

Genetic diversity among “Qamgur” varieties in China revealed by SSR markers

Jiatong Gao · Ning Li · Zhengying Xuan · Wencai Yang 

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Abstract “Qamgur”, a type of turnip (*Brassica rapa* ssp. *rapa*), is a special food of the Uyghur people living in Xinjiang, the northwest of China. In the present study, SSR markers developed for various *Brassica rapa* subspecies were tested for their transferability to turnip and used to analyze genetic diversity in “Qamgur” and other Chinese turnip varieties. A total of 18 “Qamgur” and 12 other varieties were genotyped with 170 SSR markers developed for *Brassica rapa* ssp. 80% markers could amplify products, out of which 52 produced scorable bands. The 52 markers generated 153 alleles and 39 detected polymorphisms in 30 varieties. The average Nei’s genetic distance ranged from 0.156 to 0.349 in the whole collection, but the genetic variation was narrower in “Qamgur” varieties than in other varieties. Phylogenetic analysis showed that the 18 “Qamgur” varieties were always clustered together and distinct from the other varieties, which was

supported by the data of principle component and population structure analyses. These results suggest that the SSR markers developed from other *Brassica rapa* subspecies could be used in genetic study in turnip and “Qamgur” will be valuable resources for developing new cultivars.

Keywords *Brassica rapa* ssp. *rapa* · Genetic diversity · Population structure · SSR markers

Introduction

Turnip (*Brassica rapa* ssp. *rapa*) has been cultivated over 2100 years since its first introduction into China during the Western Han Dynasty period and is probably the ancestor of Chinese cabbage (*B. rapa* ssp. *pekinensis*) (Cao 1996). China is also the center of origin of Chinese turnip rape (ssp. *oleifera*) (Li 1981), which is a unique turnip rape (oil type). Turnip is used to be grown as a popular vegetable in many regions in China with various Chinese names such as Manjing, Yuancaitou, Yuangen, Pancai, Buliuke, etc. However, with the introduction of new types and varieties of vegetables as well as the change in cultivation system, the planting area of turnip has been significantly reduced in recent years (Wang et al. 2015).

In Xinjiang Uygur Autonomous Region, the northwest of China, turnip known as “Qamgur” in Uygur is one of the popular and favorite foods of the Uygurs (Zhang et al. 2009; Ma et al. 2016). The Uygurs

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compare “Qamgur” to “renshen” (the dry root of *Panax ginseng* C.A. Mey.), a worldwide well-known traditional Chinese medicine popularly known as “ginseng”. But eating “Qamgur” does not cause inflammation while eating “renshen” could. Thus, most people in southern Xinjiang even consider “Qamgur” as an essential daily food (Ma et al. 2016). The roots and seeds of “Qamgur” are also commonly used in Uygur medicine (Aikebaierjiang et al. 2011; Xie et al. 2014). With the vigorous development of the primary industry in Xinjiang, the production area of turnip is actually increasing year by year (Ma et al. 2016). Since Xinjiang is located in the center of Eurasia, which is close to several centers where plant species originate, it has been reported that germplasm of several plant species in this region are somewhat genetically different from those in other parts of China (Wang et al. 2013, 2016). Although “Qamgur” belongs to the same species of turnip, its shape and flavor are different from other turnips (Xie et al. 2014; Ma et al. 2016). It remains unclear whether varieties of “Qamgur” in Xinjiang are genetically different from turnip varieties grown in other provinces in China.

Previous studies suggest that turnip has a higher genetic variation than other crops within *B. rapa* (McGrath and Quiros 1992; Persson et al. 2001). The genetic variation within 31 accessions including varieties, elite stocks, landraces and older turnip of slash-and-burn type collected from Nordic area was 18.7% based on 26 alleles of nine isozyme loci (Persson et al. 2001). According to 34 morphological and agronomical traits, a panel of 120 landraces consisting of turnips, turnip greens, and turnip tops collected from northwestern Spain could be classified into five clusters, suggesting that there were enough variability in these landraces to differentiate among appropriate populations for each one of the distinct crops (Padilla et al. 2005). Similar studies on 15 Chinese turnip accessions and 11 Iranian turnip accessions based on four and 16 morphological traits, respectively, also revealed high variability among accessions (Sun et al. 2007; Moghadam et al. 2014). Analysis of three European winter *B. rapa* (Turnip rapa) open-pollinated cultivars using 16 SSR markers indicated that genetic diversity within cultivars was also high (Ofori et al. 2008). However, most studies on

genetic diversity of turnip were based on morphological traits or few molecular markers.

Various molecular markers including random amplified polymorphic DNA, restriction fragment length polymorphisms (RFLP), amplified fragment length polymorphisms (AFLP), simple sequence repeats (SSR), and single nucleotide polymorphism (SNP) have been developed and used for diverse purposes in *Brassica* (Ahmad and Khalil 2013). Although large-scale discovery combining high-throughput genotyping of SNPs have shown its power in genetic study in *B. rapa* (Cheng et al. 2016a, b; Tanhuanpää et al. 2016), high costs and technical or equipment demands is still a major obstacle to breeders particularly in the developing countries to adopt this approach. On the contrary, the genotyping of SSR is relatively easy and inexpensive with a simple PCR and electrophoresis. SSR can also be analyzed with high throughput technologies and in large-scale multiplexing (Tsonev et al. 2015; Li et al. 2017). Therefore, this marker type is still an attractive tool for genetic diversity analysis and marker-assisted selection in *B. rapa*. To date, a large number of SSR markers have been discovered in the expressed sequence tags (EST) and the genomic DNA sequences in *B. rapa* (Suwabe et al. 2002; Lowe et al. 2004; Choi et al. 2007; Hobson and Rahman 2016). In addition, efforts have also been put on discovering SSR markers in ESTs from turnip (Wu et al. 2012; Luo et al. 2012). Some SSR markers have been used in genetic diversity analysis, classification of species, and marker-assisted selection (Suwabe et al. 2002; Lowe et al. 2004; Choi et al. 2007; Hobson and Rahman 2016). However, most of these studies only used a limited number of turnip accessions as plant materials to test feasibility of markers without investigating the genetic diversity within turnip accessions.

This study was initiated to evaluate (i) the feasibility of applying SSR markers derived from other *B. rapa* crops to turnip and (ii) the genetic diversity in “Qamgur” and other turnip varieties in China. The experience gained in this study will provide molecular markers for the use in understanding genetic diversity and mapping genes controlling economically important traits, and provide breeders information on the genetic relationship between “Qamgur” and other turnip varieties for cultivar improvement in turnip.

Table 1 Description of 30 turnip varieties used in this study

Code	Variety name	Class	Fleshy root color		Fleshy root shape	Location	Seed source
			Upper part	Lower part			
W1	Qamgur	Variety	Green	White	Round	Changji, Xinjiang	Xinjiang Changji City Xinke Seed Co., LTD.
W2	Qamgur	Variety	Green	White	Round	Urumqi, Xinjiang	Urumqi Changlv Yuan Seed Co., LTD.
W3	Qamgur	Variety	Green	White	Oblate	Changji, Xinjiang	Xinjiang Guoyin Seed Industry Co., LTD.
W4	Qamgur	Variety	Green	White	Round	Changji, Xinjiang	Changji Lianchuang Seeds Co., LTD.
W5	Qamgur	Variety	Green	White	Round	Changji, Xinjiang	Changji Yahua Seed Industry Co., LTD.
W6	Qamgur	Variety	Green	White	Round	Urumqi, Xinjiang	Xinjiang Lvkun Fruit and Vegetable Sci-Tech Co., LTD.
W7	Qamgur	Variety	Green	White	Round	Changji, Xinjiang	Xinjiang Jifeng Seed Industry Co., LTD.
W8	Qamgur	Variety	Green	White	Round	Urumqi, Xinjiang	Urumqi Xiyang Hongyun Agricultural Technology Co., LTD.
W9	Qamgur	Landrace	Green	White	Oblate	Kashgar, Xinjiang	Yopurga Country, Kashgar
W10	Qamgur	Landrace	Green	White	Round	Kashgar, Xinjiang	Payzawat County, Kashgar
W11	Qamgur	Landrace	Green	White	Round	Bayingol, Xinjiang	Yanqi Hui Autonomous County
W12	Qamgur	Landrace	Fuchsia	Fuchsia	Round	Hotan, Xinjiang	Pishan Farm
W13	Qamgur	Landrace	Green	White	Round	Aksu, Xinjiang	Kalpin County
W14	Qamgur	Landrace	Green	White	Round	Hotan, Xinjiang	Pishan Farm, Xinjiang
W15	Qamgur	Variety	Green	White	Round	Tianjin	Tianjin Jinke Lifeng Seed Industry Co., LTD.
W16	Qamgur	Landrace	Green	White	Round	Hotan, Xinjiang	Yutian Country, Xinjiang
W17	Qamgur	Variety	Green	White	Round	Bayingol, Xinjiang	Xinjiang Yanqi Kefeng Seed Industry Co., LTD.
W18	Texuan white turnip	Variety	Green	White	Round	Tianjin	Tianjin Jinke Lifeng Seed Industry Co., LTD.
W19	Gailiang round turnip	Variety	Red	Red	Round	Tianjin	Tianjin Jinke Lifeng Seed Industry Co., LTD.
W20	Qamgur	Variety	Green	White	Round	Changji, Xinjiang	Xinjiang Xinbao Sci-Tech Seed Industry Co., LTD.
W21	Long yellow turnip	Variety	Yellowish white	Yellowish white	Long cylindrical	Shijiazhuang, Hebei	Shijiazhuang Xianfeng Seed Industry Co., LTD.
W22	Little turnip	Variety	White	White	Round	Beijing	Beijing Dongsheng Seed Co., LTD.
W23	Yuhuan Pancai	Variety	White	White	Oblate	Suzhou, Anhui	Anhui Jingmi Seed Industry Co., LTD.

Table 1 continued

Code	Variety name	Class	Fleshy root color		Fleshy root shape	Location	Seed source
			Upper part	Lower part			
W24	Wenzhou Pancai	Variety	White	White	Oblate	Jiaxing, Zhejiang	Jiaxing Xianfeng Seed Industry Co., LTD.
W25	Red Long turnip	Landrace	Red	Red	Long cylindrical	Hebei	–
W26	Imported turnip	Variety	White	White	Round	Changsha, Hunan	Changsha Yintian Vegetable Seed Industry Co., LTD.
W27	Xinxing round turnip	Variety	Red	Red	Round	Shijiazhuang, Hebei	Shijiazhuang Xianfeng Seed Industry Co., LTD.
W28	Purple round turnip	Variety	Red	Red	Round	Beijing	Beijing Jingnong Yitong Agricultural Science and Technology Co., LTD.
W29	Long yellow turnip	Variety	Yellowish white	Yellowish white	Long cylindrical	Shijiazhuang, Hebei	Shijiazhuang Xinxing Seed Industry Co., LTD.
W30	Yellow turnip	Variety	Yellowish white	Yellowish white	Long cylindrical	Tianjin	Tianjin Jinke Lifeng Seed Industry Co., LTD.

Materials and methods

Plant materials

Thirty turnip varieties (Table 1) were used to compare genetic variation within and among populations. Of these, 18 were collected from six regions of Xinjiang Uyghur Region in northwest of China, while the remaining 12 were collected from six Provinces/Municipalities in the middle-east of China. All the 30 varieties belong to turnip root type. The seedlings were grown in 128 Square Plug Tray Deep filled with a mixture of peat soils and vermiculite (3:1) in the greenhouse for DNA isolation.

DNA isolation and marker analysis

Young leaves were collected from at least eight plants of each variety such that there was a greater than 99% probability of detecting both alleles at a given marker in a segregating variety (Huhn and Piepho 2003). Genomic DNA was isolated using the modified CTAB isolation method as described by Kabelka et al. (2002).

A total of 170 SSR markers (Table 2) were used to genotype all varieties. These markers were developed for *B. rapa* ssp. *chinensis* (Suwabe et al. 2002; Lowe et al. 2004) or *B. rapa* ssp. *pekinensis* (Choi et al.

2007) or EST sequences *B. rapa* ssp. *rapa* (Wu et al. 2012), and have been used to detect polymorphisms within the species of *B. rapa*. PCR reactions were conducted in a 10- μ l reaction volume consisting of 5 μ l 2 \times Es Taq MasterMix (Beijing ComWin Biotech Company, Beijing, China), 0.5 μ M each primer, 1 μ l (5–10 ng) genomic DNA template, and 3 μ l ddH₂O. Reactions were heated at 95 °C for 5 min, followed by 36 cycles of 30 s denaturing at 95 °C, 30 s annealing at 52–58 °C depending on the T_m values of primer pairs, and 30 s extending at 72 °C, with a final extension of 5 min at 72 °C. The PCR products were subsequently separated in 7.5% polyacrylamide gel and visualized using the silver-staining approach (Chen et al. 2009).

Genetic variation analysis

The presence or absence of each single fragment was coded by 1 or 0, respectively, and scored for a binary data matrix. Polymorphism information content (PIC) for each marker was calculated using the formula of $PIC = 1 - \sum p_i^2$ (Weir 1990), where p_i is the frequency of i^{th} allele for each marker locus. Nei's genetic distance (Nei 1972) were calculated for each pair of varieties using the program in the software package NTSYSpc 2.11a (Rohlf 2002). Unweighted

Table 2 Primer sequences, number of alleles and polymorphic information content (PIC) of 52 markers in Xinjiang Qamgur and other turnip varieties

Marker	Qamgur		Other		Forward Primer (5'-3')		Reverse Primer (5'-3')		Marker source
	No. of alleles	PIC	No. of alleles	PIC					
BrEST-09	3	0.575	3	0.515	AGCCTTCTCCCTGTTCTTGG	TTGGGTTCCCTTGTCTCTGCT	Wu et al. (2012)		
BrEST-33	5	0.461	4	0.635	CGGCGATAGGAGAGAGAGG	GTAAGGACGCTCACGGTTT	Wu et al. (2012)		
BrEST-38	2	0.000	2	0.375	CGATCCAACAGCTCCTCTTTC	CGTGGTTGACGACTTGGAGAA	Wu et al. (2012)		
BrEST-56	1	0.000	1	0.000	ATGGGTAAAGGTTACACGGTTC	CTCCAATGCGTTTCAATCATCT	Wu et al. (2012)		
BrEST-62	1	0.000	1	0.000	ATGGCGACTTCAGATTCGAG	CCTCCTCCTCCATCATCA	Wu et al. (2012)		
BrEST-86	5	0.538	5	0.699	GAGTTTCAATCGGTGGCTCT	TCTCTTTGGGCTTCTTGGTG	Wu et al. (2012)		
BRMS-001	4	0.443	6	0.497	GGTGGCTTAAATTCCTCTGA	ATCTTTCTTCAACCAACCCC	Suwabe et al. (2002)		
BRMS-005	3	0.663	3	0.561	ACCTCCTGCAGATTCGTGTC	GCTGACCTTTCTTACCCTC	Suwabe et al. (2002)		
BRMS-016	1	0.000	1	0.676	TCCCGTATCAATGGCGTAACAG	CGATGGTGACATTAATTGTGGCG	Suwabe et al. (2002)		
BRMS-017	4	0.000	4	0.000	GGAAAGGGAAGTTCATATC	CTGGAAAAGCATACACTTTGG	Suwabe et al. (2002)		
BRMS-018	4	0.389	2	0.087	TCCCAGCCTTCTAGCCTTC	ACCGGAGCTTTTCTGTGTGCC	Suwabe et al. (2002)		
BRMS-019	2	0.000	2	0.153	CCCAAACGCTTTTGACACAT	GGCACAAATCCACTCAGCTTT	Suwabe et al. (2002)		
BRMS-024	3	0.512	1	0.000	TGAATTGAAAAGGCATAAGCA	CAGCCTCCACCACCTTATTCT	Suwabe et al. (2002)		
BRMS-027	1	0.000	1	0.000	GCAGGCGTTGCCTTTAATGTA	TCGTTGGTTCGGTCACTCCTT	Suwabe et al. (2002)		
BRMS-030	3	0.000	3	0.000	TCAGCCTACCAACGAGTCAATA	AAGGTCTCATACGATGGGAGTG	Suwabe et al. (2002)		
BRMS-034	4	0.111	5	0.746	GATCAAATAACGAAACGGAGAGA	GAGCCAAGAAAAGGACCTAAGAT	Suwabe et al. (2002)		
BRMS-036	3	0.401	4	0.691	GGTCCATTCCTTTTTCATCTG	CATGGCAAGGGGTAACAAACAT	Suwabe et al. (2002)		
BRMS-037	3	0.346	4	0.735	CTGCTCGCATTTTTTATCATA	TACGCTTGGGAGAGAAAACTAT	Suwabe et al. (2002)		
BRMS-042	2	0.000	3	0.580	GGATCAGTTATCTGCACCACAA	TCGGAAATTGGATAAGAAATTCAA	Suwabe et al. (2002)		
BRMS-046	2	0.105	3	0.648	TTGGCCTTGCTAATTACGAGCTG	ATGCGCAAACCCCTAATTTTCAC	Suwabe et al. (2002)		

Table 2 continued

Marker	Qamgur		Other		Forward Primer (5'-3')	Reverse Primer (5'-3')	Marker source
	No. of alleles	PIC	No. of alleles	PIC			
BRMS-054	2	0.264	2	0.219	GAATCTCTGCAAGAAACAATAATG	TTCTCAGCATCAAGTAAACCTC	Suwabe et al. (2002)
EJU2	3	0.518	2	0.320	TTCACATCTTCTTCATCTTCC	TTGCTATTCTGTTCTCAGTCTC	Choi et al. (2007)
ENA2	5	0.282	4	0.611	GATGGTATGGTGATAGGTC	GAAGAGAAAGAGTCAGAGATG	Choi et al. (2007)
ENA6	1	0.000	1	0.000	CTCGTCTTCTCACCTACAAC	CTGACATCTTCTCACCCAC	Choi et al. (2007)
ENA7	2	0.124	2	0.000	GCTCACACCTACAAGACAGAC	ACTAATAAGCAACCAAGCAA	Choi et al. (2007)
ENA10	1	0.000	1	0.000	ATCGTCTCCTCTCATCTCAA	ATTACATCTCCACCTTCTTC	Choi et al. (2007)
ENA19	5	0.702	5	0.775	AAGTTACCAAGGAGAGACAG	AAAGGAGCTACAAGTCA	Choi et al. (2007)
ENA20	2	0.444	2	0.463	GATGGAGGAAGAAGACAAGAC	TCTGAACTACCAAAGCCAAC	Choi et al. (2007)
ENA22	2	0.000	2	0.375	TTTGTAGACGAAACAGCCACG	AGAAATCGCATTTGATGGAGG	Choi et al. (2007)
ENA28	3	0.198	3	0.654	GGAGTCCGAGCGTTATGAAT	CTTCATCGACCCACCTTGT	Choi et al. (2007)
KBRH139B23	3	0.105	3	0.607	ATCTCATGGTTGGTTCACCG	ATTTCCAAAACACACACGCA	Choi et al. (2007)
KBRH143F19	4	0.617	3	0.483	GCATGCAAGCTTGGAACTGAT	CAGTCACGCTTCTGACGAAAA	Choi et al. (2007)
KBRH143H15	2	0.278	2	0.375	TCTGCATCAAAAATGCTAAAATGA	TGATCTTTTAGAAAACAAAGATCGAG	Choi et al. (2007)
Ra1-A04	7	0.783	6	0.800	GTTTTCCAAAATTATCCCCCG	TTCCCATGGTTTCTAGAGGG	Lowe et al. (2004)
Ra1-F03	4	0.656	3	0.503	AACTCGCTTTTACCCTCGTIC	CAAGACGTGGAGCTGAAAGTG	Lowe et al. (2004)
Ra1-H08	2	0.000	2	0.375	GTCGATGATCACGGAAGAGG	CTTGACAGCTACGGTTGTCC	Lowe et al. (2004)
Ra2-A01	3	0.611	4	0.616	TTCAAAGGATAAGGGCATCG	TCTTCTCTTTTGTGTCTTCCG	Lowe et al. (2004)
Ra2-A11	2	0.180	2	0.475	GACCTATTTAATATGCTGTTTACG	ACCTCACCGGAGAGAAATCC	Lowe et al. (2004)
Ra2-B07	1	0.000	1	0.000	TTTAACTGCTGCAGGTCCG	GGGCAAAATGTATAAATCCG	Lowe et al. (2004)
Ra2-C07	1	0.000	1	0.000	ATTTCCGAATCGGGAGTTTC	ACTTGCAAAACGCACACACAC	Lowe et al. (2004)
Ra2-D04	4	0.600	4	0.685	TGGATTCTCTTTACACACGGC	CAAACCAAAATGTGTGAAGCC	Lowe et al. (2004)
Ra2-E01	3	0.504	3	0.517	TCTATATTAACGCGGACGG	GCACACACACACTCAAACCC	Lowe et al. (2004)
Ra2-E04	4	0.718	4	0.664	ACACACAACAACAAGCTCGC	AACATCAAAACCTCTCGACGG	Lowe et al. (2004)
Ra2-E07	2	0.054	2	0.375	ATTGCTGAGATTGGCTCAGG	CCTACACTTGGCATCTTCACC	Lowe et al. (2004)
Ra2-E12	4	0.725	5	0.745	TGTCAGTGTGCCACTTCCG	AAGAGAAAACCAATAAGTAGAACC	Lowe et al. (2004)
Ra2-G04	2	0.161	3	0.561	AAAACGACGTCATATTGGGC	CGCTTCTTCTCAGTCTCG	Lowe et al. (2004)
Ra2-G08	1	0.000	1	0.000	ATGTCGGATAACCGAATCC	GAAAGTTTTCAATTTTAAGTTCTCTC	Lowe et al. (2004)
Ra2-G09	2	0.153	2	0.080	ACAGCAAGGATGTGTGACG	GATGAGCCTCTGGTTCAAGC	Lowe et al. (2004)
Ra2-G10	1	0.000	1	0.000	GAGACTCTCTCTCTCTCTCT	AATAACGTGTGTGCCACCAA	Lowe et al. (2004)
Ra2-H07	2	0.000	3	0.535	ATCATCAATCTGACGAGGC	CGCGCACACACACACAC	Lowe et al. (2004)

Table 2 continued

Marker	Qamgur		Other		Forward Primer (5'-3')	Reverse Primer (5'-3')	Marker source
	No. of alleles	PIC	No. of alleles	PIC			
Ra3-D02b	1	0.000	1	0.000	CACAGGAAACCGTGGCTAGA	AACCCAAACCTCAACGCTCTTG	Lowe et al. (2004)
Ra3-E05	2	0.000	2	0.278	TTCTCATGTCTCAACCACAG	GTTTCTTCCAAGCCAAGCTG	Lowe et al. (2004)

Pair Group Method with Arithmetic Mean (UPGMA) cluster analysis was performed to develop a dendrogram. Principal component analysis (PCA) was conducted using the Past 3.13 software (Hammer et al. 2001) to estimate relationships among varieties.

Population structure of the 30 turnip varieties was estimated by a model without prior population information using a free software package of STRUCTURE2.3.4 (Pritchard et al. 2000; Falush et al. 2003, 2007). Number of populations (K) and the best K were determined by the methods described in Wei et al. (2012).

Results

Success of PCR amplification and polymorphisms of SSR markers

The success rates of PCR amplification using genomic DNA of turnip as templates varied obviously among markers originated from different *Brassica* species. The markers based on EST sequences of turnip (*B. rapa* ssp. *rapa*) yielded the lowest PCR success rate (45.5%), while the markers originated from the genomic DNA sequences of *B. rapa* ssp. *pekinensis* had the highest of 97.4% (Table 3). However, all markers from *B. rapa* ssp. *rapa* with PCR success produced scorable bands, while only 34.2% markers from *B. rapa* ssp. *pekinensis* were scorable. Of the scorable markers from three *Brassica* species, the ability of detecting polymorphisms in turnip ranged from 60.0 to 83.3% (Table 3).

Marker polymorphism and allelic variation in 30 turnip varieties

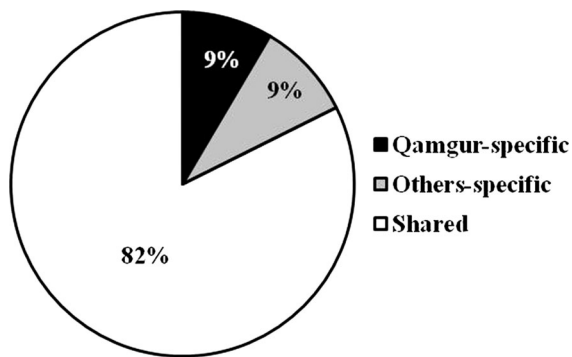
Among the 52 scorable markers, 75.0% detected polymorphisms in the whole collection of 30 turnip varieties. The proportion of polymorphic markers was lower in Xinjiang Qamgur (61.5%) than in other turnip varieties (73.1%). A total of 153 alleles were produced by the 52 markers with an average of 2.9 alleles and a range of 1–7 alleles per marker. Most alleles (82%) were shared by Xinjiang Qamgur and other varieties, while the remaining alleles were unique to either one (Fig. 1). The

Table 3 PCR success rate and polymorphisms detected by SSR markers originated from various sources

Species of marker origin	Reference	No. of markers examined	Rate of PCR success (%)	Rate of scorable markers (%) ^a	Rate of polymorphic markers (%) ^b
<i>Brassica rapa</i> ssp. <i>chinensis</i>	Suwabe et al. (2002)	33	87.9	57.6	73.3
<i>Brassica rapa</i> ssp. <i>chinensis</i>	Lowe et al. (2004)	88	73.9	48.9	75.0
<i>Brassica rapa</i> ssp. <i>pekinensis</i>	Choi et al. (2007)	38	97.4	34.2	83.3
<i>Brassica rapa</i> ssp. <i>rapa</i>	Wu et al. (2012)	11	45.5	100.0	60.0

^a The rate is based on the number of scorable markers divided by the number of markers with PCR success

^b The rate is based on the number of markers with polymorphisms to the number of scorable markers

**Fig. 1** Pie diagram shows the proportion of common alleles among Xinjiang Qamgur and other turnip varieties

average PIC of 52 markers was also lower in Xinjiang Qamgur (0.254) than in other varieties (0.379) though the range was similar (0–0.783 in Xinjiang Qamgur and 0–0.800 in other varieties). Twenty-nine markers had lower PIC values in Xinjiang Qamgur than in other varieties, while 11 markers had higher PIC values in Xinjiang Qamgur than in other varieties (Table 2).

Genetic variation in 30 turnip varieties

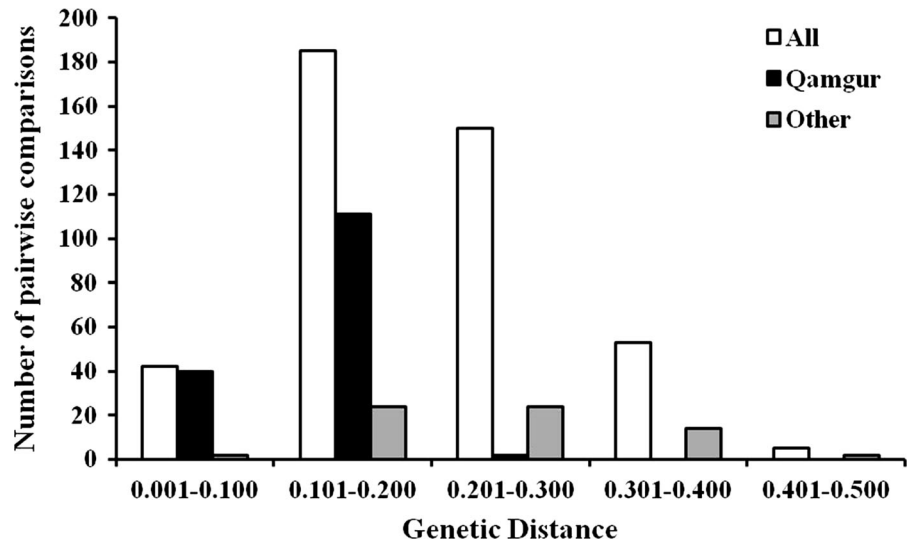
The average Nei's genetic distance for each of the 30 turnip varieties varied from 0.156 (Xinjiang Qamgur, W6 and W10) to 0.349 (Yuhuan Pancai, W23) with an average of 0.204 (Table 4). The mean genetic distance in Xinjiang Qamgur (0.127) was lower than in other varieties (0.235). Majority (77.0%) of the pairwise genetic distance in the whole collection of 30 varieties were between 0.101 and 0.300. However, the distribution of genetic distance for Xinjiang Qamgur concentrated on 0.101–0.200, while the genetic distance in other varieties had a wider distribution (Fig. 2).

The genetic variation was narrower in Xinjiang Qamgur than other varieties. Xinjiang Qamgur varieties W4 and W7 had the largest genetic distance of 0.237, while W2 and W13 had the least genetic distance of 0.047. In other varieties, Yuhuan Pancai (W23) and Wenzhou Pancai (W24) had the least genetic distance of 0.089, while Yuhuan Pancai and Little turnip had the largest genetic distance of 0.499 (Table 4).

Table 4 Statistics of Nei's genetic distance for 30 turnip varieties

Class of varieties	Genetic distance for each variety		Range of pairwise genetic distance
	Mean	Range	
Qamgur	0.127	0.098–0.160	0.047–0.237
Other	0.235	0.187–0.344	0.089–0.499
Overall	0.204	0.156–0.349	0.047–0.499

Fig. 2 Distribution of Nei's genetic distance values in 30 Xinjiang Qamgur and other turnip varieties



Genetic architecture of 30 turnip varieties

Phylogenetic tree based on the Nei's genetic distance suggested that the 30 turnip varieties could be classed into four groups at the genetic coefficient of 0.20. All varieties of Xinjiang Qamgur (W1-W17, W20) were in group I, eight varieties (W18, W19, W21, W25, W27–W30) from North China were in group II, two varieties Little turnip (W22) and Imported turnip (W26) were in group III, and two varieties Yuhuan Pancai (W23) and Wenzhou Pancai (W24) were in group IV (Fig. 3). Groups I and II had a relatively close relationship, and were more distinct from groups III and IV.

Principle component analysis was used to visualize and test relationships among the 30 turnip varieties. When Xinjiang Qamgur and other varieties were analyzed together, the first two principal components explained 30.5% of the total variation and clear clusters emerged (Fig. 4). The 30 varieties could be divided into three divergent clusters. Cluster I included all 18 varieties of Xinjiang Qamgur, cluster II contained all varieties from phylogenetic groups II and III, while cluster III consisted of two varieties Yuhuan Pancai (W23) and Wenzhou Pancai (W24).

To assign the 30 varieties to an appropriate sub-population, a series of independent runs of the data were conducted at a range of values of K from one to 10 using the software STRUCTURE. Although the best number of sub-populations was two based on the plot of ΔK against K (Fig. 5a), the log likelihood

$L(K)$ still increased till $K = 4$ when the plateau occurred (Fig. 5b). From the summary plot of membership coefficients (Q), when K increased from two to 10, all 18 Xinjiang Qamgur always stood as one sub-population (Fig. 5c), while other 12 varieties could be assigned to one to seven sub-populations. When $K = 4$, the other 12 varieties could be divided into three sub-populations (Fig. 5c), which was exactly the same as the results of phylogenetic tree analysis.

Discussion

The genetic relationship among 30 turnip varieties was investigated in this study. The data showed that the 18 varieties of "Qamgur" were always clustered together and distinct from the other turnip varieties. This could be due to the distinct shape and flavor of "Qamgur" from other turnips (Xie et al. 2014; Ma et al. 2016). Of the remaining 12 turnip varieties, eight from North China (Beijing Municipality, Tianjin Municipality and Hebei Province) formed one group, two Pancai from Eastern China (Zhejiang and Anhui Provinces) were in one group, while the remaining two clustered together. These results agree with previous findings that turnip varieties from certain regions were classified into the same cluster based on morphological, molecular or isozyme marker data (McGrath and Quiros 1992; Persson et al. 2001; Guo et al. 2014). This suggests that the long history of domestication and selection influenced the development of region-specific

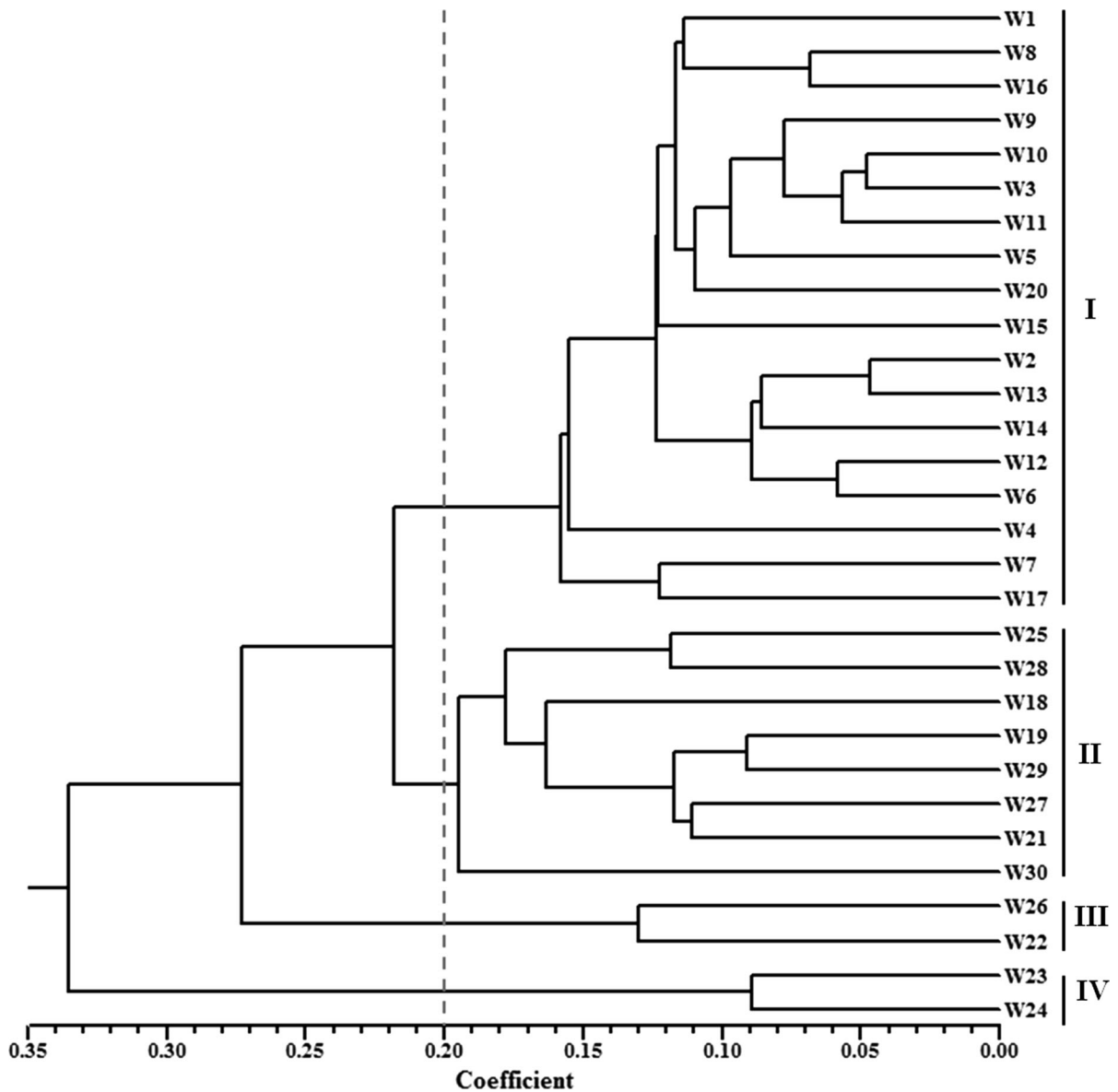


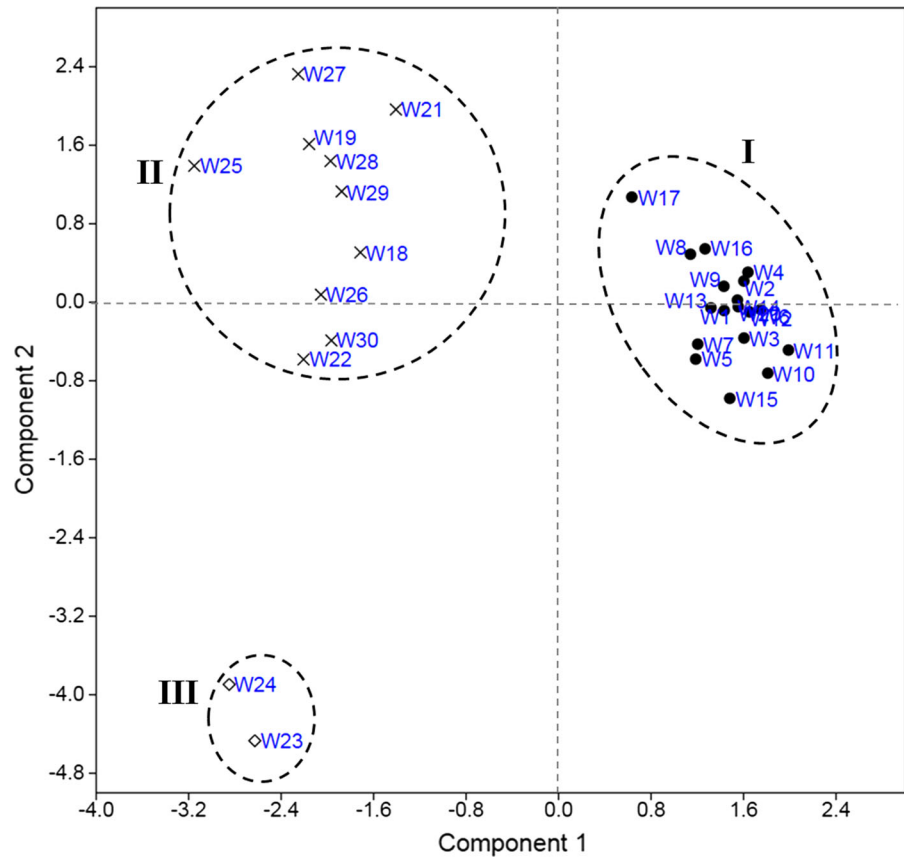
Fig. 3 UPGMA dendrogram of 30 Xinjiang Qamgur and other turnip varieties. Codes (W1–W30) for varieties can be found in Table 1

varieties. The genetic variation within the 18 varieties of “Qamgur” was low compared to the 12 varieties revealed by SSR marker data in the current study. This could be partly explained by the high morphological variation in fleshy roots in the 12 varieties than in the 18 “Qamgur” varieties. Of the 18 “Qamgur” varieties, 94.4% fleshy roots had the same color of green/white and 88.9% were round in shape, while the fleshy roots had four types of colors (white, red, yellowish white, and green) and three types of shapes (round,

oblate, and long cylindrical) in the 12 varieties (Table 1).

Previous studies on genetic divergence of turnip suggest that both Asia and Europe are geographic origins of turnips (Takahashi et al. 2016; Bird et al. 2017). Phylogenetic tree analysis in the current study showed that the 30 accessions could be divided into four groups (Fig. 3), which could be supported by population structure analysis when $K = 4$ (Fig. 5c). However, principle component analysis suggested the

Fig. 4 The genetic relationships among 30 turnip varieties revealed by principle component analysis. Codes (W1–W30) for varieties can be found in Table 1



existence of three clusters. Groups II and III in the dendrogram was in the same cluster in PCA (Fig. 4), which was consistent with the population structure when $K = 3$ (Fig. 5c). Whereas based on the best K estimation (Fig. 5a), the 30 accessions could be divided into two sub-populations (Fig. 5c, $K = 2$), one included 18 “Qamgur” accessions corresponding to group I in the dendrogram and the other contained 12 other accessions corresponding to groups II, III, and IV in the dendrogram (Fig. 3). Due to a lack of common accessions for direct comparisons between our study and others, the relationship between the 30 accessions from China and turnips from other countries cannot be determined. However, since “Qamgur” is uniquely from Xinjiang, the center of Eurasia, it is most likely that “Qamgur” is a native species there while other Chinese turnip varieties are introductions from other regions.

It has been reported that using markers developed for *B. rapa* have a higher chance of getting PCR products within subspecies of *B. rapa* than using

markers from other *Brassica* species (Lowe et al. 2004). The PCR success rates varied from 59.0 to 83.1% and the polymorphic ratio ranged from 4.26 to 20.7% (Wu et al. 2012; Luo et al. 2012). Therefore, 170 SSR markers developed for *B. rapa* were adopted to analyze genetic variation in 30 turnip varieties in this study. Both PCR success rate (80.0%) and polymorphic ratio (75.0%) were much higher than previous reports (Wu et al. 2012; Luo et al. 2012). One possible interpretation could be that both previous studies used only two cultivars while this study included 30 varieties. The larger number of samples might increase the chance of detecting more polymorphisms. In addition, the data also showed that the success rate of PCR amplification using markers derived from *B. rapa* ssp. *chinensis* and *B. rapa* ssp. *pekinensis* was higher than using markers developed for *B. rapa* ssp. *rapa* (Table 3), which was out of our expectation. Previous study suggested that designing primers using EST sequences might increase the possibility of failure in getting PCR success when the

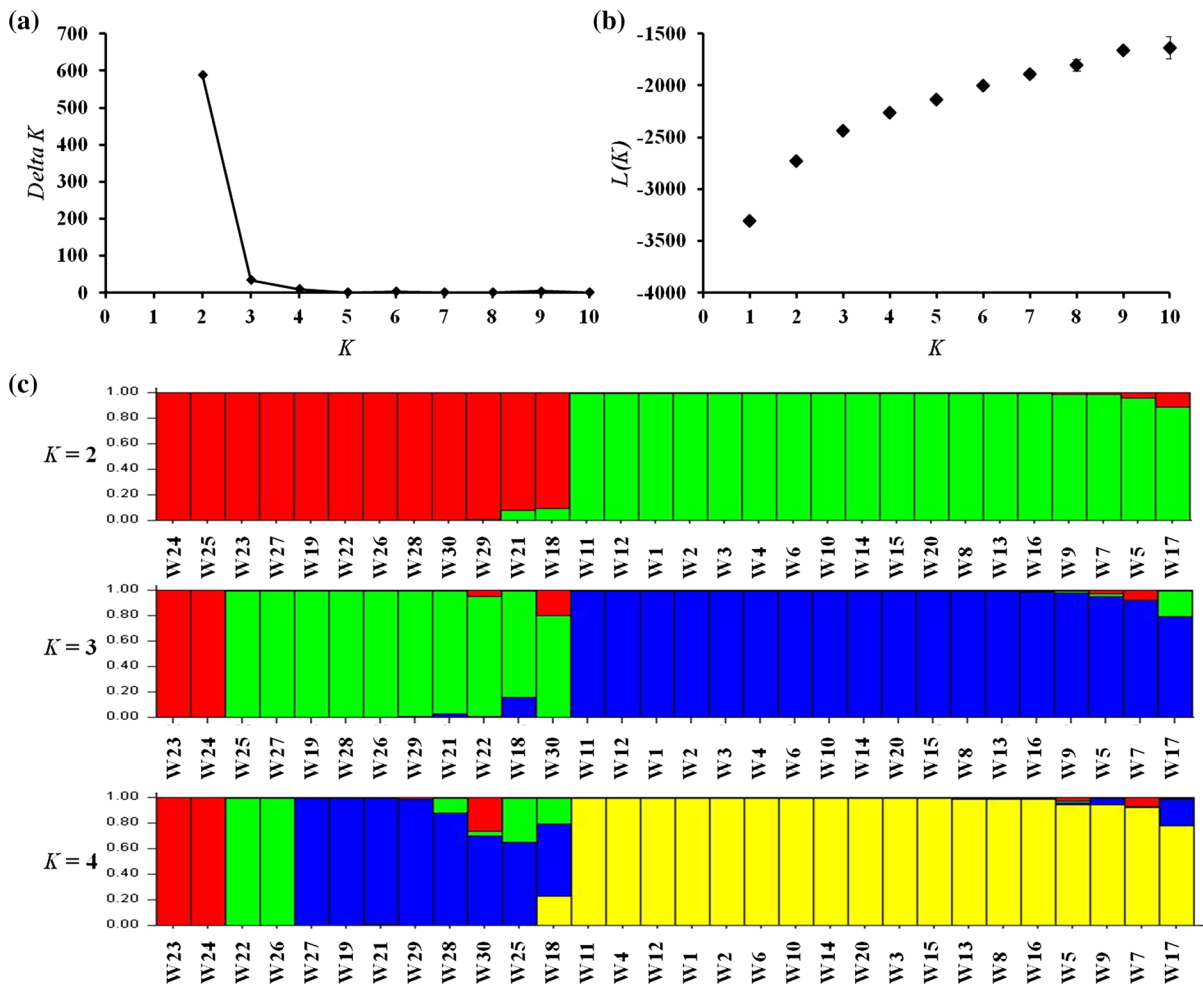


Fig. 5 Population structure of 30 Xinjiang Qamgur and other turnip varieties. Codes (W1–W30) for varieties can be found in Table 1. **a** Estimation of optimum number of groups (K). **b** The graph for the parameter $L(K)$ and number of clusters (K). **c** Population structure

PCR was conducted using genomic DNA as templates (Wang et al. 2010). In the present study, the SSR markers for *B. rapa* ssp. *chinensis* and *B. rapa* ssp. *pekinensis* were developed by mining the genomic DNA sequences (Suwabe et al. 2002; Lowe et al. 2004; Choi et al. 2007), while the SSR markers for *B. rapa* ssp. *rapa* were developed by mining the EST sequences (Wu et al. 2012). It was most likely that there were large introns between the coding sequences (EST) used for primer design resulting in large PCR products beyond the ability of *Taq* DNA polymerase to complete the reaction in a short time of 30 s.

Genetic distance in turnip accessions varied in different studies using diverse marker systems and germplasm. The genetic distance for five turnip accessions including three from Asia and two from

Europe analyzed with five isozyme and four RFLP loci ranged from 0.089 to 0.184 with an average of 0.124 (McGrath and Quiros 1992), the genetic distance in 31 turnip accessions collected from Nordic area varied from 0.016 to 0.146 based on nine isozyme (Persson et al. 2001), while the genetic distances in 30 varieties analyzed with 52 SSR markers in this study were between 0.156 and 0.349 (Table 4). This suggested that the 30 turnip varieties cultivated in China had a higher genetic variation than those in other two studies. Although “Qamgur” varieties had a low genetic distance (0.098–0.160) within the group (Table 4), they were in a unique group that was different from other Chinese varieties. This result suggests that “Qamgur” might be a special source for developing new cultivars of turnip.

In conclusion, SSR markers developed for other *B. rapa* subspecies can be used for genetic study in turnip. The markers originated from the genomic DNA sequences of *B. rapa* ssp. *pekinensis* yielded the highest PCR success (97.4%) and polymorphic rate (83.3%) in turnip (Table 3). Therefore, they might be a good source for marker development in turnip before its genomic DNA sequence is available. In addition, “Qamgur” is a valuable source for developing new turnip varieties due to its genetic distinction from other turnip varieties in China.

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Authors' contributions The experiment was conceived and designed by JG, ZX and WY and was performed by JG and NL. The data was analyzed by JG, NL and WY and the manuscript was written and edited by JG, ZX and WY.

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