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Screening of Disease-Resistant Germplasm and Its Application in Off-Season Cultivation of *Leonurus japonicus var. hunanensis*

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Abstract This study focused on screening and using the disease resistant germplasm resources of *Leonurus japonicus var. hunanensis*, and analyzed its main diseases in detail. Through field observation, common problems such as powdery mildew, downy mildew and root rot were identified, and their incidence and damage degree under off-season cultivation conditions were evaluated. In the process of off-season planting, due to the changes of temperature, humidity and management conditions, diseases are prone to outbreak, especially in warm and humid environment. The research team conducted field surveys in many planting areas and screened out a batch of disease resistant materials with good comprehensive properties. Among the selected materials with high resistance to powdery mildew, some still maintain a low incidence in high humidity environment, reducing the dependence on chemicals, reducing production costs and improving economic benefits. According to the performance of disease resistant materials, the cultivation mode and management methods were further adjusted. The planting density, water supply and fertilizer use were improved, and the planting environment was properly regulated. The use of disease resistant germplasm combined with optimized cultivation techniques can significantly reduce the occurrence of disease and improve the yield and the level of active ingredients of *Leonurus japonicus var. hunanensis* in off-season production. This not only improves the economic benefits, but also reduces the use of pesticides, helps to achieve the goal of green planting, and provides a reliable basis for the sustainable development of the industry.
Keywords *Leonurus japonicus var. hunanensis*; Disease-resistant germplasm; Off-season cultivation; Molecular marker screening; Disease control

1 Introduction

Leonurus japonicus var. hunanensis is a common medicinal plant, which is mainly used for promoting blood circulation, regulating menstruation, relieving pain and other treatments, and has a long history of application in traditional Chinese medicine (Shang et al., 2014; Li et al., 2019; Miao et al., 2019). Because of its high medicinal value and economic benefits, it has become an important object of cultivation and development.

In recent years, off-season planting has been considered as a feasible way, which can be planted outside the traditional supply period, solve the problem of off-season supply, increase farmers' income, and meet the continuous demand of the market for medicinal materials (Krishna et al., 2024). However, in the actual planting process, there are many problems with this mode, especially the high frequency of disease (Schreinemachers et al., 2016), which seriously limits the plant growth, resulting in reduced yield, weakened efficacy and damaged economic benefits.

In the off-season period, the climate fluctuates greatly, and the high humidity and temperature difference between day and night are obvious. These conditions provide a good environment for the occurrence of diseases. Diseases such as powdery mildew, downy mildew and root rot are more common, and the prevention and control are more difficult (Dahal et al., 2019; Miller et al., 2024). The disease not only affects the normal growth of plants, but also reduces the content of active ingredients such as alkaloids and flavonoids, and reduces the quality of medicinal materials.

There are many methods to solve the disease problem, among which the use of resistant germplasm materials is one of the most practical and economic ways. Screening disease resistant varieties and applying them to the planting process can reduce disease damage, reduce pesticide use, and maintain yield and quality (Scortichini, 2022; Buirs and Punja, 2024).

This study focused on the disease problems encountered in the Off-season Cultivation of *Leonurus japonicus*, screened the germplasm materials with strong disease resistance and stable growth traits, and combined with the field planting experiment, explored its performance in practical application. The study also focused on the improvement of cultivation management measures and environmental control measures, aiming to improve the growth quality and efficacy performance of *Leonurus japonicus* in unsuitable seasons, promote its stable production, meet market demand and improve planting efficiency.

2 Major Diseases of *Leonurus japonicus var. hunanensis* and Their Impact

2.1 Common diseases

Leonurus japonicus is the whole grass of *Leonurus japonicus* at seedling stage, belonging to Labiatae (Figure 1) (Wang et al., 2023a). In practice, powdery mildew is the most serious disease. The disease is caused by the fungus *Podosphaera xanthii*. When it occurs, a layer of white powder will appear on the surface of the leaves and stems. With the development of the disease, the white spots continue to spread, the leaves become weak, the photosynthetic efficiency decreases, and the plants gradually lose vitality. Powdery mildew is easy to occur in the weather conditions of high temperature in the daytime and high humidity at night. If the infection range is large, not only the yield will be reduced, but also the synthesis of important components such as alkaloids in plants will be reduced, thus reducing the quality of medicinal materials (Cho et al., 2013).

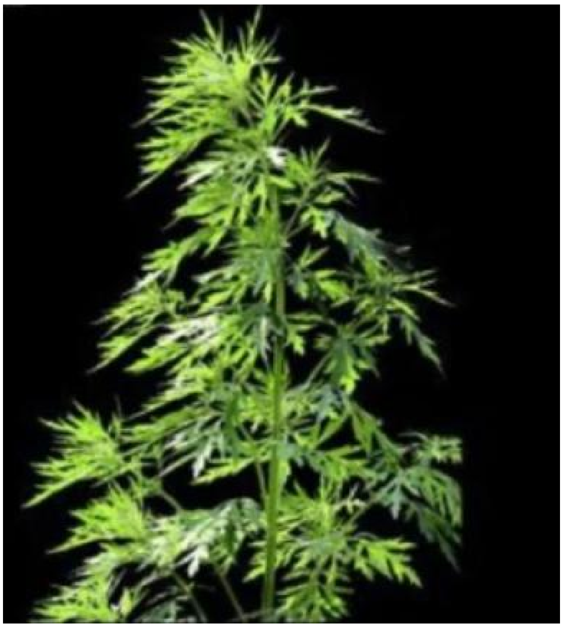

Figure 1 Leonurus japonicus seedlings (Adopted from Wang et al., 2023a)

Downy mildew is also common, and generally occurs in low temperature and high humidity climatic conditions. At the initial stage of infection, yellow or chlorotic patches will appear on the leaf surface, and gray white mold layer will grow on the back of the leaf. In off-season cultivation, especially in rainy days or humid days, downy mildew is particularly prone to outbreak. Such diseases directly affect the normal development of plants, hinder the synthesis of effective components such as *Leonurus japonicus*, and weaken the medicinal effect (Liang et al., 2018).

Root rot is one of the most troublesome problems in off-season cultivation, which is mainly caused by soil fungi such as *Fusarium* spp. and Pythium spp. (zimowska, 2008). After infection, the root will become black and rotten, and the plant is difficult to absorb water and nutrition, and gradually wither. In the soil with poor drainage or long ponding time, the incidence increased significantly, affecting the survival rate and harvest of planting (Sun et al., 2022). In addition, stem rot of Sclerotinia sclerotiorum also occurs from time to time, which can lead to stem rot, plant lodging and significant yield loss (Jiang et al., 2013).

2.2 Factors contributing to disease outbreaks

Warm and humid environments create ideal conditions for disease development. Powdery mildew thrives in warmth, while downy mildew needs high humidity for spore germination and spread. Poor ventilation and improper water management can also make diseases worse (Moparthi et al., 2017).

Diseases spread through several routes—airborne spores, contaminated water, and infected soil. Using infected seeds or plant material increases the risk of long-term pathogen presence. Pathogens like *Podosphaera xanthii* show genetic variability, making them harder to control and more likely to overcome plant resistance (Liang et al., 2018).

2.3 Impact on yield and medicinal quality

During off-season cultivation, frequent disease outbreaks cause a significant rise in plant death rates, especially with root rot or severe powdery mildew. In heavily infected fields, mortality can exceed 90%, resulting in serious economic losses for growers (Sun et al., 2022).

Diseases also reduce the synthesis of key secondary metabolites such as leonurine, lowering medicinal quality. Powdery mildew and downy mildew interfere with photosynthesis and nutrient absorption, while root rot blocks water and nutrient uptake. These combined effects lead to a clear decline in the plant's medicinal value (Yang et al., 2008; Jia et al., 2010; Cho et al., 2013).

3 Key Issues in Off-Season Cultivation of *Leonurus japonicus var. hunanensis*

3.1 Environmental conditions in off-season cultivation

Off-season cultivation of *L. japonicus var. hunanensis* is usually carried out in greenhouses or plastic tunnels. These controlled environments extend the growing season and allow flexible scheduling but also create conditions that favor disease outbreaks. High humidity, poor ventilation, and large temperature differences between day and night are common in greenhouses. These conditions are ideal for fungal pathogens such as *Podosphaera xanthii* (causing powdery mildew) and *Fusarium* spp. (causing root rot). High humidity, in particular, speeds up spore germination and fungal growth, increasing disease pressure (Liang et al., 2018).

The timing of disease and pest outbreaks is closely linked to specific features of off-season systems. For example, high planting density, used to maximize yield, can promote pathogen spread and raise infection rates. Large fluctuations in temperature between day and night can weaken plant defenses, making them more vulnerable to infections. These environmental stresses and cultivation practices interact, showing the need for targeted strategies in off-season disease management (Sun et al., 2022). Techniques like LED light treatments have been shown to improve yield and quality under low-season growing conditions (Lee et al., 2023).

3.2 Technical challenges in off-season cultivation

At present, the most troublesome problem of Off-season Cultivation is that the disease is not easy to control and the control cost is high. Although the use of pesticides, fungicides and other chemical means is common, frequent spraying not only costs money, but also easily makes the bacteria resistant. Sometimes, drug residues are left, which is not conducive to the safety and sales of medicinal materials (moparthi et al., 2017).

Another problem is the disjunction between the research and practical application of disease resistant germplasm. Although many resistant materials have been found by molecular markers and other methods, these achievements are rarely used in actual production. Many greenhouses are still under traditional manual management, and environmental control and pest control are not accurate enough. Advanced technologies such as automatic temperature and humidity regulation system and IPM (Integrated Pest Management) are not used much, which leads to frequent occurrence of diseases. To truly solve these problems, we need to combine the breeding of disease resistant materials with advanced cultivation methods, and work together from seed source to management (Gu et al., 2010).

4 Screening of Disease Resistance Germplasm Resources

4.1 Collection and evaluation of germplasm resources

The first step in screening disease resistant germplasm of *Leonurus japonicus var. hunanensis* is to collect germplasm resources from different ecological regions. Identify its main growth areas in China, and analyze its habitat characteristics and environmental adaptability. These areas include areas with different climatic conditions and disease pressures, so as to ensure the diversity of collected resources and help understand its population dynamics and migration patterns (Figure 2) (Wang et al., 2023b). Germplasm samples were collected from natural populations, cultivation sites and off-season experimental fields to comprehensively capture their genetic variability.

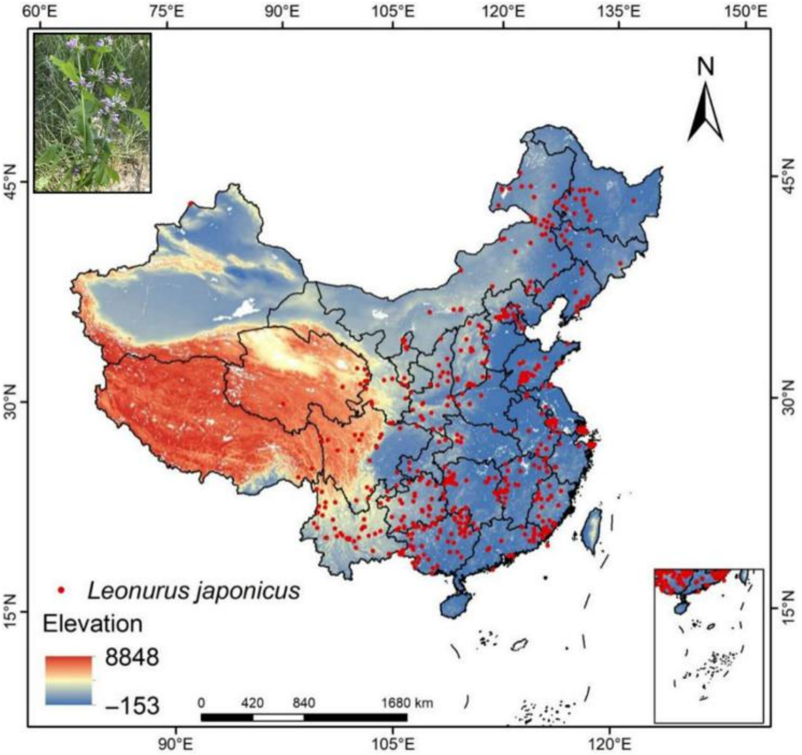

Figure 2 Geographical map of the distribution point of Leonurus japonicus (Adopted from Wang et al., 2023b)

The preliminary evaluation mainly focused on phenotypic traits, including growth rate, plant vigor and apparent resistance to powdery mildew, gray mold and leaf spot. In addition, the genetic characteristics of the collected germplasm were analyzed by molecular markers to determine the variability of the collected germplasm. The combination of phenotypic data and genetic data enables researchers to select potential Germplasms for further evaluation (Sun et al., 2022).

4.2 Evaluation method of disease resistance

The field experiment was used to analyze the difference of disease resistance of germplasm under natural conditions. These tests were conducted in areas with high disease incidence to ensure that plants were exposed to the pressure of natural diseases. The disease resistance performance was determined by evaluating the incidence and severity of powdery mildew and leaf spot disease.

In order to achieve more accurate evaluation, artificial inoculation technology is also used. The pathogen (such as powdery mildew pathogen *Podosphaera xanthii*, *Botrytis cinerea*, *Cercospora* spp.) was inoculated on the germplasm samples. This method can directly compare the disease resistance under standardized conditions. Germplasms with low susceptibility were selected for further study (Liang et al., 2018).

The disease resistance was quantified by incidence, disease index (DI) and disease resistance coefficient. The disease index is calculated according to the severity of plant population symptoms, and the disease resistance coefficient integrates multiple indicators to provide a comprehensive assessment of disease resistance. These quantitative indicators help to screen out germplasms with strong resistance to a variety of diseases (moparthi et al., 2017).

4.3 Molecular marker assisted screening

The genetic diversity of disease resistant germplasms was analyzed by molecular marker technology (including SSR and SNP markers). These markers reveal the polymorphism among germplasms and help identify germplasms with genetic characteristics related to disease resistance (Gu et al., 2010).

Candidate genes related to disease resistance were screened by marker trait association analysis. These studies linked specific genetic markers with observed disease resistance traits, and located loci related to resistance to specific diseases such as powdery mildew. This information provides support for the selection of good germplasm in breeding projects (Sun et al., 2022).

4.4 Screening results and determination of excellent germplasm

According to the results of phenotype and molecular markers, the germplasms with excellent disease resistance and comprehensive agronomic traits were identified. Selection criteria include low disease index, high disease resistance coefficient and excellent growth performance under stress conditions.

An excellent germplasm named "HJ-23" showed excellent resistance to powdery mildew, gray mold and leaf spot in field and artificial inoculation experiments. Molecular analysis showed that it carried alleles of key gene loci related to resistance, and these genes involved in defense signaling pathways (such as MYB transcription factor). HJ-23 also showed high biomass yield and stable motherwort alkali content, making it a strong candidate germplasm for Off-season Cultivation (Sun et al., 2022).

5 Application of Disease-Resistant Germplasm in Off-Season Cultivation

5.1 Seedling production and transplanting techniques

Optimizing seedling production and transplanting is key to fully using disease-resistant germplasm. In greenhouses, seedlings should be grown under controlled conditions to reduce early exposure to pathogens. Practices include disinfecting seedling trays, using sterile substrate, and maintaining optimal germination temperatures (20 ℃~25 ℃) and humidity below 70%. These measures improve survival rates and seedling vigor. In open-field cultivation, resistant germplasm should be planted in well-drained soil with added organic matter to support root development (Sun et al., 2022).

Transplanting time is as important as management. Disease resistant seedlings should be transplanted in a period with mild environmental conditions to avoid extreme humidity or temperature changes. Reasonable plant spacing (usually 30 cm × 20 cm) helps to reduce the spread of pathogens and ensure good ventilation. Inoculating seedlings with beneficial microorganisms (such as *Trichoderma* spp.) before transplanting can enhance disease resistance and promote root growth in the field (moparthi et al., 2017).

5.2 Disease management strategies

Combining disease resistant germplasm with precise control strategies can significantly enhance the effect of disease management in off-season cultivation. Physical prevention and control measures, such as greenhouse ventilation, humidity regulation and continuous monitoring of microclimate conditions, play a key role in inhibiting the spread of pathogens. Controlling the greenhouse humidity below 60% can effectively inhibit the spread of powdery mildew and downy mildew, which are most likely to breed in high humidity environment (Liang et al., 2018).

Biological control measures are complementary to disease resistant germplasms by using biological pesticides and antagonistic microorganisms to inhibit disease outbreaks (Carolan et al., 2017). Beneficial microorganisms such as *Bacillus subtilis* and *Pseudomonas fluorescens* inhibit fungal pathogens through competitive rejection and the production of antibacterial compounds. Biopesticides derived from *Beauveria bassiana* and neem oil provide eco-friendly solutions that can effectively manage fungal and bacterial infections while ensuring plant safety (Gu et al., 2010).

In a greenhouse study, the powdery mildew-resistant line “HJ-23” showed strong performance under high humidity. Compared to susceptible varieties, its disease incidence was only 10%, with a lower disease severity index (DSI). This was due to its innate resistance and compatibility with biocontrol methods. Improved ventilation and regular applications of *Bacillus subtilis*-based biopesticides further enhanced its resistance, resulting in stable growth and high biomass (Sun et al., 2022).

5.3 Effects on yield and quality

The resistant varieties were superior to susceptible varieties in yield, biomass and active ingredient content. The results of field trials showed that the biomass of resistant germplasm increased by 25% compared with non resistant varieties due to the reduction of disease-related losses. The content of leonurine and flavonoids in resistant germplasms was higher, which reflected the direct effect of pathogen pressure reduction on the synthesis of secondary metabolites (Cho et al., 2013).

In a case study evaluating yield, “HJ-23” produced 30% more during off-season cultivation than the susceptible control. Notably, its leonurine content remained stable across different environments, while susceptible varieties showed a 15%~20% drop under similar conditions. These findings highlight the potential of disease-resistant germplasm to maintain high yield and medicinal quality in challenging off-season conditions (Sun et al., 2022).

6 Optimization of Off Season Cultivation Techniques of *Leonurus japonicus*

6.1 Optimization of cultivation mode

Intercropping and rotation have been proved to be effective Off-season Cultivation Strategies in inhibiting pathogen accumulation and reducing disease occurrence. The life cycle of pathogens such as *Podosphaera xanthii* and *Botrytis cinerea* can be broken by intercropping motherwort with non host crops such as beans or grains. The use of disease resistant or non host crop rotation can effectively reduce the soil borne pathogen pool and reduce the risk of root rot outbreak. These cultivation patterns can also improve soil health and nutrient supply, thus indirectly enhancing the disease resistance of plants (Sun et al., 2022).

The adaptability analysis of different germplasm resources under these optimized cultivation modes is very important to ensure yield and disease resistance. Field trials showed that resistant germplasm such as "HJ-23" was stable in the intercropping system. Compared with susceptible varieties, its yield was high and disease index was low. This shows that the combination of agronomic measures and disease resistant germplasm is of great significance for achieving efficient Off-season Cultivation (Gu et al., 2010).

6.2 Relationship between water and fertilizer management and disease control

Precise water and fertilizer management plays a key role in controlling disease and improving the performance of disease resistant germplasm. Excessive application of nitrogen fertilizer may promote the vegetative growth of plants and increase their sensitivity to fungal pathogens such as *Botrytis cinerea*; Precise nitrogen management can enhance plant defense ability and reduce disease incidence (Cui et al., 2020; Gu et al., 2020; Lei et al., 2023). Balanced application of phosphorus and potassium fertilizer can also improve root health and plant activity, thereby enhancing plant resistance to soil borne pathogens (Liang et al., 2018).

A study on the level of nitrogen fertilizer for *Botrytis cinerea* resistant germplasms found that reducing the amount of nitrogen fertilizer by 20% and increasing potassium fertilizer can improve disease resistance and maintain high biomass yield. The disease incidence of disease resistant germplasm treated under this fertilization scheme was reduced by 35%, indicating that precise nutrient management plays an important role in disease prevention and control in off-season cultivation (Moparthi et al., 2017).

6.3 Greenhouse environment control technology

The regulation of temperature, humidity and light in greenhouse is very important for optimizing the performance of disease resistant germplasm and reducing disease occurrence (Koukounaras, 2020). The development of fungal pathogens such as *Cercospora* spp. and *Podosphaera xanthii* can be significantly inhibited by maintaining the greenhouse temperature at 20 ℃~25 ℃ and reducing the relative humidity below 60%. In the season with short illumination time, supplementary illumination can promote plant growth and maintain the resistance characteristics of germplasm (Liang et al., 2018).

A study on the control of greenhouse environment showed that the incidence of leaf spot disease of resistant germplasm planted under the optimal humidity and temperature conditions was 50% lower than that under the untreated environment. This is due to the reduction of pathogenic spore formation and the improvement of plant vitality, highlighting the importance of precise environmental control in disease management (Sun et al., 2022).

6.4 Intelligent monitoring and management

Dynamic disease monitoring and early warning technology are very important for active disease management in off-season cultivation. Sensors for real-time monitoring of temperature, humidity and soil moisture provide operable data for timely adjustment of cultivation measures. The early warning system based on machine learning algorithm can predict disease outbreaks according to environmental data and pathogen dynamics, so as to take preventive measures and minimize crop losses (Gu et al., 2010).

Integrating big data and artificial intelligence (AI) technology into disease control is an important direction of future research. The AI driven model can analyze large data sets from sensors, UAVs and weather forecasts to optimize irrigation, fertilization and pest control strategies (Maraveas, 2022; Behzadipour et al., 2024). For example, AI image recognition technology can recognize the early symptoms of powdery mildew, gray mold and other diseases, so as to achieve precise and targeted intervention. These innovations can significantly improve the efficiency and sustainability of the off-season cultivation system (Liang et al., 2018).

7 Promotion and Value of Disease-Resistant Germplasm

7.1 Breeding and promotion strategies

Breeding and promoting disease-resistant germplasm of *Leonurus japonicus var. hunanensis* is essential for sustainable production and meeting market demand. Breeding programs should focus on developing high-yielding, disease-resistant varieties that adapt to different regions. Marker-assisted selection (MAS) can be used to speed up the development of these lines. Demonstration fields should be established to test germplasm under local climate and soil conditions. These sites also serve as platforms for farmer training and technology extension (Sun et al., 2022).

To improve accessibility and usage, a germplasm database should be developed. It should include performance data, resistance traits, and regional adaptability. This would support breeders, researchers, and growers in selecting appropriate lines. Digital platforms and big data tools can help update and expand the database, encouraging collaboration and broader use of resistant germplasm (Gu et al., 2010).

7.2 Economic and ecological benefits

The use of disease resistant germplasm has brought significant economic and ecological benefits. By reducing the dependence on chemical pesticides, disease resistant germplasm reduced the cultivation cost and reduced the risk of pesticide residues in medicinal products. Planting disease resistant varieties of *Leonurus japonicus* farms can reduce the use of pesticides by up to 40%, thereby achieving significant cost savings and improving profit margins. This reduction also alleviates the impact of Off-season Cultivation on the environment and promotes the development of sustainable agricultural practices (Liang et al., 2018).

Green production practice has been further enhanced through the use of disease resistant germplasm. For example, combining these germplasm resources with precise water and fertilizer management and biological control strategies can build a low input, eco-friendly cultivation system. This method not only improves soil health and biodiversity, but also promotes long-term ecological balance by reducing the accumulation of harmful agricultural chemicals in the environment (Moparthi et al., 2017).

7.3 Enhancing industry competitiveness

The application of good germplasm has played an important role in improving the competitiveness of *Leonurus* *japonicus* industry. Disease resistant varieties meet the market demand for high-quality medicinal products by ensuring stable production and maintaining high levels of active ingredients (such as leonurine and flavonoids). This reliability in yield and quality has improved the market value of *Leonurus japonicus* and its position in the domestic and foreign markets (Cho et al., 2013).

By combining disease resistant germplasm with optimized cultivation modes (such as intercropping and greenhouse control system), regionalization and large-scale high-efficiency Off-season Cultivation technology further promoted the development of the industry. This scale can ensure that even small farmers can adopt these technologies, so as to increase production capacity and promote regional economic development. Therefore, the adoption of disease resistant germplasm provides strategic advantages for the *Leonurus japonicus* industry, supporting its sustainable development and enhancing its global competitiveness (Sun et al., 2022).

8 Challenges and Future Directions

8.1 Technical bottlenecks in disease resistance germplasm screening

There are significant technical bottlenecks in the selection of disease resistant germplasm of *Leonurus japonicus*, especially the difference between the disease inducing environment and the natural environment under experimental conditions. Artificial inoculation and control experiments usually can not fully reproduce the complexity of the natural environment, which leads to inconsistent evaluation results of germplasm resistance in field application. For example, different pathogen populations, climatic conditions and interactions with soil microbial communities are difficult to reproduce in control experiments, which limits the accuracy of resistance assessment (Sun et al., 2022).

Another bottleneck is the limitation of molecular marker techniques (such as SSR and SNP markers) in capturing the complexity of complex resistance traits. Although molecular markers perform well in identifying specific loci associated with single resistance traits, they are usually difficult to cover the polygenic characteristics associated with multiple disease resistance. This limitation hinders the development of Germplasm with broad-spectrum resistance, which is essential for sustainable production in off-season cultivation (Liang et al., 2018).

8.2 Long term utilization of disease resistant germplasm

The long-term stability of disease resistant germplasm is a key challenge, mainly due to the variation and adaptability of pathogens. Pathogens such as *Podosphaera xanthii*, which causes powdery mildew, and *Botrytis cinerea*, which causes *Botrytis cinerea*, have high genetic variability and can overcome the disease resistance mechanism of plants over time. Resistant germplasms will exert selection pressure when continuously exposed to these pathogens, thus accelerating the evolution of toxic pathogen strains. This phenomenon is called resistance breakdown, which seriously threatens the durability of resistant germplasm (moparthi et al., 2017).

The long-term use of resistant germplasm without appropriate rotation or diversification strategies will increase the vulnerability associated with monoculture. This will not only reduce the effect of disease resistance, but also aggravate the problem of soil borne diseases, highlighting the necessity of combining integrated management practices to maintain the effectiveness of disease resistant germplasm (CHO et al., 2013).

8.3 Future research directions

Future research should focus on the use of comprehensive genomics techniques (such as genomics, transcriptomics, proteomics and metabonomics) to explore the molecular mechanism of disease resistance of *Leonurus japonicus*. These methods can comprehensively reveal the regulatory networks and pathways related to resistance traits. Transcriptomic analysis under natural disease conditions can identify differentially expressed genes, and proteomic research can reveal the key post-translational modifications in resistance. By integrating these data sets through systems biology methods, new resistance genes and pathways can be found, thus promoting the development of more resistant germplasm (Sun et al., 2022).

In order to achieve variety innovation and large-scale promotion, the development of advanced molecular breeding system is very important for Disease Resistance Germplasm. Crispr/cas9 gene editing and other technologies can be used to introduce precise resistance traits, and marker assisted selection (MAS) can accelerate the breeding process by associating target traits with molecular markers (Huang, 2024). Genome selection (GS) combined with genome-wide markers can predict the breeding value of germplasm in complex resistance traits. By integrating these technologies, breeding programs can produce Germplasm with enhanced resistance, higher yield and better adaptation to Off-season Cultivation Conditions (Gu et al., 2010).

Future work should also focus on the combination of molecular breeding and precision agriculture technology. Combining disease resistant germplasm with intelligent disease monitoring system and real-time environmental control can optimize cultivation practice and further improve the performance of disease resistant varieties (Li, 2024). By solving the technical bottlenecks and long-term challenges, these advances can pave the way for the sustainable large-scale cultivation of *Leonurus japonicus* in a diversified environment.

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Conflict of Interest Disclosure
The authors affirm 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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