

# Association Analysis of Verticillium Wilt Resistance in Spinach



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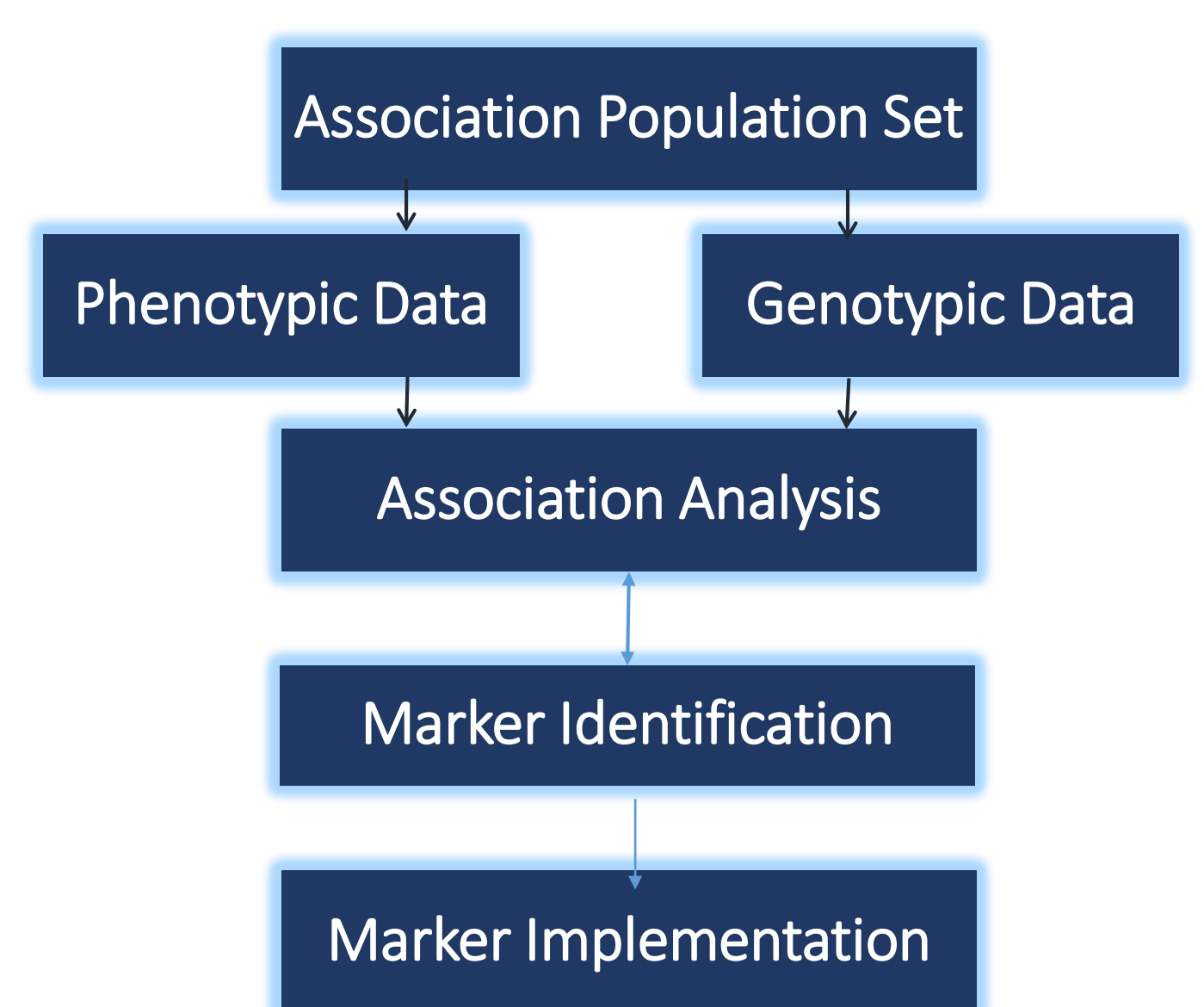
## Objective

Verticillium wilt, caused by *Verticillium dahliae* Kleb., is an important disease of spinach (*Spinacia oleracea* L.) and use of genetic resistance is the most economical method of controlling this disease. The objective of this research was to conduct molecular association analysis and identification of SNP markers for Verticillium wilt resistance in spinach.



## Materials and Methods

- Materials:** A total of 95 accessions of spinach USDA-GRIN germplasm originally collected from 10 countries were used for association analysis of Verticillium wilt resistance in spinach.
- Disease evaluation:** The *V. dahliae* inoculation and disease evaluation described by Mou et al. (2015).
- DNA extraction, GBS and SNP discovery:** Genomic DNA was extracted from dried leaves of spinach plants using the CTAB. The 90 bp double-end sequencing was performed on each spinach genotype using Genotyping By Sequencing (GBS) protocol by an Illumina HiSeq 2000 at Genomics Research Institute (BGI) in Hong Kong. GBS data assembly, mapping and SNP discovery were done using SOAP family software (<http://soap.genomics.org.cn/>) by the bioinformatics team in BGI.
- Association analysis:** Association tests are run with the general linear model (GLM) and mixed linear model (MLM) using TASSEL 5 (Bradbury et al., 2007, <http://www.maizegenetics.net/tassel>) and the analysis was also performed with compressed mixed linear model (cMLM) (Zhang et al., 2010) and enriched compressed mixed linear model (EcMLM) (Li, et al., 2014) implemented in the GAPIT R package (Lipka et al., 2012). The QGene 4.3.10 was also used to conduct Single Marker Regression (SMR) for all SNPs (Joehanes & Nelson, 2008).



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## Results

- Significant genetic variation of Verticillium wilt disease resistance was observed among the 95 spinach accessions with a wide range from 0.3 to 3.0 on a 0-4 scale (Fig.1).
- Two well-differentiated genetic populations and admixtures were postulated in the spinach panel (Fig. 2).
- A total of thirteen SNPs were identified to be associated with Verticillium wilt resistance (Table 1). Among the 13 SNPs, five SNP markers, AYZV02052595\_108, AYZV02112284\_14543, AYZV02123399\_146, AYZV02164612\_331, and AYZV02170942\_274 were identified to be strongly associated with Verticillium wilt resistance with R-squared values from 9.3 to 18.2%. These markers may provide a tool utilized in molecular spinach breeding to select Verticillium wilt resistance through marker-assisted selection.
- Eight accessions with disease severity ratings of 1.0 or less, other four accessions also showed mediate resistance with disease severity 1.3 or less. The genetic relationships among the 12 accessions were analyzed using 2878 SNPs by MEGA 6 and a phylogenetic tree was drawn (Fig 3). The results showed there was genetic diversity existed among the 12vspinach accessions and geographic effect was also observed.

Fig 1. The distribution of Verticillium wilt disease rating in 95 spinach accessions.

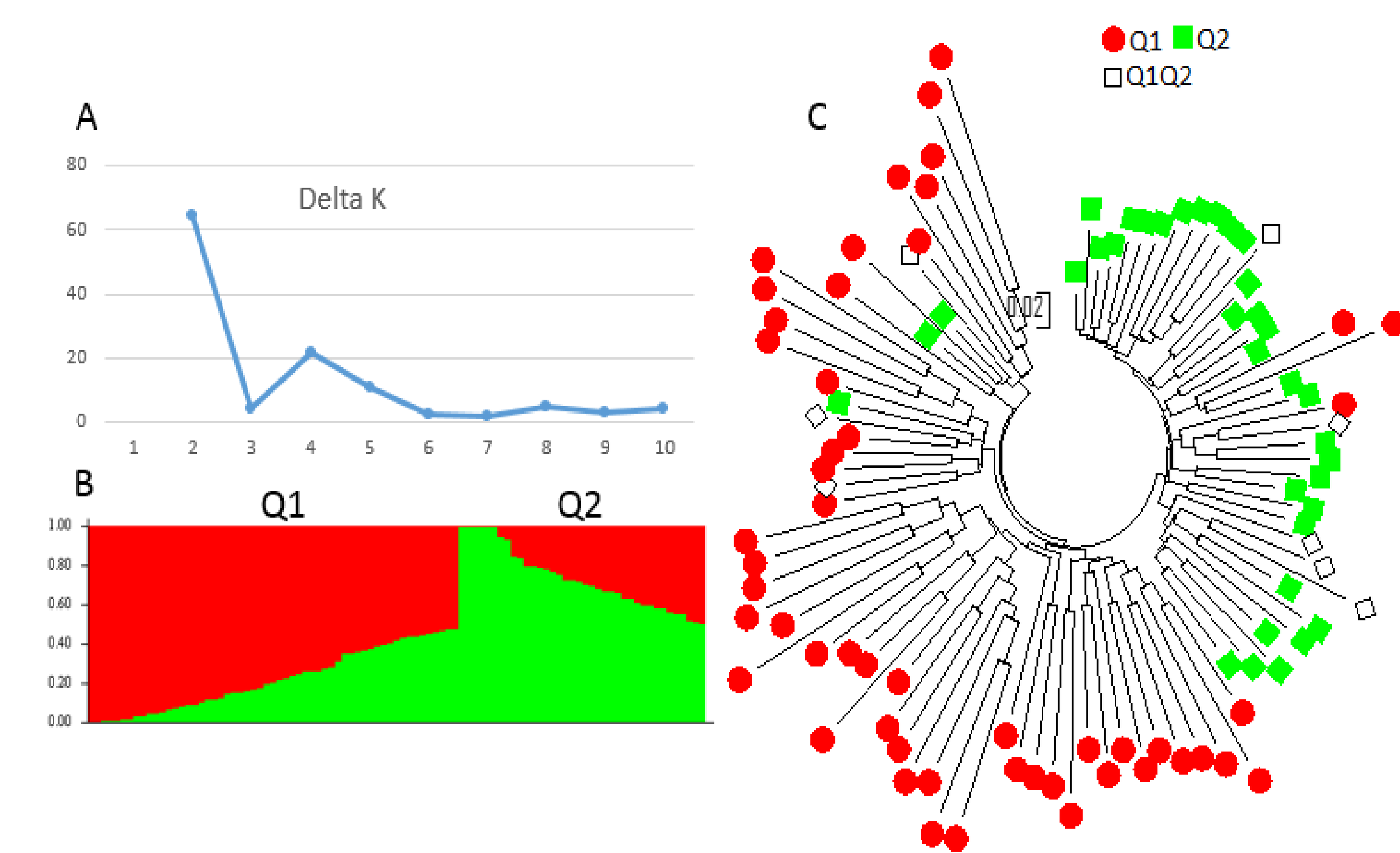
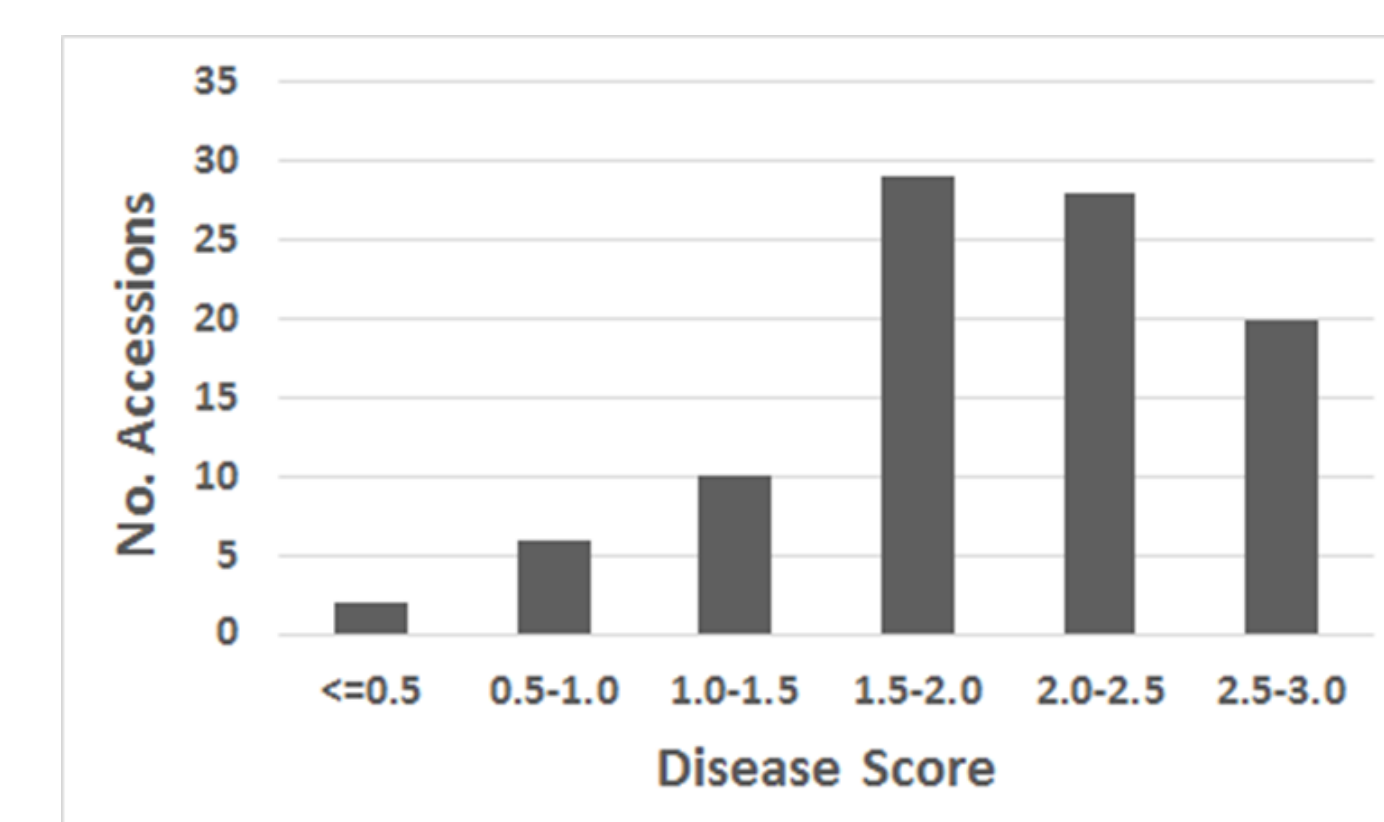


Fig 2. Model-based populations in the Verticillium wilt resistant association panel (A) Delta K values for different numbers of populations assumed (K) in the STRUCTURE analysis (B) Classification of 95 spinach accessions into two populations Q1 (red) and Q2 (green) using STRUCTURE 2.3.4. The distribution of the accessions to different populations is indicated by the color code (Q1: red and round shape, Q2: green and square shape, Q1Q2: black empty square); (C) Maximum Likelihood (ML) tree of the 95 accessions drawn by MEGA 6. The color codes for each population and admixture are consistent in the figure B and C.

Table 1. Thirteen SNP markers associated with Verticillium wilt resistance identified from five models using QGene, Tassel and GAPIT in 95 spinach accessions.

SNP name	LOD (-Log(P)) value					R-square value (%)				
	SMR	GLM	MLM	cMLM	EcMLM	SMR	GLM	MLM	cMLM	EcMLM
AYZV02052595_108	3.1	2.5	2.3	2.1	2.6	13.8	10.5	11.6	13.4	14.6
AYZV02112284_14543	4.1	2.9	2.5	2.8	3.1	18.2	9.7	9.3	17.0	17.0
AYZV02123399_146	3.4	3.1	2.8	2.7	3.1	15	13.4	14.0	16.4	17.2
AYZV02164612_331	3.4	2.1	2.0	2.1	2.4	15.1	9.3	9.5	13.4	13.6
AYZV02170942_274	2.7	2.5	2.3	2.2	2.1	12.5	10.2	10.9	13.8	12.2
AYZV02052660_2183	2.9	2.8	2.6	2.6	2.6	13.3	9.6	10.1	16.1	14.5
AYZV02064249_10266	3.1	2.8	2.6	2.9	3.1	13.8	9.4	9.6	17.3	17.0
AYZV02145765_3277	3.5	3.2	3.0	2.7	3.0	15.7	11.1	11.4	16.6	16.7
AYZV02193759_955	2.1	2.2	2.2	2.8	2.5	9.9	6.7	7.6	16.7	14.3
AYZV02194583_23976	2.4	3.0	2.9	2.2	2.8	11	10.1	10.9	14.1	15.8
AYZV02199578_156	3.3	3.1	2.8	2.9	3.0	14.8	10.2	10.2	17.2	16.7
AYZV02278250_41	2.5	3.0	2.9	2.6	2.8	11.4	10.3	11.7	16.1	15.8
AYZV02297667_1112	2.4	2.7	2.4	2.5	2.9	11.1	8.7	9.0	15.3	16.0

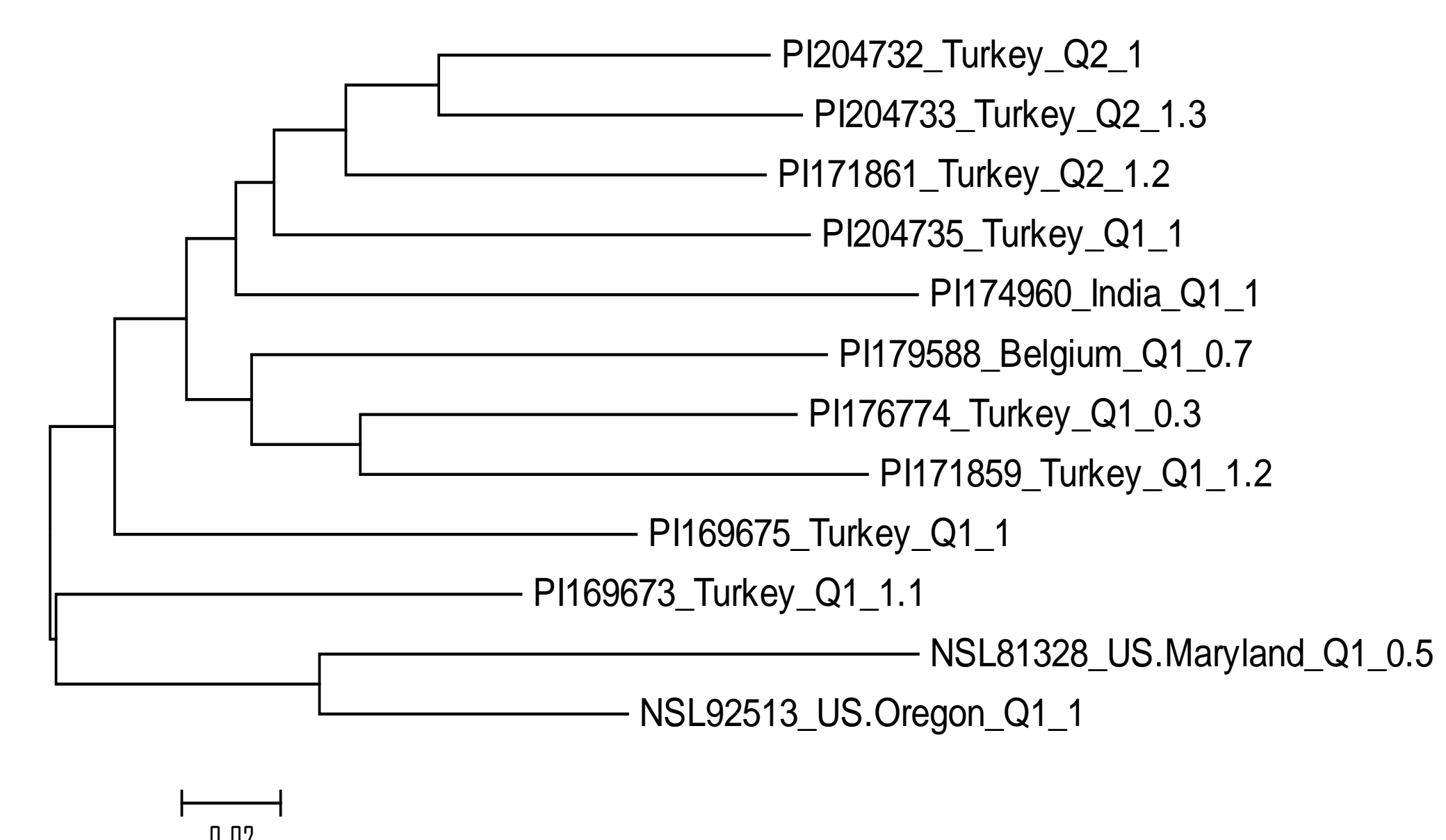


Fig 3. The phylogenetic tree among 12 Verticillium wilt spinach accessions using 2878 SNPs by MEGA 6, in which each spinach genotype was drawn using a accession\_ID combining the accession number, the original country (region), structured populations (Q1 and Q2, or mixture Q1Q2), and the scale of Verticillium wilt disease severity in order to view the information easily from the tree.