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ORIGINAL PAPER

Development of SSR markers for studies of diversity in the genus *Fagopyrum*

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Abstract The numbers of SSR markers and their utilization have not been determined and investigated as extensively in *Fagopyrum* species as compared to other crop species. The current report presents 136 new SSR markers in *Fagopyrum esculentum* ssp. *esculentum* and their application to related species in the genus *Fagopyrum*. Of the 136 SSRs, 10 polymorphic SSR markers were utilized in a genetic diversity analysis of a common buckwheat population consisting of 41 accessions of diverse origin. The study showed observed (H_O) and expected (H_E) heterozygosities ranging from 0.071 to 0.924 (mean = 0.53) and from 0.073 to 0.902 (mean = 0.412), respectively. Forty-one of the 136 SSRs amplified sequences in other *Fagopyrum* species, including the cymosum and urophyllum groups. The phylogenetic relationships revealed using the SSRs was consistent with results obtained using other marker systems, with one exception. The sequence and diversity information obtained using these new SSRs and their cross-transferability to related *Fagopyrum* species will increase our understanding of genetic structures and species relationships within the *Fagopyrum* genus.

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Introduction

Common buckwheat (*Fagopyrum esculentum* Moench), a diploid ($2n = 16$) annual crop plant, is widely cultivated in Asia, Europe, and America. Because buckwheat can be grown at high altitudes, has a short growing season, and its grains contain high-quality proteins, it is an important crop in mountainous regions of China and in other countries in the northern hemisphere. Buckwheat proteins contain a good balance of amino acids, providing an excellent supplement for cereal-grain diets (Pomeranz 1983). In addition, rutin, quercetin, kaempferol-3-rutinoside, and a trace quantity of flavonol triglycosides in buckwheat grains make them suitable for special diets (Holasova et al. 2002). However, the narrow gene pool of common buckwheat and its limited distribution make it vulnerable to potential phytopathological hazards. Thus, for future plant breeding, it is necessary to broaden the genetic base of cultivated buckwheat using the wild gene pool (Kreft 2001; Suvorova et al. 1994; Wang et al. 2005).

The genus *Fagopyrum* (Polygonaceae) consists of 16 species (Ohnishi 1998; Ohsako and Ohnishi 1998; Ohsako et al. 2002) that are classified into two monophyletic groups, the cymosum and urophyllum groups, based on morphology (Ohnishi and Matsuoka 1996). The former

group includes *F. esculentum*, *F. cymosum*, *F. tataricum*, and *F. homotropicum*; species which have large lusterless achenes incompletely covered with a persistent perianth. The latter group includes wild species that have small lustrous achenes covered completely with a persistent perianth (Yasui and Ohnishi 1996). Relationships between species as well as accessions within species have been delineated using molecular markers such as RAPDs (Iwata et al. 2005; Murai and Ohnishi 1996; Sharma and Jana 2002), allozymes (Ohnishi 1998), and AFLPs (Iwata et al. 2005; Konishi et al. 2005). These approaches have revealed discrete genetic differences between samples of common buckwheat and related species. Genetic diversity and species relationships have not been as extensively investigated using SSRs in *Fagopyrum* species as compared to other crops. Iwata et al. (2005) reported five SSR markers in common buckwheat that show a high level of polymorphism among cultivars grown in Japan. More recently, Konishi et al. (2006) developed 48 SSR markers that can be used to detect variations in common buckwheat populations and seven related *Fagopyrum* species in the cymosum group.

SSR markers are the preferred choice for certain genetic studies because they can easily detect co-dominant alleles, are highly reproducible, and have very high levels of polymorphisms (Litt and Luty 1989; Weber and May 1989). Hence, this robust genetic marker system is valuable for developing markers in minor crop species, but the requirement for flanking sequences of SSR motifs often limits their wider application in such species. Peakall et al. (1998) reported that successful cross-specific amplification is largely restricted to closely related species and genera, as found for the transferability of SSRs from legume species and soybean. In a similar study with 207 EST-SSR primers from 23 *Gossypium* species (Guo et al. 2006), 124 primer sets (60%) amplified products in all 23 species.

This report describes a new set of 136 SSRs from buckwheat and their use in examining genetic diversity in common buckwheat accessions, and discusses the feasibility of cross-species amplification in the cymosum and urophyllum groups of the *Fagopyrum* genus.

Materials and methods

Plant materials and genomic DNA extraction

A list of *Fagopyrum* species and accessions used in this study is given in Table 1. Genomic DNA was extracted from green leaves of buckwheat grown for 20–30 days in a greenhouse with a cycle of 10/24 h and 18°C/25°C (night/day). DNA was extracted using the Plant DNAzol® Reagent (Invitrogen, Carlsbad, CA, USA) with the supplier's protocols and quantified using a UV–Vis spectrophotometer (ND-1000; NanoDrop, Wilmington, DE, USA).

Construction of an SSR motif-enriched library

A modified biotin–streptavidin capture method was used to construct an SSR motif-enriched library of *F. esculentum* ssp. *esculentum* genomic DNA (Kwon et al. 2005). Genomic DNAs (10 µg) were digested with seven blunt end-producing restriction enzymes (*Eco*RV, *Dra*I, *Sma*I, *Pvu*I, *Alu*I, *Hae*III, and *Rsa*I). The fully digested DNAs were pooled and size-fractionated on 1.4% agarose gels. DNA fragments of 300–1,500 bp were eluted from the gels and purified using a gel extraction kit (Qiagen, Hilden, Germany). Approximately 1 µg of DNA fragments was ligated with 1 µg of the double-stranded adaptor (AP11/AP12). The adaptor was prepared by mixing equal molar amounts of oligonucleotides AP11 (5′-CTC TTG CTT AGA TCT GGA CTA-3′) and AP12 (5′-TAG TCC AGA TCT AAG CAA GAG CAC A-3′), heating to 94°C followed by cooling to 25°C over a period of 4–5 h. Pre-amplification of adaptor-ligated DNA fragments was performed for 15 cycles of PCR in a 50-µl reaction volume with single primer AP11 using an annealing temperature of 56°C. The pre-amplified products were hybridized with a mixture of long (40–45 nucleotides) biotin-labeled repeat probes. Hybridization was performed for 2 h at 65°C in a reaction mixture (50 µl) that included 6 × SSC, 0.1% SDS, approximately 100 ng of pre-amplified product, and 300 ng of each biotin-labeled oligo: $(GA)_{20}$, $(CA)_{20}$, $(AGC)_{15}$, $(GGC)_{15}$, $(AAG)_{15}$, $(AAC)_{15}$, $(AGG)_{15}$. The hybridized DNA fragments were captured with 400 µg of streptavidin-coated magnetic beads (Promega, Madison, WI, USA) by incubating the mixture at 65°C with gentle agitation for 30 min. The beads were separated from the liquid using a magnetic stand (Promega) and washed five times in 300 µl 6 × SSC/0.1% SDS (1 × SSC = 150 mM NaCl, 15 mM sodium citrate, pH 7.0) at room temperature with gentle agitation. After stringent washings, all the samples were briefly washed in 5 × SSC to remove the SDS, and DNA fragments were eluted with 50 µl of dH_2O at 90°C for 5 min. Final elutes (5 µl) were amplified for 15 cycles of PCR using the AP11 primer. After checking on gels, the amplified DNA products were cloned into the pGEM-T Easy Vector (Promega) and transformed into *Escherichia coli* cells through electroporation. Recombinant colonies were identified by blue/white colony selection on LB plates containing ampicillin, X-gal, and IPTG.

DNA sequencing and design of SSR primers

A total of 504 white colonies were randomly selected from the enriched library. Plasmid DNAs were purified with the

Table 1 List of *Fagopyrum* species used in this study

Fagopyrum species	Accession no.	No. of plants	Collection region, Country	*Fagopyrum* species	Accession no.	No. of plants	Collection region/country
F. esculentum ssp. *esculentum*[a]	K000696	1	Ontario, Canada	*F. esculentum* ssp. *esculentum*	104139	1	Gyeongbuk, Korea
	K000699		Ontario, Canada		103093		Jeonbuk, Korea
	187871		Yunnan, China		K003292		Gyeongnam, Korea
	K024606		Kyoto Univ, Japan		200362		Bagmati, Nepal
	213319		Kyoto Univ, Japan		K004048		Kakani, Nepal
	805069		Gyeonggi, Korea		200364		KTM market, Nepal
	105325		Jeonbuk, Korea		200363		Marpa, Nepal
	911125		Jeonnam, Korea		179842		Orel, Russia
	105254		Jeonbuk, Korea		179844		Bashkiria, Russia
	101271		Gangwon, Korea		179843		Tatarstan, Russia
	103569		Gyeongnam, Korea		199280		Vavilov Research Institute, Ukraine
	101022		Jeonbuk, Korea		199279		Vavilov Research Institute, Ukraine
	105398		Jeonbuk, Korea	*F. esculentum* ssp. *esculentum*[b]	C9106	3	Yongsheng, Yunnan, China
	102780		Gyeongbuk, Korea	*F. esculentum* ssp. *ancestralis*	C9509	2	Yanyuan, Sichuan, China
	103026		Gyeongbuk, Korea	*F. tataricum* ssp. *tataricum*	C9724	2	Baoshan, Yunnan, China
	105203		Gyeongbuk, Korea	*F. tataricum* ssp. *potanini*	C9732	2	Zhangdian, Yunnan, China
	K011766		Gangwon, Korea	*F. homotropicum*	C9740	2	Lijiang, Yunnan, China
	101091		Jeonbuk, Korea		C9139	2	Yongsheng, Yunnan, China
	K002647		Gyeongnam, Korea	*F. urophyllum*	C0281	2	Kunming, Yunnan, China
	101282		Gangwon, Korea		C9762	2	Luchen, Yunnan, China
	104904		Gyeongnam, Korea	*F. lineare*	C0296	2	Midu, Yunnan, China
	178741		Chungnam, Korea		C9758	2	Biuchuan, Yunnan, China
	104236		Gyeongbuk, Korea	*F. leptopodum*	C9780	2	Dali, Yunnan, China
	101391		Jeonbuk, Korea		C9253	2	Yongsheng, Yunnan, China
	104464		Gangwon, Korea	*F. capillatum*	C0295	2	Jinan, Yunnan, China
	104328		Gyeongbuk, Korea		C9462	2	Yongsheng, Yunnan, China
	100801		Gyeongbuk, Korea	*F. gracilipes*	C9784	2	Zhangdian, Yunnan, China
	103069		Jeonbuk, Korea		C8938	2	Kunming, Yunnan, China
	910167		Gyeongnam, Korea				

[a] The accessions analyzed with 10 polymorphic SSR loci

[b] These accessions of various species were used for cross-species amplification and subsequent species relationships

QIAprep Spin Miniprep Kit (Qiagen), and nucleotide sequencing was carried out using an ABI 3100 DNA Sequencer with a BigDye terminator kit (Applied Biosystems, Foster City, CA, USA). SSR motif elucidation and primer design were performed using the ARGOS program (Kim 2004).

PCR amplification

The "M13 tail at its 5′ end" PCR method was used to measure the size of the PCR products (Schuelke 2000). PCR amplification was carried out in a total volume of 20 µl containing 2 µl of genomic DNA (10 ng/µl), 0.2 µl of the specific primer (10 pmol/µl), 0.4 µl of M13 universal primer (10 pmol/µl), 0.6 µl of normal reverse primer, 2.0 µl of 10 × PCR buffer (Takara, Tokyo, Japan), 1.6 µl of dNTP (2.5 mM), and 0.2 µl of Taq polymerase (5 unit/µl; Takara). Conditions of the PCR amplification were as follows: 94°C (3 min), followed by 30–33 cycles at 94°C (30 s), 50–55°C (45 s), 72°C (45 s), then 15 cycles at 94°C (30 s), 53°C (45 s), and 72°C (45 s), and final extension at 72°C for 20 min. PCR was carried out in PTC-220 thermocyclers (MJ Research, Waltham, MA, USA). The PCR products of three microsatellites were mixed together in a ratio of 6-FAM:HEX:NED (fluorescent dyes) = 1:3:4, which was varied depending on the amplification intensity for individual

markers as determined on an ABI PRISM 3130 × l Genetic Analyzer (Applied Biosystems). PCR products labeled with HEX and NED were added in higher amounts, and those labeled with FAM were added in lower amounts because of the different signal intensities of these fluorescent dyes. The mixed PCR product of 1.5 µl was combined with 9.2 µl of Hi-Di formamide and 0.3 µl of an internal size standard, Genescan-500 ROX (6-carbon-X-rhodamine) molecular size standards (35–500 bp). The samples were denatured at 94°C for 3 min and analyzed with an ABI PRISM 3130 × l Genetic Analyzer (Applied Biosystems). Molecular weights, in base pairs, for microsatellite products were estimated with Genescan software ver. 3.7 (Applied Biosystems) using the local Southern method. The individual fragments were assigned as alleles of the appropriate microsatellite loci with Genotyper software ver. 3.7 (Applied Biosystems).

Data analysis

PowerMarker version 3.25 (Liu and Muse 2005) and GenAlEx version 6.1 (Peakall and Smouse 2006) were used to measure the variability at each locus: the observed heterozygosity (H_O), the gene diversity/expected heterozygosity (H_E), the polymorphism information content (PIC), the deviation from Hardy–Weinberg equilibrium (HWE), and the pairwise linkage disequilibrium (LD) (Nei and Kumar 2000; Weir 1996). The number of alleles (A) was calculated, and the PIC was estimated from the equation $\mathrm{PIC} = 1 - \sum_i P_i^2$, where P_i is the frequency of the ith allele in the sample examined (Anderson et al. 1993). A phylogenetic dendrogram was constructed using the unweighted pair-group method with arithmetic averaging (UPGMA) in the NTSYS-pc program (Rohlf 2000) and bootstrapping with Winboot (Yap and Nelson 1996). Each amplified primer was treated as a unit character and scored in a binary code of either 1 or 0 for presence or absence, respectively. Genetic variation was calculated within species and genetic similarity between species with Microsoft Excel using the NTSYS dataset.

Results

Development of SSR markers

From a random selection of 504 clones employed in this study, 54 were redundant, having the same sequences. Of the remaining 450 clones, 136 were suitable for designing primer pairs for PCR amplification of the SSR motifs. Clones of <10 nucleotides in the SSR motifs were not included in the primer design. Among the 136 clones, trinucleotide repeat motif-containing clones were dominant, with 5 dinucleotide, 102 trinucleotide, 13 tetranucleotide, 5 pentanucleotide, and 2 hexanucleotide clones. The remaining 19 clones contained composite motifs with more than one repeating motif. The number of repeating units varied from 4 to 41, and the length of the repeat region varied from 10 bp in GB-FE-017 [$(AC)_5$] to 174 bp in GB-FE-035 [$(GAY)_{14}(GGT)(GAB)_{41}$] (Supplementary Table 1).

Variations among accessions of common buckwheat

PCR amplification of the 136 SSR loci was checked on a set of 41 common buckwheat accessions representing diverse regional collections consisting of a core collection maintained at our Institute (NAC, RDA, Korea) (Table 1). The PCR amplification produced repeatable amplicons in the expected molecular size range. However, the number of SSR primer sets producing polymorphic pattern was reduced to 10 from the 136 primer sets.

A total of 59 alleles were detected with the 10 sets of SSRs, resulting in an average of 5.9 alleles per locus (Table 2). The GB-FE-035 primer pair, which had highest number of repeat motifs, produced the highest number (21) of alleles, whereas GB-FE-001, GB-FE-043, and GB-FE-055 produced only two alleles. The major allele frequency (MAF) ranged from 0.134 to 0.936 with a mean value of 0.573. The observed heterozygosity (H_O) ranged from 0.071 to 0.924 (mean = 0.530), and expected (H_E) heterozygosities ranged from 0.073 to 0.902 (mean = 0.412). Three loci deviated from HWE ($P < 0.001$), in which two (GB-FE-035, GB-FE-054) revealed higher H_O than H_E and one (GB-FE-055) exhibited less H_O than H_E (Table 2). Seven combinations of the pairwise allelic associations showed significant LD values ($P < 0.05$) (Table 3). The P value of their associations ranged from 0.0014 to 0.0338.

Cross-species transferability of the *F. esculentum* microsatellites to other *Fagopyrum* species

For cross-species transferability of the SSRs, amplification was carried out with selected two or three accessions of eight different *Fagopyrum* species (Table 1). Of the 136 SSR primer sets, 41 (30.1%) produced amplified products in all species analyzed (Table 4, Supplementary Table 1). For individual species or subspecies, the successful amplification rate ranged from 97.1% in *F. homotropicum* to 36.8% in *F. tataricum* ssp. *potanini*. *F. esculentum* ssp. *ancestralis*, which is believed to be the wild ancestor of *F. esculentum* ssp. *esculentum*, also showed a very high amplification frequency (96.3%) (Table 4). Although microsatellites were derived from *F. esculentum* ssp. *esculentum* in the cymosum group, the amplification rates of species in the urophyllum group were similar to those from

Table 2 Characteristics of 10 microsatellite loci developed for 41 accessions common buckwheat (*Fagopyrum esculentum*)

Marker	GenBank accession	Repeat motif	Primer sequence (5′-3′)	Size (bp)	Ta (°C)	MAF	*A*	H_E	H_O	PIC
GB-FE-001	EU998635	$(CAA)_7$	F: TGAAACCCAACCATCAGG	268–274	58	0.634	2	0.585	0.464	0.356
			R: CGACAGTGGCTGGAGAAC							
GB-FE-012	EU998636	$(CAG)_5(CT)(CAG)$ & $(GAK)_8$	F: ACTGCACCCCAGAGGATT	188–209	58	0.646	6	0.488	0.540	0.504
			R: GCTGTATCCATGCCCGTA							
GB-FE-014	EU998637	$(GA)_{10}C(GA)$	F: AGGAGCAGAGGTGGTGGT	198–216	59	0.488	4	0.585	0.659	0.605
			R: CGGAGCCTCTGCAACC							
GB-FE-035[a]	EU998638	$(GAY)_{14}(GGT)(GAB)_{41}$	F: TGCAATGACTTGGAGGAGA	222–404	58	0.134	21	0.220	0.924	0.919
			R: ACCACCATTCAACAAGCG							
GB-FE-043	EU998639	$(CCA)_5$	F: TTCAGCACCTGGATGGAC	186–204	58	0.768	2	0.268	0.356	0.293
			R: TGTCCCCAATGTGAAAGG							
GB-FE-054[a]	EU998640	$(TR)_{12}$	F: TGTTGGACTTCCTAGACCTG	227–247	58	0.341	9	0.122	0.783	0.754
			R: CATGAAAAGGGGATGCAA							
GB-FE-055[a]	EU998641	$(GAK)_6$ & $(GAT)_3$ & $(GAT)_2$	F: CTGCTTGGATCCCATTGA	256–259	58	0.524	2	0.902	0.499	0.374
			R: AGCCTCTCGATCCCTCTG							
GB-FE-080	EU998642	$(CST)_7$	F: CGAGGTGGGCAGTAGAGA	231–279	59	0.756	4	0.439	0.402	0.373
			R: GAGGAGGACGAGGAGGTG							
GB-FE-169	EU998643	$(ACA)_6$	F: CAACCCTATGCAGCGTTC	202–220	58	0.963	3	0.073	0.071	0.070
			R: GAGGGGAAGCTGCTTGTT							
GB-FE-191	EU998644	$(CAT)_5$	F: AATCAATGACCAGCACGC	141–220	58	0.476	6	0.439	0.597	0.516
			R: CTGATGGAGGATGCCAAA							
Mean						0.573	5.9	0.412	0.530	0.476

Ta, annealing temperature; *MAF*, major allele frequency; *A*, number of alleles; H_E, expected heterozygosity; H_O, observed heterozygosity; *PIC*, polymorphism information content. Degenerate sequences: K (G or T), Y (C or T), B (G, T or C), R (A or G), S (G or C)

[a] Loci with significant deviations from HWE

Table 3 The linkage disequilibrium associations between alleles of SSR loci in 41 common buckwheat accessions

Locus	Allele[a]	Locus	Allele	Correlation	Chi-square[b]	Probability
GB-FE-012	A	GB-FE-035	A	0.5000	10.25	0.0014
GB-FE-035	D	GB-FE-054	F	0.5000	10.25	0.0014
GB-FE-035	O	GB-FE-054	A	0.4461	8.16	0.0043
GB-FE-035	Q	GB-FE-054	B	0.5000	10.25	0.0014
GB-FE-035	J	GB-FE-080	B	0.3315	4.50	0.0338
GB-FE-035	K	GB-FE-191	F	0.3491	5.00	0.0254
GB-FE-054	G	GB-FE-191	C	0.3491	5.00	0.0254

[a] The alleles were named A to U from the smallest to largest size

[b] All Chi-square tests have 1 degree of freedom

F. tataricum ssp. *tataricum* and even higher than those from *F. tataricum* ssp. *potanini* in the cymosum group.

A total of 101 of the 136 SSR primer pairs (74.3%) had only a single SSR allele (Supplementary Table 1). The highest number of alleles was eight for GB-FE-121, which also had a high PIC value (0.54). The PIC values ranged from 0.00 in monomorphic SSRs to 0.6 for GB-FE-168 (asterisks in Supplementary Table 1 indicate polymorphic markers). However, SSRs with high PIC values were not always highly resolved SSRs for species identification, since they often failed to produce successful amplification in some species. For example, GB-FE-168 produced no amplification in *F. tataricum* ssp. *potanini*, *F. urophyllum*, *F. lineare*, or *F. capillatum*. We are not yet sure whether the amplification failure was due to the low number of accessions analyzed or to true null alleles caused by mutation(s) in the primer binding sites in these species.

Table 4 Rates of successful amplification of *F. esculentum* SSRs in other *Fagopyrum* species

Fagopyrum species	Successful amplifications (%)
F. esculentum ssp. *esculentum*	136 (100)
F. esculentum ssp. *ancestralis*	131 (96.3)
F. tataricum ssp. *tataricum*	83 (61.0)
F. tataricum ssp. *potanini*	50 (36.8)
F. homotropicum	132 (97.1)
F. urophyllum	69 (50.7)
F. lineare	68 (50.0)
F. leptopodum	82 (60.3)
F. capillatum	91 (66.9)
F. gracilipes	90 (66.2)
Fagopyrum genus (all species)	41 (30.1)

Fagopyrum species relationships obtained from SSR profiles

A phylogenetic dendrogram based on the SSR profiles revealed two major clades: one with the two *F. esculentum* species and *F. homotropicum*, and another with the five urophyllum species and two *F. tataricum* species (Fig. 1). In the former clade, *F. esculentum* ssp. *esculentum* and *F. esculentum* ssp. *ancestralis* clustered with a similarity coefficient of 0.84, which was tied with *F. homotropicum* at a similarity level of 0.70. In the latter clade, the five species in the urophyllum group clustered in a subclade with a similarity coefficient of 0.78, which was joined to the two *F. tataricum* species with a similarity coefficient of 0.49. The two *F. tataricum* species formed a subclade with a similarity coefficient of 0.63 in the urophyllum clade. The differentiation between *F. urophyllum* and *F. leptopodum* was very low, with a similarity coefficient of 0.98, which was tied with *F. lineare* with a similarity coefficient of 0.78.

Discussion

Understanding genetic structure in a given population is an important step for diversity preservation and species conservation. SSRs are the best marker system for this purpose since they can detect hypervariability in SSR motifs, which is usually selectively neutral (Amos et al. 1996; Ellegren et al. 1995; Varshney et al. 2005). Although buckwheat SSRs had been developed prior to this study (Konishi et al. 2006), the low genetic variation inherent in buckwheat requires more SSR markers to properly delineate the limited variability. The current research provided an additional 136 SSR markers overall for buckwheat. Only 10 of these 136 SSRs (7.4%) produced polymorphic patterns among accessions of common buckwheat, which is lower than the 26.7% reported from a previous study on buckwheat SSR development (Konishi et al. 2006), and also lower than in other species such as *Humulus lupulus* (41.7%) (Stajner et al. 2005) and *Acacia mangium* (40.7%) (Butcher et al. 2000).

The SSR genetic diversity observed among accessions of common buckwheat in this study can be compared with the diversities detected in buckwheat using other SSR sets (Iwata et al. 2005). The average expected heterozygosity (H_E) and observed heterozygosity (H_O) in accessions of common buckwheat in our analysis were 0.412 and 0.530, respectively; the H_E was lower than that among the indigenous 19 cultivars (0.819) used by Iwata et al. (2005), in which the PICs also differed from our results. The low detection of heterozygosity and polymorphism in this study is puzzling. It is not certain that it results from inappropriate conservation strategy. Indeed, the high number of Korean accessions (27 of 41 accessions) in our analysis might have contributed to these lower values. Murai and Ohnishi (1996) had noted a gradual decline of polymorphism with the migration from the center of origin of the species (Southern China). Founder/bottleneck effects, linked to more restricted introductions into Korea than into Japan (Murai and

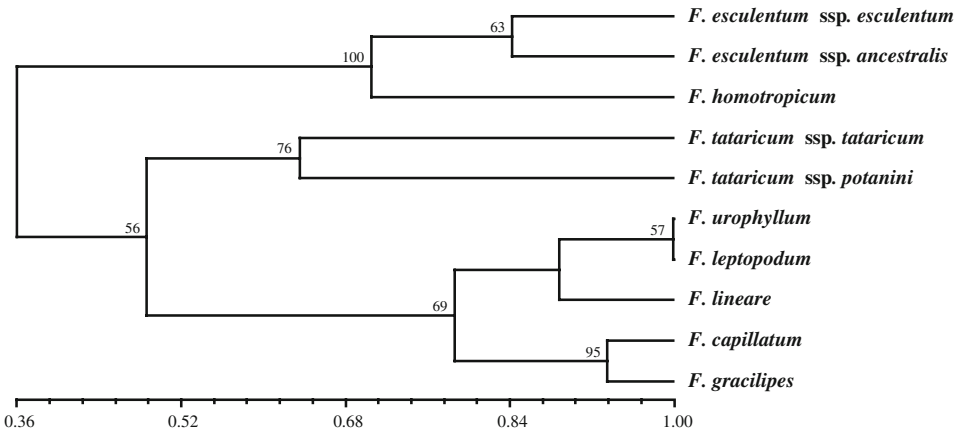

Fig. 1 Phylogenetic dendrogram of the subspecies and species in *Fagopyrum* based on SSR amplification profiles

Ohnishi 1996), could therefore account for the observed pattern. Thus, further analyses are required on the genetic diversity or variation among Korean accessions of common buckwheat because no prior studies have been undertaken. Since Konishi et al. (2006) reported an average SSR PIC value of 0.79 among a worldwide core collection of common buckwheat accession, the PIC value of 0.476 in our analysis might not reveal the full genetic diversity of common buckwheat germplasm. While most of the SSRs of Konishi et al. (2006) contained dinucleotide repeating motifs, the polymorphic SSRs in our analysis contained trinucleotide and composite-repeating motifs. A higher mutation rate in loci displaying short repeat motifs, as reported for example in maize (Vigouroux et al. 2002), could account for the higher polymorphism reported by Konishi et al. (2006). However, in a check of the polymorphic SSRs for the number of repeat motifs, the motif number did not appear to be related to polymorphism in this study. In a separate check of polymorphisms with single versus composite-repeating motifs, 4 of the 10 polymorphic primers were composite motif SSRs, suggesting that the multiple motif-containing SSRs produce higher polymorphisms.

LD is the genetic phenomenon of nonrandom association of alleles at different loci (Flint-Garcia et al. 2003). The nonrandom association was observed not only between alleles of loci on different chromosomes, but also between alleles of loci on the same chromosome (Hagenblad and Nordborg 2002; Stich et al. 2005; Tenaillon et al. 2001). Allele frequency and recombination between sites, as well as the effective population size, are important factors in LD (Weir 1996). Recombination analysis among the SSRs was not attempted in this study. Although we analyzed LDs using a limited number of accessions with only 10 SSR loci, the alleles showing high association may shed some light in germplasm management and subsequent breeding programs in buckwheat.

Although SSRs are an excellent marker system for various purposes of genetic analyses, the sequence information requirement for primer design limits its wide application in wild species or minor crop species, since few nucleotide sequences of these species have been determined. Developing an SSR motif-enriched library and subsequent nucleotide sequencing can be time-consuming procedures for minor crop species. Therefore, it is desirable to utilize SSRs from related species to examine these species of interest (Guo et al. 2006; Kuleung et al. 2004; Saha et al. 2004). However, SSR transferability is generally limited to closely related species (Peakall et al. 1998; Varshney et al. 2005). Konishi et al. (2006) first attempted cross-species transferability within species in the cymosum buckwheat group using SSRs developed from *F. esculentum* ssp. *esculentum*. In their analysis, 54 of the 180 SSR markers showed intraspecific variation among 34 accessions of common buckwheat. Among these 54 intraspecific polymorphic SSRs, the amplification transferability was 100% in *F. esculentum* ssp. *ancestralis*, 79.6% in *F. homotropicum*, and between 63 and 25.9% in *F. tataricum* ssp. *potanii*. Our SSR sets led to lower cross-amplification (2–3%) in *F. esculentum* ssp. *ancestralis* and *F. tataricum* ssp. *tataricum*, but significantly higher transferability (11–17%) in *F. homotropicum* and *F. tataricum* ssp. *potanii*. The difference might be derived from the accessions used, since our accessions of *Fagopyrum* species were all from Yunnan Province, China. Nevertheless, our attempt at transferability to species in the urophyllum group is significant since there have been no previous reports on SSR diversity in these species. The SSR markers with high PIC values in our analysis may be valuable in species identification or classification in future buckwheat collections.

The species relationships derived from our cluster analysis is consistent with those of other reports (Sharma and Jana 2002; Yasui and Ohnishi 1996, 1998a, b). However, *F. tataricum* was included in the urophyllum clade in our analysis with a loose similarity coefficient of 0.49, in contrast to previously reported results which placed it into the cymosum group. *F. tataricum* was placed within the cymosum group in previous comparative studies using the *rbcL* gene (Yasui and Ohnishi 1996) and 45S rDNA ITS sequences (Yasui and Ohnishi 1998b). The morphological–botanical features of *F. tataricum* are more similar to those of species in the cymosum group (Ohnishi and Matsuoka 1996). The lack of inclusion of *F. cymosum* in our analysis might have caused the grouping of *F. tataricum* with species in the urophyllum group, since *F. cymosum* was shown to be closely related to *F. tataricum* in a morphological classification (Ohnishi and Matsuoka 1996) and based on variations in 45S rDNA ITS sequences (Yasui and Ohnishi 1998b). It was unfortunate to exclude accessions of *F. cymosum* due to the germination failures in our analysis.

Genetic and phylogenetic studies in buckwheat have lagged behind those of other crop species. The new set of SSRs presented here, and their polymorphic information will provide valuable genetic resources for studies of *F. esculentum* and related species.

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