Identification and characterization of piperine synthase from black pepper, *Piper nigrum* L.

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Black pepper (*Piper nigrum* L.) is the world’s most popular spice and is also used as an ingredient in traditional medicine. Its pungent perception is due to the interaction of its major compound, piperine (1-piperoyl-piperidine) with the human TRPV-1 or vanilloid receptor. We now identify the hitherto concealed enzymatic formation of piperine from piperoyl coenzyme A and piperidine based on a differential RNA-Seq approach from developing black pepper fruits. This enzyme is described as piperine synthase (piperoyl-CoA:piperidine piperoyl transferase) and is a member of the BAHD-type of acyltransferases encoded by a gene that is preferentially expressed in immature fruits. A second BAHD-type enzyme, also highly expressed in immature black pepper fruits, has a rather promiscuous substrate specificity, combining diverse CoA-esters with aliphatic and aromatic amines with similar efficiencies, and was termed piperamide synthase. Recombinant piperine and piperamide synthases are members of a small gene family in black pepper. They can be used to facilitate the microbial production of a broad range of medicinally relevant aliphatic and aromatic piperamides based on a wide array of CoA-donors and amine-derived acceptors, offering widespread applications.
The Piperaceae are tropical and subtropical plants within the Piperales, a large order of the Magnoliids, showing properties of basal angiosperms. Dried fruits of several Piperaceae, specifically black pepper (Piper nigrum) have been used as popular spices by humans since antiquity, and, in the 15th and 16th century were among the driving economic forces leading to the discovery of the New World. Besides flavor, black pepper fruits show a wide range of applications in traditional and modern medicine. The pungent perception of black pepper is largely due to high concentrations of several amides, specifically piperine (1-piperoyl-piperidine), which is regarded as the basis for traditional and recent therapeutic applications. Piperine results in an oral burning sensation due to activation of the transient receptor cation channel subfamily V member 1 (TRPV-1), formerly known as the vanilloid receptor. This ion channel is also targeted by capsaicin, a structurally similar compound from Capsicum species (hot chili peppers), that are members of the Solanaceae.

Piperine was isolated 200 years ago by Hans Christian Ørstedt and numerous procedures for organic synthesis of piperamides are constantly developed. Yet, the biosynthetic steps towards piperine and piperamide formation in black pepper until recently have remained largely enigmatic. Early reports on the incorporation of -lysine and cadaverine into the piperidine heterocycle by radiolabeled tracers date back to five decades ago and were performed at that time with Crassulaceae species, rather than black pepper. The vanillloid-like aromatic part of piperine and its structural similarity to ferulic acid suggested that its extended C5-carbon side chain may be derived from the general phenylpropanoid pathway, although experimental evidence for this claim is rather poor. Feeding studies with 2-[13C]- and 2-[2H]-labeled malonic acid as well as L-valine suggested the participation of a CoA-activated malonyl coenzyme A and valine into the similar isobutylamine derived piperlongumine in Piper tuberculatum. Several piperoyl-CoA ligases capable of converting piperic acid to piperoyl-coenzyme A (piperoyl-CoA) have now been described from black pepper immature fruits and leaves, respectively. A cytochrome P450 oxidoreductase (CYP719A37) was identified in parallel from immature black pepper fruits. The enzyme catalyzes methylene-oxy bridge formation, specifically from feruperic acid to piperic acid, and not from ferulic acid or from feruloyl-CoA (Fig. 1). These recent reports corroborate earlier assumptions that amide formation is the final step in piperine biosynthesis. A corresponding piperine synthase activity (EC 2.3.1.145) from crude protein extracts of black pepper shoots capable to convert piperoyl-CoA and piperidine to piperine was already reported three decades ago. Since the presumably similar amide forming casapcin synthase that is encoded by the locus was classified as a coenzyme A dependent BAHD-type acyltransferase termed by the initials of the first four characterized transfersases of this family, BEAT, AHCT, HCBT, and DAT, a similar type of enzyme may catalyze the formation of piperine.

Next-generation sequencing technologies enable the assembly of whole transcriptomes and facilitate the identification of genes and enzymes from unknown or only partly solved biosynthetic pathways in non-model organisms. Several RNA-Seq-based transcriptome datasets from mature fruits, leaves, and roots were described from black pepper. In addition, genome information from black pepper recently suggested a series of piperamide biosynthesis candidate genes and transcripts, yet without any functional characterization. By a differential RNA-Seq approach we now demonstrate that a specific acyltransferase, termed piperine synthase, isolated from immature black pepper fruits catalyzes the decisive step in the formation of piperine from piperoyl-CoA and piperidine. This identification was based on the assumption that piperine synthase is differentially expressed in fruits, leaves, and flowers, with the highest expression levels anticipated for young fruits. Piperine synthase is dependent on activated CoA-esters and therefore, is part of the BAHD-superfamily of acyltransferases.

Results

RNA-sequencing and bioinformatics guided identification of piperine biosynthesis genes. To identify piperine biosynthesis-related genes we monitored piperine formation during fruit development of black pepper plants grown in a greenhouse over several months. Spadices of individual plants were marked and piperine amounts were quantified by LC-MS and UV/Vis-detection respectively. A time course showed...
that piperine accumulation in greenhouse-grown plants started after a lag-phase of roughly 20 days post anthesis and peaked ~3 months post anthesis at levels of 2.5% piperine calculated per fresh weight. No significant increase was observed during later stages of fruit development. Two development stages, between 20 and 30 days (stage I) and between 40 and 60 days post anthesis (stage II), were considered as ideal for collecting plant material for a comparative RNA-Seq approach assuming that transcript levels preceded the accumulation of biosynthetic product formation. Flowering spadices as well as young leaves served as negative controls. In both of these organs, either no or very low piperine amounts were detected by LC-MS analysis. The results of the RNA-Seq based differential screening approach are illustrated in Fig. 2c, supported by Supplementary Fig. S1. The first two principal components (PC) demonstrate high variability between tissue samples and high reproducibility of the datasets since samples from biological replicates cluster together (Supplementary Fig. S1). The heat map generated with the 3000 most differentially expressed genes of each statistical comparison (false discovery rate (FDR) < 0.2, |LFC| > 1) were exemplified and highlighted. RNA-Seq data were generated from individual organs in three biological replicates.

The MapMan4-based protein classification\textsuperscript{29} of the combined transcriptomes resulted in a high score for unigenes related to an “enzyme classification” bin (Supplementary Fig. S1). Specifically, the transition from fruit stage I to fruit stage II marks an over-representation of transcripts linked to enzymes of plant specialized metabolism, accentuating the initiation of terpenoid and phenylpropanoid biosynthesis. Abundant transcripts comprise terpene synthases, CoA-ligases, oxidoreductases, and several acyltransferases, the latter being potentially capable to catalyze CoA-dependent amide formation (Supplementary Table S1).

In parallel to the slope of piperine formation, expression profiles of two highly expressed acyltransferase candidate genes followed a pattern of highest abundance of piperine in fruits, stage II, versus fruits at stage I, leaves, and flowering spadices. Both are listed as number two and number five among the 30 most abundant transcripts associated to plant specialized metabolism, topped only by a terpene synthase 10-like annotation (Supplementary Table S1). The transcripts were classified as transferases with the highest sequence identity to hexen-1-ol-acetyltransferase and benzoyl-benzoate acyltransferases, respectively. Transcript abundance was further confirmed by RT-qPCR with higher expression levels of fruits at stage II (40–60 days post anthesis) as compared to fruits at stage I (20–30 days post anthesis) (Fig. 3). Transcript levels of both acyltransferases were virtually absent in leaves and flowering spadices. Minor transcript levels can be observed in roots, consistent with detectable levels of piperine in this organ (Fig. 3c). In contrast to fruits, where piperine levels just start to accumulate and rise to much higher levels, flowers exhibit detectable piperine levels already at stage I.
levels in mature fruits (see Fig. 2), root tissue was already collected at a mature level.

Functional characterization of piperine and piperamide synthases. Full-length unigenes of both candidates were assembled from sequence reads, cloned, and sequenced. Assembled sequences showed expected similarities to BAHD-type acyltransferases. The corresponding full-length cDNAs encoded proteins of 461 and 462 amino acids, with calculated molecular masses of 51 kDa in each case. The deduced protein sequences were named PipBAHD1 and PipBAHD2. Full-length cDNAs were functionally expressed in E. coli. After purification by immobilized metal affinity chromatography (IMAC), recombinant His-tagged PipBAHD1 and PipBAHD2 displayed bands around 55 kDa when separated by SDS-PAGE (Fig. 4; Supplementary Fig. S2). This slightly higher than anticipated molecular mass on SDS-PAGE is in line with previous observations of other BAHD-type acyltransferases. Enzyme activities were tested with piperoyl-CoA and piperidine as substrates by HPLC-UV/Vis as well as HPLC-MS in positive ionization mode (Fig. 4). Only purified PipBAHD2 catalyzed the efficient formation of piperine from piperoyl-CoA and piperidine (Fig. 4). We therefore termed this enzyme piperine synthase. No additional co-factors were required and a pH optimum was recorded at pH 8.0. At low µM substrate concentrations, piperine formation was poor and prominent signals of piperic acid (m/z 219) as well as two piperine isomers with identical masses, but slightly different UV-absorbance maxima compared to piperine of 345 and 330 nm were recorded (Fig. 4). The enzyme appears stable for several weeks at −80 °C and over repeated freeze-thaw cycles. DTT was included to reduce enzyme aggregation, resulting in inactive enzymes during size inclusion chromatography (Fig. S3).

When concentrations of piperidine and piperoyl-CoA were gradually increased, preferentially piperine was produced and levels of piperic acid and residual piperine isomers were reduced in parallel (Fig. 4). The activity of recombinant piperine synthase was also compared to piperine formation from crude protein preparations of young black pepper fruits 40–60 days post anthesis. A partially purified native enzyme mix, under the same
substrate concentration again showed the virtually exclusive formation of piperine, traces of stereoisomers and again, only minor levels of piperic acid (Fig. 4).

Based on classical Michaelis-Menten kinetics, recombinant piperine synthase showed an apparent $K_m$ of 342 ± 60 μM for piperoyl-CoA and 7.6 ± 0.5 mM for piperidine with a calculated $k_{cat}$ of 1.01 ± 0.16 s$^{-1}$ based on total product formation, i.e., formation of piperine and piperine isomers. Preferred piperine formation at high piperoyl-CoA concentrations is consistent with the observed presence of piperine, the 2E,4E-isomer in fresh mature and also in dried fruits. The lack of any configurational isomerism, usually observed rapidly, when piperine preparations are kept in aqueous solutions indicates a highly coordinated piperine biosynthesis and storage in vivo (Fig. 4 and Supplementary Fig. S2). Piperine synthase shows a preference, but is not specific for piperoyl-CoA and piperidine. Pyrrolidine and isobutyramine were also taken as acceptors of the activated acyl-ester although with 40 and 15% activity as compared to piperidine (Fig. 5). The corresponding mass and UV-signals of both amides, at $m/z$ 272 and $m/z$ 274 respectively, were also detected in minor quantities in commercially available dried peppercorns (Supplementary Fig. S4). 3,4-methylenedioxyxycinnamoyl-CoA is accepted as an alternative CoA-donor, yet with reduced catalytic activities.

In the case of PipBAHD1 also purified as a His-tagged protein, different piperine isomers were produced with exactly the same substrate preparations of piperoyl-CoA and piperidine (see Supplementary Fig. S2). Product peaks showed identical masses, yet different UV-absorbance spectra and retention times, and piperine were usually synthesized as a side product only, regardless of substrate concentration. Kinetic constants with piperoyl-CoA and piperidine are comparable to piperine synthase (Table 1). The unusual product formation observed for this enzyme is inconsistent with the product profile of fruits from greenhouse-grown plants and also that of dried peppercorns (Supplementary Fig. S4). Therefore, its biological relevance remains obscure and piperoyl-CoA and piperidine may not be its actual in vivo substrates. These observations are in line with higher promiscuity of PipBAHD1 for the CoA-donor and the amine acceptor compared to PipBAHD2, the actual piperine synthase. In contrast to piperine synthase, significant product formation was achieved with medium-chain aliphatic CoA-esters, hexanoyl- and octanoyl-CoA, and residual activity was also observed with benzoyl-CoA and piperidine as well as benzylamine. Piperine synthase was inactive with both substrate combinations (Fig. 5). Therefore, the broad substrate specificity of this enzyme led us to name it piperamide synthase. Piperamide synthase produced a large number of amides with a broad range of applications. Products included aromatic and aliphatic alkaloids, with diverse therapeutic, pharmacological and protective properties, covered by several, recent patent applications (e.g., PCT/US2018/054554) describing the potential application of alkaloids to treat allergic diseases and pain.

Discussion

The identification of the two major biosynthetic branches of piperine biosynthetic genes remained enigmatic for several decades, with the exception of scattered labeling studies performed to unravel piperidine heterocycle biosynthesis in Crassulaceae and a single report on the identification of a piperine synthase activity in shoots of black pepper, which was unstable and could not be further characterized. The low commercial value of pure piperine and its high abundance in black pepper may have initially contributed to the rather modest attention to decipher the biosynthesis of this universal symbol of spiciness as compared to pharmacologically more relevant indole or isoquinoline alkaloids. The limited availability of flowering and fruiting black pepper plants further impaired efforts to investigate piperine biosynthesis in this highly recalcitrant species.

We identified the key enzymatic reaction linking the aromatic branch of piperine biosynthesis to the presumably lysine-derived formation of the piperidine heterocycle. Identification of piperine synthase as a BAHD-type acyltransferase came along with a series of puzzling observations. Formation of piperic acid by piperine synthase is the predominant reaction at low substrate concentrations in vitro and, most likely is the direct consequence of the proposed reaction mechanism of BAHD-type acyltransferases where the histidine in the HXXXD motif acts as a catalytic base and in the case of piperic acid formation abstracts a proton from water rather than piperidine. The reactive hydroxyl group then attacks the carbonyl group of piperoyl-CoA as a nucleophile resulting in the hydrolysis of CoA-SH and piperic acid. Precise channeling of metabolites in a hypothetical
**Fig. 5 Metabolic grid of a large set of amides produced by piperine synthase (blue) and piperamide synthase (red).**

**a** Color intensity of catalyzed reaction is correlated to relative enzyme activity based on LC-UV/Vis and LC-ESI-MS intensities. Relative activities are shown compared to piperine formation, set to 100%, shades of blue piperine synthase, shades of red piperamide synthase. Due to the lack of available standards, and individual, different ionization intensities, relative rather than absolute values can currently be provided with confidence. The combination of CoA-esters and different amines resulted in the production of a large array of amides.

**b** Product profile of aromatic CoA-donors

**c** Product profile of aliphatic CoA-donors

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- **b** Aromatic amides; **c** Aliphatic amides. Piperine synthase appears quite specific for piperoyl-CoA and piperidine, whereas piperamide synthase is substrate promiscuous and tolerates a variety of amines and CoA-esters.
metabolon of piperine formation may reduce the risk of hydrolysis of the CoA-ester in vivo. In addition, the specific piperoyl-CoA ligase highly expressed in immature fruits with a low $K_m$ for piperic acid may serve as an anaplerotic enzyme and theoretically could replenish the pool of piperoyl-CoA at low substrate supply, reducing the accumulation of free piperic acid in the fruits. Piperine synthase is encoded by a single enzyme in the diploid black pepper genome. It is a member of a small gene family which is differentially expressed throughout individual organs. A sequence with 99% sequence identity to the piperine synthase sequence from our transcriptome data was identified from the black pepper genome dataset. In the genome, the gene is neither clustered within any other BAHD-gene nor localized close to potential and tentative pathway candidates, like a lysine/ornithine decarboxylase or the piperoyl-CoA ligase.

The role of piperamide synthase with a preference for the production of alternative stereoisomers from the identical $2E,4E$-piperoyl-CoA substrate as compared to piperine synthase remains baffling. Only piperine, rather than its isomers show up in freshly extracted peppercorns, although piperine gradually isomerizes in aqueous solutions. Since the gene encoding piperamide synthase is highly expressed in the fruits we currently assume, that it either produces the “correct” $2E,4E$-piperine isomer in vivo or, based on efficient product formation with the substrates.

### Table 1

Apparent kinetic constants of piperine synthase and piperamide synthase using piperoyl-CoA (5–2000 µM) and piperidine (1–100 mM) as substrates.

|                     | Apparent $K_m$ [mM] | Apparent $K_{cat}$ [s$^{-1}$] | $K_{cat}/K_m$ [s$^{-1}$ M$^{-1}$] |
|---------------------|---------------------|-----------------------------|----------------------------------|
| Piperine synthase   |                     |                             |                                  |
| Piperoyl-CoA        | 0.342 ± 0.060       | 1.01 ± 0.12                 | 2953                             |
| Piperidine          | 7.6 ± 0.5           | 0.47 ± 0.11                 | 16.2                             |
| Piperamide synthase |                     |                             |                                  |
| Piperoyl-CoA        | 0.196 ± 0.009       | 0.35 ± 0.01                 | 1786                             |
| Piperidine          | 8.69 ± 3.6          | 0.27 ± 0.02                 | 31.1                             |

All data were generated from three individual measurements, performed in triplicates.

### Fig. 6

Bootstrapped, unrooted cladogram of piperine and piperamide synthases among BAHD-like acyltransferases. Piperine synthase and closely related piperamide synthase from black pepper are marked in bold green. Functionally characterized benzoyl-CoA benzoate transferases (BBTs) and (Z)-hexen-1-ol acetyltransferase from Arabidopsis (in a gray box) represent clade V of BAHD-like acyltransferases but are distinct from piperine and piperamide synthases. Clusters harboring capsaicin synthase, vinorine synthase, malonoyltransferases, cocaine synthase, hydroxycinnamoyltransferases (HCTs), and spermidine hydroxycinnamoyl transferases (SHTs, SDTs and SCT) share <25% amino acid sequence identity to piperine and piperamide synthase, respectively. Either NCBI accession numbers, Arabidopsis gene entries (www.tair.org), and/or the pdb-accession number of crystallized and functionally characterized synthases are shown and are also listed in Supplementary Data 1.
hexanoyl- and octanoyl-CoA, respectively, is involved in the synthesis of medium and long-chain aliphatic amides, including (2E,4E)-N-isobutyl-decadienamide or (2E,4E,12Z)-N-isobutyl-octadecatrienamide, isolated from black pepper extracts. The unusual isomer formation in the case of piperine is strikingly similar to an observation recently reported for Arabidopsis unordinary isomer formation in the case of piperine isomers is rapidly isomerization occurs. The presence of natural deep eutectic solvents (NADES) provide a plausible explanation for the presumably high product activities can also be conserved, even if water is largely or organic acids, e.g., malic acid. Under these conditions enzyme activities were performed with edget's exact Test using a log2 fold-change (LFC) > 1 threshold and a FDR < 0.001. All further data mining and statistical analysis were performed in R (Version 3.6.2). GSEA was performed on the results obtained from HiPACH clustering by using the 3000 most differently expressed genes (FDR < 0.001, LFC > 1). The plant-specific MapMan functional BIN system was used as input ontology for the cluster-wise gene set testing profile. For RT-qPCR analysis, 200 ng of total RNA and oligo(dT)18-primers were used for cDNA synthesis with Maxima H Minus First Strand cDNA synthesis Kit (Thermo Scientific). The CDNA was diluted 1:10 with water. RT-PCR was performed with 3 µl cDNA and 2 pmol of each primer in a 10 µl reaction using qPCR Mix EvaGreen® No Rox (BioS&ell GmbH) and monitored by CFX Connect Real-Time System (Bio-Rad Laboratories, Inc.). The reference gene P. nigrum elongation factor 2B (elF2B) was described by us earlier to be fairly equally expressed in flowering plant and transgenic line, respectively. Differential gene expression analysis was performed with edget's exact Test using a log2 fold-change (LFC) > 1 threshold and a FDR < 0.001. This analysis was carried out by Trinity which employs BOWTIE and RSEM for short read alignment and transcript quantification, respectively. The results were analyzed using a |log2 fold-change (LFC)| > 1 threshold and a FDR < 0.001. All further data mining and statistical analysis were performed in R (Version 3.6.2). GSEA was performed on the results obtained from HiPACH clustering by using the 3000 most differently expressed genes (FDR < 0.001, LFC > 1). The plant-specific MapMan functional BIN system was used as input ontology for the cluster-wise gene set testing profile. For RT-qPCR analysis, 200 ng of total RNA and oligo(dT)18-primers were used for cDNA synthesis with Maxima H Minus First Strand cDNA synthesis Kit (Thermo Scientific). The CDNA was diluted 1:10 with water. RT-PCR was performed with 3 µl cDNA and 2 pmol of each primer in a 10 µl reaction using qPCR Mix EvaGreen® No Rox (BioS&ell GmbH) and monitored by CFX Connect Real-Time System (Bio-Rad Laboratories, Inc.). The reference gene P. nigrum elongation factor 2B (elF2B) was described by us earlier to be fairly equally expressed in flowering plant and transgenic line, respectively. Differential gene expression analysis was performed with edget's exact Test using a log2 fold-change (LFC) > 1 threshold and a FDR < 0.001. This analysis was carried out by Trinity which employs BOWTIE and RSEM for short read alignment and transcript quantification, respectively. The results were analyzed using a |log2 fold-change (LFC)| > 1 threshold and a FDR < 0.001. All further data mining and statistical analysis were performed in R (Version 3.6.2). GSEA was performed on the results obtained from HiPACH clustering by using the 3000 most differently expressed genes (FDR < 0.001, LFC > 1). The plant-specific MapMan functional BIN system was used as input ontology for the cluster-wise gene set testing profile.
Piperonyl-CoA was synthesized enzymatically by piperonyl-CoA ligase as previously described from coenzyme A and piperic acid. 3,4-Dimethoxybenzylcinnamyl-CoA was synthesized by the action of the double bond reduction of piperonyl-CoA. All other amines, coenzyme A, piperic acid, and CoA-esters used in enzyme assays were commercially available from Merck. All standards were dissolved in aqueous 25–50% DMSO solutions and kept in dark tubes to reduce isomerization at −20 °C until further use. Routinely, 1–2 µg of the purified enzyme was incubated with 200 µM activated ester, 4 mM amine, 30 mM Tris/HCl pH 8.0 and 1 mM DTT in a final volume of 50 µl for up to 30 min. DTT was included to prevent the formation of inactive oligomers, observed during enzyme purification by size exclusion chromatography (Supplementary Fig.S3). A reaction mix without an enzyme to 90% solvent B in 10 min. Depending on the substrate and product analyzed, a 5 cm Nucleosil C18 reverse-phase column was used at a flow rate of 0.6 mL min⁻¹ with identical solvents and similar gradient systems. Products were analyzed on an e2695 chromatography work station equipped with a photodiode array detector (PDA) and a QDA-mass detector (Waters, Eschborn, Germany). Products were recorded simultaneously by UV/Vis-detection between 280 and 380 nm (if applicable) and mass detection in a positive ionization mode between 100–1000 u. Due to the absence of commercial standards, piperine (0.1–100 µM) was used for LC-MS and UV/Vis-based quantification of product formation in the case of all piperamides produced. Kinetic constants for piperine formation were determined in three independent measurements with different enzyme preparation in three technical replicates each.

Sequence comparisons and cladogram. Protein sequences included in the cladogram (Fig. 6) were obtained by BLAST searches (Basic Local Alignment Search Tool) using the piperine synthase amino acid sequence as a query against the NCBI non-redundant protein database. Sequences with the highest sequence identities from different species are shown. Accession numbers of BAHD-like crystal structures were obtained from the PDB database (https://www.rcsb.org/). Protein sequences were aligned, accession numbers listed in the phylogenetic tree, concatenated, and are included as Supplementary Data 1.

Statistics and reproducibility. Statistical analysis of the qRT-PCR was performed using R (Version 3.6.2) as described above. For all statistical analysis, data from at least three independent measurements was used. The exact number of replicates is indicated in individual figure captions and methods.

Data availability. NCBI accession numbers and gene identifiers are listed. Sequence information of piperine synthase (MW354956) and piperamide synthase (MW354957) will be available after the publication of the manuscript. RNA-Seq data were stored in array express and are accessible under the following link: http://www.ebi.ac.uk/arrayexpress/experiments/E-MTAB-9029. All data are accessible in the public repository RADAR (https://www.radar-service.eu). Access is provided using DOI:10.22002/RAD-0087. Raw data of all files are stored and secured on local IBP-hard drives and IBP-Servers, and can also be provided by the corresponding author (T.V.).

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Author contributions

A.S. performed the major part of the experimental work and evaluated experimental data; B.A. analyzed the data of the RNA-Seq approach; K.M. performed cloning of the constructs and expression of recombinant enzymes; F.S. provided and supervised the growth of the plant material; F.C. contributed to the synthesis of standards; T.V. initiated the project, performed experimental work, and wrote the manuscript with the contribution of all co-authors.

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Competing interests

We declare the following competing financial interest: A pending international patent application PCT/EP2020/060165 (issued 9 April 2020) for producing bioactive amides by recombinant piperine synthase and piperamide synthase (illustrated in Fig. 5) has been filed by the Leibniz Institute of Plant Biochemistry (Halle (Saale), Germany), inventors: A.S., B.A., K.M. and T.V. All other authors declare no competing interests.

Additional information

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