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Genetic Diversity and Population Structure in *Solanum nigrum* Based on Single-Nucleotide Polymorphism (SNP) Markers

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Abstract: *Solanum nigrum* is a noxious weed in agricultural ecosystem that limits many crops' production. The aim of the present study was to understand the level of genetic diversity and population structure of *S. nigrum* in China. A specific-locus amplified fragment (SLAF) sequencing method was conducted to detect single-nucleotide polymorphisms (SNPs) in the genomes of *S. nigrum* from 66 populations in China. A total of 616,533 high-quality SNPs were identified from 189,840 SLAFs, with an average sequencing depth of 10.59× fold and a Q30 value of 93% and a GC content of 42.78%. It showed a considerable amount of genetic diversity and genetic variability of *S. nigrum* among samples. The genetic differentiation of *S. nigrum* indicated that there was a low level of genetic differentiation (Fst < 0.1000) among geographical populations. A cluster analysis showed that populations of *S. nigrum* were divided into two subgroups, with some samples from adjacent position roughly clustered together, which showed some correlation between geographic origins. A population structure analysis suggested the 66 *S. nigrum* samples could have originated from three different genetic clusters. The Xinjiang site was the only location where all genetic clusters were found, which suggested these populations were genetically diverse. These results showed that there was a high degree of genetic diversity and low difference among the different geographical populations of *S. nigrum*. The results from the genetic structure of the SNP markers indicated that wide genetic variability exists among the population of *S. nigrum* in China, which may contribute to the adaptation and infestation of this weed species.

Keywords: *Solanum nigrum* L.; single-nucleotide polymorphisms (SNPs); specific-locus amplified fragment (SLAF); genetic diversity; population structure

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1. Introduction

Black nightshade (*Solanum nigrum* L.) is widely distributed throughout the world. It serves as a source of leafy vegetables, fruits, or local medicinal herbs [1–3]. However, this species is considered to be a troublesome weed of agriculture in most parts of the world. *S. nigrum* causes substantial problems for crop production not only by competing for nutrients, moisture, and light with crops in an agricultural field, but also by reducing the commercial quality of crops by contaminating harvest crops because of their purple/black succulent berries. It also widespread in China, especially in the northernmost parts of China, abundant in irrigated fields in Xinjiang, Heilongjiang, and Jilin. In recent years, persistent herbicide application has resulted in herbicide-resistant *S. nigrum* populations in China [4,5]. It has become a noxious weed of crops such as cotton, corn, potato, soybean, sunflower, tomato, and vegetables, especially in cotton fields since the land is subject to irrigation in Xinjiang [4–7]. Based on our survey, more than half of cotton fields were infested with *S. nigrum*, and the number of *S. nigrum* has increased dramatically compared

with a decade ago [6]. The rapid increase in the number and spread of *S. nigrum* makes it the dominant weed in the cotton production area.

Populations of *S. nigrum* are rapidly expanding due to adapting to the environment. Genetic diversity influences populations' adaptation to cropping systems and species evolution. *S. nigrum* has accumulated many characteristics and genetic adaptations to the environment. It possesses prolific seed production, wide tolerant habits from tropical to temperate regions [1], the ability to accumulate Cd from soil [8,9], and resistance to serval herbicides [4,5,10–13]. Several PCR-based DNA markers, namely restriction fragment length polymorphism (RFLP), nuclear intron-targeting markers, and inter-sample sequence repeats (SSR), have been used to discover genetic diversity and variation among *S. nigrum* populations [14–17]. However, information on the genetic polymorphism among different *S. nigrum* populations in China has not been studied. Assessment of its genetic diversity will be helpful for better understanding the genetic structure of natural *S. nigrum* populations. An effective weed management program requires a comprehensive knowledge of the weed diversity [18–20].

Molecular markers, reflecting the actual level of genetic variation at the DNA level, have been used to estimate genetic diversity in plants because they are stable, polymorphic, readily available in the genome, and not sensitive to environmental factors [21]. SNP markers have been rapidly applied in genetics because of the abundance and accessibility of nonbiased SNPs throughout the genome [22]. They have been widely used in plants to evaluate genetic diversity, construct linkage maps, and perform association analyses [23,24]. The SLAF-seq method combining next-generation sequencing was developed for genetic mapping, polymorphism analysis, systematic evolution, and germ plasm resource identification [25–29].

In the present paper, SLAF-seq technology was used for the first time to seek molecular evidence of *S. nigrum*. Here we report on the development of SNP markers for examining the underlying population structure of *S. nigrum* based on 66 samples from a wide range in China. Based on the wide distribution and rapid increase in the number of *S. nigrum*, genetic diversity and possible differentiation within *S. nigrum* populations are hypothesized. In this study, we aimed to assess the genetic diversity and genetic structure in natural populations of *S. nigrum* by comparing the genetic distance and the number of polymorphism markers. This is the first report to use SNPs on *S. nigrum* and represent whether genetic diversity is randomly distributed or has a patterned spatial distribution.

2. Materials and Methods

2.1. Plant Materials

A total of 66 seed samples of *S. nigrum* was collected from crop fields. These seeds were obtained from different locations ranging from 34° N to 52° N and from 80° E to 133° E in China, and at elevations ranging from 50 m to 780 m (Table 1). Of these samples, 47 were from northwest China (Xinjiang), 15 were from northeast China (Heilongjiang, Liaoning, Jilin, and Inner Mongolia), 4 were from the north of China (Shaanxi, Henan, and Beijing). Ripe seeds were collected from five to ten individual plants randomly. The *S. nigrum* were cultivated in the greenhouse of the Institute of Plant Protection, Chinese Academy of Agricultural Sciences, China.

2.2. Genomic DNA Extractions and Specific-Locus Amplified Fragment Sequencing

Genomic DNA was isolated from 100 mg of fresh young leaf tissue from germinated seeds at the 3–4-leaf stage following the CTAB manufacturer's protocol. The DNA quality was then assessed using electrophoresis on a 1.2% agarose gel. A high-resolution technology named SLAF-seq was constructed for DNA sequencing as previously described [30], except that the genomic DNA was digested with the restriction enzyme HaeIII (New England Biolabs, NEB). In summary, the genomic DNA was digested into fragments with the restriction enzyme HaeIII with a size-selection window of 364–414 bp and then sequenced with the HiSeq 2500 system according to the manufacturer's protocol (Illumina Inc., San

Diego, CA, USA). It was expected to yield 113,951 unique SLAF tags. To control the quality, we screened the raw data for high-quality reads with Q30 (the ratio of bases sequencing quality value greater than 30) quality scores and guanine/cytosine content for reads 94.11% and 42.78%, respectively (Table 2). The genome size of *S. nigrum* was estimated to be 1.66 G using flow cytometry.

Table 1. Details of geographic and sampling information for *S. nigrum* populations in this study.

ID	Biogeographic Regions	Longitude	Latitude	ID	Biogeographic Regions	Longitude	Latitude
SB1	Beijing	116°16′45″	39°33′16″	SX15	Xinjiang	85°63′07″	44°19′31″
SH1	Heilongjiang	122°77′19″	52°22′92″	SX16	Xinjiang	86°50′25″	44°23′50″
SH2	Heilongjiang	133°52′53″	47°24′19″	SX17	Xinjiang	84°58′38″	44°34′17″
SH3	Heilongjiang	124°54′42″	47°08′55″	SX18	Xinjiang	86°50′25″	44°23′50″
SH4	Heilongjiang	124°28′01″	47°45′39″	SX19	Xinjiang	85°0′12″	44°27′45″
SJ1	Jilin	123°43′53″	44°10′83″	SX20	Xinjiang	86°37′30″	44°28′59″
SJ2	Jilin	124°1′57″	44°17′20″	SX21	Xinjiang	81°40′59″	45°0′14″
SJ3	Jilin	122° 55′82″	45°07′49″	SX22	Xinjiang	86°24′17″	44°30′58″
SJ4	Jilin	122°54′14″	44°36′12″	SX23	Xinjiang	85°1′36″	44°31′37
SJ5	Jilin	123°55′52″	44°17′31″	SX24	Xinjiang	80°54′50″	40°34′57″
SJ6	Jilin	123°46′35″	44°13′60″	SX25	Xinjiang	81°18′6″	40°32′37″
SJ7	Jilin	123°48′56″	44°41′54″	SX26	Xinjiang	89°08′31″	42°57′14″
SL1	Liaoning	120°50′50″	40°44′18″	SX27	Xinjiang	86°33′85″	46°19′43″
SL2	Liaoning	120°52′35″	40°46′51″	SX28	Xinjiang	85°42′23″	46°05′11″
SM1	Inner Mongolia	121°19′52″	43°45′52″	SX29	Xinjiang	84°43′12″	45°25′26″
SM2	Inner Mongolia	121°19′52″	43°45′52″	SX31	Xinjiang	84°37′17″	44°32′21″
SN1	Henan	113°41′44″	35°15′32″	SX32	Xinjiang	84°58′38″	44°34′17
SN2	Henan	114°55′22″	34°51′59″	SX33	Xinjiang	82°06′20″	44°58′02″
SS1	Shaanxi	108°13′57″	34°23′38″	SX34	Xinjiang	85°36′49″	48°08′38″
SX02	Xinjiang	81°32′6″	45°5′12″	SX35	Xinjiang	84°59′56″	44°25′45″
SX03	Xinjiang	86°24′17″	44°30′58″	SX36	Xinjiang	86°37′30″	44°28′59″
SX04	Xinjiang	82°20′33″	44°51′60″	SX37	Xinjiang	85°51′54″	47°32′53″
SX05	Xinjiang	86°51′46″	44°20′28″	SX38	Xinjiang	88°01′57″	47°14′16″
SX06	Xinjiang	82°24′30″	44°49′48″	SX39	Xinjiang	87°58′31″	47°18′39″
SX07	Xinjiang	86°37′30″	44°28′59″	SX40	Xinjiang	87°12′18″	44°07′12″
SX08	Xinjiang	82°10′35″	44°37′59″	SX41	Xinjiang	87°04′44″	42°15′58″
SX09	Xinjiang	82°27′7″	44°37′51″	SX42	Xinjiang	88°1′52 “	47°14′16 “
SX01	Xinjiang	82°5′43″	44°39′25″	SX43	Xinjiang	86°48′07″	40°10′06″
SX10	Xinjiang	82°6′22″	44°53′2″	SX44	Xinjiang	87°12′18″	44°07′12″
SX11	Xinjiang	82°8′60″	44°39′1″	SX45	Xinjiang	87°04′44″	42°15′58″
SX12	Xinjiang	85°48′55″	41°43′36″	SX46	Xinjiang	88°01′57″	47°14′16″
SX13	Xinjiang	84°58′38″	44°34′17″	SX47	Xinjiang	87°15′06″	44°08′31″
SX14	Xinjiang	86°50′25″	44°23′50″	SX48	Xinjiang	85°06′37″	44°19′03″

2.3. Sequence Alignment for SNP Calling and Quality Assessment

Taking into account the absence of a reference genome for *S. nigrum*, the SLAF reads of each sample were aligned to the *S. melongena* reference genome (ftp://ftp.solgenomics.net/genomes/Solanum_melongena_consortium/, accessed on 10 August 2020). A preliminary SLAF experiment using the genome of *S. melongena* was conducted. We called samples SNPs in the 66 *S. nigrum* individuals based on the corresponding genome-wide SLAF tags. The Genome Analysis [31] Toolkit and SAMtools [32] were used for SNP calling and the SNPs were quality-filtered with a minor allele frequency (MAF) ≥ 0.05. Based on the above parameter, 616,533 SNP markers were retained for further analysis.

2.4. Genetic Differentiation Analyses

For the estimation of the level and pattern of genetic differentiation among geographic populations, we estimated fixation index Fst (F-statistics) values for the SNPs of all pairs of populations following Weir and Cockerham's method as implemented in the GENODIVE software v2.0 [33]. Diversity parameters, the value of the observed heterozygosity (Ho), and the expected heterozygosity (He) were employed using GENODIVE software v2.0.

Table 2. Summary statistics for specific-locus amplified fragment sequence data of *S. nigrum*.

ID	Total Reads	GC Percentage (%)	Q30 Percentage (%)	ID	Total Reads	GC Percentage (%)	Q30 Percentage (%)
SB1	1,691,734	42.73	93.84	SX15	1,650,570	43.41	94.14
SH1	1,807,444	42.05	94.47	SX16	1,519,690	43.44	94.39
SH2	1,902,574	42.80	94.42	SX17	1,737,036	42.87	93.68
SH3	1,823,795	43.30	94.01	SX18	2,002,105	43.27	94.15
SH4	1,716,365	43.0	93.65	SX19	1,736,208	43.40	94.31
SJ1	2,662,305	42.15	94.57	SX20	1,681,088	43.44	94.47
SJ2	1,494,444	42.98	94.02	SX21	1,622,367	43.09	94.48
SJ3	1,735,455	43.30	94.68	SX22	1,598,613	43.41	94.58
SJ4	3,858,231	42.72	94.36	SX23	1,534,640	43.47	94.78
SJ5	1,643,277	43.10	93.62	SX24	1,941,331	43.02	94.58
SJ6	2,131,241	43.27	94.01	SX25	2,100,514	42.83	94.29
SJ7	1,588,142	42.71	93.70	SX26	1,716,587	42.76	94.53
SL1	1,883,192	43.04	94.02	SX27	1,896,964	43.15	93.89
SL2	1,616,090	43.44	94.53	SX28	2,049,341	43.24	94.14
SM1	1,657,653	42.95	94.42	SX29	1,799,983	43.29	93.80
SM2	2,166,560	42.33	93.63	SX31	1,812,148	42.8	93.90
SN1	2,109,751	42.85	93.91	SX32	1,838,587	42.82	93.44
SN2	1,676,914	43.00	94.43	SX33	1,753,477	43.08	94.06
SS1	1,620,348	42.88	94.09	SX34	1,769,883	43.0	94.23
SX01	2,450,367	41.97	94.76	SX35	1,712,473	43.29	94.27
SX02	1,723,640	42.68	93.92	SX36	1,522,925	42.49	93.27
SX03	2,213,391	41.92	94.75	SX37	1,731,599	42.72	93.57
SX04	1,973,084	42.03	94.79	SX38	1,711,985	42.73	93.68
SX05	1,958,204	42.30	94.77	SX39	1,789,567	42.51	93.37
SX06	2,121,610	42.05	94.63	SX40	1,442,651	42.36	93.34
SX07	2,053,106	42.13	94.73	SX41	1,563,989	42.78	93.21
SX08	2,393,941	41.88	94.58	SX42	1,527,690	42.56	93.11
SX09	2,205,426	42.09	94.63	SX43	1,741,900	42.61	93.55
SX10	2,023,837	42.51	94.57	SX44	1,974,449	42.57	93.60
SX11	2,404,368	42.03	94.49	SX45	1,895,990	42.50	93.53
SX12	1,750,841	42.12	94.55	SX46	1,606,552	42.57	93.42
SX13	1,863,520	42.87	94.41	SX47	1,823,353	42.65	93.87
SX14	1,451,126	43.35	94.15	SX48	1,546,745	42.65	93.81

To estimates the evolutionary divergence between sequences, analyses were conducted using the Kimura 2-parameter method [34]. Evolutionary analyses were conducted in MEGA X [35]. This analysis involved 66 nucleotide sequences. All positions with less than 80% site coverage were eliminated, i.e., fewer than 20% alignment gaps, missing data, and ambiguous bases were allowed at any position (partial deletion option). There were a total of 15,527 positions in the final dataset.

2.5. Phylogenetic Analysis

We performed multiples analyses to examine the level of genetic diversity that exists within *S. nigrum* populations. All SLAF pair-end reads with clear index information were clustered on the basis of sequence similarity. Sequence similarity was detected using one-to-one alignment with BLAST [36]. A phylogenetic tree was constructed based on the SNPs using maximum likelihood analyses [37]. Neighbor-joining trees were reconstructed in MEGA X [35] using Nei's genetic distances between pairs of samples and 1000 bootstrap replicates. Population structure analysis was performed through ADMIXTURE based on a cross-validation error ranging from k = 1 to k = 10 [38,39]. In order to obtain insight into the gene flow and evolutionary relationships among populations, TreeMix models was used to construct maximum likelihood (ML) trees based on the allele frequency covariance matrix and added migration edges [40]. The genetic drift at genome-wide polymorphisms to infer

evolutionary relationships and directionality of gene flow between *S. nigrum* populations was evaluated.

3. Results

3.1. Phylogenetic Analyses on S. nigrum Populations

A total of 189,840 high-quality SLAFs was identified and used to call the SNPs with an average sequencing depth of 10.56-fold per individual. For each sample, the average sequencing depth ranged from 8.16× fold to 14.84× fold (Table 3). Of these, 90,590 were identified as polymorphic. Finally, 616,533 SNPs with a minor allele frequency (MAF) $\geq$ 0.05 were selected for analyses of phylogenetic relationships and population structure after filtering based on SLAF-seq for the 66 samples. For each *S. nigrum* individual, the number of SNPs ranged from 266,597 to 450,693 (Table 4). The largest number of SNPs occurred on sample SJ4 (450,693 SNPs), followed by SX25 (364,002 SNPs), whereas the smallest number of SNPs was found on SX17 (266,597 SNPs).

Table 3. Summary statistics for specific-locus amplified fragment sequence data.

ID	SLAF Number	Total Depth	Average Depth	ID	SLAF Number	Total Depth	Average Depth
SB1	143,008	1,397,095	9.77	SX15	143,551	1,201,101	8.37
SH1	139,623	1,558,808	11.16	SX16	142,910	1,182,897	8.28
SH2	150,756	1,514,661	10.05	SX17	135,530	1,473,942	10.88
SH3	148,052	1,447,856	9.78	SX18	151,906	1,592,422	10.48
SH4	144,849	1,383,746	9.55	SX19	143,270	1,344,168	9.38
SJ1	143,453	2,354,446	16.41	SX20	144,038	1,352,983	9.39
SJ2	139,706	1,221,842	8.75	SX21	144,141	1,298,644	9.01
SJ3	148,056	1,347,424	9.10	SX22	145,000	1,224,795	8.45
SJ4	171,626	3,081,308	17.95	SX23	142,066	1,189,055	8.37
SJ5	146,279	1,274,606	8.71	SX24	150,371	1,563,158	10.40
SJ6	153,773	1,702,217	11.07	SX25	153,599	1,678,791	10.93
SJ7	139,864	1,326,283	9.48	SX26	148,364	1,362,955	9.19
SL1	150,344	1,497,548	9.96	SX27	151,134	1,479,202	9.79
SL2	142,746	1,290,494	9.04	SX28	152,221	1,635,532	10.74
SM1	144,398	1,334,945	9.24	SX29	145,849	1,442,431	9.89
SM2	139,926	1,877,997	13.42	SX31	146,580	1,511,018	10.31
SN1	142,636	1,782,163	12.49	SX32	147,503	1,509,826	10.24
SN2	146,239	1,327,197	9.08	SX33	148,484	1,394,929	9.39
SS1	134,045	1,351,964	10.09	SX34	147,378	1,434,272	9.73
SX01	145,563	2,152,103	14.78	SX35	146,085	1,356,216	9.28
SX02	136,493	1,474,738	10.80	SX36	139,212	1,215,151	8.73
SX03	140,107	1,955,306	13.96	SX37	141,906	1,452,165	10.23
SX04	137,242	1,734,153	12.64	SX38	142,509	1,418,841	9.96
SX05	142,744	1,662,479	11.65	SX39	144,886	1,457,086	10.06
SX06	141,570	1,858,567	13.13	SX40	135,976	1,186,188	8.72
SX07	140,762	1,798,000	12.77	SX41	106,088	1,140,162	10.75
SX08	142,846	2,119,727	14.84	SX42	137,456	1,246,916	9.07
SX09	145,038	1,910,647	13.17	SX43	141,769	1,457,711	10.28
SX10	147,941	1,680,924	11.36	SX44	144,564	1,689,305	11.69
SX11	150,815	2,067,161	13.71	SX45	144,316	1,591,007	11.02
SX12	137,667	1,510,621	10.97	SX46	137,927	1,350,467	9.79
SX13	151,122	1,475,451	9.76	SX47	146,675	1,493,755	10.18
SX14	139,948	1,141,327	8.16	SX48	138,873	1,285,432	9.26

Table 4. Summary statistics for SNP sequence data.

ID	SNP Number	Hetloci Ratio (%)	Integrity Ratio (%)	ID	SNP Number	Hetloci Ratio (%)	Integrity Ratio (%)
SB1	300,918	17.91	48.80	SX15	340,669	19.00	55.25
SH1	279,620	17.93	45.35	SX16	334,821	18.15	54.30
SH2	351,232	19.60	56.96	SX17	266,597	16.87	43.24
SH3	343,657	19.14	55.74	SX18	355,004	19.54	57.58
SH4	317,807	17.97	51.54	SX19	327,964	18.31	53.19
SJ1	276,991	18.88	44.92	SX20	322,766	17.95	52.35
SJ2	297,219	17.30	48.20	SX21	323,363	17.86	52.44
SJ3	353,923	19.30	57.40	SX22	345,938	18.78	56.11
SJ4	450,693	28.73	73.10	SX23	336,435	18.48	54.56
SJ5	341,109	18.93	55.32	SX24	346,155	19.22	56.14
SJ6	359,735	19.94	58.34	SX25	364,002	20.11	59.04
SJ7	284,537	17.15	46.15	SX26	343,732	19.22	55.75
SL1	349,578	19.69	56.70	SX27	357,266	19.79	57.94
SL2	321,662	17.92	52.17	SX28	354,589	19.76	57.51
SM1	321,841	17.88	52.20	SX29	329,492	18.55	53.44
SM2	268,565	17.91	43.56	SX31	310,206	18.43	50.31
SN1	295,616	18.08	47.94	SX32	318,428	18.67	51.64
SN2	334,074	18.23	54.18	SX33	337,137	18.91	54.68
SS1	274,926	16.70	44.59	SX34	326,911	18.22	53.02
SX01	295,832	19.07	47.98	SX35	332,924	18.43	53.99
SX02	267,309	17.33	43.35	SX36	301,753	17.80	48.94
SX03	272,426	18.23	44.18	SX37	287,012	17.57	46.55
SX04	267,987	17.77	43.46	SX38	301,108	17.77	48.83
SX05	300,601	17.99	48.75	SX39	315,850	18.54	51.23
SX06	279,522	18.32	45.33	SX40	282,203	17.29	45.77
SX07	279,250	18.17	45.29	SX41	302,783	8.39	49.11
SX08	279,198	18.55	45.28	SX42	290,009	17.36	47.03
SX09	297,579	18.89	48.26	SX43	287,487	17.25	46.62
SX10	323,345	19.13	52.44	SX44	290,581	17.74	47.13
SX11	314,870	19.83	51.07	SX45	300,420	18.20	48.72
SX12	271,604	17.61	44.05	SX46	279,457	17.04	45.32
SX13	355,317	19.87	57.63	SX47	315,801	18.34	51.22
SX14	317,163	17.48	51.44	SX48	285,131	17.00	46.24

3.2. Genetic Differences and Genetic Distances of S. nigrum

After SNP filtering, a genetic diversity study was carried out using 616,533 SNPs within 66 *S. nigrum* samples. The genetic diversity analysis showed that mean effective allele number (Ne) was 1.81. Observed (Ho) heterozygosity number ranged from 0.507 to 0.7403, with an average 0.6306. The expected (He) heterozygosity varied from 0.4048 to 0.4597 with an average 0.434. The average polymorphic information content value was 0.3353. The mean Shannon diversity index was 0.621 varied from 0.5865 to 0.6479. The average MAF was 0.3782, ranged from 0.3388 to 0.4194. Nei diversity index 0.419 to 0.613, average was 0.5019. These estimations indicated that there is a considerable amount of genetic variation among each samples.

The analysis of genetic differentiation showed that there was a low level of genetic differentiation (Fst < 0.1000) among each geographical population (Table 5). The pairwise genetic distance values among all samples ranged from 0.001 to 0.573, with an average of 0.308 (Supplementary Table S1). The minimum distance was found between SX27 and SX48 collected from two nearby locations in Xinjiang. The largest distance was observed between SJ1 and SX33, collected from the northeast (123°43′53″ E, 44°10′83″ N) and northwest (82°06′20″ E, 44°58′02″ N) of China, respectively.

Table 5. Estimates of genetic differentiation (Fst) over sequence pairs and between geographical populations.

Population	HLJ	IM	JL	LN	HN	XJ
HLJ	0.009					
IM						
JL	0.007	0.005				
LN	0.005	0.039	0.005			
HN	0.036	0.007	0.038	0.047		
XJ	0.001	0.012	0.005	0.003	0.020	

Population name HLJ: Heilongjiang, HN: Henan, IM: Inner Mongolia; JL: Jilin; LN: Liaoning; XJ: Xinjiang.

3.3. Population Structure of S. nigrum

The ML (maximum likelihood) phylogenetic tree (Figure 1) showed strong evidence for two clusters: cluster A was a Xinjiang group; cluster B was a widespread group with individuals extending from northwest to northeast China. We found some correlation between geographic origin and genetic structure among 66 individuals. In cluster B, northeast populations (JL, LN, IN) could be clustered together roughly, whereas most *S. nigrum* individuals from Xinjiang could be clustered into a subgroup (Figure 1). These two subclusters were well separated and therefore distantly related.

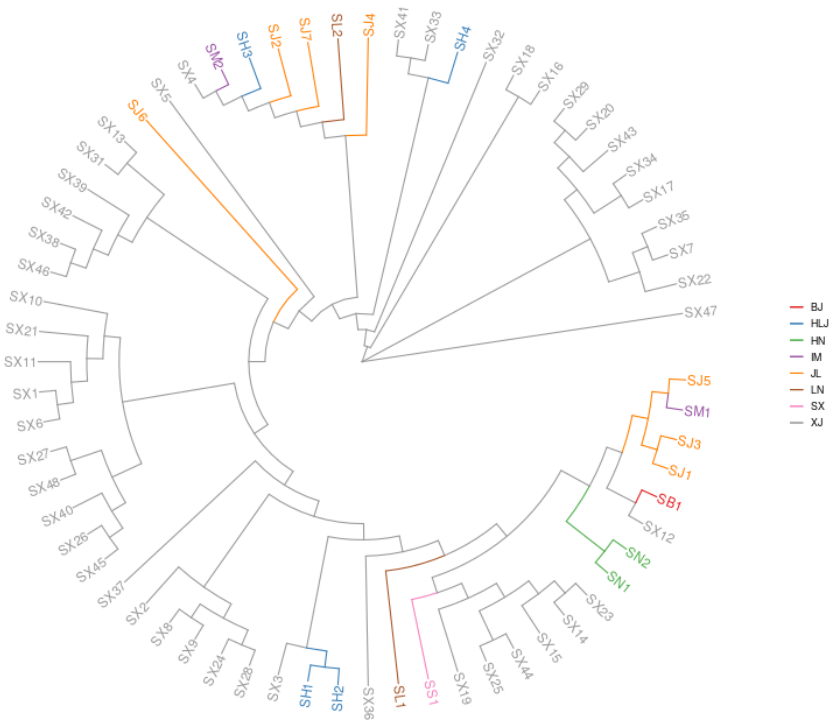
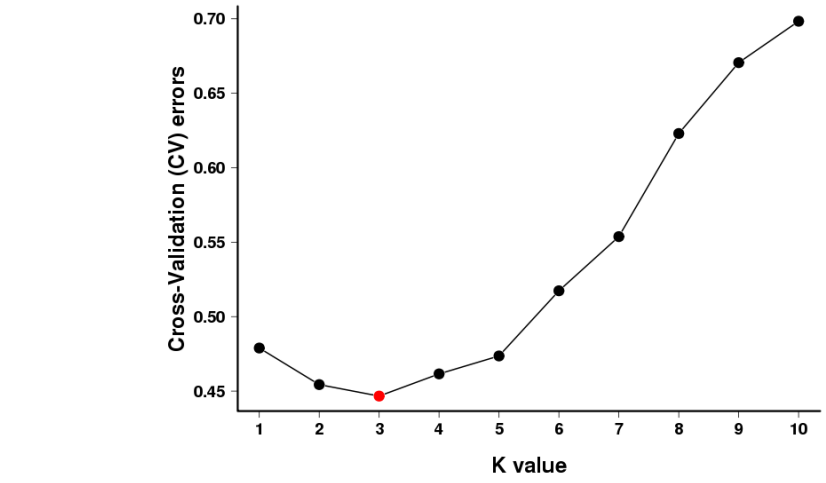


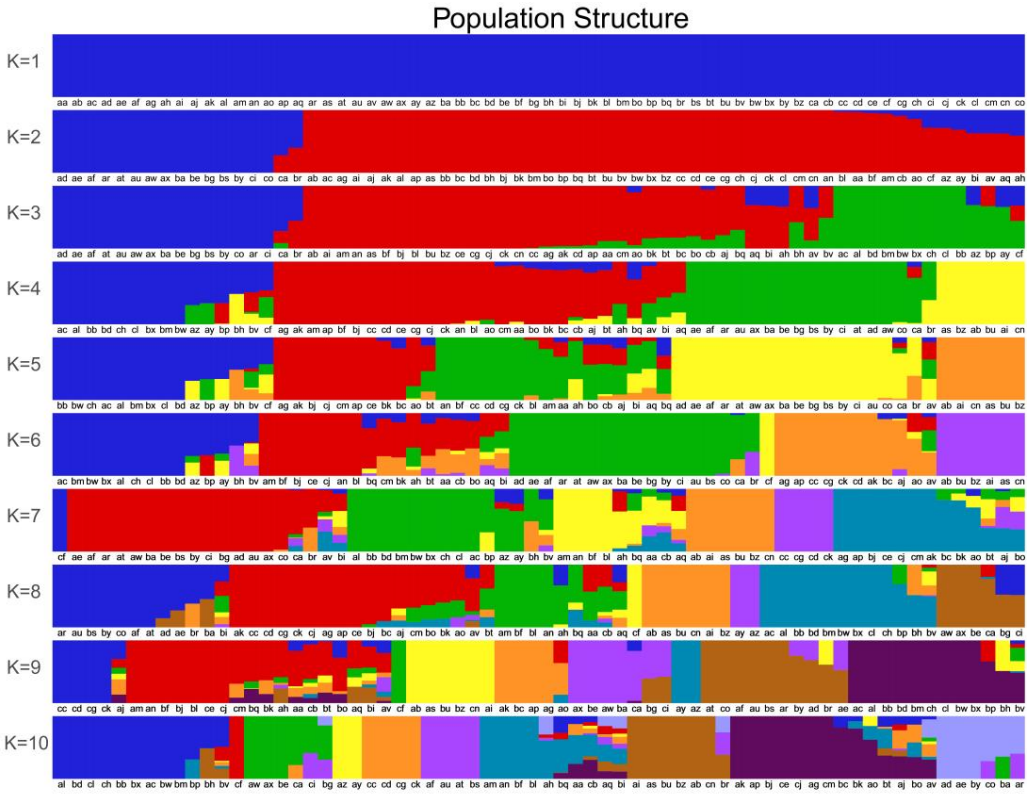
Figure 1. Phylogenetic tree constructed according to the neighbor-joining method with polymorphic SNPs based on 66 S. *nigrum* individuals. The colors of branches indicate genotypes corresponding to different sub-origin sets. BJ: Beijing; HLJ: Heilongjiang, HN: Henan, IM: Inner Mongolia; JL: Jilin; LN: Liaoning; SX: Shaanxi; XJ: Xinjiang.

To further understand the evolutionary history of *S. nigrum*, we used a Bayesian clustering algorithm with admixed models [41] to estimate the ancestral proportions for each individual. The population structure analysis was performed through ADMIXTURE based on a cross-validation error ranging from k = 1 to k = 10. The value of error ranged from 0.479 to 0.698. The lowest cross-validation error was observed at k = 3 (Figure 2). This suggests that the 66 *S. nigrum* samples could have originated from three different genetic clusters (Q1, Q2, and Q3) (Figure 2). Of three clusters, cluster II (Q2) comprised the most

germplasms with 34 samples, followed by cluster I (Q1) (18 samples) and cluster III (Q3) (14 samples). The Xinjiang site was the only location where all genetic clusters were found. A high level of admixture was detected in the Xinjiang populations. The populations from Xinjiang were distributed in three clusters, suggesting these populations were genetically diverse (Figure 3).



(**a**)



(**b**)

Figure 2. Population structure analysis of *S. nigrum* with the ADMIXTURE program using genome-wide SNP markers (n = 616,533). (**a**) The estimated cross-validation errors for different grouping results (K value); (**b**) The individuals were divided into three subgroups (there was a minimum K-value when K = 3), within each subgroup, the individuals were ordered according to the genetic component, and each line gives the sub-group value, with each accession shown as a vertical line partitioned into K colored components representing inferred membership in K genetic clusters.

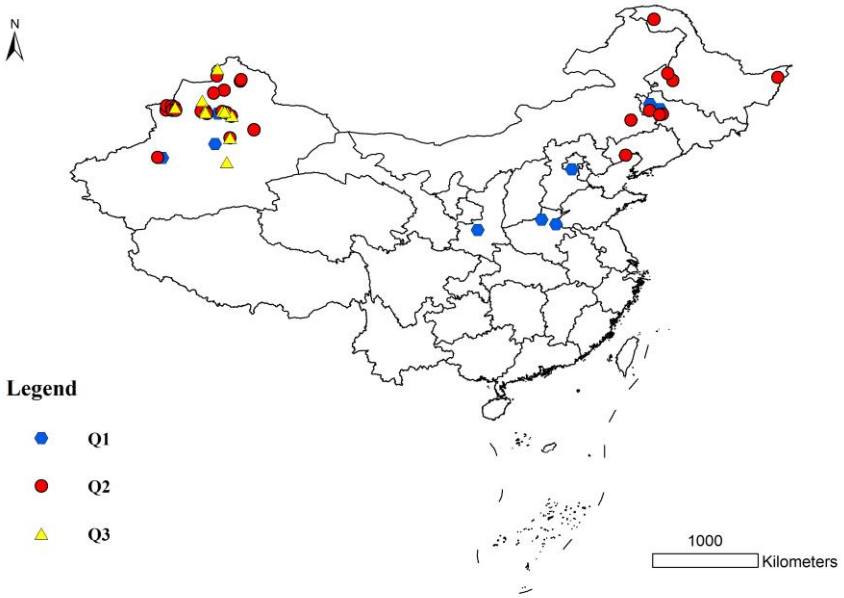


Figure 3. Distribution of *S. nigrum* used in this study and the different ancestors.

The first genetic cluster (Q1) was essentially represented by individual samples originating from Xinjiang, Beijing, Jilin, Inner Mongolia, Henan, and Shaanxi. This ancestry ranged from 0.557 to 0.999. The samples from SL1 and SX36 in Q1 had an average ancestry proportion for their major cluster below 0.75. The second genetic cluster was mainly represented by samples originating from Heilongjiang, Jilin, Liaoning, Inner Mongolia, and Xinjiang. The ancestry proportion within these populations ranged from 0.501 to 0.999. Three individuals from Heilongjiang, three samples from Xinjiang, and one from Jilin in Q2 had an ancestry proportion below 0.75. The last genetic cluster Q3 was only detected within samples collected in Xinjiang. The ancestry ranged from 0.440 to 0.999. Four samples in Q3 had an ancestry proportion below 0.75.

The geographical distances were not significantly correlated with the genetic distances among samples. Although the greatest geographic distance was between the populations from the SH2 (133°52′53″ E, 47°24′19″ N) and SX24 (80°54′50″ E, 40°34′57″ N) sites, these two samples were found to belong to the same cluster (Q2). However, the samples SM1 and SM2 from Inner Mongolia collected in very close sites were observed to belong to Q1 and Q2, respectively. The genetic distance between SM1 and SM2 was 0.428 (Supplementary Table S1), indicated that there was a significant difference between SM1 and SM2. Other samples, including SX41 and SX45, SX4 and SX44, SX13 and SX17, SX20 and SX36, and SX16 and SX14, all pairs of samples collected from very close sites, were observed to belong to different clusters (Figure 3).

To understand the history of divergence and admixture, we used TreeMix for the six geographical groups including the samples from Xinjiang, Liaoning, Heilongjiang, Jilin, Henan, and Inner Mongolia. In the TreeMix analysis (Figure 4), there was a significant gene flow event that could be found among the *S. nigrum* populations, which indicated that extensive gene flow had occurred in northeastern and western China. Between the two clusters, extensive gene flow from Liaoning (LN) to Heilongjiang (HLJ) was observed. The Inner Mongolia (IM) group showed more significant genetic similarity with the Liaoning group that differed markedly from the other groups.

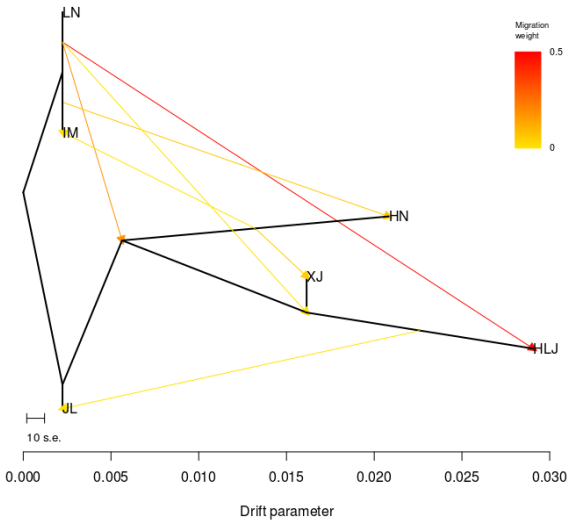


Figure 4. TreeMix analysis of the six geographic populations. The arrow corresponds to the direction of migration. Seven migration edges were allowed, as inferred using OptM. The migration edges are colored according to their weight ($\hat{w}$). The scale bar indicates ten times the average standard error of the values in the covariance matrix. LN: Liaoning population; IM: Inner Mongolia population; JL: Jilin population; XJ: Xinjiang population; HLJ: Heilongjiang population; HN: Henan population.

4. Discussion

S. nigrum is one of the most common and difficult-to-control weeds in many crops, including cotton, soybean, and sugar beet, due to biological characteristics that enhance the adaptive ability of the weed in a wide range of environments. The success of weeds in the ecosystem can be attributed to genetic diversity [42]. An effective weed management program should be based upon the diversity level of each weed species [43]. In the present study, using SLAF-seq technology, 66 *S. nigrum* individuals from a wide range in China were collected and genome-wide SNPs were obtained. The genomic data provide us novel insight into *S. nigrum*'s genetic diversity. Single-nucleotide polymorphisms are much more abundant than other molecular markers in the genome. In a previous study of 14 *Solanum* accessions, 130 polymorphic bands were identified based on ISSR [19,44]. In this study, a total of 189,840 SLAFs was detected from 66 individuals of *S. nigrum*, and almost half of the SLAF markers were polymorphic, which also suggests that the observed genetic diversity of *S. nigrum* in China is rich.

It is indicated that *S. nigrum* populations have high genetic diversity based on the observed heterozygosity (Ho > 0.5). However, the pairwise Fst comparisons showed a low level of genetic differentiation among *S. nigrum* populations. Admixture and low genetic differentiation among populations may be caused by high gene flow [45]. A high degree of gene flow can increase genetic diversity and can have significant homogenizing effects [46]. In this study we found extensive gene flow and admixture between northeast to northwest populations. This suggests that environmental factors influence the genetic diversity of *S. nigrum*.

Population structure is the result of both present and historical processes, and many factors may change the geographical distributions of plant species [47]. On the basis of the phylogenetic analysis, total 14 individuals from the Xinjiang population clustered together without other individuals, suggesting the origin of the Q3 population differs from that of the other populations. The genetic structural analysis revealed that samples from Xinjiang comprise three genotypes, which is in accordance with the phylogenetic results. Therefore the Xinjiang site could be viewed as a repository of genetic diversity.

This study highlights the value of the de novo sequencing of *S. nigrum* genomes to study the genomic patterns in a phylogenetic framework. The SLAF-seq analysis of various *S. nigrum* samples revealed the genetic diversity, which was consistent with the

results of phylogenomic analyses. Before the current study, very little was known about the population genetic structure of *S. nigrum* in China. The present results increase our understanding of *S. nigrum*'s genetic structure and diversity. The high rate of heterozygosity in every population suggested that migration of *S. nigrum* accelerated the gene flow and genetic evolution, which may facilitate *S. nigrum* to adapt to their environment.

The data presented herein may represent the basis for future studies related to *S. nigrum* genetic evolution.

5. Conclusions

In summary, we herein reveal the genetic diversity of *S. nigrum* at the population and species level. Considerable genetic differentiation was observed among individuals. A phylogenetic tree suggested the relationship between many individuals was not inconsistent with the geographical location. All individuals were clustered into three genotypes according to a structural analysis. These data provided important information about *S. nigrum* phylogenomics and will be useful for further understanding of the evolutionary genetics of weed adaptation to the agricultural environment.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13030832/s1, Table S1: Values of pairwaise genetic distance among *S. nigrum populations*.

Author Contributions: J.L. and S.W. performed data analysis and drafted the manuscript; Y.Z. (Yixiao Zhang), Z.M., and L.L. participated in the experiments; Z.H. and Y.Z. (Yuyong Zhu) provided some samples; H.H. conceived and designed the experiments and performed data analysis. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: Data available from the author.

Conflicts of Interest: The authors declare no conflict of interest.

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