**Genetic diversity and association analysis of leafminer
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Genetic diversity and association analysis of leafminer (*Liriomyza langei*) resistance in spinach (*Spinacia oleracea*)

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Abstract: Leafminer (*Liriomyza langei*) is a major insect pest of many important agricultural crops, including spinach (*Spinacia oleracea*). Use of genetic resistance is an efficient, economic and environment-friendly method to control this pest. The objective of this research was to conduct association analysis and identify single nucleotide polymorphism (SNP) markers associated with leafminer resistance in spinach germplasm. A total of 300 USDA spinach germplasm accessions were used for the association analysis of leafminer resistance. Genotyping by sequencing (GBS) was used for genotyping and 783 SNPs from GBS were used for association analysis. The distribution of leafminer resistance showed a near normal distribution with a wide range from 1.1 to 11.7 stings per square centimeter leaf area, suggesting that the leafminer resistance in spinach is a complex trait controlled by multiple genes with minor effect in this spinach panel. Association analysis indicated that five SNP markers, AYZV02040968_7171, AYZV02076752_412, AYZV02098618_4615, AYZV02147304_383, and AYZV02271373_398 were associated with the leafminer resistance with a LOD 2.5 or higher. The SNP markers may be useful for breeders to select plants and lines for leafminer resistance in spinach breeding programs through marker-assisted selection.

Key words: association mapping, germplasm, genotyping by sequencing (GBS), leafminer, single nucleotide polymorphism (SNP), spinach, *Spinacia oleracea*

Introduction

Leafminers are important insect pests of many agricultural crops throughout the world (Parrella 1987), and major leafminer species affecting vegetables include *Liriomyza brassicae* (Riley), *L. sativae* Blanchard, *L. trifolii* (Burgess), *L. huidobrensis* (Blanchard), and *L. langei* Frick. Scheffer et al. (2001) identified the leafminers in the principal spinach production area of central California to be the morphologically cryptic species *L. langei* by using polymerase chain reaction (PCR) amplification of mitochondrial DNA.

Leafminer adults are small black flies with a bright yellow triangular spot on the upper thorax between the wings. Adult flies puncture leaves to feed on plant sap and females lay white, oval eggs within the leaf tissue. Feeding and oviposition result in leaf damage as “stings” that appear as holes or bumps on the leaves, and adult feeding on cotyledons may stunt seedling growth. Larvae hatch from eggs and feed in leaves, generating the winding, whitish tunnels or mines that are initially narrow, but increase in width as the larvae grow. Larvae drop out of the mines after completing three instars and pupate in the soil or on the leaf surface, and adult flies emerge from pupae in about 8 to 11 days. The entire life cycle can be completed in less than three weeks in warm weather in California and many generations are produced each year. Damages caused by adult sting and larval mining of leaves reduce photosynthetic capacity, render spinach leaves unmarketable, and provide an entrance for disease organisms (LeStrange et al. 1999). About 75% of the spinach produced in the Salinas Valley is used for fresh market consumption (Monterey County Agricultural Commissioner’s Office 2001). The percentage of spinach acreage grown for fresh markets vs. processing markets has increased significantly in the US (Morelock and Correll 2006, 2008). Quality standards for fresh market spinach are

extremely high, so the leafminer pest poses a serious threat for growers in California and other states who need to produce defect-free products.

Use of host genetic resistance is an alternative strategy to chemical for leafminer management and resistant varieties were recognized as the most economical method to control leafminer in vegetables (Basij et al. 2011). Evaluations of germplasm for leafminer resistance have been conducted in vegetables. Mou and Liu (2003, 2004) screened more than 200 lettuce accessions, found a large range of variation in reactions to leafminer attack, and identified sources of resistance to leafminers. Trumble and Quiros (1988) did not observe any cultivated celery with resistance to leafminers (*L. trifolii*), but found that an accession of a wild species, *Apium prostratum*, was immune to the pest. Basij et al. (2011) evaluated leafminer (*L. sativae*) resistance in 17 cucumber cultivars in greenhouse conditions and found that the 17 cultivars can be divided into four groups: susceptible, semi-susceptible, semi-resistant, and resistant based the indices such as the number of leafminer stings, the number of larval mines, the proportion of larval mines to leafminer stings, and the rate of injury. In spinach, Mou (2008) screened 345 USDA accessions and commercial cultivars for resistance to leafminer, and found that no genotype was immune to leafminers, but significant genotypic differences existed for leafminer stings per unit leaf area, mines per plant, and mines per 100 g plant weight among the genotypes tested. Mou (2008) also observed some spinach accessions that had much lower levels of leafminer stings and mines than commercial cultivars and reported two accessions PI 274065 and PI 1743854 with the lowest sting density and with the fewest mines per unit plant weight, respectively among genotypes in the field. It has indicated that the leafminer resistant accessions can be used for genetic improvement of spinach for leafminer resistance. So far, two spinach germplasms with resistance to leafminer mines have been released (Mou 2007a, 2007b).

No information is available for the genetics of leafminer resistance in spinach. Leafminer resistance in spinach seems a complex trait because a large range of responses existed in spinach genotypes (Mou 2008). It would be time-consuming to transfer these complex traits through classic plant breeding approach. However, molecular plant breeding can be an efficient way to select quantitative traits through marker assisted selection (MAS). Single nucleotide polymorphism (SNP), with its abundance, cost efficiency and high-throughput scoring, has become a powerful tool in genome mapping, association studies, diversity analysis, germplasm identification, and tagging of important genes in plant genomics (Collard et al. 2005; Collard and Mackill 2008; Feng et al. 2014; Wang et al. 2015; Xu and Crouch 2008). Therefore, identification of SNP markers associated with leafminer resistance will provide breeders with a useful tool to assist in selecting for insect resistance in spinach breeding programs. Genotyping by sequencing (GBS) is one of the next-generation sequencing platforms to discover SNPs without prior knowledge of the genome in spinach (Elshire et al. 2011; He et al. 2014; Sonah et al. 2013). The spinach genome sequences AYZV01 and AYZV02 are available to the public at (<http://www.ncbi.nlm.nih.gov/Traces/wgs/?val=AYZV01> and <http://www.ncbi.nlm.nih.gov/Traces/wgs/?val=AYZV02>), and represent approximately half of the spinach genome (Dohm et al. 2014; Minoche et al. 2015). In addition, a more comprehensive version of the spinach genome assembly will be made publicly available in 2016 (van Deynze 2014; van Deynze et al. 2015; Allen van Deynze, personal communication). Recently, Xu et al. (2015) reported the comparative transcriptomes of cultivated and wild spinach postulated from RNAseq, assembled 72,151 unigenes and identified a total of ~320,000 high-quality SNPs and these information can be viewed and downloaded at <http://spinachbase.org/cgi-bin/spinach/index.cgi>. Yang et al. (2016) built a spinach genome database SpinachDB

(<http://222.73.98.124/spinachdb>), where 21,702 spinach genes were annotated; a total of 131,592 SSRs and 1,125,743 potential SNPs located in 548,801 loci of spinach genome were identified in 11 cultivated and wild spinach cultivars. These resources provide a reference for SNP discovery and association analysis in spinach.

To date, knowledge of the spinach genome is limited and few reports have been published on the use of molecular markers in spinach. Khattak et al. (2006) published a genetic linkage map with six linkage groups, constructing the map with 101 amplified fragment length polymorphisms (AFLPs) and nine simple sequence repeats (SSRs). This genetic map has a total length of 585 cM, and an average distance of 5.18 cM between markers, but does not offer a great amount of detail about the linkage groups. AFLPs and SSRs, while useful, are less specific than SNP markers. Recently, Chan-Navarrete et al. (2015) first reported a SNP genetic maps of six linkage groups (P01-P06) consisted of 283 SNP markers, ranging in size from 46 to 116 cM and identified 39 quantitative trait loci (QTLs) related to nitrogen use efficiency (NUE) in spinach. The identification of SNP markers for spinach traits of interest, including insect resistance, will provide breeders with powerful tools to develop improved spinach cultivars more efficiently. Therefore, the development of robust SNP markers and SNP genetic maps would be a valuable resource for spinach breeding efforts. The objective of this research was to conduct association analysis and identify SNP markers associated with leafminer resistance in USDA spinach germplasm.

Materials and methods

Plant materials

A total of 300 spinach genotypes were used for the association analysis of leafminer resistance in this study (Supplementary Table S1). The 300 spinach genotypes were USDA spinach germplasm accessions, originally collected from 31 countries, mainly from eight countries including Turkey, United States (US), Afghanistan, Serbia, England, Iran, China, and Belgium having 236 accessions, which consisted of 78.7% of all tested accessions, and the other 23 countries only had 64 accessions with 21.3% of the tested accessions (Supplementary Table S1). All seeds were kindly provided by the North Central Regional Plant Introduction Station, USDA-ARS, Iowa State University, Ames, Iowa.

Leafminer phenotyping

Experiment for leafminer pest evaluation was conducted at the Agricultural Research Station of the USDA, Salinas, California (Mou 2008). Sixteen seeds from each accession were planted in a plastic pot (10 · 10 · 10 cm) with 2 sand: 1 soil (by volume) in a greenhouse, and seedlings were thinned to 10 plants per pot. Plants were moved into an outdoor insect cage (2 m high · 4 m wide · 8 m deep) made of polypropylene shade cloth for resistance screening 5 weeks after planting. Lettuce leaves with leafminer mines were collected from newly harvested fields around Salinas and hung in the shade to allow leafminer larvae to emerge from the leaves and pupate. Pupae were collected and put in plastic containers to allow adult flies to emerge. Approximately 3500 flies were then released in the outdoor cage to feed on the spinach plants. After 10 d, number of stings per unit area was counted on the leaf with most leafminer stings on each plant using an optical glass binocular magnifier (OptiVisor; Donegan Optical Co., Lenexa, KS).

For each spinach genotype, the stings per square centimeter was accounted based on single plant and total of ten plants in one plot were evaluated. The mean stings per square

centimeter for each spinach genotype was used for further data analysis. The 'Tabulate' procedure in JMP Genomics 7 (SAS Institute, Cary, NC) and Microsoft (MS) Excel 2013 was used to estimate the average, range, standard deviation (SD), and standard error of leafminer stings per square centimeter leaf area. The distribution of mean leafminer stings per square centimeter in the 300 accessions was drawn using MS Excel.

DNA extraction, GBS, and SNP discovery.

Genomic DNA was extracted from leaves of spinach plants using the CTAB (hexadecyltrimethyl ammonium bromide) method (Kisha et al. 1997). DNA library was prepared using the restriction enzyme *ApeKI* following the GBS protocol described by Elshire et al. (2011). The 90 bp double-end sequencing was performed on each spinach accession using GBS protocol by an Illumina HiSeq 2000 in Genomics Research Institute (BGI) at 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. The GBS data provided by BGI averaged 3.26 M with 90 bp short-read nucleotides for each spinach sample. The short reads of the GBS data were first aligned to spinach genome reference Viroflay-1.0.1 with AYZV01 project (<http://www.ncbi.nlm.nih.gov/Traces/wgs/?val=AYZV01>) using SOAPaligner/soap2 (<http://soap.genomics.org.cn/>). After the Spinach-1.0.3 spinach genome reference released on July 22, 2015, the AYZV01 series of contig accessions were changed to AYZV02 accessions (<http://www.ncbi.nlm.nih.gov/Traces/wgs/?val=AYZV02>), all SNP information was updated to AYZV02 version. The two versions of spinach genome references were also published on web site at <http://bvseq.molgen.mpg.de/Genome/Download/Spinach/>. The SOAPsnp v 1.05 was used for SNP calling (Li 2011; Li et al. 2009). Approximately one half-million SNPs were discovered from the GBS data among the 300 spinach germplasm accessions and provided by BGI. The

spinach accessions and SNPs were filtered before conducting genetic diversity and association analyses. If the spinach accession had greater than 20% missing SNP data and the heterozygous SNP genotype > 30%, the spinach genotype was removed from the panel. The SNP data were filtered by minor allele frequency (MAF) > 2%, missing data < 7%, and heterozygous genotype < 20%. After filtering, 783 SNPs for 300 spinach accessions were used for genetic diversity and association analysis.

Population structure and genetic diversity

The model-based program STRUCTURE 2.3.4 (Pritchard et al. 2000) was used to assess the population structure of the 300 spinach accessions/cultivars based on 783 loci. In order to identify the number of populations (K) making up the structure of the data, the burn-in period was set at 10,000 with the Markov Chain Monte Carlo iterations and the run length set at 20,000 in an admixture model. The analysis then correlated allele frequencies which was independent for each run (Lv et al. 2012). Ten runs were performed for each simulated value of K, which ranged from 1 to 11. For each simulated K, the statistical value delta K was calculated using the formula described by Evanno et al. (2005). The optimal K was determined using STRUCTURE HARVESTER (Earl and von Holdt, 2012; <http://taylor0.biology.ucla.edu/structureHarvester/>). After the optimal K was determined, a Q-matrix was obtained and was used in TASSEL 5 for association analysis. Each spinach accession was then assigned to a cluster (Q) based on the probability determined by the software that the genotype belonged in the cluster. The cut-off probability for assignment to a cluster was 0.5225 for only two clusters (structure populations). Based on the optimum K, a Bar plot with 'Sort by Q' was obtained to show the visual population structure among the 300 spinach accessions.

Genetic diversity was also assessed and the phylogeny trees were drawn using MEGA 6 (Tamura et al. 2013) based on the Maximum Likelihood tree method with the following parameters. Test of Phylogeny: Bootstrap Method, No. of Bootstrap Replications: 500, Model/Method: General Time Reversible model, Rates among Sites: Gamma distributed with Invariant sites (G + I), Number of Discrete Gamma Categories: 4, Gaps/Missing Data Treatment: Use all sites, ML Heuristic Method: Subtree-Pruning-Regrafting-Extensive (SPR level 5), Initial Tree for ML: Make initial tree automatically (Neighbor Joining), and Branch Swap Filter: Moderate. In order to compare the results from the two software programs, during the drawing of the phylogeny trees by MEGA, the colored shape and branch of each spinach genotype was drawn using the same color which was located at the cluster (Q) from STRUCTUR. For sub-tree of each Q (cluster), the shape of 'Node/Subtree Marker' and the 'Branch Line' was drawn with the same color as in the figure of the Bar plot of the population clusters from the STRUCTURE analysis.

Association analysis

Association analysis was performed using TASSEL 5 software, in which the single marker regression (SMR) without structure and without kinship, the general linear model (GLM), and the mixed linear model (MLM) methods as described in TASSEL 5 (Bradbury et al. 2007; <http://www.maizegenetics.net/tassel>). Population structure (Q) was estimated using STRUCTURE 2.3.4 (Pritchard et al. 2000), and Kinship (K) was estimated by the tool Kinship with Scald_IBS method built in Tassel 5.

Results

Phenotyping of leafminer resistance

None of the genotypes tested was immune to leafminers, because all genotypes had at least a few stings. Significant genotypic differences were found for leafminer stings per unit leaf area (Supplementary Table S1). Leafminer stings per square centimeter leaf area ranged from 1.1 to 11.7 and averaged 5.2 with a near normal distribution (Table S1; Fig. 1), suggesting that the leafminer resistance in spinach is a complex trait controlled by multiple genes with minor effect in the spinach panel. The standard deviation was 1.73 with the standard error 0.0058, indicating that there were significant genetic differences of leafminer resistance among the 300 spinach accessions (Table S1).

Genetic diversity and population structure

The population structure of the 300 spinach accessions was initially inferred using STRUCTURE 2.3.4 (Pritchard et al. 2000) and the peak of delta K was observed at K=2, indicating the presence of two main populations (clusters, Q1 and Q2) in the spinach panel (Fig. 2A and 2B). The classification of accessions into populations based on the model-based structure from STRUCTURE 2.3.4 was shown in Fig. 2B and Table S1. We used Q-value = 0.525 as the value to divide the clusters, i.e. if a spinach had its Q1 value ≥ 0.525 , it would be divided into the Cluster Q1; else if its Q2 value ≥ 0.525 , it would be divided into the Cluster Q2; and the leftover ($0.475 < Q1 < 0.525$ or $0.475 < Q2 < 0.525$) would be into Q1Q2 of the admixture. In total 286 accessions (95.3%) were assigned to one of the two populations (Q1 or Q2). Population 1 and 2 (Q1 and Q2) consisted of 103 (34.3%) and 186 (61.0%) accessions, respectively. The remaining 14 accessions (4.7%) were categorized as having admixed ancestry between Q1 and Q2 called Q1Q2 (Table S1).

The genetic diversity among spinach accessions was also assessed using the Maximum Likelihood (ML) method by MEGA 6 (Tamura et al. 2013). We defined Q1 and Q2 as the two main clusters and used the same colors as the population structure Q1 (red) and Q2 (green) from the STRUCTURE 2.3.4 (Fig. 2B) to draw the subtrees of the phylogenetic tree (Fig. 2C) with Q1 (red and round shape), Q2 (green and square shape), and the admixture Q1Q2 (black empty square). Two phylogenetic trees were included: (1) Fig. 2C, without taxon names in order to compare it to the structure populations from STRUCTURE and view them easily and clearly; (2) Supplementary Fig. S1: the format of the traditional rectangular phylogenetic tree with taxon name. The phylogenetic trees from MEGA 6 (Fig. 2C and Fig. S1), were good but not fully consistent with the structure populations (Q1-Q2) from STRUCTURE 2.3.4 (Fig. 2A and 2B), indicating that there were two differentiated genetic populations and admixtures in the spinach panel, which was not completely divided into two clusters.

Association analysis

Based on the genetic diversity analysis from STRUCTURE and MEGA and viewing the phylogenetic trees from Figs. 2 and Fig. S1, the 300 spinach accessions can be organized into two structured populations. Therefore, we used the Q matrix with two structures in the association mapping in TASSEL. In total, three models in TASSEL were used to do association analysis of leafminer resistance, including SMR, GLM (Q) and MLM (Q+K). We also used a LOD value (or likely LOD $\approx -\log(P)$, where P is the P value) having equaled or greater than 2.5 as the threshold value to identify the SNP marker associated with the leafminer resistance in the study.

With LOD value of 2.5 or higher in all three models (SMR, GLM and MLM) from TASSEL, there were five SNPs showed to be associated with leafminer resistance (Table 1). Among the five SNP markers, AYZV02040968_7171, AYZV02076752_412, and AYZV02271373_398 had 2.6 or higher LOD values in all three models, and AYZV02098618_4615 and AYZV02147304_383 had a 2.5 or higher LOD in both SMR and GLM models and a 2.2 LOD value in MLM model, indicating that the five SNP markers were associated with the leafminer resistance. However, the R^2 values were very low from 3.5 to 5.5% for all five SNP markers in three models (Table 1), indicating that the markers had minor effect for the leafminer resistance. The five SNPs were located at five different contigs, which may be located at different chromosomes or different regions of chromosomes, furtherly suggesting leafminer resistance was a quantitative trait controlled by multiple genes with minor effect.

Discussion

From this research, the distribution of leafminer resistance in the tested 300 spinach genotypes showed a near normal distribution with a wide range from 1.1 to 11.7 leafminer stings per square centimeter leaf area, suggesting that the leafminer resistance in spinach was a complex trait governed by multiple genes with minor effect. Mou (2008) reported that no genotype was immune to leafminers, but significant genotypic differences were found for leafminer stings per unit leaf area, mines per plant, and mines per 100 g plant weight among the spinach genotypes tested. So far, it is not clear whether the leafminer resistance in spinach is a quantitative or qualitative trait controlled by major genes or minor genes. We did not find major QTLs for leafminer resistance in this study. All identified SNP markers had very low R-square values, further indicating that there were multiple genes with minor effect existed in these

spinach genotypes for controlling leafminer resistance. But there is no evidence either to deny that major genes exist or do not exist for leafminer resistance in spinach. For resistance to similar pest caused by *Liriomyza* spp. in other crops, major genes were identified. Dogimont et al. (1999) reported a dominant gene, *Lt* for leafminer (*L. trifolii*) resistance in melon based on F2 and back-cross progenies from a cross between a resistant line Nantais Oblong and Vedrantaïs, a Charentais line susceptible to the leafminer. However, Kennedy et al. (1978) reported that two melon accessions PI 282448 and PI 313970 had recessive, or incompletely dominant resistance to another leafminer species (*L. sativae*). In other reports, QTL and multiple genes were also identified for leafminer resistance, such as Moreira et al. (1999) identified one major QTL for leafminer resistance located on chromosome 2 using Random Amplified Polymorphic DNA (RAPD) and Restriction Fragment Length Polymorphism (RFLP) markers, and Cardoso et al. (2014) identified four candidate genes for coffee leafminer resistance. Further QTL mapping using bi-parent populations derived from highly susceptible and highly resistant spinach lines will confirm the genetics of resistance to leafminer resistance in spinach.

In this study, there was no replicates for the phenotyping experiment with the 310 spinach genotype, however, three experiments have been conducted for evaluation of leafminer resistance in spinach germplasm: one in cage and two in field during the year 2002 and 2004 (Mou 2008). The leafminer stings per square centimeter leaf area in spinach was found to be a very stable trait with strong correlations among the three experiments (Mou 2008). Mou (2008) identified the correlation coefficients (r) to be 0.770, 0.746, and 0.802 between cage and 2002 field, between cage and 2004 field, and between 2002 field and 2004 field, respectively.

Three models SMR, GLM, and MLM were used to conduct association analysis of leafminer resistance in this study. We observed that a lot of SNP showed different results in

different models (data not shown). We supposed that if it gave significant association in different models, the SNP marker should be a reliable one. Based on LOD ($-\text{Log}(P)$) values with 2.5 or higher in three models, five SNP markers were identified to be strongly associated with leafminer resistance from this study (Table 1), indicating that the three SNP markers may be used as reliable molecular markers in breeding programs through MAS.

Among the 300 spinach genotypes, six accessions, PI220121, PI274059, PI358248, PI445783, PI449353, and PI531454 had 2.0 or fewer mean stings per square centimeter leaf area. In addition, NSL6093, PI274065, PI358253, and PI531449 also had fewer mean stings per square centimeter leaf area across three experiments (Mou 2008). The ten accessions showed high resistance to leafminer and they may be used as parents in spinach breeding programs to develop leafminer resistant cultivars. Using different genetic sources will allow breeders to create a wider range of variation in a given trait among progeny derived from two parents with a broader genetic background or larger genetic distance. A phylogenetic tree among the ten spinach accessions was built using 783 SNP alleles by MEGA 6 (Fig. 3). From the phylogenetic tree, the accession PI274065 from England is merged to PI531449 from Hungary, closer to PI531454 (country: Hungary), and then clustered together with PI274059 (England) and PI449353 (Turkey); the two accessions, PI358248 (Serbia) and PI445783 (Syria) are merged together and closer to above cluster with the five accessions; another two accessions PI220121 (Afghanistan) and PI358253 (Serbia) merged together as a separate group; and the Illinois accession NSL6093 doesn't merged to anyone as a outlier but closer to PI220121 and PI358248 (Fig. 3). The phylogenetic analysis provides breeders with knowledge about how to select the ten leafminer resistant accessions in breeding program. Thus, these accessions may provide good sources of leafminer resistance to be used as parents in spinach breeding.

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Table 1. Five SNP markers associated with leaf miner resistance identified from three modes using Tassel in 300 spinach accessions.

Spinach genome Spinach-1.0.3 information				Viroflay-1.0.1		LOD (-LOG(P))			R ² (%)		
SNP name ^a	SNP Type	Contig at AYZV02 project	SNP Position	Contig at AYZV01 project	SNP Position	SMR ^b	GLM ^b	MLM ^b	SMR	GLM	MLM
AYZV02040968_7171	C/T	AYZV02040968	7171	AYZV01031587	7171	3.4	3.5	2.7	5.3	5.3	4.2
AYZV02076752_412	T/G	AYZV02076752	412	AYZV01058628	412	2.9	2.9	3.0	4.3	4.3	4.7
AYZV02098618_4615	T/C	AYZV02098618	4615	AYZV01074880	4615	3.5	3.5	2.2	5.5	5.4	3.5
AYZV02147304_383	T/C	AYZV02147304	383	AYZV01109497	383	2.5	2.5	2.2	3.7	3.7	3.5
AYZV02271373_398	A/G	AYZV02271373	398	AYZV01198119	398	2.6	2.7	3.1	4.2	4.3	5.1

^aSNP name is defined as the contig name plus the SNP position on the contig.

^bSMR = single marker regression); GLM = general linear model; and MLM = mixed linear model using TASSEL 5 (Bradbury et al. 2007; <http://www.maizegenetics.net/tassel>).

Fig. 1. The distribution of mean leafminer stings per square centimeter leaf area in 300 spinach germplasm accessions.

Fig. 2. Model-based populations in the association panel: (A) Delta K values for different numbers of populations assumed (K) in the STRUCTURE analysis. (B) Classification of 300 USDA spinach accessions into two populations using STRUCTURE 2.3.4. The distribution of the accessions to different populations is indicated by the color code (Q1: red and Q2: green). (C) Maximum Likelihood (ML) tree of the 300 accessions drawn by MEGA 6. The color codes for each population are consistent in the figure B and C, and the empty black square as the admixture Q1Q2.

Fig. 3. A phylogenetic tree drawn by MEGA 6 among ten spinach germplasm accessions with low mean stings per square centimeter leaf area.

Fig.1

154x80mm (96 x 96 DPI)

Fig.2

243x147mm (96 x 96 DPI)

Fig.3

140x60mm (96 x 96 DPI)

Draft

Supplementary Table S1. Spinach accession, original country and States in US, leafminer damage (mean strings per square centimeter leaf area), and cluster assigned in this study.

Accession No.	Original country	US State	Leaf damage ^a	Q-cluster ^b	LineID ^c
Ames23662	Afghanistan		4.5	Q2	Ames.23662_Afghanistan_Q2
NSL184378	US	Oregon	4.1	Q1	NSL184378_Oregon_Q1
NSL184380	US	California	4.9	Q1	NSL184380_California_Q1
NSL184398	US	California	3.5	Q2	NSL184398_California_Q2
NSL186328	US	Oregon	3.2	Q2	NSL186328_Oregon_Q2
NSL22003	US	California	5	Q1	NSL22003_California_Q1
NSL22149	US	California	5.9	Q2	NSL22149_California_Q2
NSL26513	US	Michigan	4.4	Q1	NSL26513_Michigan_Q1
NSL32629	US	Louisiana	3.2	Q1	NSL32629_Louisiana_Q1
NSL32678	US	NewYork	4.7	Q2	NSL32678_NewYork_Q2
NSL37031	US	Georgia	4	Q1	NSL37031_Georgia_Q1
NSL40592	US	Michigan	5.9	Q2	NSL40592_Michigan_Q2
NSL4683	US	Maryland	2.8	Q2	NSL4683_Maryland_Q2
NSL6082	US	NewYork	4.2	Q2	NSL6082_NewYork_Q2
NSL6083	US	Illinois	5.6	Q2	NSL6083_Illinois_Q2
NSL6084	US	California	4.7	Q1Q2	NSL6084_California_Q1Q2
NSL6085	US	California	2.9	Q1Q2	NSL6085_California_Q1Q2
NSL6086	US	California	7.3	Q2	NSL6086_California_Q2
NSL6087	US	NewYork	5.1	Q1	NSL6087_NewYork_Q1
NSL6088	US	NewYork	5.3	Q1	NSL6088_NewYork_Q1
NSL6089	US	Missouri	7.6	Q2	NSL6089_Missouri_Q2
NSL6090	US	Pennsylvania	5	Q2	NSL6090_Pennsylvania_Q2
NSL6092	US	NewYork	4.3	Q2	NSL6092_NewYork_Q2
NSL6093	US	Illinois	2.9	Q2	NSL6093_Illinois_Q2
NSL6094	US	Pennsylvania	5.9	Q1	NSL6094_Pennsylvania_Q1
NSL6095	US	Missouri	3.1	Q1	NSL6095_Missouri_Q1
NSL6096	US	Missouri	6	Q1	NSL6096_Missouri_Q1
NSL6097	US	Minnesota	4.1	Q1	NSL6097_Minnesota_Q1
NSL6098	US	Virginia	4.6	Q1	NSL6098_Virginia_Q1
NSL6099	US	Pennsylvania	7.1	Q1	NSL6099_Pennsylvania_Q1
NSL6557	US	Washington	4.9	Q1	NSL6557_Washington_Q1
NSL65915	US	Oregon	4.2	Q1	NSL65915_Oregon_Q1
NSL6782	Netherlands		4.2	Q1	NSL6782_Netherlands_Q1
NSL68263	US	NewYork	4.1	Q2	NSL68263_NewYork_Q2
NSL81328	US	Maryland	4.2	Q1	NSL81328_Maryland_Q1
NSL92009	US	YorkNew	6.1	Q2	NSL92009_YorkNew_Q2
NSL92513	US	Oregon	5.8	Q1	NSL92513_Oregon_Q1
PI103063	China		5.8	Q2	PI103063_China_Q2

PI163309	India	5.6	Q2	PI163309_India_Q2
PI164965	Turkey	3.9	Q1Q2	PI164965_Turkey_Q1Q2
PI164966	Turkey	3.6	Q2	PI164966_Turkey_Q2
PI165012	Turkey	4.3	Q1	PI165012_Turkey_Q1
PI165560	India	8.3	Q2	PI165560_India_Q2
PI165710	Japan	4.3	Q2	PI165710_Japan_Q2
PI165994	India	5.9	Q1	PI165994_India_Q1
PI167098	Turkey	6.3	Q1	PI167098_Turkey_Q1
PI167194	Turkey	3.5	Q1	PI167194_Turkey_Q1
PI167195	Turkey	5.6	Q2	PI167195a_Turkey_Q2
PI167434	Belgium	4.1	Q2	PI167434_Belgium_Q2
PI169026	Turkey	4.7	Q1	PI169026_Turkey_Q1
PI169668	Turkey	7.7	Q1	PI169668_Turkey_Q1
PI169669	Turkey	5.3	Q2	PI169669_Turkey_Q2
PI169671	Turkey	4.8	Q1	PI169671_Turkey_Q1
PI169673	Turkey	9	Q1	PI169673_Turkey_Q1
PI169674	Turkey	4.8	Q1	PI169674_Turkey_Q1
PI169675	Turkey	5.4	Q1	PI169675_Turkey_Q1
PI169676	Turkey	5.4	Q1	PI169676_Turkey_Q1
PI169677	Turkey	5.9	Q2	PI169677_Turkey_Q2
PI169678	Turkey	6.7	Q1	PI169678_Turkey_Q1
PI169680	Turkey	5.8	Q2	PI169680_Turkey_Q2
PI169683	Turkey	4.7	Q2	PI169683_Turkey_Q2
PI169684	Turkey	5.9	Q1	PI169684_Turkey_Q1
PI169685	Turkey	5	Q1Q2	PI169685_Turkey_Q1Q2
PI169686	US	4.9	Q2	PI169686_Maryland_Q2
PI169688	Turkey	4.6	Q2	PI169688_Turkey_Q2
PI169690	Turkey	6.3	Q1	PI169690_Turkey_Q1
PI171859	Turkey	5.7	Q1	PI171859_Turkey_Q1
PI171860	Turkey	5	Q2	PI171860_Turkey_Q2
PI171862	Turkey	10.8	Q2	PI171862_Turkey_Q2
PI171863	Turkey	6.9	Q2	PI171863_Turkey_Q2
PI171864	Turkey	4.3	Q2	PI171864_Turkey_Q2
PI171865	Turkey	6.5	Q1	PI171865_Turkey_Q1
PI171866	Turkey	8.3	Q2	PI171866_Turkey_Q2
PI173122	Turkey	6.8	Q2	PI173122_Turkey_Q2
PI173123	Turkey	6.6	Q1	PI173123_Turkey_Q1
PI173124	Turkey	6.6	Q1	PI173124_Turkey_Q1
PI173125	Turkey	6	Q1	PI173125_Turkey_Q1
PI173126	Turkey	5.1	Q2	PI173126_Turkey_Q2
PI173127	Turkey	6.3	Q1Q2	PI173127_Turkey_Q1Q2
PI173128	Turkey	4.2	Q2	PI173128_Turkey_Q2
PI173129	Turkey	3.9	Q2	PI173129_Turkey_Q2

PI173130	Turkey	7.4	Q2	PI173130_Turkey_Q2
PI173131	Turkey	8.7	Q2	PI173131_Turkey_Q2
PI173809	Turkey	3.1	Q2	PI173809_Turkey_Q2
PI173972	India	6.8	Q2	PI173972_India_Q2
PI174383	Turkey	5.3	Q1	PI174383_Turkey_Q1
PI174384	Turkey	5.9	Q2	PI174384_Turkey_Q2
PI174385	Turkey	10	Q2	PI174385_Turkey_Q2
PI174386	Turkey	6.8	Q2	PI174386_Turkey_Q2
PI174387	Turkey	5.5	Q2	PI174387_Turkey_Q2
PI174388	Turkey	4.3	Q2	PI174388_Turkey_Q2
PI174389	Turkey	6	Q2	PI174389_Turkey_Q2
PI174960	India	6.2	Q1	PI174960_India_Q1
PI175311	India	4.6	Q2	PI175311_India_Q2
PI175312	India	11.7	Q1	PI175312_India_Q1
PI175313	India	5.9	Q2	PI175313_India_Q2
PI175595	Turkey	3.1	Q2	PI175595_Turkey_Q2
PI175923	Turkey	7.1	Q1	PI175923_Turkey_Q1
PI175924	Turkey	6.4	Q2	PI175924_Turkey_Q2
PI175925	Turkey	5.3	Q1	PI175925_Turkey_Q1
PI175926	Turkey	6.5	Q1	PI175926_Turkey_Q1
PI175927	Turkey	6.5	Q2	PI175927_Turkey_Q2
PI175928	Turkey	4.4	Q1	PI175928_Turkey_Q1
PI175929	Turkey	5.6	Q2	PI175929_Turkey_Q2
PI175931	Turkey	5	Q1	PI175931_Turkey_Q1
PI175932	Turkey	4.9	Q2	PI175932_Turkey_Q2
PI176371	Italy	5.6	Q1	PI176371_Italy_Q1
PI176372	Italy	5.5	Q1Q2	PI176372_Italy_Q1Q2
PI176769	Turkey	4	Q1	PI176769_Turkey_Q1
PI176770	Turkey	4.4	Q1	PI176770_Turkey_Q1
PI176771	Turkey	7.3	Q1	PI176771_Turkey_Q1
PI176772	Turkey	3.6	Q2	PI176772_Turkey_Q2
PI176773	Turkey	5.9	Q2	PI176773_Turkey_Q2
PI176774	Turkey	6.1	Q1	PI176774_Turkey_Q1
PI176775	Turkey	4.2	Q1	PI176775_Turkey_Q1
PI176776	Turkey	4.1	Q2	PI176776_Turkey_Q2
PI176777	Turkey	5.6	Q1Q2	PI176777_Turkey_Q1Q2
PI176779	Turkey	6.7	Q2	PI176779_Turkey_Q2
PI176780	Turkey	5.9	Q2	PI176780_Turkey_Q2
PI177081	Turkey	4.9	Q1Q2	PI177081_Turkey_Q1Q2
PI177082	Turkey	5.4	Q1	PI177082_Turkey_Q1
PI177557	Turkey	4.3	Q1	PI177557_Turkey_Q1
PI177558	Turkey	7.6	Q1	PI177558_Turkey_Q1
PI179041	Turkey	3.9	Q2	PI179041_Turkey_Q2

PI179043	Turkey	5.7	Q2	PI179043_Turkey_Q2
PI179044	Turkey	5.6	Q1	PI179044_Turkey_Q1
PI179507	Syria	6.2	Q2	PI179507_Syria_Q2
PI179508	Iraq	6.2	Q2	PI179508_Iraq_Q2
PI179509	Turkey	4.3	Q1	PI179509_Turkey_Q1
PI179588	Belgium	7.2	Q1	PI179588_Belgium_Q1
PI179589	Belgium	7.3	Q2	PI179589_Belgium_Q2
PI179590	Belgium	5.3	Q2	PI179590_Belgium_Q2
PI179591	Belgium	4.5	Q1	PI179591_Belgium_Q1
PI179592	Belgium	4	Q2	PI179592_Belgium_Q2
PI179593	Belgium	4.8	Q1	PI179593_Belgium_Q1
PI179594	Belgium	3.6	Q1Q2	PI179594_Belgium_Q1Q2
PI179595	Belgium	4.4	Q2	PI179595_Belgium_Q2
PI179596	Belgium	6.9	Q2	PI179596_Belgium_Q2
PI179597	Belgium	4.3	Q1	PI179597_Belgium_Q1
PI181808	Syria	9.8	Q1	PI181808_Syria_Q1
PI181809	Syria	3.7	Q2	PI181809_Syria_Q2
PI181923	Syria	3.9	Q1	PI181923_Syria_Q1
PI181964	Syria	6.7	Q2	PI181964_Syria_Q2
PI184137	Serbia	4.5	Q1	PI184137_Serbia_Q1
PI192945	China	4.3	Q1	PI192945_China_Q1
PI193619	Ethiopia	4.7	Q1	PI193619_Ethiopia_Q1
PI200882	Afghanistan	8.4	Q2	PI200882_Afghanistan_Q2
PI204632	Turkey	5.8	Q1	PI204632_Turkey_Q1
PI204732	Turkey	5.5	Q2	PI204732_Turkey_Q2
PI204733	Turkey	8.1	Q2	PI204733_Turkey_Q2
PI204734	Turkey	7.6	Q2	PI204734_Turkey_Q2
PI204735	Turkey	6.2	Q1	PI204735_Turkey_Q1
PI204736	Turkey	5.8	Q1	PI204736_Turkey_Q1
PI205231	Turkey	4.7	Q1Q2	PI205231_Turkey_Q1Q2
PI205232	Turkey	4.7	Q2	PI205232_Turkey_Q2
PI205233	Turkey	7.2	Q2	PI205233_Turkey_Q2
PI205234	Turkey	6.8	Q2	PI205234_Turkey_Q2
PI205235	Turkey	8.8	Q1	PI205235_Turkey_Q1
PI206007	Sweden	7.8	Q2	PI206007_Sweden_Q2
PI206473	Turkey	3.9	Q1	PI206473_Turkey_Q1
PI206474	Turkey	7	Q2	PI206474_Turkey_Q2
PI206475	Turkey	6.6	Q2	PI206475_Turkey_Q2
PI206753	Turkey	9	Q1	PI206753_Turkey_Q1
PI207518	Afghanistan	4.3	Q2	PI207518_Afghanistan_Q2
PI209644	Iran	3.3	Q1	PI209644_Iran_Q1
PI209645	Iran	7.9	Q2	PI209645_Iran_Q2
PI209646	Turkey	9	Q1Q2	PI209646_Turkey_Q1Q2

PI209647	Iran	6.3	Q2	PI209647_Iran_Q2
PI211632	Afghanistan	6.2	Q2	PI211632_Afghanistan_Q2
PI212119	Afghanistan	5.7	Q2	PI212119_Afghanistan_Q2
PI212120	Afghanistan	6.4	Q2	PI212120_Afghanistan_Q2
PI212328	Afghanistan	5.2	Q2	PI212328_Afghanistan_Q2
PI217425	SouthKorea	5	Q2	PI217425_SouthKorea_Q2
PI219949	Afghanistan	3.4	Q2	PI219949_Afghanistan_Q2
PI220121	Afghanistan	1.1	Q2	PI220121_Afghanistan_Q2
PI220546	Afghanistan	6.3	Q1	PI220546_Afghanistan_Q1
PI220686	Afghanistan	4.1	Q2	PI220686_Afghanistan_Q2
PI222270	Iran	5.8	Q2	PI222270_Iran_Q2
PI222749	Iran	4.7	Q2	PI222749_Iran_Q2
PI222750	Iran	4.2	Q2	PI222750_Iran_Q2
PI222838	Iran	4.2	Q1	PI222838_Iran_Q1
PI223536	Afghanistan	11.2	Q2	PI223536_Afghanistan_Q2
PI224959	Iran	6	Q1	PI224959_Iran_Q1
PI226671	Iran	3.4	Q1	PI226671_Iran_Q1
PI227045	Iran	3.5	Q1	PI227045_Iran_Q1
PI227230	Japan	6.1	Q1	PI227230_Japan_Q1
PI227383	Iran	6.1	Q1	PI227383_Iran_Q1
PI229731	Iran	7.9	Q2	PI229731_Iran_Q2
PI229792	Iran	7.7	Q1	PI229792_Iran_Q1
PI249920	Spain	3.1	Q1	PI249920_Spain_Q1
PI251507	Iran	3.8	Q2	PI251507_Iran_Q2
PI254565	Afghanistan	5.4	Q2	PI254565_Afghanistan_Q2
PI256079	Afghanistan	3.6	Q1Q2	PI256079_Afghanistan_Q1Q2
PI261788	France	3.1	Q1	PI261788_France_Q1
PI261789	France	6.1	Q2	PI261789_France_Q2
PI262161	Spain	3.7	Q2	PI262161_Spain_Q2
PI262911	Spain	5.1	Q2	PI262911_Spain_Q2
PI263873	Greece	4.3	Q2	PI263873_Greece_Q2
PI266926	Germany	3.7	Q2	PI266926_Germany_Q2
PI274042	England	3.9	Q2	PI274042_England_Q2
PI274044	England	3.4	Q1	PI274044_England_Q1
PI274046	England	3.5	Q2	PI274046_England_Q2
PI274047	England	5.1	Q2	PI274047_England_Q2
PI274048	England	4.7	Q2	PI274048_England_Q2
PI274049	England	3.6	Q1	PI274049_England_Q1
PI274050	England	5.4	Q2	PI274050_England_Q2
PI274051	England	4.8	Q2	PI274051_England_Q2
PI274052	England	3.6	Q2	PI274052_England_Q2
PI274053	England	4.4	Q2	PI274053_England_Q2
PI274055	England	5.4	Q2	PI274055_England_Q2

PI274056	England	3.6	Q2	PI274056_England_Q2
PI274057	England	5.1	Q2	PI274057_England_Q2
PI274058	England	2.7	Q2	PI274058_England_Q2
PI274059	England	2	Q2	PI274059_England_Q2
PI274060	England	5.4	Q2	PI274060_England_Q2
PI274063	England	4.7	Q2	PI274063_England_Q2
PI274065	England	2.8	Q2	PI274065_England_Q2
PI285751	Warszawa	3.6	Q2	PI285751_Warszawa_Q2
PI296393	Iran	5.1	Q1Q2	PI296393_Iran_Q1Q2
PI303138	Netherlands	6.4	Q2	PI303138_Netherlands_Q2
PI319220	Egypt	3.1	Q2	PI319220_Egypt_Q2
PI321020	China	3.9	Q2	PI321020_China_Q2
PI339545	Turkey	2.6	Q2	PI339545_Turkey_Q2
PI339546	Turkey	3.9	Q2	PI339546_Turkey_Q2
PI339547	Turkey	4.1	Q2	PI339547_Turkey_Q2
PI339548	Turkey	3.9	Q2	PI339548_Turkey_Q2
PI358248	Serbia	1.9	Q2	PI358248_Serbia_Q2
PI358249	Serbia	5.4	Q1	PI358249_Serbia_Q1
PI358250	Serbia	4.5	Q1	PI358250_Serbia_Q1
PI358251	Serbia	4.6	Q2	PI358251_Serbia_Q2
PI358252	Serbia	5.5	Q2	PI358252_Serbia_Q2
PI358253	Serbia	2.7	Q1	PI358253_Serbia_Q1
PI358254	Serbia	4.5	Q2	PI358254_Serbia_Q2
PI358256	Serbia	7.5	Q2	PI358256_Serbia_Q2
PI358257	Serbia	6.2	Q2	PI358257_Serbia_Q2
PI358258	Macedonia	5.8	Q2	PI358258_Macedonia_Q2
PI358259	Serbia	3.2	Q1	PI358259_Serbia_Q1
PI358260	Macedonia	5.3	Q2	PI358260_Macedonia_Q2
PI360710	France	5.8	Q2	PI360710_France_Q2
PI360895	Netherlands	3.2	Q2	PI360895_Netherlands_Q2
PI361127	England	5.3	Q2	PI361127_England_Q2
PI368824	Serbia	5.7	Q2	PI368824_Serbia_Q2
PI368825	Serbia	5	Q2	PI368825_Serbia_Q2
PI368826	Serbia	7.9	Q2	PI368826_Serbia_Q2
PI370602	Serbia	9	Q2	PI370602_Serbia_Q2
PI374233	Egypt	3.7	Q1	PI374233_Egypt_Q1
PI379546	Serbia	3.6	Q2	PI379546_Serbia_Q2
PI379547	Serbia	3.8	Q2	PI379547_Serbia_Q2
PI379548	Serbia	5.5	Q2	PI379548_Serbia_Q2
PI379549	Serbia	3.7	Q2	PI379549_Serbia_Q2
PI379550	Serbia	4.5	Q1	PI379550_Serbia_Q1
PI379551	Serbia	3.9	Q2	PI379551_Serbia_Q2
PI379552	Serbia	5.2	Q2	PI379552_Serbia_Q2

PI418978	China	5.1	Q2	PI418978_China_Q2
PI419004	China	6.9	Q2	PI419004_China_Q2
PI419162	china	4.1	Q2	PI419162_china_Q2
PI419218	China	4.8	Q2	PI419218_China_Q2
PI433207	China	5.2	Q2	PI433207_China_Q2
PI433208	China	10.6	Q2	PI433208_China_Q2
PI433209	China	7.4	Q2	PI433209_China_Q2
PI433210	China	7.4	Q2	PI433210_China_Q2
PI433211	France	4.6	Q2	PI433211_France_Q2
PI433212	China	6.3	Q1	PI433212_China_Q1
PI445782	Syria	8.8	Q2	PI445782_Syria_Q2
PI445783	Syria	1.6	Q2	PI445783_Syria_Q2
PI445784	Syria	5.1	Q2	PI445784_Syria_Q2
PI445785	Syria	5.6	Q2	PI445785_Syria_Q2
PI449353	Turkey	2	Q2	PI449353_Turkey_Q2
PI478393	China	3.5	Q1	PI478393_China_Q1
PI491261	Greece	5.4	Q2	PI491261_Greece_Q2
PI491262	Greece	5	Q2	PI491262_Greece_Q2
PI499372	SovietUnion	3.2	Q2	PI499372_SovietUnion_Q2
PI499373	SovietUnion	4.1	Q1Q2	PI499373_SovietUnion_Q1Q2
PI508504	SouthKorea	6.5	Q1	PI508504_SouthKorea_Q1
PI527332	China	2.6	Q2	PI527332_China_Q2
PI531448	Hungary	3	Q2	PI531448_Hungary_Q2
PI531449	Hungary	2.7	Q1	PI531449_Hungary_Q1
PI531450	Hungary	3.1	Q2	PI531450_Hungary_Q2
PI531452	Denmark	5.1	Q1	PI531452_Denmark_Q1
PI531453	Hungary	3.5	Q2	PI531453_Hungary_Q2
PI531454	Hungary	1.9	Q2	PI531454_Hungary_Q2
PI531456	Hungary	3	Q2	PI531456_Hungary_Q2
PI531457	Hungary	2.7	Q2	PI531457_Hungary_Q2
PI535897	Poland	3.7	Q1	PI535897_Poland_Q1
PI604777	Japan	4.5	Q1	PI604777_Japan_Q1
PI604778	Japan	9.3	Q1	PI604778_Japan_Q1
PI604779	Japan	5.6	Q2	PI604779_Japan_Q2
PI604780	Japan	5.3	Q2	PI604780_Japan_Q2
PI604782	Afghanistan	4	Q1	PI604782_Afghanistan_Q1
PI604783	Afghanistan	2.5	Q2	PI604783_Afghanistan_Q2
PI604784	Afghanistan	3.3	Q2	PI604784_Afghanistan_Q2
PI604785	Mongoli	4.3	Q1	PI604785_Mongoli_Q1
PI604786	Nepal	5.5	Q1	PI604786_Nepal_Q1
PI604787	Afghanistan	2.9	Q1	PI604787_Afghanistan_Q1
PI604788	Afghanistan	4.1	Q2	PI604788_Afghanistan_Q2
PI604789	Afghanistan	2.4	Q2	PI604789_Afghanistan_Q2

PI604790	Afghanistan	3.4	Q2	PI604790_Afghanistan_Q2
PI604791	Afghanistan	2.4	Q2	PI604791_Afghanistan_Q2
PI606707	Netherlands	4.6	Q2	PI606707_NorthHolland_Q2
PI608762	Thailand	8.8	Q2	PI608762_Thailand_Q2

^aLeaf damage signifies the mean strings per square centimeter leaf area for each spinach genotype.

^bTwo population structures (Clusters) identified from Mega 6: Q1 means that the spinach accession belongs to the population group1; Q2 to group2; and Q1Q2 to group 1 or 2.

^cIn order to view the phylogenetic trees easily, the spinach accession number, the accession original country, the accession geography region, and the structure population (cluster) were merged together into one taxon name as each spinach accession in drawing the combined tree. For example, the taxon name, Ames23662_Afghanistan_Q2 includes the accession number – Ames23662, which was originally collected from Afghanistan; and assigned to cluster Q2, where there were a total of two clusters and mixtrue.

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Supplementary Fig. S1. The traditional phylogenetic tree combining structure populations (Q1 to Q2) from STRUCTURE 2 and the Maximum Likelihood (ML) method from MEGA 6. The spinach accession number, the accession original country (or the State in US), and the structure population (cluster) were merged together into one taxon name as each spinach accession in the combined tree drawn by MEGA. The colored shape and branch are one cluster that matched the structure population (red for Q1, green for Q2, and the empty black square for the admixture Q1Q2).

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