Genetic diversity of *Venturia inaequalis* isolates from the scabs in apple trees in Gansu province, China, using AFLP markers

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Apple scab is a serious disease that restricts the growth of cultivated apples. The objective of this study is to investigate the genetic variations and genetic structure of *Venturia inaequalis* in Gansu Province, China. 108 isolates of the pathogen *V. inaequalis* from the Jingning, Lingtai, Jingchuan, Xifeng, Ning and Maiji regions were collected, and their genetic diversity was analyzed using AFLP molecular marker technique. The results showed that genetic diversity was present among the isolates but was not statistically significant. Genetic distance values ranged from 0.0095 to 0.0762. Cluster analysis results showed that the 108 isolates could be divided into 2 clusters using a similarity coefficient of 0.69. 104 isolates were contained in cluster I while 4 isolates were contained in Cluster II. From the AMOVA analysis, 98% of variations were observed within the same region, while 2% were observed across different regions. The analysis of population structure showed that 108 isolates had two common ancestors, with the Jingning isolates mainly being derived from the red ancestor. PCoA analysis showed that the Jingning isolates were independent to a certain extent. The different geographical location caused the genetic difference of the isolates. The genetic diversity of apple scab in Gansu Province is greatly aided by this work, which also offers a theoretical foundation for the use of molecular markers in assisted breeding to create novel resistant types.

1 **Genetic diversity of *Venturia inaequalis* isolates from the scabs in**

2 **apple trees in Gansu province, China, using AFLP markers**

3 **Short title: Genetic Variation of *Venturia inaequalis***

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13 **Abstract**

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15 study is to investigate the genetic variations and genetic structure of *Venturia inaequalis* in

16 Gansu Province, China. 108 isolates of the pathogen *V. inaequalis* from the Jingning, Lingtai,

17 Jingchuan, Xifeng, Ning and Maiji regions were collected, and their genetic diversity was

18 analyzed using AFLP molecular marker technique. The results showed that genetic diversity was

19 present among the isolates but was not statistically significant. Genetic distance values ranged

20 from 0.0095 to 0.0762. Cluster analysis results showed that the 108 isolates could be divided into

21 2 clusters using a similarity coefficient of 0.69. 104 isolates were contained in cluster I while 4

22   isolates were contained in Cluster II. From the AMOVA analysis, 98% of variations were

23   observed within the same region, while 2% were observed across different regions. The analysis

24   of population structure showed that 108 isolates had two common ancestors, with the Jingning

25   isolates mainly being derived from the red ancestor. PCoA analysis showed that the Jingning

26   isolates were independent to a certain extent. The different geographical location caused the

27   genetic difference of the isolates. The genetic diversity of apple scab in Gansu Province is

28   greatly aided by this work, which also offers a theoretical foundation for the use of molecular

29   markers in assisted breeding to create novel resistant types.

30   **Keywords:** Genetic diversity, *Venturia inaequalis*, AFLP molecular markers

32   **Introduction**

33   The major disease known as apple scab, which is brought on by *Venturia inaequalis* ((Cooke) G.

34   Winter), affects apple industry and economic benefits. In severe cases, it will lead to a decline in

35   apple yield, especially in colder apple-growing regions around the world (*Bowen et al.*, *2011*).

36   Apple scab was discovered in China for the first time in Hebei Province in the 1920s and has

37   since gradually spread to twelve provinces (*Huang et al.*, *1997*). Apple scab pathogen *V.*

38   *inaequalis* mainly damages apple leaves and fruits, but it can also infect petioles, flowers, sepals

39   and pedicels. Furthermore, it can also infect other plants such as crabapples, loquats and

40   hawthorn (*Jha et al.*, *2009*). Apple scab is so widespread in some areas that apple production

41   losses may reach up to 70% (*MacHardy*, *1996*).

42   Researchers in many countries and regions have studied the genetic diversity of *V.*

43   *inaequalis* with different molecular tools (*Guérin et al.*, *2004*; *Mansoor et al.*, *2019*; *Gladieux et*

44   *al.*, *2010*). Amplified fragment length polymorphism (AFLP) molecular marker technique was

45   used to genotype isolates from the UK and China. Within China, there were no significant

46   differences in relation to their geography or cultivar origin, but the populations of the four

47   varieties in the UK were significantly different (*Xu et al.*, *2008*). This explains the genetic

48   differences in *V. inaequalis* among different varieties of apple trees in the UK, but not in China.

49   *V. inaequalis* isolates from two orchards in the UK were genotyped using AFLP molecular

50   marker technique and simple sequence repeats (SSR). The results showed that the isolates of an

51   individual cultivar within each orchard varied significantly, as did the isolates of an individual

52   tree of the same cultivar in the same orchard. These results indicated that resistant varieties could

53   be used for disease management in mixed orchards (*Xu et al.*, *2013*). In addition, the genetic

54   diversity of *V. inaequalis* from 28 plantations on 5 continents was examined. The comes about

55   appeared that 88% of genetic variety happened inside populaces, and it is related to widespread

56   migration within regions (*Gladieux et al.*, *2008*). At the same time, by utilizing SSR to analyze

57   the genetic diversity of *V. inaequalis* populaces within the UK and China, the results showed that

58   the genetic relationship between both regions was relatively close. This was because most of the

59   apple dwarf seedlings planted in Northwest China were from the EM system in the UK (*Li et al.*,

60   *2021*).

61   In China, Gansu Province is located in the apple-producing area of the Loess Plateau, and

62   its apple cultivation area and output rank among the top in the country. At the same time, apple

63   scab occurred less. Be that as it may, in later a long time, apple scab has gotten to be

64 commonplace in Gansu Province, driving to genuine misfortunes and posturing a noteworthy

65 danger to the feasible improvement of the apple industry (*Hu et al.*, *2008*). AFLP molecular

66 marker technique is a convenient and reliable molecular marker technology. This approach has

67 not been used to explore genetic variations and genetic structure of *V. inaequalis* in Gansu

68 Province.

69   This study collected and isolated apple scab samples from different apple-producing areas

70 in Gansu and analyzed the genetic diversity of the *V. inaequalis* population using AFLP

71 molecular marker technique. It gives a hypothetical premise for clarifying the genetic variety and

72 populace structure of *V. inaequalis* in Gansu Province.

73

74 **Materials and methods**

75 **Isolation of samples**

76 Samples collected from Gansu Province between June to September 2021 as appeared in Table

77 1. Fig. 1 gives the areas where tests were collected. The monoconidial strategy was utilized for

78 sample isolation. The spore suspension was included into water agar (WA), and air-dried. Single

79 spores were marked under the microscope after 24 h of incubation at 25℃. A sterile needle was

80 used to collect the marked areas on the WA, which were then placed in potato dextrose agar

81 (PDA) for incubation at 25℃ in dark environment. The isolate was purified to obtain the target

82 isolate when after 40 d of culture.

83

84 **DNA extraction**

85   The isolates were incubated for 40 days at 25℃ in dark environment. Approximately 100 mg of

86   fungal mycelia was then collected and ground into powder after being frozen with liquid

87   nitrogen. The Fungal Genomic DNA Extraction Kit (Omega Bio-tek, Shanghai Sangon

88   Company, China) was used to extract nucleic acid per the operating instructions of the kit, and

89   was then stored at -20°C.

90

91   **AFLP markers**

92   The AFLP molecular marker technique was modified from Vos et al (*Vos et al.*, *1995*). *Mse I*

93   and *EcoR I* were used for double digestion. Approximately 500 ng of DNA, 5 U of *Mse I*, 5 U

94   of *EcoR I* (Takara-Bio, China) and 4 μl of H Buffer (Takara-Bio, China) were used in a

95   reaction. ddH$_2$O was added to obtain a final volume of 40 μl. The digestion protocol involved

96   operation at 37℃ for 3 h followed by 80℃ for 20 min. The product of the double digestion was

97   ligated to the adapters *EcoR I* (10 μM) and *Mse I* (10 μM) via a reaction containing: 40 μl of

98   the double digestion product, 1 μl of (35 U) T4 DNA ligase (Takara-Bio, China), 1 μl of 10×T4

99   DNA ligase Buffer (Takara-Bio, China) and 1 μl of *EcoR I* and *Mse I* adapters. ddH$_2$O was

100   used to attain a final volume of 50 μl. The protocol involved operation at 16℃ for 12 h followed

101   by 80℃ for 20 min. Digestion-ligation products were visualized via patterning on 1.2% agarose

102   gel. After ligation, the product was diluted using a 1:9 ratio with ultrapure water.

103   Pre-selective amplification was performed using 10 μl of Taq PCR MasterMix (Shanghai

104   Sangon Company, China), 2 μl of a pre-selective primer mixture *E*-TTC/*M*-TAA (10 μM) (*Fu et*

105   *al.*, *2010b*), 5 μl of the ligated product and 8 μl of ddH$_2$O. Pre-selective amplification cycling

106 parameters were 94°C for 5 min followed by 31 cycles at 94°C for 45 s, 45°C for 40 s and an

107 extension at 72°C for 60 s, and 72°C for 10 min and the product was then stored at -20°C for

108 further use. After pre-selective amplification, the PCR product was diluted using a 1:9 ratio with

109 ultrapure water.

110 The 25 most common polymorphic combinations were chosen as molecular markers (*Fu et*

111 *al., 2010b*) and selectively amplified. Selective amplification was performed using 10 μl of Taq

112 PCR MasterMix (Shanghai Sangon Company, China), 2 μl of the selective primer mixture

113 *EcoR I* and *Mse I* (10 μM), 5 μl of the pre-selection amplification product and 8 μl of ddH_2O.

114 Selective amplification cycling parameters were 94°C for 5 min followed by 15 cycles at 94°C for

115 40 s and 65°C for 30 s. This was followed by an extension at 72°C for 60 s where the temperature

116 decreased by 0.7℃ after each cycle. These cycling parameters were then followed by 30 cycles

117 at 94°C for 30 s, 51°C for 30 s, and 72°C for 60 s and a final extension at 72°C for 10 min,

118 followed by storage at -20°C for further use.

119

120 **6% Polyacrylamide gel electrophoresis**

121 The SDS-PAGE Preparation kit (Shanghai Sangon Company, China) was used for

122 polyacrylamide gel electrophoresis. The electrophoresis buffer was prepared by diluting 10×TBE

123 (100 ml) in 900 ml of distilled water. The electrophoresis program began with 40 minutes of pre-

124 electrophoresis followed by 70 minutes of electrophoresis after sample loading. The sample

125 loading volume was 10 μl. The gel silver staining method was modified from Borzooeian et al

126 (*Borzooeian et al., 2018*). The method comprises of settling with 100 ml/L acetic acid

127 (CH$_3$COOH) for 20 min, washing with ddH$_2$O for 1 min and silver staining with 1 g/L of silver

128 nitrate solution (AgNO$_3$) for 20 min. The sample was then washed with ddH$_2$O for 10 s,

129 developed using 16 g/L sodium hydroxide (NaOH), 8 ml/L formaldehyde (37%) solution for 5

130 min, a wash with ddH$_2$O for 10 s and a quenching reaction via the use of 7.5 g/L sodium

131 carbonate (Na$_2$CO$_3$) for 5 min. The AFLP molecular marker bands were counted regardless of

132 their presence or absence at the same position, with their presence marked as '1' and absence

133 marked as '0'. A binary data '0-1' matrix was then established.

134

135 **Data analysis**

136 Matrix data were imported to NTSYS2.1 to analyze genetic distances (*Weir*, *1997*). Nei's genetic

137 distance among the populations was estimated using POPGENE1.32 and GenAlEx 6.5 (*Kimura*

138 *et al.*, *1964*; *Lewontin*, *1972*; *Nei*, *1973*; *Yeh et al.*, *1999*). GenAlEx 6.5 was used for analysis of

139 molecular variance (AMOVA) and analysis of principal co-ordinates (PCoA) (*Peakall et al.*,

140 *2001*). Individual genotypes were analyzed in Structure 2.3.4 (*Pritchard et al.*, *2000*). The

141 optimal K value was determined by uploading the analysis data to the platform Structure

142 Harvester (http://taylor0.biology.ucla.edu/structureHarvester/) (*Earl et al.*, *2012*).

143

144 **Results**

145 **Analysis of genetic diversity**

146 Analysis of genetic diversity for *V. inaequalis* using 6% SDS-PAGE (Fig. 2). The results of

147 Popgene1.32 are shown in Table 2. In the 108 isolates, the number of polymorphic loci (Np) was

148   301, the percentage of polymorphic loci (P) was 99.67%, and the effective number of alleles

149   (Ne) was 1.4398. Nei's gene diversity (H) was 0.2712 and Shannon's information index (I) was

150   0.4241, which shown that there were a few genetic diversities within the populace of *V.*

151   *inaequalis* in Gansu Province. The analysis results for *V. inaequalis* based on 6 geographic

152   regions show that Np values ranged from 224 to 283, P values ranged from 74.17% to 93.17%

153   while Ne values ranged from 1.3586 to 1.5506. H values ranged from 0.2206 to 0.3147 while I

154   value ranged from 0.3432 to 0.4703. Based on the analysis of H and I, Jingning isolates had the

155   highest level of genetic diversity, Maiji, Jingchuan, Xifeng, Lingtai isolates had the second

156   highest level while Ning had the lowest level of genetic diversity.

157

158   **Cluster analysis**

159   The unweighted pair group with arithmetic mean (UPGAM) method was used to cluster 108

160   isolates of *V. inaequalis* from 6 regions of Gansu Province. A tree graph (Fig. 3) was obtained,

161   which was proven to be accurate via the cophenetic correlation test ($r = 0.8969$). As can be seen

162   from Fig. 3, all the isolates tested were concentrated together when the similarity coefficient was

163   0.67. The isolates could be divided into two clusters when the similarity coefficient was 0.69.

164   104 isolates were contained in cluster I and 4 isolates were contained in Cluster II. Cluster I can

165   be further divided into two sub-clusters, with Jingning isolates included in subcluster 1. Maiji,

166   Lingtai, Jingchuan, Ning, Xifeng and several Jingning isolates were included in subcluster 2.

167   Only Jingning isolates were included in Cluster II. The results show that there is no significant

168   correlation between the isolates and their geographical locations in Gansu Province.

169

170 **Analysis of genetic distance**

171 Popgene1.32 was used to analyze Nei's genetic identity and genetic distance (Table 3). These

172 were obtained for six isolates from different geographical regions, in which isolates of Ning and

173 Jingchuan have the highest level of genetic identity (0.9906) and the closest genetic distance

174 (0.0095). This indicates that Ning and Jingchuan isolates have the smallest genetic difference.

175 Xifeng and Jingning isolates have the lowest level of genetic identity (0.9267) and the furthest

176 genetic distance (0.0762), which indicates that the genetic difference between Xifeng and

177 Jingning isolates is the largest.

178

179 **Analysis of molecular variance (AMOVA)**

180 AMOVA analysis of population variance (Table 4) showed that the coefficient of population

181 differentiation (*PHiPt*) in the 6 geographical regions is 0.027 ($P < 0.05$). This indicates that there

182 are some genetic variations for *V. inaequalis* in different regions of Gansu Province, but the

183 genetic variations are not statistically significant (*PHiPt* < 0.25). There is molecular variance in

184 108 isolates, among which the frequency of molecular variance is 98% within the same region

185 and 2% across different regions. Therefore, it can be concluded that there is greater molecular

186 variance in the same region and less gene exchange between different regions.

187

188 The results of PCoA analysis (Fig. 4) showed that Jingning had more isolates in the fourth

189 quadrant with several isolates independently distributed in the first quadrant. The genetic

190 structure of the tested isolates was analyzed, with results showing that the 6 regions in Gansu

191 Province could be divided into 2 populations (K = 2, Fig. 5). As can be seen from Fig. 6, the

192 genetic background of the Jingning isolates was mainly derived from ancestors indicated using a

193 red color, with few green components. The genetic background of the other isolates involved

194 different extents of contribution by both red and green ancestors with a high level of genetic

195 similarity.

196

197 **Discussion**

198 Apple scab was to begin with recognized in Sweden in 1819 and has since spread to Germany,

199 the United States, the United Kingdom and Australia. The disease was first discovered in China

200 in Hebei Province and has now spread to 12 provinces such as Shaanxi Province, Gansu

201 Province, Xinjiang Province and Henan Province (*Fu et al.*, *2010a*). In this places, large-scale

202 epidemics have occurred with serious economic losses.

203   AFLP is a molecular marker based on RFLP and PCR techniques created by Dutch

204 scientists Zabeau and Vos. This method combines the simplicity of RFLP and RAPD and has the

205 advantages of strong polymorphism, a simple methodology, the ability to handle a large amount

206 of information and easy applicability (*Vos et al.*, *1995*). In this study, the AFLP molecular

207 marker technique was used to analyze the genetic diversity of *V. inaequalis* from six different

208 geographical regions of Gansu Province. The results showed that the populations of Jingning,

209 Jingchuan, Lingtai, Maiji, Xifeng and Ning isolates were genetically similar and were consistent

210 with the results of previous studies in China (*Hu et al.*, *2008*; *Fu et al.*, *2010a*; *Li et al.*, *2021*).

211   The main environmental factors affecting the genetic variation of apples are the apple planting

212   area and the pathogen hosts. A higher level of genetic similarity across different geographical

213   clusters may be due to the smaller sampling amount in the same province as well as smaller

214   differences in climate and temperature (*Koopman et al.*, *2017*). The isolates of *V. inaequalis* in

215   Gansu Province are not as affected by so many factors and are therefore unlikely to cause the

216   pathogen to mutate. Furthermore, only *Fuji* and *Gala* apple varieties were involved in this

217   sampling, so the role of different hosts in the genetic variation of *V. inaequalis* remains to be

218   further explored.

219   AMOVA analysis showed that 98% of molecular variance frequency in the 108 isolates

220   were within the same region and 2% were across different regions (Table 4). The results showed

221   that the contribution of geographical location to population differentiation of *V. inaequalis* is

222   small, which is similar to that of previous studies (*Ebrahimi et al.*, *2016*; *Koopman et al.*, *2017*;

223   *Sitther et al.*, *2018*). It can also be seen that the genetic variation of apple scab was not mainly

224   caused by its geographical location in Gansu Province. The author speculates that the frequent

225   transportation of seedlings in Gansu Province may have further assimilated the genetic material

226   of *V. inaequalis* and increased gene exchange within the population.

227   Using cluster analysis, 108 isolates were grouped into 2 clusters. In genetic structure

228   analysis (Fig. 6), the isolates from 6 regions had two common ancestors, indicating a common

229   genetic basis among the isolates (*Tenzer et al.*, *1997*; *Tenzer et al.*, *1999*; *Xu et al.*, *2008*). PCoA

230   results showed that although most of the isolates were clustered together, the isolates from the

231   Jingning area had some independence, with four isolates that were dispersed. These were PLJN-

232   5, PLJN-10, PLJN-14 and PLJN-19 (Fig. 4). This may be due to the fact that Jingning is located

233   to the west of the Liupan Mountains, with other areas being to the east of the Liupan Mountains.

234   The presence of mountains makes it difficult for pathogens to spread geographically,

235   contributing to a smaller gene flow. The large apple cultivation area of Jingning, which uses

236   seedlings from all over the world has also increased the movement of pathogens between

237   regions. Large-scale planting led to frequent exchanges of pathogenic isolates in the region and

238   increased opportunities for new genetic variations. Therefore, some differences are observed in

239   the clustering of *V. inaequalis* for Jingning. However, there were only four isolates with

240   differences, so it is not possible to perform any further analysis.

241   At present, chemical fungicides are mainly used in the control of apple scab during apple

242   cultivation. The long-term use of chemical fungicides will make pathogenic isolates resistant to

243   drugs and aggravate environmental pollution. In addition, the use of different fungicides in

244   different areas also has a significant influence on genetic variations of pathogenic isolates.

245   Recently, new isolates that can overcome apple scab resistance in apple plants have been

246   discovered (*Papp et al.*, *2020*; *Parisi et al.*, *1993*). In addition, several *V. inaequalis* isolates with

247   resistance to multiple fungicides have been identified in the United States (*Lichtner et al.*, *2020*).

248   All of these variables show genuine challenges to disease control. AFLP molecular marker

249   examination of genetic diversity for *V. inaequalis* in Gansu Province was carried out in this

250   study. If we want to know the genetic variation of *V. inaequalis* globally, further sampling

251   investigations are necessary. Furthermore, this study used 6% SDS-PAGE electrophoresis to

252   discover allele differential fragments. It is possible to use a more efficient and convenient

253 capillary electrophoresis (CE) method for analysis so as to improve efficiency and data accuracy

254 (*Monnig et al., 1994*). This study provides a theoretical basis for explaining the genetic variation

255 of *V. inaequalis* in Gansu Province.

256

257 **Conflict of interest**

258 The authors declare no competing interests.

259 **Data availability statement**

260 The datasets used during the present study are available from the corresponding author on

261 reasonable request.

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266

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364   **Figures Legends**

365   **Fig. 1.** Areas from where sample collection was carried out. Six regions in Gansu were chosen

366   **Fig. 2.** Result of partial isolates using 6% SDS-PAGE electrophoresis. Primer combination E-CGC/M-GAC

367   (left), primer combination E-CGC/M-CTG (right), Marker = 2000 bp, 1 - PLJN-3; 2 - PLJN-5; 3 - PLJN-13; 4

368   - TSMJ-4; 5 - TSMJ-7; 6 - TSMJ-15; 7 - PLJC-6; 8 - PLJN-15; 9 - QYXF-4.

369   **Fig. 3.** Analysis of genetic relationship between 108 isolates of *V. inaequalis* based on 25 AFLP markers

370   **Fig. 4.** Analysis of PCoA for *V. inaequalis* isolates sampled in Gansu

371   **Fig. 5.** ΔK peak value of 41.8 among assumed K-values

372   **Fig. 6.** Analysis of *V. inaequalis* population structure shown using bar charts. Above the dividing line is the

373   red genetic background and below is the green genetic background

374   **Table Legends**

375   **Table 1.** Number of samples and information regarding collection locations

376   **Table 2.** Analysis of genetic diversity for different populations of *V. inaequalis* in Gansu Province

377   **Table 3.** Nei's genetic identity and genetic distance among six different regions

378   **Table 4.** AMOVA of *V. inaequalis* isolates grouped by geographic origin

Figure 1

Areas from where sample collection was carried out.

Six regions in Gansu were chosen

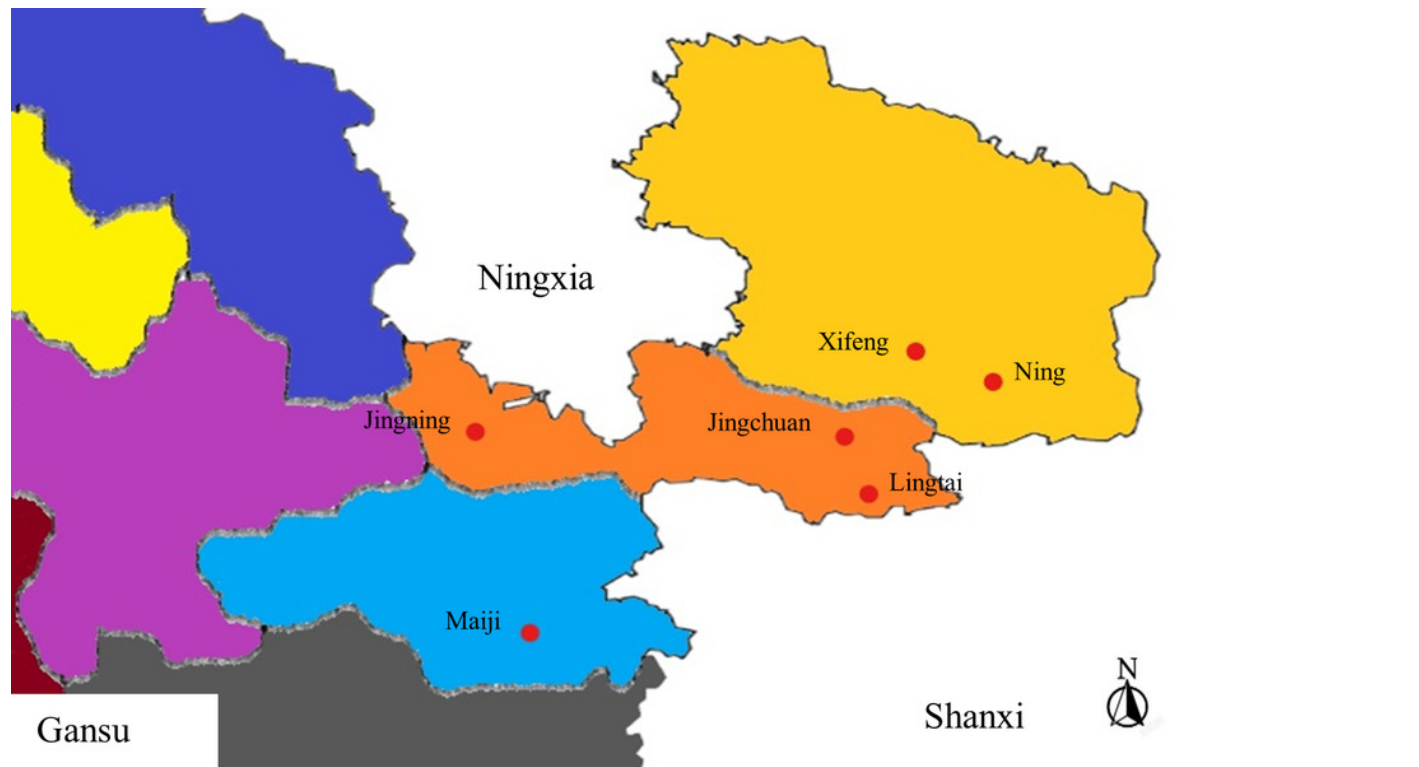



Figure 2

Result of partial isolates using 6% SDS-PAGE electrophoresis.

Primer combination E-CGC/M-GAC (left), primer combination E-CGC/M-CTG (right), Marker = 2000 bp, 1 - PLJN-3; 2 - PLJN-5; 3 - PLJN-13; 4 - TSMJ-4; 5 - TSMJ-7; 6 - TSMJ-15; 7 - PLJC-6; 8 - PLJN-15; 9 - QYXF-4.

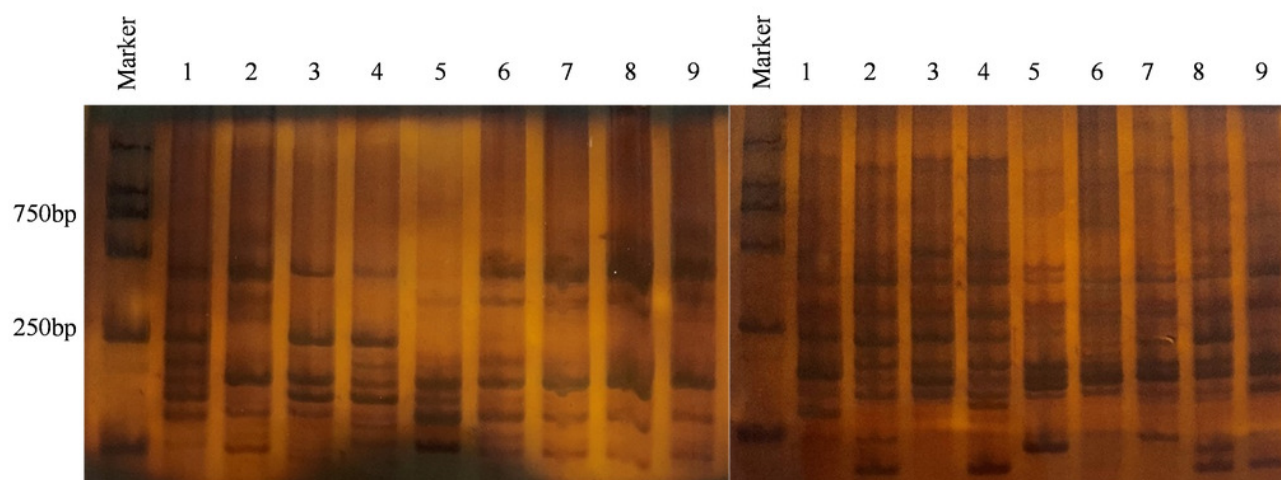



Figure 3

Analysis of genetic relationship between 108 isolates of *V. inaequalis* based on 25 AFLP markers

PLJN-1
PLJN-21
PLJN-20
PLJN-17
PLJN-3
PLJN-18
PLJN-2
PLJC-10
QYXF-3
TSMJ-4
TSMJ-14
TSMJ-8
TSMJ-9
QYNX-9
TSMJ-1
TSMJ-22
TSMJ-23
PLJC-1
QYNX-1
PLJC-15
PLLT-13
QYNX-26
PLJC-16
QYXF-9
PLLT-1
QYXF-1
QYNX-25
QYNX-2
QYNX-19
PLLT-11
QYNX-4
QYNX-24
PLLT-12
PLLT-2
QYNX-15
QYNX-17
QYNX-22
PLJC-2
QYNX-23
QYNX-5
TSMJ-2
TSMJ-21
TSMJ-12
TSMJ-11
PLJC-3
QYNX-14
QYNX-20
PLLT-3
QYNX-7
PLJC-9
QYNX-13
PLJC-4
PLJC-13
QYNX-11
PLJC-6
PLJC-14
QYNX-12
QYNX-10
PLJC-12
QYNX-21
QYXF-2
QYNX-3
QYNX-18
TSMJ-19
TSMJ-5
TSMJ-13
TSMJ-20
QYNX-16
TSMJ-17
QYNX-8
PLLT-7
QYXF-8
QYNX-6
TSMJ-7
TSMJ-18
PLJC-7
TSMJ-10
PLJC-5
PLLT-10
PLLT-4
TSMJ-3
TSMJ-6
TSMJ-16
TSMJ-15
PLLT-5
PLLT-9
PLJC-8
PLLT-8
QYXF-5
PLJN-12
PLJN-15
PLLT-6
PLJN-11
QYXF-4
PLJN-4
PLJN-8
PLJN-13
PLJN-16
PLJC-11
QYXF-7
PLJN-9
QYXF-6
PLJN-7
PLJN-6
PLJN-5
PLJN-19
PLJN-14
PLJN-10

0.67   0.74   0.81   0.88   0.95

Figure 4

Analysis of PCoA for *V. inaequalis* isolates sampled in Gansu

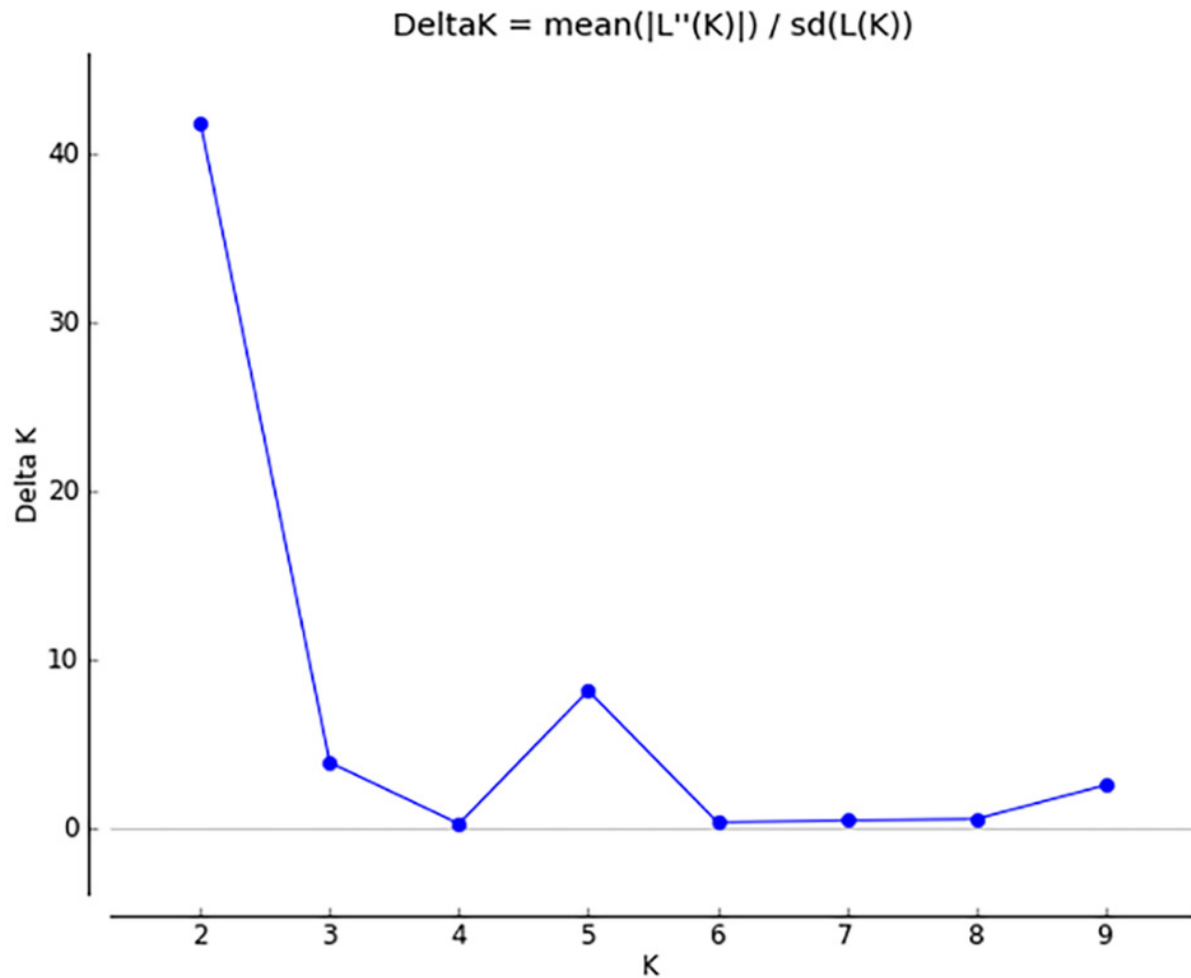



Figure 5

ΔK peak value of 41.8 among assumed K-values

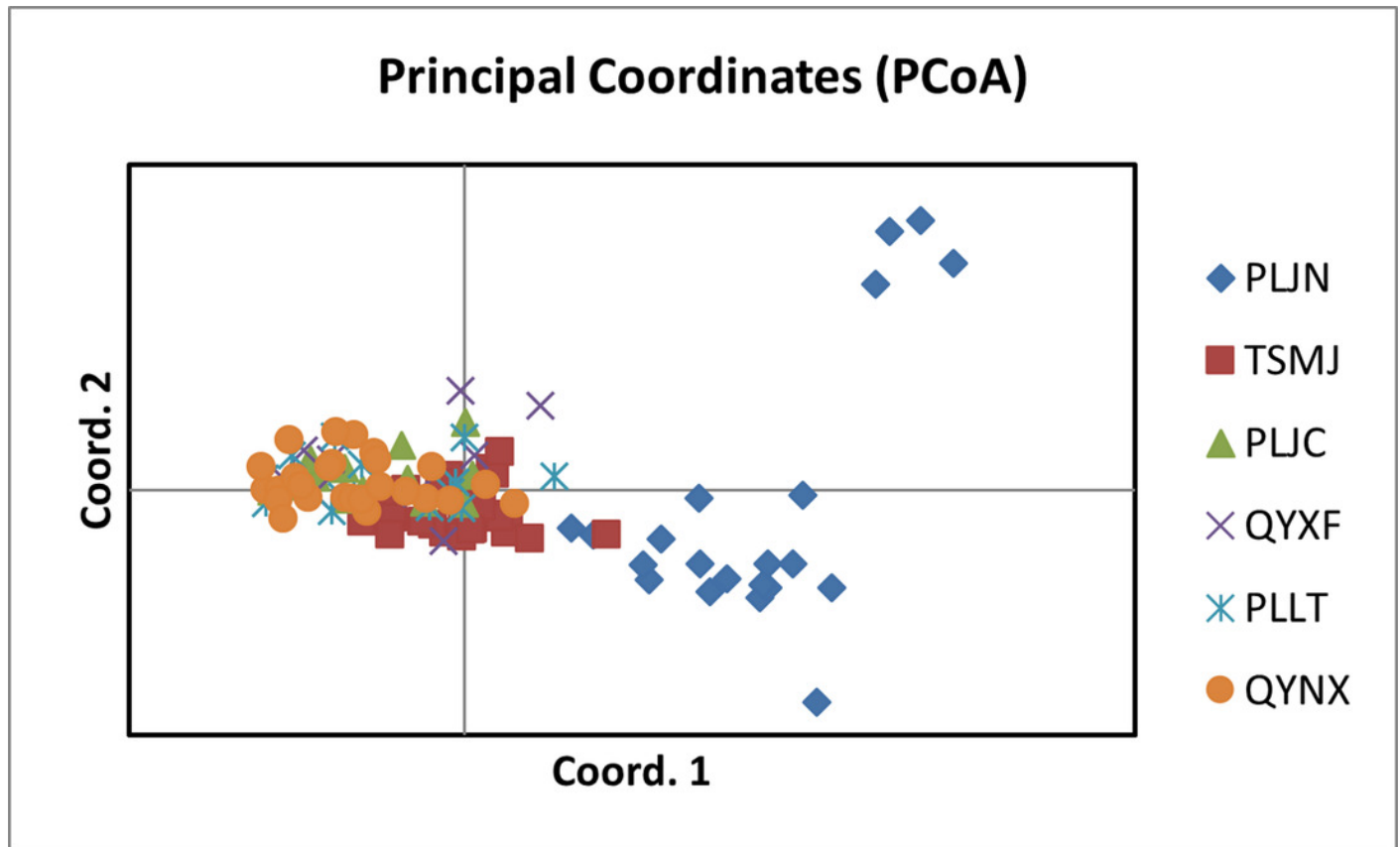
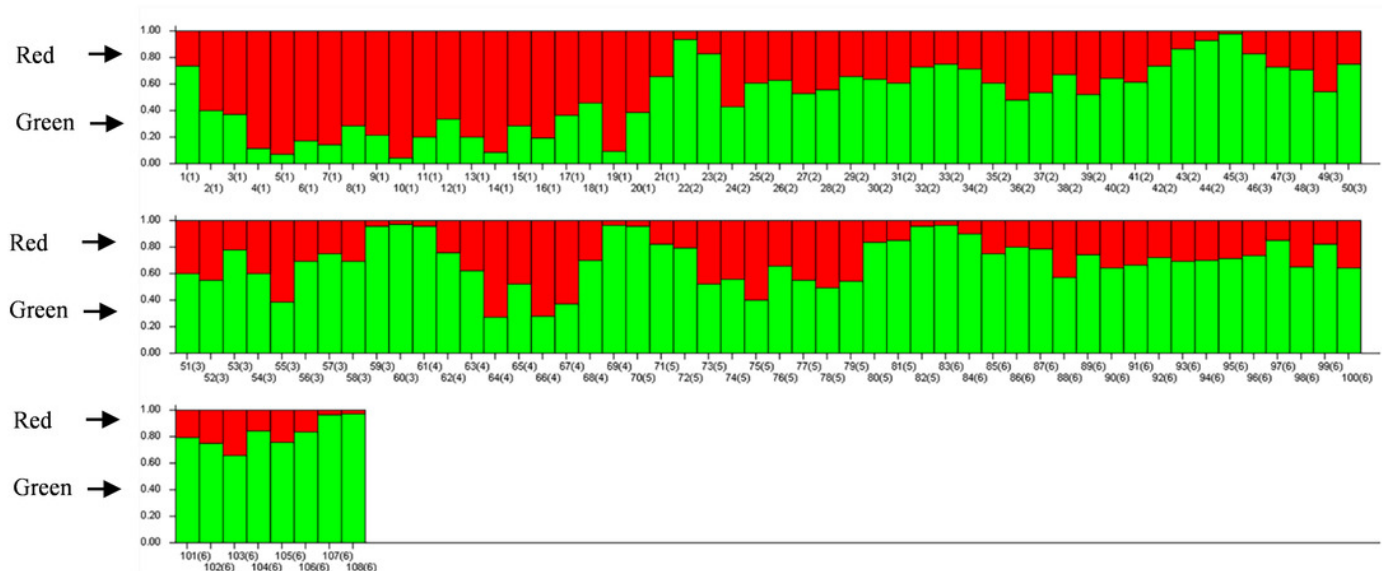



Figure 6

Analysis of *V. inaequalis* population structure shown using bar charts.

Above the dividing line is the red genetic background and below is the green genetic

background

Number of samples and information regarding collection locations

1   **Table 1.** Number of samples and information regarding collection locations

Samples	Numbers	District	Host	Longitude	Latitude	Times
PLJN	1~21	Jingning, Pingliang	Fuji	105.36° E	35.33° N	2021.6.18
PLLT	22~35	Lingtai, Pingliang	Fuji	107.33° E	34.61° N	2021.7.12
QYNX	36~41	Ning, Qingyang	Fuji	108.11° E	35.26° N	2021.7.12
TSMJ	62~84	Maiji, Tianshui	Starkrimson	106.33° E	34.36° N	2021.8.17
PLJC	85~100	Jingchuan, Pingliang	Fuji	107.36° E	35.22° N	2021.7.13
QYXF	101~108	Xifeng, Qingyang	Fuji	107.37° E	35.51° N	2021.7.13

2   Note: The isolated strains are numbered based on their region followed by a number, for example: PLJN-10,

3   TSMJ-7, etc.

Analysis of genetic diversity for different populations of *V. inaequalis* in Gansu Province

1   **Table 2.** Analysis of genetic diversity for different populations of *V. inaequalis* in Gansu Province

Code	N	Np	P (%)	Ne	H	I
Jingning	21	283	93.71	1.5506	0.3147	0.4703
Maiji	23	224	74.17	1.3787	0.2268	0.3463
Jingchuan	16	238	78.81	1.3852	0.2323	0.3569
Xifeng	9	239	79.14	1.4553	0.2650	0.3985
Lingtai	13	242	80.13	1.4169	0.2476	0.3772
Ning	26	241	79.80	1.3586	0.2206	0.3432
Total	108	301	99.67	1.4398	0.2712	0.4241

2   Notes: N = Number, Np = Number of polymorphic loci, P = Percentage of polymorphic loci, Ne = Effective

3   number of alleles (Weir et al. 1997), H = Nei's gene diversity (Kimura et al 1964), I = Shannon's information

4   index (Nei 1973).

Nei's genetic identity and genetic distance among six different regions

1   **Table 3.** Nei's genetic identity and genetic distance among six different regions

Populations	Jingning	Maiji	Jingchuan	Xifeng	Lingtai	Ning
Jingning	****	0.9387	0.9303	0.9267	0.9334	0.9353
Maiji	0.0632	****	0.9808	0.9731	0.9725	0.9787
Jingchuan	0.0722	0.0194	****	0.9893	0.9843	0.9906
Xifeng	0.0762	0.0273	0.0108	****	0.9807	0.9818
Lingtai	0.0689	0.0278	0.0159	0.0195	****	0.9876
Ning	0.0669	0.0216	0.0095	0.0184	0.0125	****

2   Notes: Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

AMOVA of *V. inaequalis* isolates grouped by geographic origin

1   **Table 4.** AMOVA of *V. inaequalis* isolates grouped by geographic origin

Variation Source	Degrees of freedom	Sum of aquares	MS	Estimate variance	Variance rate	*PHiPt*	*P*
Among Pops	5	193.355	38.671	0.654	2%	0.027	0.002
Within Pops	103	3375.000	15.617	31.250	98%		
Total	108	3568.355		31.904	100%		

2   Notes: MS = Mean-square error, *PHiPt* = Coefficient of population differentiation, p = Statistical significance.