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Study on the Genetic Diversity and Superior Gene Screening of *Lindera aggregata* Germplasm Resources in Qingchuan County

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Abstract This study analyzed the genetic diversity characteristics and ecological adaptability mechanisms of the germplasm resources of *Lindera aggregata* in Qingchuan County, and revealed the influence of different ecological environments on the genetic variation and trait expression of *Lindera aggregata*. The study identified multiple functional genes closely related to key medicinal components (volatile oils, total phenols, total flavonoids) and stress resistance (drought resistance, cold resistance, and salt resistance), some of which showed significant expression differences under specific environmental stresses. The study found that the germplasm resources of *Lindera aggregata* in Qingchuan County have rich genetic diversity, and their genetic structure characteristics are significantly correlated with the ecological environment. Based on gene function annotation, a number of core genes regulating secondary metabolic synthesis and environmental adaptability were successfully excavated. Combined with the comprehensive evaluation of multiple traits, several excellent germplasms with outstanding comprehensive traits were screened, and regional adaptive cultivation strategies were constructed accordingly. This study not only provides a necessary meal card for the protection of *Lindera aggregata* germplasm resources, but also lays a theoretical foundation for molecular breeding practice. The research results will effectively promote the quality improvement and yield stability of *Lindera aggregata*, and have important practical value for promoting the sustainable development of the Chinese medicinal materials industry.

Keywords *Lindera aggregata*; Germplasm resources; Genetic diversity; Superior gene screening; Molecular breeding

1 Introduction

As an important representative of the medicinal plants of the Lauraceae family, *Lindera aggregata* occupies a special position in the traditional Chinese medicine system due to its unique chemical composition and significant clinical efficacy. Studies have shown that the active ingredients contained in the roots and leaves of this plant can effectively regulate multiple metabolic pathways and have significant therapeutic effects on diseases such as chronic kidney disease. From ancient classic prescriptions such as "Suquan Pills" to modern medical practice, the medicinal value of *Lindera aggregata* has been continuously verified (Cai et al., 2020). It is worth noting that the unique *Lindera aggregata* germplasm resources in Qingchuan County have become key genetic materials for improving the quality of medicinal materials and promoting industrial development due to their excellent genetic characteristics (Shi et al., 2024a; Shi et al., 2024b).

In-depth analysis of the genetic diversity of *Lindera aggregata* is of great significance for the protection and development and utilization of its resources. Molecular marker analysis based on chloroplast and nuclear DNA can not only clarify the evolutionary process of species, but also provide key evidence for the study of population genetic structure (Ye and Li, 2019; Ye and Li, 2021). More importantly, screening for excellent functional genes can directly improve the medicinal quality, yield potential and stress resistance of Linderae obesa, which has a dual role in enhancing its environmental adaptability and medicinal value (Xiong et al., 2020; Shi et al., 2024a).

This study intends to use the germplasm of *Lindera aggregata* in Qingchuan County as the research core, use modern molecular biological methods to systematically analyze its genetic diversity structure, and screen functional genes closely related to pharmacodynamic properties and ecological adaptability. This study will provide theoretical support and technical reserves for the protection, utilization and resource innovation of this special medicinal plant.

2 Distribution and Ecological Environment of *Lindera aggregata* Germplasm Resources in Qingchuan County

2.1 Geographic distribution of *Lindera aggregata* in Qingchuan county

Lindera aggregata, as a representative of Lauraceae, has shown strong ecological adaptability in Qingchuan County. This species is mainly distributed in the transition zone between subtropical and temperate zones, especially in mountain forests with lush vegetation. Its natural population is mostly found in valley bottoms, gentle slopes of hills and low-altitude mountains, which create a relatively stable ecological barrier for *Lindera aggregata*. Field surveys in recent years have shown that *Lindera aggregata* mostly chooses semi-shaded and humid environments to grow in Qingchuan County, showing a high sensitivity to specific microecological conditions (Gu et al., 2010).

The core environmental factors that determine its geographical distribution include soil type, climate background and altitude. The acidic soil commonly found in Qingchuan has rich organic matter content and good drainage, which provides a suitable matrix for the growth of *Lindera aggregata*. In terms of vertical distribution, the plant is mostly concentrated in areas with an altitude of 500–1200 meters, showing obvious belt spectrum characteristics. In addition, its distribution density is closely related to the average annual temperature and precipitation. Data show that Linderae odorifera is best distributed in areas with an average annual temperature of 15–22°C, moderate precipitation, and no extreme climate disturbance (Ye et al., 2021).

2.2 Major phenotypic characteristics of *Lindera aggregata*

Lindera aggregata in Qingchuan area shows high diversity in morphological characteristics, covering differences in multiple biological traits and growth habits. This species often exists in the form of deciduous shrubs or small trees, with plant heights ranging from 2 to 5 meters. Its leaves are mostly oblong to ovate, which is one of the important external characteristics for identifying the plant. In particular, different natural populations show obvious differentiation in leaf length, width and color, suggesting that there may be rich variation resources at the genetic level (Gu et al., 2010). As the main medicinal part, the rhizome of *Lindera aggregata* is enriched with a variety of active ingredients, especially volatile oils and alkaloids (Figure 1) (Huang et al., 2023), which also provides an important material basis for its efficacy research.

Figure 1 Morphology and Medicinal Parts of *Lindera aggregata* (Adopted from Huang et al., 2023)
Image caption: (a) Whole plant, (b) medicinal portion, and (c) commercial herbal pieces of *Lindera aggregata* (Adopted from Huang et al., 2023)

The unique reproduction system of *Lindera aggregata* provides a natural guarantee for the maintenance of its population genetic diversity. The plant is dioecious, and its small yellow-green flowers rely on cross-pollination to complete the sexual reproduction process. This kind of reproduction mechanism effectively promotes gene exchange within the population, enhances the ability of genetic recombination, and lays a genetic foundation for adapting to changing environments. Through systematic observation of phenotypic characteristics such as leaf shape, floral structure and fruit morphology, the genetic mechanisms implied by these external trait variations are gradually revealed.

It is worth noting that different germplasms of *Lindera aggregata* show a continuous distribution in key agronomic traits such as fruit size, seed number and essential oil accumulation, showing significant phenotypic differences (Lv et al., 2023). This high degree of trait plasticity not only provides a broad space for the screening of excellent germplasms, but also lays a strong foundation for exploring its ecological adaptation strategy and the genetic basis for the formation of medicinal ingredients (Shi et al., 2024b).

2.3 Relationship between ecological factors and genetic diversity

The formation of the genetic pattern of *Lindera aggregata* population in Qingchuan is the result of the synergistic effect of multidimensional ecological factors. Environmental factors such as altitude gradient, temperature change and soil characteristics jointly shape the genetic variation characteristics of this species through a complex interactive network. Molecular ecological evidence shows that there is significant genetic differentiation between *Lindera aggregata* populations in different ecological zones. This spatial genetic structure is mainly due to the selection pressure driven by environmental heterogeneity (Nakamura et al., 2021). Specifically, low-altitude populations maintain high genetic diversity under superior water and heat conditions; while high-altitude populations tend to have a single genetic composition due to harsh environmental screening and geographical isolation.

Eco-genetic interaction research has important guiding value for resource conservation. Soil physical and chemical properties directly affect plant fitness by regulating water-nutrient balance, while climatic factors indirectly affect reproductive success through phenological regulation. In-depth analysis of these environmentally driven genetic adaptation mechanisms is crucial to the formulation of scientific conservation strategies. Modern conservation biology emphasizes that sustainable management of Qingchuan Linderae genetic resources can only be achieved by integrating landscape features and gene flow dynamics in a conservation framework (Xiong et al., 2020). This conservation paradigm based on the principles of eco-genetics provides a new approach to the conservation of rare medicinal plant resources.

3 Genetic Diversity Analysis of *Lindera aggregata* Germplasm Resources

3.1 Research materials and sampling methods

Through a systematic sample acquisition strategy, a full-coverage collection of Linderae obesa germplasm resources in different ecological gradient areas of Qingchuan County was carried out. The sampling area covers three representative habitats: low-altitude river valleys, medium-altitude forest belts and high-altitude mountains, with the aim of revealing the genetic differentiation trend of germplasm resources under variable environmental conditions. Drawing on the field sampling standards proposed by Gu et al. (2010), the researchers simultaneously collected leaf and seed materials to improve the comprehensiveness of genetic analysis and avoid the risk of bias caused by single materials.

During the sampling process, key phenotypic indicators such as leaf morphology, floral structure and fruit characteristics were recorded on site, providing an important reference for phenotype-genotype association studies. After sample recovery, genomic DNA was extracted according to a unified operating procedure, and the purity and integrity of nucleic acids were strictly controlled to ensure the data quality of subsequent molecular marker analysis. The multi-level and multi-dimensional data acquisition system has laid a solid foundation for the systematic evaluation of the genetic variation pattern of Linderae obesa in Qingchuan and its relationship with phenotypic diversity.

3.2 Molecular marker technologies for genetic diversity

The continuous progress of molecular marker technology has provided multi-angle technical support for the analysis of genetic diversity of P. aegypti. Among them, SSR (simple sequence repeat) markers have become one of the core means to detect population genetic variation due to their high polymorphism and co-dominance (Li, 2024). A large number of research results show that the use of this technology can clearly reveal the rich genetic heterogeneity contained in P. aegypti germplasm resources (Zhao et al., 2005; Ye and Li, 2019).

Breakthroughs in modern molecular biology techniques have revolutionized genetic research. High-throughput technologies such as genome-wide sequencing (GBS) and transcriptome sequencing can systematically detect SNP variations across the entire genome, greatly improving the accuracy of population genetic structure analysis. The latest research has successfully identified multiple population-specific genetic markers and functional sites related to ecological adaptability using these technologies (Shi et al., 2024b). These findings not only deepen our understanding of the genetic background of species, but also provide a molecular basis for the scientific protection and rational use of germplasm resources.

3.3 Methods for evaluating genetic diversity

The evaluation of the genetic diversity of Linderae obesa mainly adopts a multi-dimensional index system, including genetic similarity coefficient, genetic distance matrix and systematic cluster analysis (Ye et al., 2017). Among them, the unweighted group average method (UPGMA) as a classic clustering algorithm can effectively divide the genetic groups of populations based on molecular marker data. The study using this method found that Linderae obesa populations in different ecological regions showed obvious genetic differentiation patterns (Gu et al., 2010).

In order to accurately quantify the degree of genetic variation, researchers generally use parameter indicators such as Shannon information index and Nei gene diversity index. These indices convert genetic variation within populations into comparable numerical results through mathematical models. The measured data show that the Shannon index of Linderae obesa populations is generally higher than 0.3, which confirms that its genetic diversity is at a medium-to-high level (Bi, 2008). This kind of quantitative analysis provides a scientific basis for screening core germplasm resources with breeding value, and has important guiding significance for formulating effective protection strategies.

3.4 Genetic structure analysis

The Qingchuan Linderae population exhibits unique genetic structural characteristics, which contains the dual codes of species evolution and environmental adaptation. AMOVA analysis reveals an important rule: genetic variation is mainly enriched within the population, while differences between populations are only secondary (Nakamura et al., 2021). This distribution pattern suggests that the intensity of gene exchange within the population is significantly higher than the genetic connection between geographically isolated populations.

Further evaluation by Fst value showed that different populations showed moderate genetic differentiation. Such differentiation trends may be driven by ecological factors such as altitude differences, microenvironmental changes, and uneven soil characteristics. The results of gene flow parameters also showed that pollen dispersal and seed dispersal played a synergistic role in maintaining genetic connections between populations.

In-depth analysis of these genetic characteristics has dual value. At the theoretical level, it provides a typical case for understanding the microevolution process of species; at the application level, it points out the direction for formulating scientific protection strategies. Maintaining the ecological diversity of the native habitat can not only maintain the survival of existing genetic variation, but also create favorable conditions for the sustainable development of Linderae resources (Xiong et al., 2020). This discovery organically combines genetic principles with conservation practices, providing new ideas for resource management of rare medicinal plants.

4 Screening and Functional Analysis of Superior Genes in *Lindera aggregata*

4.1 Screening of functional genes related to medicinal ingredients

The pharmacological effects of *Lindera aggregata* mainly depend on its unique secondary metabolite system, among which the active ingredients represented by volatile oils, phenols and flavonoids are particularly critical (Chao, 2003). With the help of high-throughput transcriptome sequencing technology, researchers systematically mined and identified multiple core functional genes that regulate the above metabolic pathways. In particular, in the MVA (mevalonic acid) and MEP (methylerythrose phosphate) metabolic pathways, some genes have been clearly identified to be closely related to the synthesis of volatile oils, providing a key basis for revealing the molecular mechanism of *Lindera aggregata* quality formation (Shi et al., 2024a).

In addition, in the process of analyzing the relationship between environmental adaptability and metabolic expression, the study found that the expression levels of genes such as terpene synthase (TPS) and cytochrome P450 monooxygenase in aromatic *Lindera aggregata* populations were significantly upregulated. The high expression of these synthases not only plays a core role in the accumulation of metabolites, but also further confirms their key regulatory functions in the biosynthesis of volatile oils through transgenic functional verification methods (including overexpression and RNA interference) (Gu et al., 2010). The clear positioning and functional verification of this type of genes provide targeted resource support for future molecular breeding and high-efficiency variety improvement, and also lay a molecular foundation for improving the medicinal quality of *Linderae odorifera*.

4.2 Screening of genes related to stress resistance

Lindera aggregata exhibits excellent environmental adaptability, and its internal mechanism originates from a complex stress resistance molecular network. Transcriptome analysis identified multiple key gene families involved in abiotic stress response: dehydrins maintain cell membrane integrity to resist drought damage, heat shock proteins ensure protein homeostasis, and aquaporins precisely regulate water transport (Ye and Li, 2019). These functional units work together to form a systemic defense system for *Lindera aggregata* to cope with adversity.

The study of *Lindera aggregata* population in Qingchuan revealed the dynamic regulatory characteristics of stress signaling pathways. The core components of the ABA signaling pathway, SnRK2 kinase and MYB transcription factor, were significantly upregulated at the beginning of stress (Nakamura et al., 2021). This discovery not only clarifies the molecular mechanism of *Lindera aggregata*'s stress resistance, but also provides an important target for molecular marker-assisted breeding. In-depth analysis of the mechanism of action of these regulatory factors will accelerate the breeding of new varieties with multiple resistances.

4.3 Gene function validation technologies

The establishment of a gene function verification system is the core link in clarifying the mechanism of action of candidate genes. RT-qPCR combined with RNA-Seq technology can accurately analyze the dynamic expression characteristics of *Lindera aggregata* genes in different tissues, developmental stages and stress conditions, providing key data support for revealing its regulatory network (Shi et al., 2024a).

The innovative study by Shi et al. (2024b) systematically analyzed the structural characteristics of the mitochondrial genome of *Lindera aggregata* by integrating multi-omics data, including the precise positioning of functional elements and the distribution of repetitive sequences (Figure 2). This achievement not only improves the genome annotation information of this species, but also provides a new perspective for understanding its genetic evolution mechanism. The integrated application of multi-omics technologies is pushing the functional genomics research of *Lindera aggregata* into a new stage.

At the same time, the rapid development of gene editing technology has provided a new breakthrough for functional verification. The CRISPR/Cas9 system has been successfully applied to the functional analysis of key genes in Linderae obesa. Taking terpene synthase as an example, after specific knockout of this gene, the content of plant volatile oil decreased significantly, which clearly proved its core regulatory role in the secondary metabolic pathway (Liu et al., 2021). This type of direct correlation verification of "genetic modification-phenotypic response" has greatly improved the accuracy and practical value of functional gene analysis.

4.4 Candidate gene association analysis

Establishing the association between genotype and phenotype is an important basis for molecular breeding. Whole-genome association analysis revealed a key finding: the polymorphism of terpene synthase genes was significantly correlated with the content of volatile oils. The presence of specific alleles is often accompanied by an increase in the content of medicinal ingredients, which provides a precise target for molecular marker-assisted breeding (Xiong et al., 2020).

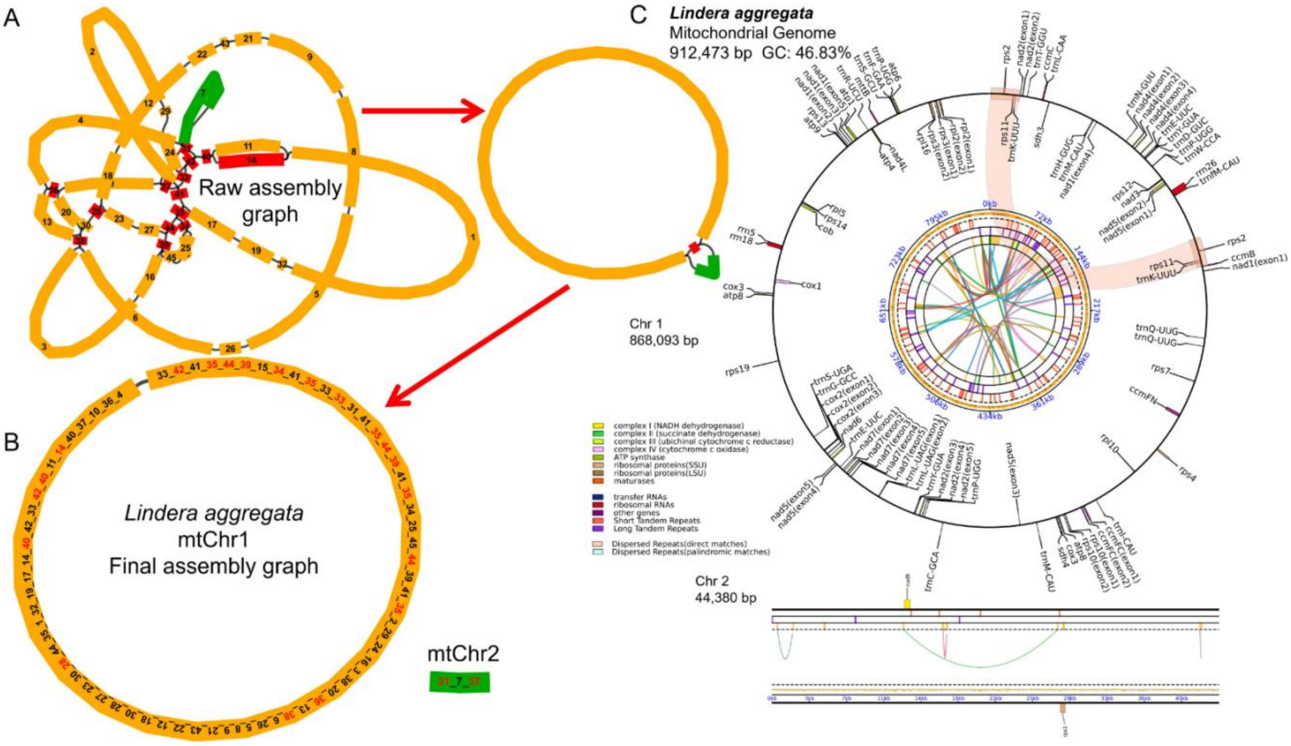

Figure 2 Map of mitochondrial genome assembly and annotation of L. aggregata (Adopted from Shi et al., 2024b)

Image caption: (A) The map of the preliminary assembly of the mitochondrial genome; (B) The master circle and linear fragment map of mitochondrial genome; Each contig is marked with different number, and the numbers order of connection is the order of unlocking circles; (C) The map of mitochondrial genome annotation; Genes with different functions are described in different colors. The colored parabola in the center circle represents the dispersed repeats (Adopted from Shi et al., 2024b)

In-depth research found that genetic variation also affects the accumulation level of total phenolic and flavonoid compounds - these substances are the material basis of the antioxidant activity of Linderae obesa. By integrating phenotypic and genomic data, researchers not only elucidated the genetic regulatory network of key metabolites, but also provided a molecular basis for the selection of new varieties with high medicinal value (Gu et al., 2010). This gene-trait association study has opened up a new path for the quality improvement of Linderae obesa.

5 Screening and Evaluation of Superior *Lindera aggregata* Germplasm in Qingchuan County

5.1 Comprehensive evaluation indicators of germplasm resources

The evaluation of Qingchuan Linderae germplasm resources adopts a three-level indicator system, which systematically integrates characteristic parameters at the three levels of phenotype, genetics and function. The phenotypic level focuses on key indicators such as growth dynamics (plant height increment, number of branches), organ morphology (leaf characteristics) and medicinal components (volatile oil, phenolic and flavonoid content). These traits show continuous variation among different geographical populations, providing an intuitive basis for preliminary screening (Gu et al., 2010).

Molecular marker technology has injected a new dimension into the evaluation system. SSR and SNP markers combined with statistical methods such as Shannon diversity index can accurately quantify genetic differences between germplasms. At the same time, functional gene expression profile analysis can effectively identify precious resources with special metabolic potential or stress resistance characteristics (Ye and Li, 2019). This "morphology-genetics-function" trinity evaluation framework not only significantly improves the screening efficiency, but also ensures the scientificity and reliability of the evaluation results.

5.2 Screening of germplasm with superior multi-traits

Multivariate statistical methods provide powerful analytical tools for the screening of superior germplasm of Linderae. Methods such as principal component analysis (PCA) and hierarchical clustering can effectively integrate multidimensional trait data and identify superior germplasm with outstanding comprehensive traits from complex variable relationships. Shi et al. (2024b) successfully established an association model between volatile oil content and key phenotypic traits through PCA analysis, and screened out excellent populations with excellent medicinal value and ecological adaptability.

Geographic ecotype research reveals the interactive effect of environment and genotype. Linderae populations in specific areas of Qingchuan County not only show high volatile oil content, but also maintain rich genetic diversity (Gu et al., 2010; Peng et al., 2020). This excellent characteristic formed by the synergistic effect of "genotype × environment" can be transformed into industrial advantages through targeted breeding, providing an important material basis for the efficient utilization of Linderae resources.

5.3 Screening of germplasm with superior adaptability

In the screening of environmentally adaptable resources, the focus is on the comprehensive evaluation of major stress resistance traits such as drought resistance, cold resistance and salt tolerance. The research strategy usually combines gene expression profile analysis with physiological response experiments to systematically reveal the potential of germplasm to adapt to adversity from multiple dimensions. The results showed that some genes closely related to osmotic regulation and key regulatory factors in the ABA signaling pathway showed a significant high expression trend in high-adaptability materials. The existence of such expression patterns gives it an important reference value as a molecular marker for resistance screening (Xiong et al., 2020).

Lindera aggregata populations show significant differences in environmental adaptability along the altitude gradient: high-altitude populations have outstanding cold tolerance, while low-altitude populations show stronger drought resistance. This ecotype differentiation is the result of long-term natural selection and provides valuable genetic resources for targeted breeding. By accurately matching germplasm characteristics with the environmental conditions of the cultivation site, the success rate and stability of artificial cultivation of *Lindera aggregata* can be significantly improved (Guo et al., 2020).

6 Development and Utilization of *Lindera aggregata* Germplasm Resources in Qingchuan County

6.1 Breeding and promotion of superior germplasm

The development of *Lindera aggregata* germplasm resources in Qingchuan requires the establishment of an efficient modern breeding technology platform. The application of biotechnologies such as tissue culture and asexual reproduction has broken through the limitations of traditional breeding methods (Bh et al., 2017; Salgotra and Chauhan, 2023). These innovative technologies can not only maintain the genetic stability of excellent traits, but also greatly improve the reproduction efficiency, providing a reliable guarantee for the large-scale promotion of high-quality germplasm (Shi et al., 2024b).

Industrial promotion should focus on selecting high-quality germplasm with high medicinal value and wide adaptability. Establishing a germplasm certification system and a regional brand strategy is an effective way to enhance market competitiveness, which can not only ensure the quality of medicinal materials, but also achieve sustainable utilization of resources (Salleh, 2020). At present, classic prescriptions with Qingchuan Linderae as the main drug, such as "Suquan Pills" for the treatment of nocturia and "Zhengqi Tianxiang Powder" for regulating gynecological diseases, are still widely used in clinical practice (Table 1) (Huang et al., 2023), demonstrating its important medicinal value.

6.2 Conservation measures for germplasm resources

Building a complete germplasm preservation system is the fundamental guarantee for maintaining the genetic diversity of Linderae obesa. Modern germplasm resource banks and gene banks have stored rich genetic materials for future breeding and scientific research by cryopreserving seeds, in vitro tissues and DNA samples (Rao and Hodgkin, 2004; Govindaraj et al., 2015; Carvalho et al., 2019). With the help of molecular marker technologies such as SSR and SNP, researchers can accurately classify the stored resources to ensure the complete preservation of genetic diversity (Guan et al., 2020).

Table 1 Examples of traditional Chinese medicine prescriptions containing *Lindera aggregata* (Adopted from Huang et al., 2023)

Preparation name	Composition	Role of LA in prescription	Traditional and clinical uses	References
Suo Quan Wan	*Lindera aggregata*, *Alpinia oxyphylla*, *Dioscorea polystachya*	Leading role	Treatment of nocturnal enuresis and frequent urination caused by kidney deficiency	Weishi Jiacangfang
Tiantai Wuyao San	*Lindera aggregata*, *Aucklandia costus*, *Foeniculum vulgare*, *Citrus reticulata*, *Alpinia officinarum*, *Areca catechu*, *Melia azedarach*, *Croton tiglium*	Leading role	Treat small intestinal hernia, reduce abdominal pain and induce testicles	Sheng Ji Zong Lu
Wu Yao Tang	*Lindera aggregata*, *Cyperus rotundus*, *Aucklandia costus*, *Angelica sinensis*, *Glycyrrhiza uralensis*	Leading role	Treatment of irregular menstruation, dysmenorrhea, premenstrual syndrome, chronic pelvic inflammatory disease, chronic hepatitis, hyperplasia of mammary glands, and chronic gastritis	Ji Yin Gangmu
Wu Mo Yin Zi	*Lindera aggregata*, *Aquilaria sinensis*, *Areca catechu*, fruit of *Citrus aurantium*, *Aucklandia costus*	Leading role	Relieve depression, treat anger and convulsion	Yifang Jijie
Zheng Qi Tian Xiang San	*Lindera aggregata*, *Cyperus rotundus*, *Citrus reticulata*, *Perilla frutescens*, *Zingiber officinale*	Leading role	Treat menstrual irregularities, chest and side pain	Yixue Gangmu
Wu Yao San	*Lindera aggregata*, *Cyperus rotundus*, *Alpinia officinarum*, *Paeonia lactiflora*	Leading role	Reconcile milk to treat children's night crying	Therapeutics of Children’s Disease
Jia Wei Wu Yao Tang	*Lindera aggregata*, *Cyperus rotundus*, *Amomum villosum*, *Aucklandia costus*	Leading role	Promote blood circulation, regulate menstruation, and relieve pain	Ji Yin Gangmu
Bai He Tang	*Lilium brownii* var. *viridulum*, *Lindera aggregata*	Supporting role	Treatment of heartache and epigastric pain	Shifang Kuoge
Bu Xin Tang	*Angelica sinensis*, *Rehmannia glutinosa*, *Paeonia lactiflora*, *Corydalis yanhusuo*, *Lindera aggregata*, *Paeonia* × *suffruticosa*, *Polygala tenuifolia*, (*Poria cocos Schw.*), *Dimocarpus longan*	Supporting role	Treatment of heartache and limb chills	Yu An
Si Mo Tang	*Panax ginseng*, *Areca catechu*, *Aquilaria sinensis*, *Lindera aggregata*	Supporting role	Treatment of chest tightness and anorexia	Ji Sheng Fang
Tong Yu Jian	*Angelica sinensis*, *Carthamus tinctorius*, *Crataegus pinnatifida*, *Lindera aggregata*, *Citrus reticulata*, *Cyperus rotundus*, *Alisma plantago-aquatica*	Supporting role	Activating blood circulation and removing blood stasis, promoting “Qi” and relieving pain	Complete Collection of Jingyue's Treatise
Nuan Gan Jian	*Angelica sinensis*, *Lycium chinense*, (*Poria cocos Schw.*), *Foeniculum vulgare*, *Cinnamomum cassia*, *Lindera aggregata*, *Aquilaria sinensis*	Supporting role	Treat liver and kidney colds, abdominal pain, and hernia	Complete Collection of Jingyue's Treatise

The protection of Linderae resources should adhere to the dual-track strategy of "combining in situ conservation with ex situ conservation". The former helps to maintain the stability of the ecosystem on which the wild population depends, while the latter preserves representative characteristic genotypes through fine management in artificial environments (Hughes et al., 2008). These two methods complement each other in function, which is not only conducive to continuing the natural evolution process of the species, but also provides controllable experimental materials and conditions for subsequent genetic improvement and functional research (Koskela et al., 2014; Fang, 2024), thus building a resource protection system with wide coverage and complete mechanisms.

6.3 Molecular breeding based on superior genes

The high-quality gene resources contained in the germplasm of *Lindera aggregata* in Qingchuan provide an important foundation for molecular breeding. Marker-assisted selection (MAS) technology achieves precise selection of excellent characteristics such as high volatile oil content and strong stress resistance by establishing a stable association between molecular markers and target traits (Upadhyaya et al., 2008; Fang, 2024). This technological breakthrough significantly improved the efficiency and accuracy of breeding.

Breakthroughs in gene editing technology have opened up new avenues for the genetic improvement of *Lindera aggregata*. The CRISPR/Cas9 system can accurately modify genes related to secondary metabolism and stress resistance. Experiments have confirmed that the volatile oil synthesis pathway can be effectively regulated by targeted editing of terpene synthase genes (Shi et al., 2024a). The integrated application of these cutting-edge technologies is pushing *Lindera aggregata* breeding into a new stage of precision and efficiency.

7 Challenges and Future Directions

7.1 Technical challenges in genetic resource analysis

There are still many technical challenges in the study of the genetic resources of Linderae obesa. Although the application of high-throughput sequencing technology provides a new perspective for analyzing genetic diversity, the genomic characteristics of the species itself - including large genome capacity, polyploidy and a high proportion of repetitive sequences - significantly increase the difficulty of data acquisition and analysis. These factors not only increase the cost of research, but also put forward higher requirements for genome assembly and homologous gene identification (Shi et al., 2024b).

The functional verification link also faces technical obstacles. Gene editing and expression regulation experiments require the establishment of an efficient genetic transformation system, and the long growth cycle of Linderae obesa greatly delays the phenotypic observation process. More importantly, there is currently a lack of standardized genetic operation platforms, which seriously restricts the depth of gene function research and the efficiency of application transformation (Salleh, 2020). Breaking through these technical bottlenecks will become an important direction for future research.

7.2 Practical bottlenecks in superior gene application

Although a series of excellent genotypes with potential have been successfully screened at the laboratory level, there are still many technical and practical obstacles to successfully transforming these achievements into real breeding materials. At present, molecular breeding methods have not yet formed an efficient connection with the traditional agricultural production system, and the lack of an innovation chain covering the entire process of "experiment-verification-promotion" has become a key constraint (Chung et al., 2023). In addition, some improved traits, such as high volatile oil content, need to be verified through cross-regional, multi-year field trials for their stability and environmental adaptability (Gu et al., 2010). However, the high human and material investment required for such long-term experiments seriously limits its feasibility in large-scale promotion.

Technology promotion also faces the problem of uneven resource allocation. Grassroots production units generally lack the necessary technical equipment and professionals, making it difficult to implement advanced breeding methods. More complicated is that the regulatory policies and intellectual property disputes of gene-edited crops have set additional obstacles to the commercialization of improved varieties (Koskela et al., 2014). To break through these difficulties, it is necessary to establish a collaborative innovation mechanism between industry, academia and research to promote the benign interaction between technological innovation and policy improvement.

7.3 Future research directions

The integration of multi-omics technologies will become the core direction of *Lindera aggregata* research. Integrating genomic, transcriptomic, proteomic and metabolomic data can systematically reveal the molecular network of active ingredient synthesis and stress response. The introduction of machine learning algorithms can mine key regulatory gene clusters from massive data (Shi et al., 2024a), providing new targets for molecular design breeding.

The construction of an interdisciplinary collaboration system is also crucial. The establishment of an innovation alliance covering genetic breeding, agronomy, ecology and industrial application can accelerate the transformation of scientific research results. The establishment of a regional research center and germplasm bank in Qingchuan will achieve efficient resource integration. Exploring the PPP model can provide continuous support for major scientific research projects and promote the industrialization of excellent germplasm (Upadhyaya et al., 2008). This multi-dimensional innovation system will comprehensively improve the research and application level of *Lindera aggregata*.

8 Concluding Remarks

Qingchuan Linderae shows rich genetic diversity, which is closely related to its ecological adaptation process. Population genetic analysis based on molecular markers such as SSR and SNP not only clarified the genetic structure characteristics of the species, but also successfully screened out characteristic germplasm with high medicinal value (such as volatile oil content) and strong stress resistance. The study also identified key genes that regulate secondary metabolism and environmental adaptation through functional genomics, laying a theoretical foundation for molecular design breeding.

To achieve sustainable utilization of *Lindera aggregata* resources, it is necessary to establish a management system that emphasizes both protection and development. Building a germplasm conservation network that coordinates multiple ecological zones is the basic guarantee for maintaining genetic diversity, and the application of modern breeding technologies such as MAS and CRISPR/Cas9 has greatly improved the efficiency of breeding excellent traits. This "protection-development" collaborative model not only ensures the continuation of species, but also meets the industry's demand for high-quality raw materials.

Technological innovation will promote industrial transformation and upgrading. Integrating cutting-edge technologies such as multi-omics analysis, intelligent breeding and ecological cultivation is expected to establish an environmentally friendly Chinese herbal medicine production system. By deepening industry-university-research cooperation, it will not only strengthen the industrial advantages of Qingchuan *Lindera aggregata*, but also achieve a win-win situation of ecological protection and economic development, and provide a demonstration for the efficient utilization of traditional medicinal resources.

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Conflict of Interest Disclosure

The author affirms that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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