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RESEARCH ARTICLE

Genetic divergence of turnip (*Brassica rapa* L. em. Metzg. subsp. *rapa*) inferred from simple sequence repeats in chloroplast and nuclear genomes and morphology

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Abstract Turnip (*Brassica rapa* L. em. Metzg. subsp. *rapa*), which is considered to be a primitive type of cultivated *B. rapa*, is cultivated worldwide as vegetable and fodder. To elucidate the phylogenetic relationships of Eurasian turnips, we examined their morphology and analyzed 6 cpSSR and 18 nuSSR loci in 87 accessions. Examination of seed coat mucilage and leaf hairs revealed existence of geographic distinctions. Two haplogroups were categorized among 12 haplotypes identified by the analysis of cpSSRs and two clusters were detected based on nuSSRs. These haplogroups and clusters were different between eastern and western Eurasia. Although morphological differences were detected between eastern and western Japan, no clear differences of haplotypes and clusters were found in Japanese turnips. Accessions from continental Asia showed various haplogroups and clusters and higher levels of genetic diversity than those from other regions. These results, in addition to previous studies suggest that central Asia is the sole geographic origin of turnips that Asian turnips did not originate as descendants of European turnips, and that almost all Japanese turnips were derived from central Asia.

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Introduction

Turnip (*Brassica rapa* L. em. Metzg. subsp. *rapa*) is a diploid (2n = 20), and an annual or biennial plant cultivated worldwide as vegetable and fodder (Rakow 2004; Hammer et al. 2013). The wild form of *B. rapa* is distributed widely from Europe to central Asia (De-Candolle 1886; Sinskaia 1928; Mizushima and Tsunoda 1967; Prakash and Hinata 1980). De-Candolle (1886) proposed that turnips were cultivated in Europe around 2500–2000 BC and spread from there to Asia (Gomez-Campo and Prakash 1999). According to leaf traits and geographical distribution, Sinskaia (1928) classified turnips into seven geographic groups; (1) Teltow turnips, (2) West European turnips with dissected leaves, (3) Asia Minor and Palestine turnips, (4) Russian turnips of the Petrovsky type, (5) Asiatic Afghanistan turnips with glabrous leaves, (6) Japanese turnips with entire glabrous leaves, and (7) European entire-leaved turnips with pubescent leaves. She proposed that Teltow and Afghanistan turnips represent a primitive group of forms very near to the wild progenitors of turnips, but that the Japanese turnips are advanced forms. Based on these observations, two hypotheses were suggested for the origin of turnips; (1) they originated in Asia or (2) their origin is

polyphyletic, with Asiatic and European turnips having developed independently in Asia and Europe.

In Japan, many landraces have been differentiated. Based on morphological traits such as seed coat mucilage and leaf hairs, the turnips of Japan are generally classified into three groups: (1) Japanese-type, which are characterized by seed coat mucilage and no leaf hairs; (2) European-type, characterized by non-seed coat mucilage and leaf hairs; and (3) intermediate-type, with segregation for seed coat mucilage and leaf hairs (Shibutani and Okamura 1954; Aoba 1958). Geographically, Aoba (1961, 1981a) reported that the European type is distributed in eastern Japan, the Japanese type in western Japan, and the intermediate type mainly in the Chubu district of central Japan. It has been suggested that Japanese-type turnips are closely related to turnips of Afghanistan, on the basis of their similar characters (Sinskaia 1928; Shibutani and Okamura 1954), whereas European-type turnips in eastern Japan, which have the same characters as European turnips, had immigrated by another route, such as northern China or Siberia, and that intermediate-type turnips in central Japan had originated from crosses between European- and Japanese-types (Aoba 1961, 1981a). Schebalina and Sazonova (1985), who classified turnips to three groups such as European, Iraqian and Asian, suggested that European group distribute widely in Eurasia from Europe to Japan.

Molecular markers are powerful tools for genetic analysis, plant systematics, and plant breeding. Among these markers, simple sequence repeat (SSR) markers have been developed for both chloroplast and nuclear genomes and have been used in various types of analysis in many crops (Kalia et al. 2011). Chloroplast SSRs (cpSSRs) show a high level of intraspecific variation and are suitable for evolutionary studies (Provan et al. 2001). Nuclear SSRs (nuSSRs) are also powerful tools for characterizing genetic differentiation in nuclear polymorphism (Wolfe and Liston 1998). Intra- and interspecific genetic relationships using SSRs have been reported in *Brassica* and related species (Flannery et al. 2006; Allender et al. 2007; Louarn et al. 2007; Yamane et al. 2009; Pino Del Carpio et al. 2011).

The purpose of the present study was to characterize the phylogenetic relationships among turnips in Eurasia based on cpSSRs and nuSSRs as well as morphology such as seed coat mucilage and leaf hairs, which have been used to classify turnips. Moreover, we attempted to test the hypothesis that the two groups of turnips in Japan (European-type in the east and Japanese-type in the west) are derived from different origins.

Materials and methods

Plant materials

Eighty-seven turnip cultivars and strains were used in this study, which were kindly provided from National Institute of Agrobiological Sciences (NIAS) in Japan, the Institute of Plant Genetics and Crop Plant Research (IPK) in Germany, the Dutch Crop Genetic Resources Center (CGN) in the Netherlands, and the Vavilov Research Institute of Plant Industry (VIR) in Russia (Table 1). These accessions were classified into six geographical groups (Europe, Russia, continental Asia, eastern Japan, central Japan, and western Japan) (Table 1).

Examinations of seed coat mucilage and leaf hairs

The presence or absence of seed coat mucilage was evaluated by easy distinction method (Yazawa et al. 1986). Twenty seeds per an accession were placed in 2 mL plastic tube containing 1 mL of distilled water for 2–3 h at room temperature, and then dipped into ethyl acetate for 30 s, air-dried for 5 min at room temperature, and dusted with 1 g cerite. A powder covering of cerite was considered as the "presence" and the absence of cerite was considered the "absence" of seed coat mucilage. Accessions with both seed types were also found. The leaf hairs of adult plants were scored at three levels: "absence," "few," and "many."

Genotyping of SSR

Total DNA was extracted from the first true leaf of a single plant of each accession using a CTAB method (Rogers and Bendich 1988). Conserved 20 primer pairs that flank cpSSRs have been selected from several species in Brassicaceae (Chung and Staub 2003; Flannery et al. 2006; Allender et al. 2007). Of these 20 primer pairs, six showed polymorphisms in eight randomly selected turnip accessions of turnips

Table 1 Eighty-seven turnips used in this study

ID	Genotypes	Source[a]	Accession no.	Subgroup	Origin	SCM[b]	LH[c]	cpSSR[d]	nuSSR[e]
eu01	Daisy	CGN	CGN07179	Europe	France	0	2	C	0.961
eu02	Unknown	CGN	CGN10995	Europe	France	0	2	C	0.989
eu03	Unknown	CGN	CGN10996	Europe	France	10	2	C	0.984
eu04	Nijmeegse lange witte	IPK	BRA1019	Europe	Netherlands	0	2	C	0.993
eu05	Lange witte depe	IPK	BRA1022	Europe	Netherlands	0	2	B	0.984
eu06	Globe witte roodkop	IPK	BRA1107	Europe	Netherlands	0	2	C	0.989
eu07	Teltower rubchen	IPK	BRA1700	Europe	Germany	0	2	C	0.994
eu08	Bayrische rube	IPK	BRA2845	Europe	Germany	0	2	B	0.991
eu09	Italiaanse witte roodkop	IPK	BRA1115	Europe	Italy	0	2	B	0.981
eu10	Cavolo verza	IPK	BRA1216	Europe	Italy	0	2	C	0.992
eu11	Rapa di milano a colletto viola	IPK	BRA1894	Europe	Italy	0	2	C	0.993
eu12	Walcowato I. H. A. R.	CGN	CGN06712	Europe	Poland	0	1	C	0.875
eu13	Rogowska	CGN	CGN06714	Europe	Poland	10	2	B	0.985
eu14	Unknown	IPK	BRA341	Europe	Hungary	0	2	C	0.984
ru01	Uralskaya	VIR	387	Russia	Russia	0	1	E	0.989
ru02	Grobovskaya	VIR	821	Russia	Russia	0	2	A	0.937
ru03	Blue local	VIR	952	Russia	Russia	0	2	C	0.992
ru04	Local from sakhalin	VIR	958	Russia	Russia	0	2	A	0.979
ru05	Local	VIR	974	Russia	Russia	0	2	B	0.992
ru06	White ball	VIR	1059	Russia	Russia	0	2	C	0.817
ru07	Yellow	VIR	1071	Russia	Russia	0	2	B	0.988
ru08	Local green head	VIR	1387	Russia	Russia	0	2	B	0.991
ru09	Namanganskaja	IPK	BRA1719	Russia	Russia	0	2	B	0.908
ru10	Kruglyj	IPK	BRA2333	Russia	Russia	0	2	B	0.976
ru11	Esti naeris	IPK	BRA2487	Russia	Russia	0	2	C	0.969
ru12	Hibinskij (Osterzundomskij)	IPK	BRA2594	Russia	Russia	0	2	C	0.985
ru13	Unknown	IU	–	Russia	Russia	0	2	B	0.993
ru14	Unknown	IU	–	Russia	Russia	0	2	C	0.992
ca01	Unknown	IPK	BRA1901	Continental Asia	Iraq	100	2	C	0.825
ca02	Kana pohkali	IPK	BRA1224	Continental Asia	Georgia	0	2	B	0.921
ca03	Belaja redka	IPK	BRA1716	Continental Asia	Georgia	0	2	C	0.891
ca04	Turneps skorospelka	IPK	BRA1892	Continental Asia	Georgia	0	2	C	0.992
ca05	Unknown	CGN	CGN20735	Continental Asia	Uzbekistan	0	2	B	0.852
ca06	Unknown	CGN	CGN20736	Continental Asia	Uzbekistan	0	1	C	0.990
ca07	Unknown	CGN	CGN20738	Continental Asia	Uzbekistan	15	1	C	0.561
ca08	Unknown	IPK	BRA2291	Continental Asia	Afghanistan	0	1	F	0.374

Table 1 continued

ID	Genotypes	Source[a]	Accession no.	Subgroup	Origin	SCM[b]	LH[c]	cpSSR[d]	nuSSR[e]
ca09	PAK-10419	NIAS	JP86234	Continental Asia	Pakistan	60	0	G	0.734
ca10	PAK-10449	NIAS	JP86236	Continental Asia	Pakistan	85	1	C	0.314
ca11	Salgam	CGN	K8266	Continental Asia	Tadzhikistan	0	2	H	0.986
ca12	Pusa chandrina	CGN	CGN06711	Continental Asia	India	45	2	J	0.993
ca13	Yuen qin cai	NIAS	JP76706	Continental Asia	China	55	2	L	0.371
ca14	Kangwha native turnip	NIAS	JP86238	Continental Asia	Korea	95	2	A	0.592
ca15	Kouka-kabu	IU	–	Continental Asia	Korea	30	2	J	0.334
ej01	Oono-beni-kabu	NIAS	JP26845	Eastern Japan	Hokaido	90	1	J	0.041
ej02	Tsutsui-kabu	IU	–	Eastern Japan	Aomori	90	1	L	0.019
ej03	Kuretsubo-kabu	IU	–	Eastern Japan	Iwate	0	0	K	0.008
ej04	Ochibo-kabu	NIAS	JP26825	Eastern Japan	Iwate	10	0	J	0.019
ej05	Atsumi-kabu	NIAS	JP26827	Eastern Japan	Yamagata	0	2	L	0.005
ej06	Hijiori-kabu	NIAS	JP46078	Eastern Japan	Yamagata	0	2	J	0.018
ej07	Terauchi-kabu	NIAS	JP46079	Eastern Japan	Yamagata	0	2	K	0.026
ej08	Miyazawa-kabu	NIAS	JP46080	Eastern Japan	Yamagata	0	2	L	0.027
ej09	Goboono-kabu	NIAS	JP46083	Eastern Japan	Yamagata	10	1	D	0.011
ej10	Jinego-kabu	NIAS	JP46084	Eastern Japan	Yamagata	95	1	J	0.012
ej11	Tooyama-kabu	NIAS	JP46085	Eastern Japan	Yamagata	25	0	J	0.037
ej12	Oguni-kabu	TU	–	Eastern Japan	Yamagata	0	2	J	0.031
ej13	Toyosato-kabu	NIAS	JP26823	Eastern Japan	Fukushima	0	2	C	0.974
ej14	Tateiwa-kabu	NIAS	JP26824	Eastern Japan	Fukushima	0	2	L	0.009
ej15	Oo-naga-kabu	NIAS	JP26813	Eastern Japan	Tohoku	0	0	K	0.009
ej16	Kanamachi-ko-kabu	NIAS	JP26852	Eastern Japan	Tokyo	0	1	L	0.009
cj01	Yorii-kabu	NIAS	JP26828	Central Japan	Nigata	100	0	K	0.041
cj02	Kanazawa-ao-maru-kabu	NIAS	JP26797	Central Japan	Ishikawa	0	0	I	0.009
cj03	Kodakari-kabu	NIAS	JP26846	Central Japan	Fukui	95	0	K	0.007
cj04	Kida-ao-kabu	NIAS	JP26847	Central Japan	Fukui	40	0	K	0.009
cj05	Suwa-beni-kabu-na	NIAS	JP26795	Central Japan	Nagano	40	0	L	0.007
cj06	Nozawa-na	NIAS	JP26796	Central Japan	Nagano	0	0	L	0.032
cj07	Gensuke-kabu	NIAS	JP26854	Central Japan	Nagano	15	0	I	0.187
cj08	Yoshino-kabu	NIAS	JP26855	Central Japan	Nagano	0	1	L	0.022
cj09	Kiso-beni-kabu	NIAS	JP26856	Central Japan	Nagano	10	2	K	0.011
cj10	Inekoki-na	NIAS	JP26857	Central Japan	Nagano	0	2	L	0.170
cj11	Fukushima-na	NIAS	JP26859	Central Japan	Nagano	70	1	L	0.014
cj12	Hida-beni-kabu	NIAS	JP26862	Central Japan	Gifu	10	2	K	0.012
cj13	Narusawa-na	NIAS	JP26861	Central Japan	Yamanashi	100	0	L	0.202
wj01	Hino-na	NIAS	JP26866	Western Japan	Shiga	100	0	J	0.115
wj02	Koizumi-kabu	NIAS	JP202058	Western Japan	Shiga	95	0	I	0.085
wj03	Yajima-kabu	NIAS	JP26874	Western Japan	Shiga	100	0	J	0.010

Table 1 continued

ID	Genotypes	Source[a]	Accession no.	Subgroup	Origin	SCM[b]	LH[c]	cpSSR[d]	nuSSR[e]
wj04	Yurugi-aka-maru-kabu	NIAS	JP43252	Western Japan	Shiga	95	1	A	0.145
wj05	Honbeni-aka-maru-kabu	NIAS	JP43263	Western Japan	Kyoto	100	0	K	0.197
wj06	Maizuru-kabu	NIAS	JP43254	Western Japan	Kyoto	95	2	K	0.019
wj07	Suguki-na	NIAS	JP26869	Western Japan	Kyoto	100	0	K	0.085
wj08	Shougoin-oo-maru-kabu	NIAS	JP43262	Western Japan	Kyoto	95	0	J	0.012
wj09	Tennouji-kabu	NIAS	JP26870	Western Japan	Ohsaka	95	0	L	0.014
wj10	Imaichi-kabu	NIAS	JP26787	Western Japan	Nara	100	0	L	0.007
wj11	Yonago-aka-kabu	NIAS	JP26890	Western Japan	Totori	100	0	J	0.007
wj12	Hatata-kabu	NIAS	JP26892	Western Japan	Shimane	15	2	C	0.011
wj13	Shougatsu-kabu	NIAS	JP26889	Western Japan	Shimane	10	2	L	0.793
wj14	Tsuda-kabu	NIAS	JP26883	Western Japan	Shimane	95	0	L	0.008
wj15	Takehisa-kabu	NIAS	JP26884	Western Japan	Yamaguchi	100	0	L	0.030

[a] *IPK* Institute of Plant Genetics and Crop Plant Research, *NIAS* National Insitute of Agrobiological Sciences, *CGN* Dutch Crop Genetic Resources Center, *VIR* Vavilov Research Institute of Plant Industry, *IU* Iwate University, *TU* Tohoku University

[b] Percentage of mucilaginous seed

[c] Glabrous (0), Intermediate (1) or Pubscent (2) for leaf hairiness

[d] Haplotypes defined by cpSSR

[e] The proportion of the membership coefficient (*Q*) for the cluster 1 on the STRUCTURE analysis (K = 2) of nuSSR

and were used for subsequent analysis (Table 2). For nuSSRs analysis, 18 primer pairs were selected from a total of 30 pairs derived from the BRMS primer set (Suwabe et al. 2006), based on the linkage group, amplification strength, and polymorphism, and covered eight of the ten linkage groups of *B. rapa* (Table 2). All cpSSR and nuSSRs loci were amplified by PCR. Each reactions was performed in 10 μL of total volume containing 10 ng of template DNA, 10 mM Tris–HCl (pH 8.3), 20 mM KCl, 1.5 mM $MgCl_2$, 100 mM dNTP, 0.05 mM forward primer with fluorescent label (HEX, FAM, VIC or TET), 0.05 mM reverse primer and 0.1 unit of Taq DNA polymerase. The thermal cycling conditions were 5 min at 94 °C, followed by 30 cycles of 94 °C for 30 s, 60 °C for 30 s and 72 °C for 1 min, and then 72 °C for 5 min. One microliter PCR product was diluted to 20 μL with Hi-Di Formamide and added 0.2 μL ROX 500 internal size standard (Applied Biosytstems) was added before loading on an ABI 310 DNA sequencer (Applied Biosystems) according to the manufacturer's instructions. Trace files from the sequencer were then scored using GeneMapper v3.5 software (Applied Biosystems).

Statistical analysis

Each accession was assigned a haplotype based on the combination of allelic information from six cpSSR loci. The relationships among haplotypes were analyzed with a median-joining network (Bandelt et al. 1999) using the NETWORK computer program (www.fluxus-engineering.com). Gene diversity *H* (Nei 1973) within geographic groups was estimated for both cpSSRs and nuSSRs using GenAlEx version 6 (Peakall and Smouse 2006). The software STRUCTURE 2.2 (Pritchard et al. 2000) was used to identify the population structure. The program was run using the admixture model with 200,000 replicates for burn-in and 200,000 replicates for analysis. The most likely number of *K* was estimated by calculating ΔK to identify the top level in the hierarchical structure (Evanno et al. 2005). Principal coordinate analysis of nuSSR data was performed with GenAlEx v. 6 program (Peakall and Smouse 2006). Neighbor-joining trees (Saitou and Nei 1987) based on Nei's genetic distance (Nei et al. 1983) were constructed using Populations 1.2.30beta (Langella 2007).

Table 2 Primer sequences, number of alleles and gene diversity *H* for cpSSR and nuSSR

Genome	Marker name	Location	Forward primers	Reverse primers	No. of alleles	Gene diversity *H*
Chloroplast (cpSSR)	ccSSR8	*ycf3*	TTGATCTTTACGGTGCTTCCTCTA	TCATTACGTGCGACTATCTCC	2	0.045
	Chloro39	Unknown	CATGAATTAGTAACTGCATCC	TCCTATTCATGGGGATTCCG	2	0.500
	MF2	*rpl16*	GGTTCCGTCGTTCCCATCGC	CATAATAATTAGATAAATCTGTTCC	2	0.045
	MF4	*psaA-ycf3*	CGGATCTATTATGACATATCC	GAAATATGAATACACTAGATTAGG	4	0.690
	MF7	*trnM-atpE*	CGGCAGGAGTCATTGGTTCAAA	GATTTTGTAACTAGCTGACG	4	0.522
	MF8	*rbcL-accD*	AATAACAATAGATGAATAGTCA	GGGCCGTTATGCTCATTACG	2	0.023
Nuclear (nuSSR)	BRMS-042	A01	GGATCAGTTATCTGCACCACAA	TCGGAATTGGATAAGAATTCAA	5	0.726
	BRMS-050	A01	AACTTTGCTTCCACTGATTTTT	TTGCTTAACGCTAAATCCATAT	12	0.800
	BRMS-158	A01	AAAAGACAAAACCATCCAAACACTA	AAGCCTTTTTGAACTTCTCTGTGAT	8	0.693
	BRMS-303	A01	ACTCAACAACCGAACAAGAAAAACA	CGGTAGAGAACAGAGGAAGCCTAAG	4	0.570
	BRMS-333	A03	ACTCTTCAGTTAGAAAAGCTAAATCT	CTCTTTCTGTGTGCTTACCGTTTTC	13	0.715
	BRMS-005	A04	ACCTCCTGCAGATTCGTGTC	GCTGACCTTTCTTACCGCTC	15	0.789
	BRMS-144	A04	CCATCTGTTGAGAGCTTCTTCTTC	AAGTTCATTTGCTCCGATGC	10	0.777
	BRMS-298	A04	CCACTGTTTTATGACTCCAGTGCTT	TGACCTGGTGAAGTAGTTGTCTCGT	6	0.550
	BRMS-051	A05	GGCCAAGCCACTACTGCTCAGA	GCGGAGAGTGAGGGAGTTATGG	9	0.688
	BRMS-037	A06	CTGCTCGCATTTTTTATCATAC	TACGCTTGGGAGAGAAAACTAT	8	0.599
	BRMS-056	A06	GATCAAGGCTACGGAGAGAGAG	CGTGACGCTAGAGTAATCGAGT	17	0.831
	BRMS-155	A06	AAGTTGAAACATTTGTCTCCTTTCA	GCCGGAAAGTGAAAGACTAAGATA	3	0.453
	BRMS-317	A06	GCAAAACAGATGAAGAAGATGGATG	GAGCTTATTGGGAGGCTTAACTCTG	5	0.672
	BRMS-033	A07	GCGGAAACGAACACTCCTCCCATGT	CCTCCTTGTGCTTTCCCTGGAGACG	15	0.840
	BRMS-019	A09	CCCAAACGCTTTTGACACAT	GGCACAATCCACTCAGCTTT	7	0.713
	BRMS-186	A09	ACAAGACACATGGAACTCTTATGC	ATATTACCAATGACCCCACTATTCA	4	0.504
	BRMS-195	A10	AATACTTTCTGAAGTTGTCCGCTAA	AACCTACGCAAGATGCTTCTACTT	13	0.829
	BRMS-276	A10	GACCGTTTTGCATTTTAAGAGCATT	TCACCACCAGTATCTTCAACAATCA	3	0.619

Results

Seed coat mucilage and leaf hairs

All turnip accessions from Europe and Russia showed absence of seed coat mucilage and many leaf hairs (Table 1, Fig. 1A, B). In those from continental Asia, three types of seed mucilage (absence, presence, and segregation) were found, and three types of leaf hairs were also found, although most accessions were characterized by many leaf hairs. Especially, such phenomena were also found in accessions in central Asia. Japanese accessions classified to the three geographical regions showed distinct geographical distributions. In eastern Japan, accessions without seed coat mucilage and/or with leaf hairs were more frequent than other types, whereas in western Japan those with seed coat mucilage and/or without leaf hairs were the most frequent. The characteristics of accessions from central Japan were intermediate between those from western and eastern Japan (Fig. 1A, B).

In general, turnips lacking seed coat mucilage tended to possess leaf hairs and those with mucilage tended to lack leaf hairs. A Chi square test of independence using the 87 accessions showed that these two characters were not independent ($\chi 2 = 32.226$, $p = 1.75 \times 10^{-6}$).

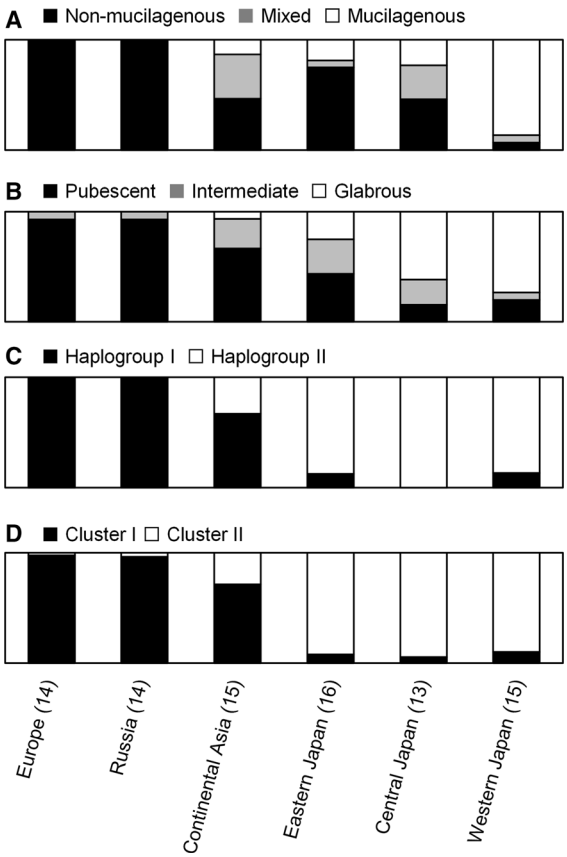

Fig. 1 Frequency distributions on 6 geographical subgroups for the seed coat mucilage (**A**), leaf hair (**B**), haplogroups of cpSSR (**C**), and overall proportion of the membership coefficient (Q) on STRUCTURE analysis ($K = 2$) (**D**). No. of accessions

Haplotypic polymorphism in cpSSR data

Analysis of six cpSSRs revealed a wide range of diversity among the turnips accessions. The numbers of alleles at cpSSR loci ranged from 2 to 4, and gene diversity H values ranged from 0.045 to 0.690 (Table 2). A total of 12 haplotypes were detected among the 87 accessions (Table 3). The median-joining network analysis showed that the haplotypes could be roughly categorized into two groups, haplogroup I (haplotypes A–F) and haplogroup II (haplotypes G–L) (Table 3, Fig. 2). All accessions from Europe and Russia had haplotypes belonging to haplogroup I, whereas almost all accessions from Japan had the haplotypes belonging to haplogroup II (Figs. 1C, 2, Table 1). The haplotypes of both haplogroups were detected in turnips of continental Asia, and also in those of central Asia. The gene diversity H of the geographical groups ranged from 0.073 to 0.363, with the highest value found in turnips from continental Asia and the lowest value in those from Europe (Table 4).

Phylogenetic analysis by nuSSR polymorphism

The number of alleles at 18 nuSSR loci in the 87 accessions varied from 3 to 17 per locus (Table 2). The gene diversity H ranged from 0.453 to 0.840 (Table 2). The genetic structure of the accessions based on STRUCTURE analysis is shown in Fig. 3. The model with $K = 2$ was selected as the most likely number of genetic clusters, because this model showed the highest ΔK [27.64 ($K = 2$), 0.84 ($K = 3$), and 0.12 ($K = 4$)]. All accessions from Europe and Russia were assigned to cluster 1, whereas all but two (Tomisato-kabu, Shogatsu-kabu) from Japan were assigned to cluster 2 (Figs. 1D, 3). Many accessions from continental Asia were assigned to cluster 1 and

Table 3 Chloroplast haplotypes based on 6 cpSSR loci in 87 landraces

Haplotype	Allele size (bp)						No. of accessions
	ccSSR8	MF2	MF4	MF7	MF8	Chloro39	
Haplogroup I							
A	234	172	140	150	243	84	4
B	234	172	141	150	243	84	12
C	234	172	142	150	243	84	23
D	234	172	142	150	243	83	1
E	234	172	142	149	243	84	1
F	235	173	142	150	243	84	1
Haplogroup II							
G	235	173	140	154	242	84	1
H	234	172	141	155	243	84	1
I	234	172	140	155	243	83	3
J	234	172	141	155	243	83	12
K	234	172	142	155	243	83	11
L	234	172	143	155	243	83	17

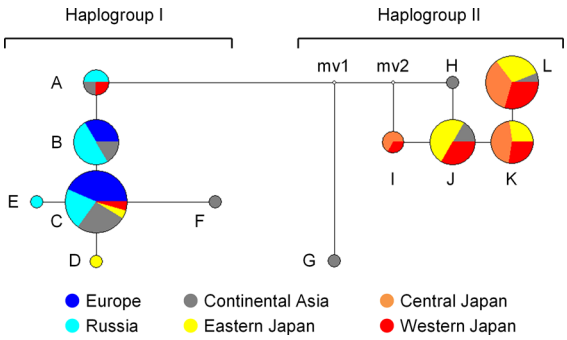

Fig. 2 Median-joining network with branch lengths proportional to the number of mutational steps between 12 chloroplast haplotypes. The size of pie charts is proportional to the frequency of haplotype and the pie charts are separated by the *colors*, which mean the geographical subgroups. The mv1 and mv2 mean median vector

some to cluster 2. In a principal coordinate analysis, the first two coordinates explained 19.7 % (14.1 % by PC1 and 5.6 % by PC2) of the total variation (Fig. 4). All accessions from Europe and Russia were scattered in the second and third quadrants, whereas almost all Japanese accessions were scattered in the first and fourth quadrants. In the Japanese accessions no geographical differences (eastern vs. western) were detected. Accessions from continental Asia showed a wide distribution. The phylogenetic trees showed the similar results (Fig. 5). The gene diversity H of the geographical groups ranged from 0.562 to 0.691, higher than those for the cpSSR markers. Despite the small differences among geographic groups, the highest value was found for continental Asia (Table 4).

Discussion

Our morphological examination of 87 landraces of turnips showed geographical differences in variation of seed coat mucilage and leaf hairs. To our knowledge, this is the first report characterizing the seed coat mucilage of extensive accessions from Europe, Russia, and continental Asia. Our results reveal that European and Russian turnips had no genetic variations in comparison with other regions. Sinskaia (1928) indicated that turnips from Europe, Russia, and Asia Minor have pubescent leaves, whereas those from Afghanistan and Japan have glabrous leaves, with turnips from Afghanistan sometimes showing pubescent leaves. However, she investigated a limited number of Japanese turnips. Via examination of seed coat mucilage and leaf characters in extensive Japanese and some European accessions, Shibutani and Okamura (1954) and Aoba (1961, 1981a) reported that Japanese turnips were classified into three types, Japanese-type (with presence of seed mucilage and glabrous leaves, and distributed in western Japan), European-type (with absence of seed mucilage and

Table 4 Gene diversity *H* of each subgroup for cpSSR and nuSSR

Genome	Europe	Russia	Continental Asia	Eastern Japan	Central Japan	Western Japan
cpSSR	0.073	0.134	0.363	0.178	0.111	0.213
Standard deviation	0.073	0.108	0.084	0.113	0.111	0.124
nuSSR	0.643	0.641	0.691	0.631	0.603	0.562
Standard deviation	0.047	0.030	0.029	0.043	0.039	0.040

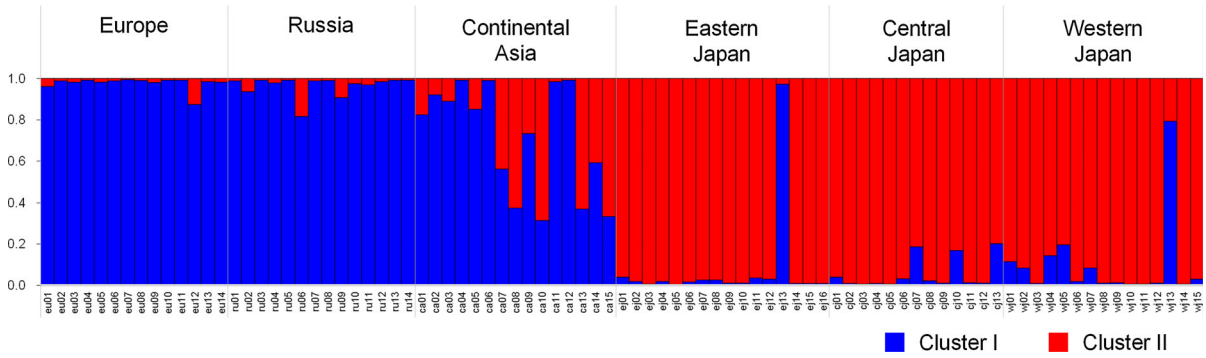

Fig. 3 Proportions of the membership coefficient (*Q*) of 87 turnips with six geographical subgroups for the clusters inferred from STRUCTURE analysis (Pritchard et al. 2000). The optimal value of *K* was determined by the highest ΔK (Evanno et al. 2005). The model with K = 2 produced the highest ΔK

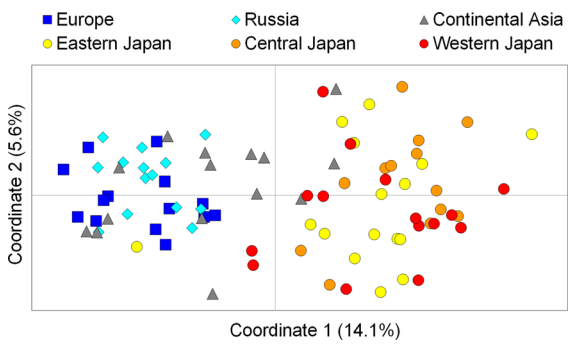

Fig. 4 Scatter diagrams of 87 turnip landraces on the first and second axis of principal coordinate analysis. The *colors* indicate six geographical subgroups

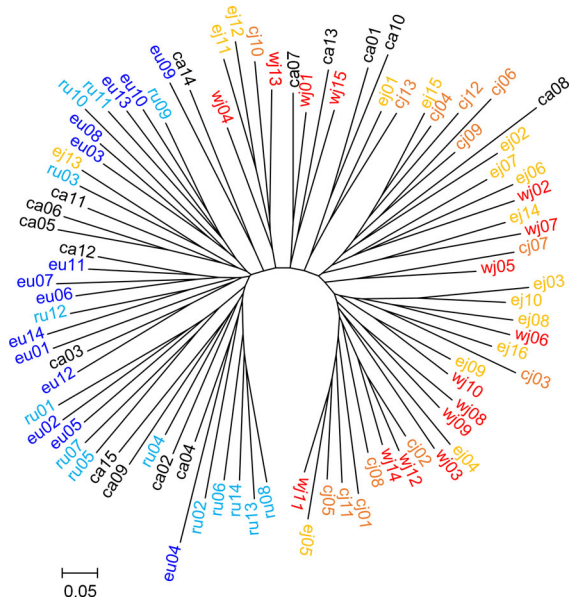

Fig. 5 Neighbor-joining (NJ) tree of 87 turnip landraces based on D_A genetic distances (Nei et al. 1983). Landraces of six geographical subgroups are also highlighted according to the *colors* given in Fig. 4

pubescent leaves, and distributed in western Japan) and intermediate-type. Our results agree with these previous studies.

Considering that no accessions with seed coat mucilage were found except in western Japan, Aoba (1981b) speculated that the seed coat mucilage character of *B. rapa* occurred with the establishment

of *B. rapa* L. em. Metzg. subsp. *niposinica* (Bailey) Hanelt in western Japan and was introduced to turnips (subsp. *rapa*). However, our results reveal that turnips with seed coat mucilage are present among accessions from central Asia, indicating that two types of seed coat mucilage were already present in turnips before their introduction to Japan.

As mentioned in the introduction, two hypotheses for the origin of turnips have been proposed: (1) the origin is monophyletic, with turnips having evolved either in Europe (De Candolle 1886; Prakash and Hinata, 1980) or in Asia (Sinskaia 1928) and (2) the origin is polyphyletic, with turnips having been cultivated in both Europe and Asia (Sinskaia 1928). Sinskaia (1928) reported that Teltow turnips (cultivated in Europe) and Afghan turnips had characters near those of the wild progenitor. Takuno et al. (2007) considered that turnips may be a primitive type of cultivated *B. rapa* that originated in central Asia or in Europe and spread to east Asia, Europe, and India. Our results of SSR analyses showed the presence of two distinct groups of haplotypes and population structures in Eurasian turnips, eastern and western, which are consistent with the results of a phylogenetic analysis of *B. rapa* using AFLP (Zhao et al. 2005; Takuno et al. 2007). Turnips in continental Asia included both types of the cpSSR haplogroup, and showed a higher level of genetic diversity than those in other regions. Wild form of *B. rapa* is distributed from Europe to central Asia (De-Candolle 1886; Mizushima and Tsunoda 1967; Gomez-Campo and Prakash 1999). These results suggest that central Asia is the sole geographical origin of turnips or one of its primary centers of origin. To correctly determine whether turnips are of monophyletic or polyphyletic origin, investigation of many accessions in central Asia and identification and comparison of the genes controlling swollen root formation in European and Asian turnips are needed.

Japan is considered as a center of turnip varietal development (Nishi 1980). Based on morphological examination, Japanese turnips are roughly divided into two types, Japanese- and European-type (Shibutani and Okamura 1954; Aoba 1961, 1981a; this study). Aoba (1961, 1981a) speculated that Japanese-type turnips distributed in western Japan migrated from Afghanistan to Japan via China or the Korean peninsula, whereas European-type turnips in eastern Japan came by way of Siberia or northern China (having originated in Europe) to eastern Japan. We expected that there are genetic differences in Japanese turnips between eastern and western Japan, and that eastern Japanese and European turnips have common genetic background. However, the analyses of cpSSR and nuSSR revealed no genetic divergence were found between eastern and western Japan, indicating that turnips in eastern Japan are different from European ones. Given that turnips in continental Asia (especially central Asia) show large genetic variations, almost all Japanese turnips are considered to be derived from central Asia. Among turnips from central Asia, those containing the chloroplast of haplogroup II and the nuclear genome of cluster 2 came to Japan. Those lacking seed mucilage and possessing leaf hairs migrated to eastern Japan and those possessing seed coat mucilage and lacking leaf hairs migrated to western Japan. There were two accessions that belonged to cluster 1, and of these one accession 'Shougatsu-kabu' grows spontaneously in field and was considered to be primitive. This suggests that turnips migrated multiple times from the Asia Continent to Japan. We examined a limited number of accessions in central Asia, including only one accession from Afghanistan. More extensive surveys of turnips in central Asia, in particular Afghanistan and neighboring countries, may reinforce our conclusion.

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