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Species relationships in *Fagopyrum* revealed by PCR-based DNA fingerprinting

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Abstract Random amplified polymorphic DNA (RAPD) markers were used to distinguish between 28 different accessions belonging to 14 species and two sub-species of *Fagopyrum*. Of the 75 random 10-mer primers tested, only 19 generated robust, easily interpretable amplification products. A total of 364 bands were observed with an average of 19.15 bands per primer, of which 99.45% were polymorphic. Primer OPN-08 produced the maximum number of fragments and UBC-183 produced the minimum number of fragments. The data were utilized to elucidate genetic relationships among 14 species and two sub-species of *Fagopyrum*. Cluster analysis using the unweighted paired group method of arithmetic means (UPGMA) showed four main clusters, two each of the cymosum and urophyllum groups. The results showed that *Fagopyrum tataricum* is closer to its wild ancestor *F. tataricum* ssp. *potanini* Batalin, closely followed by *Fagopyrum giganteum*. Cultivated common buckwheat (*Fagopyrum esculentum*) showed affinity with its putative wild ancestor *F. esculentum* ssp. *ancestrale* and the other closely related diploid species *Fagopyrum homotropicum*. In the urophyllum group, *Fagopyrum macrocarpum* and *Fagopyrum pleioramosum* formed one cluster, whereas *Fagopyrum capillatum*, *Fagopyrum gracilipes* and *Fagopyrum gilessii* clustered separately. Except for a few cases, our results correspond with previously reported studies on *Fagopyrum* using the isozyme, RFLP and RAPD methods. Species-diagnostic amplification products specific to some species in the cymosum and urophyllum groups were identified. Our results show that RAPDs can be successfully used to analyze species relationships in *Fagopyrum* and also for constructing linkage maps.

Keywords Diversity · DNA fingerprinting · *Fagopyrum* · RAPD · Species relationship

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Introduction

The genus *Fagopyrum* (Polygonaceae) consists of about 16 species, some of which have been recently discovered (Ohnishi 1998; Ohsako and Ohnishi 1998). Of the two cultivated species, *Fagopyrum esculentum* L. Moench. (common buckwheat) and *Fagopyrum tataricum* Gaertn. (tartary buckwheat), the former is cultivated in temperate Eurasia, whereas cultivation of the latter is limited mainly to southern China and the Himalayan region. Wild *Fagopyrum* species occur in mountainous regions of southern China and occasionally on the Himalayan foothills (Ohnishi 1995, 1998). The narrow gene pool of cultivated buckwheats and their limited distribution make them vulnerable to the potential phytopathological hazards. Thus, for future plant breeding, it is necessary to broaden the genetic base of cultivated buckwheats using the wild gene pool.

Phylogenetic relationships among *Fagopyrum* spp. have been studied recently using molecular data, such as isozymes and restriction fragment length polymorphism (RFLP) variation in cpDNA (Ohnishi and Matsuoka 1996), and nucleotide sequence variation in cpDNA and nuclear DNA (Yasui and Ohnishi 1998a, b; Ohsako and Ohnishi 2000). These studies led to the division of *Fagopyrum* into two major phylogenetic groups, cymosum and urophyllum. The cymosum group includes two cultivated species, *F. esculentum* and *F. tataricum* and two wild species, and the urophyllum group includes ten wild species (Ohsako and Ohnishi 2000).

The selection of an appropriate molecular technique for routine screening of accessions in gene-bank collections, and for elucidating species relationship, depends on several practical considerations. These include the speed, simplicity, cost and reproducibility of the protocol, as

well as the ability of the technique to distinguish between large numbers of closely related individuals. Random amplified polymorphic DNA (RAPD) markers (Williams et al. 1990) have been successfully used for cultivar analysis and species identification in many plants, due to the technical simplicity and speed of the RAPD methodology (Gepts 1993). The species include papaya (*Carica papaya*) (Stiles et al. 1993), rye (*Secale cereale* L.) (Iqbal and Rayburn 1994), cotton (Multani and Lyon 1995; Khan et al. 2000), lentil (Sharma et al. 1995), *Phaseolus vulgaris* (Yonghe Bai et al. 1998), *Cicer* (Ahmad 1999), Perilla crops (Nitta and Ohnishi 1999), buckwheat (Tsuji and Ohnishi 2000), groundnut (Subramanian et al. 2000), tea (*Camellia sinensis* var. *sinensis*) (Kaundun et al. 2000) and *Lachenalia bulbifera* (Kleynhans and Spies 2000). To-date, however, RAPDs have not been used exclusively to study the phylogeny of *Fagopyrum*. The present study was undertaken to: (1) analyze and quantify genetic diversity in cultivated and wild *Fagopyrum*, and (2) compare the previously published results on the phylogeny of *Fagopyrum* with alternative markers.

Materials and methods

Plant material

Twenty eight *Fagopyrum* accessions belonging to 14 species and two subspecies were used in this study (Table 1). Plants were grown in the greenhouse and 10–12 day old seedlings were harvested for DNA extraction.

Genomic DNA isolation and quantification

Total genomic DNA was extracted using a commercially available DNA extraction kit (DNeasy Plant Mini Kit, Qiagen) according to the manufacturer's instructions. This required the disruption of 1 mg of frozen leaf material by grinding in liquid nitrogen prior to cell-lysis, and an RNase-A digest in lysis buffer (supplied) at 65 °C. An optional centrifugation step was included, prior to the removal of debris and salt-precipitated polysaccharides and proteins, by column filtration. The lysates were passed through a second column matrix to which DNA binds and then washed twice with an ethanol-based buffer (supplied). DNA was removed from the column in two rinses, each using 100 µl of the elution buffer provided. DNA was quantified using a GeneQuant-II Fluorimeter (Pharmacia Biotech) and diluted to 25 ng/µl with sterile nano-pure water.

Polymerase chain reaction (PCR)

PCR was carried out in 25-µl reaction volumes containing 14.8 µl of sterile distilled water, 0.2 µl (1 unit) of *Taq* polymerase (GIBCO BRL), 2.5 µl of 10 × PCR buffer (supplied with the enzyme), 2 µl of dNTP (made from a equimolar solution of 25 µM, each of dATP, dCTP, dGTP and dTTP), 1.5 µl of 50 mM $MgCl_2$ (GIBCO BRL, supplied with the enzyme) 2 µl (2 mM) of primer (Operon Technology Inc., Alameda, Calif., USA, and the University of British Columbia, B.C., Canada) and 50 ng of template DNA. The reaction was over-laid with sterile mineral oil (VWR Scientific) prior to PCR. The PCR amplification was performed on a PTC-100 (MJ Research Inc.) thermocycler programmed for 35 cycles with an initial strand separation at 94 °C for 3 min and 94 °C for 1 min, fol-

Table 1 List of *Fagopyrum* accessions sampled for DNA extraction

No.	Species name	Accession	Source	Origin
1	*F. tataricum* Gaertn.	JCR/TRS-501	HPKV[a]	India
2	*F. tataricum* Gaertn.	IC-49678	NBPGR[b]	India
3	*F. tataricum* Gaertn.	IC-109729	NBPGR	India
4	*F. tataricum* Gaertn.	IC-37278	NBPGR	India
5	*F. tataricum* Gaertn.	EC-13162	NBPGR	Italy
6	*F. tataricum* Gaertn.	IC-108498	NBPGR	India
7	*F. tataricum* Gaertn.	IC-26595	NBPGR	India
8	*F. tataricum* Gaertn.	EC-97262	NBPGR	USSR
9	*F. tataricum* Gaertn.	B-930475	KRL[c]	Nepal
10	*F. tataricum* Gaertn.	B-930614	KRL	Nepal
11	*F. tataricum* Gaertn.	B-930598	KRL	Nepal
12	*F. giganteum* Krotov (4*x*)	B-930296	KRL	Russia
13	*F. lineare* Sam.	K-980819	KRL	China
14	*F. pilus* Chen	K-990871	KRL	China
15	*F. tataricum* ssp. *Potanini* Batalin	B-930293	KRL	China
16	*F. urophyllum* (Bur.et Franch) Gross	B-930300	KRL	China
17	*F. homotropicum* Ohnishi (4*x*)	K-950818-4	KRL	China
18	*F. macrocarpum* Ohsako et Ohnishi	K-980859	KRL	China
19	*F. cymosum* (Trev.) Meisn.	K-990875	KRL	China
20	*F. pleioramosum* Ohnishi	B-930301	KRL	China
21	*F. statice* (Levl.) Gross	K-970845	KRL	China
22	*F. homotropicum* Ohnishi (2*x*)	B-930290	KRL	China
23	*F. gilessii* Hemsl.	K-990872	KRL	China
24	*F. esculentum* ssp *ancestrale* Ohnishi	B-930292	KRL	China
25	*F. capillatum* Ohnishi	B-930299	KRL	China
26	*F. gracilipes* Dammer	B-930298	KRL	China
27	*F. esculentum* Moench	EC-453750	NBPGR	Japan
28	*F. esculentum* Moench	EC-453743	NBPGR	Japan

[a] Himachal Pradesh Krishi Vishvavidyalaya, Palampur, India
[b] National Bureau of Plant Genetic Resources, New Delhi, India
[c] Kade Research Limited, Morden, Manitoba, Canada

lowed by an annealing temperature of 37 °C for 1 min and extension at 72 °C for 1.5 min. After 35 cycles, there was a final extension of 10 min at 72 °C followed by soaking at 4 °C. Amplification products were electrophoresed in a 1.4% agarose gel (Life Technologies Inc.) and diluted by staining with ethidium bromide. A standard molecular-weight marker (a 1-kb plus DNA ladder by Life Technologies Inc.) was used in each electrophoretic run. UV transilluminated gels were photographed on polaroid film.

Gel analysis

The amplification fragments were scored as present (1) and absent (0). Faintly stained bands were not included in the analysis. Each reaction was repeated twice, hence only reproducible products were included in the study. Based on the number of shared amplification products, data for all 19 primers were assembled into a data matrix. An unweighted pair group of arithmetic means (UPGMA) cluster-analysis was performed using the NTSYS-pc software (Jaccard co-efficient, phenetic tree style).

Results and discussion

The RAPD technique provides an efficient, simple and inexpensive method of generating molecular data. For this reason, this technique has been employed in many taxonomic and phylogenetic studies (Sharma et al. 1995; Wolff and Morgan-Richards 1998; Ahmad 1999; Khan et al. 2000). We assessed the suitability and reliability of RAPD markers to study species relationships in *Fagopyrum* based on high levels of reproducible polymorphism. This approach was also used to construct the buckwheat phylogeny.

We screened 75 decamer primers to amplify the genomic DNA of *Fagopyrum*. Of these, only 19 primers generated robust and reproducible bands. These primers differed greatly in their efficiency for revealing polymorphism. Many of them failed to amplify buckwheat DNA. Since the reaction conditions were kept uniform for all primers, differences in the amplification resolution and the clarity in the banding patterns were probably due to specific requirements of the primer. The significance of the G+C content of the primer on detectable amplification products has also been reported (Fritsch et al. 1993). However, our results did not show any relation between the G+C content of the primer and the number of bands amplified. We found that if the amplification conditions (reagents and thermocycler parameters) were identical for all reactions, the results were highly reproducible.

Nineteen primers were finally selected and used to analyze all 28 accessions for polymorphism. All reactions were duplicated and only highly reproducible bands were considered. The base sequences of the primers and their G+C contents are given in Table 2. In all, 364 reproducible fragments were amplified. Primer OPN-08 amplified the highest number of bands (28), while UBC#2-183 amplified only eight bands. All bands, except two monomorphic bands generated by primers UBC#2-186 and OPM-16, were polymorphic across species. The approximate size of the largest fragment was 5.0 kb, whereas the smallest recognizable fragment was about 0.25 kb in size. Different numbers of bands were amplified in different species. A single RAPD, OPN-19-2000, which was diagnostic of the *Fagopyrum* species used here, was identified (data not shown). Such diagnostic markers are important for strain identification and cultivar characterization, and can be used to detect instances of natural interspecific gene introgression.

The primers also revealed unique banding patterns for most species, indicating the wide genetic base of the *Fagopyrum* species (Fig. 1). The presence of unique composite RAPD markers among various *Fagopyrum* species indicates the usefulness of the approach for fingerprinting purposes. With the exception of accession IC-37278, which was found to be highly polymorphic, the within-species polymorphism was small in *F. tataricum*.

The estimates of pairwise genetic distances between the 28 accessions are given in Table 3. The similarity matrix was based on Jaccard's similarity coefficient. The

Table 2 The DNA sequence of random decamer oligonucleotide primers used for *Fagopyrum* species DNA amplification, their G+C content and % polymorphism

Primer number	DNA sequence	% G+C content	Number of bands produced	% Polymorphic bands
OPN-10	5′-ACAACTGGGG-3′	60	23	100
UBC#2-185	5′-GTGTCTTCAC-3′	50	16	100
UBC#2-186	5′-GTGCGTCGCT-3′	70	20	95
OPM-02	5′-ACAACGCCTC-3′	60	26	100
OPN-19	5′-GTCCGTACTG-3′	60	22	100
OPM-05	5′-GGGAACGTGT-3′	60	26	100
OPN-20	5′-GGTGCTCCGT-3′	70	23	100
UBC#2-183	5′-CGTGATTGCT-3′	50	8	100
UBC#2-187	5′-AACGGGGGAG-3′	70	11	100
OPM-01	5′-GTTGGTGGCT-3′	60	16	100
OPM-03	5′-GGGGGATGAG-3′	70	12	100
OPN-16	5′-AAGCGACCTG-3′	60	23	100
OPN-02	5′-ACCAGGGGCA-3′	70	26	100
OPO-19	5′-GGTGCACGTT-3′	60	27	100
OPM-06	5′-CTGGGCAACT-3′	60	16	100
OPN-08	5′-ACCTCAGCTC-3′	60	28	100
OPN-10	5′-ACAACTGGGG-3′	60	13	100
OPM-10	5′-TCTGGCGCAC-3′	70	21	100
OPM-16	5′-GTAACCAGCC-3′	60	20	95

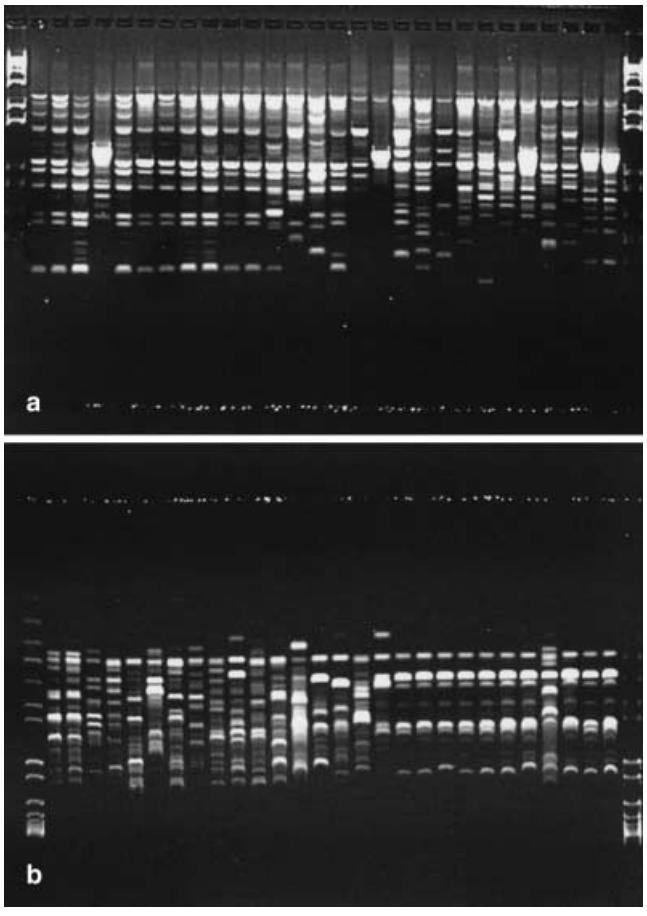

Fig. 1 Amplification profile of 28 *Fagopyrum* accessions generated using primers (*a*) OPM-16 and (*b*) UBC-186; *M* is a 1-kb plus DNA ladder

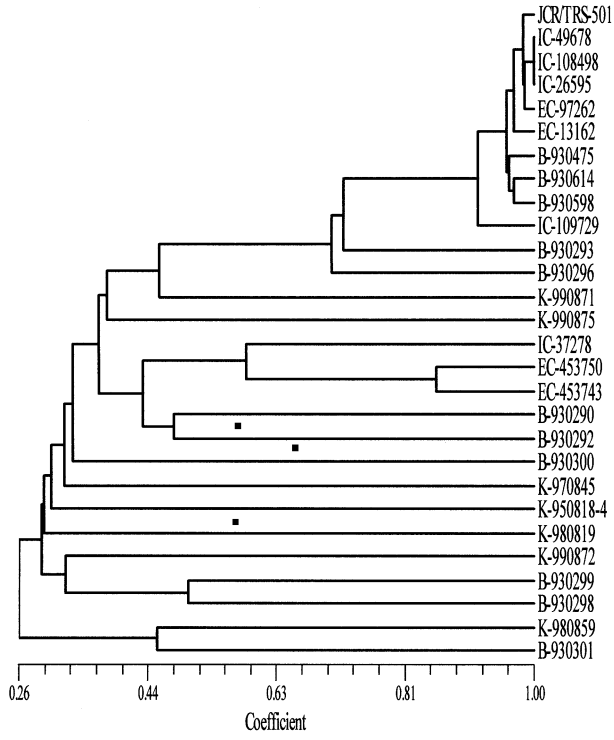

Fig. 2 Dendrogram of 16 *Fagopyrum* species and sub-species generated by RAPD data using the UPGMA method

interspecific genetic similarity indices ranged from 0.18 between *Fagopyrum lineare* (accession K-980819) and tetraploid *Fagopyrum homotropicum* (accession K-950818-4) to 0.73 between *F. tataricum* (accession B-930475) and its putative wild ancestor *F. tataricum* ssp. *potanini* (accession B-930293). In general, *Fagopyrum gilessii* showed a small level of similarity with all other species ranging between 0.19 to 0.34, thus forming an outgroup.

Cluster analysis of the genetic similarity estimates was performed using Jaccard's coefficient to generate a UPGMA dendrogram showing overall genetic relatedness among the *Fagopyrum* species (Fig. 2). Four distinct clusters, two each of the cymosum and urophyllum groups, were formed in the UPGMA dendrogram. Three species, *Fagopyrum statice*, *F. homotropicum* (4*x*) and *F. lineare*, did not cluster with any group. Most of the species clustered within their generic and sectional groups. For example, cluster 1 included all *F. tataricum* accessions, except IC-37278. Surprisingly this accession shared 57.5% of its bands with *F. esculentum* and showed 45.6% similarity with *F. tataricum*, although the seed and plant characteristics of this accession are more similar to *F. tataricum* than *F. esculentum*. This accession originated from the northeastern Himalayan region of India. The possibility of its being a chance interspecific hybrid appears remote as the natural hybridization between *F. tataricum* and *F. esculentum* is nearly impossible (personal communication, Ohmi Ohnishi). Possibly it was a mislabeled accession that warrants further investigation. The usefulness of the RAPD technique for identifying mislabeled accessions, in addition to its potential value in determining cultivar purity and genetic resource management, has been discussed previously (Sharma et al. 1995; Ahmad 1999). Cluster 1 also included *F. tataricum* ssp. *potanini*, a wild putative ancestor of tartary buckwheat and the other closely related species *Fagopyrum giganteum* (a hybrid species between *F. tataricum* and *Fagopyrum cymosum*), *Fagopyrum pilus* and *F. cymosum*.

Cluster 2 included *F. esculentum*, IC-37278, a putative wild ancestor of common buckwheat (*F. esculentum* spp. *ancestrale*) and the closely related *F. homotropicum* (2*x*). *F. homotropicum*, a wild self-fertilizing species was discovered by Ohnishi in 1992 in the Yunnan province of China (Ohnishi 1995, 1998). This recently discovered species was found to be closely related to the common wild buckwheat, *F. esculentum* ssp. *ancestrale* (Ohnishi and Matsuoka 1996; Yasui and Ohnishi 1998a). The self-fertilizing property of this species has been successfully transferred to self-incompatible *F. esculentum* (Campbell 1995; Hirose et al. 1995; Woo et al. 1995).

Table 3 Similarity matrix based on the Jaccard's similarity index

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
1	1.00																											
2	0.98	1.00																										
3	0.92	0.92	1.00																									
4	0.45	0.45	0.44	1.00																								
5	0.98	0.96	0.93	0.46	1.00																							
6	0.98	1.00	0.92	0.45	0.96	1.00																						
7	0.98	1.00	0.92	0.45	0.96	1.00	1.00																					
8	0.98	0.98	0.92	0.46	0.96	0.98	0.98	1.00																				
9	0.96	0.95	0.89	0.48	0.95	0.95	0.95	0.96	1.00																			
10	0.96	0.96	0.90	0.46	0.96	0.96	0.96	0.97	0.96	1.00																		
11	0.96	0.94	0.89	0.46	0.96	0.94	0.94	0.96	0.96	0.97	1.00																	
12	0.71	0.72	0.68	0.43	0.70	0.72	0.72	0.71	0.73	0.71	0.70	1.00																
13	0.29	0.30	0.30	0.29	0.27	0.30	0.30	0.29	0.29	0.30	0.30	0.30	1.00															
14	0.46	0.46	0.44	0.39	0.43	0.46	0.46	0.47	0.46	0.45	0.45	0.45	0.30	1.00														
15	0.72	0.71	0.70	0.45	0.71	0.71	0.71	0.71	0.75	0.73	0.73	0.65	0.32	0.44	1.00													
16	0.35	0.35	0.34	0.30	0.35	0.35	0.35	0.36	0.38	0.36	0.36	0.32	0.29	0.30	0.34	1.00												
17	0.29	0.28	0.27	0.30	0.27	0.28	0.28	0.29	0.30	0.29	0.30	0.29	0.18	0.31	0.27	0.27	1.00											
18	0.28	0.28	0.27	0.25	0.27	0.28	0.28	0.28	0.29	0.29	0.29	0.29	0.24	0.26	0.28	0.30	0.28	1.00										
19	0.38	0.38	0.36	0.32	0.35	0.38	0.38	0.39	0.39	0.38	0.37	0.38	0.24	0.41	0.35	0.32	0.34	0.25	1.00									
20	0.23	0.24	0.23	0.22	0.22	0.24	0.24	0.24	0.24	0.24	0.24	0.23	0.21	0.20	0.21	0.27	0.21	0.45	0.20	1.00								
21	0.33	0.32	0.31	0.31	0.31	0.32	0.32	0.33	0.36	0.33	0.32	0.30	0.24	0.29	0.31	0.32	0.24	0.27	0.30	0.24	1.00							
22	0.33	0.33	0.30	0.36	0.32	0.33	0.33	0.33	0.36	0.34	0.33	0.32	0.29	0.34	0.31	0.30	0.36	0.24	0.35	0.21	0.37	1.00						
23	0.26	0.26	0.25	0.24	0.26	0.26	0.26	0.27	0.29	0.27	0.26	0.27	0.20	0.27	0.27	0.23	0.19	0.25	0.25	0.21	0.30	0.30	1.00					
24	0.35	0.35	0.34	0.44	0.32	0.35	0.35	0.35	0.37	0.36	0.35	0.35	0.26	0.28	0.32	0.25	0.33	0.24	0.30	0.22	0.28	0.47	0.28	1.00				
25	0.30	0.30	0.29	0.31	0.29	0.30	0.30	0.31	0.33	0.32	0.31	0.28	0.27	0.30	0.31	0.30	0.26	0.26	0.28	0.27	0.30	0.34	0.29	0.35	1.00			
26	0.26	0.26	0.25	0.34	0.25	0.26	0.26	0.26	0.30	0.27	0.27	0.23	0.25	0.31	0.27	0.26	0.26	0.26	0.27	0.23	0.31	0.35	0.34	0.33	0.50	1.00		
27	0.34	0.34	0.34	0.57	0.32	0.34	0.34	0.35	0.36	0.36	0.35	0.33	0.32	0.31	0.34	0.29	0.32	0.30	0.35	0.24	0.29	0.40	0.24	0.48	0.30	0.37	1.00	
28	0.38	0.38	0.37	0.58	0.36	0.38	0.38	0.39	0.39	0.40	0.39	0.37	0.31	0.33	0.36	0.28	0.35	0.28	0.36	0.22	0.28	0.40	0.27	0.50	0.29	0.36	0.85	1.00

Based on the Jaccard's similarity indices and UPGMA clustering patterns, our study confirms the findings of Ohnishi (1998) that *F. tataricum* ssp. *potanini* is the putative wild ancestor of tartary buckwheat (*F. tataricum* Gaertn.), and *F. esculentum* ssp. *ancestrale* is the wild ancestor of common buckwheat (*F. esculentum* L. Moench.). The species *F. homotropicum* (2*x*) was found to be closely related to the common wild buckwheat. This could be because of their sympatric distribution along the Jhinsha river in the Yunnan province and the Yalong river in the Sichuan province of China.

Cluster 3 included *F. gilessii*, *Fagopyrum capillatum* and *Fagopyrum gracilipes*, whereas *Fagopyrum macrocarpum* and *Fagopyrum pleioramosum* formed cluster 4. The remaining species, *F. statice*, *F. homotropicum* (4*x*) and *F. lineare*, did not form clusters with any other species and formed discrete out-groups. In the earlier reports, two major *Fagopyrum* groups have been recognized; one (the cymosum group) included *F. esculentum*, *F. tataricum*, *F. cymosum* and their close relative *F. homotropicum* (Ohnishi and Matsuoka 1996). The urophyllum group included all the species that are distant from the cultivated species and those with small achnes (Ohsako and Ohnishi 2000). Both *F. cymosum* from the first group and *F. urophyllum* from the second group are heterostylous outbreeding perennial shrubs, probably representing the primitive type of each group (Ohnishi and Matsuoka 1996). Contrary to the earlier reports, *F. urophyllum* did not show much affinity with other species of the urophyllum group in our study.

The diploid and tetraploid forms of *F. homotropicum* appeared unrelated. The analysis of allozyme variation revealed that diploid populations of *F. homotropicum* were well differentiated (Ohnishi and Asano 1999), and suggested that tetraploid *F. homotropicum* was a allotetraploid of its probable diploid ancestors *F. homotropicum* and *F. esculentum* ssp. *ancestrale* (wild common buckwheat). Our results do not agree with this postulate. Isozyme and molecular phylogenetic analyses revealed that the distribution of *F. esculentum* ssp. *ancestrale* was restricted to the Yunnan province and some parts of the Sichuan province in China. The original distribution of *F. homotropicum* is also considered in these regions (Ohnishi and Asano 1999), which then extended to the Daduhe river valley of Sichuan province in the north, and gradually differentiated mainly by allelic fixation. It is also possible that the polyploidization of *F. homotropicum* occurred at more than one place and at different times. However, for a better understanding of the genetic differentiation between 2*x* and 4*x F. homotropicum*, a large number of populations from different geographical niches should be studied.

F. gilessii-F. capillatum-F .gracilipes and *F. macrocarpum-F. pleioramosum* clades were formed in the urophyllum group. Members of each of these clades share several common morphological characters. For example, both *F. gracilipes* and *F. capillatum* have heavy pubescence on the stem and stipules (Ohnishi and Matsuoka 1996; Ohsako and Ohnishi 1998). The tetraploid self-fertilizing species, *F. gracilipes*, is a sister species of the diploid outcrossing species, *F. capillatum*. Only two nucleotide substitutions have occurred in the ITS regions since the separation of *F. gracilipes* from its hypothetical ancestor (Ohsako and Ohnishi 2000). These results show

that *F. gracilipes* has recently originated from its diploid outcrossing ancestor.

F. macrocarpum and *F. pleioramosum* share heterostylous self-compatibility and larger achnes than other species in the urophyllum group (Ohsako and Ohnishi 2000). The distribution of these species is restricted to the upper Min river valley in China. Very little variation was observed among the cpDNA sequences of the five accessions of *F. pleioramosum* and *F. macrocarpum*, which is consistent with the high genetic similarity between these species revealed by isozyme analysis (Ohsako and Ohnishi 1998).

According to their molecular phylogeny, *F. lineare* and *F. urophyllum* are very close although they are morphologically quite different (Ohsako and Ohnishi 2000). Using nucleotide sequences in the cpDNA region, Yasui and Ohnishi (1998a) have shown the sister relationship between *F. lineare* and *F. urophyllum* accessions from Dali, China. We observed a low similarity index (0.29), consistent with their morphological distinctiveness. *F. lineare* might have accumulated autoapomorphic characters at both morphological and molecular levels since its divergence from its ancestor (Ohsako and Ohnishi 2000). It appears from our results that some of the species in the urophyllum group, which formed out-groups, may have been misclassified, warranting further investigation.

F. cymosum showed greater similarity with *F. tataricum* (0.37) and *F. tataricum* ssp. *potanini* (0.35) than with *F. esculentum* (0.35) and *F. esculentum* ssp. *ancestrale* (0.30). The RFLP analysis of cpDNA revealed that *F. cymosum* was more-closely related to *F. tataricum* ssp. *tataricum* than to *F. esculentum* ssp. e*sculentum* (Kishima et al. 1995). This relationship was confirmed by comparing the nucleotide sequences of the *rbcL-accD* region of cpDNA and nuclear DNA sequences (Yasui and Ohnishi 1998a, b). Since *F. cymosum* was recognized to be a primitive type of this group, it was believed that *F. esculentum* ssp. *ancestrale* differentiated from *F. cymosum* much earlier than *F. tataricum* ssp. *potanini* (Yasui and Ohnishi 1998b). These results are in agreement with the molecular systematic studies of Ohnishi and Matsuoka (1996), Ohsako and Ohnishi (1998) and Ohsako and Ohnishi (2000).

Our results agree well with the previous findings on species relationships in *Fagopyrum* using different marker approaches. We also observed a good agreement with the recognized taxonomic divisions within the genus *Fagopyrum*. However, the taxonomic position of some of the species in the urophyllum group needs further investigation. The study demonstrated the usefulness and reliability of the RAPD technique for phylogenetic studies. A recent study in *Cucumis melo* L. using AFLP, RAPD and RFLP suggests that these three types of markers may be equally effective and informative in detecting genetic polymorphism (Garcia-Mas et al. 2000).

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