Chapter 7

D-Amino Acids in Plants: New Insights and Aspects, but also More Open Questions

Üner Kolukisaoglu and Juan Suarez

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.68539

Abstract

The relevance of the homochirality of proteinogenic amino acids for life is undisputed, but also to their D-enantiomers a growing number of biological functions could be assigned. When it comes to D-amino acids in plants, information was relatively scarce for a long time. Nowadays, also in this field, knowledge is growing which will be presented and discussed in this review. In this respect, it was shown that D-amino acids are taken up by plants from soil but could also be synthesized de novo. Investigations of plant D-amino acid metabolism as well as other studies revealed a central function of D-Ala in plants, which await further elucidation. Also other D-amino acids are shown to cause physiological effects in plants, ranging from nitrogen utilization over stress adaptation to chloroplast division, and indicate that D-amino acids are responsible for a variety of yet poorly understood or even undiscovered functions in plants.

Keywords: D-amino acids in plants, D-amino acid biochemistry, functions of D-amino acids in plants and bacteria, D-alanine

1. Introduction

L-amino acids (L-AAs) are the basis of life on our planet (and maybe also on other animate ones), mainly due to their property to be the building blocks of all proteins. These proteinogenic amino acids are also one of the fundamentals of the universality of the genetic code. The limitation of protein coding sequences on 20 different L-AAs was one of the key developments in evolution to ensure the compatibility between different life forms, regardless if they belong to the same species or if their genetic material is exchanged between a bacterial pathogen and its plant host, as it is the case in the *Agrobacterium*-plant relationship.

In the course of limitation to 20 proteinogenic amino acids, also the convention of exclusive usage of L-AAs (homochirality) in the primary structure of proteins evolved to ensure the intended structure and functionality of a protein. But since the very beginning of evolution also the enantiomers of L-AAs, the D-amino acids (D-AAs) were existent. These D-AAs are mainly products of abiotic and enzymatic racemization of L-AAs [1] or synthesized by aminotransferases from other D-AAs [2]. One possibility of organisms during evolution to handle D-AAs would have been to develop mechanisms for their elimination. But instead almost all organismal classes in the tree of life learned to live with substantial amounts of D-AAs and even made use of them. One prominent example of such usage is the bacterial cell wall. It contains many layers of peptidoglycan, polysaccharide chains cross linked by oligopeptides. Parts of these oligopeptides are D-AAs, especially D-Ala and D-Glu, which protect the cell due to their resistance to cleavage by conventional proteases [3].

The decay of the bacterial cell wall is also one of the major sources of D-AAs in soil [4] and the reason why plants are specifically surrounded and challenged by D-AAs. In soil samples, almost all D-enantiomers of proteinogenic amino acids could be found in significant amounts [4–7]. For a long time, it was assumed that D-AAs are just inhibitory for plant growth and therefore plants evolved mechanisms to avoid and eliminate them. But recent studies have shown that plants are instead able to import D-AAs and metabolize them. They even synthesize D-AA themselves for physiological reasons, which raised the question about the beneficial effects of D-AAs for plants. In this review, we want to summarize the current knowledge about these processes and highlight different aspects and questions of future research with a focus on *Arabidopsis thaliana* as a model plant to investigate D-AAs in plants.

2. How do D-AAs get into the plant?

It is a widely accepted fact that plants harbour free D-AAs as they could be identified in different plant species and tissues [8–12]. In this regard, the question arose, if all these amino acids are synthesized by plants themselves or also taken up from the soil. By detecting various D-AAs in seedlings of runner and soy beans, garden and water cress, as well as alfalfa, raised on amino acid free media in Ref. [10], first indirect indications were given that these plants are able to synthesize D-AAs de novo. This hypothesis was supported by the discovery, identification and characterization of alanine, serine and isoleucine racemases from different plant species [13–16]. The toxicity of D-AAs on Arabidopsis [12, 17] and the toxic effect of D-Ser on other species [18] were first hints for a general D-AA uptake mechanism in plants. Furthermore, it was shown that almost all D-enantiomers of proteinogenic amino acids could be detected in Arabidopsis plants after their exogenous application [12, 19]. The direct uptake of D-Ala and its utilization could be demonstrated for the first time in wheat [20].

At that point, it was interesting which transporters are involved in the uptake of D-AAs and which properties they have. One of the first hints in this respect was given by the works of Ref. [21]. In an initial screen, they germinated Arabidopsis mutants on 3 mM D-Ala, a toxic D-Ala concentration for wild-type plants and found plants to survive with mutations in the *LYSINE HISTIDINE TRANSPORTER 1 (LHT1)* gene. Furthermore, the uptake for D-Ala in

http://dx.doi.org/10.5772/intechopen.68539

these plants was reduced by more than 90% in these mutants. Also the uptake of many other D-AAs was found to be reduced in *lht1* mutants [12]. This was the first evidence that a broad range specificity L-AA transporter in plants was also able to take up D-AAs from soil. A second example for plant D-AA transporters are the proline transporters of *A. thaliana* (AtProTs); they facilitate the uptake of L- and D-Pro, and mutants of *AtProT2* show reduced sensitivity against D-Pro [22].

These reports implied that transporters involved in the uptake and transport of L-AAs could also be responsible for the same processes of D-AAs. That D-AA transporting proteins are most probably not restricted to the LHT, and ProT families were given by experiments of our group: in toxicity tests, performed as described before [12], an Arabidopsis mutant of *LHT1* was confirmed to be less sensitive against D-Ala than the corresponding wild type (**Figure 1A**). A mutant of *AAP1*, belonging to the *Amino Acid Permease* family and shown to be responsible for root uptake of uncharged L-AAs [23], revealed a higher resistance against D-Met and D-Phe than Col-0 (**Figure 1B** and **C**). This result implies that AAP1 is involved in the import of D-AAs, specifically of D-Met and D-Phe.

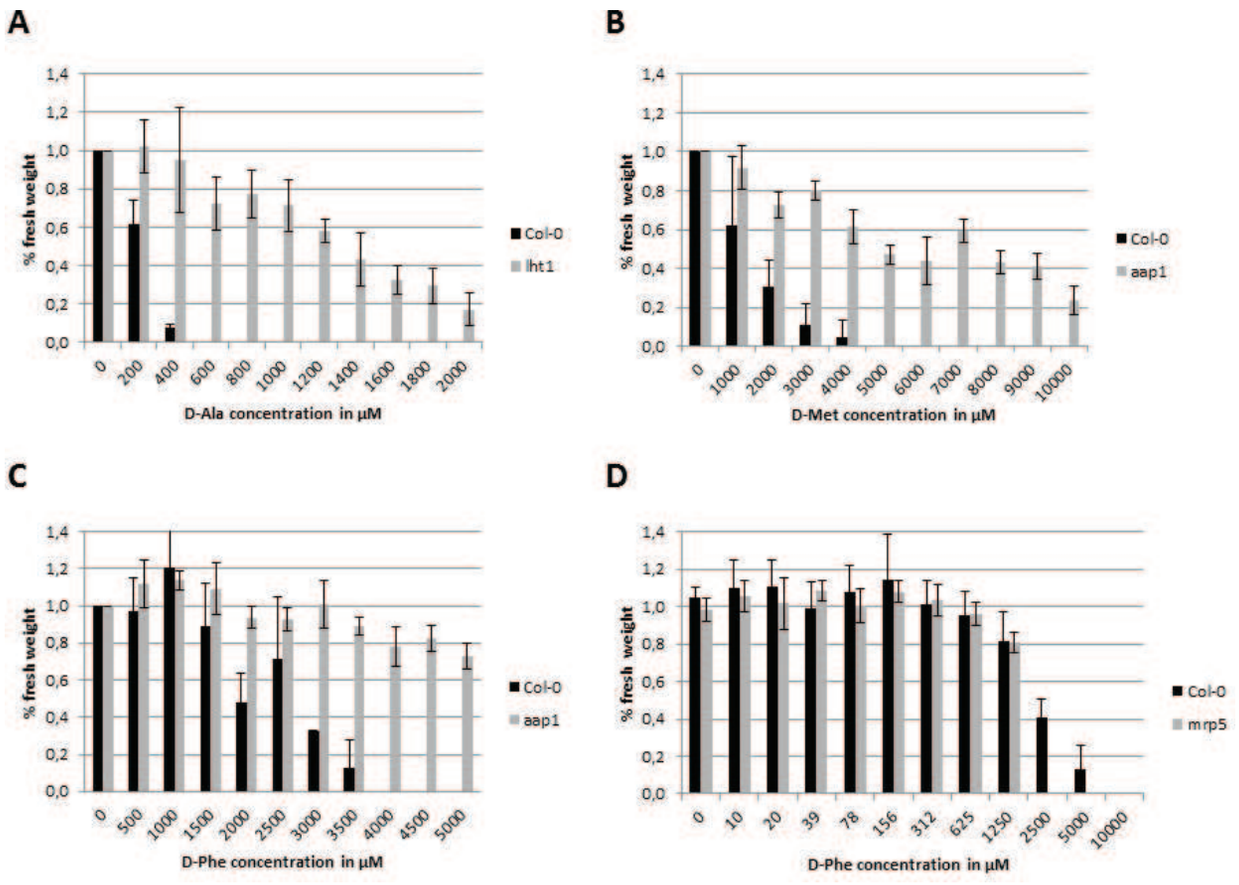

Figure 1. Seed growth inhibition of D-AAs on different transporter mutants compared to their corresponding background accession Col-0.

For these diagrams, different mutants and their corresponding background line Col-0 were germinated for 14 days in ½ MS + 1% sucrose including different D-AAs. Afterward the fresh

weight was recorded. For each measurement, three times eight seedlings were measured. In (A), *lht1* was treated with D-Ala, in (B) and (C), *aap1* was treated with D-Met and D-Phe, respectively, and in (D), *mrp5* was treated with D-Phe. Mutant values are always represented by grey blocks, control values in black blocks. All values are calculated and given in relation to the untreated control seedlings. Error bars indicate standard deviation.

But the interpretation of toxicity experiments using transporter mutants should be handled with care as another example shows: in a series of toxicity tests with different D-AAs, an Arabidopsis mutant of *AtMRP5* showed less resistance against D-Phe compared to the corresponding control (**Figure 1D**), instead of increased resistance like in the case of the tested *AAP1* mutant (**Figure 1C**). *AtMRP5* belongs to a gene family of 14 ABC transporters in the Arabidopsis genome [24] and found to transport inositol phosphate for phytate storage [25]. A functionality as amino acid transporter has not been reported for this protein, yet. Surprisingly, at *mrp5* mutant allele showed also drastically reduced root exudation of almost all L-AAs [26]. It is tempting to speculate whether the reduced D-Phe resistance of the *mrp5* mutant in our experiments may be a consequence of reduced exudation of this amino acid, which may lead to accumulation of it to toxic levels.

Altogether the presented studies in this chapter indicate that plants seem to take up D-AAs actively from their rhizosphere. As also shown above candidate transporter proteins for this uptake are found among L-AA transporting proteins like LHT1, ProT2 and AAP1. There is a certain possibility that these three transporters are not the only ones within their families to transport D-AAs. In the Arabidopsis genome, there are 10 LHTs, 3 ProTs and 8 AAPs encoded [22, 27]. This means that at least among the members of these three transporter families, further D-AA transport proteins may be found. Taking into account that members of other amino acid transporter families may also be able to do so, raises the number of candidates in Arabidopsis up to 63. Even more could be found in other plant species, as there are for instance 189 putative amino acid transporter genes encoded in the soybean genome [28].

3. What happens to D-AAs in the plant?

The fact that plants take up D-AAs from their surrounding rhizosphere leads to the question what happens to them in consequence in the plant. An approach to answer this question was given by our group by feeding Arabidopsis mutants and accessions with different D-AAs to measure the D- and L-AA contents in these plants afterwards [12, 29]. These analyses revealed two major metabolic processes which could be observed; one of them was the conversion of particular D-AAs like D-His, D-Met, D-Phe and D-Trp to their L-enantiomers. In this respect, the increase of these L-AAs was about 2–50 times compared to the untreated control plants, depending on the applied D-AA. The other one was the increase of D-Glu and D-Ala contents after treatment with any given D-AA. In this regard, D-Ala was the major compound to be found after D-AA application with concentrations more than 20 times higher than the ones of D-Glu. These observations led to speculations about the metabolic processes responsible for these effects. To explain the outcome of our amino acid profiling three different possibilities of enzymatic

http://dx.doi.org/10.5772/intechopen.68539

reaction have been discussed: racemization, deamination and transamination [4]. Recent studies of our group revealed that as well the D- to L-AA conversion as also the occurrence of D-Glu and D-Ala can be explained by the activity of a single D-AA specific transaminase in the Arabidopsis genome (Suarez et al., unpublished results).

All these studies shifted our focus towards the evolution and metabolic fate of D-Ala in plants. D-Ala appears to be the major product of D-AA metabolism in Arabidopsis, but, at the same time, it is one of the most toxic D-AAs for this species when applied exogenously [12, 17]. This raises the question how plants process D-Ala specifically and why it is the preferred product of D-AA metabolization. Several possibilities for this process are summarized in **Figure 2**.

A common feature of all possible pathways in **Figure 2** is that none of them have been characterized sufficiently to date in plants, especially in Arabidopsis. But there are a series of reports and evidences arguing for the scheme in this figure, which will be discussed in this section.

The ligation of D-Ala to its dipeptide D-Ala-D-Ala is among the best characterized ways to metabolize D-Ala (**Figure 2**). D-Ala-D-Ala could be detected in different grasses and tobacco long before [30–32], indicating the existence of a D-Ala ligating enzyme. Recently, this enzyme, D-Ala-D-Ala ligase (DDL), could be characterized physiologically for the first time from a plant source, PpDDL1 from the moss *Physcomitrella patens* [33]. As it can be seen in **Table 1** also in the Arabidopsis genome, a DDL encoding gene could be found, an orthologue of PpDDL1, which has not been characterized biochemically, yet. The situation is similar for a putative D-amino acid oxidase (DAO) from Arabidopsis: Its homologue from maize has been biochemically characterized and shown to oxidize preferably D-Ala [34], but its Arabidopsis homologue has not been characterized biochemically or physiologically, yet.

When it comes to the alanine racemase in plants (**Figure 2**), knowledge is rather scarce; Alanine racemase enzyme activity and its corresponding enzyme activity could be isolated and measured in *Chlamydomonasreinhardtii* and alfalfa [13, 35], but identification of the

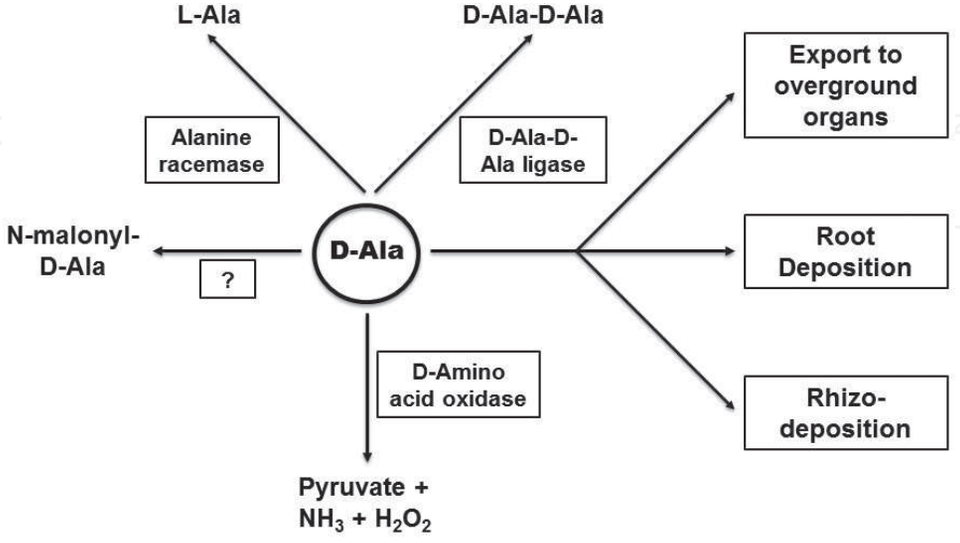

Figure 2. D-Ala as central product of D-AA metabolism and possible metabolic fates of it in plants.

corresponding gene is still pending. In the Arabidopsis genome, two genes with homologies to alanine racemases are annotated (**Table 1**), but characterization is still pending. Even less is known about malonylation of D-Ala, which is given as the fourth major enzymatic way to metabolize D-Ala in **Figure 2**: In pea seedlings, N-malonyl-D-Ala had been detected [36, 37]. Additionally, in mung bean seedlings, D-Ala malonylating activity could be shown [38], but the corresponding enzyme still awaits identification.

Apart from the enzymatic metabolization of D-Ala, other ways of its deposition have to be taken into account as depicted in **Figure 2**: The spatial distribution of D-Ala within the plant, but also of D-AAs in general, would be of interest in this respect. Then, the question could be answered if D-Ala is deposited in the root or if it is transported to other organs, in order to dilute its toxicity. Another possibility would be rhizodeposition, the exudation of metabolites from the root into soil. Rhizodeposition of L-AAs has been shown for plants several times [26, 39, 40]. This process has a strong impact on the microbial community in the rhizosphere, but reports of rhizodeposition of D-AAs are still missing.

Another look into **Table 1** reveals further D-AA processing enzymes in the Arabidopsis genome apart from either synthesizing or metabolizing D-Ala. First of all, there are four D-AA

Function	Name	AGI code	Localization	References
D-amino acid transaminase	AtDAAT1	At5g57850	chloroplast	[41]
		At3g05190	(unknown)	
		At3g54970	(unknown)	
		At5g27410	(unknown)	
Alanine racemase		At1g11930	(chloroplast)	
		At4g26860	(chloroplast)	
D-amino acid racemase	AtDAAR1	At4g02850	(unknown)	[16]
	AtDAAR2	At4g02860	(cytosol)	[16]
Serine racemase	AtSR1	At4g11640	(unknown)	[14]
Asp-Glu racemase		At1g15410	(chloroplast)	
D-aminoacyl-tRNAdeacylase	AtGEK1	At2g03800	cytosol, nucleus	[55]
D-Tyr-tRNAdeacylase		At4g18460	(unknown)	
D-amino acid oxidase		At5g67290	(chloroplast)	
D-Cysdesulfhydrase		At1g48420	mitochondria	[42]
D-Ala-D-Ala ligase		At3g08840	(chloroplast)	

Localization refers to the experimentally determined subcellular localization; localization predictions on the basis of peptide sequencing data by the Plant Proteome Database (PPD; http://ppdb.tc.cornell.edu) and unknown localizations are given in parentheses.

Table 1. Putative D-AA metabolizing genes in the Arabidopsis genome.

specific transaminases, from which just one has been shown to produce D-Ala and preferably D-Glu using various D-AAs as substrates with different affinities [41]. Beside the already mentioned alanine racemase, also three other racemases with specificities for other amino acids can be found in the Arabidopsis genome: First, there is a putative Asp-Glu racemase encoded in the Arabidopsis genome, but currently, there are no reports available about it. The second one is the serine racemase AtSR1, which catalyses the racemization of serine, but also to lesser extent alanine, arginine and glutamine. Beside its racemase activity, it acts also as a dehydratase on D- and L-serine [13]. The third one are the so-called D-amino acid racemases AtDAAR1 and AtDAAR2, which are indeed specific for Ile with D-*allo*-Ile as a product. Leu and Val were just racemized with 1 and 5% relative activity, respectively [16]. The D-Cys desulfhydrase from Arabidopsis is another example for a D-AA metabolizing enzyme with a specificity apart from D-Ala; this specificity to catabolize D-Cys to pyruvate, NH_3 and H_2S has been shown previously [42], but the physiological function of this enzyme and especially of D-Cys still remains unclear and will be discussed in the next chapter. Altogether the collection of D-AA processing enzymes in **Table 1** is a reminder that D-Ala seems to be central product of D-AA metabolism, but that there are far more putative enzyme encoding genes annotated to produce and process also other D-AAs.

4. What are the effects and functions of D-AAs in the plant?

The abundance and fate of D-AAs in plants are indicators that these compounds are actively processed and therefore play a role in the physiology of plants. This leads to the question: Which role(s) are these? In the last years, three different scenarios about the effects of D-AAs on plants were discussed. The first one was that D-AAs have either no effect on plants or even inhibit growth and therefore have to be considered as toxins for plants [19]. In contrast, it could be shown before that at least D-Ile and D-Val promoted seedling growth [17], and that for different D-AAs even the highest tested concentration did not cause growth inhibition [12]. Together with the de novo synthesis of various D-AAs in plants described above a general toxic function of all D-AAs is rather unlikely and depends on dosage, which also applies to many L-AAs.

There are also other arguments speaking against this scenario like the utilization of D-AAs as possible nitrogen source [4, 40], which is the second major postulated function of D-AAs in plants. In this respect, it could be shown that wheat plants are able to assimilate D-Ala as well as D-trialanine, which they took up from the soil [20]. This was the first evidence of direct utilization of D-AAs as a nitrogen source. Additionally, it revealed that plants are able to utilize not just free forms of D-AAs but also as oligomers, as also found as a degradation product of the bacterial cell wall. Nevertheless, more plant species and other D-AAs have to be analysed in this respect to confirm the general utilization of D-AAs as nitrogen sources for plants.

The third major complex of functions of D-AAs in plants is the ones, which have been either just recently discovered, and need to be further analysed and characterized, or which have not been discovered at all. Among these novel functions is, for instance, D-Ala as a stress signal: It has been reported that duckweed seedlings accumulate D-Ala after UV light exposure [43], but the confirmation of this finding by other groups or in other

species is still pending. Another, more prominent, example of a novel physiological function of a D-AA in plants is the impact of D-Ser on pollen tube growth in Arabidopsis [44]; In that report, the authors provided evidence that D-Ser affects pollen tube growth via its agonistic action on the glutamate receptor AtGLR1.2. Furthermore, it was shown that the loss of the serine racemase AtSR1 leads to aberrant pollen tube growth. AtGLR1.2 belongs to a protein family of 20 members in Arabidopsis with highest homologies towards the mammalian ionotropic glutamate receptors (GLRs), also known as N-methyl-D-Aspartate (NMDA) receptors [45]. In humans and other mammalians, these receptors, involved in neurotransmission, have been shown to be activated by L-Glu and D-Ser synergistically [46]. The homologous action of D-Ser on GLRs in animals and plants together with the relatively large number of GLRs in the Arabidopsis genome implies further effects of D-Ser on physiological functions in plants, especially on pathogen response, which may be regulated by GLRs, too [45].

Another type of novel functions of D-AAs was unravelled by the analyses of the structure of the chloroplast membrane of mosses [33]. The authors provided evidence that the membranes of chloroplasts from the moss *P. patens* contain the dipeptide D-Ala-D-Ala and therefore possess a major structural component of peptidoglycan, the building block of bacterial cell walls [3]. Another indication of the structural similarity of bacterial cell wall and plastidial envelopes in mosses was given by genetical experiments. Loss-of-function mutants of the Physcomitrella D-Ala-D-Ala ligase, *PpDDL1*, were not able for chloroplast division and therefore showed megachloroplasts in their protonema cells [33]. All these findings fit to the observation made before in the Physcomitrella genome, which harboured all gene homologues from bacteria to synthesize peptidoglycan including *PpDDL1* [47]. The structural similarity between bacterial cell walls and plastidial envelopes seems to be limited to cryptogamic plants, because loss-of-function mutants of *AtDDL1* did not show the megachloroplast phenotype observed in mosses [33]. This observation seems to be in concordance to the situation in the Arabidopsis genome which harbours just four homologues of the ten mentioned genes needed for peptidoglycan synthesis [47]. It is interesting in this respect that homologues of these four genes were found in all higher plant genomes [48].

Nevertheless, D-Ala seems to play a role in chloroplasts of higher plants as well. Many proteins directly involved in D-Ala metabolism in Arabidopsis were either found in the chloroplast or were predicted to be localized there (**Table 1**). Furthermore, we were able to synthesize a fluorescent D-Ala analogue, HADA (7-hydroxycoumarin-3-amino-D-alanine), according to a previously published protocol [49], and fed it to Arabidopsis seedlings. In these experiments, we could trace the HADA fluorescence evenly distributed in the chloroplasts (**Figure 3**). This targeting of the D-Ala analogue to the chloroplasts indicated a central metabolization of this compound in this compartment. The even distribution of it, in contrast to the accumulation, found in moss chloroplast envelopes [33] points to a different function of D-Ala in higher plant chloroplasts, which still awaits to be unravelled.

http://dx.doi.org/10.5772/intechopen.68539

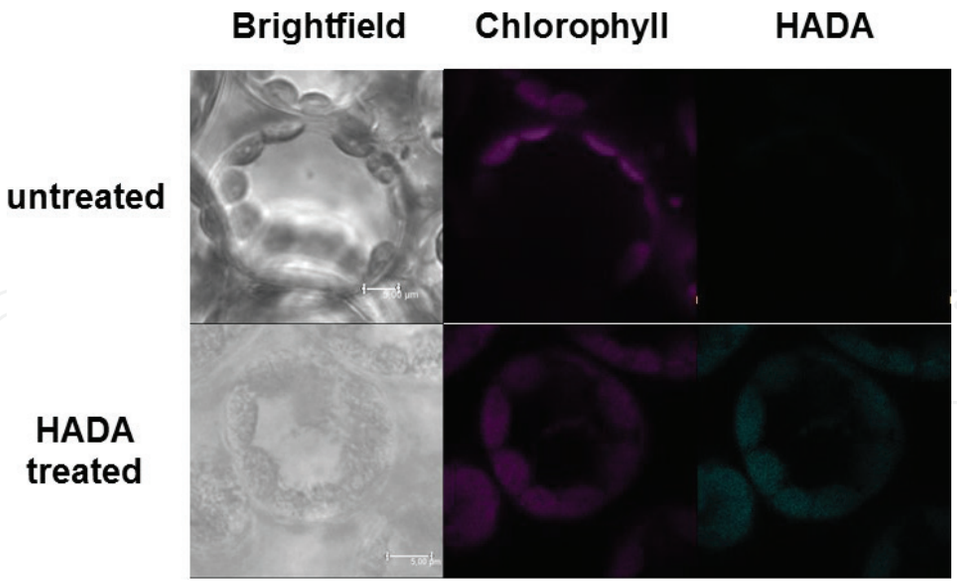

Figure 3. Fluorescent D-Ala analogue HADA accumulates in chloroplasts of Arabidopsis leaves.

Fourteen days old *Col-0* seedlings grown in liquid culture were incubated overnight in 0.1 mM HADA and then analysed microscopically. The pictures in the first column show bright field images of sponge parenchyma cells. The chlorophyll in the chloroplasts was detected by its autofluorescence (Chlorophyll, second column), and fluorescence of HADA was recorded in the DAPI channel (HADA, third column) with a laser-scanning microscope. The upper row shows cells without HADA treatment (untreated) as control, the lower one with HADA treatment (HADA treated). The size bars indicate 5 μm.

Among D-AAs with novel functions in plants, there are D-AAs to be known to affect specific proteins, but not how they cause the associated physiological reactions. One example for such a relationship is the one between D-Cys and drought resistance. As described above, a desulfhydrase specific for D-Cys could be characterized from Arabidopsis, which also produces H_2S [42]. In further experiments, it turned out that increased H_2S production leads to enhanced drought resistance [50], which can be partially assigned to increased D-Cysdesulfhydrase activity [51]. This effect seems to be related to ethylene induced stomatal aperture. Furthermore, H_2S production leads to cross adaptation of plants to several other stress factors [52]. But nevertheless, the source of D-Cys, its significance in stress signalling and adaptation and its detailed way of action still need to be elucidated.

Another example for an enigmatic relation between a D-AA and physiological response was described previously: In this case, an Arabidopsis mutant with hypersensitivity to ethanol, *gek1*, was isolated [53], where the respective mutation could be assigned to a D-aminoacyl-tRNAdeacylase (*AtGEK1*) [54]. Later, it was found that this gene encodes an active enzyme with broad substrate specificity [55]. But its overexpression led neither in *Escherichia coli* nor in yeast to an increase of ethanol tolerance [54]. Therefore, a functional explanation how the loss of *AtGEK1*, and therefore the inability to repair accidental loading of tRNAs with D-AAs, causes ethanol hypersensitivity in plants is still missing.

5. Conclusions

As discussed largely in this text, knowledge gathered in the last decade implies that D-AAs are involved in more plant physiological processes than assumed before. Furthermore, the view of D-AAs as generally toxic molecules needs to be changed to a view of them as physiologically active compounds, which can cause detrimental effects by over dosage. Therefore, the investigation of their uptake and elimination, their metabolic pathways, and their physiological functions in plants will gain more interest and significance in the future.

To understand the transport processes for uptake, distribution (intracellular, intercellular and also at long distances) as well as of possible excretion of D-AAs will be one of the major fields to be investigated. Although there are candidate transport proteins given to be analysed with the classical L-AA transporters as described above, also other, yet unknown, proteins may contribute to D-AA transport processes, as the D-Phe toxicity on *mrp5* mutants demonstrates (**Figure 1**). In this regard, one of the first questions to be solved would be the proof of active uptake of D-AAs by candidate transporters instead of indirect evidences.

When it comes to the metabolization of D-AAs, the formation of D-Ala appeared to be central, which puts also this molecule into the centre of future investigations. As it has been shown in the preceding chapter, D-Ala seems to accumulate in the chloroplast. In this respect, D-Ala may play a double role. On the one hand, it is an intermediate metabolite, which needs to be further metabolized due its toxicity in excess concentration. In this regard, the different putative metabolic pathways await elucidation. On the other hand, D-Ala is a physiologically active compound as it has been shown as a building block of moss chloroplast envelopes. In this context, the function of D-Ala in chloroplasts of higher plant and the concentration of different D-AA-related enzymes in this compartment will be of specific interest. Furthermore, it would be interesting if also other D-AAs take this way over chloroplasts and what functions they fulfil there.

Finally, which physiological role(s) the different D-AAs play in plants are the major questions to be solved. One of these questions will be, to which extent and under which circumstances D-AAs from the rhizosphere are utilized as nitrogen sources. As it was mentioned above, the accumulation of D-Ala and of D-AA-related enzymes in the chloroplasts may point to their involvement in plastid biogenesis, assembly and maintenance. The unresolved functions of D-Cys in stress resistance and the unclear involvement of AtGEK1 in ethanol resistance indicate that there is a high probability that there are still many D-AA-related functions and processes in plants waiting to be discovered.

Acknowledgements

We are grateful to Prof. Klaus Harter (Centre for Plant Molecular Biology, University of Tübingen, Germany) for the provision of research facilities, where the majority of our studies were performed. Studies of Juan Suarez on the effects of D-AAs on Arabidopsis were supported by the DeutscherAkademischerAustauschdienst (DAAD 91567028). Additionally, we would like to thank to Laura Menius for her work on the toxicity of D-AAs on the *mrp5* mutant.

Author details

Üner Kolukisaoglu* and Juan Suarez

*Address all correspondence to: uener.kolukisaoglu@zmbp.uni-tuebingen.de

Department of Plant Physiology, Center for Plant Molecular Biology (ZMBP), Eberhard Karls University of Tübingen, Tübingen, Germany

References

[1] Friedman M. Chemistry, nutrition, and microbiology of D-amino acids. Journal of Agricultural and Food Chemistry. 1999;**47**:3457–3479. DOI: 10.1021/jf990080u

[2] Radkov AD, Moe LA. Bacterial synthesis of D-amino acids. Applied Microbiology and Biotechnology. 2014;**98**:5363–5374. DOI: 10.1007/s00253-014-5726-3

[3] Typas A, Banzhaf M, Gross CA, Vollmer W. From the regulation of peptidoglycan synthesis to bacterial growth and morphology. Nature Reviews Microbiology. 2011;**10**:123–136. DOI: 10.1038/nrmicro2677

[4] Vranova V, Zahradnickova H, Janous D, Skene KR, Matharu KR, Rejsek K, Formanek P. The significance of D-amino acids in soil, fate and utilization by microbes and plants: Review and identification of knowledge gaps. Plant and Soil. 2012;**354**:21–39. DOI: 10.1007/s11104-011-1059-5

[5] Pollock GE, Cheng CN, Cronin SE. Determination of the D and L isomers of some protein amino acids present in soils. Analytical Chemistry. 1977;**49**:2–7. DOI: 10.1021/ac50009a008

[6] Amelung W, Zhang X. Determination of amino acid enantiomers in soil. Soil Biology and Biochemistry. 2001;**33**:553–562. DOI: 10.1016/S0038-0717(00)00195-4

[7] Amelung W, Zhang X, Flach KW. Amino acids in grassland soils: Climatic effects on concentrations and chirality. Geoderma. 2006;**130**:207–217. DOI: 10.1016/j.geoderma.2005.01.017

[8] Robinson T. D-amino acids in higher plants. Life Sciences. 1976;**19**:1097–1102. DOI: 10.1016/0024-3205(76)90244-7

[9] Brückner H, Westhauser T. Chromatographic determination of D-amino acids as native constituents of vegetables and fruits. Chromatographia. 1994;**39**:419–426. DOI: 10.1007/BF02278756

[10] Brückner H, Westhauser T. Chromatographic determination of L- and D-amino acids in plants. Amino Acids. 2003;**24**:43–55. DOI: 10.1007/s00726-002-0322-8

[11] Herrero M, Ibáñez E, Martín-Alvarez PJ, Cifuentes A. Analysis of chiral amino acids in conventional and transgenic maize. Analytical Chemistry. 2007;**79**:5071–5077. DOI: 10.1021/ac070454f

[12] Gördes D, Kolukisaoglu Ü, Thurow K. Uptake and Conversion of D-Amino Acids in *Arabidopsis thaliana*. Amino Acids. 2011;**40**:553–563. DOI: 10.1007/s00726-010-0674-4

[13] Ono K, Yanagida K, Oikawa T, Ogawa T, Soda K. Alanine racemase of alfalfa seedlings (*Medicagosativa* L.): First evidence for the presence of an amino acid racemase in plants. Phytochemistry. 2007;**67**:856–860. DOI: 10.1016/j.phytochem.2006.02.017

[14] Fujitani Y, Nakajima N, Ishihara K, Oikawa T, Ito K, Sugimoto M. Molecular and biochemical characterization of a serine racemase from *Arabidopsis thaliana*. Phytochemistry. 2006;**67**:668–674. DOI: 10.1016/j.phytochem.2006.01.003

[15] Fujitani Y, Horiuchi T, Ito K, Sugimoto M. Serine racemases from barley, *Hordeum vulgare* L., and other plant species represent a distinct eukaryotic group: Gene cloning and recombinant protein characterization. Phytochemistry. 2007;**68**:1530–1536. DOI: 10.1016/j.phytochem.2007.03.040

[16] Strauch RC, Svedin E, Dilkes B, Chapple C, Li X. Discovery of a novel amino acid racemase through exploration of natural variation in *Arabidopsis thaliana*. Proceedings of the National Academy of Sciences U S A. 2015;**112**:11726–11731. DOI: 10.1073/pnas.1503272112

[17] Erikson O, Hertzberg M, Näsholm T. A conditional marker gene allowing both positive and negative selection in plants. Nature Biotechnology. 2004;**22**:455–458. DOI: 10.1038/nbt946

[18] Erikson O, Hertzberg M, Näsholm T. The *dsdA* gene from *Escherichia coli* provides a novel selectable marker for plant transformation. Plant Molecular Biology. 2005;**57**:425–433. DOI: 10.1007/s11103-004-7902-9

[19] Forsum O, Svennerstam H, Ganeteg U, Näsholm T. Capacities and constraints of amino acid utilization in Arabidopsis. New Phytologist. 2008;**179**:1058–1069. DOI: 10.1111/j.1469-8137.2008.02546.x

[20] Hill PW, Quilliam RS, DeLuca TH, Farrar J, Farrell M, Roberts P, Newsham KK, Hopkins DW, Bardgett RD, Jones DL. Acquisition and assimilation of nitrogen as peptide-bound and D-enantiomers of amino acids by wheat.PLoS One. 2011;**6**:e1922. DOI: 10.1371/journal.pone.0019220

[21] Svennerstam H, Ganeteg U, Bellini C, Näsholm T. Comprehensive screening of Arabidopsis mutants suggests the lysine histidine transporter 1 to be involved in plant uptake of amino acids. Plant Physiology. 2007;**143**:1853–1860. DOI: 10.1105/tpc.106.041012

[22] Lehmann S, Gumy C, Blatter E, Boeffel S, Fricke W, Rentsch D. In planta function of compatible solute transporters of the AtProT family. Journal of Experimental Botany. 2011;**62**:787–796. DOI: 10.1093/jxb/erq320

[23] Lee YH, Foster J, Chen J, Voll LM, Weber AP, Tegeder M. AAP1 transports uncharged amino acids into roots of Arabidopsis. The Plant Journal. 2007;**50**:305–319. DOI: 10.1111/j.1365-313X.2007.03045.x

[24] Verrier PJ, Bird D, Burla B, Dassa E, Forestier C, Geisler M, Klein M, Kolukisaoglu U, Lee Y, Martinoia E, Murphy A, Rea PA, Samuels L, Schulz B, Spalding EJ, Yazaki K, Theodoulou FL. Plant ABC proteins – A unified nomenclature and updated inventory. Trends in Plant Sciences. 2008;**13**:151–159. DOI: 10.1016/j.tplants.2008.02.001

[25] Nagy R, Grob H, Weder B, Green P, Klein M, Frelet-Barrand A, Schjoerring JK, Brearley C, Martinoia E. The Arabidopsis ATP-binding cassette protein AtMRP5/AtABCC5 is a high affinity inositol hexakisphosphate transporter involved in guard cell signaling and phytate storage. The Journal of Biological Chemistry. 2009;**284**:33614–33622. DOI: 10.1074/jbc.M109.030247

[26] Zhou D, Huang XF, Chaparro JM, Badri DV, Manter DK, Vivanco JM, Guo J. Root and bacterial secretions regulate the interaction between plants and PGPR leading to distinct plant growth promotion effects. Plant and Soil. 2016;**401**:259. DOI: 10.1007/s11104-015-2743-7

[27] Tegeder M, Ward JM. Molecular Evolution of Plant AAP and LHT Amino Acid Transporters. Frontiers in Plant Science. 2012;**13**:21. DOI: 10.3389/fpls.2012.00021

[28] Cheng L, Yuan HY, Ren R, Zhao SQ, Han YP, Zhou QY, Ke DX, Wang YX, Wang L. Genome-wide identification, classification, and expression analysis of *Amino Acid Transporter* gene family in *Glycine Max*. Frontiers in Plant Science. 2016;**20**;7:515. DOI: 10.3389/fpls.2016.00515

[29] Gördes D, Koch G, Thurow K, Kolukisaoglu U. Analyses of Arabidopsis ecotypes reveal metabolic diversity to convert D-amino acids. Springerplus. 2013;**2**:559. DOI: 10.1186/2193-1801-2-559

[30] Noma M, Noguchi M, Tamaki E. Isolation and characterization of D-alanyl-D-alanine from tobacco leaves. Agricultural and Biological Chemistry. 1973;**37**:2439. DOI: 10.1271/bbb1961.37.2439

[31] Frahn JL, Illman RJ. The occurrence of D-alanine and D-alanyl-D-alanine in *Phalaristuberosa*. Phytochemistry. 1975;**14**:1464–1465

[32] Manabe H. Occurrence of D-Alanyl-D-alanine in *Oryzaaustraliensis*. Agricultural and Biological Chemistry. 1985;**49**:1203–1204. DOI: 10.1080/00021369.1985.10866877

[33] Hirano T, Tanidokoro K, Shimizu Y, Kawarabayasi Y, Ohshima T, Sato M, Tadano S, Ishikawa H, Takio S, Takechi K, Takano H. Moss chloroplasts are surrounded by a peptidoglycan wall containing D-amino acids. Plant Cell. 2016;**28**:1521–1532. DOI: 10.1105/tpc.16.00104

[34] Gholizadeh A, Kohnehrouz BB. Molecular cloning and expression in *Escherichia coli* of an active fused *Zea mays L*. D-amino acid oxidase. Biochemistry (Moscow). 2009;**74**:137–144. DOI: 10.1134/S0006297909020035

[35] Nishimura K, Tomoda Y, Nakamoto Y, Kawada T, Ishii Y, Nagata Y. Alanine racemase from the green alga *Chlamydomonasreinhardtii*. Amino Acids.2007;**32**:59–62. DOI:10.1007/s00726-006-0352-8

[36] Fukuda M, Tokumura A, Ogawa T. D-Alanine in germinating *Pisumsativum* seedlings. Phytochemistry. 1973;**12**:2893–2595. DOI: 10.1016/0031-9422(73)85061-7

[37] Ogawa T, Fukuda M, Sasaoka K. Occurrence of N-malonyl-D-alanine in pea seedlings. Biochimica et Biophysica Acta. 1973;**297**:60–69. DOI: 10.1016/0304-4165(73)90049-4

[38] Kionka C, Amrhein N. The enzymatic malonylation of 1-aminocyclopropane-1-carboxylic acid in homogenates of mung-bean hypocotyls. Planta. 1984;**162**:226–35. DOI: 10.1007/BF00397444

[39] Chaparro JM, Badri DV, Bakker MG, Sugiyama A, Manter DK, Vivanco JM. Root exudation of phytochemicals in arabidopsis follows specific patterns that are developmentally programmed and correlate with soil microbial functions. PLoS One. 2013;**8**:e55731. DOI:10.1371/journal.pone.0055731

[40] Moe LA. Amino acids in the rhizosphere: From plants to microbes. American Journal of Botany. 2013;**100**:1692–1705. DOI: 10.3732/ajb.1300033

[41] Funakoshi M, Sekine M, Katane M, Furuchi T, Yohda M, Yoshikawa T, Homma H. Cloning and functional characterization of *Arabidopsis thaliana* D-amino acid aminotransferase – D-aspartate behavior during germination. The FEBS Journal. 2008;**275**:1188–1200. DOI: 10.1111/j.1742-4658.2008.06279.x

[42] Riemenschneider A, Wegele R, Schmidt A, Papenbrock J. Isolation and characterization of a D-cysteine desulfhydrase protein from *Arabidopsis thaliana*. The FEBS Journal. 2005;**272**:1291–1304. DOI: 10.1111/j.1742-4658.2005.04567.x

[43] Monselise EB, Levkovitz A, Kost D. Ultraviolet radiation induces stress in etiolated *Landoltiapunctata*, as evidenced by the presence of alanine, a universal stress signal: a ^{15}N NMR study. Plant Biology (Stuttg). 2015;**17**(Suppl 1):101–107. DOI: 10.1111/plb.12198

[44] Michard E, Lima PT, Borges F, Silva AC, Portes MT, Carvalho JE, Gilliham M, Liu LH, Obermeyer G, Feijó JA. Glutamate receptor-like genes form Ca^{2+} channels in pollen tubes and are regulated by pistil D-serine. Science. 2011;**332**:434–437. DOI: 10.1126/science.1201101

[45] Forde BG, Roberts MR. Glutamate receptor-like channels in plants: A role as amino acid sensors in plant defence? F1000Prime Rep. 2014;**6**:37. DOI: 10.12703/P6-37

[46] Wolosker H. NMDA receptor regulation by D-serine: New findings and perspectives. Molecular Neurobiology. 2007;**36**:152–164. DOI: 10.1007/s12035-007-0038-6

[47] Machida M, Takechi K, Sato H, Chung SJ, Kuroiwa H, Takio S, Seki M, Shinozaki K, Fujita T, Hasebe M, Takano H. Genes for the peptidoglycan synthesis pathway are essential for chloroplast division in moss. Proceedings of the National Academy of Sciences U S A. 2006;**103**:6753–6758. DOI: 10.1073/pnas.0510693103

[48] van Baren MJ, Bachy C, Reistetter EN, Purvine SO, Grimwood J, Sudek S, Yu H, Poirier C, Deerinck TJ, Kuo A, Grigoriev IV, Wong CH, Smith RD, Callister SJ, Wei CL, Schmutz J, Worden AZ. Evidence-based green algal genomics reveals marine diversity

and ancestral characteristics of land plants. BMC Genomics. 2016;**17**:267. DOI: 10.1186/s12864-016-2585-6

[49] Kuru E, Tekkam S, Hall E, Brun YV, Van Nieuwenhze MS. Synthesis of fluorescent D-amino acids and their use for probing peptidoglycan synthesis and bacterial growth in situ. Nature Protocols. 2015;**10**:33–52. DOI: 10.1038/nprot.2014.197

[50] Jin Z, Shen J, Qiao Z, Yang G, Wang R, Pei Y. Hydrogen sulfide improves drought resistance in *Arabidopsis thaliana*. Biochemical and Biophysical Research Communications. 2011;**414**:481–486. DOI: 10.1016/j.bbrc.2011.09.090

[51] Hou Z, Wang L, Liu J, Hou L, Liu X. Hydrogen sulfide regulates ethylene-induced stomatal closure in *Arabidopsis thaliana*. Journal of Integrative Plant Biology. 2013;**55**:277–289. DOI: 10.1111/jipb.12004

[52] Li ZG, Min X, Zhou ZH. Hydrogen Sulfide: A Signal Molecule in Plant Cross-Adaptation. Frontiers in Plant Science. 2016;**7**:1621. DOI: 10.3389/fpls.2016.01621

[53] Hirayama T, Fujishige N, Kunii T, Nishimura N, Iuchi S, Shinozaki K. A novel ethanol-hypersensitive mutant of Arabidopsis. Plant and Cell Physiology. 2004;**45**:703–711. DOI: 10.14841/jspp.2008.0.0890.0

[54] Fujishige N, Nishimura N, Iuchi S, Kunii T, Shinozaki K, Hirayama T. A novel Arabidopsis gene required for ethanol tolerance is conserved among plants and archaea. Plant and Cell Physiology. 2004;**45**:659–666. DOI: 10.1093/pcp/pch086

[55] Wydau S, Ferri-Fioni ML, Blanquet S, Plateau P. GEK1, a gene product of *Arabidopsis thaliana* involved in ethanol tolerance, is a D-aminoacyl-tRNAdeacylase. Nucleic Acids Research. 2007;**35**:930–938. DOI: 10.1093/nar/gkl1145

IntechOpen