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GENETIC DIVERSITY AND POPULATION STRUCTURE OF WILD RADISH IN EAST ASIA

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ABSTRACT

Wild radish (*Raphanus sativus* L. var. *hortensis* f. *raphanistroides* Makino) is widely distributed in East Asia; however, its genetic diversity and population structure remain poorly understood. In our study, DNA sequence and microsatellite loci were used to analyze wild radish populations across the whole East Asia. Population genetic diversity analysis showed that the East Asian wild radish populations generally had high genetic diversity (average H_O=0.448), distinct population structure, and a positive correlation between genetic distance and geographic distance. AMOVA analysis suggested the genetic diversity among individuals in populations by far exceeded the diversity among populations/groups. The outcrossing breeding system, long geographic distance between populations and gene flow from cultivated radish populations may explain the high level of genetic diversity within wild populations. Bayesian cluster analysis identified four genetic clusters in the East Asian wild radish populations, observed by DNA sequencing analysis. The possible factors would be various aspects, such as oceanic barrier, climate fluctuations, and pollinators' characteristics. This study provides a reference for the utilization of wild radish germplasm resources and the improvement of radish varieties in East Asia.

Keywords: Wild radish, Genetic diversity, Population structure, DNA sequencing, Microsatellite loci

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INTRODUCTION

The radish (*Raphanus sativus* L), which belongs to Brassicaceae (Cruciferae) family, is one of the most important economic root vegetables in the world. It includes annual tuber vegetable crops and various wild species in East Asia. One wild radish variety, *R. sativus* L. var. *hortensis* f. *raphanistroides* Makino, also known as Hama-daikon, is mainly distributed on the dunes and sandy beaches of Japan, Korea and the southern regions of China (Huh and Ohnishi, 2001). This wild species has no juicy edible roots but slightly thick siliques consisting spongy parenchyma. The fully ripened seed of *R. sativus*, commonly known as Raphani seed, is a kind of traditional Chinese medicine with good therapeutic effects on hypertension, obesity, diabetes, constipation and cough (Tang *et al*., 2003). Wild radish has a high seed setting rate and is planted as an oil crop in the Yunnan, Sichuan, and Guizhou provinces of southern China. Wild radish is genetically related to varieties and has the potential to provide beneficial genetic traits, such as tolerance to new diseases/pests and effective seed production (Kazuhiro, 2009; Jugulam *et al*., 2014; Welles and Funk, 2020). Therefore, understanding the genetic diversity and population structure of wild radish could help in the development and breeding of this species.

Molecular research can be used to quantify genetic variation within germplasm samples. Various markers have been used in the genetic analysis of *Raphanus* populations. For example, based on chloroplast DNA sequence data, historical and current gene flow has been identified between wild and cultivated radish populations across the Cape Floristic Region (CFR), South Africa (Barnaud *et al*., 2013). Genetic diversity in radish populations has also been analyzed using inter-simple sequence repeat (ISSR), amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD) markers (Huh and Ohnishi, 2002, 2003; Muminovic *et al*., 2005; Kong *et al*., 2011). Furthermore, based on DNA sequencing and microsatellite (also known as simple sequence repeats, SSR) data, we found significant genetic variation in Japanese populations of wild radish, resulting in two distinct phylogenetic clusters (*i.e.*, southern and northern lineages in Japan) (Han *et al*., 2015, 2016). However, researches have been conducted on wild and cultivated radish varieties, the genetic variation and structure of wild radish populations in the whole East Asia remain poorly studied.

Here, we explored the genetic diversity and population structure of wild radish in various East Asian populations. The objectives of this study were to: (1)

evaluate the genetic diversity of wild radish in East Asia using DNA sequencing and SSR markers; and (2) analyze the population structure/phylogenetic relationship among wild radish populations in East Asia. This study should provide a useful reference for the utilization of wild radish germplasm resources and the long-term successful cultivation of the species.

MATERIALS AND METHODS

Plant material: 34 wild radish populations (representing ~25 individuals per population) were sampled in East Asia. And then, every three individuals per population (total 102 samples from 34 populations) were used for nuclear DNA sequencing. Meanwhile, with the exclusion of 6 populations (population 3, 5-8, and 10) owing to their limited samples size, 647 samples from the remaining 28 populations were used for SSR analysis. Details on population location and size for DNA sequencing and SSR analysis are presented in Table 1.

Molecular procedure: We extracted genomic DNA from dry leaves using the cetyltrimethyl ammonium bromide (CTAB) method with minor modifications. After preliminary screening, 14 nuclear sites were selected for sequencing (Table 2, Han *et al*., 2016). Polymerase chain reaction (PCR) volume was 10 μL and the reaction solution contained 7.25 μL of double distilled water, 0.8 μL of dNTP mixture (2.5 mM), 1 μL of 10× Ex Taq buffer, 0.25 U of Ex Taq, 0.2 μM for each primer, and 10 ng of DNA. The reaction was optimized using a thermal cycling procedure: 5 min denaturation at 94 °C, followed by 35 cycles at 94 °C for 1 min, annealing at 55 °C for 1 min, extension at 72 °C for 1.5 min, and final extension at 72 °C for 10 min. Exostar (GE Healthcare Ltd., Little Chalfont, Buckinghamshire, UK) was used to purify the PCR products. An ABI 3130 automated sequence analyzer (Applied Biosystems, Foster City, CA, USA) was used to sequence the purified PCR products directly from two directions. All sequence data were analyzed and aligned using an Auto Assembler ver. 2.3 (Applied Biosystems, Foster City, CA, USA).

SSR markers were selected from nuclear microsatellites developed for *Raphanus* (Nakatsuji *et al*., 2011; Ohsako *et al*., 2010; Hashida *et al*., 2013). PCR was conducted in a total volume of 10 μL containing 5 ng of genomic DNA, 0.5 mM dNTPs, 10× PCR buffer, 0.5 μM of forward and reverse primers and 0.4 unit of Taq DNA polymerase. PCR amplification was carried out by an initial denaturation at 94 °C for 4 min, followed by 29 cycles of 94 °C for 40 s, annealing at 56 °C for 25 s, extension at 72 °C for 30 s, and a final extension at 72 °C for 10 min. The amplified products were loaded into the ABI 3130 automatic sequencer using GeneScan Rox-350 Size Standard (Applied Biosystems), and the allele size was determined by GeneMapper Software ver. 5.0 (Applied Biosystems).

Data analysis: As for 14 nuclear genes sequencing analysis, haplotypes of all individuals were determined using PHASE with default setting in DnaSP ver. 5.10 (Librado and Rozas, 2009). The number of segregation sites (S), average number of pairwise nucleotide differences per sites (π) (Nei and Li, 1979), number of haplotypes (N_h), haplotype diversity (H_d), and degree of nucleotide polymorphism (θ_w) (Watterson, 1975) were calculated for each locus. To test the neutral evolution of each locus, Tajima'D (Tajima, 1989), Fu and Li's D^* and F^* (Li and Fu, 1993) were estimated. As for SSR data analysis, genetic diversity was estimated by calculating the number of different alleles (Na), observed heterozygosity (H_o), and expected heterozygosity (H_e) using GenAlEx V6.5.

STRUCTURE ver. 2.3.4 was used to evaluate intraspecific population structure (Pritcharda *et al*., 2010). We chose correlated allele frequencies as the admixture model, and set K values from 1 to 6 for DNA sequencing and 1 to 12 for microsatellite markers, based on previous study experience (Han *et al.*, 2015, 2016). After a burn-in period of 10^4 and 8×10^5 Markov Chain Monte Carlo (MCMC) iterations, per K value was replicated by 10 runs. To estimate the most appropriate K value, the STRUCTURE results were uploaded to the STRUCTURE HARVESTER website (http://taylor0.biology.ucla.edu/structureHarvester/) (Earl and Vonholdt, 2012). We estimated the optimal K value by evaluating delta K and maximum-likelihood values (Evanno *et al*., 2005). Ten replicate runs were grouped based on a symmetrical similarity coefficient of > 0.9 using the Greedy algorithm in CLUMPP (Jakobsson and Rosenberg, 2007) and then visualized in DISTRUCT (Rosenberg 2004).

Principle coordinate analysis (PCoA) was used to evaluate the correlation between populations using GenAlEx ver. 6.5. To calculate genetic variation within populations, among populations, within groups, and between groups, we performed analysis of molecular variance (AMOVA) using Arlequin ver. 3.5 (Excoffier and Lischer, 2010) and conducted significance tests based on 1 000 permutations. The correlation between geographic and genetic distances was evaluated using the Mantel test (Smouse *et al*., 1986) in GenAlEx ver. 6.5, with significance tested using 1 000 permutations.

Table 1. Summary of sampling localities, sample sizes, and characteristics of the populations based on the SSR markers.

Pop. Code	Locations	Samples sizes for SSR	N_a	H_O	H_E
Ryukyu Island					
1	Iriomote Isl.	27	5.111	0.395	0.556
2	Ishigaki Isl.	27	4.222	0.300	0.565
3	Miyako Isl	-	-	-	-
4	Okinawa Isl.	28	5.111	0.552	0.636
5	Yoronto Isl.	-	-	-	-
6	Okinoerabu Isl.	-	-	-	-
7	Tokuno Isl.	-	-	-	-
8	Amami Isl.	-	-	-	-
9	Yakushima Isl.	25	3.889	0.498	0.573
10	Tanega Isl.	-	-	-	-
Mainland Japan					
11	Kagoshima	25	5.444	0.489	0.661
12	Kumamoto	29	5.889	0.529	0.672
13	Kochi	28	4.333	0.377	0.525
14	Mie	16	4.444	0.500	0.527
15	Kanagawa	25	5.333	0.458	0.605
16	Shiga	55	6.444	0.440	0.624
17	Tottori	20	4.111	0.406	0.521
18	Hyogo	28	4.222	0.452	0.569
19	Fukui	22	4.667	0.485	0.518
20	Toyama	25	5.667	0.511	0.555
21	Sado Isl.	29	5.333	0.444	0.521
22	Akita	24	4.111	0.375	0.439
23	Aomori	29	5.556	0.479	0.525
24	Hokkaido	24	4.000	0.491	0.598
25	Tsushima-S	12	3.222	0.439	0.478
26	Tsushima-N	12	3.333	0.370	0.517
Korea					
27	Jeju-J	12	4.667	0.435	0.593
28	Jeju-Weo	12	4.111	0.407	0.487
29	Busan-N	24	4.889	0.491	0.616
30	Ulsan-Dae	24	3.556	0.376	0.467
China					
31	Zhejiang	18	4.556	0.429	0.630
32	Yunnan	12	5.667	0.472	0.637
33	Sichuan	17	4.222	0.537	0.627
34	Taiwan	18	3.556	0.397	0.529
Average	-	-	4.631	0.448	0.563

*N*a: average number of alleles per site; H_O, observed heterozygosity; H_E, expected heterozygosity

Table 2. Summary of nucleotide polymorphism and neutrality tests of 14 loci for wild radish.

Locus	Aligned size (bp)	*S*	θ_W	Π	N_h	H_d	Neutrality tests		
							D	*D**	*F**
PHYA	473	27	0.01044	0.01550	32	0.794	1.36592	1.18478	1.51615
PHYB	420	24	0.01055	0.02316	27	0.801	3.31984	1.48369	2.68756
PHYC	480	10	0.00357	0.00493	12	0.599	0.89806	0.55783	0.81457
PHYE	378	7	0.00341	0.00831	10	0.668	3.48344*	1.13875	2.33488*
TBL19	415	7	0.00289	0.00416	11	0.651	0.94375	1.14267	1.27895
TBL21	556	15	0.00460	0.00878	18	0.709	2.30909*	0.34622	1.31346
MYB29	441	7	0.00270	0.00515	8	0.691	1.92713	1.14029	1.68687

COL4	577	18	0.00563	0.01051	16	0.693	2.31037*	-0.38737	0.80534
COL5	357	17	0.00811	0.01361	24	0.799	1.76752	-1.74763	-0.46500
CHI	366	8	0.00371	0.00498	16	0.731	0.75304	1.20746	1.24932
VEL2	317	11	0.00590	0.00924	16	0.802	1.34964	0.64713	1.08382
LFY	235	12	0.00868	0.00265	7	0.487	-1.68923	-3.46195	-3.36023
CRY2	406	21	0.00929	0.01452	27	0.759	1.53474	0.37752	1.00858
CRY3	332	30	0.01668	0.01836	26	0.687	0.28827	0.56189	0.53893
Average	411	15	0.00687	0.01028	18	0.705			

S, number of polymorphic sites; θ_W, Watterson's estimator of θ per base pair; π, nucleotide diversity; N_h, number of haplotypes; H_d, haplotype diversity; *D*, Tajima'*D* and Fu & Li's *D** and *F**. Significant levels: *, $P \leq 0.05$

Table 3. Characteristics of the nine microsatellite loci used for this study.

Locus	Sequence	N_a	H_O	H_E
Rm19	F: GCAACCACTATCAAACTCCGTTAT R: GGGGAAAATACTAAGATGGGTGT	3.172	0.262	0.452
REL-13	F: CTAGCAATGCATACCAAACAG R: AACTTGGTCGTTGAGCAG	3.897	0.314	0.457
REL-16	F: ACAGCAACGTTTTCAAGTGCTC R: CTCACATGCAATGCAATGCATAC	4.586	0.283	0.527
RsSA014	F: AATAAGCATGTGGTGGGAAGTTA R: GGGTTTATGAAAGGGATTTTGTC	3.448	0.291	0.519
RsHR026	F: AAGCGTGTCATCAGATCCCAGA R: CATTCTCTCAATGCATAAGATTGAGC	4.241	0.617	0.636
RsSH048	F: TCGTCCGTTATGTATGTTACTCTCA R: TATGCGTACTCCGTAAGACAATGTA	2.138	0.393	0.396
RsSA078	F: AAATGCATCCTAAATGATAAAGTC R: AGAATCGGATCTAAAGGCGATAA	9.517	0.738	0.783
RsSA083	F: GCAATGGTTACAAGACAAGGTTTTA R: CTTCAGATTATTTGCAGCAGCATC	5.655	0.480	0.651
RsSA085	F: GTTATGAGTTTCTGTGGAAAGTTCG R: GACTTTTCTTTGTCGACTGTTCTTC	5.655	0.651	0.661
Average		4.701	0.448	0.565

N_a, Number of different alleles; H_O, observed heterozygosity; H_E, expected heterozygosity.

Table 4 Analysis of molecular variance (AMOVA).

Source of variance	Degree of freedom	Sum of squares	Percentage of variation	*P* value
SSR loci				
Among groups	1	156.145	9.85	<0.001
Among populations within groups	26	549.729	12.12	<0.001
Within populations	1266	3300.923	78.03	<0.001
Total	1293	400.796		
DNA sequencing				
Among groups	3	69.218	8.84	<0.001
Among populations within groups	30	185.164	11.32	<0.001
Within populations	170	567.000	79.84	<0.001
Total	203	821.382		

RESULTS AND DISCUSSION

Nucleotide polymorphism and genetic diversity: In total, 5753 base pairs (bp) of product size from 14 loci (235-577 bp/locus) (Table 2) were sequenced from the total 34 populations (Table 1). The level of polymorphism was extensive in most loci, with an average number of polymorphic sites (*S*) per locus ranging from 7 in *PHYE*, *TBL19* and *MYB29* to 30 in *CRY3*. Overall H_d was similar among the 34 wild radish populations, whereas the levels of nucleotide polymorphism(θ_W and π) differed between loci. The

highest and lowest nucleotide diversities (π) were found in *PHYB* (π = 0.02316) and *LFY* (π = 0.00265), respectively. The most and least polymorphic loci were *CRY3* (θ_W = 0.01688) and *MYB29* (θ_W = 0.00270), respectively, with an average of 0.00687 for all 14 loci. Tajima's *D* and Li's *D**and *F** statistics showed a significant departure from the mutation drift equilibrium in the sequence of *PHYE* (*D* = 3.48344, $P \leq 0.05$; *F** = 2.33488, $P \leq 0.05$), *TBL21* (*D* = 2.30909, $P \leq 0.05$) and *COL4* (*D* = 2.31037, $P \leq 0.05$), whereas other loci do not. The non-significant values, observed in other 11 nuclear genes, indicated the absence of wild radish population changes such as change in size, population expansion, population contraction, and population subdivision (Tajima, 1989; Li and Fu, 1993).

The characteristics of the nine microsatellite loci used in this study are shown in Table 3. Microsatellite marker RsSA078 had the maximum number of different alleles (N_a = 9.517), while RsSH048 had showed the least (N_a = 2.138). Observed heterozygosity (H_O) ranged from 0.262 (Rm19) to 0.738 (RsSA078), with an average of 0.448. Expected heterozygosity (H_E) ranged from 0.452 (Rm19) to 0.783 (RsSA078), with an average of 0.565 for all loci. Average N_a (4.701) was higher than that reported previously for *R.sativus* populations revealed by expressed sequence tags (ESTs) (average N_a = 3) (Wang *et al*., 2007), and lower than those generated by other EST-SSR analyses (average N_a = 7.63) (Bae *et al*., 2015). The genetic diversity parameters determined in the studied populations are presented in Table 1. Average genetic diversity of wild radish populations was high (H_E = 0.563), especially in the Kumamoto population (population 12, H_E = 0.672) (Table 1). Furthermore, the genetic diversity of most samples was relatively high, with the total population having an observed heterozygosity of > 0.3 (average of 0.448), higher than the observed allozyme diversity reported for wild radish in Japan (H_O = 0.223) and Korea (H_O = 0.214) (Huh and Ohnishi, 2001). One probable reason for the high heterozygosity is that wild radish showed insect pollinated outcrossing system, which would increase genetic diversity within population. In addition, according to previous studies (Huh and Ohnishi, 2001; Yamagishi, 2008; Yamane and Ohnishi, 2009), the conceivable gene flow from cultivated radish populations may also contribute to the high level of genetic diversity in natural populations of wild radish in East Asia.

Population structure and genetic variation: The genetic structure of wild radish was determined using STRUCTURE analysis (Figure 1). The most likely number of clusters in the microsatellites data was 2 (ΔK = 72.2768). When *K* = 2, the wild radish populations from the Ryukyu and Taiwan islands clustered together, and populations from three distanced geographical areas/country, *i.e.*, mainland Japan, Korea and the southern regions of China, comprised the other cluster. However, when *K* = 4 predicted by the Evanno *et al*., (2005) was the optimal number of clusters (ΔK = 6.4685), revealed by DNA sequencing data. The populations from the Ryukyu and Taiwan islands were placed in Group Ⅰ, consistent with the microsatellite analysis results. Group Ⅱ was mainly composed of populations from mainland Japan. Group Ⅲ (population 26 from the Tsushima Island and population 31 from Zhejiang province) and group Ⅳ (most Korean populations, Yunnan and Sichuan population) were nested within each of the Korean and Chinese populations. Population structure generally classified the wild radish groups according to their different geographic localities. Genetic mixture in structural analysis reflected the introgression of the nearest cluster/group. Obviously, wild radish in Japan were classified into two genetic clusters (Ryukyu Islands and mainland Japan cluster), which was consistent with the findings of Han *et al.* (2015). Collectively, our results indicate that wild radish populations exhibit considerable genetic differentiation among groups. In addition, although both nuclear DNA sequence and microsatellite analysis can reflect population structure related to geographic distance in wild radish populations in East Asia, but nuclear DNA sequences showed has better resolution.

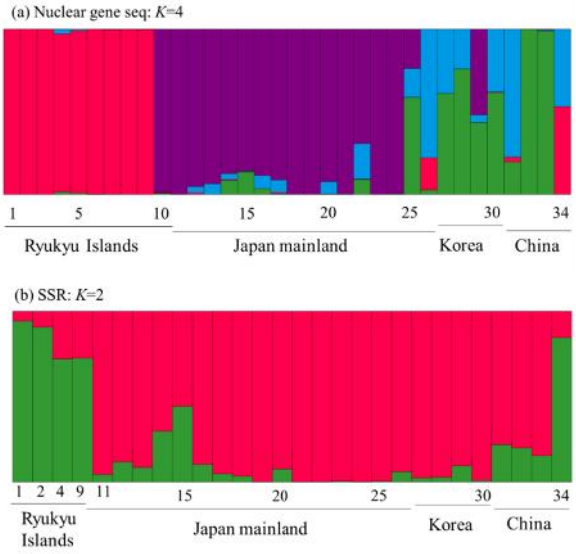

Fig. 1. Genetic structure of the East Asian wild radish populations determined by STRUCTURE analysis based on (a) nuclear genes sequence, (b) microsatellite loci (SSR) analysis. Each bar represents one population. Population codes correspond to those in Table 1.

Population in Taiwan Island exhibited a closer genetic relationship with the Ryukyu Islands wild radish populations than other Japanese populations, consistent with the conclusions of previous research (Han *et al*., 2016). Kuroshio Current (also called Japan Current), beginning at east of the Philippines then flows in a northeastward direction past Taiwan and Japan, was reported to promoted population exchange between the Taiwan and Ryukyu islands (Han *et al*., 2016). Chinese and Japanese wild radish populations diverged genetically, which mainly attributed to separation by oceanic barrier and the limited gene flow or secondary contact after original migration. In other words, geographical ocean barriers likely shaped the heterogeneity of different habitat and produced different natural selection forces to restrict gene flow among populations in divided zones. In addition, climate oscillations and characteristics of pollinator may also contribute to their genetic differentiation. Further studies combing ecological or geographic factors with larger DNA datasets will be helpful to clarify the population demography and speciation processes of wild radish in East Asia.

Genetic differentiation among populations was further analyzed by PCoA (Figure 2), which illustrated clear population genetic divergence. Figure 2b shows two distinct groups, one represented by the Ryukyu and Taiwan island populations, the other by populations from mainland Japan, Korea, and southern regions of China. Thus, as identified by STRUCTURE analysis, our results showed very strong genetic differentiation between the groups. PCoA-based on the 14 nuclear genes (Figure 2a) assigned the populations into four separate groups. Populations from the Ryukyu and Taiwan islands were confined to one group, and populations from mainland Japan tended to form another group, similar to that observed from cluster analysis. And populations from Korea and China did not segregate into distinguishable clusters, the frequent gene flow or historical population migrations were supposed to contribute to the close genetic relationship between Chinese and Korean wild radish populations (Yamane *et al.*, 2005, 2009). A positive relationship was observed between pairwise genetic distance and geographic distance over total 28 populations using SSR analysis, indicating the geographically close populations tended to be genetically similar. The Mantel correlation ($r = 0.245$, $P \leq 0.05$) was lower than those obtained in Korean wild radish populations ($r = 0.52$, $P \leq 0.05$), and higher than those generated in Japanese population ($r = 0.12$, $P \leq 0.05$) by allozyme analysis (Huh and Ohnishi, 2001).

The clustered groups obtained by STRUCTURE analysis provided a logical basis for organizing data for hierarchical AMOVA (Table 4). DNA sequencing was highly consistent with the hierarchical genetic differentiation patterns of the microsatellite loci. Hierarchical AMOVA of DNA sequencing data indicated that most of the changes occurred within populations (79.84%), while the genetic variance among groups and among populations within groups accounted for 8.84% and 11.32% (Table 4). As for microsatellite data, AMOVA analysis showed that intergroup variation (F_{ST}) was 9.85%, while variation among individuals within groups was 12.12% (Table 4). In addition, the most (78.03%) of the variation occurred within populations, which further highlighted the genetic diversity was higher between individuals within populations than between populations. One possible explanation for the high genetic variance within populations is that the self-incompatibility of breeding system of East Asian wild radish, which leads to the prevalent mating among neighboring plants (Huh and Ohnishi, 2001). The other possibility would be the long geographic distance between populations and the short dispersal distance of wild radish's siliques, which limited the gene flow between populations of different countries (Tokunaga and Ohnishi, 1992). Furthermore, gene flow from cultivated populations to natural populations of wild radish may explain the high level of genetic diversity within populations (Yamane *et al*., 2005; Lü *et al*., 2008; Wang *et al*., 2015).

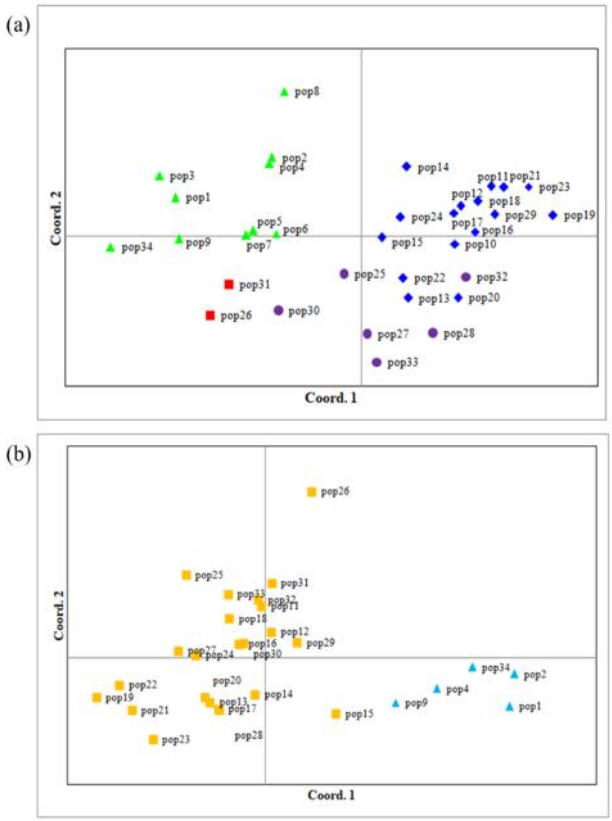

Fig. 2. Principal Coordinate Analysis of DNA sequence (a) and microsatellite loci (b) study of 34 populations of wild radish in East Asia

based on their genetic distances. Colours in the dendrogram correspond to population structure as identified in structure analysis.

Conclusions: The genetic variation in wild radish provided an abundant resource for genetic improvement and genetic diversity identification, which could be conducive to the utilization and improvement of radish germplasm resources. DNA sequencing and microsatellite loci analysis showed that the East Asian wild radish populations were highly genetically diverse at the population level, which may be related to the strong outcrossing system, limited gene flow between populations and possible genetic exchange with local cultivated radish. Our study also revealed a distinct population structure, with genetic distance essentially related to geographic distance, which may be due to various factors, such as oceanic barrier, climate fluctuations, and insects' characteristics. Further research on larger DNA datasets at a larger and more precise geographical scale will be instrumental in revealing the origin and speciation of wild radish in East Asia. This research is currently in progress.

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REFERENCES

Bae, K.M., S.C. Sim, J.H. Hong, K.J. Choi, D.H. Kim and Y.S. Kwon (2015). Development of genomic SSR markers and genetic diversity analysis in cultivated radish (*Raphanus sativus* L.). Hortic. Environ. Biotechnol. 56(2): 216-224. https://doi.org/10.1007/s13580-015-0089-y.

Barnaud, A., J.M. Kalwij, M.A. McGeoch and B.J. van Vuuren (2013). Patterns of weed invasion: evidence from the spatial genetic structure of *Raphanus raphanistrum*. Biol. Invasions, 15: 2455-2465. https://doi.org/10.1007/s10530-013-0465-4.

Earl, D.A. and B. M. VonHoldt (2012). STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv. Genet. Resour. 4: 359-361. https://doi.org/10.1007/s12686-011-9548-7.

Evanno, G., S. Regnaut and J. Goudet (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol. Ecol. 14: 2611-2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x.

Excoffier, L. and H.E.L. Lischer (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. https://doi.org/10.1111/j.1755-0998.2010.02847.x.

Han, Q., H. Higashi, Y. Mitsui and H. Setoguchi (2015). Distinct phylogeographic structures of wild radish (*Raphanus sativus* L. var. *raphanistroides* Makino) in Japan. PloS one.https://doi.org/10.1371/journal.pone.0135132.

Han, Q., H. Higashi, Y. Mitsui and H. Setoguchi (2016). Lineage isolation in the face of active gene flow in the coastal plant wild radish is reinforced by differentiated vernalisation responses. BMC Evol. Biol. 16: 84. https://doi.org/10.1186/s12862-016-0655-7.

Hashida, T., R. Nakatsuji, H. Budahn, O. Schrader, H. Peterka, T. Fujimura, N. Kubo and M. Hirai (2013). Construction of a chromosome-assigned, sequence-tagged linkage map for the radish, *Raphanus sativus* L. and QTL analysis of morphological traits. Breed. Sci. 63(2): 218-226. https://doi.org/10.1270/jsbbs.63.218.

Huh, M.K. and O. Ohnishi (2001). Allozyme diversity and population structure of Japanese and Korean populations of wild radish, *Raphanus sativus* var. *hortensis* f. *raphanistroides* (Brassicaceae). Genes Genet. Syst. 76(1): 15-23. https://doi.org/10.1266/ggs.76.15.

Huh, M.K. and O. Ohnishi (2002). Genetic diversity and genetic relationships of East Asian natural populations of wild radish revealed by AFLP. Breed. Sci. 52(2): 79-88. https://doi.org/10.1270/jsbbs.52.79.

Huh, M.K. and O. Ohnishi (2003). Genetic diversity and relationships among natural and cultivated populations of radish in Korea revealed by RAPD. Genes Genom. 25(2): 119-126.

Jakobsson, M. and N.A. Rosenberg (2007). CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics, 23(14): 1801-1806.https://doi.org/10.1093/bioinformatics/btm233.

Jugulam, M., M. Walsh and J.C. Hall (2014). Introgression of phenoxy herbicide resistance from *Raphanus raphanistrum* into *Raphanus*

sativus. Plant Breed. 133(4): 489-492. https://doi.org/10.1111/pbr.12168.

Kazuhiro, S. (2009). Evaluation of salt tolerance in Japanese wild radishes (*Raphanus sativus* f. *raphanistroides* Makino). Bull. Minamikyushu Univ. Natural Science, 39A: 79-88.

Kong, Q., X. Li, C. Xiang, H. Wang, J. Song and H. Zhi (2011). Genetic diversity of radish (*Raphanus sativus* L.) germplasm resources revealed by AFLP and RAPD markers. Plant Mol. Biol. Rep. 29: 217-223. https://doi.org/10.1007/s11105-010-0228-7.

Li, H. and X. Fu (1993). Statistical tests of neutrality of mutations, Genetics, 133(3): 693-709. https://doi.org/10.1093/genetics/133.3.693.

Librado, P. and J. Rozas (2009). DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics, 25(11): 1451-1452. https://doi.org/10.1093/bioinformatics/btp187.

Lü, N., K. Yamane and O. Ohnishi (2008). Genetic diversity of cultivated and wild radish and phylogenetic relationships among *Raphanus* and *Brassica* species revealed by the analysis of trnK/matK sequence. Breed. Sci. 58(1): 15-22. https://doi.org/10.1270/jsbbs.58.15.

Muminovic, J., A. Merz, A.E. Melchinger and T. Lubberstedt (2005). Genetic structure and diversity among radish varieties as inferred from AFLP and ISSR analyses. J Am. Soc. Hortic. Sci. 130(1): 79-87. https://doi.org/10.21273/JASHS.130.1.79.

Nakatsuji, R., T. Hashida, N. Matsumoto, M. Tsuro, N. Kubo and M. Hirai (2011). Development of genomic and EST-SSR markers in radish (*Raphanus sativus* L.). Breed. Sci. 61(4): 413-419. https://doi.org/10.1270/jsbbs.61.413.

Nei, M. and W.H. Li (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. P Natl. Acad. Sci. USA. 76(10): 5269-5273. https://doi.org/10.1073/pnas.76.10.5269.

Ohsako, T., M. Hirai and M. Yamabuki (2010). Spatial structure of microsatellite variability within and among populations of wild radish *Raphanus sativus* L. var. *hortensis* Backer f. *raphanistroides* Makino (Brassicaceae) in Japan. Breed. Sci. 60(3): 195-202. https://doi.org/10.1270/jsbbs.60.195.

Pritcharda, J.K., X. Wen and D. Falush (2010). Documentation for structure software: Version 2.3. University of Chicago, Chicago, IL.

Rosenberg, N.A. (2004). DISTRUCT: a program for the graphical display of population structure. Mol. Ecol. Notes. 4(1): 137-138. https://doi.org/10.1046/j.1471-8286.2003.00566.x.

Smouse, P.E., J.C. Long and R.R. Sokal (1986). Multiple regression and correlation extensions of the Mantel test of matrix correspondence. Syst. Zool. 35(4): 627-632. https://doi.org/10.2307/2413122.

Tajima, F. (1989). Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics, 123(3): 585-595. https://doi.org/10.1101/gad.3.11.1801.

Tang, J., L. Zhang and C. Peng (2003). Study on mechanism of semen Raphanus in moving qi and removing food retention. Chin. J. Integr. Tradit. West Med. Dig. 11(5): 287-289 (in Chinese).

Tokunaga, T. and O. Ohnishi (1992). Spatial autocorrelation analysis of allozyme variants within local sites of wild radish population. Japanese J. Genet. 67(3): 209-216. https://doi.org/10.1266/jjg.67.209.

Wang, N., J. Hu, R. Ohsawa, M. Ohta and T. Fujimura (2007). Identification and characterization of microsatellite markers derived from expressed sequence tags (ESTs) of radish (*Raphanus sativus* L.). Mol. Ecol. Notes. 7(3): 503-506. https://doi.org/10.1111/j.1471-8286.2006.01635.x.

Wang, Q.B., L. Zhang and P.J. Zheng (2015). Genetic diversity and evolutionary relationship analyses within and among *Raphanus* species using EST-SSR markers. Mol. Breed. 35(2): 62. https://doi.org/10.1007/s11032-015-0261-1.

Watterson, G.A. (1975). On the number of segregating sites in genetical models without recombination. Theor. Popul. Biol. 7(2): 256-276. https://doi.org/10.1016/0040-5809(75)90020-9.

Welles, S. R. and J. L. Funk (2020). Patterns of intraspecific trait variation along an aridity gradient suggest both drought escape and drought tolerance strategies in an invasive herb. Ann. Bot. 127(4): 461-471. https://doi.org/10.1093/aob/mcaa173.

Yamagishi, H. (2008). Assessment of cytoplasmic polymorphisms by PCR-RFLP of mitochondrial orfB region in wild and cultivated radishes (*Raphanus*). Plant Breed. 123(2): 141-144. https://doi.org/10.1046/j.1439-0523.2003.00899.x.

Yamane, K., N. Lü and O. Ohnishi (2005). Chloroplast DNA variations of cultivated radish and its wild relatives. Plant Sci. 168(3): 627-634. https://doi.org/10.1016/j.plantsci.2004.09.022.

Yamane, K., N. Lü and O. Ohnishi (2009). Multiple origins and high genetic diversity of cultivated radish inferred from polymorphism in

chloroplast simple sequence repeats. Breed. Sci. 59(1): 55-65. https://doi.org/10.1270/jsbbs.59.55.

Supplementary information

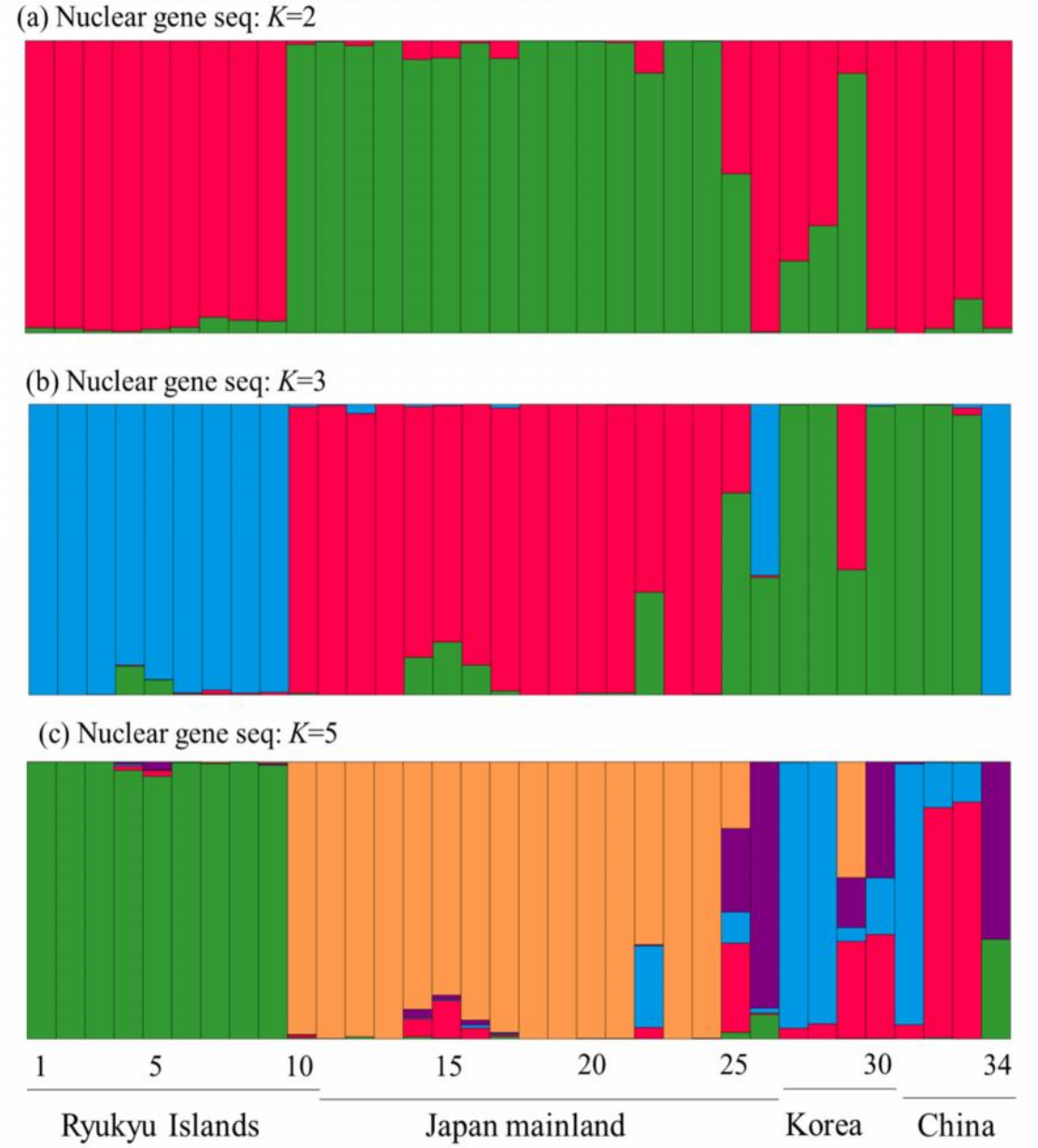

Fig. S1. Population assignment via STRUCTURE analysis using models with $K = 3$, $K = 4$ and $K = 5$, revealed by 14 nuclear genes sequencing. The numbers of each population are shown beneath the bars. Population information is described in detail in Table 1.

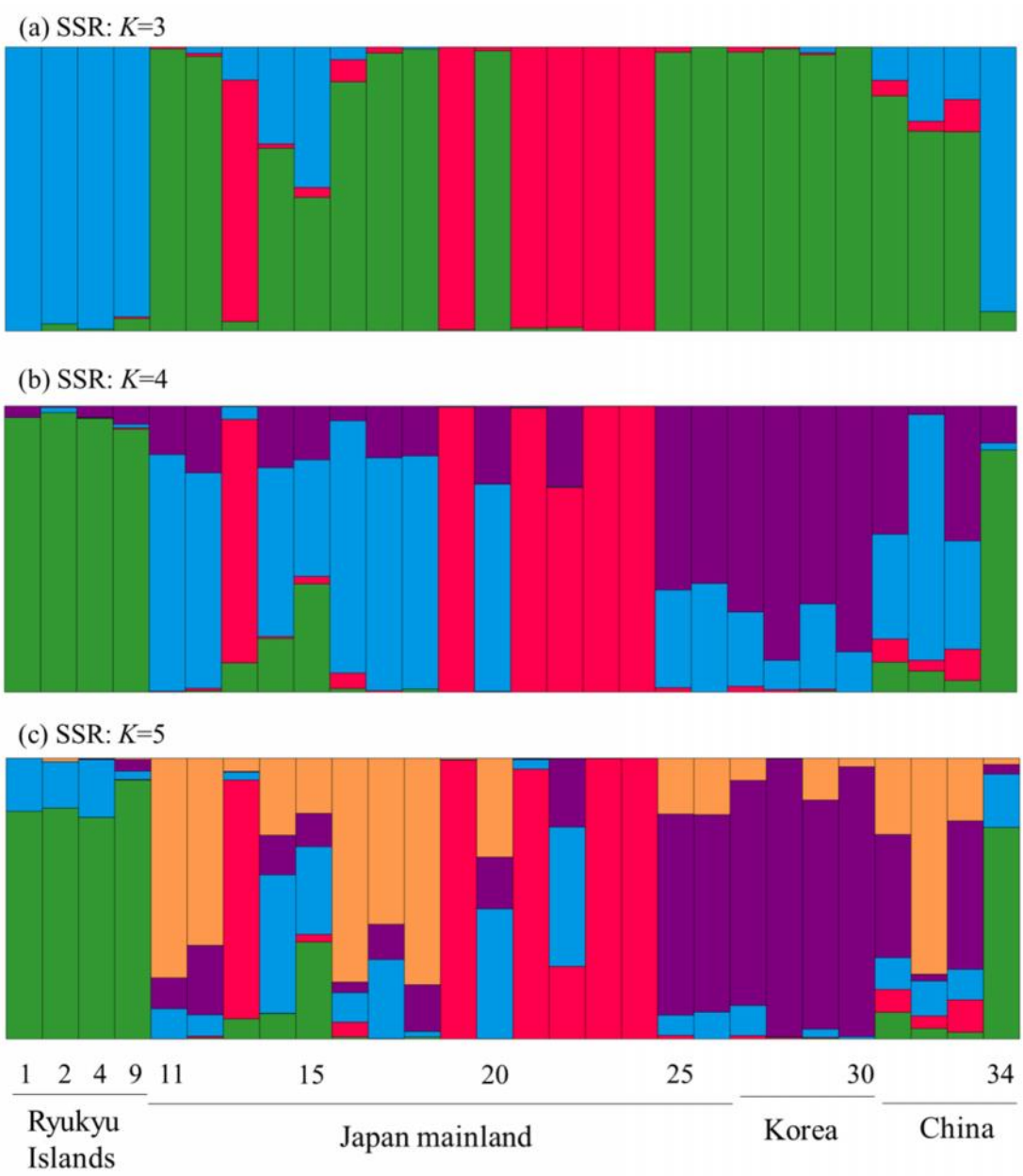

Fig. S2. Population assignment via STRUCTURE analysis using models with K = 3, K = 4 and K = 5, revealed by nine SSR markers. The numbers of each population are shown beneath the bars. Population information is described in detail in Table 1.