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# D-amino acids metabolism reflects the evolutionary origin of higher plants and their adaptation to the environment

Jaime Porrás-Dominguez<sup>1</sup> | Jérémy Lothier<sup>1</sup> | Anis M. Limami<sup>1</sup>  |  
Guillaume Tcherkez<sup>1,2</sup> 

<sup>1</sup>Institut de Recherche en Horticulture et Semences, INRAE, Université d'Angers, Beaucouzé, France

<sup>2</sup>Research School of Biology, Australian National University, Canberra, Australia

## Correspondence

Guillaume Tcherkez  
Email: [guillaume.tcherkez@anu.edu.au](mailto:guillaume.tcherkez@anu.edu.au)

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## Abstract

D-amino acids are the D stereoisomers of the common L-amino acids found in proteins. Over the past two decades, the occurrence of D-amino acids in plants has been reported and circumstantial evidence for a role in various processes, including interaction with soil microorganisms or interference with cellular signalling, has been provided. However, examples are not numerous and D-amino acids can also be detrimental, some of them inhibiting growth and development. Thus, the persistence of D-amino acid metabolism in plants is rather surprising, and the evolutionary origins of D-amino acid metabolism are currently unclear. Systemic analysis of sequences associated with D-amino acid metabolism enzymes shows that they are not simply inherited from cyanobacterial metabolism. In fact, the history of plant D-amino acid metabolism enzymes likely involves multiple steps, cellular compartments, gene transfers and losses. Regardless of evolutionary steps, enzymes of D-amino acid metabolism, such as D-amino acid transferases or racemases, have been retained by higher plants and have not simply been eliminated, so it is likely that they fulfil important metabolic roles such as serine, folate or plastid peptidoglycan metabolism. We suggest that D-amino acid metabolism may have been critical to support metabolic functions required during the evolution of land plants.

## KEYWORDS

aminotransferase, metabolic pathways, racemase, signalling, stereoisomer

## 1 | INTRODUCTION

The 20 major proteinogenic amino acids have been selected by evolution for their specific chemical properties, which are essential for protein structure and function. They share a crucial chemical feature, the stereochemical configuration L (as opposed to D). This feature has probably been selected early on during the chemical evolution of living organisms. For example, it has been demonstrated

that L-amino acids can catalyse the synthesis of D-carbohydrates in solution, suggesting stereospecificity appeared first in amino acids and preconditioned sugar metabolism to use D stereoisomers (Breslow & Cheng, 2010). Having said that, the most intriguing feature in amino acid chemistry is the natural occurrence of their symmetrical counterparts, that is, D-amino acids. They are mostly present in prokaryotic micro-organisms where they participate in cell wall (peptidoglycan) structure. However, D-amino acids can also be

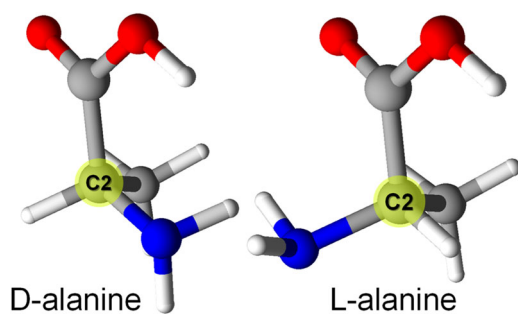
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found in plants, often to easily detectable amounts. What is their biological function and why enzymes of D-amino acid metabolism can be found in plants? To date, this question has not been answered satisfactorily. In this brief Review, we compare enzymes across different lineages and propose a scenario to explain how enzymes of D-amino acid metabolism have been kept by plants, providing insights on plant cell evolutionary origin.

## 2 | THE PARADOX OF D-AMINO ACIDS IN PLANTS

Amino acids are not only a component of proteins or peptides in living organisms, but also act as a free form as signal molecules or precursor of nonprotein molecules. D-amino acids (Figure 1), which are not incorporated into proteins, have different functions especially in bacteria in the synthesis of antibiotics, surfactants, various polymers and crucially, peptidoglycans, which are rich in D-alanine and D-glutamate (Kolukisaoglu, 2020). D-amino acids can be taken up from the environment, a D-amino acid transaminase (DAT) transferring the amino group to a molecule of, typically, pyruvate, yielding D-alanine. Also, D-alanine D-alanine ligase (DDL) is used to produce a D-alanine dimer which is in turn transferred onto the peptidoglycan backbone by MurF (Egan et al., 2020). Many prokaryotes possess racemases (glutamate racemases [GR] and alanine racemases [AR]) and thus D-glutamate is produced via racemization of L-glutamate by GR. D-glutamate is then added to the peptidoglycan backbone by MurD (Egan et al., 2020). The resulting complicated peptidoglycan structure protects the cell, typically against conventional L-peptidases, but also forms a rigid structure that helps cell division. Unsurprisingly, engineering of antibiotics has been focused on the design of drugs targeting Mur proteins or DDL, to inhibit peptidoglycan biosynthesis (Lu et al., 2020). In plants, D-amino acid metabolism could be linked to organelles: considering



**FIGURE 1** 3D representation of L and D-amino acids, with the example of alanine. L and D enantiomers are like mirror images of each other (here shown with fixed CH<sub>3</sub> and COOH positions), with an asymmetric centre at C2 (highlighted in yellow). This representation highlights the fact that with fixed COOH and CH<sub>3</sub> positions, the orientation of the NH<sub>2</sub> group for transaminase activity is opposite and thus impacts on active site geometry. Grey, blue, white and red balls stand for carbon, nitrogen, hydrogen and oxygen, respectively. [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1111/pce.14826)]

the cyanobacterial origin of plastids, biochemical reactions involving D-amino acids could be simply viewed as remnants of cyanobacterial metabolism, perhaps reflecting ancestral peptidoglycan chemistry (Aliashkevich et al., 2018; Gincel & Shoshan-Barmatz, 2004). However, the implication of D-amino acids in plant cells seems to be dual:

On the one hand, D-amino acids can be detrimental since their addition to culture medium may inhibit plant cell growth (Forsum, 2016; Forsum et al., 2008). In effect, growth of *Arabidopsis* seedlings has been found to be inhibited by D-amino acid addition (including D-alanine, D-aspartate and D-glutamate), with the exception of D-isoleucine which had no visible effect—while D-glutamine, D-asparagine, D-lysine and D-valine have an effect only at high (10 mM) concentration (Gördes et al., 2011).

On the other hand, D-amino acid metabolism seem to be involved in several processes (detailed below) and furthermore, enzymes involved in D-amino acid metabolism have been found, like DAT in *Arabidopsis*. This plastid enzyme exhibits amino group transfer activity from D-aspartate to pyruvate (or  $\alpha$ -ketoglutarate), yielding D-alanine (D-glutamate, respectively), along with aminotransferase activity with other D-amino acids (Funakoshi et al., 2008). D-Ala is a component of proto-peptidoglycan produced via an enzyme located in chloroplasts in mosses, where the synthesis of the D-alanine dimer is important for both the structure and division of chloroplasts (Hirano et al., 2016). Recently, evidence for the presence of (proto) peptidoglycan in higher plants plastids has been provided (discussed further below) (Tran et al., 2023). In addition, higher plants have genes encoding for Mur enzymes, DDLs and DATs (Garcia et al., 2008). Taken as a whole, higher plant genomes have genes encoding for enzymes of D-amino acid metabolism and therefore, D-amino acids likely fulfil important metabolic functions, despite the detrimental effect of some of them.

## 3 | D-AMINO ACIDS AND PLANT RESPONSES TO THE ENVIRONMENT

Soil micro-organisms can involve D-amino acid metabolism and thus D-amino acids are present in the rhizosphere and in fact, plants can use some D-amino acids as a nitrogen source (reviewed in Aliashkevich et al., 2018). For example, the incorporation of D-amino acids has been found using several *Arabidopsis* ecotypes (Gördes et al., 2013). Conversely, plants can produce D-amino acids (see below for the description of enzymes involved therein), which could be used by soil micro-organisms, although exudation probably occurs at a low rate (Hener et al., 2018). Also, D-amino acids are believed to be involved in signalling pathways or regulations in the response to the environment; for example, D-methionine interacts with ethylene signalling (reviewed in Kolukisaoglu, 2020). In fact, D-methionine addition competes with 1-aminocyclopropan-1-oic acid (ethylene precursor) for malonylation (and thus D-methionine addition favours ethylene production) (Suarez et al., 2019). Also, added D-cysteine seems to be broken down to H<sub>2</sub>S, which in turn stimulates

tolerance to heavy metals, regulates stomatal closure or impacts on ethylene (Li et al., 2018). It should also be noted that metabolic changes caused by environmental conditions could favour the biosynthesis of D-amino acids. For example, potassium deficiency leads to an enhancement of the C<sub>5</sub>-branched acid pathway, which could potentially generate D-amino acids: C<sub>5</sub>-branched intermediates itaconate, citraconate and citramalate (Cui, Abadie, et al., 2019; Cui, Davanture, et al., 2019) can easily form either L- or D-methylmalate, which could in turn be converted into L- or D-aspartate, respectively. However, in practice, the impact of environmental conditions on D-amino acid accumulation is not very well known since the vast majority of metabolomics technologies do not separate D and L enantiomers.

## 4 | SPECIALISED D-AMINO ACID METABOLISM IN PLANTS

At least four cases where D-amino acid metabolism has been documented in plants can be described: D-(cyclo)serine, 4-amino deoxychorismate metabolism (folate metabolism), D-tryptophan utilisation and peptidoglycan chemistry.

### 4.1 | D-(cyclo)serine metabolism

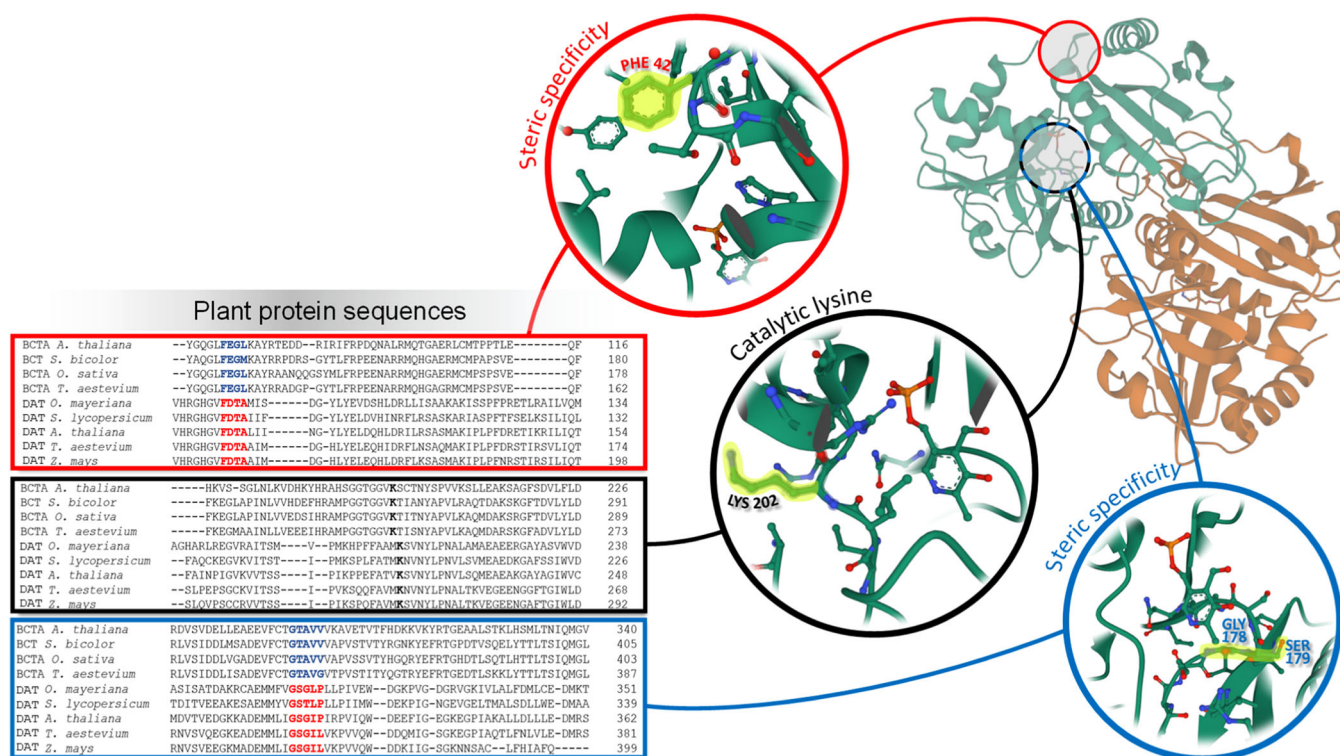
D-serine is a known inhibitor of several DATs and as such, it is undetectable in most prokaryotes since it would inhibit peptidoglycan biosynthesis (Martinez del Pozo et al., 1989). However, some bacteria of the genus *Streptomyces* produce antibiotics based on D-serine, such as D-cycloserine. D-cycloserine is a strong inhibitor peptidoglycan synthesis, targeting not only DATs, but also ARs and DDLs (Noda et al., 2004). D-cycloserine is also an inhibitor of moss and higher plants DDLs, with consequences on chloroplast morphology and division (Katayama et al., 2003; Tran et al., 2023). D-serine synthesis in *Streptomyces* involves racemisation by serine racemases (SRs) which are absent in most bacteria (Yoshimura & Esak, 2003). D-cycloserine has a very different structure from D-serine, the term "D-cycloserine" coming from the fact its biosynthesis starts with L-serine. L-serine is first acetylated, then a molecule of hydroxyurea is added onto the OH group producing O-ureido-L-serine, then converted by a specific racemase to O-ureido-D-serine; after decarboxylation and cyclisation, it yields D-cycloserine (Svensson & Gatenbeck, 1982). In *Streptomyces*, the detrimental effect of D-cycloserine is avoided via resistant AR and DDL forms, that is, unaffected by usual D-cycloserine concentrations (Noda et al., 2004).

SRs are only present in plants and animals where D-serine is produced in situ to activate ionic glutamate-like receptors, which in turn control Ca<sup>2+</sup> fluxes. D-serine has also been found to play a role in the amoeba *Dictyostelium discoïdum* (Ito et al., 2018). In plants, this process is believed to be involved in pollen tube formation (Forsum et al., 2008; Michard et al., 2011). Interestingly, SRs can catalyse two reactions, serine racemisation and

### BOX 1

While BCATs contain a Phe-Glu-Gly (FEG) conserved motif in the N-ter region located near the lysine catalytic residue and Gly-Thr-Ala-Val-Val in the C-ter region, the DATs conserved motif is Phe-Asp-Thr/Val (FD<sup>T/V</sup>) and Gly-Ser-Gly-Ile/Leu-Pro/Leu in C-ter region (Figure 2). For example, the glycine residue has been replaced by threonine in *A. thaliana* DAT and valine in *Bacillus* DAT (Walton & Chica, 2013). Interestingly, both residues are geometrically similar. Both threonine and valine form a steric hindrance to avoid binding of L-amino acids and match  $\alpha$ -keto acid geometry, facilitating amino group transfer following the D-configuration. In the case of BCATs, the residue conferring chiral specificity and creating steric hindrance is also a threonine but quite understandably, it is separate from the catalytic FEG motif: because of the L-configuration of products, it faces the glycine residue of the FEG conserved motif. Its biochemical role remains the same, that is, it avoids binding of D-amino acids and creates an adapted geometry for amino group transfer following the L-configuration. Systematic analysis of available sequences in Uniport carrying the FD<sup>T/V</sup> conserved motif shows that this group of proteins are found only in bacteria and plants (some of them being annotated 'putative BCATs') while transaminases with the FEG conserved motif are found in all kingdoms of life, with occasional changes to another hydrophobic residue, either tyrosine or leucine. Multi-alignment phylogenetic analysis of BCATs and DATs from all taxonomic groups further shows a complete separation these two enzyme types. Still, it is striking that plant DATs are closer to their bacterial counterparts than to plant BCATs (Figure 4). In addition, prokaryotes that appear to be close to plant DATs do not include cyanobacterias. In fact, it is not possible to find any DAT with the FD<sup>T/V</sup> motif in cyanobacterias, closest relatives being BCATs with a special L-enantiospecific FGG motif, where glutamate is substituted by glycine.

serine dehydration, the latter producing pyruvate and ammonium. With the *Arabidopsis* enzyme, the racemisation-to-dehydration ratio is high (0.26 with D-serine as a substrate) compared to enzymes known (i.e., annotated) as serine dehydratases (Fujitani et al., 2006). Unlike bacteria and animals, plants lack enzymes with pure serine dehydratase activity, and, therefore, it is not very likely that plant SR comes from duplication and functional divergence from plant genes encoding serine dehydratase. The absence of serine dehydratase is also surprising because plants contain threonine dehydratase (with which plant SR shares some structural homology, with the difference that threonine



**FIGURE 2** Alignment of plant D-amino acid transaminases (DATs) and branched-chain amino transferases (BCATs) showing conserved motifs for steric specificity and the catalytic lysine residue. The 3D structure is illustrated with the crystallised DAT homodimer from *Bacillus* (reference number 1DAA in Protein Data Bank) with magnifications showing key residues (highlighted in yellow; residue numbering in *Bacillus*) and their position with respect to the PLP cofactor. PLP, pyridoxal phosphate. [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

dehydratase has an extra N-ter domain (Szamosi et al., 1994) (Supporting Information S1: Figure S1). Phylogenetic analysis suggests that plant SR closest relatives are bacterial serine dehydratases, perhaps reflecting horizontal transfer of a gene encoding initially a serine dehydratase that subsequently evolved to a racemase. In terms of plant physiology, the concurrent reactions catalysed by SR (racemization and dehydration) probably explain why D-serine does not accumulate to high levels, thus avoiding inhibition of DATs involved in the metabolism of other D-amino acids.

## 4.2 | Folate metabolism: A link with DATs?

DATs share the same folding and catalytic conserved motif Phe-Asp-Thr (FDT) with another group of enzymes, 4-amino deoxychorismate lyases (ADCLs). ADCLs are involved in the biosynthesis of folate since they catalyse the production of the biosynthetic intermediate *para*-amino benzoic acid; this reaction takes place in the chloroplast (Basset et al., 2004). Folate synthesis only occurs in bacteria, plants and fungi (folate being a vitamin obtained from food in animals). In the conserved motif FDT, ADCLs contain an amino acid residue that is important for steric purposes, threonine, which can be occasionally replaced by valine, isoleucine or leucine. Also, in some cases, the second residue (aspartate) is replaced by glutamate, like in BCATs.

Phylogenetic analyses including plant ADCL sequences show that plant ADCLs are much closer to bacterial DATs and ADCLs than cyanobacterial or fungal ADCLs (Supporting Information S1: Figure S2). In *Arabidopsis*, three genes are annotated as DAT or DAT-like. One of them (encoded by At5g57850) has been shown experimentally to encode an enzyme with DAT activity (AtDAT1), with a rather broad substrate spectrum (Funakoshi et al., 2008; Suarez et al., 2019). The same enzyme has also been shown to exhibit ADCL activity, like its homolog in tomato (Basset et al., 2004). Interestingly, AtDAT1 has also been shown to be involved in D-methionine metabolism and ethylene production (Suarez et al., 2019). In terms of chemistry, DATs and ADCLs catalyse quite different reactions, suggesting that DATs probably form a group of promiscuous enzymes with versatile catalytic functions. If true, this could explain why DATs have been kept during evolution, including in plants, to fulfil multiple metabolic functions where specific amino acid chemistry is required.

## 4.3 | D-tryptophan: A natural precursor of auxin?

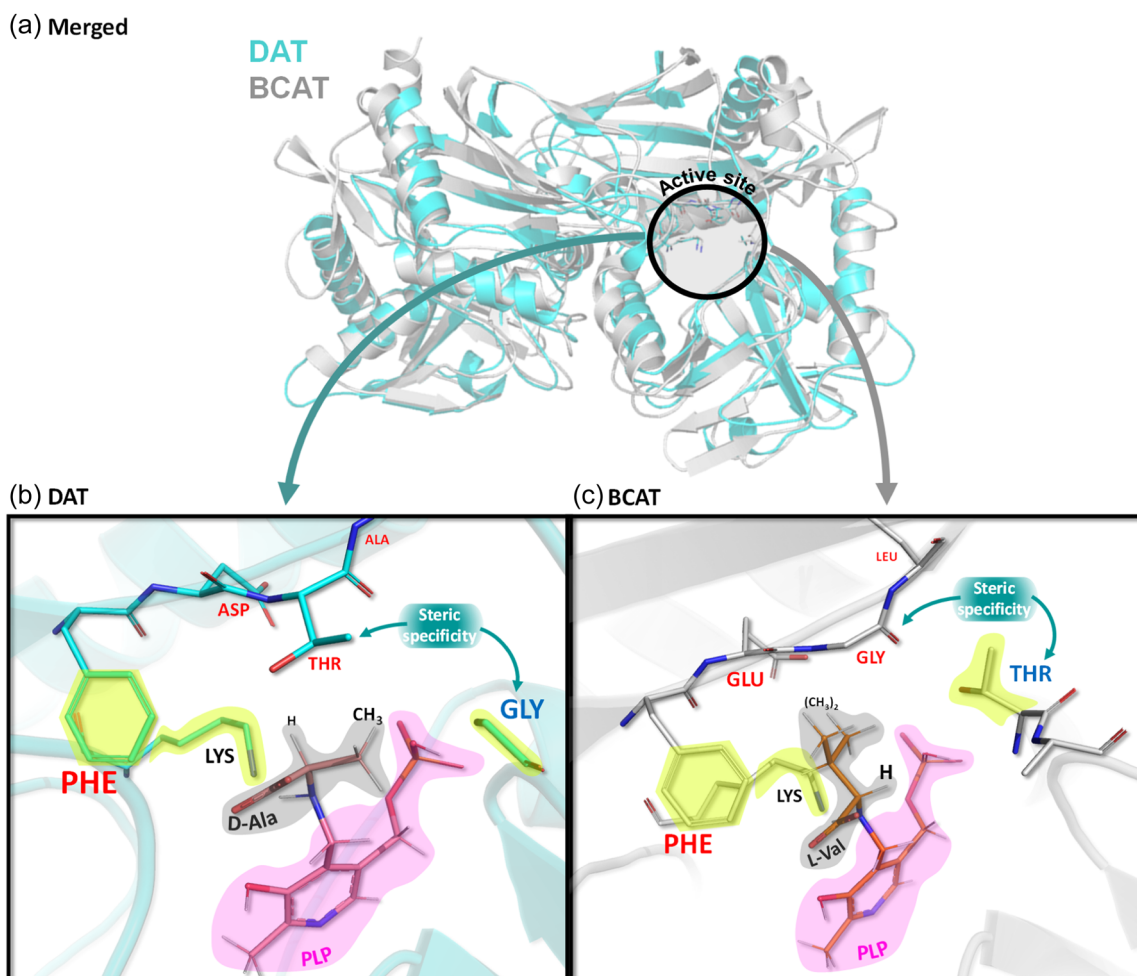
L-tryptophan is the biosynthetic precursor of auxin, indole-3-acetic acid (IAA), through deamination and decarboxylation (Won et al., 2011). Interestingly, in Alaska peas and wheat, it has been reported that the aminotransferase that performs deamination to

produce indolepyruvic acid is more effective with D-tryptophan than L-tryptophan (McQueen-Mason & Hamilton, 1989; Rekoslavskaya et al., 1999). This observation raises two questions: (i) why has D-tryptophan higher specificity than L-tryptophan? (ii) Is there a source of D-tryptophan in *planta*? In wheat and tomato, the occurrence of N-malonyl-D-tryptophan has been reported and one might speculate that the chemical modification (malonylation) is a strategy to store D-tryptophan for IAA synthesis while avoiding detrimental effects of free D-tryptophan (Gamburg et al., 1991; Rekoslavskaya et al., 1999). Interestingly, tryptophan racemase (TR) activity has been detected in wheat and tobacco where there is a high activity L- to D-tryptophan conversion activity during first days of germination; TR activity is localised in the plastid, where the synthesis of both L-tryptophan and IAA also occurs (Miura & Mills, 1971; Rekoslavskaya et al., 1999). However, to date, there is no report of a specific protein sequence or gene associated with TR in plants. Collectively, experimental support for a crucial role of

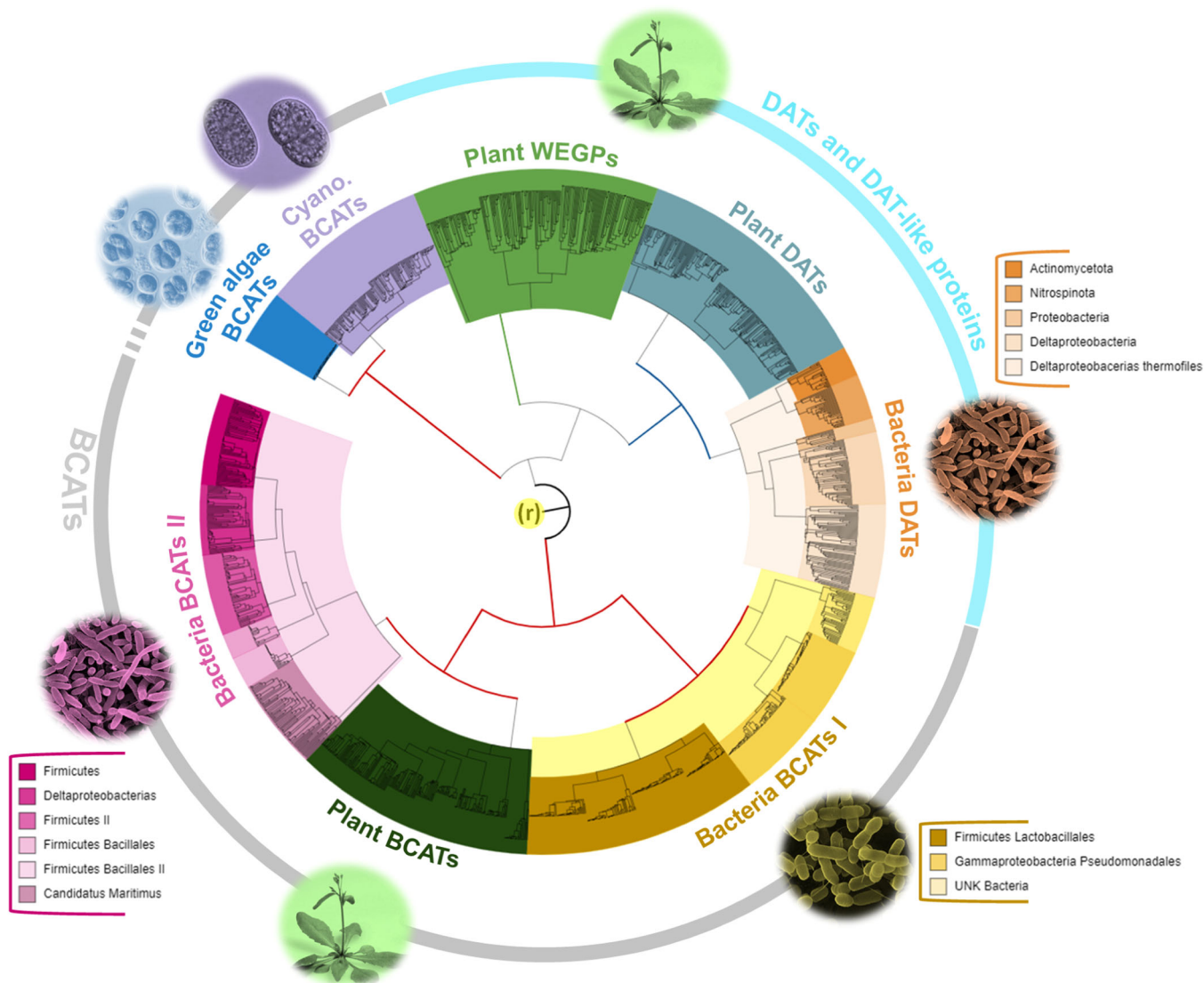
D-tryptophan is presently rather thin, and thus further research on D-tryptophan metabolism is necessary to better understand how it can feed IAA biosynthesis and thus impact on plant development.

#### 4.4 | Plant chloroplastic peptidoglycan

At first, it was believed that in *Arabidopsis*, there was little peptidoglycan-associated metabolism since DDL known-out lines did not show chloroplastic alteration (Hirano et al., 2016). This was in line with the presumption of peptidoglycan absence in higher plants, in contrast to Glaucophytes, Charophytes or Lycopphytes (Takano & Takechi, 2010). However, it has recently been shown that higher plant chloroplast envelope (*Arabidopsis* and tobacco) contains a peptidoglycan layer (Tran et al., 2023). In effect, amino acid 'click chemistry' coupled to fluorescence microscopy provided evidence for the presence of peptidoglycan in chloroplasts, which nearly



**FIGURE 3** Structure of the active site in plant D-amino acid transaminase (DAT) and branched-chain aminotransferase (BCAT). (a) Overlapped 3D folding of DAT and BCAT from *Arabidopsis thaliana*. (b) DAT active site structure model with the covalent reaction intermediate N-D-alanyl-PLP (grey and pink). (c) BCAT active site structure with the covalent reaction intermediate N-L-valyl-PLP (grey). Both protein models were generated with Swiss Model using *Bacillus* DAT as a training template (PDB reference number 3DAA). Figures were created using Pymol. Key residues mentioned in Box 1 are highlighted in yellow. PDB, protein data bank; PLP, pyridoxal phosphate. [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]



**FIGURE 4** Phylogenetic tree of  $\alpha$ -amino acid transaminases (DATs) and branched-chain amino transferases (BCATs). The tree includes DAT-like fold IV proteins containing a motif WEG and referred to as WEGPs. Note (i) the subdivision of BCATs into two distinct, nonsister clades: plant + bacteria, and green algae + cyanobacteria, (ii) closest relatives to plant BCATs are bacterial BCATs II including  $\delta$ -proteobacterial forms. The tree was rooted with fold I transaminases (symbol r highlighted in yellow). The phylogenetic tree was generated using iTOL (details are provided in supplementary material, legend of Supporting Information S1: Figure S1). WEGPs, WEG-motif containing proteins. [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

disappeared when  $\alpha$ -cycloserine is added (Tran et al., 2023). The lack of chloroplastic phenotype in DDL knock-out lines has been proposed to be due to remaining DDL activity despite protein truncation (Tran et al., 2023). It has been suggested that across photosynthetic lineages, there is a link between the structure of the protein machinery of chloroplastic division and the presence of a peptidoglycan layer (Miyagishima & Kabeya, 2010). Accordingly, in *Physcomitrella*, a physical link between peptidoglycan synthase penicillin-binding protein (PBP) and plastid division 2, an outer envelope component of the chloroplast division complex, has been shown recently (Chang et al., 2023). Also, in the same study, PBP deletion is shown to affect plastid division. Taken as a whole,  $\alpha$ -amino acid metabolism, and peptidoglycan synthesis appear to

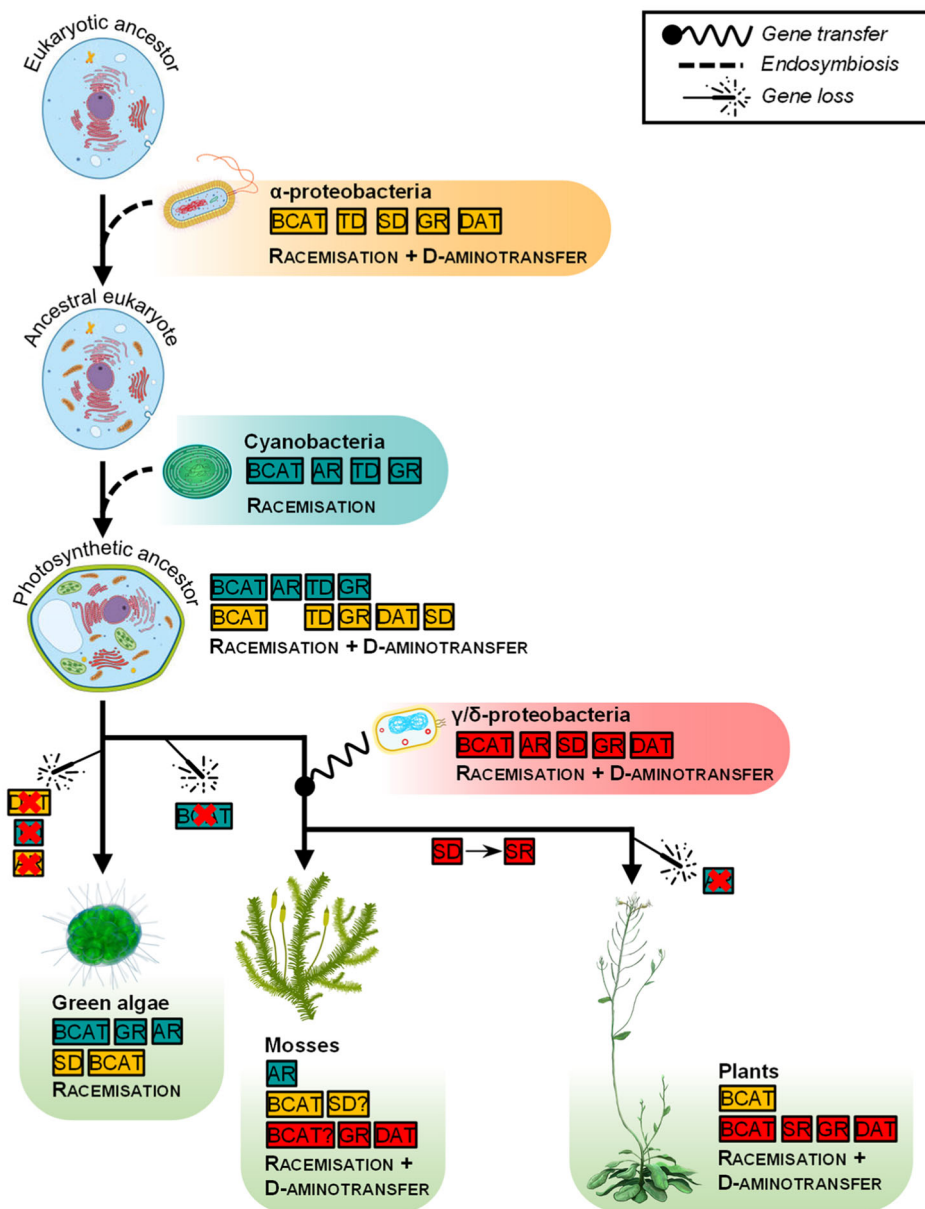
be crucial for chloroplast development and division, including in higher plants.

## 5 | WHERE DO PLANT ENZYMES OF $\alpha$ -AMINO ACID METABOLISM COME FROM?

DATs belongs to the fold IV transaminases family, which also includes branched-chain amino transferases (BCATs). BCATs transfer the amino group of an  $\alpha$ -amino acid onto an  $\alpha$ -keto acid to produce valine, leucine and isoleucine (Hutson, 2001). BCATs can be found in all taxonomic groups due to the importance of branched-chain amino acids, while DATs are only present in bacteria and plants. Owing to

similarities in sequence and protein folding between them (Pavkov-Keller et al., 2016), DATs and BCATs are phylogenetically related, DATs likely emerging from a BCAT ancestor. Here, the comparison of active site stereochemistry across enzymes and clades is informative (Box 1): BCAT have a conserved motif Phe-Glu-Gly associated with a separate threonine residue, while DATs have a  $FD^T/V$  motif, reflecting stereochemistry reversion (Figures 2 and 3). This

comparison does not concern cyanobacteria, which are devoid of DATs or any other  $FD^T/V$  motif-containing enzyme. Cyanobacteria nevertheless synthesise peptidoglycans and use another metabolic pathway to produce D-amino acids. In effect, cyanobacteria possess ARs, suggesting the source of D-alanine is racemic equilibration with L-alanine, in clear contrast with DATs which transfer  $NH_2$  from D-amino acids to yield D-alanine (Cava et al., 2011). AR utilisation also



**FIGURE 5** Hypothetical evolutionary scenario associated with D-amino acid metabolism. Genes encoding for enzymes involved in D-amino acid metabolism in green photosynthetic Eukaryotes come from both α-proteobacterial (mitochondrion) and cyanobacterial (chloroplast) endosymbiotic events. In plants, they are also inherited from gene transfer from a prokaryote of the γ/δ-proteobacteria group. Also, gene losses took place (red crosses) and therefore, there is presently limited genetic material associated with D-amino acid metabolism from cyanobacteria in plants. It should be noted that some uncertainty nevertheless remains as to whether mosses have a serine dehydratase and a BCAT of γ/δ-proteobacterial origin due to limited available sequences in this group. Also, available sequences suggest there is no glutamate racemases inherited from α-proteobacteria in green algae, mosses and plants. This figure shows taxonomic groups discussed in the text and do not include other groups (such as other algae and fungi) so as to focus on plant D-amino acid metabolism. AR, alanine racemase; BCAT, branched-chain amino acid transaminase; DAT, D-amino acid transaminase; GR, glutamate racemase; SD, serine dehydratase; SR, serine racemase; TD, threonine dehydratase. [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1111/pce.14826)]



occurs in green algae, which also lack DATs and exhibit BCATs sequences of the same clade as cyanobacterial BCATs, that is, far from higher plants (Figure 4). Conversely, ARs are not present in higher plant genomes (although some AR activity can be found in some plant species, for example, in alfalfa seedlings (Ono et al., 2006)) but are present in mosses and green algae (Figure 5).

BCATs are present in all living organisms, suggesting they have a common ancestor originating billions of years ago. Nevertheless, plant BCATs belong to the same clade as a specific group of bacterial enzymes, rather distinct from other BCATs, including green algae BCATs (Figure 4). Furthermore, BCATs from cyanobacteria are not the closest relatives to plant BCATs, suggesting that plastid ancestors were not at the origin of plant BCATs. Rather, it suggests a horizontal gene transfer after endosymbiotic events that led to plastids occurred or alternatively, that this specific type of BCATs was lost in photosynthetic clades other than plants (this alternative is, however, phylogenetically less parsimonious). It is appealing that Firmicutes including Bacillales and Clostridiales are the most represented bacteria in closest plant BCATs relatives. These organisms often live in soil in a symbiotic relationship with plants, perhaps facilitating horizontal transfer of genes associated with branched-chain amino acid metabolism (for a similar discussion with Rhizobia, see Andrews et al. (2018)). Plant DATs closest relatives are a group of bacterial DATs (Figure 4), including species different from plant BCATs closest relatives. In fact, this group is represented by Actinomycetes, Nitrospina, Proteobacteria and  $\delta$ -proteobacteria. Some of these are bacteria with specialised N metabolism (including D-amino acids), often involved in symbioses with plants (Moe, 2013; Radkov et al., 2016).

Taken as a whole, plant BCATs and DATs do not seem to have the same ancestry, and there were potentially two separate horizontal gene transfers from noncyanobacterial, prokaryotic organisms. Given that DATs likely originated from BCATs as enzymes specialised in D-configuration chemistry (see above), it suggests that these two transfers were not synchronous but rather separated by millions of years.

## 6 | EVOLUTION OF D-AMINO ACIDS METABOLIC STRATEGIES: A POSSIBLE SCENARIO

Different strategies seem to have been retained to synthesise D-amino acids in prokaryotic and eukaryotic lineages, and this allows us to propose an evolutionary scenario (Figure 5). Since ARs are present in various organisms, from cyanobacteria to organisms of the green lineage, we may hypothesise that the initial source of D-alanine was its L-enantiomer using AR activity, the origin of which in mosses is probably cyanobacterial via endosymbiosis. Later (or concurrently), functional divergence from a bacterial BCAT ancestor led to DAT activity, which spread across many bacterial lineages. Depending on organisms, this innovation replaced or accompanied AR activity to sustain D-amino acid metabolism in plants which do not have AR.

Then, a specific group of bacteria with symbiotic characteristics transferred this activity to plants ancestors. Also, a distinct gene transfer took place, providing plants with some BCATs—likely in addition to BCATs coming from the  $\alpha$ -proteobacterial endosymbiotic event that generated mitochondria.

Although complicated, this multiple-transfer scenario is supported by phylogenetic analysis of other enzymes involved somehow in peptidoglycan chemistry. First, it has been found that in plants, some Mur genes do not have a cyanobacterial or  $\alpha$ -proteobacterial origin, indicating another horizontal transfer after endosymbiotic events that generated mitochondria and plastids (Sato, 2021). Second, even though D-amino acid ligases are present in both bacteria and plants, they do not share similarities at the sequence level, as we observed with DATs, suggesting that their origin is distinct and unrelated to ancestors at the origin of plant DATs. Third, plant GR are very close to GR from  $\alpha$ -proteobacteria, showing probable inheritance of GR from the endosymbiotic event at the origin of mitochondria (Supporting Information S1: Figure S1). This is in clear contrast with DATs and BCATs, meaning that alternative origins (such as late horizontal transfer events) are more likely.

## 7 | CONCLUSIONS AND PERSPECTIVES

Plants enzymes involved in D-amino acid metabolism have an evolutionary connection with bacterial enzymes, suggesting multiple gene transfer events. It is particularly striking that unlike plants, cyanobacteria and green algae are devoid of DATs, suggesting that DAT activity was acquired via gene transfer from bacteria. This raises the question of whether D-amino acid metabolism (DAT activity) was crucial to support metabolic functions required during land plants evolution (such as pollen tube growth and vitamin and auxin biosynthesis, detailed above). Therefore, D-amino acid metabolism seems to have significant roles in plant biology, not only nitrogen metabolism but also for signalling and plant development. However, there is a rather poor knowledge of D-amino acid function in plants in metabolic studies, because most metabolomics techniques do not differentiate D- and L-enantiomers. Metabolomics by gas chromatography- or liquid chromatography-mass spectrometry coupled to chiral chromatography should solve this issue. Specific flux analyses (with e.g., isotope tracing) are necessary to appreciate the specific fate of D-amino acids in plant metabolism and also, further characterisation of potential enzymes of D-metabolism to check their enantioselectivity. This would be essential to clarify why enzymes like DATs that originated secondarily from a BCAT ancestor have been selected for and kept by plant evolution.

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### CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

### DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no data sets were generated or analysed during the current study.

### ORCID

Anis M. Limami  <http://orcid.org/0000-0002-9985-2363>

Guillaume Tcherkez  <http://orcid.org/0000-0002-3339-956X>

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Additional supporting information can be found online in the Supporting Information section at the end of this article.

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