLKAVKKVVAKTEKDAQIRLQLYDPSEFHV VNPLKKTRIGNPVGYKLVPGATAASLLDPEDPPQKRAAFTNNQIWVTPYNKSEQWAGG LFAYQSKGDDTLQVWSNRLLIF
LcDAO	P49252 http://www.uniprot.org/uniprot/P49252	N/A	667	>P49252 KFALFSVLTLLSFHAVFSFTPLHTQHPLDPITKEEFLAVQTIVQNKVPISNNKLAFHYIGV DDPEKDLVLKVETSPTLISIPRKIFVVAIINSQTHEILIDLTIKSIVSDNIHNGYGFPVLSAAE QFLAIDLPLKVPPFIASVNKRGLNISEIVCSSFTMGWFGEEKNSRTVPVDCFMKESTVNI YVRPTTGITIVADLDLMKIVEYHDRDTEAVPTAENTEYQVSKQSPPFGPKQHSLTSHQPQ GPGFQINGTSVSWANWKFHIGFDVRAGIVISLASIYDLEKHKSRRVLYKGYISELFVPYQ DPTEEFYFKTFFDSGEFGFGLSTVSLIPNRDCPPHAQFIDTYIHSADGTPIFLENAICVFEQ YGNIMWRHTETGIPNESIEESRTEVDLAIRTVVTVGNYDNVLDWEFKTSGWMKPSIALS GILEIKGTNIKHKDEIKEDLHGKLVSANSIGIYHDHFYIYYLDFDIDGTQNSFTKSLKTVRI VDGGSKRKSYWTTETQTAKTESDAKITIGLAPAELVVVNPNIKTAVGNEVGYRLIPAIPA HPLLTEDDYPQIRGAFTNYNVWVIPYNRTEKWAGGLYVDHSRGDDTLAVWTKKNREI VNKDIVMWHVVGIHHVPAQEDFPIMPLLSTSFELRPTNFFERNPVLKTLPPRDFTWPGC SN >P49252 KFALFSVLTLLSFHAVFSFTPLHTQHPLDPITKEEFLAVQTIVQNKVPISNNKLAFHYIGV DDPEKDLVLKVETSPTLISIPRKIFVVAIINSQTHEILIDLTIKSIVSDNIHNGYGFPVLSAAE QFLAIDLPLKVPPFIASVNKRGLNISEIVCSSFTMGWFGEEKNSRTVPVDCFMKESTVNI YVRPTTGITIVADLDLMKIVEYHDRDTEAVPTAENTEYQVSKQSPPFGPKQHSLTSHQPQ GPGFQINGTSVSWANWKFHIGFDVRAGIVISLASIYDLEKHKSRRVLYKGYISELFVPYQ DPTEEFYFKTFFDSGEFGFGLSTVSLIPNRDCPPHAQFIDTYIHSADGTPIFLENAICVFEQ YGNIMWRHTETGIPNESIEESRTEVDLAIRTVVTVGNYDNVLDWEFKTSGWMKPSIALS GILEIKGTNIKHKDEIKEDLHGKLVSANSIGIYHDHFYIYYLDFDIDGTQNSFTKSLKTVRI VDGGSKRKSYWTTETQTAKTESDAKITIGLAPAELVVVNPNIKTAVGNEVGYRLIPAIPA HPLLTEDDYPQIRGAFTNYNVWVIPYNRTEKWAGGLYVDHSRGDDTLAVWTKKNREI VNKDIVMWHVVGIHHVPAQEDFPIMPLLSTSFELRPTNFFERNPVLKTLPPRDFTWPGC SN
MdDAO1	A0A096ZNR5 http://www.uniprot.org/uniprot/A0A096ZNR5	N/A	727	>A0A096ZNR5 MEGRRFLRFFSLSLCIGLVLLFTWSHLPYAPPYVSDLLDCTANSAWCSSKNRIQAKPPIP TRRLKDHSSDTPHHPLDPLTIQELNAVRTILNSHALFTSTSSYALHSIELQEPDKSLVLGW KHGDPLFPRKASIVARVDSKSLVLTVDLTTSEVTVHETGSHVGVPTMTLEELAAATSPL KNADFNRTIVERGVDLADLGCLPLSTGWYGADENRRLIKVQCYSTKDTVNFYMRPIE RLTVLVDLDTQQVVEISDKGRGIPIPKAANTDYRYSAQRPNQVKKLIKPISIEQPDGPSFT VENDHLVKWANWEFHLKPDARAGVIVSRAKVWDPETGELRDVMYKGFTSELFVPYM DPTDAWYFKTYMDAGEYGFGLQAMPLEPLNDCPRNAYYMDGVFAAADGKPVYRSN MVCIFERYTGDIGWRHTECPITGMEVREVRPKVTLVVRMAASVANYDYIVDWEFQTD GLIRVKVGLSGILMVKGTSYENMNQVSSSQENLYGTLSENVIGVHHWHYVTFHLDMDV DGSDNSFMKVNLQRQSNSPTESPRKSYLKATKTVAKTEKDAQIKLKLYDPSEFHVNPS KKTRVGNPVGYKLVPGGTAASLLDLDDPPQKRGAFTNNQIWVTPYNKSEQWAGGLFV DQSTGEDTLAVWSNRDRPIENKDIVVWYTLGFHHIPCQEDFPIMPTVSSSFDIKPVNFFE SNPILRTPPNVATDLPVCTAAASA
MdDAO2	A0A096ZNU5 http://www.uniprot.org/uniprot/A0A096ZNU5	N/A	775	>A0A096ZNU5 MASASKKTTPRDAASAESSALLRKAGEPVRNWTGSASDLDSDPIRTRASVPTLIRPMES LPAPSTNTAATKGIPVMLRAQSSHPLEPLSAAEISVAVATVRAAGATPEVRDSMRFVEV ALVEPDKRVVALADAYFFPPFQPSLLPRTKGGPMIPSKLPPRQARLVVYNKKSNETSIWI VELSEVHAATRGGHHRGKVISSEVVPDVQPPMDAVEYAECEAVVKNFPPFREAMKKR GIEDMDLVMVDPWCTGYHSGADAPSRRLAKPLIFCRTESDCPLENGYARPVEGIRVLV DMQNMVVLEFEIRKLVPLPPADPLRNYTPGETRGGVDRSDVKPLQHQPFGPSFRVNGH FVEWQKWNFRIGFTSKEGLVIYSVAYIDGSRGRRPVAHRLSFVEMVVPYGDPNDPHYR KNAFDAGEDGLGKNAHSLKKGCDCLGYIKVFDANFTNFTGGVETIENCVCLHEEDHGIL WKHQDWRTGLAEVRRARRLTVSFICTVANYEYGFYWHFYQDGKIEAEVG LSGILMVKGSPYVNMNQVNQNEYLYGTLLSENIIGVHHDHYITFHLDMDIDGSNNSFVK VNLQKAMTSPGESPRKSYLKAVRNVAKTEKDAQIKLKLYDPSEFHVINPNKKSRVGNP VGYKPVPGTAASLLDLDDPPQKRGAFTNNQIWVTPYNESEQWAAGLFVYQSQGDDTL AVWSNRDRPIENKDIVLWYTLGFHHIPCQEDFPIMPTVSSSFEIKPVNFFESNPILXIPPNS PNDLPICKAAASA >A0A096ZNU5 MASASKKTTPRDAASAESSALLRKAGEPVRNWTGSASDLDSDPIRTRASVPTLIRPMES LPAPSTNTAATKGIPVMLRAQSSHPLEPLSAAEISVAVATVRAAGATPEVRDSMRFVEV ALVEPDKRVVALADAYFFPPFQPSLLPRTKGGPMIPSKLPPRQARLVVYNKKSNETSIWI VELSEVHAATRGGHHRGKVISSEVVPDVQPPMDAVEYAECEAVVKNFPPFREAMKKR GIEDMDLVMVDPWCTGYHSGADAPSRRLAKPLIFCRTESDCPLENGYARPVEGIRVLV DMQNMVVLEFEIRKLVPLPPADPLRNYTPGETRGGVDRSDVKPLQHQPFGPSFRVNGH FVEWQKWNFRIGFTSKEGLVIYSVAYIDGSRGRRPVAHRLSFVEMVVPYGDPNDPHYR KNAFDAGEDGLGKNAHSLKKGCDCLGYIKVFDANFTNFTGGVETIENCVCLHEEDHGIL WKHQDWRTGLAEVRRARRLTVSFICTVANYEYGFYWHFYQDGKIEAEVG LSGILMVKGSPYVNMNQVNQNEYLYGTLLSENIIGVHHDHYITFHLDMDIDGSNNSFVK VNLQKAMTSPGESPRKSYLKAVRNVAKTEKDAQIKLKLYDPSEFHVINPNKKSRVGNP VGYKPVPGTAASLLDLDDPPQKRGAFTNNQIWVTPYNESEQWAAGLFVYQSQGDDTL AVWSNRDRPIENKDIVLWYTLGFHHIPCQEDFPIMPTVSSSFEIKPVNFFESNPILXIPPNS PNDLPICKAAASA
NtDAO	A0A075EZT1 http://www.uniprot.org/uniprot/A0A075EZT1	cuAO	723	>A0A075EZT1 MEGRNFLRVLFFLMSILLLFFTFLNLPSPLSHTTELLDCTTYSPWCSSRKPFFLRNSNKD KKHYADFPNRPLDPLTQEIQKVKKHNSLDDFKVKGSYVLHSVVLEEPEKEVVLNWRKG QRLPSRKASVVARAHGDVHVLTVDIEADRVTHREMDHLSGYPILTIEDMITATAAPLGN SDFNRTIIQRGVDLGDLACLPISTGWYGKVEEKRRVIKVQCYTMKDTINFYMRPIEGLT VLLDLDTKQVIEILDEGKNIPIPKAADTDYRFSTQKNTQALNLLKPISIEQPNGPSFTIEDN HVKWANWEFHLKPDPRAGVISRVMVQDPDTGKMRNVMVKGFTSELFVPYMDPSDA WYFKTYMDAGEYGFGLQAMPLDPLNDCPRNAYYMDGVFVAADGTPVVRSNMICVFE SYAGDIGWRHAESPITGLPIREVRPKVTLVVRMAASVANYDYIVDWEFQTDGLIRVKVG LSGILMVKGSPYVNMNQVNQNEYLYGTLLSENIIGVHHDHYITFHLDMDIDGSNNSFVK VNLQKAMTSPGESPRKSYLKAVRNVAKTEKDAQIKLKLYDPSEFHVINPNKKSRVGNP VGYKPVPGTAASLLDLDDPPQKRGAFTNNQIWVTPYNESEQWAAGLFVYQSQGDDTL AVWSNRDRPIENKDIVLWYTLGFHHIPCQEDFPIMPTVSSSFEIKPVNFFESNPILXIPPNS PNDLPICKAAASA >A0A075EZT1 MEGRNFLRVLFFLMSILLLFFTFLNLPSPLSHTTELLDCTTYSPWCSSRKPFFLRNSNKD KKHYADFPNRPLDPLTQEIQKVKKHNSLDDFKVKGSYVLHSVVLEEPEKEVVLNWRKG QRLPSRKASVVARAHGDVHVLTVDIEADRVTHREMDHLSGYPILTIEDMITATAAPLGN SDFNRTIIQRGVDLGDLACLPISTGWYGKVEEKRRVIKVQCYTMKDTINFYMRPIEGLT VLLDLDTKQVIEILDEGKNIPIPKAADTDYRFSTQKNTQALNLLKPISIEQPNGPSFTIEDN HVKWANWEFHLKPDPRAGVISRVMVQDPDTGKMRNVMVKGFTSELFVPYMDPSDA WYFKTYMDAGEYGFGLQAMPLDPLNDCPRNAYYMDGVFVAADGTPVVRSNMICVFE SYAGDIGWRHAESPITGLPIREVRPKVTLVVRMAASVANYDYIVDWEFQTDGLIRVKVG LSGILMVKGSPYVNMNQVNQNEYLYGTLLSENIIGVHHDHYITFHLDMDIDGSNNSFVK VNLQKAMTSPGESPRKSYLKAVRNVAKTEKDAQIKLKLYDPSEFHVINPNKKSRVGNP VGYKPVPGTAASLLDLDDPPQKRGAFTNNQIWVTPYNESEQWAAGLFVYQSQGDDTL AVWSNRDRPIENKDIVLWYTLGFHHIPCQEDFPIMPTVSSSFEIKPVNFFESNPILXIPPNS PNDLPICKAAASA
OsDAO1	A3AUU3 http://www.uniprot.org/uniprot/A3AUU3	OsJ_15179	838	>A3AUU3 MAATQEKAPVVVCCGGGGAAAARRVDGGPGSSSRGAIVAAPAAAAAAGKVSMVSGD DPRVAAAAGGGGGAVMEEIAAAVQPTTAKVSSKGKRVKCLKWTMPVQRWTNRLVD YVNHGAISNMESALYLYAPLGTGRIPIMTRAQRSHPLDPLSAAEIAVAIATVRAAGKSPE ERDGMREVEVVLLEPEKNVVALADAYFFPPFQPSLLPRTKGSAVIPSRLPPRRAKLTVY NRQSNETSIWIVELSEVHAATRGGHHRGKVISSEVVPEVQPPMDAMEYAECEATVKSY PPFEAMKRRGVDDMELVMVDAWCAGYYSDADADPSRRLGKPLIFCRTDSDSPMENGY ARPVEGHNVIVDMQNMVVIEFEDRKLVPLPPPDHLRNYTPGETRGGVDRSDLKPLINQP EGPSFRINGYFVEWQKWNFRIGFTPKEGLVIYSVAYVDGSRGRRPIAHRLSFVEMVVPY GEPNEPHYRKNAFDAGEDGLGKNAHSLKRGCDCLGFIKVFDAIFTNFTGGVETIENCV CLHEEDHGILWKHQDWRTGLAEVRRSRRLTVSFICTVANYEYGFYWHFYQDGKIEAEV KLTGILSLGALMPGESRKYGTTIAPGLYAPVHQHFFVARMDMAVDCKPNEAHNQVVEV NVKVENAGTHNVHNNAFYAEEKLLKSELQAMRDCDPSSARHWIVRNTRAVNRTGQPT GYRLVPGSNCLPLALPEAKFLRRAGFLKHNLWVTQYKSDEVFPGGEFPNQNPRIHEGLA TWVKDDRPLEETDIVLWYVFGLTHIPRLEDWPVMPVERIGFMLMPHGFFNCSPAVDVP PGSSDADIKEAESPKAIQNGLISKL
OsDAO2	Q7XWW0 http://www.uniprot.org/uniprot/Q7XWW0	Os04g0269600	702	>Q7XWW0 MDHSTSLLRLIFLALGAALVVLVVRSAFRLPGDIDTTTSLFDDGNGGSCTRFSPWACR QGRGDPRSNPSNPRRPSHESDVPRHPLDPLTVREVNRVRELLRAHPLFASAPSSLFVHS LELDEPEKSVVKSWRKGADPLPRRRAVAVVRFRGESHVLGVDLSEGDGAVTPLPVPAS GYPMMNMDEQTSLCFAPFKDEAFNASLLRRGVRASDVACLPISLGWYGPAEENRRLIK SQCFSTEGTANFYMRPIEGLTVLVDMDTAEVLHVSDRGAGIPIPAAANTDYRIHGHSAPT PAEAQAQGRHGVQTDPATGEHRDVMVKGMAASELFVPVMDPTEAWYFKTYMDAGEYG FGLQAMPLVPLNDCPRHARYLDAVFVAADGRPVVRENMICVFERYAGDIAWRHSESPI TGMDIRESRPKVTLVARMAASVANYDYIVDWEFQMDGLVRIKVGLSGILMVKGTQYS HMNQVHQNDNMVGTLLSENVIGVHHDHFVTFRLDMDIDGADNSFVKVAMARQNTGAG ESPRKSYLKATHIVARTEKDAQNRLKLYEPSEFHIVNPMKKTRVGNPVGYKVVPAGTA ASLLDPEDPPQKRGAFTNNQIWVTPYNKTEEWAGGLFVYQSKGEDTLATWSERDRPIEN KDLVLWYTLGFHHVPCQEDFPIMPTVSSSFDLKPVNFFESNPILGQRPTQENDLPVCAA AATTS >Q7XWW0 MDHSTSLLRLIFLALGAALVVLVVRSAFRLPGDIDTTTSLFDDGNGGSCTRFSPWACR QGRGDPRSNPSNPRRPSHESDVPRHPLDPLTVREVNRVRELLRAHPLFASAPSSLFVHS LELDEPEKSVVKSWRKGADPLPRRRAVAVVRFRGESHVLGVDLSEGDGAVTPLPVPAS GYPMMNMDEQTSLCFAPFKDEAFNASLLRRGVRASDVACLPISLGWYGPAEENRRLIK SQCFSTEGTANFYMRPIEGLTVLVDMDTAEVLHVSDRGAGIPIPAAANTDYRIHGHSAPT PAEAQAQGRHGVQTDPATGEHRDVMVKGMAASELFVPVMDPTEAWYFKTYMDAGEYG FGLQAMPLVPLNDCPRHARYLDAVFVAADGRPVVRENMICVFERYAGDIAWRHSESPI TGMDIRESRPKVTLVARMAASVANYDYIVDWEFQMDGLVRIKVGLSGILMVKGTQYS HMNQVHQNDNMVGTLLSENVIGVHHDHFVTFRLDMDIDGADNSFVKVAMARQNTGAG ESPRKSYLKATHIVARTEKDAQNRLKLYEPSEFHIVNPMKKTRVGNPVGYKVVPAGTA ASLLDPEDPPQKRGAFTNNQIWVTPYNKTEEWAGGLFVYQSKGEDTLATWSERDRPIEN KDLVLWYTLGFHHVPCQEDFPIMPTVSSSFDLKPVNFFESNPILGQRPTQENDLPVCAA AATTS
PsDAO	Q43077 http://www.uniprot.org/uniprot/Q43077	N/A	674	>Q43077 MASTTTMRLALFSVLTLLSFHAVVSVTPLHVQHPLDPLTKEEFLAVQTIVQNKVPISNNR LAFHYIGLDDPEKDIHVLRYETHPTLVSIPRKIFVVAIINSQTHEILINLRIRSIVSDNIHNGY GFPILSVDEQSLAIKLPLKYPPFIDSVKKRGLNLSEIVCSSFTMGWFGEEKNVRTVRLDC FMKESTVNIYVRPTTGITIVADLDLMKIVEYHDRDIEAVPTAENTEYQVSKQSPPFGPKQ HSLTSHQPQGPGFQINGHSVSWANWKFHIGFDVRAGIVISLASIYDLEKHKSRRVLYKG YISELFVPYQDPTEEFYFKTFFDSGEFGFGLSTVSLIPNRDCPPHAQFIDTYVHSANGTPIL LKNAICVFEQYGNIMWRHTENGIPNESIEESRTEVNLIVRTIVTVGNYDNVIDWEFKASG SIKPSIALSGILEIKGTNIKHKDEIKEDLHGKLVSANSIGIYHDHFYIYYLDFDIDGTHNSFE KTSLKTVRIKDGSSKRKSYWTTETQTAKTESDAKITIGLAPAELVVVNPNIKTAVGNEVG YRLIPAIPAHPLLTEDDYPQIRGAFTNYNVWVIPYNRTEKWAGGLYVDHSRGDDTLAV WTKQNREIVNKDIVMWHVVGIHHVPAQEDFPIMPLLSTSFELRPTNFFERNPVLKTLSP RDVAWPGCSN
SlDAO	K4CNU3 http://www.uniprot.org/uniprot/K4CNU3	543808	721	>K4CNU3 MEGRNLFFLMSIILLIFTFLNLPSPLSHTTDQLFDCTTYSPWCSSKKPFFLRQPENSKNH YADFPNRPLDPLTGEIQKVKKHNSIAEFRKGCVLHSVVLEEPEKEVVLNWRRGRLLP PRKAAVVARALGVVHVLSVDIETGRVTRRETGSVSGVPILTIEDMISATSAPFANSDFNR SHQRGVDLADLACLPVAAGWYGKIEEKRRVIKVQSFTLKDTINFYMRPIEGLTVLLDLD TQQVIEIFDEGESIPIPKAANTDYRYSRIKKNKQKINLLKPISIEQPNGPSFTIENNHLVKW ANWEFHLKPDPRAGVISRVMFEDPDTGKMRNVMVKGFTSELFVPYMDPSDAWYFKT YMDAGEYGFGLQAMPLDPLNDCPRNAYYMDGVFVAADGTPVYRSNMICVFERYAGD IGWRHAECPITGLPIREVRPKVTLVVRMAASVANYDYIVDWEFQNDGLIRVKVGLSGIL MVKGSPYVNMNEVNQNEYLYGTLLSENIVGVHHDHYVTFHLDMDIDGPSNNSFVKVNL QKEMTSSGESPRRSYLKAVRNVAKTEKDAQIKLKLYDPSEFHVINSNKKSRVGNPVGY KVVPGGTASSLLDINEDPPQKRAAFTNNQIWVTPYNESEQWAAGLFVYQSQGDDTLAV WSDRDRAIENKDIVLWYTLGFHHIPCQEDFPIMPTVSSSFEIKPVNFFESNPILNIPPNSPK DLPICKAASA >K4CNU3 MEGRNLFFLMSIILLIFTFLNLPSPLSHTTDQLFDCTTYSPWCSSKKPFFLRQPENSKNH YADFPNRPLDPLTGEIQKVKKHNSIAEFRKGCVLHSVVLEEPEKEVVLNWRRGRLLP PRKAAVVARALGVVHVLSVDIETGRVTRRETGSVSGVPILTIEDMISATSAPFANSDFNR SHQRGVDLADLACLPVAAGWYGKIEEKRRVIKVQSFTLKDTINFYMRPIEGLTVLLDLD TQQVIEIFDEGESIPIPKAANTDYRYSRIKKNKQKINLLKPISIEQPNGPSFTIENNHLVKW ANWEFHLKPDPRAGVISRVMFEDPDTGKMRNVMVKGFTSELFVPYMDPSDAWYFKT YMDAGEYGFGLQAMPLDPLNDCPRNAYYMDGVFVAADGTPVYRSNMICVFERYAGD IGWRHAECPITGLPIREVRPKVTLVVRMAASVANYDYIVDWEFQNDGLIRVKVGLSGIL MVKGSPYVNMNEVNQNEYLYGTLLSENIVGVHHDHYVTFHLDMDIDGPSNNSFVKVNL QKEMTSSGESPRRSYLKAVRNVAKTEKDAQIKLKLYDPSEFHVINSNKKSRVGNPVGY KVVPGGTASSLLDINEDPPQKRAAFTNNQIWVTPYNESEQWAAGLFVYQSQGDDTLAV WSDRDRAIENKDIVLWYTLGFHHIPCQEDFPIMPTVSSSFEIKPVNFFESNPILNIPPNSPK DLPICKAASA

Table 3: Continuation.

Diamine oxidase	Uniprot Entry	Locus	Lenght	FASTA
TpDAO	A0A2K3NT42 http://www.uniprot.org/uniprot/A0A2K3NT42	L195_g002668	733	>A0A2K3NT42 PTPSTLRNQKPRHESDVPHHPLDPLTIQEFNEVRAVLSTHPLFKYSNSYTFNSIVLEEPDK ELVLKWKKGQPLLPRKASVVARVKCVTHTLTVDLSTSQVTNHETRSPSSGYPTMTIEEM TSVLDVPFKSDEFNRTINNRGVNLADLACLPISSGWYGTPVEENRRLIKVQCYSRKGTV NFYMKPIEGLTVLVDMDKKEVVSITDNGLNIPVANGIDTDYRYSVQKLNGELNLINPISL EQPKGPSFTVDGHLVKWANWEFHLKPDPRAGTIISRAKVRDPDTLEFRNVIYKGFTSEL FVPYMDPTDQWYFKTYMDAGEYGFGLQAMPLDPLNDCPRNAYYMDGVFASADGTPF IQPNMICIFESYAGDIAWRHAECPTTDLKVTEVRPKVTLVVRMAAAVANYDYIMDWEF QTDGLIRSKVGLSGILMVKGTTYENMNQVPDEEYLYGTLLSENIIGVIHDHFITYYLDMD IDGSDNSFVKVNIKKQQTSPGESPRKSYLKAVRKVAKTEKEAQIKLKLYDPSEFHVVNPL KKTRVGNPVGYKLVPGATAASLLDHDDPPQKRAAFTNNQIWVTPYNKSEQWAGGLLV YQSQGDDTLQVWSDRDSPIENKDIVLWYTVGFHHVPCQEDYPIMPTVSSSFDLKPVNFF ERNPILRMPPNFKEDLPVCKAQDSA
TrDAO	AQQ81871 http://www.uniprot.org/uniprot/A0A247ZT15	DAO	665	>AQQ81871 ASTTIKFALFSALTLLSLQAVVSVTPLHFQHPLDPLTKEEYSLVQKIVLHKYPKVAFHYIG LDDPEKDAILKWESFKPSVITIPRKAFVIAIINHQSHELILNLRLKSIVSDTIYTGNGFPTLS VDEQSVAIELPFKVPPFIASVKKRGLNISEVVCSTFTMGWFGEEKSTRTARLDCFMKETT VNIYVRPTIGITVVDLGLLKIVEYHDRNVEAVPTAENTEYQLSKQSPPFGPKQHSLASHQ PQGPGFQINGHSVSWANWKFHIGFDVRAGVIISLASIYDLEKHKSRRVLYKGYISELFVP YQDPSDEFYFKTFFDAGEFGFGLSTVSLIPNRDCPPNAQFIDTYIHGADGSPIPLKNAVCV FEQYNSIMWRHTETGIPNEFIEESRTEVNLIVRSVVTVGNYDNIIDWEFKASGSIKPAIAL SGILEIKGTNIKHKDEIKEDLHGTLVSENSIGIYHDHFYIYYLDLDIDGTHNSFEKTSLKTV RITDGSSKRKSYWTTETQTAKTESEAKITLGVAPGELAIVNPNKKTAVGNEVGYRLIPAIP AHPLLVEDDYPQIRGAFTNFNVWVIPYNRTEKWAGGLFVDHSHGDDTLAVWTKKNRD IVNKDIVMWHVVGIHHVPAQEDFPIMPLLSTAFELRPTNFFDRNPVLKTLSPADVVGC
ZmDAO	A0A096SGQ3 http://www.uniprot.org/uniprot/A0A096SGQ3	100383438	780	>A0A096SGQ3 MAAAKEKASVCCRDAPARVAGAPVVPMRAIAASPVGKVVALAAGGGERVSASAAGS SGAVIEEIAAVQPTTAKASSKGIPIMTRAQRCHPLDPLSAAEIAVAVATVRVAGRSPEER DSMRFVEAVLLEPEKNVVALADAYFFPPFQPSLLPRSKGSAVIPSRLPPRRARLVVYNK QSNETSIWIVELSEVHAATRGGHHRGKVISSEVIPDVQPAMDAMEYAECEATVKSYPPFI EAMKRRGVDDMDLVMVDAWCAGYYSEADAPSRRLGKPLIFCRTESDSPMENGYARP VEGIHVVVDMQNNAVIEFEDRKLVPLPPPDHLRNYTPGETRGGVDRSDVKPLIINQPEGP SFRINGYFVEWQKWNFRIGFTPKEGLVIYSVAAYVDGSRGRRPIAHRLSFVEMVVPYGDP SEPHYRKNAF DAGEDGLGKNAHSLKKGCDCLGYIKYFDAHFTNFTGGVETIENCVCLHEEDHGILWKH QDWRTGLAEVRRSRRLTVSFICTVANYEYGFYWHFYQDGKIEAEVKLTGILSLGALMP GESRKYGTTIAPGLYAPVHQHFFVARMDMAVDCKPNEAHNQVVEVNVKVESAGTHNV HNNAFYAEEKLLKSELQAMRDCDPSSARHWIVRNTRTVNRTGQPTGYRLVPGSNCLPL ALPEAKFLRRAGFLKHNLWVTQYKRGEMFPGGEFPNQNPRIHEGLPTWVKNDRPLEET DIVLWYVFGLTHIPRLEDWPVMPVEHIGFMLMPHGFFNCSPAVDVPPSSSDADVKEAE SPKAIQNSLISKL

Table 4: Orthologous identified polyamine oxidases. *The locus, length of amino acid sequences, and FASTA sequences were obtained when orthologous proteins were researched against Uniprot database.*

Polyamine oxidase	Uniprot Entry	Locus	Length	FASTA
AtPAO1	Q9FNA2 http://www.uniprot.org/uniprot/Q9FNA2	At5g13700	472	>Q9FNA2 MSTASVIIGAGISGISAAKVLVENGVEDVLILEATDRIGGRIHKQNFGDVPVELGAGWIAGVGGKESNPV WELASRFNLRTCFSDYTNARFNIYDRSGKIFPTGIASDSYKKAVDSAILKLKSLEAQCSGQVAEEAPSSP KTPIELAIDFILHDFEMAEVEPISTYVDFGEREFLVADERGYECLLYKMAEEFLVTSHGNILDYRLKLNQV VREVQQSRNGVVVKTEDGSVYEANYVIVSASIGVLQSDLLSFQPLLPRWKTEAIQKCDVMVYTKIFLKF PQCFWPCGPGQEFFIYAHEQRGYFTFWQHMENAYPGSNILVVTLTNEQSKRVEAQSDQETIMKEAMSV LRDMFGATIPYATDILVPRWWNNRFQRGSYSNYPMISDNQLLQNIKAPVGRIFFTGEHTSEKFSGYVHG GYLAGIDTSKSLLEEMKQSLLLQPLLAFTESLTLTHQKPNNSQYTNVKFISGTS
AtPAO2	Q9SKX5 http://www.uniprot.org/uniprot/Q9SKX5	At2g43020	490	>Q9SKX5 MESRKNSDRQMRRANCFSAGERMKTRSPSVIVIGGGFGGISAARTLQDASFQVMVLESRDRIGGRVHT DYSFGFPVDLGASWLHGVCKENPLAPVIGRLGLPLYRTSGDNSVLYDHDLESYALFDMDGNQVPQELV TQIGVTFERILEEINKVRDEQDADISISQAFSIVFSRKPELRLEGLAHNVLQWYVCRMEGWFAADAETIS AKCWDQEELPGGHGLMVRGYRPVINTLAKGLDIRVGHRVTKIVRRYNGVKVTTENGQTFVADAAVIA VPLGVLKSGTIKFEPKLPEWKQEAINDLGVGIENKIILHFEKVFWPKVEFLGVVAETSYGCSYFLNLHKA TGHPVLVYMPAGQLAKDIEKMSDEAAANFAVLQLQRILPDALPPVQYLVSRWGSDVNSMGSYSYDIV GKPHIDLYERLRVPVDNLFFAGEATSSSFPGSVHGAYSTGLMAAEDCRMRVLERYGELDLFQPVMGEE GPASVPLLISRL
AtPAO3	Q9LYT1 http://www.uniprot.org/uniprot/Q9LYT1	At3g59050	488	>Q9LYT1 MESGGKTNRQLRKAICVSTDEKMKKKRSPSVIVIGGGMAGISAARTLQDASFQVVVLESRDRIGGRVH TDYSFGFPVDLGASWLHGVCKENPLAAVIGRLGLPLYRTSGDNSVLYDHDLESYALFDKAGNQVSQEL VTKVGENFEHILEEICKVRDEQDEDMSIAQAFSIVFKRNPELRLEGLAHNVLQWYLCRMEGWFAADAE TISAKCWDQEELPGGHGLMVRGYRPVINTLSKGLDIRLSHRITKISRRYSGVKVTTEKGDTFVADAAVI ALPLGVLKSGMTFEPKLPQWKQEAINDLGVGIENKIILNFDNVFWPNVEFLGVVAETSYGCSYFLNLHK ATSHPVLVYMPAGQLARDIEKKSDEAAANFAFSQLQKILPDASSPINYLVSRWGSDINSLGSYSYDIVNK PHDLYERLRVPLDNLFFAGEATSSSYPGSVHGAYSTGVLAAEDCRMRVLERYGELEHEMEEEAPASVP LLISRM
AtPAO4	Q8H191 http://www.uniprot.org/uniprot/Q8H191	At1g65840	497	>Q8H191 MDKKKNSFPDNLPEGTISELMQKQNNVQPSVIVIGSGISGLAAARNLSEASFKVTVLESRDRIGGRIHTD YSFGCPVDMGASWLHGVSDENPLAPIIRRLGLTLYRTSGDDSILYDHDLESYGLFDMHGNKIPPQLVTK VGDAFKRILEETEKIRDETANDMSVLQGISIVLDRNPELRQEGMAYEVLQWYLCRMEAWFAVDANLIS LKCWDQDECLSGGHGLMVQGYEPVIRTIAKDLDIRLNHRVTKVVRTSNNKVIVAVEGGTNFVADAVIIT VPIGVLKANLIQFEPELPQWKTSAISGLGVGNENKIALRFDRAFWPNVEFLGMVAPTSYACGYFLNLHK ATGHPVLVYMAAGNLAQDLEKLSDEATANFVMLQLKKMFPDAPDPAQYLVTRWGTDPNTLGCYAYD VVGMPEDLY PRLGEPVDNIFFGGEAVNVEHIQGSAHGAFLAGVSASQNCQRYIFERLGAWEKLKLVSLMG NSDILETATVPLQISRM
AtPAO5	Q9SU79 http://www.uniprot.org/uniprot/Q9SU79	At4g29720	533	>Q9SU79 MAKKARIVIIGAGMAGLTAANKLYTSSNNTFELSVVEGGSRIGGRINTSEFSSEKIEMGATWIHGIGGSP VYRIAKETGSLVSDEPWECMDSTIDKAKTFAEGGFEIEPSIVESISGLFTALMELAQGKEISQSDADLSRL AHIYETATRVCSKGSSTSVGSFLKSGFDAYWDSISNGGEEGVKGYGKWSRKSLEEAIFTMFSNTQRTYT SADELSTLDFAAESEYQMFPGEETIAKGYLSVIHHLASVLPQQVIQLNRKVTKIEWQSNEVKLHFSDGS VVFADHVIVTVSLGVLKAGIETDAELFSPPLPDFKSDAIRRLGVGVVNKLFVEMSQRKFPSLQLVFDRLED SEFRFVKIPWWMRRTATITPFHSNSKVLLSWFAGKEALELEKLTDEEIKDAVMTTISCLTGKEVKNDTAK PLTNGSLNDDDEAMKITKVLKSKWGSDPLFRGSYSYVAVGSSGDDLDAMAEPLPKINKKVGQVNGHD QAKVHELQVMFAGEATHRTHYSTTHGAYYSGLREANRLLKHYKCNF
GsPAO1	A0A0B2QXT4 http://www.uniprot.org/uniprot/A0A0B2QXT4	glysoja_032252	578	>A0A0B2QXT4 MVVKKPRIVIIGAGMAGLTAAKLYTATASKDLFELCVVEGGTRIGGRINTSEFGGDRIEMGATWIHGIG GSPIHKIAQEIHSLHSDQPWECMDGNTDEAITIAEGGFHLHPSIVDPITKLFNTLMEYSQGKKTLTEATSK GAELESYHKLAALAAKLASASASAYNNNNLSVGSFLRQGLEAYQVSKEQEEVKGCGNWSRKLLEEAIF AMHENNQRTYTSADDLLTLDYGAESEYRMFPGEEITIAKGYLSIIESLASVLPPGFVQLGRKVTRIEWQL DDEKRKGAVENGCCSSRPVKLHFCDGSVMSADHVIVTVSLGVLKAASDDDDDSGMFCPPLPPSKTEA ISRLGFGVVNKLFMQLSPTHEFGKHGNIHSNKGFPFLQMVFHSPQSIMGHKDPWWMRRTATLFPYYN NSSVLLSWFVGEEALALESLKDEEIINGVSSTVSCTLQHSQWQKGSTSSHKLCNGNVNSEERSHDQNEV KFSKVMKSKWGTDPLFLGSYSYVAVGSSGDDLDTMAEPLPKDNSCQPPAASSPLQILFAGEATHRTHY STTHGAYFSGLREANRLLQHYHWIYNN
HvPAO1	Q93WM8 http://www.uniprot.org/uniprot/Q93WM8	bpao1	495	>Q93WM8 MKPTTATAALVLALTLAHHASIAAAAGPRVIIVGAGMSGISAGKRLSEAGITDLVILEATDRVGGRMHK QSFGGINVEVGANWVEGVNGAGRMNPIWPLVNSTLKLKNFRSDFDGLADNVYKENGGVYERAYVQK RLDRWGEVEEGGEKLSAKLRPSGQDDMSILAMQRLNDHLPNGPTSPVDMVLDYFKHDYEFAEPPRVTS SLQNVVPLATFTDFGDDVYFVADQRGYEAVVYYLAGQYLKADKSGNIVDPRLQLNKVVTEISHSGGGV TVRTEDAKVYKADYVMVSTSVGVLQSDLIQFKPRLPTWKVLSIYQFDMAVYTKIFVKFPRKFWPQGKG REFFLYASSRRGYGVWQEFEAQVPDANVLLVTVTDDESRRIEQQSDNQTKAEIVEVLRSMFPGEDVP DATDLVPRWWSDRFYRGTFSNWPGVNRYEYDQLRAPVGRVYFTGEHTSEHYNGYVHGAYLSGIDSA DILIKCAQKRMCKYHSPGKFD
HvPAO2	Q93WC0 http://www.uniprot.org/uniprot/Q93WC0	bpao2	503	>Q93WC0 MKPSFVTAIAALLLIAAQHASIVAAGKGPRVIIVGAGMSGISAGKRLWDAGVRDLLILEATDRVGGRMH KHNFGGLNVEIGANWVEGLNGDKTNPIWPMVNSTLKLRNFYSDFDGVVANVYKESGGLYDEEFVQKR MDRADEVEELGGKFAAKLDPSGRDDESLAMQRLFNHQPNGPTIPVDMALDYYKYDYEFAEPPRVTSL QCITEPTATFADFGDDANFVADQRGFETIIYHIAGQYLRSDKSGNIDPRVKLNKVVREQSYNDKGVVVTT EDNSAYSADYVMVSTSLGVLQSDLIQFKPQLPAWKIMAIYRFDMAVYTKIFLKFPKKFWPTGPGKQFF VYASSRRGYYGMWQSFEKEYPGANVLLVTVTDVESRRIEQQPDNVTMAEAVGVLRNMFPDRDVPDA TDIYVLRWSNRFFKGSYSNWPIGVNRYEYDQLRAPVGGRVYFTGEHTSEHYNGYVHGAYLAGIHSADI LMNKASNNVDFKVRPKYDDELKAEAK
MdPAO	A0A1C8M5I8 http://www.uniprot.org/uniprot/A0A1C8M5I8	N/A	493	>A0A1C8M5I8 MDPKDPFSAVLFDGSVSSHIERQQNACPSVIVIGGGISGIAAARILHDASFKVLLLESRDRLGGRIHTDYS FGCPVDMGASWLHGVCNENPLAPLJRRLGLTLYRTSGDDSVLYDHHLESFALFDTDGHQVPQKMVVE VGDTFKKILRETEKVRNEHTDDMSVSQAISVVMDRHPELRQNGLAHEVLQWCICRMEAWFAADADAI SLKCWDQDEHFVLSGGHGLMVQGYDPHIKALAKDHDVRLNHRVTKILNQSNKVMVTIEDGRNFIADAAIT VPHGILKAKLIEFVPQLPEWKVAAISDLGVGNENKVAPRFEKVFWPNVELLGIVAPNSYACGYFLNLHK ATGHPVLVYMAAGRFAYDLEKLTDDAAVNLVMLQLKKMLPDATEPVQYLLSRWGTDLNSLGCYALD LVGKPGDIYER LRAPLGNLFFGGEAVSMDHQGSVHGAYSAGVMAAENCQQHLLKKLGNLETPQHAYLMEEVLEAATVP LQISRM
OsPAO1	Q5NAI7 http://www.uniprot.org/uniprot/Q5NAI7	Os01g0710200	512	>Q5NAI7 MVAKKPRVVVVGAGISGLAAAHRLCGAGGDRFEVAVVEAGDRVGGRILTSEFAGHRVEMGATWVQG VVGSPVYALARDAGALGEEEGRGLPYERMDGFPDRVLTVAEGGEVVDADTVAGPIEELYRGMMEAAR AGEAGGGGGVEEYLRRGLRAYQAARSAGGGGGGGKELEEVDEALLAMHINRERTDTSADDLGDLDLT AEGEYRDFPGEHVTPGGYSRVVERLAAALPPGTVRLGLRRLKWGGTPVRLHFADGAPPLTADHVIL TVSLGVLKASLGNKDTAGVGAAAIFDPPLPPFKREAVARLGFGVVNKLFMEVEAVAPSEPEDVAGVQ PAAAGFPFLHMAFRGHVSKIPWWMRGTESICPVHAGSTVALAWFAGREAAHLESLPDDDVIRGAHAT LDSFLPAAPRWRVRRIKRSGWATDPLFLGSYSYVAVGSSGDDLDRMAEPLPRGPDAAADERPPSPRLL FAGEATHRTHYSTTHAAYLSGVREANRLLQHYRGGANHTT
OsPAO2	Q0DUC7 http://www.uniprot.org/uniprot/Q0DUC7	Os03g0193400	351	>Q0DUC7 MDSLPNGPSSPVDMVVDYYLYDYEYAEPPRVTSLQNAVPQRTFSDFGDDVYFVADKRGYESVVHYLA GQYLNTDDSGNVADPRLQLNKVVREISYSSSGVTVKTEDGSVYQADYRHGLCQLGSPAERSYTVQATA ACKFRVLCPARFSRIASDRCVLHVFDQKWKILAIYEFDMAVYTKIFVKFPKRFWPEGEGREFFLYASTR RGYYGIWQEFEKQVPDSNVLLVTVTDKESRRIEQQSDNQTKAEIMEVLRNMFPDQDVPDATDILVPRW WSNRFYKGTFSNWPIGVNRYEYDQLRAPIERVYFTGEHTSEYYNGYVHGGYLAGIDSAEILIDCAQNQ MCKYHVQGKYD

Table 4: Continuation.

Polyamine oxidase	Uniprot Entry	Locus	Length	FASTA
OsPAO3	Q7X809 http://www.uniprot.org/uniprot/Q7X809	Os04g0623300	484	>Q7X809 MANNSSYGENVRRKSHTPSAIVIGSGFAGIAAANALRNASFEVVLLESRDRIGGRIHTDYSFGFPVDLGA SWLHGVCEENPLAPIIGRLGLPLYRTSGDDSVLFDHDLESYALYDTKGHQVPQELVEKIGKVFETILEETG KLREETKEDISIAKAIAIVMERNPHLRQEGIAHDVLQWYLCRMEGWFATDADAISLQGWDQEVLLPGG HGLMVRGYRPVINTLAKGLDIRLGHRVVEIVRHRNRVEVTVSSGKIFVADAAAVIAVPLGVLKANTIKFE PRLPEWKEEAIRELSVGVENKIILHFSEVFWPNVEFLGVVSSTTYGCSYFLNLHKATGHPVLVYMPAGR LACDIEKLSDEAAAQFAFSQLKKILPNAAEPHHYLVSHWGSDENTLGSYTFTDVGKPRDLYEKLRIPVDN LFFAGEATSVQYTGTVHGAFSTGLMAAEECRMRVLERFRELDMLEMCHPAMGEQTATVSVPLLISRL
OsPAO4	Q7XR46 http://www.uniprot.org/uniprot/Q7XR46	Os04g0671200	487	>Q7XR46 MDPNSLKTGGLLLPTIERQCASPPSVIVIGGGISGVAAARALSNASFEVTVLESRDRVGGRVHTDYSFGC PIDMGASWLHGVCNENSLAPLIGYLGLKLYRTSGDNSVLYDHDLESYALFDKAGHQVSKETVAKVEET FERILDETVKVRDEQEHDMPLLQAISLVLERHPHLKLDGLQYEVLQWCICRLEAWFATDVDNISLKNW DQEHVLTGGHGLMVNGYYPIIQALAQGLDIRLNQRVTKIARQFNGVTVTTEDGTSYSADACIITVPLGVL KANIIKFEPELPSWKSSAIADLGVGIENKIAMHFDTVFWPNVEVLGMVGPTPKACGYFLNLHKATGNPV LVYMAAGRFAQEVEKLSDKEAVDLVMSHLKKMLPDATEPTKYLVSRWGSDPNSLGSYSCDLVGKPAD VSAREFAAP VENLYFAGEAASADHSGSVHGAYSSGIAAADECRKRILMQKGIPDLVQVKAYEEMAGVIAPLQICRT
OsPAO5	Q0J954 http://www.uniprot.org/uniprot/Q0J954	Os04g0671300	492	>Q0J954 MDQPSNGFAAGGLFLRHIDGQNASPPSVIVIGGGISGIAAARALSNASFKVTLLESRDRLGGRVHTDYSF GCPIDMGASWLHGVCNENSLAPLIRLLGLRLYRTSGDNSVLYDHDLESYALFDKDGRQVPQEIVTKVGE TFEKILKETVKVRAEHEDDMPLIQAISIVLRHNPHLKLDGLQYEVLQWCICRLEAWFATDVDNISLKNW DQEHVLTGGHGLMVHGYDPVIKALAQDLDIRLNHRVTKIHQRVNKTIVCVEDGTSFVADAAIITVPLGVL KANIIKFEPELPDWKLSSISDLGIGIENKIALRFNSVFWPNVEVLGRVAPTSNACGYFLNLHKATGHPVLV CMVAGRFAYEFEKLSDEESVNFVMSQLKKMLPGATEPVQYLVSRWGTDPNSLGSYSCDLVGKPADLY ERFCAPVGNLFFAGEAACIDHSGSVHGAYSSGIVAAEDCRRHLSTQLGISDLFQVGKIIMREEM TEVMVPFQISRL
OsPAO6	Q0J291 http://www.uniprot.org/uniprot/Q0J291	Os09g0368200	540	>Q0J291 MEENKVSMLLLDGWWHMVLMNVMLINVFLDMGRYNFFGSMEPHRFVWLKNEWIEKRNVLKVHRY RLFMTLKLNLRSEIRKGKHLTCISAGKRIWEAGIADVLILEATDRIGGRMHKQSFAGVNVEIGANWVEG VNGEKKNPIWPIVNSTLKLRSFRSDFDSLAQNVYKDGGLCDEAYVQKRMDRADEVDKSGENLSATLHP SGRDDMSILSMQRLNDHLPNGPSSPVDMAVDYFTYDYEFAEPPRVTSLQNTVPLPTFTDFGDDTYFVA DQRGYESVVHHLAGQYLNADKSGNIADARLKLNKVVREISYSSTGVTVKTEDNSTYQADYVMVSASLG VLQSDLIQFKPQLPSWKILAIYQFDMAVYTKIFVKFPKKFWPEGAGREFFLYASTRRGYYGVWQEFEKQ YPDANVLLVTVTDEESRRIEQQPDSQTKAEIMEVVRCMFPDEDVPDATDILVPRWWSDRFFRGSFSNW PIGVSRYEYDQLRAPVGRVYFTGEHTSERYNGYVHGAYLAGIDSAEILINCAQKMCKYNVGGKHG
OsPAO7	Q0J290 http://www.uniprot.org/uniprot/Q0J290	Os09g0368500	474	>Q0J290 MTKPTTMAIFLSIVLLSMAQLPSLVAGTGRPRVIIIGAGISGISAGKRLSEAGITDILILEATDHIGGRMHK QRFAGVNVEIGANWVEGVNGEKMNPIWPIVNSTLKLRNFLSDFDSLAQNVYKDGGLCDAAYVQKRIDL ADEADKSGENLSATLHPSGRDDMSILSMQRLNNHLPNGPSSPVDMVVDYFTYDYEFAEPPRVTSLRNT VPLPTFTDFGDDNYFVADQRGYEAVVYYLAGQYLEADKSGNIVDARLQLNKVVREISYSSTGVTVKTE DNSTYQADYVMVSASLGVLQSDLIQFKPQLPSWKILAIYQFDMAVYTKIFVKFPKKFWPEGAGREFFLY ASTRRGYYGVWQEFEKQYPDANVLLVTVTDEESRRIEQQPDSQTKAEIMEVVRSMFPDEDVPDATDIL VPRWWSDRFFQGSFSNWPIGVSRYEHDQLRAPVGRVYFTGEHTSERYNGYVHGAYLAGIYA
SmPAO	A6U6Y8 http://www.uniprot.org/uniprot/A6U6Y8	Smed_0562	549	>A6U6Y8 MQADFVIIGSGSAGSALAYRLSEDGANSVVVLEFGGSDVGPFIQMPAALAWPMSMNRYNWGYLSEPE PNLNNRRITAPRGKVIGGSSSINGMVYVRGHSEDFDRWEELGAKGWAYADVLPYYKRMEHSHGGEEG WRGTDGPLHVQRGPVKNPLFHAFIEAGKQAGFEVTEDYNGSKQEGFGLMEQTTWRGRRWSAASAYLR PALKRPNVELVRCTARKIVIENGRATGVEIERGGRTEVVRANREVIVSASSFNSPKLLMLSGIGPAAHLQ EMGIDVKADRPGVGQNLQDHMEFYFQQVSTKPVSLYSWLPWFWQGVAGAQWLFFKRGLGISNQFES CAFLRSAPGVKQPDIQYHFLPVAISYDGKAAAKSHGFQVHVGYNLSKSRGDVTLRSSDPKADPVIRFNY MSHPEDWEKFRICVRLTREIFGQKAFDLYRGPEIQPGEKVRTDEEIDAFLREHLESAYHPCGTCKMGA KDDPMAVVDPETRVIGVDGLRVADSSIFPHITYGNLNAPSIMTGEKAADHILGKQPLARSNQEPWINPR WAVSDR
TpPAO1	A0A2K3P3K3 http://www.uniprot.org/uniprot/A0A2K3P3K3	L195_g006434	751	>A0A2K3P3K3 MATPSSDESVSRRASLRKKVNLRNYNEGLIDDDFFDEKLDTSLKKKKGKSKEDLQKEANTESMIAFSLG FPIDALLENEIRAGVVGQLGGKEQNDYIVVRNHILALWRGNVRRWLTRGRVRETVSNEFAHLINNAYDF LLYNGYINFGVLPSFTSQLPEATEGTVVIGAGLAGLAAARQLISFGYKVAVLEGRNRPGGRVYTQKIGK EGNFAAMDLGGSVITGIHANPLAVLARQLSIPLHTVRANCPLYKPNGIPVDKEIDSSVEFVFNKLLDKV MELRKIMGGFSSDTSLGSVFETLKSLYGVAQSTDEKQLLDWHLANLEYANAGCLSNISAAHWDQDDPF EMGGDHCFLAGGNWRLIKAMCEGVPIFYGKTVNTIRYGDEGVEIIAGDQVFQADFALCTVPLGVLKKK AISFQPELPARKLESIERMGFGLLNKVAMVFPHVFWGEDLDAFGCLNEKSHDRGEFFLFYGYHTVSGGS GLIALVAGEAAQTFETTDPTILLHRVLTTLKGIFQPKGITVPDPIQSICTRWGSDPLSYGSYSHVSVQSSGK DYDILAENVGNRLFFAGEATCRQYPATMHGAFMSGLREASRIYHLTRVQQAYPKKALSKNIEQSTSILL NLFKRPDLEFGNFAFVFDLSSEDMQSKAILQLTFGGTEENYKDILTCYPNTTKLPLQLYTIISREQVHQM QETTGDENRLSYLTKNLGLKLMGINALLITGNTVIDNIAGFRKTIKRYRTVITFQQPYNQ
TpPAO2	A0A2K3JRK6 http://www.uniprot.org/uniprot/A0A2K3JRK6	L195_g050004	212	>A0A2K3JRK6 YLPVINTLAKGLDIRLGHRVTNVVRRYNGVKVTVENGKTFHADAAIVAVPLGVLKAKRISFEPKLPDWK EAAIAELGVGLENKIILHFENVFWPNVEFLGVVADTSYGCSYFLNLHKAAXHPVLVYMPSGRLAKDIEK MSDEAAANFAFVQLKKILPDASSPIQYLVSRWGSDINSLGEATSMCYPGSVHGAYSTGMMAAEDCRM RVLERYG
TrPAO	A0A286QIQ2 http://www.uniprot.org/uniprot/A0A286QIQ2	PAO	486	>A0A286QIQ2 MESRIKTNSRKGFYYTNVDQQSERSPSVIVIGGGMAGIAAARALHDASFQVVLLESRDRLGGRIHTDYS FGFPVDLGASWLHGVCNENPLAPLIGRLGLPLYRTCEDNSVLYDHDLESYALFDMDGNQVPQELVTEV GKIFEMILQETDNVRQEFSEDMSILHALSIVFERKPELRLKGLSHKVLQWVLCRMEGWFAADADSISLK CWDQEELLPGGHGLMVRGYLPVIHTLAKGLDIRLGHRVTKIVRYNQVKVTTENGKTFVADAAIAVPL GVLKANVIKFEPKLPEWKEAAIADIGVGVENKIILHFKNVFWPNVEFLGVVSETSYGCSYFLNLHKAAG HNVLVYMPAGRLAKDIEKMSDEAAADFAFAQLKKILPDASSPIQYLVSRWGTDINSLGSYSYDAVGKP HGLYERLRVPVDNLFFAGEATSVLYTGSVHGAYSTGTMAAEDCRMRVLERYGELDIFQPVLEEGSVIPL LISRI
ZmPAO	O64411 http://www.uniprot.org/uniprot/O64411	PAO	500	>O64411 MSSSPSFGLLAVAALLLALSLAQHGSLAATVGPRVIVVGAGMSGISAAKRLSEAGITDLLILEATDHIGG RMHKTNFAGINVELGANWVEGVNGGKMNPIWPIVNSTLKLRNFRSDFDYLAQNVYKEDGGVYDEDYV QKRIELADSVEEMGEKLSATLHASGRDDMSILAMQRLNEHQPNGPATPVDMVVDYYKFDYEFAEPPR VTSLQNTVPLATFSDFGDDVYFVADQRGYEAVVYYLAGQYLKTDDKSGKIVDPRLQLNKVVREIKYSP GGVTVKTEDNSVYSADYVMVSASLGVLQSDLIQFKPKLPTWKVRAIYQFDMAVYTKIFLKFPRKFWPE GKGRFFFLYASSRRGYYGVWQEFEKQYPDANVLLVTVTDEESRRIEQQSDEQTKAEIMQVLRKMFPGK DVPDATDILVPR WWSDRFYKGTFSNWPVGVNRYEYDQLRAPVGRVYFTGEHTSEHYNGYVHGAYLSGIDSAEILINCAQ KKMCKYHVQGKYD