

REVIEW

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Harnessing genomic and molecular biology resources for genetic improvement of lotus: current achievements and future directions

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Abstract

Lotus, an economically significant aquatic crop within the genus *Nelumbo* (family *Nelumbonaceae*), includes the two extant species *Nelumbo nucifera* and *Nelumbo lutea*. Renowned for its ornamental, culinary, and medicinal value, lotus has seen notable advancements in genomics and molecular biology, particularly in reference genome sequencing and assembly, bioactive compound biosynthetic pathways, and molecular mechanisms underlying key traits. Critical genes linked to horticultural characteristics, edible quality, medicinal properties, and stress resilience have been identified and functionally validated. This review presents a thorough overview of recent molecular developments in lotus research, emphasizing genome sequencing and functional gene analysis. Additionally, it addresses prevailing challenges and future directions, providing valuable insights for foundational research and genetic enhancement of lotus.

Keywords *Nelumbo*, Lotus, Genome, Molecular biology, Molecular mechanism, Genetic improvement

Introduction

The lotus, a basal eudicot of substantial medicinal, cultural, and agricultural importance, has been cultivated extensively in China, which serves as a primary global distribution center and a prominent hub for lotus cultivation and scientific study. Revered as a significant horticultural ornament, the lotus flower is distinguished by its vivid colors and diverse flower shapes, offering high ornamental value (Song et al. 2024a). Beyond its aesthetic appeal, lotus is a key resource for food and medicine; its seeds and rhizomes are nutrient-rich and edible, while the leaves, flowers, seedpods, and plumules are packed with bioactive compounds like alkaloids, flavonoids, and polyphenols, which have broad medicinal applications. In Asia, lotus seeds have been traditionally used as both a vegetable and herbal medicine, especially within medical systems such as Ayurveda in India and traditional Chinese medicine (Bishayee et al. 2022). Furthermore, unique features such as the exceptional longevity of lotus seeds and the self-cleaning properties of lotus leaves continue to intrigue botanists (Wang 2023).

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In the past decade, lotus research has seen remarkable strides, including advancements in genome sequencing and assembly, quantitative trait loci (QTL) mapping for key traits, and the exploration of underlying molecular mechanisms. These scientific efforts enhance our understanding of the lotus’s complex biological attributes and support its genetic enhancement through molecular breeding approaches. This review provides a thorough summary of recent developments in lotus genetic resources, genomics, and the molecular mechanisms that regulate essential traits. Additionally, it discusses prevailing challenges and proposes future research directions,

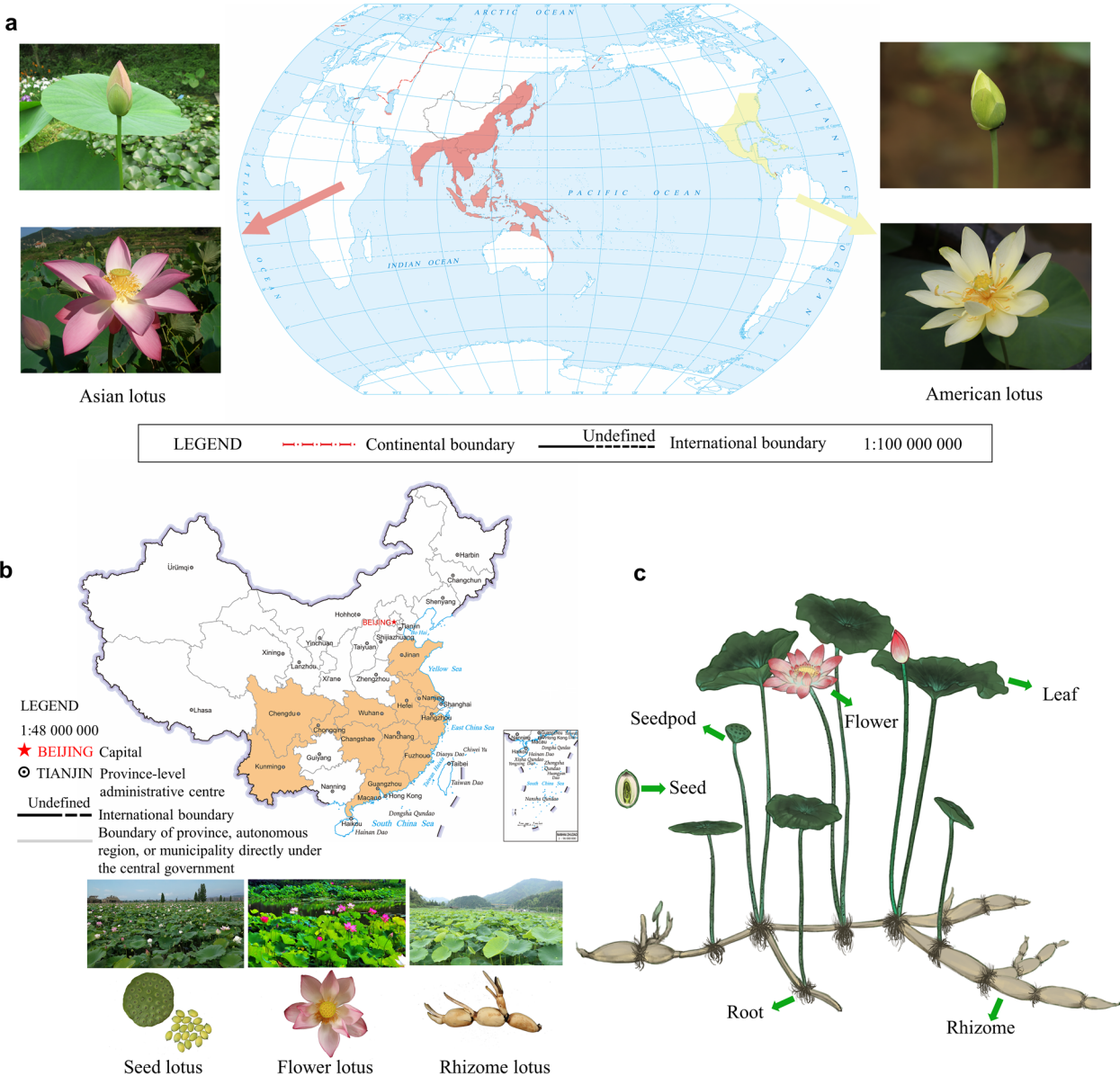


Fig. 1 Geographical distribution, classification, and structural characteristics of lotus. **a** Geographic distribution of lotus. The left and right images depict the buds and flowers of the Asian and American lotus, respectively. The red areas on the world map mark the range of Asian lotus, including China, Japan, Thailand, India, and northern Australia. Yellow regions indicate the range of the American lotus, covering North America, the Atlantic, and the Caribbean. This figure references the standard map with censor code: GS(2016)2962. **b** Distribution and classification of cultivated lotus species in China. The orange areas on China’s map denote the main lotus cultivation regions. Flower lotus varieties are cultivated primarily for ornamental purposes, valued for their wide array of flower shapes and colors. Seed lotus varieties are grown mainly for their seeds, while rhizome lotus varieties, with typically fewer flowers, are cultivated for their edible rhizomes. This figure references the standard map with censor code: GS(2019)1673. **c** Typical structural features of lotus, showcasing the leaf, flower, seedpod, seed, root, and rhizome

aiming to serve as a comprehensive reference for molecular research and potential applications in lotus breeding.

Systematic classification of lotus

Lotus exhibits an Asian-North American disjunctive distribution pattern (Fig. 1a), the genus *Nelumbo* encompasses two species throughout its evolutionary history: the Asian lotus (*N. nucifera*) and the American lotus (*N. lutea*) (Lin et al. 2019). Predominantly cultivated in Asia, the Asian lotus features a range of flower colors, including red, pink, white, and variegated varieties, and is distributed across numerous Asian regions (Fig. 1a). In contrast, the American lotus is characterized by a shorter stature, yellow flowers, and dark green, nearly circular leaves, and is primarily found in North America (Fig. 1a). Separated by the Pacific Ocean, the Asian and American lotus exhibit considerable genetic divergence, as shown by whole-genome resequencing analysis (Zheng et al. 2022a). Despite this genetic differentiation, reproductive isolation is absent, as both species share the same chromosome count of eight. In Asia, traditional hybridization breeding has led to the development of over a thousand lotus varieties (Zhu et al. 2019b).

The Asian lotus comprises two distinct ecological types: temperate and tropical (Yang et al. 2015). The temperate lotus thrives in subtropical and temperate regions with a clearly defined annual growth cycle, sprouting in spring, blooming over three months in summer, forming swollen rhizomes in autumn, and entering dormancy in winter. In contrast, the tropical lotus, found in tropical regions, lacks a dormant period, bearing evergreen leaves and supporting a flowering period of nearly five months, with rhizomes that do not swell (Gao et al. 2021). Asian cultivation of lotus has spanned thousands of years, leading to extensive artificial domestication and the selection of three main cultivation types: seed lotus, rhizome lotus, and flower lotus, each distinguished by specific morphological traits and applications (Fig. 1b) (Chen et al. 2023c; Song et al. 2024a). Population genetic analyses suggest multiple genetic origins, with potential matrilineal

introgression influencing Asian lotus domestication. Furthermore, the seed and rhizome lotus groups likely have a more complex multisource origin rather than a single ancestral source (Liu et al. 2020; Li et al. 2020; Wang et al. 2022). In China, lotus stands as the most widely cultivated aquatic economic crop, valued both for its medicinal and culinary uses, and holds substantial growth potential in the consumer market. It is distributed extensively across regions south of the Yangtze River (Fig. 1b). Nearly every part of the lotus, including flowers, leaves, seedpods, seeds, rhizomes, and roots, finds application as food or in traditional medicine (Fig. 1c).

Sequencing and assembly of lotus genomes

Lotus, a diploid organism ($2n=2x=16$) with a relatively small genome, exhibits unique gene duplication events. High-quality reference genomes are essential for identifying genes linked to key agronomic traits, accurately assessing germplasm resources, and developing molecular breeding systems. The first genome sequence of *Nelumbo*—the ‘China Antique’ cultivar—was published by Ming et al. (2013), revealing a genome size of 804 Mb with a contig N50 of 38.8 Kb (Table 1). In the same year, a genome map of another Chinese wild lotus, ‘Chinese Tai-zi,’ was completed, with a genome size of 792 Mb and a contig N50 of 39.3 Kb (Wang et al. 2013). These publications marked the onset of lotus genomics. Recent advancements in third-generation sequencing and high-throughput chromosome conformation capture (Hi-C) technologies have propelled the lotus genome to the chromosomal level. Utilizing BioNano optical mapping, Gui et al. (2018) anchored 97.9% of the ‘Chinese Tai-zi’ genome scaffolds to lotus’s eight chromosomes, identifying centromeric regions and increasing the scaffold N50 from 0.98 Mb to 1.48 Mb. Shi et al. (2020) achieved chromosome-level assembly of the ‘China Antique’ genome using PacBio long-read sequencing and Hi-C, resulting in a genome size of 807.6 Mb, with 799.7 Mb anchored to the eight chromosomes. In 2022, sequencing of the subspecies ‘Taikonglian No.3’ using Nanopore

Table 1 Comparative statistics of different versions of lotus genome assemblies

Variety	Sequencing technology	Genome size	Contig N50	Chromosome number	Busco	Protein-coding gene number	Reference
China Antique	Illumina	804 Mb	38.8 Kb			26,685	(Ming et al. 2013)
Chinese Tai-zi	Illumina	792 Mb	39.3 Kb			36,385	(Wang et al. 2013)
Chinese Tai-zi	BioNano, Hi-C	645 Mb	0.33 Mb	8	90.10%	30,378	(Gui et al. 2018)
China Antique	PacBio, Hi-C	808 Mb	484.3 Kb	8	94.60%	32,124	(Shi et al. 2020)
Taikonglian No.3	Nanopore, Hi-C	807 Mb	5.1 Mb	8	94.24%	28,274	(Zheng et al. 2022b)
<i>Nelumbo lutea</i>	PacBio, Hi-C	843 Mb	1.3 Mb	8	90.70%	31,382	(Zheng et al. 2022a)
China Antique	Falcon, AllHiC	817.9 Mb	44.31 Mb	8		27,981	(Qi et al. 2023)

technology provided further chromosomal-level refinement, enhancing the genome's utility for genetic variation and evolutionary studies (Zheng et al. 2022b). Additionally, Zheng et al. (2022a) completed the first assembly of the American lotus genome, totaling 843 Mb with PacBio and Hi-C technologies, which showed high collinearity with the Asian lotus genome but an increase in repetitive sequences. The divergence between Asian and American lotus species is estimated at around 13.86 million years ago. Qi et al. (2023) generated the highest-quality lotus reference genome to date, reassembling the lotus genome using 163.29 Gb of PacBio long reads with FALCON and ALLHiC, anchoring 98.7% of the data onto the eight chromosomes (Table 1). While these advancements provide invaluable resources for lotus research, further efforts are needed to close gaps in the current genome assemblies, which will be essential for supporting ongoing scientific investigations into lotus biology and improvement.

Molecular mechanisms of lotus flowering

Flowering represents a key biological milestone in the anthophyte life cycle, marking the transition from vegetative to reproductive growth. This shift from apical meristem to floral meristem is regulated by various meristem determinant genes that activate the expression of genes essential for floral organ development. Comparative transcriptome analysis of flower buds across developmental stages in temperate and tropical lotus has identified 147 flowering-related genes, including *constitutive photomorphogenesis 1* (*COP1*), *circadian clock associated 1* (*CCA1*), *late elongated hypocotyl* (*LHY*), and *constans-like* (*COL*) genes (Yang et al. 2014a). Molecular markers linked to initial and population flowering periods in lotus have also been developed through association analysis (Yang et al. 2014b). The florigen-encoding gene *FLOWERING LOCUS T* (*FT*) is pivotal in regulating flowering timing, ensuring prompt initiation. The interaction of NnFTIP1 (FT-INTERACTING PROTEIN1) with NnFT1 facilitates its transport from leaves to the shoot apical meristem (SAM), promoting early flowering in transgenic *Arabidopsis* (Zhang et al. 2021b). Overexpression of *NnFT* homologs, *NnFT2* and *NnFT3*, has been shown to rescue the late flowering phenotype in the *Arabidopsis ft-10* mutant, effectively inducing flowering (Song et al. 2024a). Additionally, abscisic acid (ABA) treatment enhances the inhibition of *NnSnRK1* expression, which helps reduce bud abortion in lotus (Cao et al. 2021b). Although significant progress has been made in elucidating flowering regulatory mechanisms in model plants, the complex genetic networks and pathways governing flowering in lotus remain largely uncharted, necessitating further in-depth research.

Molecular mechanisms of lotus flower color formation

Flower color significantly enhances the ornamental appeal of lotus, which typically displays shades of white, pink, and red, with occasional yellow flowers. Lotus petal coloration is closely linked to anthocyanin content, including key glycosides such as delphinidin, cyanidin, petunidin, and peonidin, though pelargonidin is absent. Red petals achieve their vibrant hues primarily through anthocyanins, with deeper tones reflecting higher anthocyanin concentrations. Yellow petals contain primarily carotenoids and flavonols, while white petals lack anthocyanins and are characterized by carotenoids and flavonols instead (Zheng et al. 2022a).

Anthocyanin biosynthesis in lotus petals follows a conserved pathway beginning with phenylalanine metabolism (Fig. 2a), regulated by transcription factors, chiefly R2R3-MYB, bHLH, and WD40, which function independently or form complexes to control key gene expression. To date, 116 R2R3-type MYB transcription factors have been identified in lotus, with several *MYB* genes implicated in anthocyanin biosynthesis (Deng et al. 2016a). Proteins such as NnMYB5, NnbHLH1, and NnTTG1 interact to modulate lotus flower color (Sun et al. 2016), while the bHLH transcription factor *NnTT8* plays a role in anthocyanin and proanthocyanidin biosynthesis, influencing petal coloration (Deng et al. 2021). Comparative studies between red-flowered Asian lotus and American lotus revealed three allelic variants of *NnMYB5*. In American lotus, two non-functional alleles result in premature translation termination in the second exon, preventing anthocyanin synthesis and accumulation. Transient expression of *NnMYB5* in white lotus petals induces anthocyanin accumulation, turning the petals red (Zheng et al. 2022a). Liu et al. (2023) recently elucidated the molecular mechanisms behind anthocyanin accumulation differences between red and white Asian lotus flowers (Fig. 2b). In red lotus, NnMYB5 activates *NnGST2*, facilitating anthocyanin transport from the endoplasmic reticulum to vacuoles, producing the red color. In white lotus, the absence of NnMYB5 prevents *NnGST2* activation, resulting in white flowers (Fig. 2b). Additionally, cis- and trans-regulatory modifications impact selective constraints and gene expression inheritance patterns in lotus petal color differentiation (Gao et al. 2023). While the regulatory mechanisms for red, white, and yellow lotus flowers are well-established, the factors influencing chimeric and composite petal colors remain uncertain. Expanding the color spectrum in lotus petals may require the integration of anthocyanin and other pigment biosynthesis pathways.

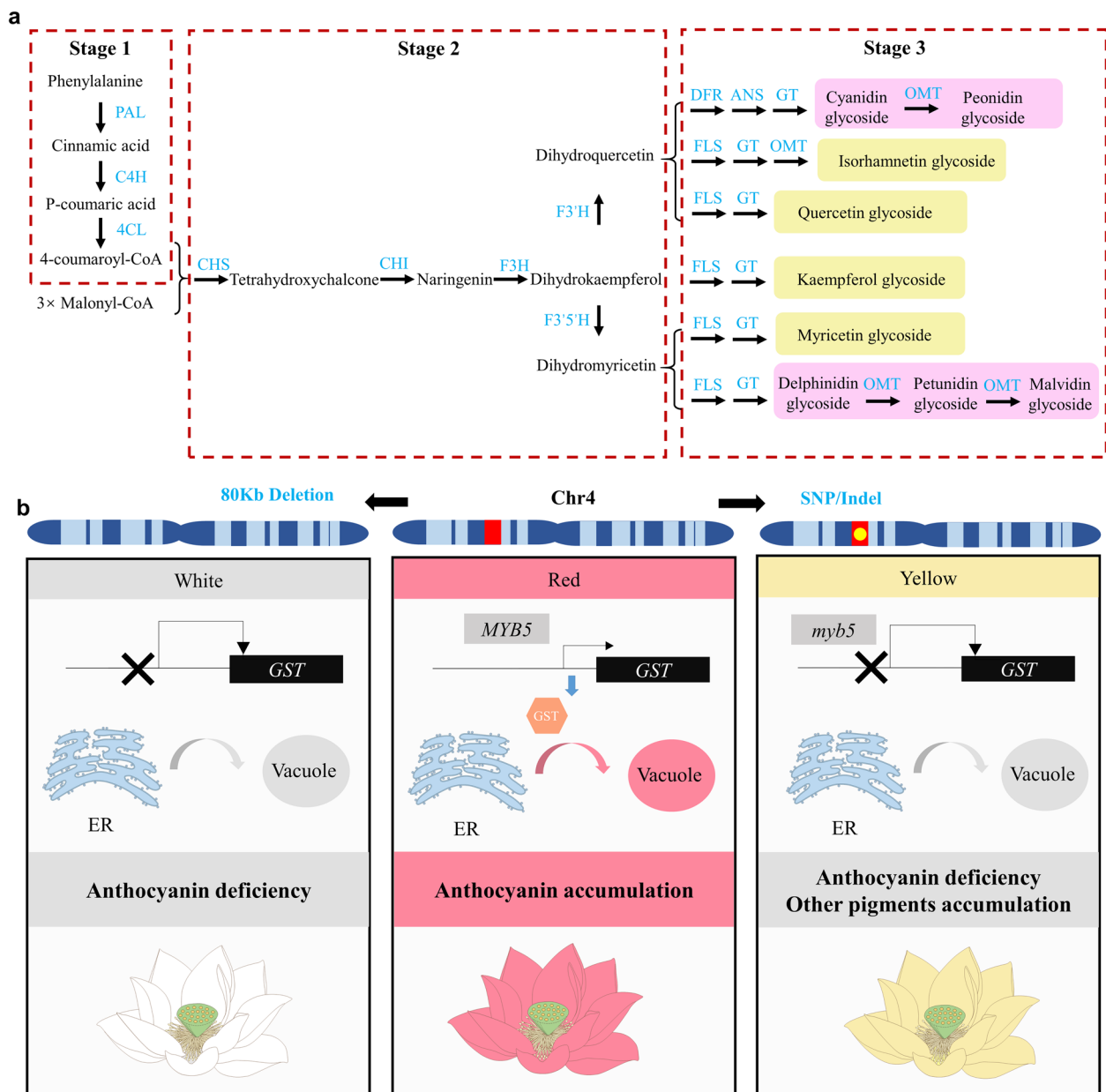


Fig. 2 Molecular model of lotus flower color formation. **a** Schematic diagram illustrating the anthocyanin biosynthesis pathway in lotus petals. PAL: Phenylalanine ammonia lyase; C4H: Cinnamate 4-hydroxylase; 4CL: 4-Coumaroyl CoA ligase; CHS: Chalcone synthase; CHI: Chalcone isomerase; F3H: Flavanone 3-hydroxylase; F3'H: Flavonoid 3'-hydroxylase; F3'5'H: Flavonoid 3'5'-hydroxylase; DFR: Dihydroflavonol reductase; FLS: Flavonol synthase; ANS: Anthocyanidin synthase; GT: Glycosyltransferases; OMT: O-Methyltransferase. **b** Genetic mechanisms underlying petal color variation in lotus. In red-flowered Asian lotus, MYB5 activates *GST2* transcription, enabling anthocyanin transport from the endoplasmic reticulum (ER) to vesicles. In white-flowered Asian lotus, an 80-kb presence/absence variant (PAV) deletion containing the functional *MYB5* gene results in *GST2* inactivation and pigment loss. In American lotus, SNPs within the *MYB5* exon cause protein truncations, preventing anthocyanin accumulation. Red boxes on Chr 4 mark the presence of *MYB5*, while the yellow circle indicates SNPs in *MYB5*. Black crosses represent the failure to activate *GST2*. *MYB5*: *v-myb avian myeloblastosis viral 5*; *GST2*: *Glutathione S-transferase 2*. The diagram is redrawn based on Liu et al. 2023

Molecular mechanisms of lotus flower shape formation

Lotus flowers are predominantly single-petaled, though variations in morphology are evident across different varieties, ranging from few-petaled to semi-double,

double, multi-staminate, and thousand-petaled forms. These morphological variations primarily result from the transformation of stamens and pistils into petal-like structures (Lin et al. 2020; Kubo et al. 2009). In

single-petaled lotus flowers, the perianth develops typically, with fewer than 20 segments, as observed in most wild varieties. Semi-double flowers exhibit partial stamen petalization, resulting in 21 to 50 perianth segments, while double flowers display extensive stamen petalization, often exceeding 50 segments. Multi-staminate flowers not only have petalized stamens but also include carpel structures within the pistil that resemble petals. Thousand-petaled forms involve the complete transformation of stamens and pistils into petal-like structures, with the receptacle absent, resulting in over 800 perianth segments.

Research on lotus flower morphology remains limited, and the mechanisms driving specialized petal structures are not well understood. Lin et al. (2021) conducted a comparative transcriptomic analysis of two double-petaled lotus varieties, 'Pink Climber' and 'Sleeping Beauty,' identifying 1,568 differentially expressed genes (DEGs) associated with petaloid carpel formation. MADS-box transcription factors, notably the *AGAMOUS* (*AG*) flower homeotic gene, play a central role in the transformation of stamens and carpels into petal-like structures and have been identified as candidate genes for petaloid development in lotus. Further, Gao et al. (2022) conducted a genome-wide association study identifying a 2.75-kb genomic fragment associated with stamen petaloid variation and petal number. This region, enriched with MITE transposons bound by siRNAs, was linked to expression differences in a nearby candidate gene, distinguishing few-petaled from double-petaled lotus varieties. Despite these findings, a comprehensive understanding of gene functions and regulatory pathways influencing flower morphology in lotus is still lacking. Future research directions should focus on the relationship between gene expression and cell differentiation, particularly the transformation from stamen and pistil cells to petal cells, to deepen our understanding of lotus flower morphology.

Mechanisms of lotus seed development

Lotus seeds, the fruit of the lotus plant, comprise four main parts: pericarp, seed coat, cotyledon, and plumule (Chen et al. 2023b, 2023c; Sun et al. 2020, 2022). Maturation occurs approximately 30 days post-pollination (DAP), progressing through four developmental stages: organ formation (1–3 DAP), cell expansion (4–9 DAP), material accumulation (10–25 DAP), and dehydration (26–30 DAP) (Sun et al. 2020) (Fig. 3a). During development, the pericarp transitions from green to dark brown and hardens, providing mechanical protection. The mature seed coat turns purple-red or milky white, tightly surrounding starch-rich cotyledons, with a green plumule embedded inside. Transient overexpression of

NnEXPA2 has been shown to promote early cell expansion in cotyledons (Li et al. 2024). Starch, comprising 40%–60% of lotus seed dry matter, is predominantly high-amylose, making it a promising candidate for functional foods aimed at blood sugar management and low glycemic index products (Sun et al. 2020; Wang et al. 2016) (Fig. 3b).

Metabolic analysis reveals active metabolism in lotus seeds between 10 and 20 DAP, shifting from active metabolism to nutrient storage, with soluble sugar content peaking at 15 DAP (Wang et al. 2016). Significant starch accumulation and peak protein abundance of ADP-glucose pyrophosphorylase (AGPase) occur at 15 and 21 DAP, respectively (Sun et al. 2020). During seed development, expression levels of enzymes involved in starch biosynthesis, such as AGPase, soluble starch synthase (SS), and starch branching enzyme (SBE), change significantly (Sun et al. 2020; Zhu et al. 2019a). Song et al. (2024b) demonstrated that transient overexpression of *NnSBE1* in cotyledons increases amylopectin and total starch content. Additionally, Sun et al. (2021) investigated the molecular mechanisms underlying post-harvest quality decline in fresh seeds, observing rapid increases in starch and protein content alongside reduced soluble sugars. The lotus sucrose synthase (*SUS*) gene family consists of five members, with *NnSUS1* co-expressing with multiple genes involved in starch and sucrose metabolism. Overexpression of *NnSUS1* in cotyledons notably elevates total soluble sugar levels, including sucrose and fructose (Song et al. 2024c). The *SWEET* gene family, related to sugar transport, also plays a role in sugar and starch metabolism; overexpression of *NnSWEET14* significantly increases soluble sugar content in cotyledons (Chen et al. 2024a). Focused research on lotus seed development and nutrient accumulation in cotyledons is expected to drive substantial growth in the lotus seed industry in the near future.

Lotus plumules predominantly accumulate bisbenzylisoquinoline alkaloids, including liensinine, isoliensinine, and neferine, whereas the leaves primarily contain aporphine alkaloids such as nuciferine, *N*-nornuciferine, and *O*-nornuciferine (Wei et al. 2024). In recent years, several synthase genes involved in alkaloid biosynthesis in lotus—such as *NCS*, *6OMT*, *NDM*, *ODM*, *7OMT*, *CYP80A*, *CYP80G*, and *CYP719*—have been identified through genome-wide association studies (GWAS), transcriptomics, metabolomics, and enzyme function analysis (Deng et al. 2016b, 2018; Yu et al. 2023). These studies have led to proposed biosynthetic pathways for lotus alkaloids. Notably, alkaloid synthesis in lotus predominantly follows the (*R*)-configuration, with enzymes like *6OMT* and *CYP80G* favoring (*R*)-configuration intermediates, such as (*R*)-norcoclaurine and

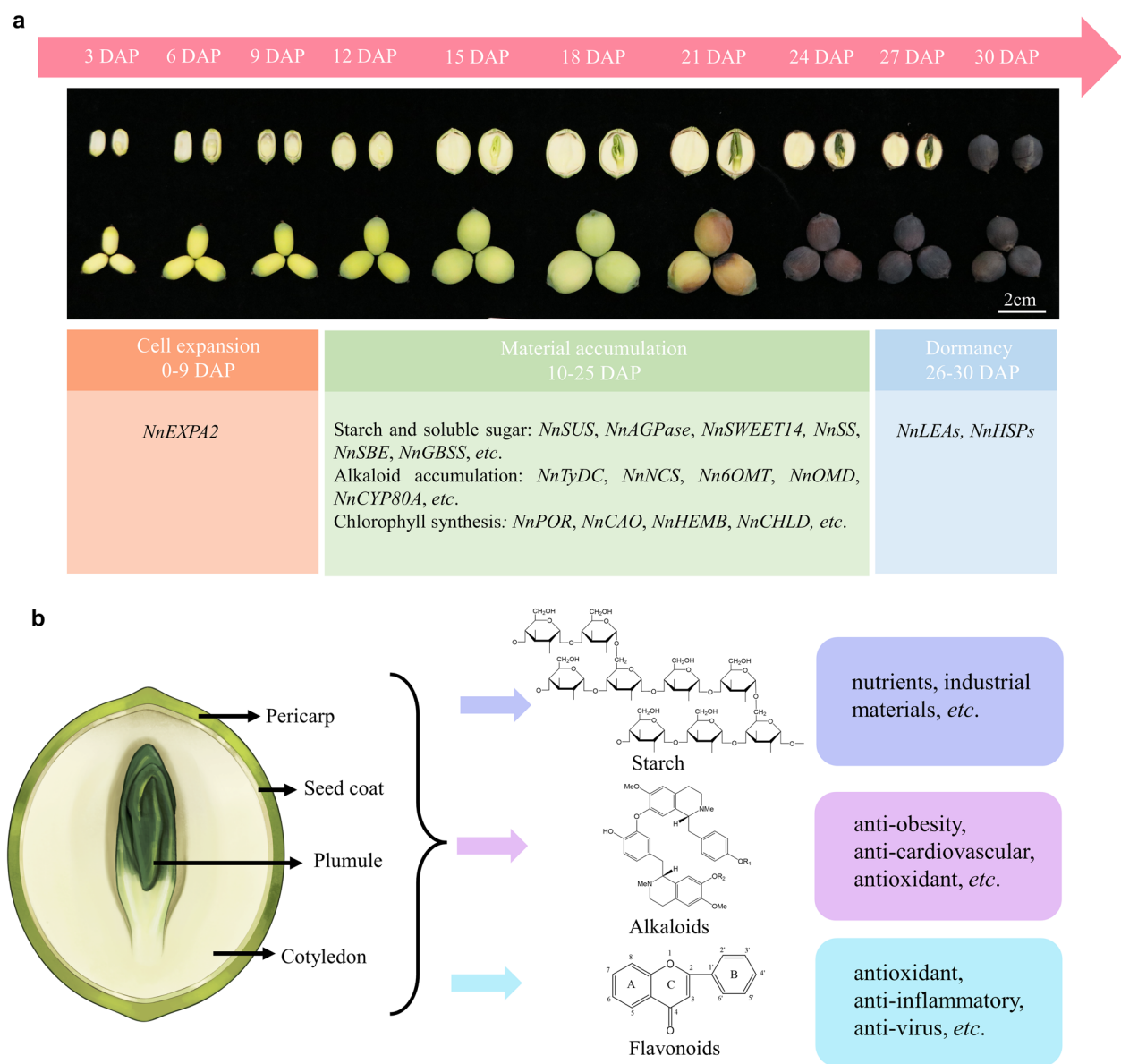


Fig. 3 Morphological changes during lotus seed development and functional components. **a** Morphological changes in developing lotus seeds from the variety 'Jianxuan17'. Scale bar = 2 cm. Highlighted boxes indicate genes associated with regulatory roles at different developmental stages. *NnEXPA2*, *Nelumbo nucifera* *EXPA* (*Expansin* 2); *NnSUS*, *Nelumbo nucifera* *SUS* (*Sucrose synthase*); *NnAGPase*, *Nelumbo nucifera* *AGPase* (*ADP-glucose pyrophosphorylase*); *NnSWEET14*, *Nelumbo nucifera* *SWEET* (*Sugars will eventually be exported transporter* 14); *NnSS*, *Nelumbo nucifera* *SS* (*Soluble starch synthase*); *NnSBE*, *Nelumbo nucifera* *SBE* (*Starch branching enzyme*); *NnGBSS*, *Nelumbo nucifera* *GBSS* (*Granule-bound starch synthase*); *NnTyDC*, *Nelumbo nucifera* *TyDC* (*Tyrosine decarboxylase*); *NnNCS*, *Nelumbo nucifera* *NCS* (*Norcoclaurine synthase*); *Nn6OMT*, *Nelumbo nucifera* *6OMT* (*6-O-methyltransferase*); *NnODM*, *Nelumbo nucifera* *ODM* (*O-demethylase*); *NnCYP80A*, *Nelumbo nucifera* *CYP* (*Cytochrome P450 monooxygenase*) 80A; *NnPOR*, *Nelumbo nucifera* *POR* (*Cytochrome P450 Oxidoreductase*); *NnCAO*, *Nelumbo nucifera* *CAO* (*Chlorophyll a oxygenase*); *NnHEMB*, *Nelumbo nucifera* *HEMB* (*HemB delta-aminolevulinic acid dehydratase*); *NnCHLD*, *Nelumbo nucifera* *CHLD* (*Mg chelatase D subunit*); *NnLEAs*, *Nelumbo nucifera* *LEAs* (*Late embryogenesis abundant*); *NnHSPs*, *Nelumbo nucifera* *HSPs* (*Heat shock protein*). **b** Structural components of the lotus seed and the corresponding functions of each part

(*R*)-*N*-methylcoclaurine (Menéndez-Perdomo and Facchini 2018, 2023; Kashiwada et al. 2005). (*S*)-*N*-methylcoclaurine is considered a common branching point, serving as a substrate for aporphine alkaloid synthesis in leaves and bisbenzylisoquinoline alkaloids in plumules (Deng et al. 2018). Absence of an active CYP80B enzyme in lotus contributes to this pathway's functional differentiation, with tissue-specific expression

of enzymes like CYP80 driving the synthesis of distinct alkaloid types (Deng et al. 2018). Recent findings indicate that NnCYP80A catalyzes C–O coupling in both (*R*)-*N*-methylcoclaurine and (*S*)-*N*-methylcoclaurine, forming bisbenzylisoquinoline alkaloids with diverse linkages, while NnCYP80G enables C–C coupling in aporphine alkaloids, exhibiting broad substrate flexibility by utilizing (*R*)-*N*-methylcoclaurine, (*S*)-*N*-methylcoclaurine, coclaurine, and reticuline (Hao et al. 2024).

Flavonoids, another significant class of compounds in lotus plumules, are characterized by two phenolic rings (A and B rings) connected by a three-carbon linker (C6–C3–C6). These compounds exhibit a wide range of biological activities, including hypoglycemic, hypolipidemic, antioxidant, anti-inflammatory, and antitumor properties. Lotus plumules are especially rich in secondary metabolites such as rutin, hyperoside, and schaftoside. Research on lotus plumule flavonoids has largely centered on identification, isolation, purification, and biological activity assessment, while the biosynthesis pathways remain underexplored. Feng et al. (2021) identified two *C-glycosyltransferase* (*CGT*) genes, *NnCGT1* and *NnCGT2* (also known as *UGT708N1* and *UGT708N2*), whose transient expression and enzymatic activity in tobacco leaves suggest their role in flavonoid di-*C*-glycoside biosynthesis in lotus plumules. Prior studies have shown that transcription factors such as R2R3-MYB, basic helix-loop-helix (bHLH), and WD-repeat proteins contribute to flavonoid biosynthesis by regulating structural gene expression (Koes et al. 2005). For instance, in tobacco, R2R3-MYB transcription factors directly activate genes like *chalcone isomerase* (*CHI*) and *chalcone synthase* (*CHS*), thereby modulating the flavonoid biosynthetic pathway (Huang et al. 2016). Although flavonoid biosynthesis has been extensively studied in various species, comprehensive investigations into its biosynthetic and transcriptional regulatory mechanisms in lotus seeds remain limited.

Molecular mechanisms of lotus seed longevity

Lotus seeds rank among the longest-lived seeds globally, with a record survival time exceeding 1,300 years (Shen-Miller et al. 1995). The lotus seed pericarp, reinforced by a protective waxy layer, exhibits exceptional robustness, serving as a critical barrier that preserves structural integrity and significantly extends seed lifespan (Shen-Miller et al. 2002). Within lotus seeds, a suite of heat-resistant proteins supports essential biological functions under extreme conditions. Prominent among these are heat shock protein 80 (HSP80), protein l-isoleucine methyltransferase 1 (PIMT1), and methionine synthase, all of which remain stable at temperatures up to 110 °C, enabling self-repair and protective responses

(Shen-Miller et al. 2013) (Table 2). Copper/zinc superoxide dismutase (CuZn-SOD), another vital protein, preserves cellular integrity and activity under high-temperature stress (Shen-Miller et al. 2013). PIMT1 maintains its repair function even at 100 °C by removing detrimental isoleucyl residues from seed proteins, thus enhancing seed viability and longevity (Oge et al. 2008). Additionally, overexpression of the annexin *NnANN1* gene in *Arabidopsis* enhances seed thermotolerance and promotes germination (Chu et al. 2012). The NnCAT protein offers cellular protection from oxidative stress by modulating intracellular reactive oxygen species (ROS) levels (Dong et al. 2015). The 1-cys peroxiredoxin gene *NnPER1*, abundantly expressed in lotus seeds, significantly contributes to longevity and stress resilience when heterologously expressed in *Arabidopsis* (Chen et al. 2016). Furthermore, the overexpression of lotus metallothionein genes *NnMT2a* and *NnMT3* improves seed germination vigor in *Arabidopsis* (Zhou et al. 2012c).

The presence of HSPs and late embryogenesis abundant (LEA) proteins in mature lotus seeds is instrumental in promoting seed longevity (Table 2). A tandem mass tag (TMT) labeled proteomics study on the desiccation mechanism of lotus seeds highlighted the potential roles of LEA proteins and HSPs in enhancing storage tolerance (Zhang et al. 2021a). Specifically, the *NnHSP17.5* gene, encoding a cytosolic class II small heat shock protein, is involved in oxidative stress responses during germination, thereby enhancing germination vigor and thermotolerance in transgenic *Arabidopsis* seeds (Zhou et al. 2012b). Recent genomic analysis has identified and characterized lotus *NnLEA* genes, some of which are highly expressed in later stages of seed development. These genes are hypothesized to support seed storage stability by facilitating dehydration during seed maturation (Chen et al. 2023c). Elucidating the molecular mechanisms underlying lotus seed longevity will offer vital insights for improving seed storage and production practices.

Mechanisms of lotus rhizome development

The two ecotypes of Asian lotus, temperate and tropical, are primarily distinguished by rhizome morphology differences. In temperate lotus, rhizome enlargement begins in late summer or early autumn, accumulating high levels of proteins, polysaccharides, and phenolic compounds, which are economically valuable as a vegetable crop (Gao et al. 2021). Acting as a key storage organ, the lotus rhizome stores sucrose to fuel energy needs during bud dormancy and winter, supporting asexual reproduction (Cheng et al. 2016). Transcriptomic analysis of temperate and tropical lotus rhizomes at various developmental stages has revealed that rhizome enlargement is regulated by multiple genes involved in carbohydrate

Table 2 Lotus genes and their functions

Biological process	Gene	Functions	References
Lotus flowering	<i>NnFT2/FT3</i>	<i>FLOWERING LOCUS T</i> genes successfully rescued the late-flowering phenotype of the <i>Arabidopsis ft-10</i> mutant, promoting flowering induction	(Song et al. 2024a)
	<i>NnFT1</i>	The <i>FLOWERING LOCUS T</i> gene promotes early flowering in transgenic <i>Arabidopsis</i>	(Zhang et al. 2021b)
	<i>NnSnRK1</i>	The Sucrose nonfermenting-1 (SNF1)-related protein kinase 1 (SnRK1) plays a role in influencing bud abortion in lotus	(Cao et al. 2021b)
	<i>COP1</i>	Constitutive photomorphogenesis plays a central role in regulating lotus flowering by integrating signals from photoperiodic, vernalization, and gibberellin pathways	(Yang et al. 2014a)
	<i>CCA1</i>	Circadian clock-associated 1 is a pivotal regulator in the photoperiodic, vernalization, and gibberellin pathways that govern lotus flowering	(Yang et al. 2014a)
	<i>LHY</i>	Late elongated hypocotyl acts as a central regulator in the photoperiodic, vernalization, and gibberellin pathways involved in lotus flowering	(Yang et al. 2014a)
	<i>COL</i>	Constans-like functions as a key regulator within the photoperiodic, vernalization, and gibberellin pathways controlling lotus flowering	(Yang et al. 2014a)
Lotus flower color and shape formation	<i>NnMYB5</i>	The transient expression of <i>NnMYB5</i> in white lotus petals induces anthocyanin accumulation	(Zheng et al. 2022a)
	<i>NnGST2</i>	Glutathione s-transferase mediates the transport of anthocyanins from the endoplasmic reticulum to the vacuoles	(Liu et al. 2023)
	<i>NnTT8</i>	The bHLH transcription factor regulates the biosynthesis of anthocyanins and proanthocyanidins	(Deng et al. 2021)
Lotus seed development	<i>NnEXPA2</i>	As a member of the expansin gene family, this gene plays a critical role in promoting cell expansion during the early developmental stages of lotus seed cotyledons	(Li et al. 2024)
	<i>NnAGPase</i>	ADP-glucose pyrophosphorylase is a key enzyme in starch biosynthesis	(Sun et al. 2020)
	<i>NnSS</i>	Soluble starch synthase is essential in starch biosynthesis	(Sun et al. 2020)
	<i>NnSBE1</i>	Starch branching enzyme is crucial in amylopectin and total starch biosynthesis	(Song et al. 2024b)
	<i>NnSUS1</i>	A member of the sucrose synthase gene family plays a significant role in starch and sucrose metabolism, contributing to an increase in total soluble sugar levels	(Song et al. 2024c)
	<i>NnSWEET14</i>	The sugar transport-related SWEET gene family plays a critical role in sugar and starch metabolism, significantly increasing soluble sugar content	(Chen et al. 2024a)
	<i>NCS</i>	Norcochlorine synthase is involved in lotus alkaloid biosynthesis	(Sun et al. 2022)
	<i>6OMT</i>	6-O-methyltransferase is involved in lotus alkaloid biosynthesis	(Sun et al. 2022)
	<i>NDM</i>	N-demethylase is involved in lotus alkaloid biosynthesis	(Sun et al. 2022)
	<i>ODM</i>	O-demethylase is involved in lotus alkaloid biosynthesis	(Sun et al. 2022)
	<i>7OMT</i>	O-methyltransferase is involved in lotus alkaloid biosynthesis	(Sun et al. 2022)
	<i>CYP719</i>	Cytochrome P450 gene is involved in lotus alkaloid biosynthesis	(Sun et al. 2022)
	<i>NnCYP80A/G</i>	The cytochrome P450 monooxygenase gene catalyzes C-O coupling reactions in both (R)-N-methylcochlorine and (S)-N-methylcochlorine	(Hao et al. 2024)
	<i>NnCGT1/2</i>	Two C-glycosyltransferase (CGT) genes are involved in flavonoid di-C-glycosides biosynthesis in lotus plumules	(Feng et al. 2021)

Table 2 (continued)

Biological process	Gene	Functions	References
Lotus seed longevity	<i>NnPER1</i>	The 1-cys peroxiredoxin gene functions in scavenging excess reactive oxygen species (ROS), thus safeguarding seeds against oxidative stress	(Chen et al. 2016)
	<i>NnLEAs</i>	Late embryogenesis abundant (LEA) proteins contribute to the desiccation process during the later stages of lotus seed development, thereby enhancing the storage tolerance of lotus seeds	(Chen et al. 2023c)
	<i>NnHSP17.5</i>	The cytosolic class II small heat shock protein gene is involved in oxidative stress responses, thereby enhancing seed tolerance	(Zhou et al. 2012b)
	<i>CuZn-SOD</i>	Copper/zinc superoxide dismutase (CuZn-SOD) maintains seed viability and safeguards cell integrity under high temperature treatment	(Shen-Miller et al. 2013)
	<i>HSP80</i>	Heat shock proteins (HSPs) enhance plant resistance	(Shen-Miller et al. 2013)
	<i>NnANN1</i>	<i>Annexin</i> genes protect membranes from peroxidation and regulate seed thermotolerance and germination	(Chu et al. 2012)
	<i>PIMT1</i>	L-isoleucyl-O-methyltransferase exhibits protein-repair activity after exposure to temperatures of 100 °C	(Shen-Miller et al. 2013)
	<i>NnCAT</i>	<i>Catalase</i> genes play a critical role in mitigating oxidative stress during its early stages	(Dong et al. 2015)
Lotus rhizome development	<i>NnMT2a /MT3</i>	<i>Metallothionein</i> genes enhance seed resistance to stresses	(Zhou et al. 2012c)
	<i>NnCOL5</i>	The COL family gene plays a critical role in the rhizome enlargement process	(Cao et al. 2021a)
	<i>NnNF-YB1</i>	Nuclear factor-Y transcription factor increases the starch content in lotus rhizomes	(Zhao et al. 2023)
Response to diverse stresses	<i>NnGBSSII</i>	Granule-bound starch synthase gene is involved in starch biosynthesis	(Zhao et al. 2023)
	<i>NnDREB1</i>	This gene enhances resistance to both drought and salt stress	(Zhou et al. 2012a)
	<i>NnPIP1-2</i>	This gene enhances resistance to both drought and salt stress	(Zhou et al. 2012a)

metabolism and hormone signaling pathways (Yang et al. 2015). For example, the COL family gene *NnCOL5* shows increased expression during rhizome enlargement, and heterologous overexpression of *NnCOL5* has been shown to enhance starch content and biomass in potato tubers under short-day conditions (Cao et al. 2021a). Additionally, recent studies have identified *NnNF-YB1*, an NF-YB transcription factor induced by potassium fertilizer, which directly regulates *NnGBSSII* expression. Transient overexpression of *NnNF-YB1* in lotus rhizomes increases total starch, amylose, and amylopectin content (Zhao et al. 2023).

Rhizome enlargement in lotus is not only an essential adaptation for cold resistance but also a critical determinant of rhizome yield. Huang et al. (2021) conducted the first QTL mapping for rhizome yield-related traits using an F₂ population derived from hybridization between temperate and tropical lotus, creating a genetic map with 12,113 SNP markers. This study identified 22 QTLs associated with rhizome yield, providing valuable markers for marker-assisted breeding. Subsequently, Liu et al. (2022) identified QTLs linked to rhizome enlargement, focusing

on the rhizome enlargement index and the number of enlarged nodes. Functional validation of the candidate gene *NnBEL6* through heterologous expression in potatoes demonstrated its significant role in tuber formation. Starch, the primary nutrient stored in lotus seeds and rhizomes, exhibits considerable variation in content and composition between these two tissues (Zhu 2017). Transcriptome analysis by Zhu et al. (2022) identified 11 key genes involved in starch biosynthesis, with genes associated with amylopectin biosynthesis showing higher expression in rhizomes, while those related to amylose biosynthesis were more highly expressed in seeds. This differential gene expression suggests that these key genes may contribute to the observed differences in starch properties between lotus seeds and rhizomes.

Mechanisms of response to diverse stress in lotus

The growth cycle of lotus spans six developmental stages: germination, leaf expansion, flowering, fruiting, rhizome development, and dormancy. Across these stages, lotus faces various abiotic and biotic stresses that influence its economic and ornamental value. As a thermophilic

plant, lotus demonstrates high tolerance to elevated temperatures, thriving optimally between 22 °C and 32 °C. Temperatures below 15 °C induce dormancy, interrupting its growth cycle (Li et al. 2022). During dormancy, lotus leaves display reduced water and chlorophyll content, accompanied by elevated proline and malondialdehyde levels. Enzymatic activity, particularly of peroxidase (POD), also adjusts, enhancing cold resistance. Cold acclimation is the process through which plants gradually increase their tolerance to low temperatures. Li et al. (2022) found that cold-acclimated lotus shows markedly elevated levels of osmotic adjustment substances, including proline, soluble proteins, and soluble sugars. Concurrently, the activity of antioxidant enzymes and expression of related genes, such as *CAT1*, *GPX*, *APX*, and *MSD*, are significantly upregulated. This cold acclimation process effectively boosts lotus cold tolerance, extending its growth cycle and enhancing its ornamental appeal. Current research on lotus cold tolerance primarily emphasizes physiological and biochemical responses, highlighting the need for further genetic studies in this area.

Lotus, a perennial aquatic plant typically cultivated in ponds and lakes, has evolved an interconnected pore network to facilitate ventilation throughout the plant. Despite this adaptation, lotus exhibits relatively low tolerance to full submergence compared to other aquatic species (Matthews and Seymour 2014). In response to complete submersion, lotus employs two distinct strategies (Deng et al. 2022). Initially, it activates an “escape strategy,” relying on anaerobic respiration to conserve energy, which allows rapid leaf elongation to break the water surface, along with an increase in stomatal density. During this phase, plant hormone levels shift, with a slight increase in gibberellin (GA) and a decrease in ABA levels, alongside significant upregulation of jasmonic acid (JA) response genes. In later stages of submergence, lotus transitions to a “quiescent strategy,” reducing anaerobic respiration to adapt more effectively to prolonged water-logging. However, without sufficient energy reserves, lotus leaves may fail to reach the water surface, resulting in rapid plant decline (Deng et al. 2022). Submersion for 12 h induces rapid and substantial mRNA expression changes in approximately 10% of lotus genes (Wang et al. 2018). Genes associated with the MAPK cascade pathway are active across various lotus tissues and developmental stages, influencing seed development and complete submergence responses (Chen et al. 2023b). Additionally, multiple lotus tissues—including rhizomes, stems, and leaves—exhibit potential for remediating heavy metal-contaminated water. Soluble proteins in lotus enhance intracellular osmotic potential and form complexes with heavy metals, mitigating their toxic effects. Several lotus genes responsive to drought and salt stress have also

been identified; for instance, overexpression of *NnDREB1* and *NnPIP1-2* in *Arabidopsis* enhances resistance to both drought and salt stress (Zhou et al. 2012a).

Challenge and perspective

Lotus occupies a significant place in the traditional cultures of Asian countries, and its unique aesthetic and multifunctional applications have drawn increasing scientific interest (Song et al. 2024a). Although primarily widespread in Asia, lotus remains relatively unfamiliar in other regions. This review systematically compiles recent advancements in lotus molecular biology, particularly focusing on the identification and functional analysis of genes linked to key traits (Fig. 4).

Currently, molecular research on lotus is still in an early phase, with many regulatory mechanisms of essential traits yet to be fully understood. To expedite the breeding of new lotus varieties and improve resource utilization, future research should prioritize two main objectives: (1) the effective application of emerging technologies, and (2) elucidating the mechanisms behind important traits while exploring valuable genetic resources (Fig. 4).

Emerging biological research techniques present new opportunities for advancing lotus molecular biology research

Recent improvements in lotus genomic data have opened new pathways for molecular studies. Although chromosome-level assemblies have been achieved for the main lotus species, *N. nucifera* and *N. lutea*, remaining gaps and inaccuracies in these assemblies have constrained further progress (Ming et al. 2013; Zheng et al. 2022a). A complete telomere-to-telomere (T2T) genome assembly would provide chromosomal-scale continuity, offering a more accurate chromosome map that facilitates a deeper understanding of agronomic traits. The successful assembly of T2T genomes in crops like rice, kiwifruit, capsicum, and maize underscores the importance of a gapless T2T genome for lotus to resolve complex biological questions beyond the scope of the current genome (Shang et al. 2023; Han et al. 2023; Chen et al. 2024b, 2023a).

Pan-genomes compile the complete genetic repertoire of a single species across diverse varieties through genome sequencing, assembly, and integration, providing a sophisticated reference for identifying genetic variations within individual genomes (Bayer et al. 2020; Shi et al. 2023). The intricate genetic relationship between *N. nucifera* and *N. lutea* has long been debated, and the development of lotus pan-genomes holds potential for delivering more refined genetic insights to address this issue. Additionally, pan-genomes will advance molecular breeding in lotus by efficiently uncovering valuable

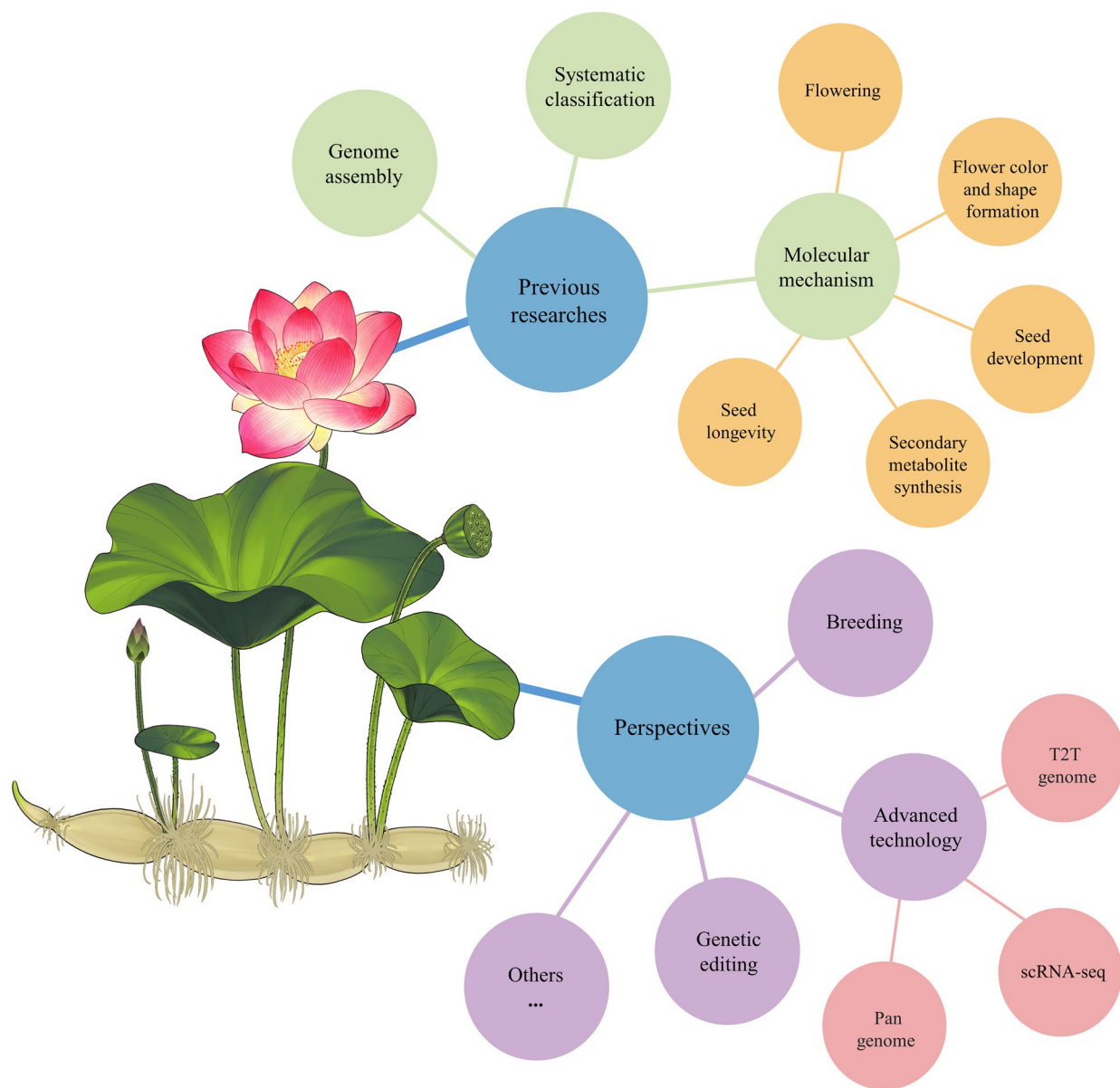


Fig. 4 A summary model for previous research and future perspectives on lotus

genetic resources linked to key agronomic traits, thus accelerating innovation in lotus germplasm.

Emerging research techniques like single-cell RNA sequencing (scRNA-seq) are also anticipated to play a significant role in future lotus molecular studies. ScRNA-seq, a high-throughput sequencing technology, offers powerful capabilities in identifying cellular heterogeneity, profiling gene expression, and constructing transcriptional regulatory networks (Baysoy et al. 2023). This technique has already been applied successfully across various species, including animals and model plants, and lotus research stands to benefit from

the spatial and temporal insights it provides. ScRNA-seq promises to illuminate biosynthetic pathways of key metabolites in lotus, such as alkaloids and flavonoids, laying a cellular and genetic foundation for in-depth investigations into the biosynthesis, transport, and storage of bioactive components. Furthermore, integrating multi-omics approaches with scRNA-seq is poised to open new avenues in lotus molecular biology, enhancing our understanding of its genetic complexity and potential applications.

Exploring valuable gene resources will greatly expedite the molecular breeding process of lotus

Fresh lotus seeds are highly regarded for their sweet and crisp taste, making them a popular summer fruit. However, their quality and flavor degrade rapidly post-harvest, significantly limiting storage and transportability (Sun et al. 2021). Investigating the physiological changes and molecular mechanisms that occur during postharvest storage to extend shelf life remains a critical challenge. In contrast, dry lotus seeds, rich in nutrients, are commonly used in soups and are valued as a healthful tonic. Additionally, lotus plumules contain abundant bioactive compounds with promising pharmacological properties for disease treatment. Targeted breeding of lotus varieties optimized for consumption or processing through genetic improvement will accelerate advancements in the lotus seed industry.

The lotus flower holds substantial ornamental value, ranking among China's top ten traditional flowers. However, *N. nucifera* flower colors are primarily limited to red and white. Expanding the color range could be achieved by incorporating carotenoid and other pigment synthesis pathways. Understanding the mechanisms underlying variegated flower colors, as seen in varieties like 'Sajin,' is essential for advancing knowledge of lotus color formation. The floral scent of lotus, composed of volatile organic compounds (VOCs), plays a pivotal role in attracting pollinators and deterring pests (Muhlemann et al. 2014). Despite this, research on the specific VOCs in lotus has been limited. Uncovering the molecular regulatory mechanisms of floral scent biosynthesis and the functional roles of VOCs could drive the commercial production of lotus-scented products. Extracting lotus floral scent into essential oils or pure extracts, or blending it with other aromatic plants like rose and jasmine, offers potential for new aroma products and perfumes to meet the rising demand for personalized, high-quality fragrances.

In the context of intensifying global climate change and environmental pressures, understanding plant responses to stress is essential for fostering sustainable agriculture and maintaining ecosystem stability. As an aquatic species, lotus is particularly sensitive to stresses such as salinity, low temperatures, submergence, pests, and diseases. Despite this, studies on the environmental stress resistance of lotus and other aquatic plants remain relatively limited. Deciphering the molecular pathways involved in lotus stress responses can yield valuable insights into plant stress tolerance at the molecular level. Identifying and harnessing key genes that regulate stress resilience in lotus is critical for developing high-tolerance varieties, enabling effective utilization and sustainable growth of lotus resources. Gene editing technology provides a powerful approach for exploring gene functions and underlying molecular mechanisms. However, the lack of a stable

genetic transformation system in lotus represents a significant barrier to advancing molecular biology research and implementing molecular breeding techniques in this species.

Conclusion

In conclusion, ongoing advancements in lotus genome assembly toward greater precision and completeness have substantially accelerated molecular biology research in lotus. Insights into the fundamental mechanisms governing key agricultural traits—such as flower color and morphology, seed yield, edible quality, and bioactive compound production—offer promising target genes for future breeding and genetic enhancement through molecular biotechnology. These achievements are set to significantly drive the development of new lotus varieties with enhanced and desirable traits.

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Authors' contributions

All authors contributed to the study conception and design. H.S., G.Q.D., and M.Y. conceived of the idea; H.S., J.X., H.Y.S., and L.C. collected and analyzed the literature; H.S., J.X., H.Y.S., and L.C. wrote the first draft of the manuscript; D.Y., H.Y., X.B.D., J.L., R.C., and Y.Y.S. reviewed and prepared the final draft of the manuscript. All authors read and approved the final manuscript.

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The authors declare that they have no competing interests.

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