Plant Science xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Plant Science

journal homepage: www.elsevier.com/locate/plantsci

Review

# Amino acids – A life between metabolism and signaling

Q1 Rainer E. Häusler*, Frank Ludewig, Stephan Krueger

*Department of Botany II, University of Cologne, Cologne Biocenter, Zülpicherstr. 47B, 50674 Cologne, Germany*

ARTICLE INFO

*Article history:*
Received 24 July 2014
Received in revised form
18 September 2014
Accepted 19 September 2014
Available online xxx

*Keywords:*
Serine
GABA
Neolignans
Hydroxycinnamic-acid-amides
Signaling

ABSTRACT

Amino acids serve as constituents of proteins, precursors for anabolism, and, in some cases, as signaling molecules in mammalians and plants. This review is focused on new insights, or speculations, on signaling functions of serine, γ-aminobutyric acid (GABA) and phenylalanine-derived phenylpropanoids. Serine acts as signal in brain tissue and mammalian cancer cells. In plants, *de novo* serine biosynthesis is also highly active in fast growing tissues such as meristems, suggesting a similar role of serine as in mammalians. GABA functions as inhibitory neurotransmitter in the brain. In plants, GABA is also abundant and seems to be involved in sexual reproduction, cell elongation, patterning and cell identity. The aromatic amino acids phenylalanine, tyrosine, and tryptophan are precursors for the production of secondary plant products. Besides their pharmaceutical value, lignans, neolignans and hydroxycinnamic acid amides (HCAA) deriving from phenylpropanoid metabolism and, in the case of HCAA, also from arginine have been shown to fulfill signaling functions or are involved in the response to biotic and abiotic stress. Although some basics on phenylpropanoid-derived signaling have been described, little is known on recognition- or signal transduction mechanisms. In general, mutant- and transgenic approaches will be helpful to elucidate the mechanistic basis of metabolite signaling.

© 2014 Published by Elsevier Ireland Ltd.

## Contents

1. Introduction ........ 00
2. Serine, a key regulator for development? ........ 00
   2.1. Serine, an indispensable metabolite ........ 00
   2.2. Serine, a metabolic signal? ........ 00
3. GABA signaling in plants ........ 00
   3.1. Putative plant GABA receptors ........ 00
   3.2. The role of GABA in plant sexual reproduction ........ 00
   3.3. GABA and cell elongation ........ 00
   3.4. A role for GABA in patterning and cell identity ........ 00
4. Signal molecules deriving from aromatic amino acids ........ 00
   4.1. Phosphoenolpyruvate, an important link between primary metabolism and aromatic amino acid-based signaling ........ 00
   4.2. Lignans and neolignans deriving from phenylpropanoid metabolism act as signal molecules ........ 00
   4.3. Transgenic and mutant plants with impaired neolignan signaling ........ 00
   4.4. Hydroxycinnamic acid amides (HCAA) as metabolic signals ........ 00
   4.5. HCAAs have multiple functions in plants and mammalians ........ 00
5. Conclusions and future perspectives ........ 00
   5.1. Serine ........ 00
   5.2. GABA ........ 00
   5.3. Phenylpropanoids and HCAAs ........ 00
   Acknowledgements ........ 00
   References ........ 00

* Corresponding author. Tel.: +49 0221 4702340; fax: +49 0221 4705039.
*E-mail address:* rainer.haeusler@uni-koeln.de (R.E. Häusler).

http://dx.doi.org/10.1016/j.plantsci.2014.09.011
0168-9452/© 2014 Published by Elsevier Ireland Ltd.

UNCORRECTED PROOF

## 1. Introduction

All life depends on a constant flow of metabolites that provide building blocks as well as energy and reducing power for growth, development, and reproduction. Beside of their role in biochemistry, metabolic intermediates can also serve as signaling molecules contributing to the complex regulatory network that eventually adapts gene expression to altered requirements during the life cycle, or as a response to a changing environment. In this review we focus on the dual functions of certain amino acids and their derivatives as metabolic intermediates/end-products and signaling molecules. Such dual functions are well documented in the medical/mammalian field, and evidence for similar functions is also emerging for the plant system.

The amino acid serine has recently been suggested to act as a signal controlling the proliferation of mammalian cancer cells [1,2]. As the demand for nutrients in fast growing cells is high, the nutritional state determines the rate of cell proliferation. In plants, *de novo* serine biosynthesis is highly active in fast growing tissues, such as meristems [3] suggesting a similar role of serine as signaling molecule in plants.

Likewise, in the mammalian brain glutamate-derived γ-amino butyric acid (GABA) is an inhibitory neurotransmitter that exerts its signaling effect after binding to specific receptors [4]. In plants, evidence for GABA-dependent signal transduction pathways exists and awaits a detailed characterization.

Besides their role as constituents of proteins, the aromatic amino acids phenylalanine, tyrosine and tryptophan are the precursors for a variety of secondary products [5,6] among them compounds with signaling function. The phenylpropanoid pathway, starting from phenylalanine delivers, for instance, the neolignan dehydrodiconiferyl alcohol glucoside (DCG), which has been shown to exert cytokinine-like effects in plants [7–9]. Likewise, amines and polyamines deriving from the amino acid arginine together with the phenylpropanoid *p*-coumaric acid converge in the synthesis of hydroxycinnamic acid amides (HCAAs). HCAAs are involved in stress- and pathogen responses and might also act as signaling molecules during developmental processes [10].

Fig. 1 shows an overview on the compartmentation of anabolic and catabolic pathways in a mesophyll cell including branch points leading to those metabolic signals that are highlighted in this review. In contrast to catabolism, which is mainly localized in the cytosol or mitochondria, the majority of the anabolic reaction sequences are initiated in the plastid stroma. Chloroplasts are the site of $CO_2$-, ammonia- and sulphur assimilation and of a variety of pathways leading to the biosynthesis of building blocks like fatty acids [11], aromatic amino acids [5,6], branched chain amino acids [12], isoprenoids *via* the mevalonate-independent way (methylerythritol 4-phosphate pathway; [13], serine [3,14], and arginine [15]). The glycolytic intermediate phosphoenolpyruvate (PEP) obviously plays a central role both in anabolism and catabolism [16] and hence also in the production of amino acid derived signaling molecules.

In this review we elucidate the dual or multiple functions of serine, GABA, neolignans like DCG as well HCAAs with respect to metabolism and signaling. Mutant plants impaired in the biosynthesis of amino acids or downstream products might help to dissect the involvement of amino acid metabolism in cellular signaling processes.

## 2. Serine, a key regulator for development?

### 2.1. Serine, an indispensable metabolite

In addition to its role as constituent of proteins, L-serine is a precursor for the biosynthesis of a multitude of metabolites. For instance, it is required for the biosynthesis of the amino acids glycine, cysteine and tryptophan (for the latter see Fig. 1), or for the biosynthesis of lipids like sphingolipids and phosphatidylserine [17,18]. In addition L-serine delivers one-carbon units for the tetrahydrofolate metabolism [19]. In most organisms L-serine is synthesized by the glycolytic or 'phosphorylated' pathway, in which 3-phosphoglycerate is converted to phosphoserine and subsequently to L-serine [3]. However, in plants, L-serine is predominantly generated during the overall process of photorespiration [14]. As photorespiration is tightly coupled to photosynthesis, this path of L-serine production is restricted to autotrophic tissues. In addition to photorespiratory L-serine biosynthesis, plants contain all genes essential for the 'phosphorylated' pathway [3,20]. These genes are highly expressed in non-photosynthetic tissues like roots or in the regions of primary meristems, where cell proliferation takes place. Mutant plants deficient in the 'phosphorylated' pathway are embryo lethal, underlining the importance of this path of L-serine biosynthesis. Moreover, even if the activity of this pathway was only diminished by artificial silencing of genes involved, it resulted in severely impaired leaf and root development [3,20]. However, these developmental constraints cannot be explained by a general decrease in L-serine contents, because these remain unaltered in transgenic plants [3,20]. At the present state it has not been resolved yet whether the observed developmental constraints are simply based on metabolic limitations or whether L-serine functions as a growth-regulating signal itself as it has been reported for other organisms.

### 2.2. Serine, a metabolic signal?

Recently it has been shown that D-serine, synthesized by serine racemase from proteinogenic L-serine, occurs in plants. D-Serine functions as a signaling molecule in the communication between male gametophytes and the pistil by regulating a glutamate receptor-like $Ca^{2+}$ channel in the apical region of pollen tubes [21]. This regulatory mechanism resembles those known from mammalians, where D-serine functions as neurotransmitter in the brain and regulates the activity of the N-methyl-D-aspartate receptor, a non-selective ion channel [22]. In plants, not only D-serine, but also L-serine is supposed to act as metabolic signal. Deletion of the gene encoding the photorespiratory enzyme hydroxypyruvate reductase1 only affected the L-serine content in the respective mutants, but not the contents of most metabolites. Moreover, the mutation in this gene leads to a considerable change in expression of photorespiration-related genes. Similar alterations in gene expression pattern have been observed for wild-type plants grown on a medium supplemented with physiological concentrations of L-serine [23]. Nevertheless, it remains elusive whether or not L-serine is directly or indirectly responsible for the deregulation of photorespiratory genes.

Recently notable advances have been made on the path to understand the regulatory function of L-serine in mammalian cancer cells [24]. L-Serine plays an important role in controlling cell proliferation during cancer progression. On the one hand, the flux of 3-phosphoglycerate to L-serine synthesis *via* glycolysis is enhanced, to provide sufficient L-serine required for protein synthesis in the cancer cells, and on the other hand, also as carbon donor for one-carbon ($C_1$) metabolism. $C_1$-metabolism is the source for a large number of molecules essential for regeneration and proliferation of cells, such as S-adenosylmethionine, an important methyl-group donor, and purine bases required for DNA and RNA synthesis [1]. In proliferating cancer cells, L-serine controls the flux into $C_1$ metabolism by balancing the carbon flow between glycolysis and its own biosynthesis. Beside its signaling potential, L-serine functions as an allosteric activator of pyruvate kinase M2, an isoenzyme specific for embryo and tumor cells. In these cells

UNCORRECTED PROOF

Please cite this article in press as: R.E. Häusler, et al., Amino acids – A life between metabolism and signaling, Plant Sci. (2014), http://dx.doi.org/10.1016/j.plantsci.2014.09.011

the glycolytic flux into the tricarboxylic acid cycle is diminished because of the low activity of pyruvate kinase M2. However, the activity of this enzyme is enhanced in the presence of high endogenous L-serine concentrations.

Deficiency in L-serine activates the expression of L-serine biosynthesis genes *via* the 'general control of the non-derepressible 2 kinase-activating transcription factor 4'-pathway [24]. Moreover, L-serine activates 'mammalian target of rapamycin complex 1' (mTORC1), a master regulator integrating nutrient availability and cell growth. Whether or not L-serine has similar functions in plants is not yet known. The *Arabidopsis thaliana* genome contains one gene encoding a 'general control non-derepressible 2 kinase-like' enzyme (AtGCN2). This enzyme seems to be functional as it is capable of complementing a yeast mutant deficient in the endogenous kinase [25]. Plants lacking the 'non-derepressible 2 kinase' enzyme are more susceptible to herbicide-induced amino acid starvation [26]. In yeast the 'non-derepressible 2 kinase'-pathway is known to induce the expression of multiple genes involved in the biosynthesis of a variety of different amino acids in response to amino acid starvation [27]. However, in plants only the expression of nitrate reductase, the key enzyme of nitrogen assimilation, seems to be regulated by the 'non-derepressible 2 kinase-like' enzyme [26,28]. These results indicate that the function of the 'non-derepressible 2 kinase'-pathway in plants appears to be similar, but not identical to that observed in other organisms. It remains to be shown whether or not L-serine homeostasis in proliferating cells of plant meristems is regulated by the 'non-derepressible 2 kinase'-pathway.

Another possible mechanism to integrate L-serine signaling in plants could be the 'target of rapamycin' (TOR) pathway. TOR is a relatively large protein kinase associated with other regulatory proteins in two high mass complexes (TORC1 and TORC2) [29]. The TOR pathway functions as regulatory integrator of environmental signals, like the availability of nutrients, and conveys this information to adjust cellular processes such as metabolism, protein synthesis, and cell proliferation. In plants, *TOR* is expressed in the endosperm, the embryo, and primary meristems [30]. Homozygous *tor* mutant embryos are arrested in development, and inducible silencing of *TOR* leads to a retardation in growth, induction of premature senescence, and accumulation of amino acids [31,32]. It has been demonstrated that TOR deficiency caused by an inducible RNA interference approach mimics nitrogen and carbon starvation responses in plants leading, among other effects, to a massive increase in the content of free amino acids [33,34]. Because TOR regulates loading of ribosomes with amino acids and recycling of cellular components, amino acid accumulation in TOR deficient plants has been attributed to a diminished protein biosynthesis combined with an enhanced protein degradation by autophagy [34].

A link between TOR signaling and L-serine homeostasis has recently been discovered in proliferating human lung carcinoma cells (H1299) [24]. Down-regulation of the 'phosphorylated' L-serine biosynthesis pathway in H1299 cells inhibits the phosphorylation of the ribosomal S6 kinase, a prominent target of TOR leading to a reduced cell proliferation [24]. Although there is no direct evidence for the regulation of TOR by L-serine in plants, there are some indirect indications. (1) The 'phosphorylated' serine biosynthesis pathway seems to be more restricted to proliferating cells in the primary meristems, and hence overlaps with TOR expression. In addition, (2) plants deficient in the 'phosphorylated' pathway as well as *tor* mutant plants are embryo lethal, and down-regulation of the 'phosphorylated' pathway leads to similar growth defect as observed for plants with a diminished TOR kinase activity [3,20]. (3) Plants deficient in the 'phosphorylated' pathway accumulate amino acids in a similar way as observed for inducible *TOR*-silencing plants [3,20,30]. However, whether or not TOR signaling is regulated by L-serine in plants still remains elusive.

## 3. GABA signaling in plants

The four carbon, non-proteinogenic amino acid GABA is well-known as main inhibitory neurotransmitter in the central nervous system of mammalians. Nevertheless, GABA has also been found in some non-neuronal cells [35] as well as in plants, which of course also lack neurons. GABA rapidly accumulates in plant tissues as a response to abiotic or biotic stresses and it is important for sexual reproduction and cell elongation. Moreover, GABA and/or its derivatives play an important role in defining cell identity in leaves and the shoot apical meristem. Furthermore, GABA is involved in the interaction of plants with bacteria and insects [36–38]. However, the latter aspects belong to a different topic and will hence not be covered by this review.

GABA is mainly formed in the cytosol as decarboxylation product of glutamate catalyzed by various glutamate decarboxylases. It can be degraded by the reaction sequence of the so-called GABA shunt inside the mitochondrial matrix (Fig. 1). The import of GABA into the mitochondria is mediated by GABA permease [39], which is encoded by a single copy gene in *A. thaliana*. The lack of any strong phenotype of the *gabp* mutant, defective in this GABA permease, suggests that its function can probably be taken over by unspecific amino acid permeases. In mitochondria, the amino group of GABA is transferred to pyruvate by GABA transaminase yielding alanine and succinic semialdehyde. The latter is oxidized, and thereby detoxified, by succinic semialdehyde dehydrogenase yielding succinate, which can be further metabolized in the tricarboxylic acid cycle (Fig. 1). Apart from its important role in primary metabolism, *i.e.* at the intersection of nitrogen and carbon metabolism [40], GABA also functions as signal molecule. This function will be focused on in this part of the review.

### 3.1. Putative plant GABA receptors

Exposure to various abiotic and biotic stresses leads to a rapid accumulation of GABA, which can be partially explained on the basis of the regulatory properties of glutamate decarboxylases, in particular their interaction with $Ca^{2+}$/calmodulin. The concentrations of cytosolic $Ca^{2+}$ and concomitantly of $Ca^{2+}$/calmodulin are strongly increased in response to various stresses. As glutamate decarboxylases are activated when $Ca^{2+}$/calmodulin binds to a C-terminal auto-inhibitory domain, $Ca^{2+}$ indirectly triggers GABA formation from glutamate [41]. Thus $Ca^{2+}$ signaling can be transduced by GABA. However, in order to exert a signaling function itself, GABA has to be recognized by receptors. Evidence for the existence of GABA receptors in plants emerged from experiments with the GABA agonist baclofen (β-(4-chlorophenyl)-GABA) and the two antagonists picrotoxin and bicuculline, which are also used in pharmacological studies with mammalians. Treatment of duckweed (*Lemna*) with either baclofen or the antagonists resulted in a promotion or an inhibition of plant growth, respectively [42]. The GABA-dependent growth promotion in the *Lemna* system was due to an increased ion uptake into the plant [42]. In the mammalian system, GABA is capable of regulating the activities of ion channels *via* $GABA_A$- and $GABA_B$-receptors. It is likely, but not yet proven, that a similar mechanism exists in plants. However, plants lack $GABA_A$- and $GABA_B$-like receptors. If GABA regulates ion channels in plants in a similar way as in mammalians, different receptor types have to be involved in GABA binding. Two different functions of transmembrane proteins are currently discussed as being GABA-dependent in plants. These are (1) a putative GABA-gated $Ca^{2+}$ channel (permease) and (2) GABA-dependent aluminum-activated malate transporters. In any case experimental evidence for the direct involvement of GABA is still lacking.

(1) Plant homologues of mammalian ionotropic glutamate receptors [43,44] have been speculated to mediate the permeation

Please cite this article in press as: R.E. Häusler, et al., Amino acids – A life between metabolism and signaling, Plant Sci. (2014), http://dx.doi.org/10.1016/j.plantsci.2014.09.011

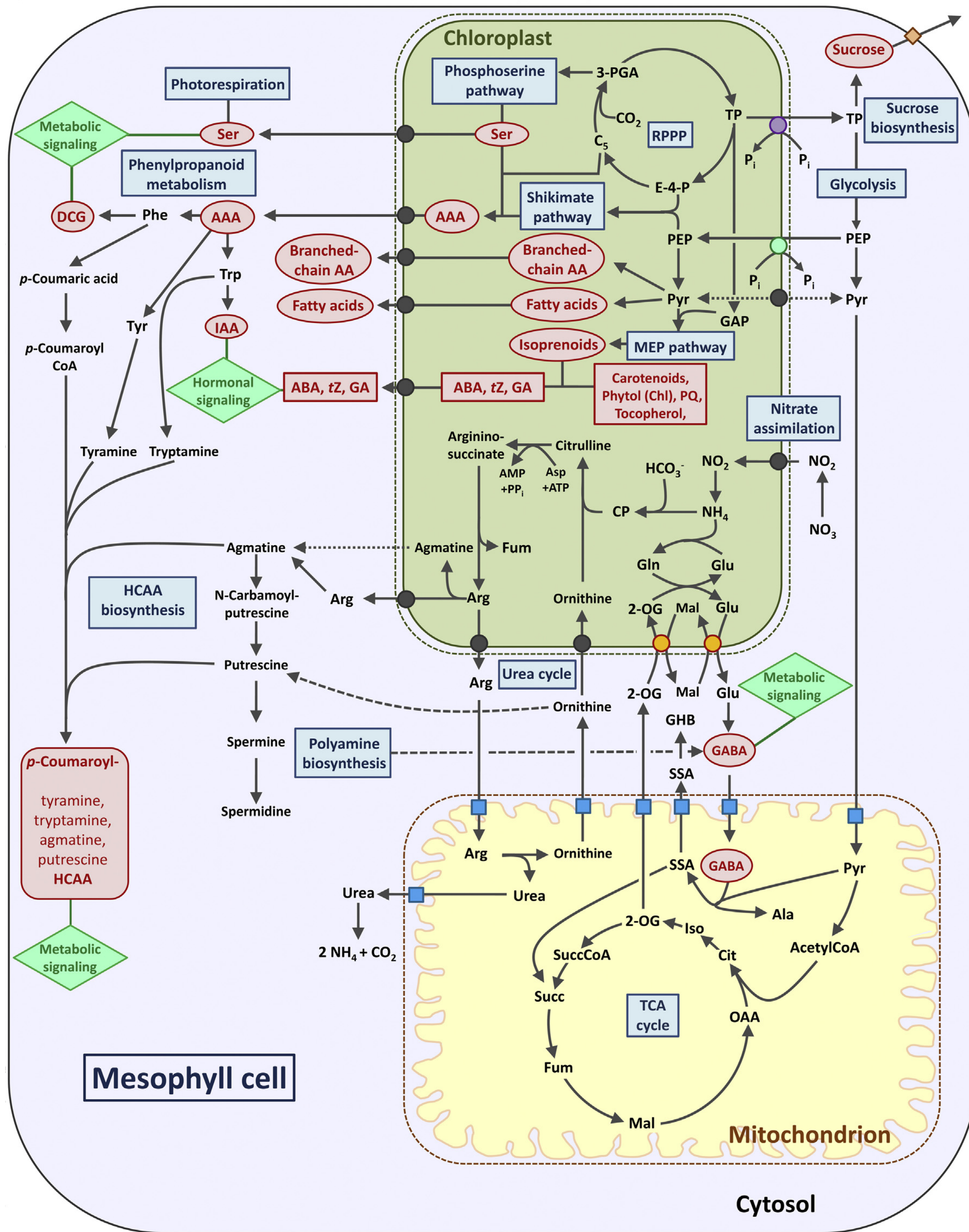


**Fig. 1.** Overview on metabolic pathways leading to amino acid-based metabolic signals and selected phytohormones in a mesophyll cell. The blue boxes, red ellipses, and green rhombuses represent major metabolic pathways, end products or important precursors, and metabolic or hormonal signals, respectively. In the chloroplasts, $CO_2$ is assimilated *via* the Calvin–Benson Cycle (reductive pentose phosphate pathway; RPPP) leading to triose phosphates (TP), which are exported by the TP/phosphate translocator (TPT; purple circle) to support sucrose biosynthesis in the cytosol and the subsequent export to the sinks. Another part of photoassimilates is subjected to glycolysis in order to form, for instance, phosphoenolpyruvate (PEP), which is either further metabolized to pyruvate or imported by the PEP/phosphate translocator (green circles) into the stroma as a substrate for the shikimate pathway, from which the aromatic amino acids (AAA) phenylalanine (Phe), tyrosine (Tyr) or tryptophan (Trp) derive. For the synthesis of Trp the amino acid serine (Ser) is required. Ser can also act as a metabolic signal. In photosynthetic tissues, photorespiration is the main source of Ser formation, followed by the phosphoserine pathway starting from 3-phosphoglycerate (3-PGA). Following its export to the cytosol, the AAA Phe can be de-aminated to cinnamic acid by Phe-ammonia lyase and forms the starting point for phenylpropanoid metabolism, from which flavonoids and lignin derive. The compound dehydrodiconiferyl alcohol glucoside (DCG) is a neolignan with a signaling potential. Plastidial PEP can be converted to pyruvate and serves as a substrate for the *de novo* synthesis of fatty acids, branched chain amino acids, or together with glyceraldehyde 3-phosphate (GAP) for isoprenoid biosynthesis *via* the methylerythritol 4-phosphate (MEP) pathway. Plastidial isoprenoids provide carbon skeletons for photosynthetic components such as carotenoids, phytol and the prenyl residues of plastoquinone (PQ) or tocopherol, but they are also involved in the biosynthesis of phytohormones like abscisic acid (ABA), *trans*-zeatin (*tZ*), or gibberellic acid (GA). Nitrate reduction combined with ammonia assimilation *via* the glutamine synthetase/glutamate synthase (GS/GOGAT) cycle leads to the formation of glutamate (Glu) from 2-oxoglutarate (2-OG). Decarboxylation of Glu delivers γ-amino butyric acid (GABA), which can act as a metabolic signal. In the GABA shunt, GABA is imported into the mitochondrial matrix and transaminated to succinic semialdehyde (SSA),

of cations, such as $Ca^{2+}$ upon GABA binding [45]. Twenty homologues have been identified in *A. thaliana*. However, only a few of these proteins have been characterized to date, and their role as amino acid-gated $Ca^{2+}$-channels has been substantiated only in the recent years ([46] and references therein). However, GABA has not been tested frequently as a ligand. Probably this sparse testing of GABA as a possible ligand of ion channels and other membrane proteins is the reason for the lack of information on GABA binding to plant glutamate receptors or any GABA-eliciting effect on electrogenic transport [47]. Considering the large number of putative glutamate receptor homologues in plants and their interplay among each other [48], it is still likely that a combination of glutamate receptors might be found that binds GABA and elicits $Ca^{2+}$ uptake into the cytosol. In such a scenario, the presence of GABA would strongly amplify the $Ca^{2+}$ signal in a feed-forward fashion, *i.e.* $Ca^{2+}$ stimulates GABA formation *via* binding of $Ca^{2+}$/calmodulin to glutamate decarboxylases and, in turn, GABA elicits further $Ca^{2+}$ uptake. GABA has to be exported in order to get access to its extracellular binding site of the glutamate receptor. Such an export can be accomplished by low-affinity (ProTs) [49,50] as well as high-affinity (GAT1) plasma membrane GABA transporters [51].

(2) The group of aluminum-activated malate transporters (AlMTs) can be considered as second type of transmembrane proteins acting as putative GABA receptors [52,53]. However, members of this group have characteristics of anion channels rather than transporters, *e.g.* AlMT9, a malate-gated $Cl^-$ channel of guard cell tonoplasts [54] and AlMT12, also known as QUAC1 (quick anion channel 1; [55,56]), functions as malate and sulfate outward rectifier in guard cells. AlMT proteins contain putative GABA binding sites that are homologous to GABA binding motifs of mammalian $GABA_A$ receptors [53]. The binding of GABA to the putative binding site occurs at the intracellular side of the plasma membrane and inhibits the conductivity of these channels for anions. Site-directed mutagenesis of a conserved phenylalanine (or tyrosine) residue within this sequence abolished binding of GABA. This was demonstrated by transient expression of the mutagenized wheat AlMT1 in *Xenopus laevis* oocytes and the determination of ion flux-dependent currents. In contrast to its native version, the mutagenized protein was functional as an ion channel in the presence of GABA.

However, it is puzzling that none of the suggested topologies of AlMTs predict the complete putative GABA binding site to be intracellular [57–59] questioning either the topology predictions, binding site predictions or the experimental setup. Taken together, GABA might be recognized by anion- and cation-conducting channels in plants. As binding of GABA to the 'receptors' modulates ion flow across membranes, GABA might be regarded as messenger for cellular communication, *i.e.* for signaling in plants.

### 3.2. The role of GABA in plant sexual reproduction

Apart from stress signaling, GABA is involved in plant sexual reproduction in that it influences pollen tube growth and guidance [60]. This part will be focused on the role of GABA in reproduction. Of course, there is much more needed than functional GABA metabolism or signaling to ensure successful double fertilization, as described in a recent review [61]. Indeed, it is not yet clear whether the aspects on the role of GABA in sexual reproduction discussed below should be regarded as signaling or as a consequence of metabolic imbalances, which eventually lead to the accumulation of toxic intermediates of GABA metabolism. Generally, it is a big challenge to differentiate between both options.

In an *A. thaliana* mutant deficient in GABA transaminase (*pop2-1*) sperm cells, delivered by the pollen tubes, were impaired in efficient self-fertilization of mutant ovules [60]. In contrast, *pop2-1* pollen tubes were capable of fertilizing wild-type ovules and *vice versa*. These findings are interesting due to their inherent complexity. The GABA concentration determines pollen tube growth both *in vitro* and *in vivo* (*i.e.* in the carpels). An increased GABA concentration resulted in enhanced elongation of *A. thaliana*, tobacco and lily pollen tubes. However, GABA concentrations in excess inhibited pollen tube elongation almost completely [60,62,63]. In the carpels of *A. thaliana* wild type a GABA gradient is established with increasing concentrations from the stigma *via* the stylar tissue to the micropyle of the ovary [60,63]. This gradient is disrupted in the carpels of *pop2-1* because GABA cannot be degraded and hence accumulates. The absence of a proper GABA gradient within the carpels leads either to a complete growth arrest of pollen tubes or, in rare cases, to a misguided growth of pollen tubes. Hence the *pop2-1* mutant is not completely sterile, as some pollen tubes and the sperm cells therein manage to accomplish their mission. It is, however, remarkable that wild-type pollen tubes manage to grow from the stigma to the ovaries of the *pop2-1* mutant despite of high GABA concentrations and the concomitant absence of a GABA gradient. Indeed, the resulting heterozygous mutant plants lack any decrease in fertility [60,62]. This finding can be explained if it is assumed that wild-type pollen tubes are capable of degrading excess GABA in the extracellular matrix and by this prevent GABA-induced growth arrest. However, for its degradation GABA has to be taken up by the pollen tubes. This uptake of GABA is probably mediated by the low-affinity GABA transporter ProT1 [49,50]. According to the eFP browser (http://bbc.botany.utoronto.ca/efp/; [64]) this transporter is strongly expressed in germinating pollen. Conversely, *pop2-1* pollen tubes also efficiently deliver their sperm cells to wild-type ovules. The presumed high GABA concentration inside the mutant pollen tubes is obviously not deleterious for pollen tube growth, when carpels contain moderate GABA concentrations and a GABA gradient exist. The mechanism as to how GABA mediates pollen tube growth remains elusive. However, there are approaches targeting this issue. For instance, putative GABA binding sites have been detected on the membranes of pollen protoplasts using GABA-coated fluorescent probes called quantum dots [65,66]. Moreover, it was possible to elicit $Ca^{2+}$ influx into pollen tubes upon GABA application [63,66]. $Ca^{2+}$ currents were stimulated by moderate GABA concentrations that would usually stimulate pollen tube growth, but were inhibited by high GABA concentrations that restrict pollen tube growth. Glutamate receptors could be excluded as being responsible for the GABA-induced currents by the application of a specific inhibitor for ionotropic glutamate receptors; 6-cyano-nitroquinoxaline 2,3-dione. As a positive control, glutamate-stimulated $Ca^{2+}$ currents were blocked by the inhibitor. Moreover, there are only few candidate genes

which then enters the tricarboxylic acid (TCA) cycle after oxidation to succinate (Succ). Other TCA cycle intermediates are fumarate (Fum), malate (Mal), oxaloacetate (OAA), citrate (Cit), isocitrate (Iso), and succinyl-CoA (SuccCoA). Alternatively SSA can be exported and reduced to γ-hydroxy butyric acid (GHB). Plastidial ammonium together with bicarbonate can be used for the synthesis of carbamoyl phosphate (CP) leading to the synthesis of the amino acid arginine (Arg), which can be further metabolized *via* the urea cycle or decarboxylated to agmatine. Agmatine together with putrescine, the decarboxylation product of ornithine, enters polyamine biosynthesis or the formation of hydroxycinnamic acid amides (HCAAs), which can have signaling functions in plants. The acid moiety of HCAAs derives from Phe in form of (*e.g.*) *p*-coumaric acid, the amide moiety from the amines, agmatine, putrescine, or tyramine and tryptamine as decarboxylation product of Tyr and Trp. The circles, squares or diamonds represent metabolite transporters in the inner envelope membrane of chloroplasts, the cristae membrane of mitochondria or the plasma membrane, respectively. For the sake of clarity enzyme names have been omitted.

Please cite this article in press as: R.E. Häusler, et al., Amino acids – A life between metabolism and signaling, Plant Sci. (2014), http://dx.doi.org/10.1016/j.plantsci.2014.09.011

encoding glutamate receptors that are expressed in pollen tubes and that might be responsible for the observed $Ca^{2+}$ currents [64].

Taken together, some light has been shed on how GABA might be involved in sexual reproduction of plants, especially in the delivery of sperm cells to the ovaries. Nevertheless, much more remains to be discovered, mainly on the mechanism of how plants exactly manage this important issue. It would be interesting to analyze whether a GABA gradient is indeed necessary for the guidance of pollen tubes to the micropyle or whether the arrest of pollen tube elongation- or misguidance in *pop2* mutants is just a matter of extremely high GABA tissue levels. Different approaches are required to tackle this issue in a way complementary to the *pop2* mutants. For instance, mutants or transgenic plants lacking GABA in floral organs would be ideal to study the GABA dependency of pollen tube growth. The decarboxylation of glutamate represents the main path of GABA formation. Mutants like *glutamate decarboxylase 5* might probably lack GABA in pollen tubes, as other glutamate decarboxylases are not expressed. If it were possible to generate plants that completely lack GABA in floral organs both the absolute requirement of certain GABA levels and/or a GABA gradient for proper pollen tube growth and fertilization could be tested.

### *3.3. GABA and cell elongation*

GABA is also involved in the elongation of cells other than pollen tubes. Exogenous supply of GABA resulted in a diminished growth of etiolated hypocotyls [62] or roots [62,67]. This GABA-dependent growth restriction was, in both cases, more pronounced in the *pop2* mutants defective in GABA transaminase. A closer inspection of hypocotyl epidermal cells as well as root cortical cells revealed that the growth retardation was based on a restriction of cell elongation rather than a decreased cell number [62]. Moreover, the suppression of root growth by E-2-hexenal, one of the major $C_6$-volatiles produced in *Arabidopsis* in response to wounding or herbivore attack, was accompanied by an increase in GABA contents in wild-type roots. However, an inhibition of root growth was absent in the so-called *E-2-hexenal response1* mutant. Strikingly this mutant is allelic to *pop2*, *i.e.* it lacks GABA transaminase activity. This failure to inhibit root growth in the presence of E-2-hexenal was completely unexpected and awaits an explanation. Probably, a threshold level of GABA has to be exceeded to confer E-2-hexenal resistance [68]. This would either mean that extremely high GABA concentrations promote rather than inhibit root growth or that the susceptibility of putative GABA receptors is decreased in mutants lacking GABA transaminase.

### *3.4. A role for GABA in patterning and cell identity*

Derivatives of GABA also seem to be involved in signaling. Accumulation of the transamination product of GABA, succinic semialdehyde, led to a severe growth retardation phenotype in *ssadh* mutants defective in succinic semialdehyde dehydrogenase. These plants accumulated reactive oxygen species, which may be partly responsible for the phenotype [69]. A simultaneous knock-out of the *GABA transaminase* gene upstream of the succinic semialdehyde dehydrogenase reaction almost completely rescued this phenotype [70]. However, a closer inspection of cotyledons of the *enf1-1* allele of mutations in the *succinic semialdehyde dehydrogenase* gene [71] revealed the absence of a full rescue by the simultaneous knock out of *GABA transaminase*. Cotyledons of *enf1-1* contained a white sector that also persisted in the *gaba transaminase/enf1-1* double mutant [71]. The *enlarged fil expression domain1* (*enf1*) mutation was detected in an EMS-mutagenized M2 generation that carried a *FIL* promoter-GFP construct. The *FIL* gene is expressed on the abaxial side of the leaf primordia [72,73]. The size of the FIL expression domain changed more frequently in both directions in *enf1-1* mutants, compared to the wild type, *i.e.* more mutant plants had abnormally small or large FIL expression domains. Robust leaf patterning along the adaxial–abaxial (upper–lower) axis was impaired in these plants [74]. Interestingly, the size of the FIL expression domain also changed in *gaba transaminase* mutants in that it increased significantly compared to the wild type. In the *enf1-1/gaba transaminase* double mutant, the size of the FIL expression domain was reduced back to wild-type size, *i.e.* the *enf1-1* mutation rescued the increased FIL domain phenotype of the *gaba transaminase* mutant. Moreover, the application of succinic semialdehyde at a position of the shoot apical meristem destined to develop the next leaf primordium resulted in leaves with abnormal adaxial–abaxial polarity. Some plants showed complete reversions of the abaxial and adaxial sides of the leaves, *i.e.* they carry more trichomes on the ‘lower’ side of the leaf. The identity of the ‘shoot apical meristem organizing center’ was disrupted in a way that several of the *enf1-1* mutant plants either lack a meristem, or have smaller, bigger or even multiple meristems. This has been exemplified in plants expressing a *Wuschel* promoter-GUS construct [74]. *Wuschel* can be considered as a marker gene for the ‘shoot apical meristem organizing center’.

Taken together, GABA and derivatives seem to be involved in a variety of metabolic (not discussed) and signaling functions. Still most of the underlying mechanisms have not been discovered despite the obvious importance of GABA in stress responses, sexual reproduction and development of plants. Even more puzzling, it is unclear how the absence of a hypostatic gene such as *succinic semialdehyde dehydrogenase* (compared to *GABA transaminase*) should lead to the rescue of a given phenotype, *i.e.* the reduced size of the FIL expression domain in *enf1-1/gaba transaminase* double mutants compared to the *gaba transaminase* single mutant. Two possible explanations should be analyzed in more detail in the future: The first is the existence of a source for succinic semialdehyde other than GABA, the second is a specific spatio-temporal expression of a different aminotransferases (*i.e.* not GABA transaminase) capable of using GABA as substrate.

## 4. Signal molecules deriving from aromatic amino acids

The aromatic amino acids phenylalanine, tyrosine, and tryptophan are essential for the diet of humans and animals because only bacteria, yeast, fungi and plants are capable of their *de novo* biosynthesis [5]. Beside of their role as constituents of proteins, aromatic amino acids are the precursors for the biosynthesis of large varieties of secondary products, among them compounds with hormonal or signaling function.

Phenylalanine is the precursor for phenylpropanoid metabolism (Fig. 1), which leads, for instance, to quantitatively important lignin in woody plants [75] or the blue to red pigments of the anthocyanin class as well as other flavonoids of the flavonol class [5,76]. The latter function as UV-shield and thus represented an important prerequisite for plants on the way to colonize the land during evolution [77]. Moreover, the flavonoid naringenin has been proposed to be involved in the regulation of auxin transport and is hence indirectly involved in hormonal signaling [78,79].

Phenolics deriving from the shikimate pathway intermediate chorismate, such as salicylic acid, are involved as signals in the response of plants to pathogens in a process termed systemic acquired resistance [80].

Tryptophan is the precursor for the synthesis of the auxin indole acetic acid [81] and, in Brassicacean species, for indole glucosinolates, which play a profound role in the defense against herbivores together with aliphatic glucosinolates [82].

UNCORRECTED PROOF

### 4.1. Phosphoenolpyruvate, an important link between primary metabolism and aromatic amino acid-based signaling

In plants, the synthesis of aromatic amino acids *via* the shikimate pathway takes place exclusively inside the plastids [5,6,83] and starts from erythrose 4-phosphate and PEP (see Fig. 1). As most plastids lack a complete glycolysis [84] and are hence unable to produce PEP inside the stroma, it has to be imported from the cytosol *via* a PEP-specific phosphate translocator (PPT; [85]).

The *chlorophyll a/b binding protein underexpressed1* (*cue1*) mutant defective in one of the two PPTs of *A. thaliana* [86], exhibits a developmental phenotype characterized by reticulate leaves and stunted roots [87,88]. A complete loss of PEP supply to plastids in a double mutant lacking both PPT1 and plastidial enolase [84] resulted in gametophytic lethality of the double homozygous mutant plants [89]. In an earlier report, a general restriction of the shikimate pathway by limiting PEP supply has been assumed [86], a view that seemed to be oversimplified, as some downstream products of the shikimate pathway appeared to be decreased whereas others were increased in *cue1* [90]. The complex developmental phenotype of the *cue1* mutant cannot be solely explained by an impaired metabolism, but suggests constraints in signaling pathways as well.

Probably not only metabolic intermediates and signals deriving directly from the shikimate pathway and downstream products are affected in *cue1*, but also anabolic sequences starting from plastidial pyruvate [16], like *de novo* fatty acid or plastidial isoprenoid biosynthesis (see Fig. 1). Beside of carotenoids or the phytol-chain of chlorophyll the latter also generates the precursors of the phytohormones gibberellic acid and abscisic acid [91] as well as the prenyl side-chain of the active cytokinin *trans*-zeatin [92]. Thus, in *cue1*, hormonal signaling might be affected beside of metabolic signaling. It would therefore be challenging for future experiments to dissect such hormonal and metabolic signaling pathways in similar mutant systems.

### 4.2. Lignans and neolignans deriving from phenylpropanoid metabolism act as signal molecules

Products of the shikimate pathway play multifaceted roles in primary and secondary metabolism. Lignans and neolignans represent signal molecules deriving from phenylpropanoid metabolism. They are products of the oxidative dimerization of two phenylpropanoid molecules [77], whereby the $C_3$ side chains of the monomers are linked by C—C bonds either tail-to-tail (lignans) or head-to-tail (neolignans). Neolignans have been discussed as putative signal molecules in plants for approximately 20 years starting from the time point when the infection mechanism of *Agrobacterium tumefaciens* and its potential for plant transformation had been unraveled [93,94]. However, at the end of the 1990s neolignans almost completely disappeared from the focus of plant science, but experienced a renaissance thereafter based on their pharmaceutical potential, in particular as both substance classes appear to have anti-cancer properties [95,96].

At the end of the 1970s the mechanism of infection of host plants by *A. tumefaciens* had been resolved in detail. Axenic cultures of tumor cells generated from the crown galls of infected plants exhibited an apparently phytohormone-independent growth. This is due to the fact that biosynthesis genes for auxins and cytokinins are contained on the transferred DNA of the bacterial tumor inducing plasmid, which is stably integrated in the host genome after infection [97]. There were, however, early indications for other, yet unidentified components synthesized by *Vinca rosea* crown galls [98]. Based on its capacity to replace the cytokinin requirement of axenic tobacco pith cultures, a new compound has been isolated from *V. rosea* crown gall tissue, dehydrodiconiferyl alcohol glucoside (DCG; see Figs. 1 and 2A), that seemed to be linked to cytokinin accumulation [7]. Various enantiomers of DCG have either been purified or were chemically synthesized and they all exhibited growth-stimulating effects on tobacco pith cultures albeit to a different extent [8,9]. From a structural point of view DCG or its aglycon DCA resemble cell wall components, which can be formed as side products of lignin biosynthesis [8]. In *Zinnea elegans* the accumulation of DCG coincides with tracheary element differentiation [99]. Indeed the biosynthesis of DCA starts from the phenylpropanoid coniferyl alcohol. Dimerization of coniferyl alcohol monomers can occur non-enzymatically in the presence of $H_2O_2$. The glucose moiety is then added by glycosyl transferases [100]. More recent studies in flax cells revealed that four major di-lignols could be identified when cell suspension cultures were fed with $^{13}$C labeled coniferyl alcohol, *i.e.* the lignan lariciresinol diglucoside, and the neolignans DCG as well as the erythro- and threo-forms of guaicylglycerol-β-coniferyl ether glucoside [101].

The cytokinin-like role of DCG has been re-inforced following the infection of tobacco leaf discs with an *A. tumefaciens* strain that lacks the cytokinin biosynthesis locus *tumor morphology r* (*tmr*).

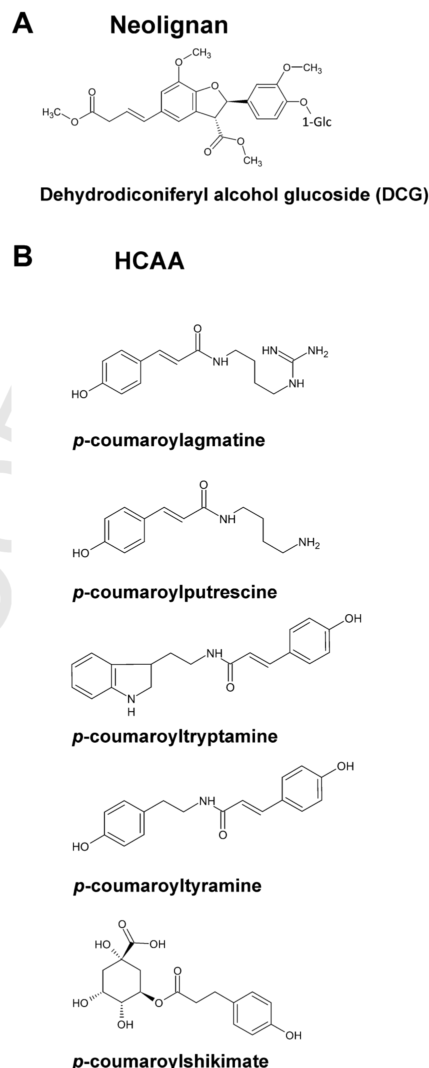


**Fig. 2.** Structures of putative signal molecules belonging to the neolignan and hydroxycinnamic acid amide (HCAA) class deriving from phenylpropanoid metabolism (*i.e.* from phenylalanine) and arginine (*i.e. p*-coumaroylagmatine and *p*-coumaroylputrescine).

Please cite this article in press as: R.E. Häusler, et al., Amino acids – A life between metabolism and signaling, Plant Sci. (2014), http://dx.doi.org/10.1016/j.plantsci.2014.09.011

Instead of developing a rooty phenotype, indicative for a high auxin to cytokinin ratio, the explants produced fast growing, unorganized tumors suggesting that DCG can substitute cytokinins in callus growth [102]. DCG belongs to the about 40 low molecular weight phenolics that are capable of inducing virulence genes of *A. tumefaciens*, and might therefore determine the susceptibility of the host toward *A. tumefaciens* infection [103]. In the meantime pathways, by which lignans and neolignans are synthesized *in planta* have been studied in more detail, *e.g.* [101].

### 4.3. Transgenic and mutant plants with impaired neolignan signaling

Transgenic tobacco plants overexpressing the weak MYB transcription factors AmMYB308 and AmMYB330 from *Antirrhinum major* exhibited a perturbed phenylpropanoid metabolism due to the replacement of strong endogenous tobacco MYB factors from their target genes. The transgenic plants showed stunted growth, reticulate leaves and less lignification in their stems [104]. A detailed analysis of the leaf phenotype revealed a reduced size of mesophyll cells accompanied by increased intercellular air spaces [105]. Both characteristics resemble the leaf- and growth phenotype that had been previously reported for the *A. thaliana cue1* mutant [86,87].

A further analysis of the phenylpropanoid composition of the AmMYB308 and AmMYB330 overexpressing lines compared to the control plants revealed that the contents of DCG and its aglycon DCA were diminished [105]. In a cell culture system the aberrant rod-shaped mesophyll cells of the transgenic lines could be rescued by the application of either 100 μM DCA or 10 μM DCG, suggesting that the lowered content of this substance might be linked to the phenotype [105].

In addition, tobacco antisense lines with a diminished activity of cytosolic enolase show a similar phenotype as the MYB factor overexpressing lines [106]. In the case of the antisense plants, PEP generation further upstream of phenylpropanoid metabolism or PEP import by the PPT is impaired.

The *reduced epidermal fluorescence8* (*REF8*) locus of *A. thaliana* encodes a *p*-coumarate hydroxylase [107]. The corresponding mutant accumulates *p*-coumarate esters instead of sinapylmalate like wild-type plants [108]. It has been speculated, but not shown, that diminished contents of DCG and other phenylpropanoid derivatives might be responsible for the severe growth retardation of the mutant.

Recently we could demonstrate that DCG, but not DCA is capable of rescuing the reticulate leaf phenotype of *cue1* [88]. However, unlike other soluble compounds, DCG cannot be taken up by the roots of the mutants. Hence roots had to be excised and the substance was then fed *via* the cut-edge of the stems.

The major obstacle for further elucidating the mechanism by which DCG or similar compounds interfere with plant growth is the lack of commercial availability. Indeed DCG had to be either purified from tissues that produce substantial amounts, such as roots of a certain *Linum usitassimum* variety [96] or it has to be chemically synthesized. As a matter of fact, the feeding studies conducted with the *cue1* mutant [88] were done with DCG or DCA preparations used for the transgenic tobacco plants [105] and these were obtained from David Lynn's lab in the first place. The lack of availability is probably one major reason for the decreased interest in mechanistic studies of this substance class in plant metabolism, development or signaling.

In the meantime a large number of different lignans and neolignans have been isolated and their chemical structure unraveled, for instance, those involved in the biosynthesis of the lignin hinokinin in *Linum corymbulosum* [109] including neolignans like DCA or lignans like lariciresinol or pinoresinol. However, neither DCA nor both lignans had any rescuing effect in the *A. thaliana cue1* mutant [88].

### 4.4. Hydroxycinnamic acid amides (HCAA) as metabolic signals

Hydroxycinnamic acids also belong to the class of phenylpropanoids originating from the aromatic amino acid phenylalanine. The metabolic fate of hydroxycinnamic acids is tightly coupled with the biosynthesis of polyamines [110], which starts from the amino acid arginine (see Fig. 1) or other urea cycle intermediates [111]. Following the decarboxylation of arginine by arginine decarboxylase, its product agmatine can be converted to putrescine by two enzymatic steps accompanied by the release of urea. Likewise, the arginine precursor ornithine can be directly decarboxylated to putrescine. The chain elongation of putrescine to spermidine or spermine involves the decarboxylation product of S-adenosylmethionine [111].

For arginine biosynthesis, carbamoyl phosphate is required as a substrate in the step from ornithine to citrulline catalyzed by ornithin transcarbamoylase (Slocum, 2005; see Fig. 1). Surprisingly, the *A. thaliana venosa3/6* (*ven3/6*) double mutant, which is impaired in two different subunits of carbamoyl phosphate synthase, exhibits a similar reticulate leaf phenotype as the *cue1* mutant [112]. Obviously defects in completely different metabolic pathways lead to similar developmental constraints.

The agmatine, putrescine and polyamines are substrates for the synthesis of hydroxycinnamic acid amides (HCAA) such as *p*-coumaroylagmatine or *p*-coumaroylputrescine (Fig. 2B). HCAAs are widely distributed among the plant kingdom, but their physiological function is still controversially discussed. As previously summarized [10], the function of HCAAs during development ranges from induction of flowering, and sexual differentiation to tuber induction of potato plants, as well as cell division, and photomorphogenesis. A function of HCAA and its polymers as an integral constituent of pathogen defense in the cell wall appears to be generally accepted. A detailed analysis of the impact of these compounds is awaited.

Genes involved in the synthesis of HCAA in *A. thaliana* have been partially annotated. For instance, the transferase, which catalyzes the agmatine-dependent synthesis of *p*-coumaroylagmatine has been functionally and molecularly characterized in barley [113]. Beside of *p*-coumaroylCoA this enzyme also uses other hydroxycinnamic acid CoA esters such as feruloylCoA or caffeoylCoA as substrates. A comparison of the amino acid sequence of the agmatine O-hydroxycinnamoyl transferase from barley with those of other organisms revealed that this protein belongs to a highly diverse transferase superfamily. There is for instance no clear sequence homology of the gene encoding the barley enzyme with genes from *A. thaliana*. However, the shikimate/quinate O-hydroxycinnamoyl transferase of *A. thaliana*, leading for instance to *p*-coumaroylshikimate (Fig. 2B), belongs to the same family. A knockout of this transferase leads to an inhibition of lignin biosynthesis combined with an increase in flavonoid contents and a de-regulation of auxin effects [114]. Moreover, the mutant is severely compromised in growth. Enzymes involved in the biosynthesis of hydroxycinnamic acid conjugates with spermidine have recently been identified in *A. thaliana* [115].

### 4.5. HCAAs have multiple functions in plants and mammalians

Like lignans and neolignans HCAAs stimulate *vir* genes in *A. tumefaciens* [116]. One of the most profound functions of polyamines and their conjugates is the role in plant pathogen interactions [117,118]. For instance, in winter wheat the antifungal component *p*-coumaroylagmatine accumulates when the plants are covered with snow. This compound is probably induced by cold

Please cite this article in press as: R.E. Häusler, et al., Amino acids – A life between metabolism and signaling, Plant Sci. (2014), http://dx.doi.org/10.1016/j.plantsci.2014.09.011

stress and protects the plant from snow mold by a yet unknown mechanism [119].

Some more recent publications provide examples that demonstrate the impact HCAAs have on the response of plants toward pathogens. In tomato plants infection with the bacterium *Pseudomonas syringae* resulted in the accumulation of HCAAs, among them novel compounds such as *p*-coumaroyldopamine and feruloyldopamine [120]. Similarly, the hypersensitive response elicited by the pathogen *Cladosporium fulvum* is accompanied by a massive alteration in the transcriptome and metabolome in tomato plants giving rise to the accumulation of phenylpropanoids including HCAAs [121]. HCAAs are also induced when *A. thaliana* plants are infected with pathogens like *Alternaria brassicicola* [122]. Torenia plants overexpressing agmatine coumaroyl transferase, the enzyme catalyzing the final step in the synthesis of the HCAA *p*-coumaroylagmatine, were resistant against the fungus *Botrytis cinerea*, but not against herbivores [123]. Late blight is a serious pathogen in potato crops and can lead to complete crop loss. Several quantitative trait loci have been identified that confer resistance to late blight. Factors involved in this resistance could recently be identified by an undirected metabolome approach. Among the phenylpropanoids, HCAAs play a profound role in the resistance to this pathogen [124]. Undirected metabolome and proteome analyses of another quantitative trait locus in wheat reveal HCAAs as major factors involved in the resistance against the fungus *Fusarium graminearum* [125].

Similar approaches were conducted with tobacco plants in response to inoculation with pathogens. Again HCAAs were among the compounds that accumulated [126]. The importance of tryptophan-derived secondary compounds such as conjugates of the decarboxylation product tryptamine or serotonin in pathogen defense [127] has been tested by inhibition of tryptophan decarboxylase in rice plants infested by the fungus *Bipolaris oryzae* [128].

Wounding is another abiotic stress that leads to the accumulation of HCAAs. The composition of wound-induced HCAAs formation has been further studied in transgenic tobacco plants overexpressing tryptophan- or tyrosine decarboxylases [129]. Although tryptamine accumulated in tryptophan decarboxylase overexpressors after wounding, they lack the accumulation of hydroxycinnamic acid conjugates. In contrast overexpression of tyrosine decarboxylase led to the accumulation of wound-induced hydroxycinnamic acid conjugates with tyramine suggesting that tyrosine decarboxylase is a rate limiting step in their synthesis [129]. Similarly, a combined constitutive overexpression of tyrosine decarboxylase and tyramine hydroxycinnamoyl transferase also led to an increase in tyramine conjugates of hydroxycinnamic acid [130].

Polyamines, their conjugates and HCAAs are highly abundant in flowers. Flower-specific HCAAs have been analyzed in pollen of *A. thaliana* wild-type plants compared to a mutant lacking spermidine hydroxycinnamoyl transferase [131]. Particularly high concentrations of HCAAs were found in the tapetum of the stamen. The tapetum-localized spermidine hydroxycinnamoyl transferase plays a key role. The loss of this enzyme results in a strong depletion of HCAAs in anthers and pollen grains [132].

HCAAs have antiviral, antioxidative, and anti-inflammatory potentials in humans [133,134]. Hydroxycinnamic acid conjugates with serotonin have been shown to exert cytoprotective effects in mammalian cell cultures against oxidative stress [135]. In this context it appears interesting that transgenic rice plants produce coumaroylserotonin and feruloylserotonin when they express hydroxycinnamoyl-CoA:serotonin N-(hydroxycinnamoyl) transferase from pepper under the control of a constitutive maize ubiquitin promoter [136]. By chemical fusion of pharmacologically active compounds such as the aporphine alkaloid glaucin, which itself has some antioxidative and antiviral potential, with cinnamic acid or hydroxcinnamic acids, products have been obtained that showed enhanced individual effects [137].

Apart from pharmacological approaches another focus is a survey on the occurrence and contents of amines and HCAAs such as the antioxidant and anti-inflammatory compounds feruloyltyramine and *p*-coumaroylserotonin, which are beneficial for human diet, in a number of vegetables such as tomato or pepper fruits [138]. Changes in the composition of phenylpropanoids and enzymes involved in their metabolism during potato tuber growth suggest varying nutritional values depending upon the developmental stage [139].

Like for neolignans and lignans mechanistic studies are required that will help to understand the impact HCAAs have on the performance and development of plants.

## 5. Conclusions and future perspectives

### 5.1. Serine

The function of L-serine as signaling molecule is currently subject of intense debate in the fields of cancer research and plant biology. In proliferating cancer cells, L-serine has been identified as a regulator of TOR kinase activity. In plants, the 'phosphorylated' serine and the TOR pathways are highly active in meristems. A regulation of TOR kinase by L-serine similar to the mammalian system can be assumed and would hence represent a promising target for future studies on the signaling function of L-serine.

The activity of TOR kinase is usually measured as change in the phosphorylation state of its target protein, *i.e.* the ribosomal S6 kinase, by antibodies specific for the phosphorylation site. In turn, the S6 kinase phosphorylates the ribosomal protein S6, a critical component of the 40S ribosomal subunit. A possible impact of L-serine on TOR kinase activity could be studied by determination of the S6 kinase phosphorylation state either after treatment of plants with physiological concentrations of L-serine or in plants deficient in the 'phosphorylated' serine biosynthesis pathway. This approach would not only shed light on the question if L-serine is a signaling molecule in plants, but it would also help to understand how growth and development is regulated by metabolite signals in plants.

### 5.2. GABA

It is generally challenging to discriminate between a metabolic and a putative signaling function of a substance. In case of GABA the metabolic role is beyond dispute. As an amino acid it is located at the intersection of nitrogen and carbon metabolism. In order to assign a signaling function to GABA, it has to be recognizable in the first place. Receptor proteins can usually perceive a signal, and as outlined above, there are indications that among glutamate receptors and AlMTs there might also be GABA receptors. A role for GABA or derivatives as signal has been discussed in sexual reproduction, cell elongation, patterning and cell identity. In all these cases novel insights were obtained from exogenous GABA feeding or from mutants that accumulate GABA or its derivatives. Both approaches have an increased GABA content in common. A significant advance in understanding could be achieved by the creation and analysis of GABA-deficient plants. With help of these plants the problems in distinguishing between metabolic and signaling functions of GABA could finally be broken up.

Provided that GABA has no extraordinary importance for primary metabolism unlike *e.g.* glutamate, strong metabolic phenotypes would not be expected in GABA deficient mutant plants. In contrast, the absence of a signal might elicit strong phenotypes, especially when plants are grown under conditions where GABA

Please cite this article in press as: R.E. Häusler, et al., Amino acids – A life between metabolism and signaling, Plant Sci. (2014), http://dx.doi.org/10.1016/j.plantsci.2014.09.011

usually is increased. Access to GABA-free mutants would certainly improve GABA-related research and should therefore be strongly promoted in the future.

### 5.3. Phenylpropanoids and HCAAs

As already mentioned in Section 4.3, the lack of commercial availability of lignans, neolignans or HCAAs might hamper more detailed analyses on the signaling functions of these compounds in the future. Probably scientists interested in this branch of research should communicate with each other and bring forward sources, where such compounds of interest can be obtained from, or information on who might be capable of synthesizing and/or purifying larger amounts of these substances. Mutants of *A. thaliana* or transgenic tobacco plants might be a helpful tool. For instance, the *ref2* mutant of *A. thaliana* impaired in a P450-dependent monooxygenase and consequently in phenylpropanoid metabolism might help to unravel the complex interplay of plant phenolics [140]. Moreover, metabolic mutants that are impaired in different pathways, but share a common developmental phenotype might be used as tools to unravel putative signal molecules or signaling pathways. As an example, reticulate mutants that show wild-type like vascular bundles and bundle sheath cells, but are affected in the size and density of mesophyll cells and chloroplasts therein might share common de-regulated signaling pathways.

The *cue1* mutant is defective in PEP provision to the shikimate pathway in most plastid types and is hence partially impaired in secondary metabolism [90], probably including the generation of metabolic signals [88]. Another reticulate mutant, *small organ1* (*smo1*) allelic to *trp2*, is defective in tryptophan biosynthesis due to a lesion in the β-subunit of tryptophan synthase [141]. This mutant shares not only reticulated leaves with *cue1*, but also growth retardation of aerial parts and stunted roots [88]. The *low cell density1* (*lcd1*) mutants, which is allelic to *reticulata* [142], is impaired in a not yet functionally characterized chloroplast membrane protein [143]. It shares only the reticulate leaf phenotype with *cue1* [88]. The reticulate mutant *ven3/6* is defective in carbamoyl phosphate synthase and hence in the production of arginine [112]. Indeed citrulline, the precursor of arginine synthesis, is severely decreased in *ven3/6*. Interestingly, *cue1* shows increased levels of arginine [86,88]. It is hence tempting to speculate that HCAA synthesis might be impaired in *cue1* or *ven3/ven6* mutants by an inhibited provision with either hydroxycinnamic acids or with agmatine (the decarboxylation product of arginine), respectively.

For most genes involved in phenylpropanoid metabolism mutants are available. However, to the knowledge of the authors none of these mutants shows a reticulate leaf phenotype. Hence the developmental constraints observed in reticulate mutants are probably based on a combination of defects in metabolism and hormonal- as well as metabolic signaling. Moreover, a crosstalk between phenylpopanoids and transcription factors exists in both di- and monocotyledonous species (like grasses), as has been recently summarized [144].

Combined transcriptome and metabolome analyses of mutant, transgenic and wild-type plants will help to unravel detailed mechanisms of metabolic signaling. So far, neither interaction partners nor *cis* or *trans* elements of metabolite triggered gene regulation have been identified.

## Acknowledgements

Q2 We thank the 'Deutsche Forschungsgemeinschaft' for funding projects dealing with certain aspects of this paper, *i.e.* the roles of serine (KR4245/1-1), GABA (LU1199/2-1), and transporters, such as the phosphoenolpyruvate/phosphate translocator (FL126/23-1). We also thank the German-Israeli Foundation for Scientific Research and Development (GIF) for funding another grant dealing with GABA (I-933-239.12/2006).

## References

[1] J.W. Locasale, Serine, glycine and the one-carbon cycle: cancer metabolism in full circle, Nat. Rev. Cancer 13 (2013) 572–583, http://dx.doi.org/10.1038/nrc3557.Serine.
[2] R. Possemato, K.M. Marks, Y.D. Shaul, M.E. Pacold, D. Kim, K. Birsoy, et al., Functional genomics reveal that the serine synthesis pathway is essential in breast cancer, Nature 476 (2011) 346–350, http://dx.doi.org/10.1038/nature10350.
[3] R.M. Benstein, K. Ludewig, S. Wulfert, S. Wittek, T. Gigolashvili, H. Frerigmann, et al., Arabidopsis phosphoglycerate dehydrogenase1 of the phosphoserine pathway is essential for development and required for ammonium assimilation and tryptophan biosynthesis, Plant Cell 25 (2013) 5011–5029, http://dx.doi.org/10.1105/tpc.113.118992.
[4] R. Olsen, W. Sieghart, $GABA_A$ receptors: subtypes provide diversity of function and pharmacology, Neuropharmacology 56 (2009) 141–148, http://dx.doi.org/10.1016/j.neuropharm.2008.07.045.GABA.
[5] V. Tzin, P.B. Division, T. Samuel, R. Noble, Shikimate Pathway and Aromatic Amino Acid Biosynthesis, eLS. John Wiley Sons, Ltd, Chichester, 2012, pp. 1–10, http://dx.doi.org/10.1002/9780470015902.a0001315.pub2.
[6] H. Maeda, N. Dudareva, The shikimate pathway and aromatic amino acid biosynthesis in plants, Annu. Rev. Plant Biol. 63 (2012) 73–105.
[7] D.G. Lynn, R.H. Chen, K.S. Manning, H.N. Woodt, Structural characterization of endogenous factors from *Vinca rosea* crown gall tumors that promote cell division of tobacco cells, Proc. Natl. Acad. Sci. U. S. A. 84 (1987) 615–619.
[8] A.N. Binns, R.H. Chen, H.N. Wood, D.G. Lynn, Cell division promoting activity of naturally occurring dehydrodiconiferyl glucosides: do cell wall components control cell division? Proc. Natl. Acad. Sci. U. S. A. 84 (1987) 980–984.
[9] R.A. Teutonico, M.W. Dudley, J.D. Orr, D.G. Lynn, A.N. Binns, Activity and accumulation of cell division-promoting phenolics in tobacco tissue cultures, Plant Physiol. 97 (1991) 288–297.
[10] P.J. Facchini, J. Hagel, K.G. Zulak, Hydroxycinnamic acid amide metabolism: physiology and biochemistry, Can. J. Bot. 80 (2002) 577–589, http://dx.doi.org/10.1139/B02-065.
[11] J.B. Ohlrogge, J.G. Jaworski, Regulation of fatty acid synthesis, Annu. Rev. Plant Physiol. Plant Mol. Biol. 48 (1997) 109–136, http://dx.doi.org/10.1146/annurev.arplant.48.1.109.
[12] B.K. Singh, D.L. Shaner, Biosynthesis of branched chain amino acids: from test tube to field, Plant Cell 7 (1995) 935–944, http://dx.doi.org/10.1105/tpc.7.7.935.
[13] H.K. Lichtenthaler, The 1-deoxy-D-xylulose-5-phosphate pathway of isoprenoid biosynthesis in plants, Annu. Rev. Plant Physiol. Plant Mol. Biol. 50 (1999) 47–65, http://dx.doi.org/10.1146/annurev.arplant.50.1.47.
[14] H. Bauwe, M. Hagemann, A.R. Fernie, Photorespiration: players, partners and origin, Trends Plant Sci. 15 (2010) 330–336, http://dx.doi.org/10.1016/j.tplants.2010.03.006.
[15] R.D. Slocum, Genes, enzymes and regulation of arginine biosynthesis in plants, Plant Physiol. Biochem. 43 (2005) 729–745, http://dx.doi.org/10.1016/j.plaphy.2005.06.007.
[16] U.-I. Flügge, R.E. Häusler, F. Ludewig, M. Gierth, The role of transporters in supplying energy to plant plastids, J. Exp. Bot. 62 (2011) 2381–2392, http://dx.doi.org/10.1093/jxb/erq361.
[17] M. Chen, G. Han, C.R. Dietrich, T.M. Dunn, E.B. Cahoon, The essential nature of sphingolipids in plants as revealed by the functional identification and characterization of the *Arabidopsis* LCB1 subunit of serine palmitoyltransferase, Plant Cell 18 (2006) 3576–3593, http://dx.doi.org/10.1105/tpc.105.040774.
[18] Y. Yamaoka, Y. Yu, J. Mizoi, Y. Fujiki, K. Saito, M. Nishijima, et al., Phosphatidylserine synthase1 is required for microspore development in *Arabidopsis thaliana*, Plant J. 67 (2011) 648–661, http://dx.doi.org/10.1111/j.1365-313X.2011.04624.x.
[19] A.D. Hanson, S. Roje, One-carbon metabolism in higher plants, Annu. Rev. Plant Physiol. Plant Mol. Biol. 52 (2001) 119–137.
[20] B. Cascales-Miñana, J. Muñoz-Bertomeu, M. Flores-Tornero, A.D. Anoman, J. Pertusa, M. Alaiz, et al., The phosphorylated pathway of serine biosynthesis is essential both for male gametophyte and embryo development and for root growth in *Arabidopsis*, Plant Cell 25 (2013) 2084–2101, http://dx.doi.org/10.1105/tpc.113.112359.
[21] E. Michard, P.T. Lima, F. Borges, A.C. Silva, M.T. Portes, J.E. Carvalho, et al., Glutamate receptor-like genes form $Ca^{2+}$ channels in pollen tubes and are regulated by pistil D-serine, Science 332 (2011) 434–437, http://dx.doi.org/10.1126/science.1201101.
[22] J.P. Mothet, A.T. Parent, H. Wolosker, R.O. Brady, D.J. Linden, C.D. Ferris, et al., D-Serine is an endogenous ligand for the glycine site of the N-methyl-D-aspartate receptor, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 4926–4931.
[23] S. Timm, A. Florian, M. Wittmiß, K. Jahnke, M. Hagemann, A.R. Fernie, et al., Serine acts as a metabolic signal for the transcriptional control of photorespiration-related genes in *Arabidopsis*, Plant Physiol. 162 (2013) 379–389, http://dx.doi.org/10.1104/pp.113.215970.
[24] J. Ye, A. Mancuso, X. Tong, P.S. Ward, J. Fan, J.D. Rabinowitz, Pyruvate kinase M2 promotes de novo serine synthesis to sustain mTORC1 activity

UNCORRECTED PROOF

and cell proliferation, Proc. Natl. Acad. Sci. U. S. A. 109 (2012) 6904–6909, http://dx.doi.org/10.1073/pnas.1204176109.
[25] Y. Zhang, J.R. Dickinson, M.J. Paul, N.G. Halford, Molecular cloning of an *Arabidopsis* homologue of GCN2, a protein kinase involved in co-ordinated response to amino acid starvation, Planta 217 (2003) 668–675, http://dx.doi.org/10.1007/s00425-003-1025-4.
[26] Y. Zhang, Y. Wang, K. Kanyuka, M.A.J. Parry, S.J. Powers, N.G. Halford, GCN2-dependent phosphorylation of eukaryotic translation initiation factor-2alpha in *Arabidopsis*, J. Exp. Bot. 59 (2008) 3131–3141, http://dx.doi.org/10.1093/jxb/ern169.
[27] A.G. Hinnebusch, Translational regulation of GCN4 and the general amino acid control of yeast, Annu. Rev. Microbiol. 59 (2005) 407–450, http://dx.doi.org/10.1146/annurev.micro.59.031805.133833.
[28] E.H. Byrne, I. Prosser, N. Muttucumaru, T.Y. Curtis, A. Wingler, S. Powers, et al., Overexpression of GCN2-type protein kinase in wheat has profound effects on free amino acid concentration and gene expression, Plant Biotechnol. J. 10 (2012) 328–340, http://dx.doi.org/10.1111/j.1467-7652.2011.00665.x.
[29] S. Wullschleger, R. Loewith, M.N. Hall, TOR signaling in growth and metabolism, Cell 124 (2006) 471–484, http://dx.doi.org/10.1016/j.cell.2006.01.016.
[30] B. Menand, T. Desnos, L. Nussaume, F. Berger, D. Bouchez, C. Meyer, et al., Expression and disruption of the *Arabidopsis* TOR (target of rapamycin) gene, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 6422–6427, http://dx.doi.org/10.1073/pnas.092141899.
[31] D. Deprost, L. Yao, R. Sormani, M. Moreau, G. Leterreux, M. Nicolaï, et al., The *Arabidopsis* TOR kinase links plant growth, yield, stress resistance and mRNA translation, EMBO Rep. 8 (2007) 864–870, http://dx.doi.org/10.1038/sj.embor.7401043.
[32] C.S. Ahn, J.-A. Han, H.-S. Lee, S. Lee, H.-S. Pai, The PP2A regulatory subunit Tap46, a component of the TOR signaling pathway, modulates growth and metabolism in plants, Plant Cell 23 (2011) 185–209, http://dx.doi.org/10.1105/tpc.110.074005.
[33] M. Moreau, M. Azzopardi, G. Clément, T. Dobrenel, C. Marchive, C. Renne, et al., Mutations in the *Arabidopsis* homolog of LST8/GβL, a partner of the target of rapamycin kinase, impair plant growth, flowering, and metabolic adaptation to long days, Plant Cell 24 (2012) 463–481, http://dx.doi.org/10.1105/tpc.111.091306.
[34] C. Caldana, Y. Li, A. Leisse, Y. Zhang, L. Bartholomaeus, A.R. Fernie, et al., Systemic analysis of inducible target of rapamycin mutants reveal a general metabolic switch controlling growth in *Arabidopsis thaliana*, Plant J. 73 (2013) 897–909, http://dx.doi.org/10.1111/tpj.12080.
[35] N.J. Tillakaratne, L. Medina-Kauwe, K.M. Gibson, gamma-Aminobutyric acid (GABA) metabolism in mammalian neural and nonneural tissues, Comp. Biochem. Physiol. A: Physiol. 112 (1995) 247–263.
[36] D.H. Park, R. Mirabella, P.A. Bronstein, G.M. Preston, M.A. Haring, C.K. Lim, et al., Mutations in γ-aminobutyric acid (GABA) transaminase genes in plants or *Pseudomonas syringae* reduce bacterial virulence, Plant J. 64 (2010) 318–330, http://dx.doi.org/10.1111/j.1365-313X.2010.04327.x.
[37] R. Chevrot, R. Rosen, E. Haudecoeur, A. Cirou, B.J. Shelp, E. Ron, et al., GABA controls the level of quorum-sensing signal in *Agrobacterium tumefaciens*, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 7460–7464, http://dx.doi.org/10.1073/pnas.0600313103.
[38] B.J. Shelp, A.W. Bown, D. Faure, Extracellular gamma-aminobutyrate mediates communication between plants and other organisms, Plant Physiol. 142 (2006) 1350–1352, http://dx.doi.org/10.1104/pp.106.088955.
[39] S. Michaeli, A. Fait, K. Lagor, A. Nunes-Nesi, N. Grillich, A. Yellin, et al., A mitochondrial GABA permease connects the GABA shunt and the TCA cycle, and is essential for normal carbon metabolism, Plant J. 67 (2011) 485–498, http://dx.doi.org/10.1111/j.1365-313X.2011.04612.x.
[40] A. Batushansky, M. Kirma, N. Grillich, D. Toubiana, P.A. Pham, I. Balbo, et al., Combined transcriptomics and metabolomics of *Arabidopsis thaliana* seedlings exposed to exogenous GABA suggest its role in plants is predominantly metabolic, Mol. Plant 7 (2014) 1065–1068, http://dx.doi.org/10.1093/mp/ssu017.
[41] C.P. Trobacher, A. Zarei, J. Liu, S.M. Clark, G.G. Bozzo, B.J. Shelp, Calmodulin-dependent and calmodulin-independent glutamate decarboxylases in apple fruit, BMC Plant Biol. 13 (2013) 144, http://dx.doi.org/10.1186/1471-2229-13-144.
[42] A.M. Kinnersley, F. Lin, Receptor modifiers indicate that 4-aminobutyric acid (GABA) is a potential modulator of ion transport in plants, Plant Growth Regul. 32 (2000) 65–76.
[43] H.M. Lam, J. Chiu, M.H. Hsieh, L. Meisel, I.C. Oliveira, M. Shin, et al., Glutamate-receptor genes in plants, Nature 396 (1998) 125–126, http://dx.doi.org/10.1038/24066.
[44] B. Lacombe, D. Becker, R. Hedrich, R. DeSalle, M. Hollmann, J. Kwak, et al., The identity of plant glutamate receptors, Science 292 (2001) 1486–1487, http://dx.doi.org/10.1126/science.292.5521.1486b.
[45] N. Bouché, B. Lacombe, H. Fromm, GABA signaling: a conserved and ubiquitous mechanism, Trends Cell Biol. 13 (2003) 607–610.
[46] B.G. Forde, M.R. Roberts, Glutamate receptor-like channels in plants: a role as amino acid sensors in plant defence? F1000Prime Rep. 6 (2014) 37, http://dx.doi.org/10.12703/P6-37.
[47] Z. Qi, N.R. Stephens, E.P. Spalding, Calcium entry mediated by GLR3.3 an *Arabidopsis* glutamate receptor with a broad agonist profile, Plant Physiol. 142 (2006) 963–971, http://dx.doi.org/10.1104/pp.106.088989.
[48] E.D. Vincill, A.E. Clarin, J.N. Molenda, E.P. Spalding, Interacting glutamate receptor-like proteins in phloem regulate lateral root initiation in *Arabidopsis*, Plant Cell 25 (2013) 1304–1313, http://dx.doi.org/10.1105/tpc.113.110668.
[49] R. Schwacke, S. Grallath, K.E. Breitkreuz, E. Stransky, H. Stransky, W.B. Frommer, et al., LeProT1, a transporter for proline, glycine betaine, and gamma-amino butyric acid in tomato pollen, Plant Cell 11 (1999) 377–392.
[50] S. Grallath, T. Weimar, A. Meyer, C. Gumy, M. Suter-Grotemeyer, J.-M. Neuhaus, et al., The AtProT family. Compatible solute transporters with similar substrate specificity but differential expression patterns, Plant Physiol. 137 (2005) 117–126, http://dx.doi.org/10.1104/pp.104.055079.1.
[51] A. Meyer, S. Eskandari, S. Grallath, D. Rentsch, AtGAT1, a high affinity transporter for gamma-aminobutyric acid in *Arabidopsis thaliana*, J. Biol. Chem. 281 (2006) 7197–7204, http://dx.doi.org/10.1074/jbc.M510766200.
[52] S. Ramesh, S. Tyerman, P. Ryan, M. Gilliham, GABA-gated anion channels in plants – they exist and have important physiological roles, in: 16th International Workshop on Plant Membrane Biology, 2013.
[53] M. Gilliham, S. Tyerman, S. Ramesh, GABA responsive motif, Patent – Int. Publ. No WO 2014/094073 A1 (2014).
[54] A. De Angeli, J. Zhang, S. Meyer, E. Martinoia, AtALMT9 is a malate-activated vacuolar chloride channel required for stomatal opening in *Arabidopsis*, Nat. Commun. 4 (2013) 1804, http://dx.doi.org/10.1038/ncomms2815.
[55] S. Meyer, P. Mumm, D. Imes, A. Endler, B. Weder, K.A.S. Al-Rasheid, et al., AtALMT12 represents an R-type anion channel required for stomatal movement in *Arabidopsis* guard cells, Plant J. 63 (2010) 1054–1062, http://dx.doi.org/10.1111/j.1365-313X.2010.04302.x.
[56] R. Hedrich, Ion channels in plants, Physiol. Rev. 92 (2012) 1777–1811, http://dx.doi.org/10.1152/physrev.00038.2011.
[57] P. Mumm, D. Imes, E. Martinoia, K. Al-Rasheid, D. Geiger, I. Marten, et al., C-terminus mediated voltage gating of *Arabidopsis* guard cell anion channel QUAC1, Mol. Plant 6 (2013) 1550–1563.
[58] I. Dreyer, J.L. Gomez-Porras, D.M. Riaño-Pachón, R. Hedrich, D. Geiger, Molecular evolution of slow and quick anion channels (SLACs and QUACs/ALMTs), Front. Plant Sci. 3 (2012) 263, http://dx.doi.org/10.3389/fpls.2012.00263.
[59] H. Motoda, T. Sasaki, Y. Kano, P. Ryan, E. Delhaize, H. Matsumoto, et al., The membrane topology of ALMT1, an aluminum-activated malate transport protein in wheat (*Tritcum aestivum*), Plant Signal. Behav. 2 (2007) 467–472.
[60] R. Palanivelu, L. Brass, A.F. Edlund, D. Preuss, Pollen tube growth and guidance is regulated by *POP2*, an *Arabidopsis* gene that controls GABA levels, Cell 114 (2003) 47–59.
[61] T. Dresselhaus, N. Franklin-Tong, Male-female crosstalk during pollen germination, tube growth and guidance, and double fertilization, Mol. Plant 6 (2013) 1018–1036, http://dx.doi.org/10.1093/mp/sst061.
[62] H. Renault, A. El Amrani, R. Palanivelu, E.P. Updegraff, A. Yu, J.-P. Renou, et al., GABA accumulation causes cell elongation defects and a decrease in expression of genes encoding secreted and cell wall-related proteins in *Arabidopsis thaliana*, Plant Cell Physiol. 52 (2011) 894–908, http://dx.doi.org/10.1093/pcp/pcr041.
[63] G.-H. Yu, J. Zou, J. Feng, X.-B. Peng, J.-Y. Wu, Y.-L. Wu, et al., Exogenous γ-aminobutyric acid (GABA) affects pollen tube growth via modulating putative $Ca^{2+}$-permeable membrane channels and is coupled to negative regulation on glutamate decarboxylase, J. Exp. Bot. 65 (2014) 3235–3248, http://dx.doi.org/10.1093/jxb/eru171.
[64] D. Winter, B. Vinegar, H. Nahal, R. Ammar, G.V. Wilson, N.J. Provart, An "Electronic Fluorescent Pictograph" browser for exploring and analyzing large-scale biological data sets, PLoS ONE 2 (2007) e718, http://dx.doi.org/10.1371/journal.pone.0000718.
[65] G. Yu, M. Sun, Deciphering the possible mechanism of GABA in tobacco pollen tube growth and guidance, Plant Signal. Behav. 2 (2007) 393–395, http://dx.doi.org/10.1016/j.chembiol.2006.05.007.
[66] G. Yu, J. Liang, Z. He, M. Sun, Quantum dot-mediated detection of gamma-aminobutyric acid binding sites on the surface of living pollen protoplasts in tobacco, Chem. Biol. 13 (2006) 723–731, http://dx.doi.org/10.1016/j.chembiol.2006.05.007.
[67] M.R. Roberts, Does GABA act as a signal in plants? Hints from molecular studies, Plant Signal. Behav. 2 (2007) 408–409, http://dx.doi.org/10.1111/j.1365-3040.2006.01526.x.
[68] R. Mirabella, H. Rauwerda, E.A. Struys, C. Jakobs, C. Triantaphylidès, M.A. Haring, et al., The Arabidopsis *her1* mutant implicates GABA in E-2-hexenal responsiveness, Plant J. 53 (2008) 197–213, http://dx.doi.org/10.1111/j.1365-313X.2007.03323.x.
[69] A. Fait, A. Yellin, H. Fromm, GABA shunt deficiencies and accumulation of reactive oxygen intermediates: insight from *Arabidopsis* mutants, FEBS Lett. 579 (2005) 415–420, http://dx.doi.org/10.1016/j.febslet.2004.12.004.
[70] F. Ludewig, A. Hüser, H. Fromm, L. Beauclair, N. Bouché, Mutants of GABA transaminase (POP2) suppress the severe phenotype of *succinic semialdehyde dehydrogenase* (*ssadh*) mutants in *Arabidopsis*, PLoS ONE 3 (2008) e3383, http://dx.doi.org/10.1371/journal.pone.0003383.
[71] K. Toyokura, M. Hayashi, M. Nishimura, K. Okada, Adaxial–abaxial patterning: a novel function of the GABA shunt, Plant Signal. Behav. 7 (2012) 705–707, http://dx.doi.org/10.4161/psb.20346.
[72] S. Sawa, K. Watanabe, K. Goto, Y.G. Liu, D. Shibata, E. Kanaya, et al., *FILAMENTOUS FLOWER*, a meristem and organ identity gene of *Arabidopsis*, encodes a protein with a zinc finger and HMG-related domains, Genes Dev. 13 (1999) 1079–1088.

UNCORRECTED PROOF

Please cite this article in press as: R.E. Häusler, et al., Amino acids – A life between metabolism and signaling, Plant Sci. (2014), http://dx.doi.org/10.1016/j.plantsci.2014.09.011

[73] K.R. Siegfried, Y. Eshed, S.F. Baum, D. Otsuga, G.N. Drews, J.L. Bowman, Members of the *YABBY* gene family specify abaxial cell fate in *Arabidopsis*, Development 126 (1999) 4117–4128.
[74] K. Toyokura, K. Watanabe, A. Oiwaka, M. Kusano, T. Tameshige, K. Tatematsu, et al., Succinic semialdehyde dehydrogenase is involved in the robust patterning of *Arabidopsis* leaves along the adaxial–abaxial axis, Plant Cell Physiol. 52 (2011) 1340–1353, http://dx.doi.org/10.1093/pcp/pcr079.
[75] E.A. Rennie, H.V. Scheller, Xylan biosynthesis, Curr. Opin. Biotechnol. 26 (2014) 100–107, http://dx.doi.org/10.1016/j.copbio.2013.11.013.
[76] V. Tzin, G. Galili, New insights into the shikimate and aromatic amino acids biosynthesis pathways in plants, Mol. Plant 3 (2010) 956–972, http://dx.doi.org/10.1093/mp/ssq048.
[77] V. Cheynier, G. Comte, K.M. Davies, V. Lattanzio, S. Martens, Plant phenolics: recent advances on their biosynthesis, genetics, and ecophysiology, Plant Physiol. Biochem. 72 (2013) 1–20, http://dx.doi.org/10.1016/j.plaphy.2013.05.009.
[78] A. Murphy, W.A. Peer, L. Taiz, Regulation of auxin transport by aminopeptidases and endogenous flavonoids, Planta 211 (2000) 315–324.
[79] D.E. Brown, A.M. Rashotte, A.S. Murphy, J. Normanly, B.W. Tague, W.A. Peer, et al., Flavonoids act as negative regulators of auxin transport in vivo in *Arabidopsis*, Plant Physiol. 126 (2001) 524–535.
[80] H. Suzuki, Y. Xia, R. Cameron, G. Shadle, J. Blount, C. Lamb, et al., Signals for local and systemic responses of plants to pathogen attack, J. Exp. Bot. 55 (2004) 169–179, http://dx.doi.org/10.1093/jxb/erh025.
[81] Y. Mano, K. Nemoto, The pathway of auxin biosynthesis in plants, J. Exp. Bot. 63 (2012) 2853–2872, http://dx.doi.org/10.1093/jxb/ers091.
[82] I.E. Sønderby, F. Geu-Flores, B.A. Halkier, Biosynthesis of glucosinolates – gene discovery and beyond, Trends Plant Sci. 15 (2010) 283–290, http://dx.doi.org/10.1016/j.tplants.2010.02.005.
[83] J. Schmid, N. Amrhein, Molecular organization of the shikimate pathway in higher plants, Phytochemistry 39 (1995) 737–749.
[84] V. Prabhakar, T. Löttgert, T. Gigolashvili, K. Bell, U.-I. Flügge, R.E. Häusler, Molecular and functional characterization of the plastid-localized phosphoenolpyruvate enolase (ENO1) from *Arabidopsis thaliana*, FEBS Lett. 583 (2009) 983–991, http://dx.doi.org/10.1016/j.febslet.2009.02.017.
[85] K. Fischer, B. Kammerer, M. Gutensohn, B. Arbinger, A. Weber, R.E. Häusler, et al., A new class of plastidic phosphate translocators: a putative link between primary and secondary metabolism by the phosphoenolpyruvate/phosphate antiporter, Plant Cell 9 (1997) 453–462, http://dx.doi.org/10.1105/tpc.9.3.453.
[86] S.J. Streatfield, A. Weber, E.A. Kinsman, R.E. Häusler, J. Li, D. Post-Beittenmiller, et al., The phosphoenolpyruvate/phosphate translocator is required for phenolic metabolism, palisade cell development, and plastid-dependent nuclear gene expression, Plant Cell 11 (1999) 1609–1621.
[87] H. Li, K. Culligan, R.A. Dixon, J. Chory, CUE1: a mesophyll cell-specific positive regulator of light-controlled gene expression in *Arabidopsis*, Plant Cell 7 (1995) 1599–1610, http://dx.doi.org/10.1105/tpc.7.10.1599.
[88] P. Staehr, T. Löttgert, A. Christmann, S. Krueger, C. Rosar, J. Rolčík, et al., Reticulate leaves and stunted roots are independent phenotypes pointing at opposite roles of the phosphoenolpyruvate/phosphate translocator defective in *cue1* in the plastids of both organs, Front. Plant Sci. 5 (2014) 126, http://dx.doi.org/10.3389/fpls.2014.00126.
[89] V. Prabhakar, T. Löttgert, S. Geimer, P. Dörmann, S. Krüger, V. Vijayakumar, et al., Phosphoenolpyruvate provision to plastids is essential for gametophyte and sporophyte development in *Arabidopsis thaliana*, Plant Cell 22 (2010) 2594–2617, http://dx.doi.org/10.1105/tpc.109.073171.
[90] L. Voll, R.E. Häusler, R. Hecker, A. Weber, G. Weissenbock, G. Fiene, et al., The phenotype of the *Arabidopsis cue1* mutant is not simply caused by a general restriction of the shikimate pathway, Plant J. 36 (2003) 301–317, http://dx.doi.org/10.1046/j.1365-313X.2003.01889.x.
[91] A. Staniek, H. Bouwmeester, P.D. Fraser, O. Kayser, S. Martens, A. Tissier, et al., Natural products – modifying metabolite pathways in plants, Biotechnol. J. 8 (2013) 1159–1171, http://dx.doi.org/10.1002/biot.201300224.
[92] H. Kasahara, K. Takei, N. Ueda, S. Hishiyama, T. Yamaya, Y. Kamiya, et al., Distinct isoprenoid origins of cis- and trans-zeatin biosyntheses in *Arabidopsis*, J. Biol. Chem. 279 (2004) 14049–14054, http://dx.doi.org/10.1074/jbc.M314195200.
[93] J. Schell, M. Montagu, The Ti-plasmid of *Agrobacterium tumefaciens*, a natural vector for the introduction of nif genes in plants? Basic Life Sci. 9 (1977) 159–179.
[94] P. Zambryski, H. Joos, C. Genetello, J. Leemans, M.V. Montagu, J. Schell, Ti plasmid vector for the introduction of DNA into plant cells without alteration of their normal regeneration capacity, EMBO J. 2 (1983) 2143–2150.
[95] R.R.J. Arroo, A.W. Alfermann, M. Medarde, M. Petersen, N. Pras, J.G. Woolley, Plant cell factories as a source for anti-cancer lignans, Phytochem. Rev. 1 (2002) 27–35.
[96] J. Attoumbré, S. Charlet, S. Baltora-Rosset, C. Hano, S. Raynaud-Le Grandic, F. Gillet, et al., High accumulation of dehydrodiconiferyl alcohol-4-beta-D:-glucoside in free and immobilized *Linum usitatissimum* cell cultures, Plant Cell Rep. 25 (2006) 859–864, http://dx.doi.org/10.1007/s00299-006-0137-2.
[97] E.W. Nester, M.P. Gordon, R.M. Amasino, M.F. Yanofsky, Crown gall: a molecular and physiological analysis, Annu. Rev. Plant Physiol. 35 (1984) 387–413.
[98] H.N. Wood, A.C. Braun, H. Brandes, H. Kendet, Studies on the distribution and properties of a new class of cell division promoting substances from higher plant species, Proc. Natl. Acad. Sci. U. S. A. 62 (1969) 349–356.
[99] N. Tokunaga, N. Sakakibara, T. Umezawa, Y. Ito, H. Fukuda, Y. Sato, Involvement of extracellular dilignols in lignification during tracheary element differentiation of isolated Zinnia mesophyll cells, Plant Cell Physiol. 46 (2005) 224–232, http://dx.doi.org/10.1093/pcp/pci017.
[100] J.D. Orr, D.G. Lynn, Biosynthesis of dehydrodiconiferyl alcohol glucosides: implications for the control of tobacco cell growth, Plant Physiol. 98 (1992) 343–352.
[101] V. Beejmohun, O. Fliniaux, C. Hano, S. Pilard, E. Grand, D. Lesur, et al., Coniferin dimerisation in lignan biosynthesis in flax cells, Phytochemistry 68 (2007) 2744–2752, http://dx.doi.org/10.1016/j.phytochem.2007.09.016.
[102] R.C. Black, A.N. Binns, C.F. Chang, D.G. Lynn, Cell-autonomous cytokinin-independent growth of tobacco cells transformed by *Agrobacterium tumefaciens* strains lacking the cytokinin biosynthesis gene, Plant Physiol. 105 (1994) 989–998.
[103] M.E. Duban, K. Lee, D.G. Lynn, Strategies in pathogenesis: mechanistic specificity in the detection of generic signals, Mol. Microbiol. 7 (1993) 637–645.
[104] L. Tamagnone, A. Merida, A. Parr, S. Mackay, F. Culianez-Macia, K. Roberts, et al., The AmMYB308 and AmMYB330 transcription factors from Antirrhinum regulate phenylpropanoid and lignin biosynthesis in transgenic tobacco, Plant Cell 10 (1998) 135–154.
[105] L. Tamagnone, A. Merida, N. Stacey, K. Plaskitt, A. Parr, C. Chang, et al., Inhibition of phenolic acid metabolism results in precocious cell death and altered cell morphology in leaves of transgenic tobacco plants, Plant Cell 10 (1998) 1801–1816.
[106] L.M. Voll, M.R. Hajirezaei, C. Czogalla-Peter, W. Lein, M. Stitt, U. Sonnewald, et al., Antisense inhibition of enolase strongly limits the metabolism of aromatic amino acids, but has only minor effects on respiration in leaves of transgenic tobacco plants, New Phytol. 184 (2009) 607–618, http://dx.doi.org/10.1111/j.1469-8137.2009.02998.x.
[107] R. Franke, J.M. Humphreys, M.R. Hemm, J.W. Denault, M.O. Ruegger, J.C. Cusumano, et al., The *Arabidopsis* REF8 gene encodes the 3-hydroxylase of phenylpropanoid metabolism, Plant J. 30 (2002) 33–45.
[108] R. Franke, M.R. Hemm, J.W. Denault, M.O. Ruegger, J.M. Humphreys, C. Chapple, Changes in secondary metabolism and deposition of an unusual lignin in the *ref8* mutant of *Arabidopsis*, Plant J. 30 (2002) 47–59.
[109] U. Bayindir, A.W. Alfermann, E. Fuss, Hinokinin biosynthesis in *Linum corymbulosum* Reichenb., Plant J. 55 (2008) 810–820, http://dx.doi.org/10.1111/j.1365-313X.2008.03558.x.
[110] J.-E. Bassard, P. Ullmann, F. Bernier, D. Werck-Reichhart, Phenolamides: bridging polyamines to the phenolic metabolism, Phytochemistry 71 (2010) 1808–1824, http://dx.doi.org/10.1016/j.phytochem.2010.08.003.
[111] R.K. Kakkar, V.K. Sawhney, Minireview: polyamine research in plants – a changing perspective, Physiol. Plant. 116 (2002) 281–292.
[112] A. Mollá-Morales, R. Sarmiento-Mañús, P. Robles, V. Quesada, J.M. Pérez-Pérez, R. González-Bayón, et al., Analysis of *ven3* and *ven6* reticulate mutants reveals the importance of arginine biosynthesis in *Arabidopsis* leaf development, Plant J. 65 (2011) 335–345, http://dx.doi.org/10.1111/j.1365-313X.2010.04425.x.
[113] K. Burhenne, B.K. Kristensen, S.K. Rasmussen, A new class of N-hydroxycinnamoyltransferases. Purification, cloning, and expression of a barley agmatine coumaroyltransferase (EC 2.3.1.64), J. Biol. Chem. 278 (2003) 13919–13927, http://dx.doi.org/10.1074/jbc.M213041200.
[114] S. Besseau, L. Hoffmann, P. Geoffroy, C. Lapierre, B. Pollet, M. Legrand, Flavonoid accumulation in *Arabidopsis* repressed in lignin synthesis affects auxin transport and plant growth, Plant Cell 19 (2007) 148–162, http://dx.doi.org/10.1105/tpc.106.044495.
[115] J. Luo, C. Fuell, A. Parr, L. Hill, P. Bailey, K. Elliott, et al., A novel polyamine acyltransferase responsible for the accumulation of spermidine conjugates in *Arabidopsis* seed, Plant Cell 21 (2009) 318–333, http://dx.doi.org/10.1105/tpc.108.063511.
[116] K. Berthelot, D. Buret, B. Guérin, D. Delay, J. Negrel, F.M. Delmotte, *Vir*-gene-inducing activities of hydroxycinnamic acid amides in *Agrobacterium tumefaciens*, Physiol. Plant. 49 (1998) 1537–1548.
[117] D.R. Walters, Polyamines and plant disease, Phytochemistry 64 (2003) 97–107, http://dx.doi.org/10.1016/S0031-9422(03)00329-7.
[118] R. Alcázar, F. Marco, J.C. Cuevas, M. Patron, A. Ferrando, P. Carrasco, et al., Involvement of polyamines in plant response to abiotic stress, Biotechnol. Lett. 28 (2006) 1867–1876, http://dx.doi.org/10.1007/s10529-006-9179-3.
[119] S. Jin, M. Yoshida, T. Nakajima, A. Murai, Accumulation of hydroxycinnamic acid amides in winter wheat under snow, Biosci. Biotechnol. Biochem. 67 (2003) 1245–1249.
[120] L. Zacarés, M.P. López-Gresa, J. Fayos, J. Primo, J.M. Bellés, V. Conejero, Induction of p-coumaroyldopamine and feruloyldopamine, two novel metabolites, in tomato by the bacterial pathogen *Pseudomonas syringae*, Mol. Plant Microbe Interact. 20 (2007) 1439–1448, http://dx.doi.org/10.1094/MPMI-20-11-1439.
[121] D.W. Etalo, I.J.E. Stulemeijer, H.P. van Esse, R.C.H. de Vos, H.J. Bouwmeester, M.H.A.J. Joosten, System-wide hypersensitive response-associated transcriptome and metabolome reprogramming in tomato, Plant Physiol. 162 (2013) 1599–1617, http://dx.doi.org/10.1104/pp.113.217471.
[122] A. Muroi, A. Ishihara, C. Tanaka, A. Ishizuka, J. Takabayashi, H. Miyoshi, et al., Accumulation of hydroxycinnamic acid amides induced by pathogen infection and identification of agmatine coumaroyltransferase in *Arabidopsis thaliana*, Planta 230 (2009) 517–527, http://dx.doi.org/10.1007/s00425-009-0960-0.
[123] A. Muroi, K. Matsui, T. Shimoda, H. Kihara, R. Ozawa, A. Ishihara, et al., Acquired immunity of transgenic torenia plants overexpressing agmatine

UNCORRECTED PROOF

coumaroyltransferase to pathogens and herbivore pests, Sci. Rep. 2 (2012) 689, http://dx.doi.org/10.1038/srep00689.
[124] K.N. Yogendra, D. Pushpa, K.A. Mosa, A.C. Kushalappa, A. Murphy, T. Mosquera, Quantitative resistance in potato leaves to late blight associated with induced hydroxycinnamic acid amides, Funct. Integr. Genomics 14 (2014) 285–298, http://dx.doi.org/10.1007/s10142-013-0358-8.
[125] R. Gunnaiah, A.C. Kushalappa, R. Duggavathi, S. Fox, D.J. Somers, Integrated metabolo-proteomic approach to decipher the mechanisms by which wheat QTL (Fhb1) contributes to resistance against *Fusarium graminearum*, PLoS ONE 7 (2012) e40695, http://dx.doi.org/10.1371/journal.pone.0040695.
[126] K. Cho, Y. Kim, S.J. Wi, J.B. Seo, J. Kwon, J.H. Chung, et al., Nontargeted metabolite profiling in compatible pathogen-inoculated tobacco (*Nicotiana tabacum* L. *cv.* Wisconsin 38) using UPLC-Q-TOF/MS, J. Agric. Food Chem. 60 (2012) 11015–11028.
[127] A. Ishihara, Y. Hashimoto, C. Tanaka, J.G. Dubouzet, T. Nakao, F. Matsuda, et al., The tryptophan pathway is involved in the defense responses of rice against pathogenic infection *via* serotonin production, Plant J. 54 (2008) 481–495, http://dx.doi.org/10.1111/j.1365-313X.2008.03441.x.
[128] A. Ishihara, T. Nakao, Y. Mashimo, M. Murai, N. Ichimaru, C. Tanaka, et al., Probing the role of tryptophan-derived secondary metabolism in defense responses against *Bipolaris oryzae* infection in rice leaves by a suicide substrate of tryptophan decarboxylase, Phytochemistry 72 (2011) 7–13, http://dx.doi.org/10.1016/j.phytochem.2010.11.001.
[129] G. Guillet, V. de Luca, Wound-inducible biosynthesis of phytoalexin hydroxycinnamic acid amides of tyramine in tryptophan and tyrosine decarboxylase transgenic tobacco lines, Plant Physiol. 137 (2005) 692–699, http://dx.doi.org/10.1104/pp.104.050294.and.
[130] J.M. Hagel, P.J. Facchini, Elevated tyrosine decarboxylase and tyramine hydroxycinnamoyltransferase levels increase wound-induced tyramine-derived hydroxycinnamic acid amide accumulation in transgenic tobacco leaves, Planta 221 (2005) 904–914, http://dx.doi.org/10.1007/s00425-005-1484-x.
[131] V. Handrick, T. Vogt, A. Frolov, Profiling of hydroxycinnamic acid amides in *Arabidopsis thaliana* pollen by tandem mass spectrometry, Anal. Bioanal. Chem. 398 (2010) 2789–2801, http://dx.doi.org/10.1007/s00216-010-4129-2.
[132] C. Fellenberg, J. Ziegler, V. Handrick, T. Vogt, Polyamine homeostasis in wild type and phenolamide deficient *Arabidopsis thaliana* stamens, Front. Plant Sci. 3 (2012) 180, http://dx.doi.org/10.3389/fpls.2012.00180.
[133] I. Stankova, K. Chuchkov, S. Shishkov, K. Kostova, L. Mukova, A.S. Galabov, Synthesis, antioxidative and antiviral activity of hydroxycinnamic acid amides of thiazole containing amino acid, Amino Acids 37 (2009) 383–388, http://dx.doi.org/10.1007/s00726-008-0165-z.
[134] I. Stankova, M. Spasova, Hydroxycinnamic acid amides with oxazole-containing amino acid: synthesis and antioxidant activity, Zeitschrift für Naturforschung 64 (2009) 176–178.
[135] J.-Y. Choi, H. Kim, Y.-J. Choi, A. Ishihara, K. Back, S.-G. Lee, Cytoprotective activities of hydroxycinnamic acid amides of serotonin against oxidative stress-induced damage in HepG2 and HaCaT cells, Fitoterapia 81 (2010) 1134–1141, http://dx.doi.org/10.1016/j.fitote.2010.07.015.
[136] S. Jang, A. Ishihara, K. Back, Production of coumaroylserotonin and feruloylserotonin in transgenic rice expressing pepper hydroxycinnamoyl-coenzyme A: serotonin N-(hydroxycinnamoyl)transferase, Plant Physiol. 135 (2004) 346–356, http://dx.doi.org/10.1104/pp.103.038372.346.
[137] M. Spasova, S. Philipov, L. Nikolaeva-Glomb, A.S. Galabov, T. Milkova, Cinnamoyl- and hydroxycinnamoyl amides of glaucine and their antioxidative and antiviral activities, Bioorg. Med. Chem. 16 (2008) 7457–7461, http://dx.doi.org/10.1016/j.bmc.2008.06.010.
[138] D. Ly, K. Kang, J. Choi, A. Ishihara, K. Back, S. Lee, Hydroxycinnamic acid amides of serotonin and tyramine in food vegetables, J. Med. Food 11 (2008) 385–389, http://dx.doi.org/10.1089/jmf.2007.514.
[139] D.A. Navarre, R.S. Payyavula, R. Shakya, N.R. Knowles, S.S. Pillai, Changes in potato phenylpropanoid metabolism during tuber development, Plant Physiol. Biochem. 65 (2013) 89–101, http://dx.doi.org/10.1016/j.plaphy.2013.01.007.
[140] M.R. Hemm, M.O. Ruegger, C. Chapple, The Arabidopsis *ref2* mutant is defective in the gene encoding CYP83A1 and shows both phenylpropanoid and glucosinolate phenotypes, Plant Cell 15 (2003) 179–194, http://dx.doi.org/10.1105/tpc.006544.et.
[141] Y. Jing, D. Cui, F. Bao, Z. Hu, Z. Qin, Y. Hu, Tryptophan deficiency affects organ growth by retarding cell expansion in *Arabidopsis*, Plant J. 57 (2009) 511–521, http://dx.doi.org/10.1111/j.1365-313X.2008.03706.x.
[142] R. González-Bayón, E.A. Kinsman, V. Quesada, A. Vera, P. Robles, M.R. Ponce, et al., Mutations in the *RETICULATA* gene dramatically alter internal architecture but have little effect on overall organ shape in *Arabidopsis* leaves, J. Exp. Bot. 57 (2006) 3019–3031, http://dx.doi.org/10.1093/jxb/erl063.
[143] C. Barth, P.L. Conklin, The lower cell density of leaf parenchyma in the *Arabidopsis thaliana* mutant *lcd1-1* is associated with increased sensitivity to ozone and virulent *Pseudomonas syringae*, Plant J. 35 (2003) 206–218, http://dx.doi.org/10.1046/j.1365-313X.2003.01795.x.
[144] J. Gray, D. Caparrós-Ruiz, E. Grotewold, Grass phenylpropanoids: regulate before using!, Plant Sci. 184 (2012) 112–120, http://dx.doi.org/10.1016/j.plantsci.2011.12.008.

UNCORRECTED PROOF

Please cite this article in press as: R.E. Häusler, et al., Amino acids – A life between metabolism and signaling, Plant Sci. (2014), http://dx.doi.org/10.1016/j.plantsci.2014.09.011