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Gene Discovery in *Gelsemium* Highlights Conserved Gene Clusters in Monoterpene Indole Alkaloid Biosynthesis

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Genome mining is a routine technique in microbes for discovering biosynthetic pathways. In plants, however, genomic information is not commonly used to identify novel biosynthesis genes. Here, we present the genome of the medicinal plant and oxindole monoterpene indole alkaloid (MIA) producer *Gelsemium sempervirens* (Gelsemiaceae). A gene cluster from *Catharanthus roseus*, which is utilized at least six enzymatic steps downstream from the last common intermediate shared between the two plant alkaloid types, is found in *G. sempervirens*, although the corresponding enzymes act on entirely different substrates. This study provides insights into the common genomic context of MIA pathways and is an important milestone in the further elucidation of the *Gelsemium* oxindole alkaloid pathway.

Almost all biosynthetic pathways of secondary or specialized metabolites in bacteria and fungi are physically clustered.[1,2] In contrast, gene clustering in plant-specialized metabolism occurs more rarely, and many plant biosynthetic pathways that are co-regulated are not clustered.[3] Therefore, gene discovery in plants is a slow process and typically relies solely on co-expression analysis and the identification of homologues of known genes. Although plant genome data are becoming more readily available, we are still not able to predict which natural product pathways will be clustered and which will not. Moreover, although conserved gene clusters have been observed in closely related species that produce chemically similar products,[4–6] it is not known how well gene clusters would be conserved in less closely related species that produce chemically divergent compounds. An example is the monoterpene indole alkaloids (MIAs), which are produced by plants of the Apocynaceae, Gelsemiaceae, Loganiaceae, Nyssaceae and Rubiaceae families. The MIAs comprise approximately 3000 compounds, with chemical scaffolds so different that it is not always obvious that the chemicals are biosynthetically related.[7] This enormous chemical complexity stands in contrast to only three sequenced MIA producers, namely *Catharanthus roseus* (Apocynaceae, order Gentianales),[8] *Rhazya stricta* (Apocynaceae, order Gentianales)[9] and *Camptotheca acuminata* (Nyssaceae, order Cornales).[10] Only a small number of the genes accounting for the enormous diversity of MIAs are known so far, and these are primarily from *C. roseus*. Therefore, the genomic context of MIA biosynthesis is largely unknown and has only been systematically investigated in *C. roseus*; this revealed two small gene clusters composed of previously identified MIA pathway genes.[8] Given the vast chemical diversity of MIAs, we wondered whether these gene clusters would be conserved in MIA-producing plants with different chemical profiles, and whether they might potentially be useful for accelerating biosynthetic gene discovery. As a case study, we selected the MIA producer Carolina jasmine (*Gelsemium sempervirens*, Gelsemiaceae), which produces a wide variety of oxindole alkaloids (Figure 1).[11]

Gelsemium alkaloids have potent biological activities.[11,12] However, due to low production levels in planta and their complex chemical structures, they are not readily available for further research. Elucidation of the underlying biosynthetic pathway would permit metabolic engineering strategies to harness the medicinal potential of *Gelsemium* alkaloids, yet no gene of this MIA pathway has been identified.

Herein, we present a high-quality genome assembly and annotation of *G. sempervirens* along with a revised, much more contiguous genome for *C. roseus* for comparison. Using this data, we rapidly identified five *Gelsemium* pathway genes. We show that gene clusters between these plant species from two different plant families are conserved, and that gene clustering in combination with classical homology searches can accelerate gene discovery in non-model plants across family borders and for structurally divergent compounds.

Our first task was to obtain a suitable genome of *G. sempervirens*. An initial assembly of *G. sempervirens* (v1, 219 Mb) was previously constructed by using Illumina-derived paired end libraries and low-coverage synthetic long-reads;[13] this assembly was fragmented as indicated by the small N_{50} scaffold size (29 kb). Thus, we constructed three mate-pair libraries and used the ALLPATHS-LG assembler[14] to generate a significantly improved *G. sempervirens* genome sequence (v3) of 244 Mb with an N_{50} scaffold size of 411 072 bp. Annotation of the *G. sempervirens* v3 genome by using RNA-sequencing-derived transcript assemblies and protein evidence yielded 22 617 genes. Quality assessments of the *G. sempervirens* v3 genome

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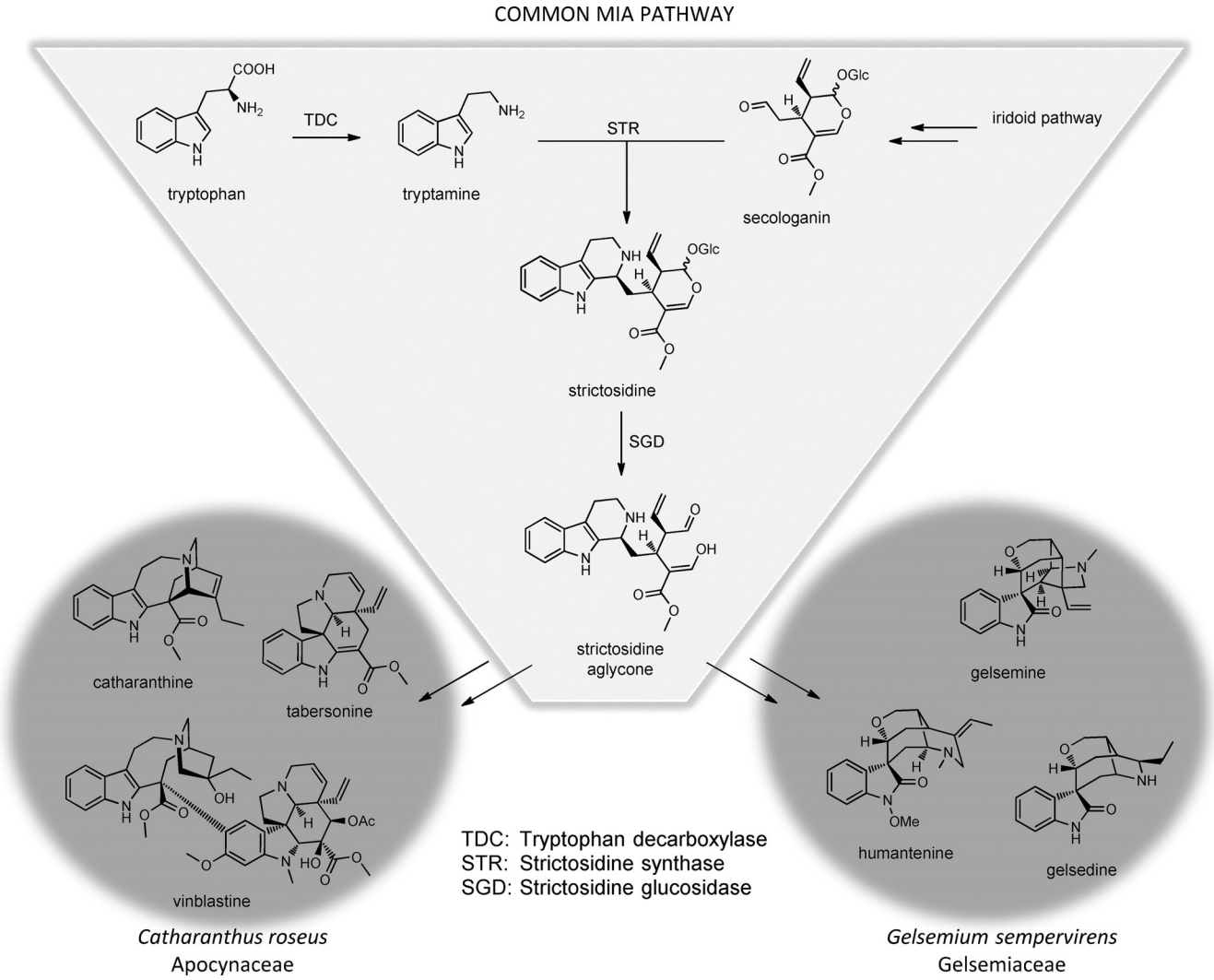

Figure 1. Key steps in MIA biosynthesis, catalyzed by the enzymes tryptophan decarboxylase (TDC), strictosidine synthase (STR) and strictosidine glucosidase (SGD). The pathway diverges after strictosidine aglycone and leads to very different alkaloids in the plants *C. roseus* and *G. sempervirens*. Representative alkaloids for each plant are shown.

assembly revealed robust representation of the genome and gene space with 88% of the paired-end genomic reads aligning to the assembly in the correct orientation and 93.1% of the BUSCO orthologues present (86.1% complete, 7.0% fragmented).[15] Although a genome assembly is available for *C. roseus*,[16] it has limited contiguity (N_{50} scaffold length 27.3 kb; total size 506 Mb, 41176 scaffolds), and thus, we generated an improved genome assembly for *C. roseus* to enable genomic comparisons between the two species. Using a set of paired-end and mate pair libraries, we generated a 541 Mb assembly (v2) of *C. roseus* composed of 2090 scaffolds with an N_{50} scaffold size of 2579454 bp, a significant improvement in assembly metrics; re-annotation of v2 of the genome yielded 34363 genes. Alignment of Sanger-derived expressed sequence tags to v2 of the genome revealed robust representation of genic sequences (96.7%) that was further supported by the detection of 93.3% complete BUSCO core orthologues (91.6% single copy, 1.7% duplicated) and 1.9% fragmented BUSCO core orthologues. An *ortho* group and synteny comparison of both genomes indicated substantial similarities (Figure S12 in the Supporting Information).

With both genomes in hand, we tested whether an early pathway gene cluster observed in *C. roseus* is conserved in *G. sempervirens*. Strictosidine synthase (STR) and tryptophan decarboxylase (TDC) are key enzymes for the production of strictosidine (Figure 1). In *C. roseus* the corresponding genes *CrSTR* and *CrTDC* form a gene cluster,[8] along with a MATE transporter gene (*CrMATE1*) for which the target substrate has not yet been identified (Figure 2B).[17] *GsSTR* and *GsTDC* have not been reported, but orthologues are known from several species,[7,18,19] and it has been shown that *Gelsemium* oxindole alkaloids are derived from strictosidine.[20] To identify target genes, we performed a BLAST search of known *TDC* and *STR* genes and their protein sequences against the predicted *G. sempervirens* proteome. We identified a *GsTDC* candidate gene with 77% (gene ID 147-3.2) and a *GsSTR* gene with 55% (147-3.18) amino acid sequence identity compared to *CrTDC* and *CrSTR*, respectively. *GsSTR*, *GsTDC* and two genes annotat-

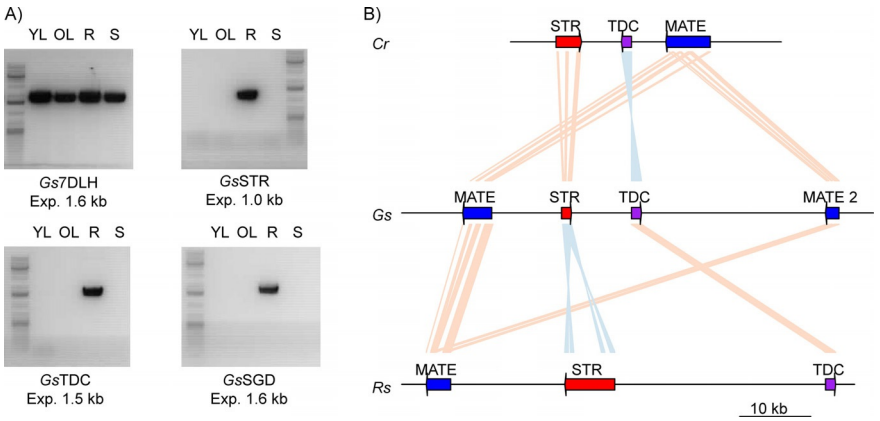

Figure 2. GsTDC, GsSTR and GsSGD are conserved key enzymes in *Gelsemium sempervirens* and are exclusively produced in roots. A) RT-PCR results for *GsSTR*, *GsTDC* and *GsSGD*. A gene putatively coding for 7-deoxyloganic acid hydroxylase (7DLH)[21] was used as a positive control. YL: young leaves, OL: old (mature) leaves, R: roots, S: stem. B) Synteny between *TDC*/*STR*/*MATE* gene clusters from *C. roseus* (*Cr*), *G. sempervirens* (*Gs*) and *Rhazya stricta* (*Rs*).

ed as MATE efflux family proteins (147-3.6 and 147-3.7) with 78 and 73% nucleotide sequence identity to *CrMATE1*[17] (Figure 2B) were present on a single scaffold (scaffold 147) in the *G. sempervirens* genome, in a similar arrangement to that in *C. roseus*.[8] In *R. stricta*, the only other sequenced MIA producer[9] that utilizes strictosidine, we found a similar *TDC*/*STR*/*MATE* gene cluster (Figure 2B).

Using RT-PCR, we showed that *GsTDC* and *GsSTR* are expressed exclusively in roots; this is consistent with the location of these alkaloids (Figure 2A). The third key MIA biosynthesis gene, strictosidine glucosidase (*SGD*),[22–24] is not part of a gene cluster in the *C. roseus* genome. A BLAST search of *CrSGD* with the *G. sempervirens* annotated gene set and phylogenetic analyses (Figure S1) identified three *GsSGD* candidates (68-0.7, 13-14.56 and 13-14.57), none of them on the same scaffold as *GsTDC* and *GsSTR*. Of these, only one (13-14.56) was exclusively expressed in roots (Figure 2A; data for others not shown). The biochemical function of GsTDC, GsSTR and GsSGD (13-14.56) was confirmed by heterologous production in *Escherichia coli* and enzymatic analysis (Figure S2A–C). The other two GsSGD candidates were inactive under assay conditions. Accordingly, *GsTDC*, *GsSTR* and *GsSGD* are co-regulated, whereas only *GsTDC* and *GsSTR* are physically clustered. This finding is consistent with the genomic arrangement in *C. roseus*[8] and is in agreement with previous studies that show that co-expression is more prevalent than gene clustering in plant-specialized metabolism.[3]

All MIAs are derived from strictosidine, so even though it is notable that the *STR*/*TDC* gene cluster is conserved across plant species, this is perhaps not surprising. However, the alkaloid scaffolds of *C. roseus* and *G. sempervirens* are structurally distinct, so we wondered how the organization of enzymes downstream of the point of structural divergence compared. Both plants produce MIAs derivatized with a methoxy group at the same position of the indole nucleus (C-16 of indole, C-11 of oxindole alkaloids). The most prominent examples are tabersonine (**1**) in *C. roseus,* and humantenirine (**2**) and its N-methylated analogue 11-methoxyhumantenine (**3**)[11,25] in *G. sempervirens* (Figure 3A). Despite the common O-methylation pattern, tabersonine and the humantenines are structurally distinct and not easily recognizable as biosynthetically related (Figure 1). In *C. roseus* a cluster of three genes involved in the methoxylation of tabersonine has been identified;[8] it consists of two genes encoding catalytically equivalent P450 monooxygenases[26] and one encoding an *O*-methyltransferase (OMT; Figure 3A).[27] We hypothesized that genes homologous to *CrT16H1/2* and *Cr16OMT* are involved in the hydroxylation and methylation of this oxindole scaffold based on the similar chemistry. Using BLAST searches, we identified 15 potential *G. sempervirens* P450 monooxygenase genes with amino acid sequence identities between 55 and 63%, and three *G. sempervirens* OMT genes with 53 to 62% amino acid identity to *CrT16H1* and *Cr16OMT*, respectively. Two of the P450 and one of the OMT candidate genes were co-located on the same *G. sempervirens* scaffold (scaffold 505; Figure 3B). Additionally, the intron–exon structures of all genes were identical to their *C. roseus* homologues (Figure S11). We therefore prioritized these gene candidates for functional characterization and named them *RH11H1/2* (505-0.45 and 505-0.58) and *RH11OMT* (505-0.42) (rankinidine and humantenine-11-hydroxylase or -*O*-methyltransferase).

The lack of authentic standards is one of the biggest limitations for studying the *Gelsemium* pathway. Using the exact masses of the desired compounds as a guideline, we separated *G. sempervirens* leaf and stem material by acid–base extraction, column chromatography and semipreparative HPLC, and succeeded in obtaining about 600 μg humantenirine (**2**), 100 μg 11-methoxyhumantenine (**3**), and ≈5 μg rankinidine (**4**) as well as ≈5 μg humantenine (**5**; Figure S3). The structural identity of **2** was confirmed by using ^{1}H and HSQC NMR spectroscopy, and of that of **3** by ^{1}H spectroscopy, in comparison to literature data (Figures S4–S6; Tables S2 and S3).[25,28] Compounds **4** and **5** were not obtained in sufficient amounts for NMR analysis, though both compounds showed the expected high-resolution mass (Table S1) and had similar chromatographic behavior as their methoxylated counterparts (Figure S3). To confirm that these compounds have unsubstituted oxindole cores, we analyzed their UV spectra (Figure S7). Both compounds showed a maximum absorbance at ≈250–260 nm, whereas **2** and **3** have

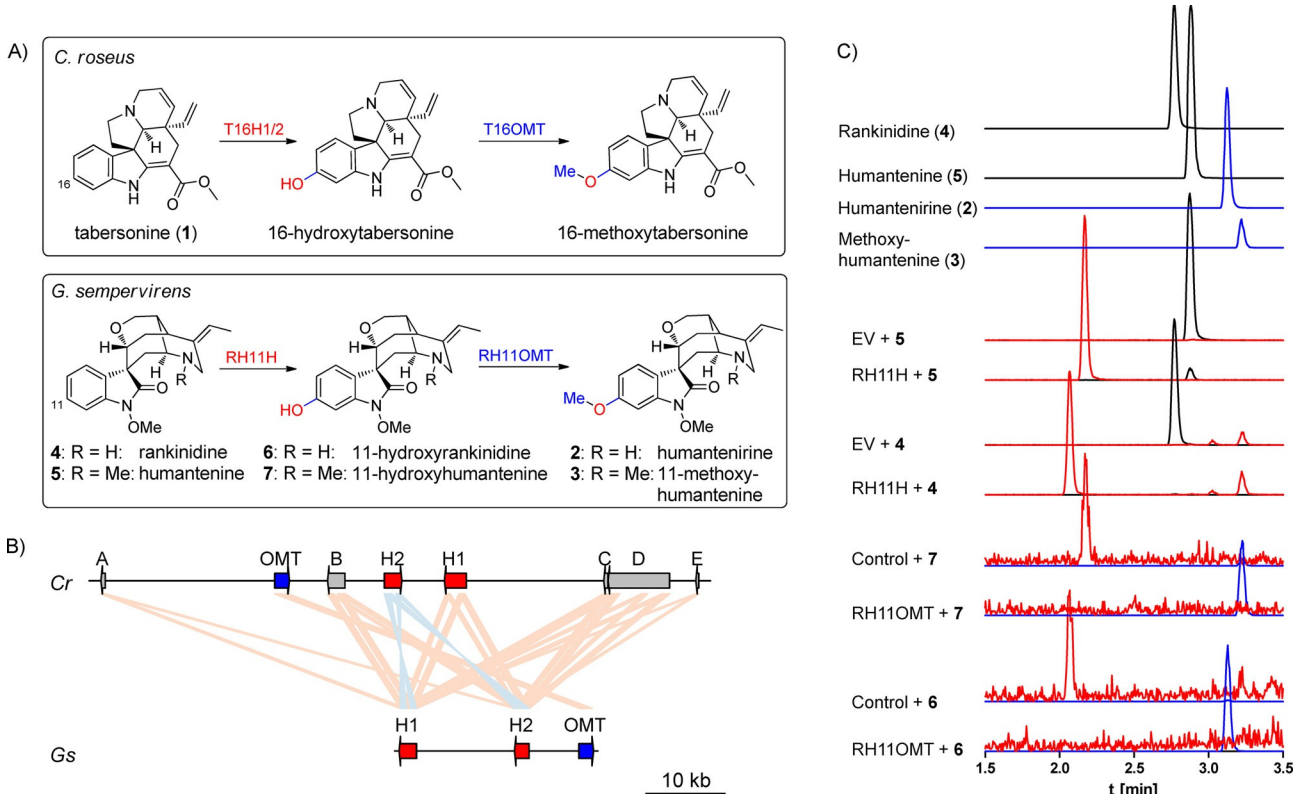

Figure 3. A conserved gene cluster is responsible for C-11 methoxylation of humantenine and rankinidine in *G. sempervirens*. A) Structures and reactions of indole methoxylation of *C. roseus* and *G. sempervirens* alkaloids. B) Synteny between the *C. roseus* and *G. sempervirens* scaffolds involved in indole methoxylation. H1/2 refers to T16H1/2 in *C. roseus* and RH11H1/2 in *G. sempervirens*. A/B/C/D/E share (partial) sequence similarities with T16H1/2 but have not yet been characterized. C) LCMS profiles of in vitro assays showing oxindole C-11 hydroxylation of rankinidine/humantenine, catalyzed by microsomes enriched with RH11H3, and subsequent methylation of the intermediates by RH11OMT. Traces shown are MRMs (**4**/**5** in black, **2**/**3** in blue), daughter scans of 357 for **6** and of 371 for **7** (both in red). EV: empty vector control microsomes.

maxima at ≈280–290 nm due to the bathochromic effect of the C-11 methoxy group (Figure S7, Table S1).[25,29,30]

We then attempted to clone *RH11H1/2* genes from *G. sempervirens* root-derived cDNA. Only a single set of sequences with nucleotide sequence identities of 99.3–100.0% could be repeatedly amplified from root cDNA, which had 97.5–98.2% and 93.1–93.3% nucleotide sequence identity to the predicted genome sequences of *RH11H2* and *RH11H1*, respectively. The most abundant sequence (5 of 9 clones) was 97.6% identical to *RH11H2*. This gene, which we named *RH11H3*, was expressed in *Saccharomyces cerevisiae*, and microsomes enriched in the RH11H3 protein were obtained. *RH11OMT* was successfully expressed in *E. coli* in soluble form.[27] We observed complete consumption of **4** and **5** when incubated with RH11H3, along with the formation of new compounds, **6** and **7**, with an increased molecular weight corresponding to the addition of one oxygen, as confirmed by HRMS (Figure 3C). Then, both compounds **6** and **7** were used as substrates for subsequent assays containing purified RH11OMT. Both were completely consumed and resulted in the formation of new compounds that showed identical mass and retention times to the isolated reference standards for **2** and **3** (Figure 3C). When we combined RH11H3 and RH11OMT in a single assay, we observed no hydroxylated intermediates, only starting substrate and methoxylated product. The substrate of CrT16H, **1**, was also tested with RH11H3, but no reaction was observed, thus highlighting the substrate specificity of this CYP.

In summary, RH11H3 and RH11OMT are responsible for the C-11 methoxylation of humantenine alkaloids in *G. sempervirens* and are therefore the first pathway-specific enzymes from *Gelsemium* oxindole alkaloid biosynthesis to be discovered. Notably, neither RH11H3 nor RH11OMT is the closest homologue of CrT16H or CrT16OMT (Figure S13). Although they could have been discovered without gene cluster analysis, this would have required the screening of a much larger number of candidates, more time and larger quantities of limited substrate.

Although we could not determine where RH11H3 is located on the *G. sempervirens* genome, we assume that the methoxylation gene cluster is conserved between *C. roseus* and *G. sempervirens*, given the high identity of RH11H3 and RH11H2 (97.6%), the conserved intron–exon structure of all genes and the similar gene arrangement of the clusters. This is unexpected, considering the fact that these methoxylation reactions occur at least six enzymatic steps after the last shared intermediate. This suggests that gene clusters are potentially useful for gene discovery in other distantly related plant species and pathways that share a common evolutionary origin.

Current models for gene cluster maintenance state that selection primarily aims to maintain the function of a gene cluster, regardless of rearrangements.[31,32] This is well reflected by

the plasticity that we observed for the MIA gene clusters: both the order and the orientation of genes was variable. Also, gene duplication was observed several times, for example, for *GsMATE1/2*, *RH11H1/2/3* or *CrT16H1/2*. In the case of *CrT16H1/2*, it has been shown that this gene duplication has led to neofunctionalization to perform the hydroxylation of tabersonine in different *C. roseus* tissues.[26] In contrast, in *G. sempervirens*, the catalytic and physiological role of *GsRH11H1* and *GsRH11H2* remain unclear. Notably, the amino acid sequence identities of the duplicated gene pairs are much higher (CrT16H1/2 82%, GsRH11H1/2 88%) than the interspecies comparison (CrT16H1 vs. GsRH11H2 55%), thus suggesting that gene duplication has occurred independently in both species.

The identification of plant biosynthetic genes typically relies on co-expression analysis, often leading to a large number of gene candidates that have to be screened.[33] Herein, we have shown that gene clusters in MIA metabolism are conserved across family borders and can help to accelerate gene discovery when combined with traditional approaches. We expect that this additional layer of information will prove fruitful for the discovery of further genes in plant-specialized metabolism. This is especially important for non-model plants, for which multi-tissue transcriptome datasets or reference genes for co-expression analysis are not available. This holds true for the *Gelsemium* oxindole alkaloids—the identification of the first five pathway genes, *GsTDC*, *GsSTR*, *GsSGD*, *RH11H3* and *RH11OMT*, along with initial expression data reported herein now facilitates future coexpression studies. It is therefore an important milestone for harnessing the pharmacological potential of the *Gelsemium* oxindole alkaloids.

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Conflict of Interest

The authors declare no conflict of interest.

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[1] N. Ziemert, M. Alanjary, T. Weber, *Nat. Prod. Rep.* **2016**, *33*, 988 – 1005.
[2] M. H. Medema, M. A. Fischbach, *Nat. Chem. Biol.* **2015**, *11*, 639 – 648.
[3] J. H. Wisecaver, A. T. Borowsky, V. Tzin, G. Jander, D. J. Kliebenstein, A. Rokas, *Plant Cell Online* **2017**, *29*, 944 – 959.
[4] Y. Zhou, Y. Ma, J. Zeng, L. Duan, X. Xue, H. Wang, T. Lin, Z. Liu, K. Zeng, Y. Zhong, et al., *Nat. Plants* **2016**, *2*, 16183.
[5] M. Itkin, U. Heinig, O. Tzfadia, A. J. Bhide, B. Shinde, P. D. Cardenas, S. E. Bocobza, T. Unger, S. Malitsky, R. Finkers, et al., *Science* **2013**, *341*, 175 – 179.
[6] Y. Shang, Y. Ma, Y. Zhou, H. Zhang, L. Duan, H. Chen, J. Zeng, Q. Zhou, S. Wang, W. Gu, et al., *Science* **2014**, *346*, 1084 – 1088.
[7] S. E. O'Connor, J. J. Maresh, *Nat. Prod. Rep.* **2006**, *23*, 532 – 547.
[8] F. Kellner, J. Kim, B. J. Clavijo, J. P. Hamilton, K. L. Childs, B. Vaillancourt, J. Cepela, M. Habermann, B. Steuernagel, L. Clissold, et al., *Plant J.* **2015**, *82*, 680 – 692.
[9] J. S. M. Sabir, R. K. Jansen, D. Arasappan, V. Calderon, E. Noutahi, C. Zheng, S. Park, M. J. Sabir, M. N. Baeshen, N. H. Hajrah, et al., *Sci. Rep.* **2016**, *6*, 33782.
[10] D. Zhao, J. P. Hamilton, G. M. Pham, E. Crisovan, K. Wiegert-Rininger, B. Vaillancourt, D. DellaPenna, C. R. Buell, *GigaScience* **2017**, *6*, gix065.
[11] G.-L. Jin, Y.-P. Su, M. Liu, Y. Xu, J. Yang, K.-J. Liao, C.-X. Yu, *J. Ethnopharmacol.* **2014**, *152*, 33 – 52.
[12] B. Yu, D.-Q. Yu, H.-M. Liu, *Eur. J. Med. Chem.* **2015**, *97*, 673 – 698.
[13] J. A. Stapleton, J. Kim, J. P. Hamilton, M. Wu, L. C. Irber, R. Maddamsetti, B. Briney, L. Newton, D. R. Burton, C. T. Brown, et al., *PLoS One* **2016**, *11*, e0147229.
[14] S. Gnerre, I. MacCallum, D. Przybylski, F. J. Ribeiro, J. N. Burton, B. J. Walker, T. Sharpe, G. Hall, T. P. Shea, S. Sykes, et al., *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 1513 – 1518.
[15] F. A. Simão, R. M. Waterhouse, P. Ioannidis, E. V. Kriventseva, E. M. Zdobnov, *Bioinformatics* **2015**, *31*, 3210 – 3212.
[16] F. Kellner, F. Geu-Flores, N. H. Sherden, S. Brown, E. Foureau, V. Courdavault, S. E. O'Connor, *Chem. Commun.* **2015**, *51*, 7626 – 7628.
[17] R. M. E. Payne, D. Xu, E. Foureau, M. I. S. T. Carqueijeiro, A. Oudin, T. D. de Bernonville, V. Novak, M. Burow, C.-E. Olsen, D. M. Jones, et al., *Nat. Plants* **2017**, *3*, 16208.
[18] T. Charoonratana, J. Wungsintaweekul, N. Keawpradub, R. Verpoorte, *Acta Physiol. Plant.* **2013**, *35*, 2611 – 2621.
[19] W. Liu, R. Chen, M. Chen, H. Zhang, M. Peng, C. Yang, X. Ming, X. Lan, Z. Liao, *Planta* **2012**, *236*, 239 – 250.
[20] N. Nagakura, M. Rüffer, M. H. Zenk, *J. Chem. Soc. Perkin Trans. 1* **1979**, 2308 – 2312.
[21] K. Miettinen, L. Dong, N. Navrot, T. Schneider, V. Burlat, J. Pollier, L. Woittiez, S. van der Krol, R. Lugan, T. Ilc, et al., *Nat. Commun.* **2014**, *5*, 3606.
[22] I. Gerasimenko, Y. Sheludko, X. Ma, J. Stöckigt, *Eur. J. Biochem.* **2002**, *269*, 2204 – 2213.
[23] N. Yerkes, J. X. Wu, E. McCoy, M. C. Galan, S. Chen, S. E. O'Connor, *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3095 – 3098.
[24] G. Guirimand, V. Courdavault, A. Lanoue, S. Mahroug, A. Guihur, N. Blanc, N. Giglioli-Guivarc'h, B. St-Pierre, V. Burlat, *BMC Plant Biol.* **2010**, *10*, 182.
[25] L.-Z. Lin, G. A. Cordell, C.-Z. Ni, J. Clardy, *J. Nat. Prod.* **1989**, *52*, 588 – 594.
[26] S. Besseau, F. Kellner, A. Lanoue, A. M. K. Thamm, V. Salim, B. Schneider, F. Geu-Flores, R. Höfer, G. Guirimand, A. Guihur, et al., *Plant Physiol.* **2013**, *163*, 1792 – 1803.
[27] D. Levac, J. Murata, W. S. Kim, V. De Luca, *Plant J.* **2008**, *53*, 225 – 236.
[28] N. Kogure, H. Kobayashi, N. Ishii, M. Kitajima, S. Wongseripipatana, H. Takayama, *Tetrahedron Lett.* **2008**, *49*, 3638 – 3642.
[29] A. W. Sangster, K. L. Stuart, *Chem. Rev.* **1965**, *65*, 69 – 130.
[30] Y. Wang, S. Wu, Z. Chen, H. Zhang, W. Zhao, *J. Ethnopharmacol.* **2015**, *166*, 66 – 73.
[31] L. D. Hurst, C. Pál, M. J. Lercher, *Nat. Rev. Genet.* **2004**, *5*, 299 – 310.
[32] F. Al-Shahrour, P. Minguez, T. Marqués-Bonet, E. Gazave, A. Navarro, J. Dopazo, *PLoS Comput. Biol.* **2010**, *6*, e1000953.
[33] A. Goossens, *Mol. Plant* **2015**, *8*, 2 – 5.

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