Search for the wild ancestor of buckwheat II. Taxonomy of *Fagopyrum* (Polygonaceae) species based on morphology, isozymes and cpDNA variability

Ohmi Ohnishi^{1,*} and Yoshihiro Matsuoka²

¹*Plant Germ-Plasm Institute, Faculty of Agriculture, Kyoto University, Mozume-cho, Mukoh city, Kyoto 617, and*

²*Department of Bioscience, Fukui Prefectural University, 4-1-1 Kenjojima, Matsuoka, Fukui 910-11, Japan*

(Received 30 August, accepted 23 January 1997)

Four newly discovered and seven previously known *Fagopyrum* species were classified based on their morphology, isozyme variability, and RFLP of chloroplast DNA (cpDNA). Three independently constructed phylogenetic trees showed good agreement and we had sufficient confidence in them to assert that they probably reflect the phylogenetic relationships among the *Fagopyrum* species. The new classification differs from Steward (1930)'s classification in the position of *Fagopyrum tataricum* and *F. gracilipes*; *F. tataricum* is very close to *F. cymosum*. The new classification resolved several problems which we suffered from, when we used Steward's classification. Three new species, *F. pleioramosum*, *F. callianthum* and *F. capillatum* are closely related to *F. gracilipes*. New species *F. homotropicum* is very close to *F. esculentum* in morphology as well as in isozyme variability.

INTRODUCTION

In the previous paper of this series (Ohnishi, 1997a), the senior author described four new species (*Fagopyrum pleioramosum*, *F. capillatum*, *F. callianthum* and *F. homotropicum*) and one new subspecies (*F. esculentum* ssp. *ancestralis*) of the genus *Fagopyrum*, and gave their distribution in China and the Himalayan hills. During the genetic study of these new and previously described species using isozymes and chloroplast DNA (cpDNA), we found difficulties in the traditional taxonomic arrangement of the *Fagopyrum* species, in three respects; (1) the taxonomic position of *F. tataricum* Gaertn. (cultivated tatar buckwheat) which has been distinguished from all the other *Fagopyrum* species by kernel morphology (see Steward, 1930). However, morphologically *F. tataricum* appears to be closely related to both *F. esculentum* Moench (cultivated common buckwheat) and *F. cymosum* Meisn. (2) the phylogenetic relationships among the three species, *F. esculentum*, *F. tataricum*, and *F. cymosum*. It has been thought that *F. cymosum* is more closely related to *F. esculentum* than to *F. tataricum*, but the recent analysis of cpDNA by Kishima et al. (1995) showed that *F. tataricum* and *F. cymosum* are very closely related. Isozyme analysis on these three species (Ohnishi, 1983) supports this view. (3) the taxonomic position of *F. gracilipes* Dommer.

Many of the newly found species are morphologically similar to *F. gracilipes* (Ohnishi, 1997a) which is considered to be closely related to *F. esculentum* (Steward, 1930). Thus many species should be closely related to cultivated common buckwheat, *F. esculentum*, but in fact this is probably not the case.

Furthermore, the key characters used for the genus *Fagopyrum* (e.g. Munshi and Javeid, 1986) are apparently no longer valid; such criteria as laterally long thick cotyledons, and exerted achenes, not covered with persistent perianths are not applicable for many of the *Fagopyrum* species. If we retain these key characters, then only four species, *F. esculentum*, *F. tataricum*, *F. cymosum* and the newly found *F. homotropicum* remain in the genus *Fagopyrum*.

Recently, Ye and Guao (1992) classified buckwheat species. They took annual vs. perennial, shrubby vs. herbaceous and heterostylous outbreeding vs. homostylous self-pollinating as the key characters to divide the species. We believe that their classification is convenient for the practical division of the species in *Fagopyrum*, but it probably does not reflect the true phylogenetic relationships.

In the present study, we attempt to classify *Fagopyrum* species based on morphology, isozymes and cpDNA variability. Three independently constructed phylogenetic trees showed fairly good agreement and we had sufficient confidence in them to assert that they probably reflect the phylogenetic relationships among the *Fagopyrum* species.

* Corresponding author.

MATERIALS AND METHODS

Plant materials used for morphological analyses were grown in the green house, starting from seed samples collected by the senior author during his expeditions to China in 1988–1992. Identification of species was done by comparing our samples to the herbaria of Gray Herbarium, Harvard University (GH) and United States Herbarium of Smithsonian Institute (US). Those herbaria identified by Steward (1930) were primarily used. The details of herbaria used were described in Ohnishi (1997a).

Morphological variability was checked or measured for 5–10 plants in two pots for each population in 1992 and in 1993. The populations used for each species are shown in Table 1. All the *Fagopyrum* species described in Steward (1930), except *F. gilesii* Hemsl., *F. statice* (Levl.) Gross and *F. suffruticosum* Schmidt were included in this analysis, as well as the four newly discovered *Fagopyrum* species and one subspecies mentioned in Ohnishi (1997a). To classify *Fagopyrum* species based on morphological variability, we mainly took into account the cotyledons, achenes and ochreae and pubescence on the ochreae and stems.

Since *F. urophyllum* (Bur. et Franch.) Gross shows remarkable morphological differences between the samples from eastern Yunnan and from western Yunnan, two samples, one from Kunming (eastern Yunnan), and the other from Dali (western Yunnan) were used for cpDNA analyses. *F. lineare* Sam. was included in morphological and isozyme studies, but because of shortage of biomass, it

was not included in cpDNA analyses. For other species, only one population was used for each species in cpDNA analysis as shown in Table 1. *Polygonum thunbergii* Sieb. et Zucc. (a common weed in Japan) was used as an outgroup sample.

As for *F. suffruticosum*, it has been believed to be distributed in Sakhalin, and to be very closely related to *F. tataricum*. However, no one has confirmed its existence in Sakhalin. *F. gilesii* was originally classified to *Cephalophilon* (now included in *Persicaria*) by Hooker (1886), and is distributed in southern China to the north-western Himalayas, but we have never seen it. *F. statice* is distributed in the central parts of Yunnan province in China (Wu et al., 1986; you can see a sketch of this plant as well as that of *F. gilesii* in Ye and Guo, 1992; the senior author has recently found it in Yunnan province), but we could not include it in the present study.

For the electrophoretic analyses of isozymes, the method for *F. esculentum* described in Ohnishi and Nishimoto (1988) was applied to the other *Fagopyrum* species. Minor procedural modifications were necessary for some species, mostly in the choice of the extraction buffer, either Tris-HCl buffer (pH 8.0), or a buffer containing 1% mercaptoethanol. We examined 12 enzymes encoded by 19 loci.

The results of the allozyme survey have been already reported for *F. esculentum* (see for examples Ohnishi, 1985, 1988, 1993). Similar experiments have already been completed for *F. tataricum*, *F. tataricum* ssp. *potanini*, *F. cymosum*, *F. gracilipes* and *F. leptopodum*

Table 1. Plant materials used in this study

Species	Natural (or cultivated) populations used for		
	Morphology	cpDNA	Allozyme
<i>F. esculentum</i> Moench	Yongsheng (Y*) Maerkan (S)	Maerkan (S)	Yongsheng (Y) and other 20 populations (Y, S)
<i>F. esculentum</i> ssp. <i>ancestralis</i> Ohnishi	Yongsheng (Y)	Yongsheng (Y)	Yongsheng (Y) Lijiang (Y)
<i>F. homotropicum</i> Ohnishi	Yongsheng (Y)	Yongsheng (Y)	Yongsheng (Y)
<i>F. tataricum</i> Gaertn.	Wenchuan (S) Zhaojiao (S)	Zhaojiao (S)	Zhaojiao (S) and other 15 populations (S, Y)
<i>F. tataricum</i> ssp. <i>potanini</i> Batalin	Lhasa (T)	Lhasa (T)	Lhasa (T) and other 10 populations (Y, S, T)
<i>F. cymosum</i> Meisn.	Kunming (Y) Duijiangyan (S)	Duijiangyan (S)	Duijiangyan (S) and other 4 populations (Y, S)
<i>F. urophyllum</i> Bur. et Franch.	Kunming (Y) Dali (Y)	Kunming (Y) Dali (Y)	Kunming (Y) Dali (Y)
<i>F. leptopodum</i> Diels	Yongsheng (Y) Hanyuan (S)	Binchuan (Y)	Binchuan (Y) and other 9 populations (Y, S)
<i>F. lineare</i> Sam.	Binchuan (Y)		Binchuan (Y)
<i>F. gracilipes</i> Hemsl.	Kunming (Y) Dali (Y)	Dali (Y)	Kunming (Y) and other 11 populations (Y, S)
<i>F. pleioramosum</i> Ohnishi	Wenchuan (S)	Wenchuan (S)	Wenchuan (S)
<i>F. capillatum</i> Ohnishi	Yongsheng (Y)	Yongsheng (Y)	Yongsheng (Y)
<i>F. callianthum</i> Ohnishi	Lixian (S)	Lixian (S)	Lixian (S)

* Y, S and T stand for Yunnan province, Sichuan province and Tibet (all in China), respectively.

(Ohnishi, unpublished). For the remaining species, one or two populations were investigated (see Table 1) and only ten individuals were assayed for each population. Based on these ten samples, the gene frequencies were estimated as usual. Based on the gene frequency data, the genetic distances (Nei, 1972) between species were calculated and a phylogenetic tree was constructed by the UPG method (Sneath and Sokal, 1973). Intraspecific variability was completely ignored in this analysis.

For the cpDNA analysis, intact chloroplast was extracted by the same method as Ogihara and Tsunewaki (1982) from ca. 100g foliage leaves of one to several individuals for each population studied, and DNA was purified by the method of Kolodner and Tewari (1975). Chloroplast DNA was digested by six endonucleases, *Kpn*I, *Pst*I, *Pvu*II, *Sal*I, *Sma*I and *Xho*I, then electrophoretically separated in 0.7% agarose gels at 20V for 40 h. Resulting band patterns were detected by staining with ethidiumbromide and the band patterns were photographed under UV irradiation. For *F. esculentum*, *F. tataricum* and *F. cymosum*, the band patterns were exactly those expected from the physical maps obtained by Kishima et al. (1995). The base substitution rate was estimated for each pair of species according to Nei (1987), and a phylogenetic tree was constructed from the substitution rate matrix by both the UPG method (Sneath and Sokal, 1973) and by the neighbours joining (NJ) method (Saitou and Nei, 1987).

RESULTS

Morphological classification. By adopting Nakai's criteria on the characteristics of the genus *Fagopyrum* (1926), and primarily taking the characters of the embryo, the cotyledons and the achenes, and also considering the perianths, the pubescence of the ochreae and the stems, we arrived at a new classification of *Fagopyrum*. This classification is slightly different from those of Steward

(1930), Hedberg (1946), and Ye and Guao (1992).

The most significant difference between the present classification and that of Steward (1930) is the position of *F. tataricum* and *F. gracilipes*. Steward (1930) first separated *F. tataricum* from the other species based on kernel morphology. If we ignore the kernel morphology, then *F. tataricum* and *F. cymosum* are morphologically similar in many aspects.

The key characters for the new morphological classification of *Fagopyrum* are shown in Appendix.

Candidates of wild ancestors of cultivated buckwheat, *F. esculentum* ssp. *ancestralis* Ohnishi and *F. tataricum* ssp. *potanini* Batalin (see Ohnishi, 1991, 1997b) were classified as subspecies of two cultivated species, respectively, because they are different only in dormancy and shattering habit which are the key characters distinguishing cultivated species from its wild relatives.

A phylogenetic tree can be drawn by cladistic separations of species by key characters mentioned in Appendix, and it is shown in Fig. 1. The new species, *F. pleioramosum* Ohnishi, *F. callianthum* Ohnishi, and *F. capillatum* Ohnishi, are all closely related to *F. gracilipes*. *F. gracilipes* is a tetraploid and a completely self-fertilizing species (Ohnishi, 1989); however, its relatives are diploid and some (*F. pleioramosum* and *F. callianthum*) are predominantly self-fertilizing. *F. capillatum* is a definitely heterostylous cross-pollinating species as are many other species in *Fagopyrum*.

The taxonomic positions of *F. statice* and *F. gilesii* which are not included in this analysis will be discussed later.

Classification based on isozyme variability. The genetic distance between the species, based on allozyme variability is shown in Table 2. The genetic distance between populations within species has been estimated for some species, but variability within species was ignored in this analysis, hence the genetic distance within species is omitted from Table 2. Based on the genetic distances, a

Fig. 1. A phylogenetic tree for *Fagopyrum* species based on morphological cladistics (see Appendix)

Table 2. Genetic distance for each pair of taxa

Species	1	2	3	4	5	6	7	8	9	10	11	12	13
1		0.0348	0.0930	0.7176	0.6304	0.5410	1.0388	0.5888	0.7655	0.6413	0.9632	0.8207	1.0197
2			0.0944	0.8115	0.7227	0.5736	1.1042	0.6534	0.7588	0.6555	0.9736	0.8516	1.0297
3				0.7470	0.6757	0.5916	0.9996	0.6354	0.9473	0.8300	1.0364	0.8780	1.0926
4					0.0648	0.2932	0.6361	0.6302	1.1247	0.8117	1.2434	1.0513	1.3055
5						0.2288	0.6848	0.6292	0.9424	0.6591	1.0337	0.8731	1.0909
6							0.6195	0.6880	0.8740	0.6760	0.9784	0.9767	1.0405
7								0.5916	0.4917	0.6972	0.9196	0.9767	1.0405
8									0.4989	0.4700	0.6191	0.6483	0.6205
9										0.4879	0.5815	0.5785	0.8240
10											0.2897	0.3144	0.5014
11												0.1104	0.5795
12													0.6529
13													

Species: 1. *F. esculentum* 2. *F. esculentum* ssp. *ancestralis* 3. *F. homotropicum* 4. *F. tataricum* 5. *F. tataricum* ssp. *potanini*
 6. *F. cymosum* 7. *F. urophyllum* 8. *F. leptopodum* 9. *F. lineare* 10. *F. gracilipes* 11. *F. pleioramosum*
 12. *F. capillatum* 13. *F. callianthum*

Fig. 2. UPG phylogenetic tree based on isozyme variability.

phylogenetic tree was constructed by the UPG method (Sneath and Sokal, 1973), and it is given in Fig. 2. Apparently *Fagopyrum* species can be divided into two groups; one includes *F. esculentum*, *F. homotropicum*, *F. tataricum*, and *F. cymosum* (the cymosum group), the other includes all the remaining species (the urophyllum group). *F. urophyllum* and *F. leptopodum* formally belong to the second group; however, we note that they are morphologically intermediate between the two groups. The three newly found species and *F. gracilipes* are very closely related as we expected from morphological investigation. We found that it is difficult to separate the *F. tataricum* - *F. cymosum* clade from the *F. esculentum* - *F. homotropicum* clade on the basis of morphology, but using isozymes they are clearly resolved as shown in Fig. 2.

Classification based on RFLP of cpDNA. The molecular size of the total chloroplast genome in *Fagopyrum* is approximately 155 kbp in all the species examined. This is similar to the findings of Kishima et al. (1995).

The number of base pair substitutions/site (d) was estimated from the banding patterns for each pair of species, and it is given in Table 3. From these figures, a phylogenetic tree for *Fagopyrum* species was obtained by the UPG (Fig. 3) and NJ methods (Fig. 4). The two methods gave almost the same results, which are quite similar to that obtained from isozyme data (Fig. 2), however, they differ in the position of *F. leptopodum* with respect to *F. gracilipes* and the three newly discovered species. *F. leptopodum* is so closely related to *F. gracilipes* by cpDNA analysis that the difference between them is smaller than the differences observed within *F. esculentum* and *F. urophyllum*. Two *F. urophyllum* samples, one from Dali (western Yunnan) and one from Kunming (eastern Yunnan), show the remarkable differences in cpDNA; they also differ in morphology and in fertility in nature.

Table 3. Estimated number of substitutions/site d ($\times 10^4$) between pairs of taxa

Species	1	2	3	4	5	6	7	8	9	10	11
2	9.96										
3	101.45	90.10									
4	101.45	90.10	0.00								
5	114.44	102.80	16.73	16.73							
6	256.71	253.31	235.92	235.92	242.82						
7	303.07	299.71	259.62	259.62	277.55	50.92					
8	292.94	289.53	260.10	260.10	267.42	35.01	47.31				
9	270.82	267.42	239.29	239.29	246.21	21.98	40.72	18.67			
10	267.42	264.01	235.92	235.92	242.82	6.21	44.25	28.45	15.60		
11	274.19	270.82	242.64	242.64	249.59	25.07	24.51	28.13	15.42	18.67	
12	256.71	264.01	235.92	235.92	242.82	6.21	44.25	28.45	15.60	0.00	18.67

Species: 1. *F. esculentum* 2. *F. esculentum* ssp. *ancestralis* 3. *F. tataricum* ssp. *potanini* 4. *F. tataricum*
 5. *F. cymosum* 6. *F. leptopodum* 7. *F. urophyllum* (Kunming) 8. *F. urophyllum* (Dali)
 9. *F. callianthum* 10. *F. gracilipes* 11. *F. pleioramosum* 12. *F. capillatum*

Fig. 3. UPG phylogenetic tree based on RFLP of cpDNA. Numbers in Figs. 3, 4 are d (substitutions/site $\times 10^4$)

Fig. 4. NJ phylogenetic tree based on RFLP of cpDNA.

DISCUSSION

Fagopyrum is often included in *Polygonum* (sensu lato) as in Steward (1930); however, many taxonomists consider that *Fagopyrum* should be separated from *Polygonum* for various reasons; including general morphology (Nakai, 1926), pollen morphology (Hedberg, 1946; Doida, 1962a, b), and chromosome number (Doida, 1962a, b; Munshi and Javeid, 1986; $x = 8$ for *Fagopyrum*, $x = 10$, 11, 12 for other *Polygonum*).

What then is the key character for the genus *Fagopyrum*? Apparently, the criteria for *Fagopyrum* given by Graham and Wood (1965) and by Munshi and Javeid (1986) are incomplete. They considered only the cymosum group of our new classification; it may be sufficient if we know only the buckwheat species of the *F. cymosum* group as European taxonomists in 19th and early 20th centuries. We prefer Nakai's opinion on this account (1926) and take as the key character that thick plaited cotyledons are accumbently embedded in the center of the endosperm. By doing this, we unambiguously include all the *Fagopyrum* species and exclude species of *Polygonum* (sensu stricto), *Tiniaria* and other closely related genera.

As for *F. gilesii*, Hooker (1886) originally put it into *Cephalophylon* (now usually included in *Persicaria*) and some taxonomists included it in *Fagopyrum* (Gross, 1913; Steward, 1930). Unfortunately, we could not include this species in our samples, hence we could not decide its taxonomic position. We believe that we will be able to examine this species and give a taxonomic position in the near future.

When we compare our classification and Steward's (1930), we find that they are not so very different; in the classification of Steward (1930, pp. 113–114), if we put *F. tataricum* next to *F. cymosum* by virtue of kernel morphology and ignore Steward's key character (a) "surfaces of the achenes grooved", then we have a classification almost the same as ours.

Recently Hirose et al. (1994) commented on the phylogenetic relationships among *Fagopyrum* species using interspecific crossability (actually they measured pollen tube growth in styles and used this as an index of crossability). Their data are clearly consistent with our classification.

Both of the phylogenetic trees obtained from the isozyme and cpDNA variability show that *F. cymosum* and *F. tataricum* are much more closely related than are *F. esculentum* and *F. cymosum*. This was first noticed by Kishima et al. (1995). However, we had some difficulty finding the morphological characters similar between *F. cymosum* and *F. tataricum*, but different between *F. cymosum* and *F. esculentum*. More careful comparisons between *F. esculentum* ssp. *ancestralis*, *F. cymosum* (2x form) and *F. tataricum* ssp. *potanini* should be done in future analyses. Recently, more data have accumulated

relevant to this issue (Yasui and Ohnishi, 1995); by comparing the DNA sequence of the rubisco large subunit (*rbcL*) gene in the cpDNA, the close relationship between *F. tataricum* and *F. cymosum* has been confirmed.

Discrepancy between classification by numerical analyses of isozyme or DNA polymorphism data and classification by orthodox morphological key characters is still a subject of controversy (see for example Buth, 1984; Wiley, 1981). However, if we get the same results by both methods, then those classifications deserve considerable confidence. Our classification resolved discrepancies and problems mentioned before. Two major groups are recognized; one (the cymosum group) includes *F. esculentum*, *F. tataricum* and *F. cymosum* and their close relative *F. homotropicum*. The other group (the urophyllum group) includes those *Fagopyrum* species with small achenes, and all the species that are remotely related to cultivated species. *F. cymosum* from the first group and *F. urophyllum* from the second group are both typical heterostylous outbreeding shrubby perennial species, and may be the primitive types for each group. In this regard, *F. statice* which unfortunately was not included in this project is of some interest. It is a small perennial species and it is a heterostylous outbreeder (see a sketch in Ye and Guo, 1992). Steward (1930) classified it as a close relative of *F. leptopodum*. We speculate that its taxonomic position is intermediate between the *F. cymosum* and *F. urophyllum* groups and close to *F. leptopodum* and *F. lineare*.

Cultivated common buckwheat is a heterostylous self-incompatible species, as is well known, and so are many wild species in the genus *Fagopyrum*. However, several cultivated and wild species are homostylous self-fertilizing species; *F. tataricum*, *F. homotropicum* and *F. gracilipes* are examples. *F. pleioramosum* and *F. callianthum* are heterostylous, yet they are self-fertilizing. Judging from the phylogenetic relationships we obtained, the breakdown of heterostylous self-incompatibility occurred relatively recently. This is because no large group of species is entirely self-fertilizing. For every self-fertilizing species, there is a heterostylous species closely related to it.

Another significant event in the evolution of *Fagopyrum* is polyploidization. This is a very common phenomenon in the plant kingdom (see de Wet, 1979). In *Fagopyrum*, it occurred in *F. gracilipes* (4x), and in *F. cymosum* (both 2x and 4x exist). Again, there is no large group of species that is entirely tetraploid. No critical experiments have been done to study the possibility of autotetraploidy in *F. cymosum* and *F. gracilipes*, but the segregation of variant alleles at isozyme loci fits the idea that both are autotetraploids (Ohnishi, unpublished).

Based on the classification of *Fagopyrum* species including the wild forms of the cultivated species *F. esculentum* and *F. tataricum*, we can infer the ancestors of cultivated buckwheat, common buckwheat and tatary buckwheat.

This issue is treated in the next article (Ohnishi, 1997b).

The authors are grateful to Michael J. Simmons, the University of Minnesota, for his reading the manuscript and correcting the English and making numerous useful suggestions. Contribution No. 77 from Plant Germ-Plasm Institute, Faculty of Agriculture, Kyoto University.

REFERENCES

- Buth, D. G. (1984) The application of electrophoretic data in systematic studies. *Annu. Rev. Ecol. Syst.* **15**, 501–522.
- De Wet, J. M. J. (1979) Origins of polyploidy. In: *Polyploidy* (ed.: W. H. Lewis), pp. 3–15. Plenum, New York.
- Doida, Y. (1962a) Consideration on the intrageneric differentiation in *Polygonum*. I. *J. Jpn. Bot.* **37**, 3–12. (in Japanese)
- Doida, Y. (1962b) Consideration on the intrageneric differentiation in *Polygonum*. II. *J. Jpn. Bot.* **37**, 81–88. (in Japanese with English summary)
- Graham, S. A. and Wood Jr., C. W. (1965) The genera of Polygonaceae in the southeastern United States. *J. Arnold Arb. Harv. Univ.* **46**, 91–121.
- Gross, M. H. (1913) Remarques sur les Polygonees de l'Asie Orientale. *Bull. Geogr. Bot.* **23**, 7–32.
- Hedberg, O. (1946) Pollen morphology in the genus *Polygonum* l.s.lat. and its taxonomic significance. *Sven. Bot. Tidskr.* **40**, 371–404.
- Hirose, T., Ujihara, A., Kitabayashi, H. and Minami, M. (1994) Interspecific cross-compatibility in *Fagopyrum* according to pollen tube growth. *Breed. Sci.* **44**, 307–314.
- Hooker, J. D. (1886) *Flora of British India. v. Polygonaceae.* Reeve & Co., London.
- Kishima, Y., Ogura, K., Mizukami, K., Mikami, T. and Adachi, T. (1995) Chloroplast DNA analysis in buckwheat species: phylogenetic relationships, origin of the reproductive systems and extended inverted repeats. *Plant Sci.* **108**, 173–179.
- Kolodner, R. and Tewari, K. K. (1975) The molecular size and conformation of the chloroplast DNA from higher plants. *Biochim. Biophys. Acta* **402**, 372–390.
- Munshi, A. H. and Javeid, G. N. (1986) *Systematic Studies in Polygonaceae of Kashmir Himalaya.* Scientific Pub. Jodhpur.
- Nakai, T. (1926) A new classification of Linnean *Polygonum*. *Rigakkai* **24**, 289–301. (in Japanese)
- Nei, M. (1972) Genetic distance between populations. *Am. Nat.* **106**, 283–297.
- Nei, M. (1987) *Molecular Evolutionary Genetics.* Columbia University Press, New York.
- Ogihara, Y. and Tsunewaki, K. (1982) Molecular basis of the genetic diversity of the cytoplasm in *Triticum* and *Aegilops*. I. Diversity of the chloroplast genome and its lineage revealed by the restriction fragment analysis. *Theor. Appl. Genet.* **76**, 321–332.
- Ohnishi, O. (1983) Isozyme variation in common buckwheat, *Fagopyrum esculentum* Moench, and its related species. *Proc. 2nd Int. Symp. Buckwheat at Miyazaki*, 39–50.
- Ohnishi, O. (1985) Population genetics of cultivated common buckwheat, *Fagopyrum esculentum* Moench. IV. Allozyme variability in Nepali and Kashmirian populations. *Jpn. J. Genet.* **60**, 293–305.
- Ohnishi, O. (1988) Population genetics of cultivated common buckwheat, *Fagopyrum esculentum* Moench. VII. Allozyme variability in Japan, Korea and China. *Jpn. J. Genet.* **63**, 507–522.
- Ohnishi, O. (1989) Cultivated buckwheat species and their relatives in the Himalaya and southern China. *Proc. 4th Int. Symp. Buckwheat at Orel*, 562–571.
- Ohnishi, O. (1991) Discovery of the wild ancestor of common buckwheat. *Fagopyrum* **11**, 5–10.
- Ohnishi, O. (1993) Population genetics of cultivated common buckwheat, *Fagopyrum esculentum* Moench. IX. Concluding remarks on worldwide survey of allozyme variability. *Jpn. J. Genet.* **68**, 317–326.
- Ohnishi, O. (1997a) Search for the wild ancestor of buckwheat. I. Description of new *Fagopyrum* species and their distribution in China. *Fagopyrum* **15**, (in press)
- Ohnishi, O. (1997b) Search for the wild ancestor of buckwheat. III. The wild ancestor of cultivated common buckwheat and of tatar buckwheat. *Econ. Bot.* **50**, (in press)
- Ohnishi, O. and Nishimoto, T. (1988) Population genetics of cultivated common buckwheat, *Fagopyrum esculentum* Moench. X. Further studies on allozyme variability in the Indian and Nepali Himalaya. *Jpn. J. Genet.* **63**, 51–66.
- Sneath, P. and Sokal, R. (1973) *Numerical Taxonomy.* Freeman, San Francisco.
- Saitou, N. and Nei, M. (1987) The neighbor joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **4**, 406–425.
- Steward, A. N. (1930) The Polygoneae of eastern Asia. *Contrib. Gray Herb. Harv. Univ.* **88**, 1–129.
- Wiley, E. O. (1981) *Phylogenetics: the theory and practice of phylogenetic systematics.* Wiley, New York.
- Wu, C. Y., Yin, W. Q., Rao, S. Y., Tao, D. D., Yuan, S. H., Deng, X. F., Yuan, S. X., You, H. Z. and Lin, Q. (1986) *Index Florae Yunnanensis.* The People's Pub. House, Yunnan. (in Chinese)
- Yasui, Y. and Ohnishi, O. (1995) Phylogenetic relationship among three *Fagopyrum* species (*Fagopyrum esculentum*, *F. tataricum* and *F. cymosum*) revealed by nucleotide sequencing of *rbcL* gene. *Curr. Adv. Buckwheat Res.* **1**, 155–162.
- Ye, N. G. and Guo, G. Q. (1992) Classification, origin and evolution of genus *Fagopyrum* in China. *Proc. 5th Int. Symp. Buckwheat at Taiyuan*, 19–28.

APPENDIX

The key characters for the new classification of *Fagopyrum*.

- (1) thick plaited cotyledons lie in the center of the achene. (*Fagopyrum*) ..... (2)
- (2) a cotyledons horizontally long, large lusterless achene is partially covered with persistent perianths. .... (3)
  - b cotyledons laterally long or round, small lustrous grains are completely covered with persistent perianths. .... (4)
- (3) a cotyledons in endosperm are colorless, blade's veins are not transparent. .... (5)
  - b cotyledons in endosperm are yellowish, blade veins are transparent. .... (6)
- (5) a heterostylous and cross-pollinating species. .... *F. esculentum*
  - b homostylous, self-fertilizing species. .... *F. homotropicum*
- (6) a surface of achene is smooth. .... *F. cymosum*
  - b surface of achene is rough, with a canal in the center. .... *F. tataricum*
- (4) a five perianths are equal in size, the lower two lack a green stripe. .... (7)
  - b perianths consist of two smaller, and three larger ones; the lower small perianths have a greenish stripe. .... (8)
- (7) a blades are ovate or cordate. .... *F. leptopodium*
  - b blades are linear. .... *F. lineare*
- (8) a ochrea is green and not transparent. .... *F. urophyllum*
  - b ochrea is transparent with greenish stripes. .... (9)
- (9) a ochrea is not pubescent, main blade-vein number is 5. .... *F. callianthum*
  - b ochrea is pubescent, main blade-vein number is 7. .... (10)
- (10) a ochrea is not heavily pubescent, stem is not pubescent, it has many creeping branches. .... *F. pleioramosum*
  - b ochrea is heavily pubescent, stems are also pubescent. .... (11)
- (11) a ochreae and stems heavily pubescent, blades cordate or sagitate, inflorescences are drooping. .... *F. gracilipes*
  - b pubescence in ochreae and stems is not so heavy as (11-a), blades are cordate or ovate, branches are erect. .... *F. capillatum*