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Original Article

Analysis of Genetic Diversity of *Botrytis* Pathogens Causing Broad Bean Chocolate Spot in Qinghai Province

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Abstract:
This study investigated the cultural characteristics and pathogenicity isolates of broad bean chocolate spot pathogens collected from 352 fields across 143 villages, 66 towns, and 4 prefectures in Qinghai Province, where broad beans are predominantly cultivated. Genetic diversity analysis was conducted on the dominant Botrytis pathogens causing broad bean chocolate spot, with populations grouped by species and geographical origin. Results showed that the growth rate of Botrytis strains primarily ranged between 20-25 mm/d (68 strains) and 15-20 mm/d (96 strains), indicating an overall rapid growth rate. Based on colony characteristics, spore-type pathogens were the most prevalent. Pathogenicity assays revealed that most pathogens exhibited strong to moderate virulence, with variations in virulence distribution among strains from different geographical origins. Using 15 selected ISSR primers, 235 Botrytis strains were amplified, yielding 166 loci with 100% polymorphism. Analyses indicated genetic differences among strains from different geographical regions, which correlated with their geographical locations, alongside evidence of gene flow between regional populations. Genetic diversity analysis by pathogen species showed scattered relationships and significant genetic differences among populations. This study provides a critical basis for understanding the genetic evolution of Botrytis, the dominant pathogen of broad bean chocolate spot.

Keywords: Broad bean chocolate spot, Cultural characteristics, Pathogenicity, Genetic diversity, ISSR markers

1. Introduction

Broad bean (*Vicia faba* L.) is a characteristic cash crop in Qinghai Province, one of China's major broad bean-producing regions (Liu. 2018). Rich in high-quality protein, carbohydrates, minerals, vitamins, and trace elements (Cui. et al. 2012; Zhuang. et al. 2018), broad beans serve as food (Li. et al. 2015), medicinal resources(Wang. et al. 2007a), and a crucial component of dietary diversification. They are also used as feed to improve fish meat quality (Fu. et al. 2024) and contribute to soil fertility, soil structure improvement, and enhanced nitrogen fixation when cultivated (Fu. et al. 2024; Zhu. et al. 2023). However, broad bean production is vulnerable to various pests and diseases (Zhou. et al. 2016), among which chocolate spot is a primary threat, severely hindering the development of the broad bean industry and requiring urgent resolution (Yu. et al. 2021; Yu. 1979; Xu. et al. 2016; Wang. et al. 1997).

Broad bean chocolate spot is a significant fungal disease caused by a complex of Botrytis species, primarily Botrytis cinerea, Botrytis fabae, and Botrytis fabiopsis (Deverall. et al. 1961; Zhang. et al. 2010). B. cinerea exhibits high morphological variability in culture (Zhang. et al. 2018), with single strains capable of forming sclerotia, hyphae, and diverse cultural forms, enhancing its adaptability to the environment. The pathogenicity of strains varies among different strains and different crops (Bi.et al.2023). A study by Brauna et al (Brauna.et al. 2023) showed that the pathogenicity of broad bean chocolate spot is affected by the type of crop. At present, research on the pathogen of broad bean chocolate spot in Qinghai Province is in the preliminary stage. However, Qinghai Province is an area with complex ecological environment and changeable climatic conditions, and whether there are regional differences in the pathogen of broad bean chocolate spot remains an unsolved scientific problem.

ISSR (Inter-simple sequence repeat) is an amplification reaction using single long oligonucleotides anchored to simple sequence repeats at both ends, which was first described by Meyer et al(Meyer. et al.1993). The ISSR molecular marker technology has been widely applied in research fields such as the population genetic structure, genetic diversity, pathogen classification, and genetic relationship between strains of plant pathogens (Wen. 2010). At present, there are few reports on the genetic diversity analysis of the pathogen causing broad bean chocolate spot at home and abroad. Huang Yan (Huang. 2012) found that there are certain genetic variations among the populations of *Botrytis* pathogens in different geographical regions. The results of Aouzal et al (Aouzal. et al. 2022) showed that the *Botrytis cinerea* population in Morocco exhibits high genetic diversity. Samuel et al (Samuel. et al. 2012) conducted studies on the pathogenicity and genetic diversity of the pathogen causing broad bean chocolate spot in different agricultural ecological zones in Ethiopia, and evaluated the quantity and distribution of genetic variations within and between broad bean populations from the humid and semi-humid agricultural ecological zones in northern Ethiopia.

In this study, the dominant pathogen *Botrytis* causing broad bean chocolate spot in Qinghai Province was divided into different populations according to their geographical origin, cultural characteristics, growth rate, etc., for genetic diversity analysis. The purpose is to clarify the genetic relationship of the pathogen causing broad bean chocolate spot in Qinghai Province, understand the genetic relationship between various populations, and thus provide a preliminary theoretical basis for the development, utilization, and control of such fungal resources.

Materials and Methods

Pathogen Sources

The 235 Botrytis isolates used in this study were collected and identified from 352 fields across 143 villages, 66 towns, and 4 prefectures in Qinghai Province between 2020 and 2023, encompassing 11 Botrytis species (Annex 1).

Determination of Cultural Characteristics

Cultural characteristics of 235 *Botrytis* pathogens were observed. The purified pathogens were inoculated onto new PDA medium and cultured at 25°C in the dark. The colony diameter was measured using the cross method on the 2nd and 4th days (Hui. et al. 2021), and after the measurement on the 4th day, the colony morphology, color, and conidial conditions were observed and recorded. In one replicate test, each strain was tested in 3 dishes. After the measurement, the culture was continued until the 15th day, and the presence or absence of sclerotia was observed and recorded.

Pathogenicity Assay

The pathogenicity of the isolated and identified *Botrytis* pathogens was determined using the detached leaf method (Castro. et al. 2003). The mycelial discs were inoculated onto fresh and healthy broad bean leaves (Yun-122 and Lingxi 1 Cun), with pure PDA used as the blank control (all the above operations were carried out in a clean bench). After 3 days of cultivation, observations were made and recorded, and the diameter of the lesion at the inoculation site was measured using the cross method. Referring to the method of Huang Yan(Huang. 2012), the disease severity was statistically analyzed according to the size of the lesion diameter.

Establishment of ISSR-PCR Reaction System

DNA was extracted from 235 isolates using the CTAB method (Doyle. et al. 1987).

PCR Amplification System: A 10 μL reaction mixture containing 5 μL Premix Taq, 1 μL of 2 μmol/L primer, 1 μL of 50 ng/μL DNA, and ddH_2O to volume.

PCR Program: 95°C pre-denaturation for 4 min; 35 cycles of 94°C denaturation for 40 s, annealing for 40 s, and 72°C extension for 1 min; final extension at 72°C for 10 min.

Electrophoresis: Amplified products were separated on 8% polyacrylamide gels at 300 V and 150 mA for 90 min, then photographed.

ISSR Primer Screening

Six random isolates were used to screen 10 reference primers from Wang et al (Wang. et al. 2007b) and Huang (Huang. 2012), plus 10 additional polymorphic primers for Botrytis designed by Yangling Tianrun Aoke Biotechnology Co. Ltd. Primers yielding clear, reproducible bands were selected for genetic diversity analysis. For low-clarity bands, an 8-gradient annealing temperature test (±6°C from the initial temperature) was performed to determine optimal annealing temperatures.

Data Statistics and Analysis

Statistical analysis was performed using Excel for data processing. Amplified bands were converted into a binary matrix (1 = clear band, 0 = absent or unclear band) and saved as an Excel file. Popgene32 software (Nei. 1973; Yeh. et al. 1999) was used to calculate genetic diversity indices: Shannon's index, Nei's index, number of alleles, and effective alleles within and between populations. Nei's genetic distances among populations grouped by geographical origin. SAHN clustering in NTSYSpc 2.10e (Rohif. 2000) was used to construct dendrograms, and GenAlEx was used for PCoA analysis based on genetic similarity coefficients. MEGA7.0 (Kumar. et al. 2016) was used to build individual-based phylogenetic trees.

Results and Analysis

Analysis of Cultural Characteristics

Colony Morphology of Botrytis **Isolates**

After 14 days of cultivation on PDA, the colonies of *Botrytis* pathogens showed significant differences. Based on the colony characteristics, the pathogens were classified into sclerotial type, mycelial type, and spore type, as shown in Figure 1a. There were 80 sclerotial-type, 20 mycelial-type, and 135 spore-type pathogens. The distribution of the three cultivation morphologies of *Botrytis* pathogens in 12 districts and counties is shown in Figure 1b.

Analysis of colony growth rate of *Botrytis* pathogens.

According to the colony growth rate, they were divided into 7 grades, and the distribution of strains in each growth interval was counted. As shown in Figure 1c, the number of strains with a growth rate of 20-25 mm/d was the largest, reaching 96. Overall, Xunhua County had the most abundant strains, with all 7 growth rate grades represented. Generally, the strains from Hualong County all had relatively fast growth rates (Figure 1d).

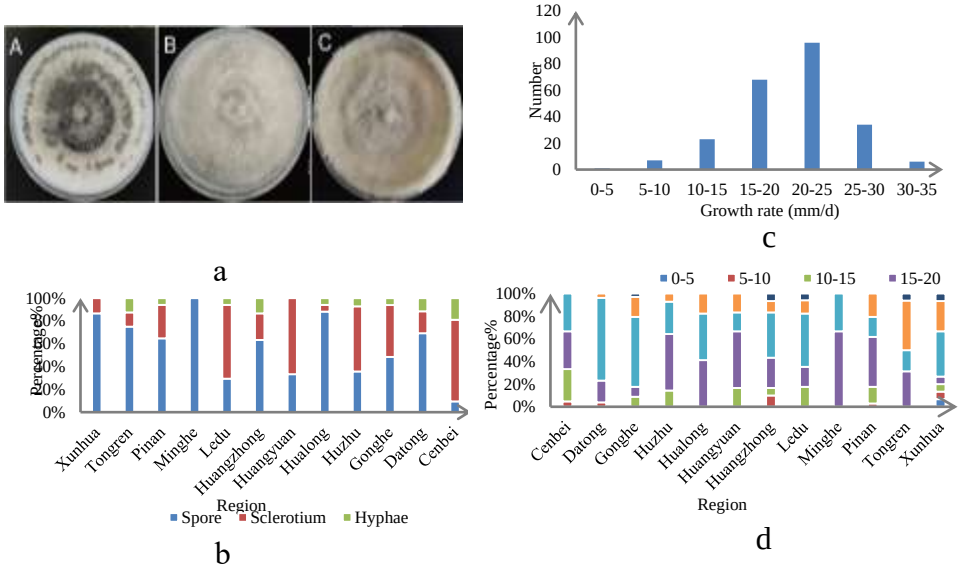


Fig a Three culture types of *Botrytis* spp. isolates

Note: A. Sclerotial type (ZS031A); B. Hyphal type (ZS048D); C. Conidial type (PY015) The sclerotial type produces a large number of sclerotia, with a small amount of aerial hyphae and little or no sporulation. The hyphal type does not produce sclerotia, has vigorous aerial hyphae, and produces little or no spores. The conidial type has vigorous aerial hyphae, produces a large number of spores during cultivation, and forms few or no sclerotia.

Fig b The distribution of culture morphology of three Botrytis pathogens in 12 districts and counties

Fig c Statistical figure of the number of pathogens in different growth rate ranges of Botrytis spp.

Fig d Growth rate of Botrytis spp. pathogens in 12 counties

Figure 1 Physiological characteristics of *Botrytis* spp under different classification scenarios.

Pathogenicity Analysis

Analysis of Pathogenicity of Botrytis Pathogens on Different Varieties

Detailed results of the pathogenicity of 235 *Botrytis* strains are shown in Annex 1. 235 strains exhibited significantly stronger pathogenicity on Yun-122 than on Lingxi 1 Cun, and *B. fabae* showed the strongest pathogenicity (Annex 3). The pathogenicity of the strains on Yun-122 was stronger than that on Lingxi 1 Cun (Figure 2a). When the same strain was inoculated onto detached leaves of the two broad bean varieties in the laboratory, the pathogenicity identification results showed that the lesion size on Yun-122 was significantly larger than that on Lingxi 1 Cun, with an obvious difference (Figure 2b).

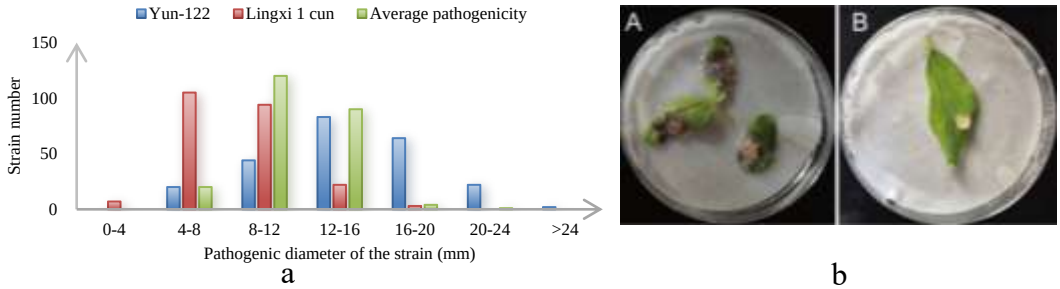


Fig a Statistics of the diameter of different pathogens after inoculation of different varieties

Fig b The results of differentiating the pathogenicity of different faba bean varieties inoculated with test isolate

Note: A is the symptom of the pathogen inoculated on Yun-122; b is the symptom of the pathogen inoculated on Lingxi 1 cun, and the same pathogen was inoculated.

Figure 2 Pathogenic status of *Botrytis* pathogens.

Screening of ISSR Primers and Determination of Annealing Temperatures

From 20 ISSR primers, 15 primers that produced clear, bright(Annex 2), and highly reproducible electrophoresis bands were selected for subsequent ISSR genetic diversity analysis of Botrytis pathogens. These 15 ISSR primers were then used for PCR amplification of 235 samples, resulting in a total of 166 polymorphic bands with a polymorphic locus percentage of 100%.

Genetic Polymorphism Analysis of ISSR Target Fragments in Broad Bean Chocolate Spot Pathogens

Results from 12 geographical populations showed that the average percentage of polymorphic bands was 91.22%. Based on the calculation results of Nei's diversity index and Shannon's diversity index, the total genetic diversity related to different geographical populations of *Botrytis* pathogens was 0.3745 and 0.5538, respectively (Table 1). The above results indicate that there is abundant genetic diversity among different geographical populations of *Botrytis* pathogens causing broad bean chocolate spot in Qinghai Province.

Table 1 Genetic diversity of 12 *Botrytis* spp. populations by ISSR analysis

Population	PPB (%)	*Na*	*Ne*	*H*	*I*
Xunhua	93.98	1.9398	1.6226	0.3526	0.5189
Tongren	95.78	1.9578	1.6338	0.3615	0.5326
Pingan	98.80	1.9880	1.6314	0.3676	0.5456
Minhe	81.33	1.8133	1.5208	0.2984	0.4426
Ledu	87.95	1.8795	1.5377	0.3163	0.4724
Huangzhong	96.39	1.9639	1.6048	0.3495	0.5192
Huangyuan	75.30	1.7530	1.4743	0.2745	0.4084
Hualong	93.98	1.9398	1.6137	0.3548	0.5243
Huzhu	88.55	1.8855	1.5626	0.3248	0.4817
Gonghe	95.18	1.9518	1.6149	0.3536	0.5232
Datong	96.39	1.9639	1.6183	0.3561	0.5273
Chenbei	90.96	1.9096	1.5742	0.329	0.4879
Average	91.22	1.9122	1.5841	0.3366	0.4987
Total	100.00	2.0000	1.6495	0.3745	0.5538

Note: PPB: Polymorphic band ratio. *Na*: Number of observed alleles. *Ne*: Effective numbers of alleles. *H*: Nei 's gene diversity index. *I*: Shannon's information index.

Genetic Differentiation of Broad Bean Chocolate Spot Pathogens

Analysis of molecular variance (AMOVA) of ISSR revealed that among the 12 geographical populations, 4.503% of the genetic variation existed among populations, and 95% of the genetic variation existed within populations (Table 2).

Table 2 Molecular variance analysis (AMOVA) between and within populations of 12 different geographical sources of *Botrytis* spp.

Source of variation	Df	Sum of square	Variance component	Percentage of variation	P value
Among pops	11	634.048	1.460	5%	<0.001
With pops	223	6601.825	29.605	95%	
Total	234	7235.872	31.065	100%	

Homology Analysis of Broad Bean Chocolate Spot Pathogens

It can be seen from Annex 4 that the average values of genetic distance and genetic similarity

were calculated for the 12 populations of pathogens causing broad bean chocolate spot from different geographical sources in Qinghai Province. The average genetic distance among the 12 populations was 0.0542, and the average genetic similarity was 0.8642. The genetic distance between Gonghe County and Huzhu County was the smallest (0.0263). The results of Huangyuan County and Xunhua County showed that the farther the genetic distance, the lower the genetic similarity. Based on the comprehensive analysis of the above results, it can be concluded that there is a certain relationship between genetic distance and genetic similarity.

Analysis of Population Structure of Broad Bean Chocolate Spot

Using NTSYS-pc software, a UPGMA cluster analysis was performed based on the 12 geographical populations (Figure 3). The results showed that there was an obvious clustering phenomenon in the populations. At a similarity coefficient level of 0.94, the 12 geographical populations were divided into 3 groups and 6 subgroups. The genetic distance between Gonghe County and Datong County was close, with the smallest genetic difference. At a genetic similarity coefficient of 0.96, Ledu District and Chengbei District formed independent branches, each being a subgroup, and their genetic distances from other populations were also relatively large.

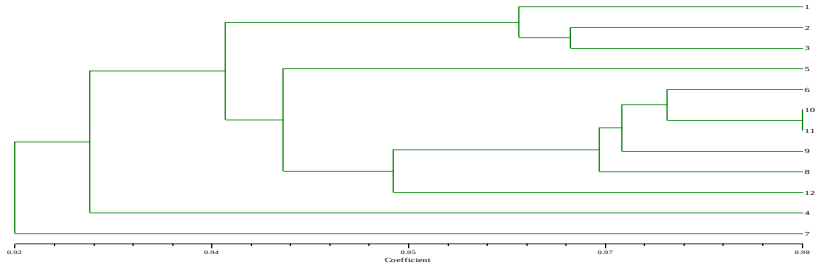
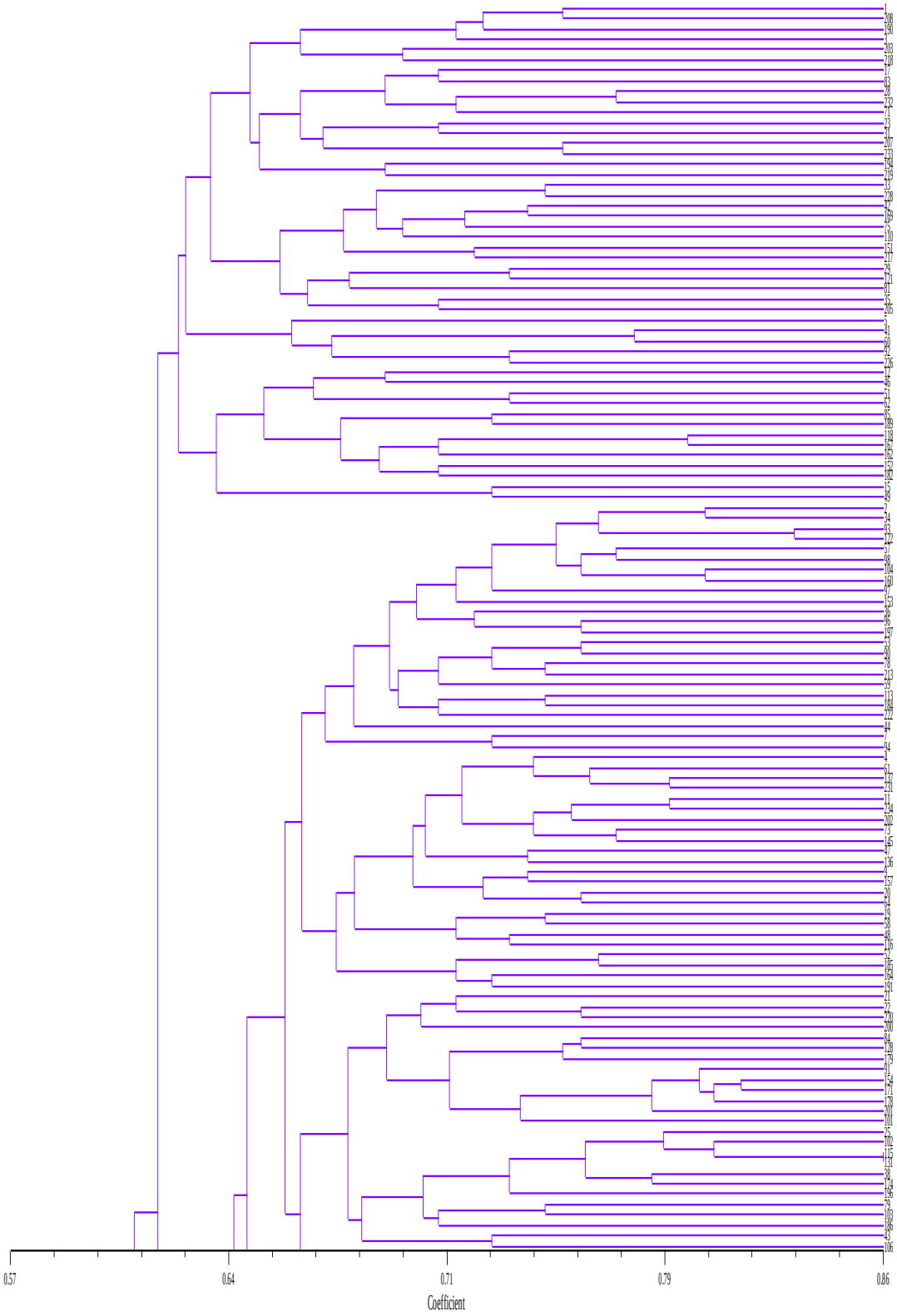
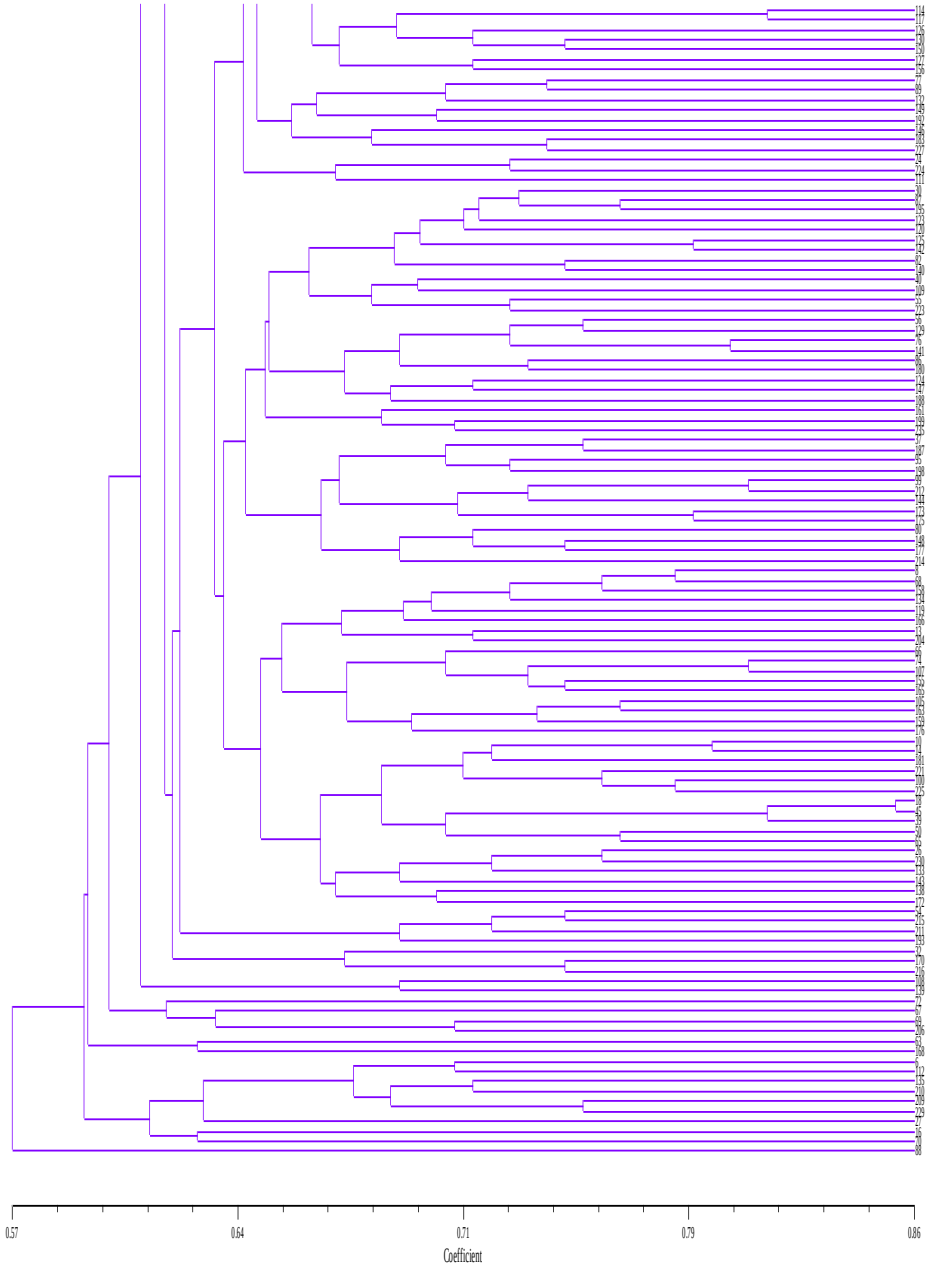


Figure3 Cluster Analysis of 12 Geographical Populations of *Botrytis* spp.

Note : population 1 is Xunhua County, population 2 is Tongren City, population 3 is Ping 'an District, population 4 is Minhe County, population 5 is Ledu District, population 6 is Huangzhong District, population 7 is Huangyuan County, population 8 is Hualong County, population 9 is Huzhu County, population 10 is Gonghe County, population 11 is Datong County, population 12 is Chengbei District.

Cluster analysis of 235 *Botrytis* strains was performed using NTsys2.10e software, and the results are shown in Suppl 1. At a similarity coefficient level of 0.605, all 235 strains were divided into 5 groups (I, II, III, IV, V). Among them, Subgroup 1 mainly included pathogens from Gonghe County and Huangzhong District, indicating that the adjacent *Botrytis* pathogens from different geographical populations have a close genetic relationship with a high genetic similarity coefficient, and there is a certain degree of gene flow between different pathogen species.


0.57
0.64
0.71
0.79
0.86
Coefficient



Suppl 1Cluster analysis of 235 isolates of *Botrytis* spp.

Note : The pathogen number is the number used in the previous sequencing.

Based on the ISSR experimental data, a PCoA (Principal Coordinate Analysis) was performed on 235 individuals from 12 geographical populations (Figure 4). In the two-dimensional scatter plot, most individual plants from different populations formed overlapping distribution areas. The individual plants from Gonghe County, Datong County, and Huangzhong District clustered together with mutual overlaps, showing a relatively close relationship. The two populations from Minhe County and Huangyuan County were relatively distant and scattered, with only some individuals overlapping partially with those from other populations. The overlapping populations from Tongren County and Ping'an District gradually showed differentiation. Meanwhile, Hualong County and Huzhu County were connected through their overlapping populations. These results are consistent with those of the UPGMA cluster analysis. Compared with the PCoA analysis, the UPGMA cluster analysis revealed that, in terms of genetic relationship, the analysis at the individual plant level shows a stronger tightness than that at the inter-population level.

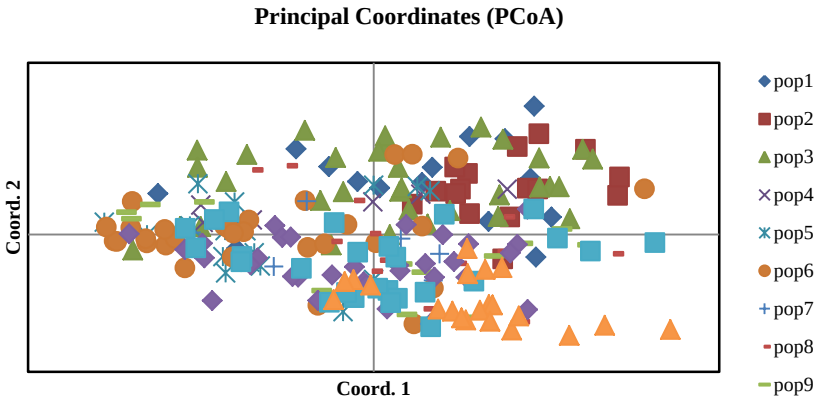


Figure.4 PCoA analysis of 235 individuals from 12 geographical populations of *Botrytis* spp.

Note : population 1 is Xunhua County, population 2 is Tongren City, population 3 is Ping 'an District, population 4 is Minhe County, population 5 is Ledu District, population 6 is Huangzhong District, population 7 is Huangyuan County, population 8 is Hualong County, population 9 is Huzhu County, population 10 is Gonghe County, population 11 is Datong County, population 12 is Chengbei District.

Population structure analysis based on mathematical models was performed on 235 strains, and the appropriate number of populations was selected according to the maximum likelihood value corresponding to K. When K=4, ΔK showed an obvious peak (Figure 5a), and the pathogenic fungi had the highest similarity. Therefore, K=4 was selected as the final number of populations according to the principle of the maximum likelihood value. The 235 strains were divided into 4 groups with different genetic compositions, where different colors represent different genetic compositions. Most individuals in the 4 groups could be distinguished, but there were also many interactive phenomena (Figure 5b), indicating frequent gene exchange of pathogenic fungi among different geographical populations, which may be caused by factors such as gene flow and mutation.

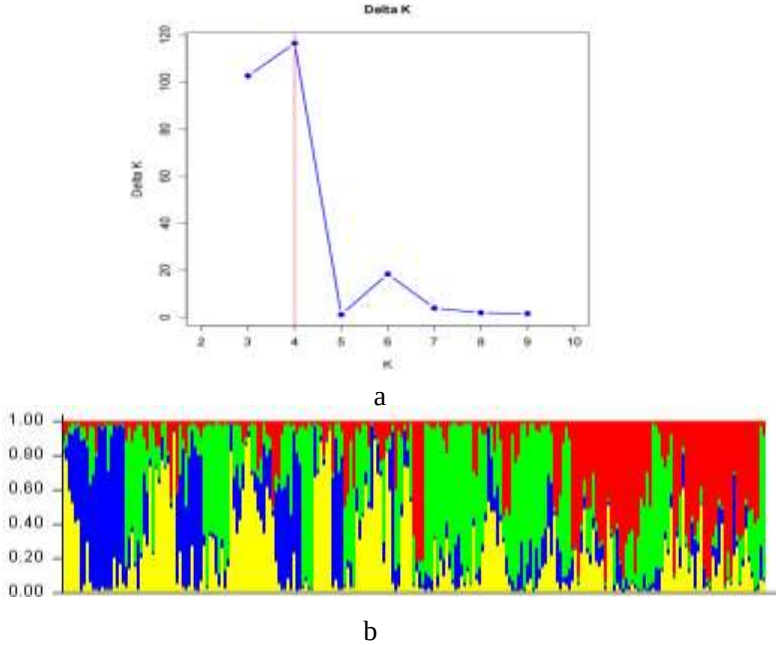


Fig a Line chart of K value changing with ΔK sequence

Fig b Population structure result of 235 Botrytis fabae.

Figure 5 Analysis of the population structure of *Botrytis* pathogens

Discussion

Broad bean chocolate spot is a disease caused by complex infections, with Botrytis pathogens being the main causative agents (Alemneh. et al. 2024). Previous studies have shown that Botrytis pathogens exhibit different growth morphologies on culture media, such as sclerotial type and mycelial type (Zhang . et al. 2018). In this study, we also observed three distinct morphologies on the culture medium: spore type, sclerotial type, and mycelial type, among which the spore type was dominant. Many studies have indicated that the pathogenicity of fungi varies across different crop varieties (Chen. et al. 2024; Zheng. et al. 1993). When we tested the pathogenicity of Botrytis pathogens using two different broad bean varieties, we also found differences in the strains between the two varieties. Specifically, when comparing the strains overall, their pathogenicity was weaker on 'Lingxi Yicun' and stronger on 'Yun-122'. Analysis of the pathogenicity of different pathogen types revealed that *B. fabae* had the strongest pathogenicity. From a geographical perspective, the pathogenicity of Botrytis pathogens varied across each district and county. However, areas such as Tongren, Gonghe, and Xunhua had a relatively high proportion of highly pathogenic strains, while Hualong County had the highest proportion of weakly pathogenic strains. We speculate that different physiological races may be prevalent in different regions, and the differentiation in pathogenicity among fungal populations is attributed to differences in physiological races. Although geographical distance and variations in environmental conditions may be the main factors contributing to the differentiation of physiological races, the differentiation in pathogenicity within fungal populations suggests the possibility of population migration and gene exchange between adjacent regions.

As an efficient molecular marker technique (Gemmill. et al. 2021), ISSR markers have shown unique advantages in studying the genetic diversity of pathogens (Gramaje. et al. 2014; Abadio. et al. 2012). Based on the characteristics of microsatellite sequences in the genome, it amplifies the regions between repetitive sequences using anchored primers, enabling rapid detection of polymorphic loci within pathogen populations (Abadio. et al. 2012; Wang. 2002). In this study, ISSR primers amplified rich band polymorphisms among strains from different geographical origins and populations, with the polymorphic band ratio reaching 100%, confirming that this technique can effectively reveal the genetic variation of pathogens. Compared with traditional morphological identification and biochemical analysis, ISSR markers can directly reflect genetic differences at the DNA level, overcoming the limitation that phenotypic characteristics are easily affected by the environment, thus providing a stable and reliable basis for analyzing the population structure of pathogens. The results of genetic diversity of broad bean chocolate spot pathogens revealed by ISSR markers showed that there was no significant difference in the fungal species whether viewed from geographical origin or population. However, there was a certain degree of exchange between the pathogens. The reason for this result may be the exchange of broad bean seeds between regions in Qinghai Province. Another possibility is that the seeds used by cooperatives engaged in large-scale broad bean cultivation are purchased, and factors such as variety differences and seed coating treatments may change the fungal community structure. Additionally, differences in climatic conditions lead to a complex and chaotic occurrence of broad bean chocolate spot. Furthermore, AMOVA (Analysis of Molecular Variance) indicated that 95% and 96% of the genetic variation exists within populations, suggesting that random mutations during the asexual reproduction of pathogens contribute significantly to genetic diversity, while genetic recombination resulting from sexual reproduction may only play a key role in local regions.

ISSR marker analysis provides theoretical support for the precise prevention and control of broad bean chocolate spot. It can monitor the dynamics of pathogen populations (Alemneh. et al. 2024), evaluate the impact of fungicide selection pressure on genetic structure, and prevent the spread and enrichment of resistant strains. Future research may involve joint analysis of ISSR markers and phenotypic data (Brauna. et al. 2023) to establish prediction models for genetic diversity and disease prevalence (McDonald. et al. 2002; Taliadoros. et al. 2023), thereby providing more accurate theoretical support for the intelligent

prevention and control of broad bean chocolate spot.

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References

1. Abadio AK, Lima SS, Santana MF, et al. 2012. Genetic diversity analysis of isolates of the fungal bean pathogen Pseudocercospora griseola from central and southern Brazil. Genet Mol Res. 11:1272-9. doi: 10.4238/2012.May.14.1.
2. Alemneh HT, Molla AE, Makinde OD. 2024 Cost-effective and optimal control analysis for mitigation strategy to chocolate spot disease of faba bean. Sci Rep. Oct 25;14(1):25360. doi:10.1038/s41598-024-74121-0.
3. Aouzal S, Krimi Bencheqroun S, Zelmat L, et al. 2022; Genetic diversity and population structure of Moroccan *Botrytis* spp. strains, causing chocolate spot disease in faba bean. Archives of Phytopathology and Plant Protection. 55:761-772. https://doi.org/10.1080/03235408.2022.2047368.
4. Bi K, Liang Y, Mengiste T, et al. 2023; Killing softly: a roadmap of *Botrytis cinerea* pathogenicity. Trends Plant Sci. 28:211-222. doi: 10.1016/j.tplants.2022.08.024.
5. Brauna-Morževska E, Stoddard FL, Bankina B, et al. 2023; Evaluation of pathogenicity of *Botrytis* species isolated from different legumes. Front Plant Sci. 14:1069126. https://doi.org/10.3389/fpls.2023.1069126.
6. Castro M, Kramer K, Valdivia L, et al. 2003; A double-stranded RNA mycovirus confers hypovirulence-associated traits to *Botrytis cinerea*. FEMS Microbiol Lett. 228:87-91. doi: 10.1016/S0378-1097(03)00755-9.
7. Chen ZH, Yi XT, Liu QB, et al. 2024; Investigation of Gray Mold in Grapevine Greenhouse and Pathogenicity Analysis of *Botrytis Cinerea* in Air. Sino-Overseas Grapevine & Wine. (05):62-68. doi:10.13414/j.cnki.zwpp.2024.05.008.
8. Cui ZH, Liu SJ, Chai ST, et al. . 2012; Evaluation of Associate Effects of Alfafa Green Hay and Crops Straws in Qinghai Plateau. Acta Agriculturae Boreali-occidentalis Sinica. 21:146-152. doi:10.7606/j.issn.1004-1389.2012.2.030.
9. Deverall BJ, Wood RKS. 1961. Infection of bean plants (*Vicia faba* L.) with *Botrytis cinerea* and *B. fabae*. Annals of Applied Biology, 49 (3):461-472. doi:10.1016/0048-4059(80)90046-6.
10. Doyle JJ, Doyle JL. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical bulletin. .
11. Fu B, Li Y, Wang G, et al. 2024; The effect of broad bean diet on structure, flavor and taste of fresh grass carp: A comprehensive study using E-nose, E-tongue, TPA, HS-SPME-GC-MS and LC-MS. Food Chemistry. 15:436. doi:10.1016/j.foodchem.2023.137690.
12. Gemmill CEC, Grierson ERP. 2021; Inter-Simple Sequence Repeats (ISSR), Microsatellite-Primed Genomic Profiling Using Universal Primers. Methods Mol Biol. 2222:249-262. doi:10.1007/978-1-0716-0997-2_14.
13. Gramaje D, León M, Santana M, et al. 2014; Multilocus ISSR markers reveal two major genetic groups in Spanish and South African populations of the grapevine fungal pathogen Cadophora luteo-olivacea. PLoS One. 9:e110417. doi: 10.1371/journal.pone.0110417.
14. Huang Y. 2012; Identification of Pathogens Causing Chocolate Spot on Broad Bean(vicia fabae) and Resistance Resource Screening. Hebei Normal University of Science & Technology. doi:10.7666/d.y2154840.
15. Hui H, Zhang XK, Yang DY, et al. 2021, Culture characteristics and pathogenicity differentiation of Verticillium dahliae of cotton in Xinjiang. Acta Phytopathologica Sinica, 51(04):592-606. doi：10.13926/j.cnki. apps. 000705.
16. Kumar S, Stecher G, Tamura K. 2016; MEGA 7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular biology and evolution. 33(7): 1870-1874. doi:10.1093/molbev/msm092.
17. Li HG, Tang WQ, Yang BG, et al. 2015; High-efficiency cultivation technology for pollution-free broad beans. Journal of Changjiang Vegetables, 19:35-36.doi:cnki:sun:cjsc.0.2015-19-020.
18. Liu YJ. 2018. Adjust the structure, transform the mode, and promote the transformation and upgrading of Qinghai's broad bean industry. Qinghai Science and Technology. 25:35-37.doi:cnki:sun:qkkj.0.2018-01-009.

19. McDonald BA, Linde C. 2002; Pathogen population genetics, evolutionary potential, and durable resistance. Annu Rev Phytopathol. 40:349-79. doi: 10.1146/annurev.phyto.40.120501.101443.
20. Meyer W, Mitchell TG, Freedman EZ, et al 1993; Hybridization probes for conventional DNA fingerprinting used as single primers in the polymerase chain reaction to distinguish strains of Cryptococcus neoformans. Journal of Clinical Microbiology. 31:2274-80. https://doi.org/10.1128/jcm.31.9.2274-2280.1993.
21. Nei M. 1973; Analysis of gene diversity in subdivided populations. Proc Natl Acad Sci USA . 70:3321–3323. doi: 10.1073/pnas.70.12.3321.
22. Rohif FJ. 2000. NTSYS-pc: Numerical taxonomy andmultivariate analysis system, version 2.1. Setauket: Exeter Software.
23. Sahile S, Abang MM, Fininsa C, et al. 2012; Pathogenic and genetic diversity of *Botrytis fabae* Sand. isolates from faba bean fields in different agro-ecological zones of Northern Ethiopia. Archives of Phytopathology and Plant Protection. 45: 1218–1236. https://doi.org/10.1080/03235408.2012.664710.
24. Taliadoros D, Stukenbrock EH. 2023; The use of evolutionary analyses to predict functionally relevant traits in filamentous plant pathogens . Curr Opin Microbiol. 73:102244. doi: 10.1016/j.mib.2022.102244.
25. Wang JB. 2002; [ISSR markers and their applications in plant genetics]. Yi Chuan. 24:613-6. Chinese. PMID: 16135460.
26. Wang SY, Nan ZB, Liu F. 1997, Studies on the damage of broad bean chocolate spot, annular spot and is economic threshold in gansu province. Journal of Plant Protection, 24(4):371-372.doi:cnki:sun:zwbf.0.1997-04-017.
27. Wang XM, Zhu ZD, Duan CX. 2007a. Identification and Control Techniques for Broad Bean and Pea Pests and Diseases. Bei Jing:China Agricultural Press.
28. Wang ZY, Wang YC, Zhang ZG, et al. 2007b, Genetic relationships among Chinese and American isolates of Phytophthora sojae by ISSR markers.Biodiversity Science, 15(3):215–223. doi:10.3321/j.issn:1005-0094.2007.03.001.
29. Wen J. 2010; Application of ISSR Molecular Marker Technique in Plant Pathogens Research.Journal of Anhui Agricultural Sciences. 38:20658-20660. doi：10.13989/j.cnki.0517-6611.2010.36.075.
30. Xu S, Li YZ. 2016, Research advances on fungal diseases of Vicia sativa.Acta Prataculturae Sinica, 25(7):203-214.doi:10.11686/cyxb2015478.
31. Yu DF. 1979. Broad bean diseases. Bei Jing:Science Press.
32. Yeh FC, Yang RC, Boyle TJ, et al. 1997. Popgene ver. 1.32, The user-friendly shareware for population genetic analysis. Molecular biology and Biotechnology centre, University of alberta, Edmonton, Canada..
33. Yu MB, Zhang G, Hou L. 2021. Evaluation of Resistance of 34 Broad Bean(Vicia faba L.) Resources to Chocolate Spot Disease in Qinghai Province.Molecular Plant Breeding, 19(13):4504-4516. doi:10.13271/j.mpb.019.004504.
34. Zhang H, Ma LJ, Feng MY, et al. 2018; Identification and Biological Characteristics of Brown Spot Pathogen in Broad Bean. Northern Horticulture. 19:4. doi:cnki:sun:bfyy.0.2018-19-006.
35. Zhang J, Wu MD, Li GQ, et al. 2010; *Botrytis fabiopsis*, a new species causing chocolate spot of broad bean in central China. Mycologia. 102:1114-1126. https://doi.org/10.3852/09-217.
36. Zheng XL, Li P, Ren JG, et al. 1993, Exploration on Toxins and Pathogenicity of *Botrytis squamosa* and *Botrytis cinerea*.Plant Protection, (01):20-21.
37. Zhou JL, Zhang HJ. 2016; Analysis of the World Broad Bean Production and Trade Situation. World Agriculture. doi：10.13856/j.cn11-1097/s.2016.11.019.
38. Zhuang YQ, Tu JL, Fei WY, et al. 2018; Comparison of the Yield Traits and Nutritional Ingredients among Variety of Fresh Broad Beans. Journal of Anhui Agricultural Sciences. 46:52-54. doi:10.3969/j.issn.0517-6611.2018.32.016.
39. Zhu JH, Jiao J, Pu Y. 2023; Symbiotic Compatibility and Growth Promotion Effect of Nitrogen Fixing Rhizobia on Hainan Vegetable-type Soybean. Soybean Science. 42:441-450. doi:10.11861/j.issn.1000-9841.2023.04.0441.