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Progress on synthesis of benzylisoquinoline alkaloids in sacred lotus (*Nelumbo nucifera*)

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Abstract

Sacred lotus (*Nelumbo nucifera*) is a 2,000-year-old perennial rhizome aquatic crop that is primarily employed as a food and drug dual-use crop in East Asia. One of the key bioactive components of sacred lotus is benzylisoquinoline alkaloids (BIAs). Existing research has demonstrated that they have therapeutic and preventive benefits on obesity, diabetes, cancer, and cardiovascular disease. Despite their broad pharmacological relevance, the metabolism of BIA in sacred lotus has received little attention. We reviewed the biosynthetic process of the BIA in sacred lotus in this research. We concluded that a thorough functional characterization of BIAs biosynthesis enzymes provides a wide range of significant therapeutic applications for sacred lotus.

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Introduction

China has cultivated the sacred lotus (*Nelumbo nucifera*), a perennial rhizome aquatic plant in the Nelumbonaceae family, for over 2,000 years on 330,000 hectares[1]. Two species of *Nelumbo* exist: *nucifera* and *lutea*. *N. nucifera* inhabits Asia and Oceania[2]. *N. lutea* inhabits North and Northern South America[3,4]. *N. nucifera* and *N. lutea* are only geographically separated, not reproductively[5]. Hybrid breeding of *N. nucifera* and *N. lutea* may enhance sacred lotus diversity. As a food-drug dual, sacred lotus is popular in East Asia, especially China[2]. According to the 2020 edition of the 'Pharmacopoeia of the People's Republic of China'[6], sacred lotus leaves, flowers, seeds, stamens, receptacles, and internodes are commonly used medicinal materials and have important medicinal value. For example, the lotus leaf may clear heat, relieve summer heat, and send clarity (pure) upward. Lotus has been shown to promote blood circulation and hemostasis, as well as to remove dampness and wind and nourish the heart and kidney. The lotus seed can tonify the spleen and kidney, alleviate diarrhoea, and stop seminal secretions[6]. Lotus plumules can clear the mind and clear the heart, as well as restore appropriate heart-kidney coordination, boost essence, and stop bleeding[7–9].

BIAs with medical potential and healthcare benefits are being studied. BIAs are various plant-specific tyrosine-derived metabolites[10]. Most sacred lotus alkaloids are 1-benzylisoquinoline, aporphine, and bisbenzylisoquinoline. Norcoclaurine, a typical 1-benzylisoquinoline alkaloid, treats heart failure, arrhythmia, bradycardia, myocardial ischemia-reperfusion injury, and cardiac fibrosis in traditional Chinese medicine[11,12]. Norcoclaurine has anti-inflammatory, anti-arrhythmic, and antithrombotic properties and is a β2-adrenergic receptor agonist[13]. Neferine and isoliensinine, the main bisbenzylisoquinoline alkaloids in sacred lotus plumule extract, are pharmacologically significant[7]. Neferine possesses anti-inflammatory, anti-oxidative, anti-hypertensive, anti-arrhythmic, anti-platelet, anti-thrombotic, anti-amnesic, anti-anxiety, and anti-cancer characteristics. Isoliensinine is anti-tumor, cardioprotective, antioxidant, antidepressant, anti-HIV, and anti-Alzheimer's[14,15]. According to recent research, bisbenzylisoquinoline alkaloids may cure new coronavirus pneumonia[16]. Lotus leaves contain high-purity aporphine alkaloid nuciferine (NF). NF is anti-obesity, anti-hyperlipidemia, hypoglycemia, hypouricemic, anti-inflammatory[17], and otherwise therapeutic[18–20].

The metabolic pathways, biosynthesis, and corresponding enzymes involved in the formation of benzylisoquinoline alkaloids derived from the sacred lotus plant have yet to be elucidated, despite their significant pharmacological properties. Currently, the primary focus of research on the biosynthesis of benzylisoquinoline alkaloids (BIAs) lies in opium poppy (*Papaver somniferum*) and other related species within the Ranunculales order. Extensive investigations have successfully revealed the complete biosynthetic pathways of various alkaloids possessing significant pharmacological properties, including morphine (morphinan), noscapine (phthalideisoquinoline), and sanguinarine (benzophenanthridine)[21]. Although the structure of BIAs in members of the Ranunculales order is characterised by complexity and diversity, it is important to note that all BIAs share a common biosynthetic origin. Specifically, metabolites derived from *L*-tyrosine, dopamine, and 4-hydroxyphenylacetaldehyde (4-HPAA) undergo a Pictet-Spengler condensation catalysed by norcoclaurine synthase (NCS), resulting in the formation of (*S*)-norcoclaurine. Subsequently, this compound is transformed into the key intermediate (*S*)-reticuline through the action of three methyltransferases (6OMT, CNMT, 4'OMT) and one cytochrome P450 monooxygenase (CYP), known as *N*-methylcoclaurine 3'-hydroxylase (NMCH)[22–26]. The

processes outlined above are often known as the upstream universal synthesis pathway. Subsequently, a series of oxidative enzymes facilitate the specific coupling of C-C and C-O bonds, leading to the transformation of (*S*)-reticuline into protoberberine, which serves as a precursor for the synthesis of benzophenanthridines and phthalideisoquinolines. Additionally, the conversion of (*S*)-reticuline gives rise to the formation of aporphine and morphinan alkaloids.

In the BIAs biosynthetic pathway of sacred lotus, from *L*-tyrosine to dopamine and 4-hydroxyphenylacetaldehyde (4-HPAA) to the formation of *N*-methylcoclaurine and reticuline is common to the synthesis pathway of Ranunculales species such as opium poppy, and the synthesis pathway is clear. However, the synthesis of bisbenzylisoquinolines (liensinine, neferine), the different methylation modifications between bisbenzylisoquinoline alkaloids, and the synthesis of aporphine compounds (nuciferine, etc.) are not clear. Therefore, it is crucial to investigate the pharmacological importance of these particular chemicals by studying the sacred lotus' functional enzymes. Hence, it is essential to conduct a comprehensive investigation on the functional enzymes present in the sacred lotus in order to elucidate the pharmacological potential of these distinct substances. Furthermore, it is worth noting that several benzylisoquinoline alkaloids (BIAs) derived from the sacred lotus have a conformation mostly composed of the *R*-enantiomer. This is in stark contrast to the prevalent *S*-enantiomer conformation seen in BIAs derived from opium poppy and plants connected to the Ranunculales order. Hence, the investigation of the atypical stereochemistry of BIAs in the sacred lotus has significance in terms of its molecular and biochemical aspects. Furthermore, the study of the metabolism and biosynthesis of angiosperms, which constitute the fundamental group of flowering plants, has significant implications for the understanding of plant evolution.

Occurrence of BIAs in sacred lotus

The sacred lotus contains three forms of BIAs: 1-benzylisoquinoline, aporphine, and bisbenzylisoquinoline alkaloids (Table 1). Their structure, concentration, and physiological functions in sacred lotus have been extensively studied. Their chemical formula, stereo configuration and distribution in sacred lotus organs are shown in Table 1.

1-Benzylisoquinoline alkaloids

1-Benzylisoquinoline alkaloids are traced in lotus leaves, flowers, embryos, and seeds (Table 1). The 1-benzylisoquinoline alkaloids in sacred lotus mainly include norcoclaurine, coclaurine, norjuziphine, isococlaurine, *N*-methylcoclaurine, 6-demethyl-4'-*O*-methyl-*N*-methylcoclaurine, norarmepavine, *N*-methylisococlaurine, norroefractine, juziphine, armepavine, 4'-*O*-methyl-*N*-methylcoclaurine, lotusine, isolotusine, 4'-*O*-methylarmepavine.

The pharmacological effects of these 1-benzylisoquinoline alkaloids are diverse. Norcoclaurine's pharmacological action is one of the most extensively researched. It possesses antioxidant, anti-HIV, and anti-Alzheimer's disease pharmacological actions[46], as well as cardiovascular pharmacological activities such as treating heart failure, lowering myocardial ischemia injury, and reducing pathological cardiac fibrosis and dysfunction[27,31,46]. Other 1-benzylisoquinoline alkaloids' pharmacological properties are also noteworthy. Armepavine, for example, inhibits melanin formation and regulates the immunological system[36]. Furthermore, it has been shown that this therapeutic approach may be used for the treatment of autoimmune disorders, including systemic lupus erythematosus and crescentic glomerulonephritis[47]. Lotusine contains anti-wrinkle, neuroprotective, and liver-protective properties[48,49].

Aporphines

The aporphine and pre-aporphine compounds found in sacred lotus are caaverine, asimilobine, glaziovine, *O*-nornuciferine, *N*-nornuciferine, lirinidine, *N*-methylasimilobine, roemerine, dehydronuciferine, dehydroanonaine, dehydroroemerine, pronuciferine, nuciferine, 7-hydroxydehydronuciferine, lysicamine, cepharadione B, anonaine, liriodenine. Among them, the pharmacological effect of NF is the most concerning which has anti-obesity, anti-hyperlipidemia, anti-diabetes, anti-arteriosclerosis, anti-tumor and other effects[50–52].

Among them, aporphine and pre-aporphine chemicals found in sacred lotus, such as lirinidine, asimilobine, *N*-methylasimilobine, and pronuciferine, *O*-nornuciferine, have anti-Alzheimer's disease properties[53,54]; Lirinidine in lotus petals has an anti-cervical cancer effect[33].

Bisbenzylisoquinolines

Bisbenzylisoquinoline alkaloids are mainly accumulated in the seed embryo of sacred lotus. The main bisbenzylisoquinoline compounds are included nelumboferine, liensinine, isoliensinine, dauriciline, 6-hydroxynorisoliensinine, *N*-norisoliensinine, nelumborine, dauricinoline, neferine, dauricine. There are several investigations being conducted on bisbenzylisoquinoline alkaloids at the moment. The most noteworthy is that bisbenzylisoquinoline alkaloids have the potential to be exploited as therapeutic agents for new coronavirus pneumonia. Neferine, in particular, can prevent SARS-CoV-2 infection by inhibiting Ca^{2+}-dependent membrane fusion[16]. Furthermore, neferine possesses anti-tumor, anti-inflammatory[25], anti-hypertension, anti-diabetes, anti-arrhythmia, anti-platelet, anti-thrombosis, neuroprotective, anti-amnesia, anti-anxiety, and other properties[55–58]. Neferine anti-tumor research has been on the rise in recent years. Isoliensinine and liensinine have notable pharmacological actions. Isoliensinine provides several health benefits, including anti-tumor, heart protection, anti-oxidation, anti-depression, anti-HIV, and anti-Alzheimer's disease[14,15].

BIAs biosynthesis

The latest research progress and difficulties of BIAs biosynthesis

Because of the monophyletic evolution of BIAs biosynthesis in angiosperms, the selection of genes related to BIAs biosynthesis in sacred lotus can be guided by the opium poppy BIAs metabolic pathway. Hence, it is anticipated that the biosynthetic route of benzylisoquinoline alkaloids (BIAs) in the sacred lotus involves the condensation of dopamine and 4-hydroxyphenylacetaldehyde (4-HPAA) catalysed by NCS, followed by the enzymatic conversion of (*R,S*)-norcoclaurine into various substituted 1-benzylisoquinoline, protoaporphine, aporphine, and bisbenzylisoquinoline alkaloids. This conversion is facilitated by specific enzymes such as *O*-methyltransferase (OMT), *N*-methyltransferase (NMT), cytochrome P450 oxidoreductases (CYPs), and others, which belong to a restricted enzyme

Table 1. Benzylisoquinoline alkaloids (BIAs) were identified in several organs of *Nelumbo nucifera*, together with their respective chemical formulas and stereochemical properties. L, lotus leaf; E, lotus embryo; F, lotus flower; S, lotus seed; R, lotus rhizome.

No.	Alkaloid	Formula	Enantiomer	Organ	Reference
1-Benzylisoquinoline					
1	Norcoclaurine	$C_{16}H_{17}NO_3$	(+)-R and (−)-S	L, E	[27–30]
2	Coclaurine	$C_{17}H_{19}NO_3$	(+)-R	L, E, F	[27,29,31]
3	Norjuziphine	$C_{17}H_{19}NO_3$	NS	F	[32]
4	Isococlaurine	$C_{17}H_{19}NO_3$	NS	F	[33]
5	*N*-Methylcoclaurine	$C_{18}H_{21}NO_3$	(−)-R	L, E, F	[27,29,31]
6	6-Demethyl-4'-*O*-methyl-*N*-methylcoclaurine	$C_{18}H_{21}NO_3$	NS	E	[29]
7	Norarmepavine	$C_{18}H_{21}NO_3$	(+)-R	F	[31]
8	*N*-Methylisococlaurine	$C_{18}H_{21}NO_3$	NS	L, E	[29,34]
9	Norroefractine	$C_{18}H_{21}NO_3$	NS	F	[33]
10	Juziphine	$C_{17}H_{19}NO_3$	NS	F	[33]
11	Armepavine	$C_{19}H_{23}NO_3$	(−)-R and (+)-S	L, E, S	[29,31,35,36]
12	4'-*O*-Methyl-*N*-methylcoclaurine	$C_{19}H_{23}NO_3$	NS	E	[29]
13	Lotusine	$C_{19}H_{24}NO_3^+$	NS	E	[29]
14	Isolotusine	$C_{19}H_{24}NO_3^+$	NS	E	[29]
15	4'-*O*-Methylarmepavine	$C_{20}H_{25}NO_3$	NS	L	[37]
Aporphine					
16	Caaverine	$C_{17}H_{17}NO_2$	(−)-R	L	[35,38]
17	Asimilobine	$C_{17}H_{17}NO_2$	(−)-R	L, F	[31,38,39]
18	Glaziovine	$C_{18}H_{19}NO_3$	N/A	F	[33]
19	*O*-Nornuciferine	$C_{18}H_{19}NO_2$	(−)-R	L, F	[13,38,40]
20	*N*-Nornuciferine	$C_{18}H_{19}NO_2$	(−)-R	L, E, F	[13,29,38]
21	Lirinidine	$C_{18}H_{19}NO_2$	(−)-R	L, F	[13]
22	*N*-Methylasimilobine	$C_{18}H_{19}NO_2$	N/A	F	[32]
23	Roemerine	$C_{18}H_{17}NO_2$	(−)-R	L, F	[38,40–42]
24	Dehydronuciferine	$C_{19}H_{19}NO_2$	N/A	L, R	[13,34,41]
25	Dehydroanonaine	$C_{17}H_{13}NO_2$	N/A	L	[34]
26	Dehydroroemerine	$C_{18}H_{15}NO_2$	N/A	L	[34]
27	Pronuciferine	$C_{19}H_{21}NO_3$	(+)-R and (−)-S	L, E, F	[13,29,35,37]
28	Nuciferine	$C_{19}H_{21}NO_2$	(−)-R	L, E, F	[29,31,38,40]
29	7-Hydroxydehydronuciferine	$C_{19}H_{19}NO_3$	N/A	L	[38]
30	Lysicamine	$C_{18}H_{13}NO_3$	N/A	L, F	[13]
31	Cepharadione B	$C_{19}H_{15}NO_4$	N/A	L	[32]
32	Anonaine	$C_{17}H_{15}NO_2$	(−)-R	L, F	[38,41]
33	Liriodenine	$C_{17}H_9NO_3$	N/A	L	[38]
Bisbenzylisoquinoline					
34	Nelumboferine	$C_{36}H_{40}N_2O_6$	NS	E, S	[41,43]
35	Liensinine	$C_{37}H_{42}N_2O_6$	1R,1'R	L, E, F, S	[39–41,44]
36	Isoliensinine	$C_{37}H_{42}N_2O_6$	1R,1'S	E	[40,44]
37	Dauriciline	$C_{36}H_{40}N_2O_6$	NS	S	[5]
38	6-Hydroxynorisoliensinine	$C_{36}H_{40}N_2O_6$	NS	E	[29]
39	*N*-Norisoliensinine	$C_{36}H_{40}N_2O_6$	NS	E	[29]
40	Nelumborine	$C_{36}H_{40}N_2O_6$	NS	E	[43]
41	Dauricinoline	$C_{37}H_{42}N_2O_6$	NS	S	[5]
42	Neferine	$C_{38}H_{44}N_2O_6$	1R,1'S	E, S	[40,41,44]
43	Dauricine	$C_{38}H_{44}N_2O_6$	NS	S, R	[45]
Tribenzylisoquinoline					
1	Neoliensinine	$C_{63}H_{70}N_3O_{10}$	1R,1'S,1''R	E	[44]

family[59]. In contrast to the preponderance of *S*-conformational BIAs in Ranunculales, the majority of BIAs observed in sacred lotus are *R*-conformational. As a result of the presence of this anomalous stereochemistry in sacred lotus, it is possible that the biosynthesis of sacred lotus will contain unique pathways or homologous enzymes. The most recent study conducted by Menéndez-Perdomo and J. Facchini has provided further validation that dopamine and 4-HPAA, both derived from *L*-tyrosine, serve as the precursors for the synthesis of (*R,S*)-norcoclaurine in the sacred lotus plant. Conversely, it was observed that in other plant species, the production of (*R*)-norcoclaurine by-products was predominantly favoured due to the presence of *R*-enantiospecific methyltransferase and CYPs. The presence of these enzymes has been shown to have a role in the synthesis of diverse 1-benzylisoquinolines inside the sacred lotus plant. The study also shown that the enzymes accountable for the production of *R*-enantiomers of pre-aporphine (*Nn*CYP80Q1) and bisbenzylisoquinoline (*Nn*CYP80Q2), as well as the incorporation of methylenedioxy bridges on the aporphine substrate (*Nn*CYP719A22), exhibit identical characteristics[59].

Nevertheless, there are still unresolved matters pertaining to the examination of the biosynthetic pathway of the sacred lotus' benzylisoquinoline alkaloids (BIAs). Firstly, it is observed that BIAs mostly occur in *R*-enantiomers, whereas *S*-enantiomers are more prevalent in the order Ranunculales. The enantioselective synthesis of (*S*)-norcoclaurine has been facilitated by NCS catalysis. However, it has been shown that both (*R*)-norcoclaurine and (*S*)-norcoclaurine are present in sacred lotus, suggesting the presence of diastereoselective enzymes or two distinct NCS orthologs that selectively favour either the R or S enantiomer[59]. Secondly, it was observed that no benzylisoquinoline alkaloids (BIAs) bearing a 3'-hydroxyl group were detected in the benzyl moiety. This absence may be attributed to the absence of the NMCH enzyme. This suggests that *N*-methylcoclaurine serves as a pivotal intermediary in the production of proaporphine, aporphine, and bisbenzylisoquinoline alkaloids in the sacred lotus[59]. Thirdly, in contrast to the direct conversion of (*S*)-reticuline bases to aporphine alkaloids through C-C and C-O coupling in plants of the order Ranunculales, the appearance of proaporphine in the sacred lotus plant indicated that 1-benzylisoquinoline substrates were indirectly transformed into apophine in the absence of ortho- or para- substituents in the phenyl moiety[59]. Fourthly, it was shown that bisbenzylisoquinolines exist as head-to-tail dimers in the sacred lotus, but only tail-to-tail couplings were detected in plants belonging to the Ranunculales order. The occurrence of aporphine and bisbenzylisoquinoline alkaloids in Ranunculales plants is attributed to the intramolecular C-C and intermolecular C-O coupling of BIAs. These coupling reactions are facilitated by enzymes belonging to the CYP80 family. The major aporphine alkaloid found in sacred lotus has an isoquinoline component that is characterised by the presence of a methylenedioxy bridge. The production of protoberberine in Ranunculales involves the participation of many enzymes belonging to the CYP719A subfamily, which catalyse the formation of methylenedioxy bridges. Notably, this biosynthetic pathway is absent in the sacred lotus plant[59].

Possible biosynthetic pathways

According to the study of BIAs biosynthesis, the possible BIAs biosynthesis pathway of sacred lotus can be obtained, which is mainly divided into three parts (Fig. 1). Firstly, the common biosynthetic pathway of sacred lotus and Ranunculales species is the Pictet-Spengler condensation of two *L*-tyrosine derivatives dopamine and 4-hydroxyphenylacetaldehyde (4-HPAA) to produce (*R,S*) -norcoclaurine. It was catalyzed by *Nn*OMT1 (6-*O*-methyltransferase) to generate coclaurine, which was then catalyzed by *N*-methyltransferase to generate *N*-methylcoclaurine. It also served as a central branch point for the biosynthesis of various BIAs. *N*-methylcoclaurine was catalyzed by cytochrome P450 monooxygenase 80B to generate 3'-hydroxy-*N*-methylcoclaurine, and it was catalyzed by 4'-*O*-methyltransferase to generate reticuline. Secondly, the synthesis pathway of bisbenzylisoquinoline alkaloids in sacred lotus heart and aporphine alkaloids in sacred lotus leaves: *N*-methylcoclaurine was catalyzed by *Nn*CYP80Q1 in sacred lotus to nelumboferine (bisbenzylisoquinoline alkaloids). *N*-methylcoclaurine is catalyzed by *Nn*CYP80Q2 to *N*-methylcrotsparine (proaporphine), which may generate lirinidine through reduction, dehydration and aromatic ring rearrangement, and generate

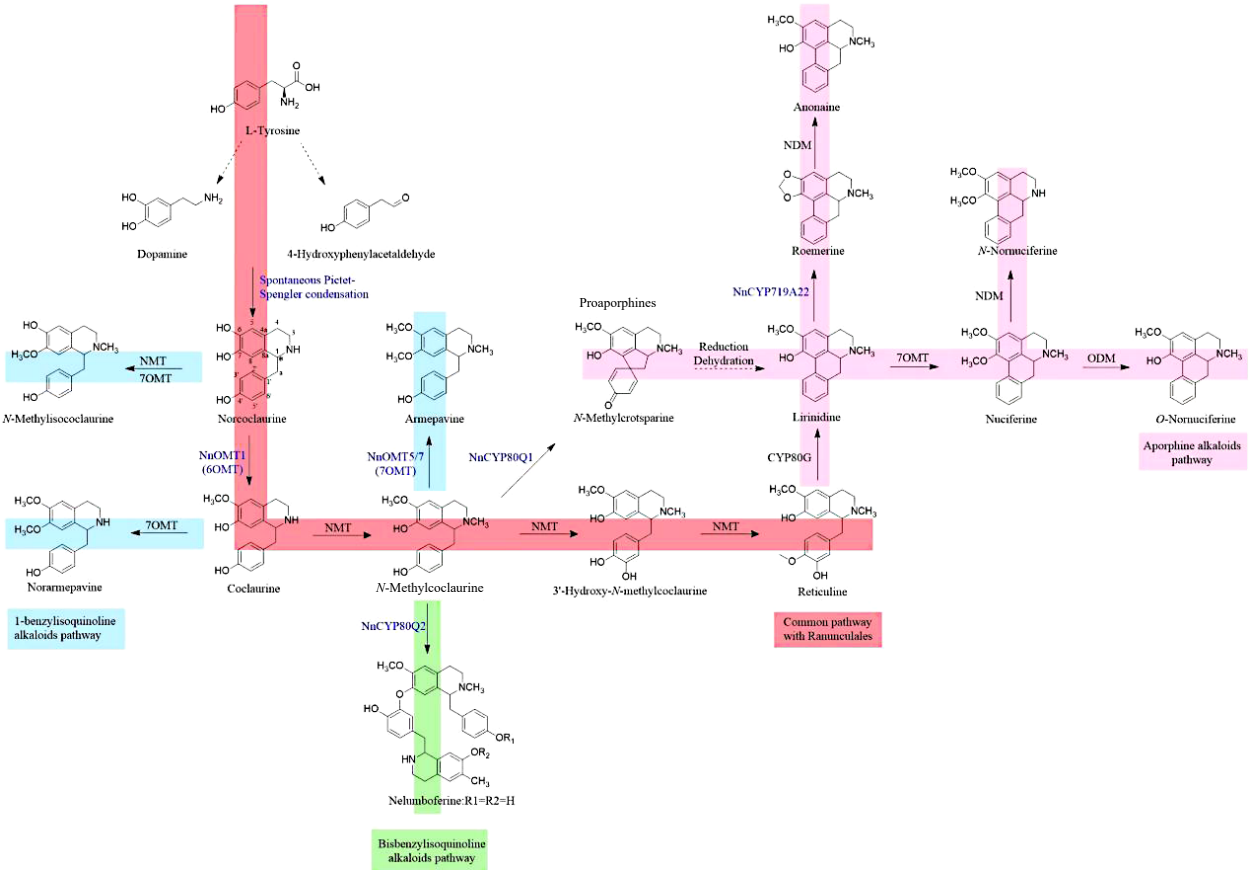

Fig. 1 Possible biosynthetic pathways of BIAs in sacred lotus.

anonaine under the action of *Nn*CYP719A22. In addition, it is speculated that the aporphine alkaloids in sacred lotus may also come from reticuline and generate various aporphine alkaloids under the catalysis of CYP80G, 7OMT, ODM, NDM and other enzymes. Thirdly, the synthesis of 1-benzylisoquinoline alkaloids, *N*-methylcoclaurine was catalyzed by *Nn*OMT5 / 7 (7OMT) to armepavine.

BIA biosynthetic genes and enzymes in the sacred lotus

Norcoclaurine synthase

The enzymatic pathway leading to the surprising diversity of benzylisoquinoline derivatives has been shown to originate from a common route, in which the first step is the NCS-catalyzed PictetSpengler condensation of dopamine with (4-HPAA) to produce (*S*)-norcoclaurine[60,61]. However, (*R*)- and (*S*)-norcoclaurine were both detected in sacred lotus. NCS selectively catalyzed the formation of (*S*)-norcoclaurine, which indicated that there may be diastereoselective enzymes or two different *R*- and *S*-enantiomerically selective NCS orthologs[59]. Recently, the study of Menéndez-Perdomo & Facchini proposed a new possibility that the formation of (*R*)- and (*S*)-desmethylhengzhouaconitine in lotus is a spontaneous, non-enzymatic Pictet-Spengler condensation reaction of dopamine and 4-HPAA[59]. It can be seen that the study of NCS in lotus is of great significance to the interpretation of lotus-specific R configuration, and the study of NCS needs to be further promoted.

Methyltransferases

Methylation, a frequent biological change in plants, plays an important role in the structural and functional diversity of BIAs. By adding methyl groups, BIAs' chemical characteristics, including as steric effects, overall hydrophobicity, and electronic properties, can be altered, resulting in a shift in biological activity. Methylation processes known as methyltransferases employed *S*-adenosyl-*L*-methionine as a methyl donor[62]. The widespread terminal alteration on BIAs of sacred lotus by methyltransferases, including *O*-methylation and *N*-methylation, is also a source of its variety.

OMT

So far, OMTs in sacred lotus have largely been studied in terms of gene expression, with little functional characterisation of the encoded proteins[63–65]. Despite the fact that BIAs were largely active metabolites in *N. nucifera*, only three OMTs engaged in the 1-BIA upstream biosynthetic pathway in *N. nucifera* were discovered *in vitro*[66]. Two OMTs implicated in BIA metabolism in sacred lotus, which catalysed the 6-*O* and 7-*O*-methylation of the 1-benzylisoquinoline backbone, have been functionally characterised. In sacred lotus, the 1-benzylisoquinoline backbone was mostly *O*-methylated at the C6, C7, and/or C4' locations, yielding a range of 1-benzylisoquinoline alkaloid compounds[66]. Our lab discovered a new and regiospecific *O*-methyltransferase (*Nn*OMT6) that methylated monobenzylisoquinoline 6-*O*/7-*O*, aporphine skeleton 6-*O*, phenylpropanoid 3-*O*, and protoberberine 2-*O*[67]. Monobenzylisoquinoline was converted into aporphine and bisbenzylisoquinoline alkaloids in sacred lotus. However, no reports of OMTs catalysing the aporphine and bisbenzylisoquinoline backbones in sacred lotus have been found.

NMT

It is unknown how BIAs are *N*-methylated in sacred lotus. According to chemical structural suggestions, the N position occurred in once or twice methylation to form tertiary amine or quaternary amine (e.g., *N*-methylcoclaurine and lotusine). Based on transcriptome analysis, two *N*-methyltransferases, *Nn*CNMT1 and *Nn*CNMT2, were identified from sacred lotus[68]. However, the role of the *N*-methyltransferase involved in the production of BIAs in sacred lotus has not yet been determined. It is critical to identify the NMT in the biosynthesis of BIAs.

Cytochrome P450 monooxygenases

Cytochrome P450 monooxygenases (CYPs) include a heterogeneous collection of heme proteins that facilitate a multitude of reactions within plant-specific metabolic pathways. NADPH-cytochrome P450 reductase, an enzyme responsible for transferring a pair of electrons from NADPH, facilitates the activation of these enzymes[69]. The formation of sacred lotus benzylisoquinoline alkaloids (BIA) is believed to be influenced by two primary cytochrome P450 (CYP) families, namely CYP80 (subfamilies A and G) and CYP719A[69,70].

Menéndez-Perdomo & Facchini's most recent study characterised the functions of *Nn*CYP80Q1, *Nn*CYP80Q2, and *Nn*CYP719A22, which were responsible for the formation of pre-aporphine *R*-enantiomers, dibenzylisoquinoline *R*-enantiomers, and the formation of methylenedioxy bridges on the aporphine substrate[59]. Based on predictions, the catalytic mechanism of cytochrome P450 enzymes (CYPs) involves several key reactions. Firstly, an intramolecular C-C phenol coupling occurs between the C8 and C1' positions of 1-benzylisoquinoline substrates, resulting in the formation of the corresponding pro-aporphine compound. Additionally, an intermolecular head-to-tail C-O phenol coupling reaction takes place between the C7-hydroxyl and C3' positions of two 1-benzylisoquinoline substrates, leading to the production of the corresponding bisbenzylisoquinoline compound. Furthermore, the oxidative cyclisation of the ortho-hydroxyl group of the isoquinoline moiety in the aporphine substrate, along with the methoxy-substituted aromatic ring, results in the formation of a methylenedioxy bridge[59].

Conclusions

The extraordinary therapeutic potential of BIAs is one of the reasons why they have garnered so much interest. In contrast to the *S*-conformation seen in Ranunculaceae, the sacred lotus, which belongs to an ancient group of aquatic basal plants, has an exceptionally high number of BIAs that have an *R*-conformation. The investigation of the *in vitro* synthesis and the pharmacological efficacy of BIAs will be helped along by the discovery of important genes and functional enzymes connected to the BIAs biosynthesis.

Author contributions

The authors confirm contribution to the paper as follows: conceptualization and supervision: Chen S; draft manuscript and figure preparation: Chen Z; manuscript review and editing: Zhao H. All authors reviewed and approved the final version of the manuscript.

Data availability

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

Conflict of interest

The authors declare that they have no conflict of interest.

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References

1. Li Z, Liu X, Gituru RW, Juntawong N, Zhou M, et al. 2010. Genetic diversity and classification of *Nelumbo* germplasm of different origins by RAPD and ISSR analysis. *Scientia Horticulturae* 125:724−32
2. Zhang Y, Lu X, Zeng S, Huang X, Guo Z, et al. 2015. Nutritional composition, physiological functions and processing of lotus (*Nelumbo nucifera* Gaertn.) seeds: A review. *Phytochemistry Reviews* 14:321−34
3. Limwachiranon J, Huang H, Shi Z, Li L, Luo Z. 2018. Lotus flavonoids and phenolic acids: Health promotion and safe consumption dosages. *Comprehensive Reviews in Food Science and Food Safety* 17:458−71
4. Hu P, Ge X, Gao MT, Wang XZ, Zhang YY, et al. 2022. *Nelumbo nucifera* Gaertn: An updated review of the antitumor activity and mechanisms of alkaloids. *Pharmacological Research-Modern Chinese Medicine* 5:100167
5. Pei H, Su W, Gui M, Dou M, Zhang Y, et al. 2021. Comparative analysis of chemical constituents in different parts of lotus by UPLC and QToF-MS. *Molecules* 26:1855
6. National Pharmacopoeia Committee. (Eds.) 2020. *Pharmacopoeia of the People's Republic of China*. Beijing: China Pharmaceutical Science and Technology Press. pp. 285−87
7. Chen S, Li X, Wu J, Li J, Xiao M, et al. 2021. Plumula Nelumbinis: A review of traditional uses, phytochemistry, pharmacology, pharmacokinetics and safety. *Journal of Ethnopharmacology* 266:113429
8. Lin S, Wang Z, Lin Y, Ge S, Hamzah SS, et al. 2019. Bound phenolics from fresh lotus seeds exert anti-obesity effects in 3T3-L1 adipocytes and high-fat diet-fed mice by activation of AMPK. *Journal of Functional Foods* 58:74−84
9. Wang Z, Hu J, Hamzah SS, Ge S, Lin Y, et al. 2019. *n*-Butanol extract of lotus seeds exerts antiobesity effects in 3T3-L1 preadipocytes and high-fat diet-fed mice via activating adenosine monophosphate-activated protein kinase. *Journal of Agricultural and Food Chemistry* 67:1092−103
10. Ziegler J, Facchini PJ. 2008. Alkaloid biosynthesis: Metabolism and trafficking. *Annual Review of Plant Biology* 59:735−69
11. Hudzik TJ, Patel M, Brown A. 2021. β2-Adrenoceptor agonist activity of higenamine. *Drug Testing And Analysis* 13:261−67
12. Wen J, Li M, Zhang W, Wang H, Bai Y, et al. 2022. Role of higenamine in heart diseases: A mini-review. *Frontiers in Pharmacology* 12:798495
13. Nakamura S, Nakashima S, Tanabe G, Oda Y, Yokota N, et al. 2013. Alkaloid constituents from flower buds and leaves of sacred lotus (*Nelumbo nucifera*, Nymphaeaceae) with melanogenesis inhibitory activity in B16 melanoma cells. *Bioorganic & Medicinal Chemistry* 21:779−87
14. Bharathi Priya L, Huang CY, Hu RM, Balasubramanian B, Baskaran R. 2021. An updated review on pharmacological properties of neferine-A bisbenzylisoquinoline alkaloid from *Nelumbo nucifera*. *Journal of Food Biochemistry* 45:e13986
15. Cheng Y, Li HL, Zhou ZW, Long HZ, Luo HY, et al. 2021. Isoliensinine: A natural compound with "drug-like" potential. *Frontiers in Pharmacology* 12:630385
16. He CL, Huang LY, Wang K, Gu CJ, Hu J, et al. 2021. Identification of bis-benzylisoquinoline alkaloids as SARS-CoV-2 entry inhibitors from a library of natural products. *Signal Transduction and Targeted Therapy* 6:131
17. Bai X, Liu X, Li S, An H, Kang X, et al. 2022. Nuciferine Inhibits TMEM16A in Dietary Adjuvant Therapy for Lung Cancer. *Journal of Agricultural and Food Chemistry* 70:3687−96
18. Kang EJ, Lee SK, Park KK, Son SH, Kim KR, et al. 2017. Liensinine and nuciferine, bioactive components of *Nelumbo nucifera*, inhibit the growth of breast cancer cells and breast cancer-associated bone loss. *Evidence-based Complementary and Alternative Medicine* 2017:1583185
19. Wan Y, Xia J, Xu JF, Chen L, Yang Y, et al. 2022. Nuciferine, an active ingredient derived from lotus leaf, lights up the way for the potential treatment of obesity and obesity-related diseases. *Pharmacological Research* 175:106002
20. Zhang L, Gao J, Tang P, Chong L, Liu Y, et al. 2018. Nuciferine inhibits LPS-induced inflammatory response in BV2 cells by activating PPAR-γ. *International Immunopharmacology* 63:9−13
21. Singh A, Menéndez-Perdomo IM, Facchini PJ. 2019. Benzylisoquinoline alkaloid biosynthesis in opium poppy: An update. *Phytochemistry Reviews* 18:1457−82
22. Stadler R, Kutchan TM, Zenk MH. 1989. (*S*)-Norcoclaurine is the central intermediate in benzylisoquinoline alkaloid biosynthesis. *Phytochemistry* 28:1083−86
23. Minami H, Dubouzet E, Iwasa K, Sato F. 2007. Functional analysis of norcoclaurine synthase in Coptis japonica. *Journal of Biological Chemistry* 282:6274−82
24. Lee EJ, Facchini P. 2010. Norcoclaurine synthase is a member of the pathogenesis-related 10/Bet v1 protein family. *The Plant Cell* 22:3489−3503
25. Li J, Lee EJ, Chang L, Facchini PJ. 2016. Genes encoding norcoclaurine synthase occur as tandem fusions in the Papaveraceae. *Scientific Reports* 6:39256
26. Sheng X, Himo F. 2019. Enzymatic Pictet-Spengler reaction: Computational study of the mechanism and enantioselectivity of norcoclaurine synthase. *Journal of The American Chemical Society* 141:11230−38
27. Kashiwada Y, Aoshima A, Ikeshiro Y, Chen YP, Furukawa H, et al. 2005. Anti-HIV benzylisoquinoline alkaloids and flavonoids from the leaves of *Nelumbo nucifera*, and structure-activity correlations with related alkaloids. *Bioorganic & Medicinal Chemistry* 13:443−48
28. Koshiyama H, Ohkuma H, Kawaguchi H, Hsu H, Chen Y. 1970. Isolation of 1-(p-hydroxybenzyl)-6,7-dihydroxy-1 2,3,4-tetrahydroisoquinoline (demethylcoclaurine), an active alkaloid from *Nelumbo nucifera*. *Chemical and Pharmaceutical Bulletin* 18:2564−68
29. Lin Z, Yang R, Guan Z, Chen A, Li W. 2014. Ultra-performance LC separation and quadrupole time-of-flight MS identification of major alkaloids in plumula nelumbinis. *Phytochemical Analysis* 25:485−94
30. Hong HX, Lee YI, Jin DR. 2010. Determination of *R*-(+)-higenamine enantiomer in *Nelumbo nucifera* by high-performance liquid chromatography with a fluorescent chiral tagging reagent. *Microchemical Journal* 96:374−79
31. Morikawa T, Kitagawa N, Tanabe G, Ninomiya K, Okugawa S, et al. 2016. Quantitative determination of alkaloids in lotus flower (flower buds of *Nelumbo nucifera*) and their melanogenesis inhibitory activity. *Molecules* 21:930
32. Wang Z, Li Y, Ma D, Zeng M, Wang Z, et al. 2021. Alkaloids from lotus (*Nelumbo nucifera*): recent advances in biosynthesis, pharmacokinetics, bioactivity, safety, and industrial applications. *Critical Reviews in Food Science and Nutrition* 30:4867−900
33. Maneenet J, Omar AM, Sun S, Kim MJ, Daodee S, et al. 2021. Benzylisoquinoline alkaloids from *Nelumbo nucifera* Gaertn. petals with antiausterity activities against the HeLa human cervical cancer cell line. *Zeitschrift Fur Naturforschung Section C* 76:401−6

34. Kunitomo J, Yoshikawa Y, Tanaka S, Imori Y, Isoi K, et al. 1973. Alkaloids of *Nelumbo nucifera*. *Phytochemistry* 12:699−701
35. Do TCMV, Nguyen TD, Tran H, Stuppner H, Ganzera M. 2013. Analysis of alkaloids in Lotus (*Nelumbo nucifera* Gaertn.) leaves by nonaqueous capillary electrophoresis using ultraviolet and mass spectrometric detection. *Journal of Chromatography A* 1302:174−80
36. Ka SM, Kuo YC, Ho PJ, Tsai PY, Hsu YJ, et al. 2010. (*S*)-armepavine from Chinese medicine improves experimental autoimmune crescentic glomerulonephritis. *Rheumatology* 49:1840−51
37. Guo Y, Chen X, Qi J, Yu B. 2016. Simultaneous qualitative and quantitative analysis of flavonoids and alkaloids from the leaves of *Nelumbo nucifera* Gaertn. using high-performance liquid chromatography with quadrupole time-of-flight mass spectrometry. *Journal of Separation Science* 39:2499−507
38. Liu CM, Kao CL, Wu HM, Li WJ, Huang CT, et al. 2014. Antioxidant and anticancer aporphine alkaloids from the leaves of *Nelumbo nucifera* Gaertn. cv. *Rosa-plena*. *Molecules* 19:17829−38
39. Grienke U, Mair CE, Saxena P, Baburin I, Scheel O, et al. 2015. Human ether-à-go-go related gene (hERG) channel blocking aporphine alkaloids from lotus leaves and their quantitative analysis in dietary weight loss supplements. *Journal of Agricultural and Food Chemistry* 63:5634−39
40. Zhou M, Jiang M, Ying X, Cui Q, Han Y, et al. 2013. Identification and comparison of anti-inflammatory ingredients from different organs of *Lotus nelumbo* by UPLC/Q-TOF and PCA coupled with a NF-κB reporter gene assay. *PLoS ONE* 8:81971
41. Deng X, Zhu L, Fang T, Vimolmangkang S, Yang D, et al. 2016. Analysis of isoquinoline alkaloid composition and wound-induced variation in *Nelumbo* using HPLC-MS/MS. *Journal of Agricultural and Food Chemistry* 64:1130−36
42. Agnihotri VK, ElSohly HN, Khan SI, Jacob MR, Joshi VC, et al. 2008. Constituents of *Nelumbo nucifera* leaves and their antimalarial and antifungal activity. *Phytochemistry Letters* 1:89−93
43. Itoh A, Saitoh T, Tani K, Uchigaki M, Sugimoto Y, et al. 2011. Bisbenzylisoquinoline alkaloids from *Nelumbo nucifera*. *Chemical Pharmaceutical Bulletin* 59:947−51
44. Yang GM, Sun J, Pan Y, Zhang JL, Xiao M, et al. 2018. Isolation and identification of a tribenzylisoquinoline alkaloid from *Nelumbo nucifera* Gaertn, a novel potential smooth muscle relaxant. *Fitoterapia* 124:58−65
45. Zhao X, Shen J, Chang KJ, Kim SH. 2014. Comparative analysis of antioxidant activity and functional components of the ethanol extract of lotus (*Nelumbo nucifera*) from various growing regions. *Journal of Agricultural and Food Chemistry* 62:6227−35
46. Khan S, Khan HU, Khan FA, Shah A, Wadood A, et al. 2022. Anti-Alzheimer and antioxidant effects of *Nelumbo nucifera* L. alkaloids, nuciferine and norcoclaurine in alloxan-Induced diabetic albino rats. *Pharmaceuticals* 15:1205
47. Liu CP, Tsai WJ, Shen CC, Lin YL, Liao JF, et al. 2006. Inhibition of (*S*)-armepavine from Nelumbo nucifera on autoimmune disease of MRL/MpJ-*lpr*/*lpr* mice. *European Journal Of Pharmacology* 531:270−79
48. Xu J, Zhang X, Yan L, Zhang Z, Wei J, et al. 2022. Insight into Lotusine and Puerarin in Repairing Alcohol-Induced Metabolic Disorder Based on UPLC-MS/MS. *International Journal of Molecular Sciences* 23:10385
49. Ryu TK, Roh E, Shin HS, Kim JE. 2022. Inhibitory effect of lotusine on solar UV-induced matrix metalloproteinase-1 expression. *Plants* 11:773
50. Yu Y, Lu J, Sun L, Lyu X, Chang XY, et al. 2021. *Akkermansia muciniphila*: A potential novel mechanism of nuciferine to improve hyperlipidemia. *Biomedicine & Pharmacotherapy* 133:111014
51. Pan Y, Cai B, Wang K, Wang S, Zhou S, et al. 2009. Neferine enhances insulin sensitivity in insulin resistant rats. *Journal of Ethnopharmacology* 124:98−102
52. Xiao M, Xian C, Wang Y, Qi X, Zhang R, et al. 2023. Nuciferine attenuates atherosclerosis by regulating the proliferation and migration of VSMCs through the Calm4/MMP12/AKT pathway in ApoE$^{(-/-)}$ mice fed with High-Fat-Diet. *Phytomedicine* 108:154536
53. Yang ZD, Zhang X, Du J, Ma ZJ, Guo F, et al. 2012. An aporphine alkaloid from *Nelumbo nucifera* as an acetylcholinesterase inhibitor and the primary investigation for structure-activity correlations. *Natural Product Research* 26:387−92
54. Yano M, Nakashima S, Oda Y, Nakamura S, Matsuda H. 2020. BBB-permeable aporphine-type alkaloids in *Nelumbo nucifera* flowers with accelerative effects on neurite outgrowth in PC-12 cells. *Journal of Natural Medicines* 74:212−18
55. Sengking J, Oka C, Yawoot N, Tocharus J, Chaichompoo W, et al. 2022. Protective effect of neferine in permanent cerebral ischemic rats via anti-oxidative and anti-apoptotic mechanisms. *Neurotoxicity Research* 40:1348−59
56. Lin TY, Hung CY, Chiu KM, Lee MY, Lu CW, et al. 2022. Neferine, an alkaloid from lotus seed embryos, exerts antiseizure and neuroprotective effects in a kainic acid-induced seizure model in rats. *International Journal of Molecular Sciences* 23:4130
57. Zhong Y, He S, Huang K, Liang M. 2020. Neferine suppresses vascular endothelial inflammation by inhibiting the NF-κB signaling pathway. *Archives of Biochemistry and Biophysics* 696:108595
58. Poornima P, Weng CF, Padma VV. 2014. Neferine, an alkaloid from lotus seed embryo, inhibits human lung cancer cell growth by MAPK activation and cell cycle arrest. *Biofactors* 40:121−31
59. Menéndez-Perdomo IM, Facchini PJ. 2023. Elucidation of the (*R*)-enantiospecific benzylisoquinoline alkaloid biosynthetic pathways in sacred lotus (*Nelumbo nucifera*). *Scientific Reports* 13:2955
60. Facchini PJ, St-Pierre B. 2005. Synthesis and trafficking of alkaloid biosynthetic enzymes. *Current Opinion In Plant Biology* 8:657−66
61. Stadler R, Zenk MH. 1990. A revision of the generally accepted pathway for the biosynthesis of the benzyltetrahydroisoquinoline alkaloid reticuline. *Liebigs Annalen der Chemie* 6:555−62
62. Liscombe DK, Louie GV, Noel JP. 2012. Architectures, mechanisms and molecular evolution of natural product methyltransferases. *Natural Product Reports* 29:1238−50
63. Yang, M, Zhu L, Li L, Li J, Xu L, et al. 2017. Digital gene expression analysis provides insight into the transcript profile of the genes involved in aporphine alkaloid biosynthesis in lotus (*Nelumbo nucifera*). *Frontiers in Plant Science* 8:80
64. Meelaph T, Kobtrakul K, Chansilpa NN, Han Y, Rani D, et al. 2018. Coregulation of biosynthetic genes and transcription factors for aporphine-type alkaloid production in wounded lotus provides insight into the biosynthetic pathway of nuciferine. *ACS Omega* 3:8794−802
65. Deng X, Zhao L, Fang T, Xiong Y, Ogutu C, et al. 2018. Investigation of benzylisoquinoline alkaloid biosynthetic pathway and its transcriptional regulation in lotus. *Horticulture Research* 5:29
66. Menéndez-Perdomo IM, Facchini PJ. 2020. Isolation and characterization of two *O*-methyltransferases involved in benzylisoquinoline alkaloid biosynthesis in sacred lotus (*Nelumbo nucifera*). *Journal Of Biological Chemistry* 295:1598−612
67. Yu Y, Liu Y, Dong G, Jiang J, Leng L, et al. 2023. Functional characterization and key residues engineering of a regiopromiscuity *O*-methyltransferase involved in benzylisoquinoline alkaloid biosynthesis in *Nelumbo nucifera*. *Horticulture Research* 10:uhac276
68. Esau K, Kosakai H. 1975. Laticifers in *Nelumbo nucifera* Gaertn.: Distribution and structure. *Annals of Botany* 39:713−19
69. Nelson DR. 2009. The cytochrome P450 homepage. *Human Genomics* 4:59
70. Nelson DR, Schuler MA. 2013. Cytochrome P450 genes from the sacred lotus genome. *Tropical Plant Biology* 6:138−51