Genet Resour Crop Evol (2019) 66:47–59
https://doi.org/10.1007/s10722-018-0694-6

RESEARCH ARTICLE

Characterization of genetic diversity and structures in natural *Glycine tomentella* populations on the southeast islands of China

Xu-Dong Wang · Xiang-Hua Li · Zheng-Wei Zhang · Ke-Jing Wang

Received: 2 June 2018 / Accepted: 11 September 2018 / Published online: 25 September 2018

Abstract *Glycine tomentella* Hayata is a species of *Glycine* Willd. subgenus *Glycine*, and in China it is distributed along the southeast coast. In this study, 11 natural *G. tomentella* populations were collected and their genetic diversity levels and population structures were analyzed using 25 simple sequence repeat (SSR) markers. The number of alleles per locus averaged 7.16 and ranged from 2 to 17. The expected heterozygosity (H_e) per locus averaged 0.60, varying from 0.19 to 0.86. The *G. tomentella* populations on these Chinese islands showed a greater average genetic variation (60.96%) among populations and gene differentiation index (G_{st}= 0.607), and a lower average within-population genetic variation (33.47%) and gene flow (N_m= 0.162). In this study, these *G. tomentella* island populations were characterized by a relatively greater average multilocus outcrossing rate of 5.74%, which may the result of heterogeneity owing to the perennation of *G. tomentella*. A spatial autocorrelation analysis revealed that populations within a radius of approximately 30.45 km had positive and significant genetic relationships. The Neighbor-Joining (NJ) and STRUCTURE analyses strongly showed a pattern of 'island differentiation' for the populations on southeast islands of China and also suggested that some genetic interconnection occurred along the southeast coast of China. The *F*-statistics suggested that geographically different *G. tomentella* populations had specific population structures. We propose that when collecting this species as a genetic resource, every *G. tomentella* population should be sampled.

Keywords Chinese *Glycine tomentella* · Genetic diversity · Genetic structure · *Glycine tabacina* · SSR

X.-D. Wang · X.-H. Li · Z.-W. Zhang · K.-J. Wang (✉)
Institute of Crop Science, Chinese Academy of Agricultural Sciences, Beijing 100081, People's Republic of China
e-mail: wangkj@caas.net.cn

Introduction

The genus *Glycine* Willd. is widely distributed in Australia, the western Pacific Islands, and the surrounding islands, including Taiwan (Chung and Singh 2008). The genus *Glycine* contains two subgenera, termed subgenus *Soja*, which contains two annual species [the cultivated soybean *Glycine max* (L.) Merr. and its progenitor wild soybean *Glycine soja* Sieb. et Zucc.], and subgenus *Glycine*, which contains 26 perennial species. Species *Glycine tomentella* Hayata is one of these perennial species and has the complex karyotype containing 2n = 38, 40, 78, or 80 (Singh et al. 1987). To date, *G. tomentella* has been identified as having several genomes. The 2n = 38 *G. tomentella* contains the EE genome (D_1 and D_2 isozyme types); 2n = 40 *G. tomentella* contains the DD (D_3 isozyme type), D_2D_2 (D_5A) and H_2H_2 (D_5B) genomes; 2n = 78

G. tomentella contains the AE (T_5), D_3E (T_1) and EH_2 (T_6) genomes; and 2n = 80 *G. tomentella* contains the DA_6 (T_2), DD_2 (T_3) and DH_2 (T_4) genomes (Doyle and Brown 1985; Doyle et al. 1986; Brown et al. 2002; Chung and Singh 2008). A T7 isozyme type was found in an Indonesian tetraploid *G. tomentella* (2n = 80) (Kollopara et al. 1994). In phylogenetic studies of *G. tomentella*, Tindale (1986) classified the original *G. tomentella* (2n = 40, D_6 isozyme type) as a species of *Glycine arenaria* Tindale (genome H), while Pfeil et al. (2006) recognized the original *G. tomentella* (2n = 40, D_4 isozyme type) as a new species, *Glycine syndetika* B. E. Pfeil et Craven (genome A_6). Moreover, one of the T_2-isozyme typed *G. tomentella* (2n = 80, D_1A, or DA_6) was regarded as the species *G. dolichocarpa* Tateishi and Ohashi (2n = 80, D_1A) (Tateishi and Ohashi 1992; Chung and Singh 2008).

Glycine tomentella is also recorded in the flora of China (Hayata 1920); however, no relevant taxonomic studies on genome classifications of these species have been performed. Two chromosome-based observational studies of *G. tomentella* from several places in Fujian Province, China showed that the number of chromosomes was 80 (Bau et al. 1993; Gao et al. 2002), but the genome and isozyme types of the Chinese 80-chromosome *G. tomentella* remain unclear.

As a wild relative of cultivated soybean, *G. tomentella* can enhance the genetic basis of soybean breeding and, therefore, is an important germplasm for soybean innovation (Singh et al. 1990, 1993, 1998, 2010; Singh and Nelson 2015). *G. tomentella* is also used as a Chinese herbal medicine for treating rheumatism and bone pain (Zhang et al. 2011). However, there have been limited studies regarding the genetic diversity of the subgenus *Glycine*'s perennial species. Currently, only one small-scale study of the ISSR marker-based genetic diversity in 16 *G. tomentella* individuals collected from three small areas in China has been performed (Chen et al. 2013). Therefore, there is a lack of geographical and genetic information on the Chinese *G. tomentella* complex species.

Presently, subgenus *Glycine* plants growing in China are being threatened from coastal exploration, nuclear power plant construction, and tourism, with the number and area of existing surviving populations declining. To effectively protect genetic resources of *G. tomentella* species in China, the genetic diversity and structure of their natural populations are required. Here, the objective was to investigate the genetic diversity and structure of the natural *G. tomentella* species' populations on the southeast islands of China to establish a genetic conservation strategy.

Materials and methods

Material sampling

Leaves were sampled from on-the-spot field plants as the experimental materials. The leaves were stored with silica gel in zip-lock plastic bags for later analyses. In total, 291 *G. tomentella* individuals from 11 populations were collected on five coastal islands, Nanao, Dongshan, Jingan, Meizhou and Pingtan, along the southeast coast of China (Table 1 and Fig. 1). The geographical coordinates of each population were recorded (Table 1). The sampling distance between individuals within populations was more than 5 m, and 14–30 plants were sampled from each population, depending on population size.

DNA extraction and SSR genotyping

Total genomic DNA was extracted using a modified CTAB method (Narzary et al. 2015). Zou et al. (2004) shows that the use of soybean SSR makers is a rapid and reliable method to detect *G. tomentella* chromosomes. In the species having less genome sequence information, we selected usable soybean SSRs identified from 930 markers (Cregan et al. 1999; Song et al. 2010), and 25 soybean SSR markers with polymorphisms and high levels of stability were used to assess the genetic diversity of *G. tomentella*. The rest of 905 SSR markers were unavailable (nil-amplified, single amplification, smeared, or uncountable multi-bands). PCR amplification was carried out on a TaKaRa TP650 PCR thermal cycler (TaKaRa Bio, Japan) with a 10-μL total volume of reaction mixture, containing 2 μL of template DNA (30 ng/μL), 5 μl of 2 × Taq PCR StarMix (Genstar Biosolusiond Co., Ltd., Beijing, CN), 0.5 μL of each primer (10 mM), and 2 μL of ddH_2O. PCR amplification reactions were performed with an initial denaturation at 95 °C for 5 min, 34 cycles of 30 s denaturation at 94 °C, 30 s annealing at 54 °C (the annealing temperature and cycle number varied according to different SSR primers used), and

Table 1 Information for natural populations of perennial *G. tomentella* sampled on the southeast islands of China

Population	Location	Latitude (N)	Longitude (E)	Sample size	Area	Island length (km)
Pop-1	Nanao Island-1	23°24′27.47″	117°08′14.72″	30	1100	20.01
Pop-2	Nanao Island-2	23°25′00.06″	117°08′20.29″	14	150	
Pop-3	Dongshan Island-1	23°43′13.84″	117°23′37.13″	30	850	28.37
Pop-4	Dongshan Island-2	23°41′30.54″	117°25′49.93″	21	560	
Pop-5	Jinan Island-1	23°46′52.75″	117°41′13.72″	26	200	1.50
Pop-6	Jinan Island-2	23°47′07.80″	117°41′00.71″	28	260	
Pop-7	Jinan Island-3	23°47′08.14″	117°40′45.49″	30	200	
Pop-8	Meizhou Island-1	25°04′07.06″	119°07′53.73″	30	2000	9.44
Pop-9	Meizhou Island-2	25°04′03.23″	119°07′59.25″	30	30,000	
Pop-10	Pingtan Island-1	25°37′09.74″	119°48′07.67″	30	2000	29.00
Pop-11	Pingtan Island-2	25°37′05.70″	119°47′53.77″	22	400	
Total	–	–	–	291		

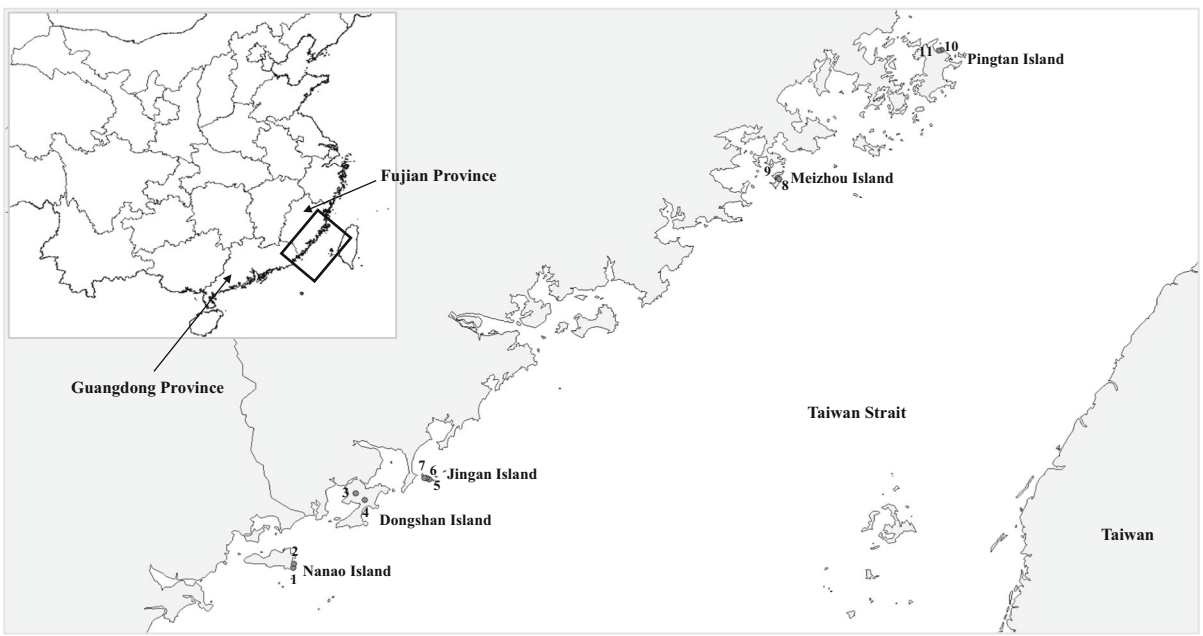


Fig. 1 A sketch map showing the sampling sites of the 11 natural *G. tomentella* populations on five islands along the southeastern coast of Fujian and Guangdong Provinces, China

30 s extension at 72 °C. This was followed by 10 min at 72 °C for the final extension, and samples were stored at 4 °C. The PCR products were electrophoresed on an 8% non-denaturing polyacrylamide gel and visualized by silver nitrate staining. Because most of the markers exhibited more than one band, we chose the brightest bands, clearly legible and polymorphic, and recorded them as alleles.

Data analysis

The software POPGENE1.32 (Yeh et al. 1999) was used to calculate genetic diversity parameters, including the number of alleles (N_a), number of effective alleles (N_e), expected heterozygosity (H_e), observed heterozygosity (H_o), percentage of polymorphic loci (P), mean number of alleles per locus (A),

polymorphism information content (*PIC*), genetic differentiation index (G_{st}), and gene flow (N_m), for each locus. The number of genotypes (haplotypes) was estimated using Power Marker (Liu and Muse 2005). The software FSTAT, version 2.9.3 (Goudet 2001) was used to estimate the fixing index (F_{is}) for each locus and population. The outcrossing rate (t) was calculated from $t = (1 - F_{is})/(1 + F_{is})$ (Weir and Cockerham 1984). The *F*-statistics evaluating the genetic differentiation among populations and the molecular variance (AMOVA), and evaluating the among- and within-population genetic variation amounts, were calculated using ARLEQUIN, version 3.5 (Excoffier and Lischer 2010). The relationship dendrogram was constructed using the Neighbor-Joining (NJ) method based on the allele frequencies and genetic distance (Nei et al. 1983), with a bootstrap number of 1000 and a 0.95 confidence interval (CI), using PowerMarker, version 3.25 (Liu and Muse 2005). The online software IBDWS, version 3.23 (Jensen et al. 2005) (http://ibdws.sdsu.edu/~ibdws/aboutibdws.html) was used to detect the correlation between genetic and geographic distances among populations based on the Mantel test. STRUCTURE, version 2.1 (Pritchard et al. 2000) was run for the population structure analysis using a model without prior population information. Values of 1–11 *K* (the number of assumed clusters) were used to infer the number of clusters. Each run was conducted 20 times with independent simulations for each *K* value under the conditions of a 100,000 burn-in period and 100,000 MCMC (Markov Chain Monte Carlo method) replications. Furthermore, the optimal K value was inferred by the online program Structure Harvester (Earl and vonHoldt 2012) (http://taylor0.biology.ucla.edu/structureHarvester). Based on the optimal *K* values, the program CLUMPP, version 1.1.2 (Jakobsson and Rosenberg 2007) was used to find the optimal alignments of 20 independent runs produced by STRUCTURE. The spatial autocorrelation of the overall geographical populations was analyzed using software GenAlEx, version 6.502 (Peakall and Smouse 2012). The autocorrelogram was set using variable distance classes that spanned the full range of geographic distances among the populations. An assignment test was applied to infer the possible foreign individuals in a population using Geneclass 2 (Piry et al. 2004).

Results

Performance of soybean SSR markers

In this study, 25 pairs of SSR markers could be amplified with polymorphisms, and they produced 179 bands (alleles) in the 291 *G. tomentella* individuals. The mean N_a was 7.16 per loci, ranging from 2 (Satt153, Satt504 and TAB 16) to 17 (sat_142). The number of genotypes (haplotype) was 11.32 per locus, ranging from 3 (Satt153, Satt504 and TAB 16) to 25 (Satt563). The mean H_e and *PIC* per locus showed similar trends. The mean H_e value was 0.595 per locus, with a range of 0.186 (Satt421) to 0.862 (Satt339 and Satt563), and the mean *PIC* value was 0.562 per locus, with a range of 0.174 (Satt421) to 0.846 (Satt339 and Satt563) (Table 2). The fixation index (F_{is}) for the degree of allelic fixation was 0.842 per loci, ranging from 0.649 (Sat_421) to 1.00 (Sat_142) among loci.

Population genetic variation

In the 11 populations, the mean *P* was 58.6% for populations, ranging from 24% (pop. 7) to 100% (pop. 4). These populations averaged 13.82 haplotypes, ranging from 3 (pop. 7) to 30 (pop. 3). The mean *A* for populations was 2.18, ranging from 1.24 (pop. 7) to 4.28 (pop. 3). The H_e per population was 0.232, ranging from 0.020 (pop. 7) to 0.545 (pop. 3). The average *PIC* was 0.203 for the populations, ranging from 0.02 (pop. 7) to 0.50 (pop. 3). Among these populations, pops-3, 4, and 9 had the greatest levels of genetic variation, as characterized by their greater genetic parameters (P = 88.0–100.0, H_{ap} = 19–30, A = 3.400–4.280, H_e = 0.408–0.545, H_o = 0.080–0.156 and *PIC* = 0.369–0.497, Table 3). Additionally, they usually occupied greater land areas (Table 1).

Relatively greater outcrossing rate

Outcrossing can cause genetic recombination and heterogeneity in species and populations. The outcrossing rate is different among plant species or categories. This is related to the species' nature and is also affected by geographical distance, weather, and pollinators. Single locus (t_s) and multiloci (t_m) outcrossing rates were estimated for populations

Table 2 Estimates of genetic variation at 25 SSR loci for the Chinese *G. tomentella* materials

Marker	Genetic diversity index									
	N_a	G_t	N_e	H_e	H_o	PIC	G_{st}	F_{is}	N_m	t_s (%)
Sat_111	4	7	1.301	0.232	0.014	0.211	0.755	0.712	0.078	0.149
Sat_142	17	17	6.228	0.841	0.000	0.822	0.587	1.000	0.165	0.000
Sat_193	11	15	4.775	0.792	0.024	0.762	0.592	0.918	0.162	0.041
Sat_262	13	22	6.342	0.844	0.031	0.825	0.393	0.934	0.0353	0.034
Sat_347	5	7	1.975	0.495	0.014	0.408	0.591	0.940	0.162	0.031
Sat_397	5	10	1.438	0.305	0.041	0.291	0.483	0.749	0.250	0.134
Sat_421	5	7	2.630	0.621	0.076	0.557	0.669	0.649	0.118	0.186
Satt137	5	9	1.925	0.481	0.038	0.452	0.555	0.809	0.188	0.100
Satt153	2	3	1.358	0.264	0.003	0.229	0.656	0.951	0.124	0.025
Satt304	5	8	3.996	0.751	0.069	0.710	0.710	0.676	0.098	0.170
Satt323	13	18	4.843	0.795	0.052	0.768	0.595	0.846	0.160	0.079
Satt346	9	17	2.994	0.667	0.041	0.630	0.732	0.771	0.087	0.118
Satt399	10	19	7.193	0.862	0.041	0.846	0.555	0.882	0.188	0.061
Satt421	3	4	1.228	0.186	0.003	0.174	0.490	0.970	0.241	0.015
Satt460	7	13	4.602	0.784	0.038	0.753	0.704	0.846	0.100	0.079
Satt481	15	24	5.712	0.826	0.062	0.807	0.498	0.844	0.235	0.081
Satt504	2	3	1.324	0.245	0.017	0.215	0.693	0.739	0.105	0.136
Satt563	11	25	7.187	0.862	0.082	0.846	0.555	0.794	0.188	0.108
Satt619	5	9	1.848	0.460	0.024	0.410	0.693	0.817	0.105	0.094
Satt636	7	12	4.707	0.789	0.052	0.754	0.638	0.822	0.134	0.092
Satt654	6	11	3.743	0.734	0.028	0.689	0.523	0.916	0.213	0.043
Satt713	4	7	1.583	0.369	0.031	0.337	0.585	0.813	0.167	0.097
BARCSOYSSR_04_0001	2	3	1.315	0.240	0.003	0.211	0.747	0.957	0.081	0.022
BARCSOYSSR_09_0585	4	6	3.618	0.725	0.024	0.673	0.620	0.910	0.144	0.046
BARCSOYSSR_18_0002	9	17	3.495	0.715	0.072	0.675	0.547	0.777	0.194	0.117
Mean	7.16	11.32	3.494	0.595	0.035	0.562	0.607	0.842	0.162	0.082

N_a number of alleles, G_t number of genotypes, N_e effective number of alleles, H_e expected heterozygosity, H_o observed heterozygosity, PIC polymorphism information content, G_{st} genetic differentiation index, N_m gene flow, F_{is} fixation index, t_s single locus outcrossing rate

(Table 3). The t_s showed a mean of 8.2% per locus, ranging from 0 (sat_142) to 18.6% (sat_421). The t_m exhibited a mean of 5.74% per population, ranging from 0 (pops. 1, 7) to 16.7% (pop. 3). This value seemed to be greater for self-pollinating plants. The mean F_{is} across loci in populations was 0.897. Three populations, pops. 2, 3, and 4, had lower F_{is} values (0.772–0.787) and two populations, pops. 1 and 7, were fixed (F_{is}= 1.00) in the loci. Only two populations, pops. 1 and 7, had no occurrence of outcrossing, suggesting that there was genetic recombination within most populations.

Population genetic differentiation

Population genetic differentiation in *G. tomentella* species on the southeast islands of China was estimated. It was characterized by an average G_{st} of 0.607 per locus among populations, ranging from 0.393 (sat_262) to 0.755 (sat_111) (Table 2). The number of loci with a G_{st} value over 0.5 was 21, implying that an overwhelming majority of the loci had greater among-population variation levels.

The AMOVA analysis showed that 60.96% of genetic variation in the *G. tomentella* species on the southeast islands occurred among populations

Table 3 Genetic parameter estimates for 11 *G. tomentella* populations on the southeast islands of China

Population or genetic structure group	Sample size	Genetic diversity index							
		P (%)	H_{ap}	*A*	H_e	H_o	F_{is}	t_m (%)	*PIC*
Population									
Pop-1	30	44.0	4	1.480	0.185	0.000	1.000	0.00	0.150
Pop-2	14	24.0	7	1.240	0.038	0.009	0.772	12.84	0.034
Pop-3	30	92.0	30	4.280	0.545	0.156	0.714	16.70	0.497
Pop-4	21	100.0	19	3.400	0.439	0.093	0.787	11.91	0.395
Pop-5	26	60.0	9	1.800	0.223	0.006	0.972	1.40	0.183
Pop-6	28	52.0	7	1.640	0.155	0.006	0.963	1.88	0.130
Pop-7	30	24.0	3	1.240	0.020	0.000	1.000	0.00	0.019
Pop-8	30	48.0	8	1.720	0.148	0.008	0.946	2.77	0.128
Pop-9	30	88.0	29	3.480	0.408	0.080	0.804	10.87	0.369
Pop-10	30	68.0	21	2.040	0.202	0.015	0.927	3.77	0.170
Pop-11	22	44.0	15	1.640	0.187	0.004	0.981	0.98	0.155
Mean	26	58.6	14	2.178	0.232	0.034	0.897	5.74	0.203

P percentage of polymorphic loci, H_{ap} number of haplotype within population, *A* mean number of alleles per locus, H_e expected heterozygosity (gene diversity), H_o observed heterozygosity, F_{is} genetic differentiation index, t_m multilocus outcrossing rate (%)

(d.f. = 10; $P < 0.0001$), while 33.47% of genetic variation existed within populations (d.f. = 280; $P < 0.0001$) (Table 4). The result was in accordance with the G_{st} value, indicating a greater among-population genetic variation than within-population genetic variation in this *G. tomentella* species.

F-statistics for population differentiation showed significant differences between populations (Table 5), suggesting that all of the populations had their own genetic structures, even those at shorter distances (197 m between pops. 8 and 9; 408 m, pops. 10 and 11; 436 m, pops. 6 and 7; and 602 m, pops. 5 and 6; Table 5).

Population structure and spatial distribution

The structure analysis showed that there were two sharp peaks at k = 2 and k = 5 (Fig. 2). When k = 2 (Fig. 3a, b), group-1 included pops. 1, 2, 8, 10, 11 and a majority of pops. 3 and 4, and a minority of pop. 9 and group-2 included pops. 5, 6, 7 and a minority of pops. 3 and 4, and a majority of pop. 9. The results by STRUCTURE analysis could be understood as having two geographical groups, i.e. northeastern group (pops. 8, 10 and 11) and southwestern group (pops. 5, 6 and 7) (Fig. 1). The southwestern pops. 1 and 2 were only genetically consanguineous to the northeastern group; pops. 3 and 4 on Dongshan Island and pop. 9 on Meizhou Island were consanguineously

Table 4 Analysis of molecular variance (AMOVA) among *G. tomentella* natural populations

Source	df	SS	MS	Est. var	%	*P* value
Among-population	10	2593.48	259.35	4.81	60.96	$P < 0.0001$
With-population	280	1602.49	5.72	2.64	33.47	$P < 0.0001$
Within-individual	291	128.10	0.44	0.44	5.57	$P < 0.0001$
Total	581	4323.97		7.89	100.00	

df degree of freedom

SS sun of squares

MS mean square

Est. Var. estimator of variance

Table 5 *F*-statistics (lower) and geographical distances (meter, upper) between *G. tomentella* natural populations on the southeast islands of China

Pop	Pop-1	Pop-2	Pop-3	Pop-4	Pop-5	Pop-6	Pop-7	Pop-8	Pop-9	Pop-10	Pop-11
Pop-1		1016	43,405	43,423	69,719	69,710	69,360	273,586	273,623	364,542	364,170
Pop-2	0.308*		42,521	42,597	69,000	68,982	68,632	272,792	272,831	363,748	363,375
Pop-3	0.438*	0.493*		4938	30,665	30,423	29,996	231,004	231,048	321,915	321,539
Pop-4	0.596*	0.641*	0.209*		27,977	27,813	27,408	230,280	230,321	321,233	320,860
Pop-5	0.672*	0.756*	0.417*	0.533*		602	929	204,414	204,443	295,355	294,988
Pop-6	0.738*	0.831*	0.445*	0.592*	0.665*		436	204,333	204,364	295,280	294,912
Pop-7	0.837*	0.961*	0.559*	0.717*	0.751*	0.777*		204,652	204,684	295,603	295,234
Pop-8	0.777*	0.854*	0.533*	0.631*	0.733*	0.804*	0.882*		197	90,985	90,615
Pop-9	0.523*	0.598*	0.248*	0.402*	0.470*	0.540*	0.638*	0.490*		90,950	90,580
Pop-10	0.659*	0.764*	0.419*	0.539*	0.604*	0.703*	0.798*	0.721*	0.463*		408
Pop-11	0.672*	0.779*	0.386*	0.546*	0.614*	0.705*	0.846*	0.730*	0.456*	0.415*	

* Significance at 0.05 level

The geographical distances was estimated by Google earth

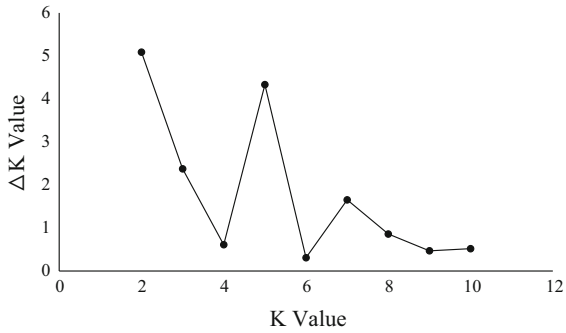


Fig. 2 Magnitude of Δk for each K value, for *G. tomentetta* population on islands off the southeastern coast of China

mixed populations, being assigned to the northeastern and southwestern groups, respectively, for their individual plants.

When k = 5 (Fig. 3c, d), all 291 *G. tomentella* plant samples were assigned to five genetic structural groups just as island populations (Fig. 1), group-1 (pops. 10 and 11, Pingtain Island), group-2 (pops. 1 and 2, Nanao Island), group-3 (pops. 5, 6 and 7, Jingan Island), group-4 (pops. 3 and 4, Dongshan Island) and group-5 (pops. 8 and 9, Meizhou Island), which genetically demonstrated 'island differentiation'. However, most populations exhibited geographical genetic exchange; pops. 5 and 9 were genetically more complicated (Fig. 3d), and included genetic composition from the southwest and northeast.

The NJ clusters based on Nei et al.'s (1983) genetic distance revealed that the 11 populations were first clustered as the sampling islands geographically (Fig. 4), suggesting that there was a genetically stronger island differentiation effect. A closer genetic kinship could be observed between geographically far northeastern and southwestern regions (Nanao, Meizhou and Pingtan Islands) (Fig. 4b), suggesting that there was a genetic connection between the northeastern and southwestern *G. tomentella*.

A Mantel test was performed to determine whether genetic distances were related to the geographic distances among *G. tomentella* populations. A significant correlation ($r = 0.295^*$, $P < 0.001$) existed between the two, and the regression analysis (y) also showed that the correlation decreased as the geographical distances increased between populations (Fig. 5). The spatial autocorrelation analysis showed a positive correlation within 40.59 km, and a significant positive correlation within 30.45 km (Fig. 6).

Discussion

Distribution of perennial *G. tomentella* species in China

Four subgenus *Glycine* species have been reported to exist in Taiwan, i.e. *G. tabacina* (Labill.) Benth.

(2n = 80, BB_1, BB_2 B_1B_2), 80-chromosome typed *G. tomentella* Hayata (2n = 80, DA_6; DH_2), *G. pescadrensis* Hayata (2n = 80, AB_1), and *G. dolichocarpa* Tateishi et Ohashi (2n = 80, D_1A) (Pfeil et al. 2006; Tateishi and Ohashi 1992; Chung and Singh 2008). Thus far, only one 80-chromosome typed *G. tomentella* has been reported to grow on the southeast islands of China (Bau et al. 1993; Gao et al. 2002), while the 38-chromosome, 40-chromosome and 78-chromosome *G. tomentella* have not been found. Our partial cytological analyses showed that the *G. tomentella* plants from the southeast islands of China were also 80-chromosomes, but that their genome types remain unknown. Geographically, the southeast islands of China were close to Taiwan. Since genome-DA_6, and DH_2 typed *G. tomentella* (2n = 80) exists in Taiwan, the *G. tomentella* that appears on the southeast islands of China could also belong to the DA_6, or/and DH_2 genomes (Chung and Singh 2008), this remains to be confirmed in our future research. However, based on morphological comparison, particularly pods, the *G. tomentella* on the islands of southeastern China greatly differs from the *G. dolichocarpa* in Taiwan, (Pfeil et al. 2006; Tateishi and Ohashi 1992; Chung and Singh 2008).

Our field survey in 2013–2017 found that the distribution range of *G. tomentella* in China was contoured by about 114°44′07″–119°52′57″E, for the

Fig. 4 Neighbor-Joining clustering of the 11 *G. tomentella* populations based on allele frequencies and Nei et al.'s (1983) distance with 1000 bootstrap replicates (0.95 confidence interval). The island populations of Chinese *G. tomentella* had a genetically stronger island differentiation pattern (**a**). Genetic kinship appeared to exist between the northeastern and southwestern regions (Nanao, Meizhou, and Pingtan Islands) (**b**)

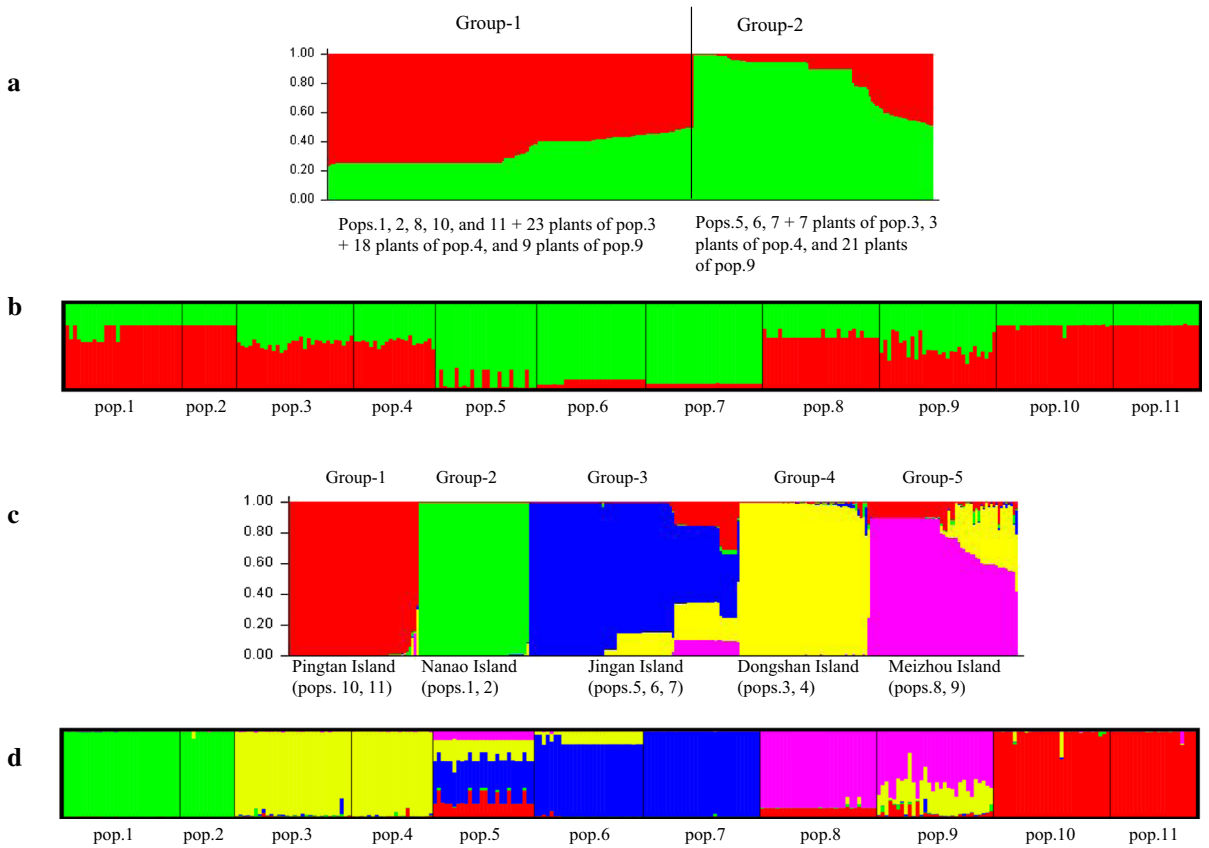
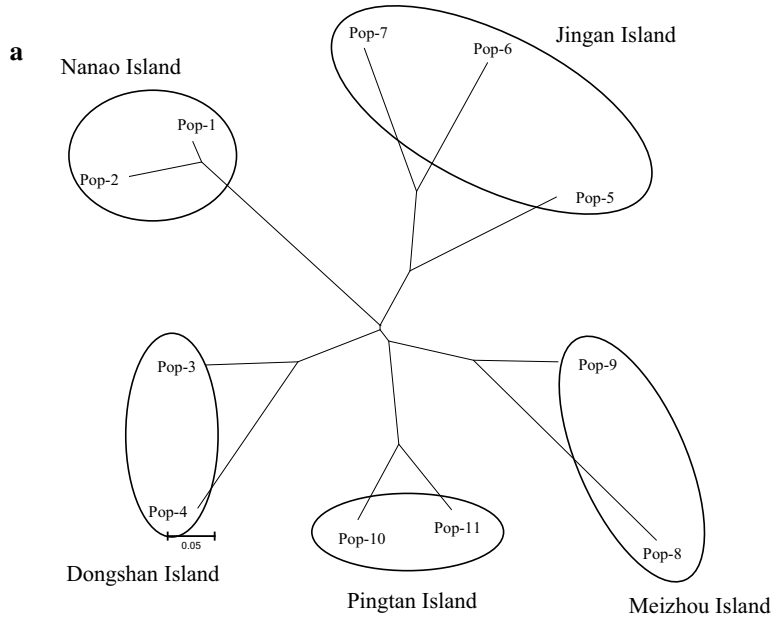
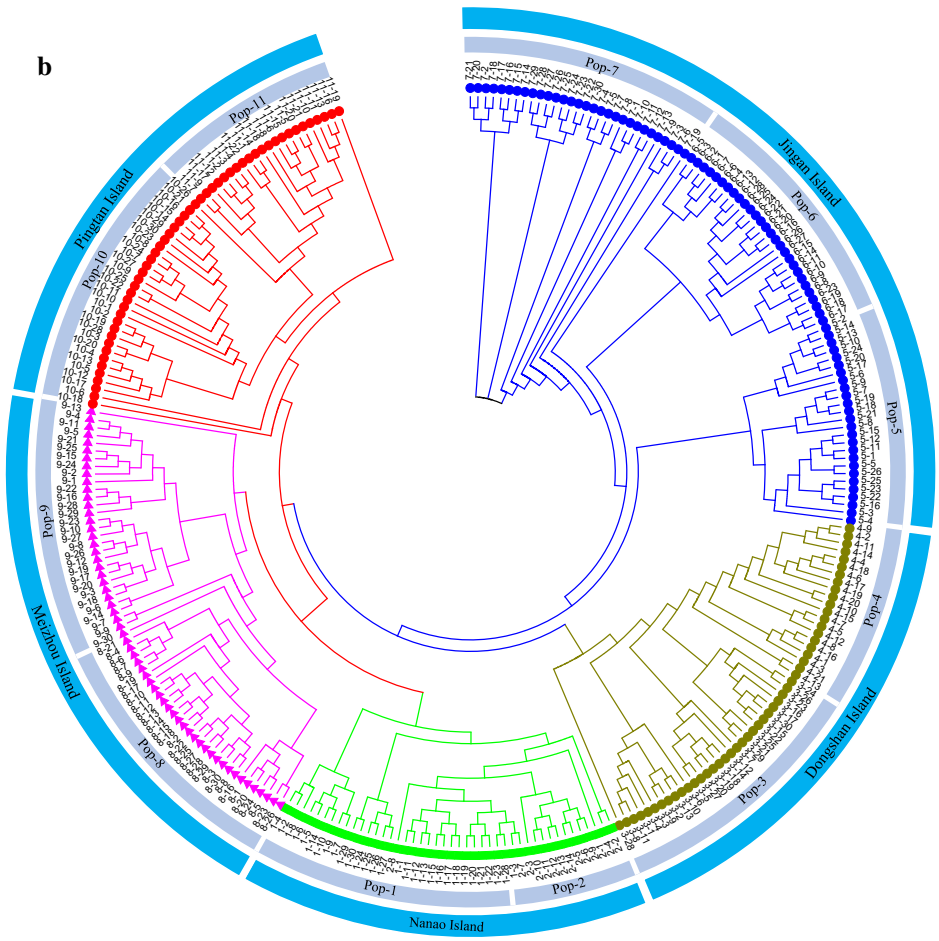


Fig. 3 Genetic structure analysis under a model without prior population information for the 11 natural *G. tomentella* populations on the islands off the southeastern coast of China. When k = 2, there were two genetic structural groups (**a**, **b**); when k = 5, all plants were assigned to five genetic structural groups just as island populations, group-1 (pops. 10, 11), group-2 (pops. 1, 2), group-3 (pops. 5, 6, 7), group-4 (pops. 3, 4) and group-5 (pops. 8, 9) (**c**, **d**)


a
Nanao Island
Jingan Island
Pop-1
Pop-2
Pop-7
Pop-6
Pop-5
Pop-3
Pop-4
0.05
Pop-9
Pop-10
Pop-11
Pop-8
Dongshan Island
Pingtan Island
Meizhou Island


b
Pop-11
Pingtan Island
Pop-10
Pop-9
Meizhou Island
Pop-8
Pop-7
Jingan Island
Pop-6
Pop-5
Pop-4
Dongshan Island
Pop-3
Pop-1
Pop-2
Nanao Island

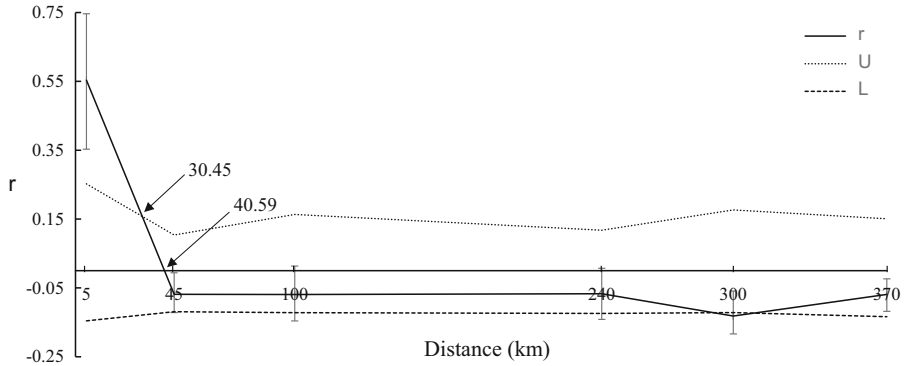


Fig. 6 Spatial autocorrelogram for *G. tomentella*'s geographical distribution on the southeast islands of China. A positive correlation occurred within a radius of 40.59 km, and a significantly positive correlation occurred within a radius of 30.45 km. The *r* indicates correlation coefficient; U and L indicate upper and lower 95% confidence intervals of the null hypothesis, respectively

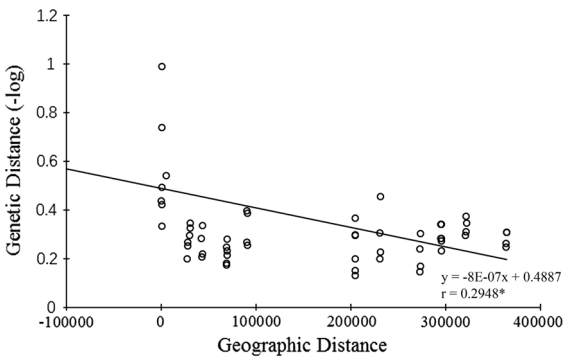


Fig. 5 Relationships between the genetic and geographical distances of populations. Genetic and geographical distances showed significant correlations

west–east limit, on the eastern Guangdong coast (Dayawa Bay) to the southeastern Fujian coast (Pingtan Island) and the westernmost site was located in Dayawan (22°35′18″N, 114°44′07″E). Here, we only sampled the natural populations on the islands (Fig. 1), but Pingtain and Meizhou Islands also had distributions of *Glycine tabacina* species. Another Chinese perennial species, *G. tabacina* has a small geographical range, being confined to the southeast coast of Fujian Province, whereas *G. tomentella* has a more extensive geographical range, owing to its stronger environmental adaptability. Chinese *G. tomentella* has strong drought and salt tolerance levels, and prefers sunny locations. It grows in a wide variety of environments, such as roadsides, sand dunes, grass, thickets, hillsides, rocky hills and graveyards on the islands, but it rarely grows in shady places, tall grasses, or little shrubs. Commonly, *G. tomentella* has stoloniferous stems, with plant heights of about 10–200 cm, procumbent or creeping along the ground. In the field, *G. tomentella* grows commonly prostrate along the ground, and rarely twists or climbs on accompanying plants.

Does the creeping growth habit reduce genetic diversity in Chinese *G. tomentella* compared with the sympatric *G. tabacina* (Labill.) Benth.?

In general, the natural *G. tomentella* populations had a relatively lower mean genetic diversity (P = 58.6%, H_e= 0.232 and PIC = 0.203) than the *G. tabacina* populations (P = 78.57%, H_e= 0.272 and PIC = 0.237) in this sympatric region (all the analytic results about *G. tabacina* will appear elsewhere). The causes of this lower genetic diversity level in *G. tomentella* than in *G. tabacina* is unknown. However, *G. tomentella* and *G. tabacina* grow in the same ecological environments in the same region, but the two species exhibited differences in genetic diversity levels. The creeping growth habit of *G. tomentella* beneath the companion species, which was inferior for its N_m, may influence seed dispersal and lead to the lower genetic diversity in comparison with that of the sympatric *G. tabacina*.

The implications of a relatively high outcrossing rate and lower N_m in Chinese *G. tomentella*

Commonly, self-pollinating plants have a lower outcrossing rate, such as annual wild soybean (*G. soja*), which has an outcrossing rate of less than 4% (Kiang et al. 1992; Fujita et al. 1997; Kuroda et al. 2006; Wang and Li 2012). Compared with the sympatric perennial *G. tabacina* in the same island zone, *G. tabacina* had a greater t_m of 6.75% and an N_m of 0.315. In this study, *G. tomentella* also had a relatively high average t_m of 5.74% (Table 3). Of 11 populations, pops. 2, 3, 4, and 9 had exceptionally high t_m values of 10.87–16.70%, and pop. 1 had no occurrence of outcrossing (Table 3). This study did not consider the age structure of individual plants in the populations because determining age is difficult for plants when sampling in the field. Additionally, an isogenous family, or lineage samples of assorted ages, would decrease the detected outcrossing rate. We hypothesize that the relatively high outcrossing rate did not always reflect the actual outcrossing occurring among *G. tomentella* populations, and it may have resulted from heterogeneity owing to the perennation that maintains historical mutations and genetic recombination in *G. tomentella*.

N_m is composed of the components of seed dispersal or individual migration and introgression by hybridization. The relatively lower N_m (mean 0.162 N_m, Table 3) implied that individual dispersal seemed be restricted among populations, particularly islands, as shown by the *F*-statistics in which all populations had significant genetic differentiation (Table 5). Additionally, the NJ-clustering showed a pattern of stronger island differentiation (Fig. 4).

We hypothesize that the stoloniferous habit of *G. tomentella* along the ground limits the exchanges between populations or islands through the activities of birds, animals and natural factors. This influences the N_m, resulting in a lower N_m (Table 2).

Population genetic variation and differentiation in Chinese *G. tomentella*

A typical feature of autogamous plants is that they have greater inter-population than intra-population genetic variation (Reif et al. 2003; Guo et al. 2012; He et al. 2012; Wang et al. 2014, 2017), in contrast to typical allogamous plants (Persson and Bothmer 2002; Hang et al. 2004; Costa et al. 2013; Maggioni et al. 2014; Hao et al. 2015). This study revealed that island populations of Chinese *G. tomentella* had the typical feature of autogamous plants in terms of genetic variation, with a high inter-populations genetic variation of 60.96% (Table 4; and 60.7% variation in G_{st}, Table 2). This differed from *G. tabacina*, which exhibited the opposite trend, like an allogamous plant, in terms of population genetic variation.

Table 6 Assignment of possible foreign plants within the *G. tomentella* natural populations on the southeast islands of China

Population	Number of foreign plants*	From possible foreign population sources	Geographical distance from foreign population (km)	A_f (%)
Pop-1	15	Pop-2	1.02	50.00
Pop-2	0	–	–	
Pop-3	0	–	–	
Pop-4	1	Pop-3	4.94	4.76
Pop-5	0	–	–	
Pop-6	0	–	–	
Pop-7	0	–	–	
Pop-8	0	–	–	
Pop-9	0	–	–	
Pop-10	0	–	–	
Pop-11	0	–	–	

*Number was inferred using Geneclass 2 (Piry et al. 2004)

A_f proportion (%) of foreign individuals in populations

The NJ cluster and STRUCTURE assignation showed that island populations of Chinese *G. tomentella* had a genetically stronger island differentiation pattern based on the clustering of populations and individual samples (Figs. 3c, 4b). The closer genetic kinship between northeastern and southwestern regions (Nanao, Meizhou, and Pingtan Islands) in *G. tomentella* suggested that some genetic interconnection occurred along the southeast coast of China in ancient times. The spatial autocorrelation analysis showed a significant positive correlation within a radius of about 30.45 km (Fig. 5). The assignment test inferred possible individual dispersal (Table 6). The STRUCTURE analysis showed some geographical consanguinity (Fig. 3a). *G. tomentella* populations might have spread among the islands along the coast while the southeast islands of China and the Taiwan Strait were joined as a continuous land form during the last glacial period (approximately 70,000–11,500 years ago) (Lin 1980), and the island differentiation pattern was formed as the islands separated at the end of the glacial period, approximately 15,000 years ago (Yao et al. 2009). It is possible that some dispersal would be disseminated by birds and sea seawater or by human activities.

The *F*-statistics suggested that geographically different *G. tomentella* populations had unique structures, even distance–near neighboring populations in the same islands, such as pops. 1 and 2, and pops. 3 and 4 (Table 5). Based on the idiosyncrasies of the genetically-based geographical differentiation among populations of *G. tomentella*, we propose that when collecting genetic resources of this species, every *G. tomentella* population should be sampled.

Acknowledgements This work was financially supported by project "Sci and Tech Innovation Program of Chinese Academy of Agricultural Sciences" and "Crop Germplasm Resources Protection (Item No. 2130135-09) from the Ministry of Agriculture of the People's Republic of China.

References

Bau T, Xu B, Zhuang BC (1993) A brief report of cytology study on Chinese subgenus *Glycine*. J Jilin Agric Univ 15:218 (**in Chinese**)

Brown AHD, Doyle JL, Grace JP, Doyle JJ (2002) Molecular phylogenetic relationships within and among diploid races of *Glycine tomentella* (Leguminosae). Aust Syst Bot 15:37–47

Chen LL, Liu XD, Zhao HK, Yuan CP, Wang YN, Xu MZ, Wang YM (2013) Genetic diversity of *G. tabacina* from Meizhou Island of Fujian Province. Soybean Sci 32:286–290 (**in Chinese**)

Chung G, Singh RJ (2008) Broadening the genetic base of soybean: a multidisciplinary approach. Crit Rev Plant Sci 27:295–341

Costa TRD, Filho PSV, Gonçalvesvidigal MC, Galván MZ, Lacanallo GF, Silva LID, Kvitschal MV (2013) Genetic diversity and population structure of sweet cassava using simple sequence repeat (SSR) molecular markers. Afr J Biotechnol 12:1040–1048

Cregan PB, Jarvik T, Bush AL, Shoemaker RC, Lark KG, Kahler AL, Kaya N, VanToai TT, Lohnes DG, Chung J, Specht JE (1999) An integrated genetic linkage map of the soybean genome. Crop Sci 39:1464–1490

Doyle MJ, Brown AHD (1985) Numerical analysis of isozyme variation in *Glycine tomentella*. Biochem Syst Ecol 13:413–419

Doyle MJ, Grant JE, Brown AHD (1986) Reproductive isolation between isozyme groups of *Glycine tomentella* (Leguminosae), and spontaneous doubling in their hybrids. Aust J Bot 34:523–535

Earl DA, vonHoldt BM (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv Genet Resour 4:359–361

Excoffier L, Lischer HEL (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol Ecol Resour 10:564–567

Fujita R, Ohara M, Okazaki K, Shimamoto Y (1997) The extent of natural cross-pollination in wild soybean (*Glycine soja*). J Hered 88:124–128

Gao X, Qian J, Ma YH, Zheng SZ (2002) Research on chromosomes of perennial wild soybeans in China. J Fudan Univ (Nat Sci) 41:717–719 (**in Chinese**)

Goudet J (2001) FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). My Publications

Guo J, Liu YF, Wang YS, Chen JJ, Li YH, Huang HW, Qiu LJ, Wang Y (2012) Population structure of the wild soybean (*Glycine soja*) in China: implications from microsatellite analyses. Ann Bot 110:777–785

Hang Y, Jin Y, Lu BR (2004) Genetic diversity of the endangered species *Psathyrostachys huashanica* in China and its strategic conservation. J Fudan Univ (Nat Sci) 43:260–266 (**in Chinese**)

Hao D, Zhang Z, Cheng Y, Chen G, Lu H, Mao Y, Shi M, Huang X, Zhou G, Xue L (2015) Identification of genetic differentiation between waxy and common maize by SNP genotyping. PLoS ONE 10:e0142585

Hayata B (1920) Icones Plantarum Formosanarum nec non et Contributiones ad Floram Formosanam; or, Icones of the Plants of Formosa, and Materials for a Flora of the Island, based on a study of the collections of the botanical survey of the Government of Formosa, vol 9. Bureau of Forestry, Industries, Government of Formosa, Taihoku, p 26

He S, Wang Y, Volis S, Li D, Yi T (2012) Genetic diversity and population structure: implications for conservation of wild soybean (*Glycine soja* Sieb. et Zucc.) based on nuclear and

chloroplast microsatellite variation. Int J Mol Sci 13:12608–12628

Jakobsson M, Rosenberg NA (2007) CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics 23:1801–1806

Jensen JL, Bohonak AJ, Kelley ST (2005) Isolation by distance, web service. BMC Genet 6:13

Kiang YT, Chiang YC, Kaizuma N (1992) Genetic diversity in natural populations of wild soybean in Iwate Prefecture, Japan. J Hered 83:325–329

Kollopara KP, Singh RJ, Hymowitz T (1994) Genomic diversity and multiple origins of tetraploid (2n = 78, 80) *Glycine tomentella*. Genome 37:448–459

Kuroda Y, Kaga A, Tomooka N, Vaughan DA (2006) Population genetic structure of Japanese wild soybean (*Glycine soja*) based on microsatellite variation. Mol Ecol 15:959–974

Lin DG (1980) Eustacy in Fujian during the Quaternary Period. Chin Sci Bull 25:1134–1136 (**in Chinese**)

Liu KJ, Muse SV (2005) POWERMARKER: integrated analysis environment for genetic marker data. Bioinformatics 21:2128–2129

Maggioni L, von Bothmer R, Poulsen G, Branca F, Bagger Jørgensen R (2014) Genetic diversity and population structure of leafy kale and *Brassica rupestris* Raf. in south Italy. Hereditas 151:145–158

Narzary D, Verma S, Mahar KS, Rana TS (2015) A rapid and effective method for isolation of genomic DNA from small amount of silica-dried leaf tissues. Nat Acad Sci Lett 38:441–444

Nei M, Tajima F, Tateno Y (1983) Accuracy of estimated phylogenetic trees from molecular data II. Gene frequency data. J Mol Evol 19:153–170

Peakall R, Smouse PE (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update. Bioinformatics 28:2537–2539

Persson K, Bothmer RV (2002) Genetic diversity amongst landraces of rye (*Secale cereale* L.) from northern Europe. Hereditas 136:29–38

Pfeil BE, Craven LA, Brown AHD, Murray BG, Doyle JJ (2006) Three new species of northern Australian *Glycine* (Fabaceae, Phaseolae), *G. gracei*, *G. montis-douglas* and *G. syndetika*. Austral Syst Bot 19:245–258

Piry S, Alapetite A, Cornuet JM, Paetkau D, Baudouin L, Estoup A (2004) GENECLASS2: a software for genetic assignment and first-generation migrant detection. J Hered 95:536–539

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945–959

Reif JC, Melchinger AE, Xia XC, Warburton ML, Hoisington DA, Vasal SK, Srinivasan G, Bohn M, Frisch M (2003) Genetic distance based on simple sequence repeats and heterosis in tropical maize populations. Crop Sci 43:1275–1282

Singh RJ (2010) Methods for producing fertile crosses between wild and domestic soybean species: US, US7842850

Singh RJ, Nelson RL (2015) Intersubgeneric hybridization between *Glycine max* and *G. tomentella*: production of F1, amphidiploid, BC1, BC2, BC3, and fertile soybean plants. Theor Appl Genet 128:1117–1136

Singh RJ, Kollipara KP, Hymowitz T (1987) Polyploid complexes of *Glycine tabacina* (Labill.) Benth. and *G. tomentella* Hayata revealed by cytogenetic analysis. Genome 29:490–497

Singh RJ, Kollipara KP, Hymowitz T (1990) Backcross-derived progeny from soybean and *Glycine tomentella* Hayata intersubgeneric hybrids. Crop Sci 30:871–874

Singh RJ, Kollipara KP, Hymowitz T (1993) Backcross (BC2–BC4)-derived fertile plants from *Glycine max* and *G. tomentella* intersubgeneric hybrids. Crop Sci 33:1002–1007

Singh RJ, Kollipara KP, Hymowitz T (1998) Monosomic alien addition lines derived from *Glycine max* (L.) Merr. and *G. tomentella* Hayata: production, characterization, and breeding behavior. Crop Sci 38:1483–1489

Song QJ, Jia GF, Zhu YL, Grant D, Nelson RT, Hwang EY, Hyten DL, Cregan PB (2010) Abundance of SSR motifs and development of candidate polymorphic SSR markers (BARCSOYSSR_1.0) in soybean. Crop Sci 50:1950–1960

Tateishi Y, Ohashi H (1992) Taxonomic studies on *Glycine* of Taiwan. J Jpn Bot 67:127–147

Tindale M (1986) Taxonomic notes on three Australian and Norfolk Island species of *Glycine* Willd. (Fabaceae: Phaseolae) including the choice of a neotype for *G. clandestina* Wendl. Brunonia 9:179

Wang KJ, Li XH (2012) Genetic characterization and gene flow in different geographical-distance neighbouring natural populations of wild soybean (*Glycine soja* Sieb. & Zucc.) and implications for protection from GM soybeans. Euphytica 186:817–830

Wang KJ, Li XH, Yan MF (2014) Microsatellite markers reveal genetic diversity of wild soybean in different habitats and implications for conservation strategies (*Glycine soja*) in China. Conserv Genet 15:605–618

Wang Y, Ghouri F, Shahid MQ, Naeem M, Baloch FS (2017) The genetic diversity and population structure of wild soybean evaluated by chloroplast and nuclear gene sequences. Biochem Syst Ecol 71:170–178

Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. Evolution 38:1358–1370

Yao YT, Harff J, Meyer M, Zhan WH (2009) Reconstruction of paleocoastlines for the northwestern South China Sea since the last glacial maximum. Sci China Ser D Earth Sci 52:1127–1136

Yeh FC, Yang RC, Boyle T (1999) Popgene version 1.32: Microsoft Windows-based freeware for population genetic analysis. University of Alberta, Edmonton

Zhang R, Liu JQ, Yang M, Zhu GH (2011) The extracts from *Glycine tomentella* roots used to produce traditional Chinese medicine for the treatment of rheumatoid arthritis: Patent, China, CN102119954A (**in Chinese**)

Zou JJ, Singh RJ, Hymowitz T (2004) SSR marker and ITS cleaved amplified polymorphic sequence analysis of soybean × *Glycine tomentella* intersubgeneric derived lines. Theor Appl Genet 109:769–774