ISSN 0352-3020

FAGOPYRUM

NOVOSTI O AJDI - NAJNOWSZE INFORMACJE O GRYKE
BUCKWHEAT NEWSLETTER - БЮЛЛЕТЕНЬ ''ГРЕЧИХА''
NOVOSTI O HELJDI - LES ANNALES DE SARRASIN
कूटू: समाचार - पत्रक ソバ ニュースレター

FAGOPYRUM (LJUBLJANA), Vol. 13, 1993, 1–44

FAGOPYRUM (NOVOSTI O AJDI), zvezek 13, Ljubljana 1993.
Izdajatelj: Biotehniška fakulteta, Oddelek za agronomijo, Center za rastlinsko biotehnologijo in žlahtnjenje, Jamnikarjeva 101, 61111 Ljubljana
Za publikacijo odgovarja: Ivan Kreft
Oblikovanje teksta in ilustracij: Borut Bohanec. Tekst urejen s programom za namizno založništvo STEVE avtorja Primoža Jakopina, Trnovska 2, Ljubljana
Lektoriranje angleščine: Martin Cregeen
Tisk: Tiskarna Planprint d.o.o. Ljubljana, Rožna dolina c. IV/32-36, cena izvoda 100 SIT.
V publikacijo so vključena znanstvena dela (izvirna dela in pregledni članki), vsako pred objavo pregledata vsaj dva mednarodno priznana strokovnjaka. Bibliografske in druge informacije niso recenzirane.
Po mnenju Ministrstva za znanost in tehnologijo št. 2839/92 z dne 5. 3. 1992 šteje revija Fagopyrum med proizvode, za katere se plačuje 5 % davek od prometa proizvodov na osnovi 13. točke tarifne številke 3 tarife davka od prometa proizvodov in storitev.
Pri finansiranju revije Fagopyrum sodeluje Ministrstvo za znanost in tehnologijo Republike Slovenije.

FAGOPYRUM (BUCKWHEAT NEWSLETTER), Vol. 13, Ljubljana 1993.
Published under the auspices of the International Buckwheat Research Association (IBRA).
Editor: Ivan KREFT (Biotechnical Faculty, Agronomy Department, Jamnikarjeva 101, 61111 Ljubljana, Slovenia)
Advisory committee:
Taiji ADACHI (Fac. of Agriculture, Miyazaki University, Gakuen Kibanadai Nishi 1-1, Miyazaki 889-21, Japan),
N.V. FESENKO (All-Union Research Institute of Legume and Groat Crops, Orel, Russian Federation),
Toshiko MATANO (Fac. of Agriculture, Shinshu University, Ina, Nagano-Ken, Japan),
Takashi NAGATOMO (7075 Funahiki, Kiyotake-cho, Miyazaki, 889-16, Japan),
Marek RUSZKOWSKI (Institute of Soil Science and Plant Cultivation, 24-100 Pulawy, Poland)
V.P. SINGH (School of Studies in Botany, Vikram University, Ujjain 456 010, India).
Language editor: Martin Cregeen
Managing editor: Borut Bohanec
Published by: Biotechnical Faculty, Agronomy Department, 61111 Ljubljana, Jamnikarjeva 101, Slovenia
Subscription Information: One volume per year (one issue in 1993).
Subscription prices for 1993 are 30.00 USD for individuals and scientific institutions.

FAGOPYRUM (Buckwheat Newsletter) is open to everyone interested in buckwheat and will cover all aspects of buckwheat research: genetics, cytology, breeding, cultivation, nutrition, utilization, biochemistry and other. No special priority is given to any language. Scientific papers, reviews, research notes on work in progress or on results not yet published: comments and speculations related to buckwheat; list of stock materials wanted or available; lists of names, addresses and field of work of scientists who have expressed the desire to receive the Newsletter; lists of publications which are related to buckwheat and which have appeared during preceding years; announcements concerning the promotion of research on buckwheat (workshop, symposia, and so on); abstracts and/or contents of published papers/books on buckwheat; bibliographies and other information related to buckwheat or buckwheat research will be published. In order to facilitate the elaboration of the bibliography scientists are asked to send reprints of their own publications to the editor of Fagopyrum.

Front page photo: Cultivation of buckwheat in Tibet: harvest of common buckwheat at Shigatse and flowering of common buckwheat, at 10km east of Shigatse (See paper of O. Ohnishi, pp. 3-10)

KAZALO - CONTENTS

The publication of this issue of Fagopyrum was supported by:

The Ministry of Science and Technology of Slovenia;
Japanese members of IBRA (International Buckwheat Research Association) and by
Mr. Satsuma, of Mimiu, Osaka and Tokyo;
The Centre of Plant Biotechnology and Breeding, Agronomy Department, Biotechnical Faculty, University of Ljubljana

Fagopyrum 13 (1993): 3 - 10

A memorandum on the distribution of buckwheat species in Tibet and the Himalayan hills: has buckwheat crossed the Himalayas?

Ohmi Ohnishi
Laboratory of Genetics, Faculty of Agriculture, Kyoto University, Kyoto 606, Japan

Key words: *Fagopyrum cymosum*, *F. gracilipes*, *F. tataricum*, allozyme variability, buckwheat cultivation in Tibet, wild form of tartary buckwheat.

Abstract

Cultivation of common and Tartary buckwheats is rare in central and western parts of Tibet, while it is relatively extensive in eastern parts of Tibet. As for the wild species, wild Tartary is widely distributed throughout Tibet, mainly along the Yalutsangpu river. The distribution of *F. cymosum* is limited only in eastern parts of Tibet. No other wild species can be seen. Cultivated buckwheats, both common and Tartary, diffused from southern China, their original birth place, along the southern slopes of the Himalayas and two wild species, *F. cymosum* and *F. gracilipes*, took the same route in their diffusion. Wild Tartary has spread westward along the Yalutsangpu river in Tibet and arrived at Kashmir and Karakoram. Transmittance of buckwheat species across the Himalayas has been extremely limited, if any.

Raziskava razširjenosti vrst ajde v Tibetu in na območju Himalaje: ali je ajda prečkala Himalajo?

V osrednjih in zahodnih predelih Tibeta le redko pridelujejo navadno in tatarsko ajdo, medtem, ko jo relativno pogosto pridelujejo v vzhodnih predelih. Od divjih vrst je divja tatarska ajda razširjena po vsem Tibetu, predvsem ob reki Yalutsangpu. *F. cymosum* je le v vzhodnih predelih Tibeta. Druge divje vrste niso našli. Gojena ajda, tako navadna kot tatarska, sta se sem razširili iz južne Kitajske, od koder izvirata, po južnih pobočjih Himalaje. Tudi divji vrsti *F. cymosum* in *F. gracilipes* sta se širili po isti poti. Divja tatarska ajda se je razširjala ob reki Yalutsangpu vse do Kašmirja in Karakorama. Prehoda vrst ajde preko Himalaje ni bilo, ali pa je bil le v zelo majhnem obsegu. (Prevod uredništva).

Introduction

Travelling in Tibet is not an easy task for foreigners, because of the poor transportation system and of the governmental restriction policy against foreigners. There is, therefore, no recent report on any cultivated crops in Tibet by European scientists. I had opportunities to travel in Tibet in 1990 and 1992, and had chances to see buckwheat cultivation and the distribution of wild buckwheat species. Wang (1989) has already reported on the cultivation and distribution of buckwheat in Tibet, mainly the eastern parts of Tibet, and commented on the rich buckwheat genetic resources in this area.

This article overviews buckwheat cultivation and the distribution of wild species in the central parts of Tibet. I entirely rely on Wang (1989) for information on the distribution in eastern

parts of Tibet. Nothing is reliably known for western parts of Tibet. By comparing wild species distribution in Tibet and the Himalayan hills, and by a comparative isozyme study of cultivated common and Tartary buckwheat in these two regions, I concluded that buckwheat species, both cultivated and wild ones, have never crossed the Himalayas; cultivated common and Tartary buckwheat diffused along the southern slopes of the Himalaya (see, Ohnishi 1992b), while wild Tartary buckwheat travelled westward along the Yalutsangpu (Brahmaputra) river in Tibet and arrived in Kashmir and Karakoram, where we can now see it.

Cultivated and wild buckwheat species in Tibet

My travel route in Tibet is given in Figure 1. At present, foreigners are completely excluded from eastern parts of Tibet, Changdu and Linzhi districts; so I quote Wang (1989) on the distribution of buckwheat in this region. Fig. 1 also gives the distribution of buckwheat species in Tibet and the Himalayan hills.

I saw no buckwheat cultivation in the Lhasa district in 1990 and 1992; wheat and barley supplemented by vegetables such as potatoes, beans, radish and rape are the main crops in this area (maize is lacked in Tibet) . The same is also true in the Shannan district (Zedang, Gongga; see Fig. 1), where the climate is slightly warmer and drier than Lhasa. Wild Tartary (*F. tataricum* ssp. *Potanini* Batalin), however, can be seen commonly on road sides and farmer's fields both in Lhasa and Shannan.

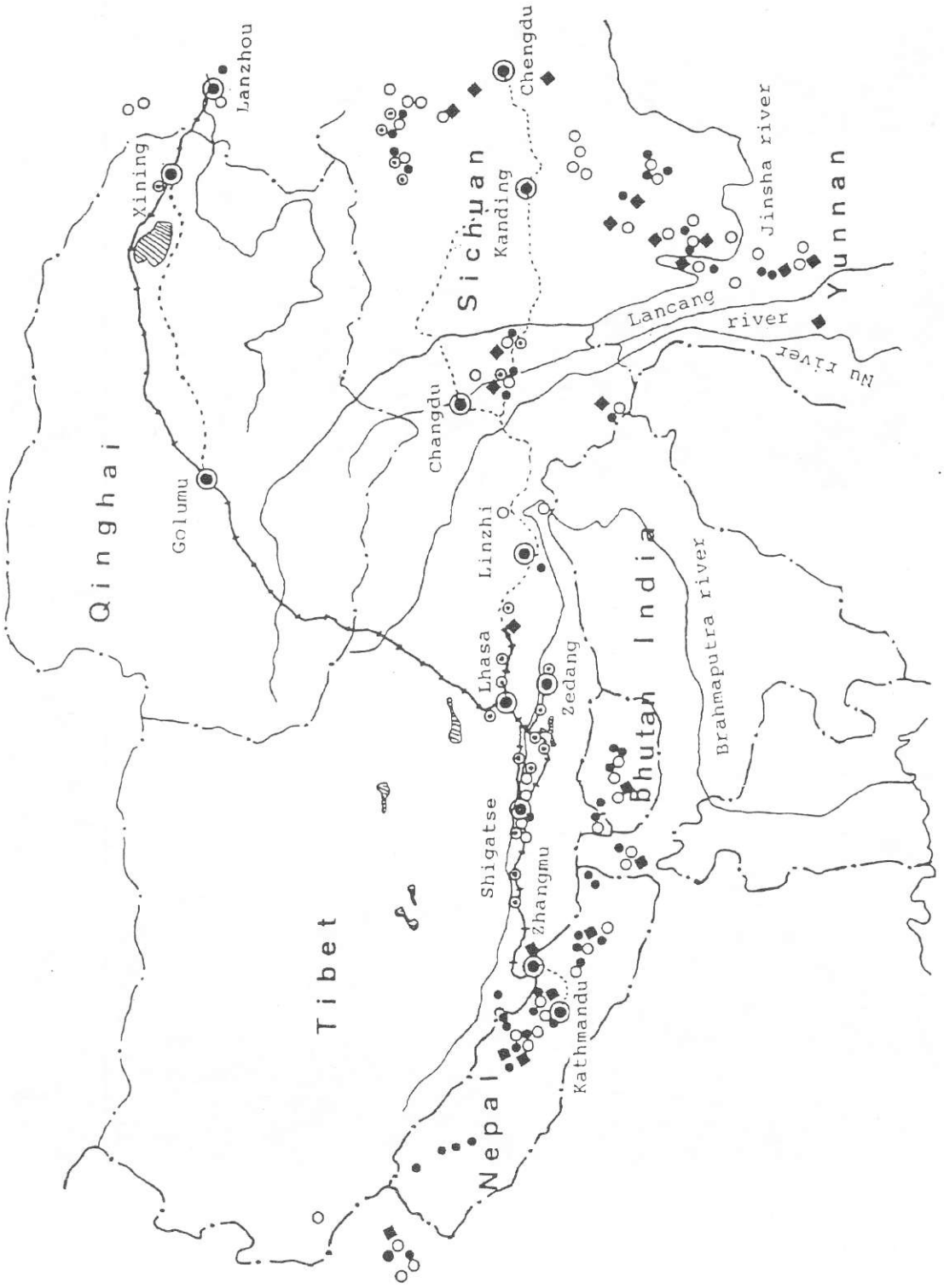
In contrast, in Shigatse I saw cultivation of common buckwheat (*F. esculentum* Moench) and occasionally Tartary buckwheat (*F. tataricum* Gaertn.), too (see Fig. 2). Chinese farmers rather than Tibetans mainly cultivated them. Because of the cool climatic conditions, the plant is only 30-60 cm tall. It is sown in July and harvested in late September. It is consumed as a tsampa like food and never as noodles. Wild Tartary buckwheat could be also seen in Shigatse, primarily in barley or buckwheat fields. No other wild buckwheat species was found in Lhasa and Shigatse districts.

Wang (1989) reported cultivation of common buckwheat along the Yalutsangpu river from Mangkan in the east to Zhada in the west. However, the distribution along the river is apparently discontinuous, there being a major disruption in the Lhasa area. Furthermore, I can not believe common cultivation in western parts of Tibet, around Zhada in the Ali district. He also mentioned that Tartary buckwheat is cultivated in almost the same regions as common buckwheat; i.e. along the Yalutsangpu river and more extensively in eastern parts of Tibet.

As for wild Tartary, he mentioned a distribution up to 4900 m above sea level at Mt. Mila and it forms populations at lower altitudes, below 3000 m. Plant height is lower, several tens of cm in the high altitude zone, but at a lower altitude, it may be higher than 100 cm, as I saw in Sichuan province. He distinguished several grain types, grey, black, notched winged, but I believe that they all belong to the same subspecies, *F. tataricum* ssp. *Potanini* Batalin, the wild form of tartary buckwheat.

He also reported the distribution of *F. cymosum* Meisner in south eastern parts of Tibet; he described large leafed and small leafed *F. cymosum*. I cannot decide which scientific subspecies or variety correspond to each, or whether the difference in leaf size is due only to soil or climatic conditions. *F. cymosum* is seen mainly in warmer districts as a cultivated buckwheat species. He noticed the rich genetic resources of cultivated buckwheat and *F. cymosum* in Tibet and mentioned that the history of cultivation in eastern Tibet is very old, more than 4600 years in the Lancang river valley near Changdu (The Lancang river is the upper stream of the Mekon river, see Fig. 1).

Fig.1: Distribution of Fagopyrum species in Tibet and the Himalayan hills.
+++++ Travelling route
o: F. esculentum
•: F. tataricum (cultivated)
⊙: F. tataricum ssp. Potanini (wild form)
◆: F. cymosum



A

B

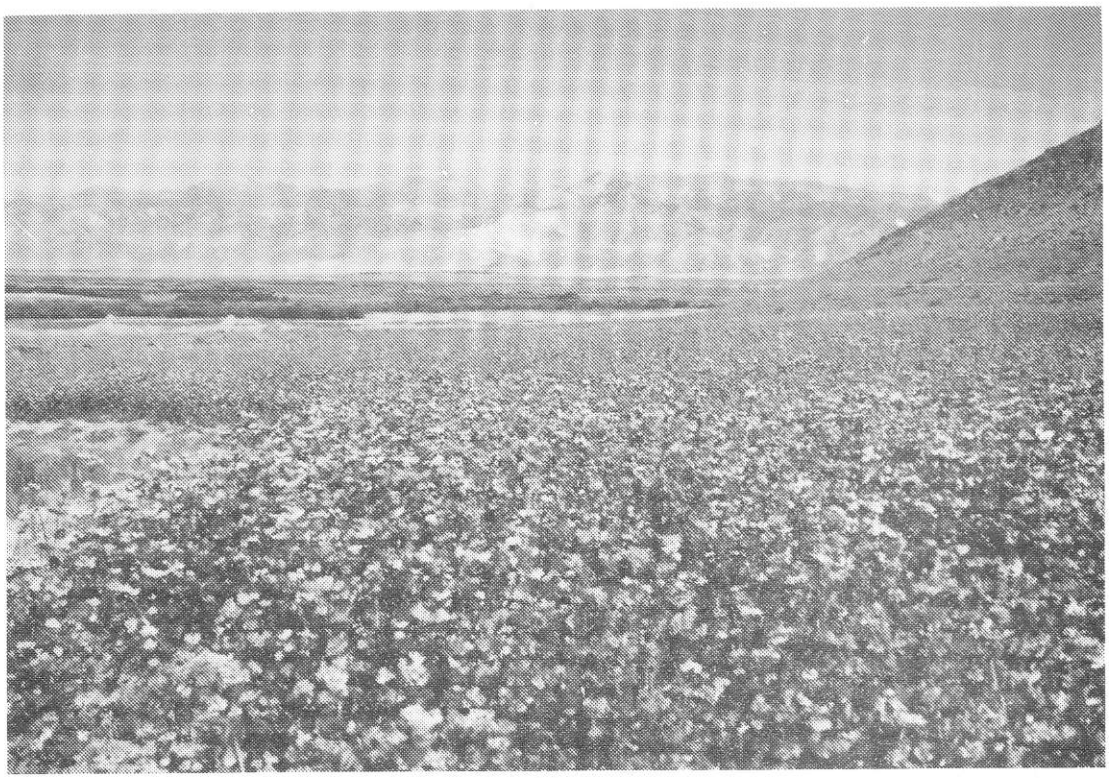


Fig. 2: Cultivation of buckwheat in Tibet: A: Harvest of common buckwheat at Shigatse, B: Flowering of common buckwheat, at 10km east of Shigatse.

Table 1. Allele frequency(%) at polymorphic loci of common buckwheat populations in Tibet

Locus / Allele / Population	6-PDH-1			PGM-1			PGM-2			ADH			MDH-1	
	S	N	F	S	N	F	S	N	F	S	N	F	N	F
Shigatse W-1	9.3	90.7	0.0	6.5	93.5	0.0	4.8	90.4	4.8	0.0	98.7	1.3	46.7	53.3
" W-2	2.3	97.7	0.0	6.3	93.7	0.0	7.3	89.2	3.5	0.0	98.0	2.0	51.2	48.8
" E-1	3.0	96.7	0.3	6.5	93.5	0.0	7.0	87.7	5.3	0.0	99.7	0.3	49.7	50.3
" E-2	5.0	95.0	0.0	5.8	94.2	0.0	7.3	87.7	5.0	0.0	100.0	0.0	48.5	51.5
Average of Tibet[1)]	4.9	95.0	0.1	6.3	93.7	0.0	6.6	88.7	4.7	0.0	99.1	0.9	49.0	51.0
Sichuan[2)]	2.8	96.6	0.6	12.5	87.4	0.1	13.6	77.8	8.6	0.1	97.2	2.7	57.5	42.5
Yunnan[2)]	5.0	94.5	0.5	12.6	87.3	0.1	12.9	78.3	8.8	0.4	97.4	2.2	63.1	35.9
Bhutan[3)]	2.8	97.1	0.1	9.4	90.1	0.6	9.0	87.0	4.0	0.3	98.1	1.6	59.4	40.6
East Nepal[4)]	5.3	94.4	0.3				11.5	81.0	7.5	0.6	99.4	0.0	69.5	30.5

Locus / Allele / Population	MDH-3				SDH-1			GOT-2			DIA-2			PGI-1	
	U	S	N	F	U	S	F	U	S	N	S	N	F	S	N
Shigatse W-1	0.3	18.8	67.1	13.8	2.8	56.5	40.7	4.0	47.3	48.7	1.5	98.5	0.0	0.0	98.7	1.3
" W-2	0.0	17.5	68.0	14.5	0.3	57.3	42.4	7.3	55.4	37.3	1.8	98.2	0.0	0.3	99.7	0.0
" E-1	0.8	17.8	64.4	17.0	1.3	57.2	41.5	7.3	56.8	35.9	2.5	97.5	0.0	0.0	99.2	0.8
" E-2	2.0	16.5	71.0	10.5	0.3	56.5	43.2	7.5	55.3	37.2	1.0	99.0	0.0	0.0	99.7	0.3
Average of Tibet[1)]	0.8	17.7	67.6	13.9	1.2	56.9	41.9	6.5	53.7	39.8	1.7	98.3	0.0	0.1	99.3	0.6
Sichuan[1)]	1.0	22.7	61.8	14.5	0.2	47.6	52.2	10.0	47.6	42.4	5.3	94.3	0.4	0.2	99.4	0.4
Yunnan[2)]	1.3	24.0	62.7	12.0	0.2	52.7	46.1	10.2	54.2	35.6	4.8	94.6	0.6	0.2	99.4	0.4
Bhutan[3)]	1.0	17.6	61.9	19.5	0.1	54.5	45.4	12.6	47.0	40.4	9.9	90.1	0.0	0.1	99.5	0.4
East Nepal[4)]	0.0	19.2	68.2	12.6	0.1	59.7	40.2	8.2	46.4	45.4	4.3	92.5	3.2			

1) Average of 6 populations(Ohnishi unpublished) 2) Average of 5 populations(Ohnishi unpublished) 3) Average of 5 populations(Ohnishi 1992) 4) Average of 3 populations (Ohnishi and Nishimoto 1988)

Allozyme variability in Tibetan buckwheat

In Table 1, the frequency of different alleles at ten polymorphic loci is given for four common buckwheat populations in Tibet. For the procedures of electrophoresis and designation of isozyme loci, see Ohnishi and Nishimoto (1986), Ohnishi (1990). The Tibetan populations have similar gene frequency to populations in Sichuan province, Nepal and Bhutan. In such a narrow geographical range as Tibet, southern China, Nepal and Bhutan, no local differentiation of gene frequency is expected (see Ohnishi 1988). Losses of alleles with a low frequency are the most popular genetic events that may occur during the diffusion of buckwheat cultivation. The F allele at 6-P 1, the S allele at ADH and the F allele at DIA-2 have been lost in central Tibet.

Buckwheat cultivating farmers in Shigatse are usually Chinese, as already mentioned. The food and cooking customs among Tibetan Chinese usually come from eastern Tibet or Sichuan province. I suspect that buckwheat cultivation in central Tibet is of relatively recent origin and probably came from eastern Tibet or Sichaun province, although we have already been informed that buckwheat cultivation has a long history in eastern parts of Tibet.

Table 2 gives the allele frequency of Tibetan Tartary buckwheat, both cultivated and wild forms, along with data on other regions. Tibetan Tartary buckwheat has exactly the same allozyme constitution as cultivated Tartary in southern China, and wild tartary from Kashmir and Pakistan. The variant F allele at Lap locus found in Bhutan and Nepal (see Ohnishi 1992b) has never been found in Tibet. The allozyme data on Tartary buckwheat suggest that Tartary buckwheat in Tibet came from the original birth place, Sichuan province, and went farther westward to Kashmir and Karakoram; but we have no positive data on migration of buckwheat across the Himalayas.

Table 2. Allele frequency (%) at polymorphic loci in wild and cultivated tartary buckwheat populations

Locus	Allele	Wild population				Cultivated population			
		Lixian	Markan	Central Tibet	Pakistan	Northern China[1]	Bhutan[2]	Nepal[3]	Southern China[4]
PGM-2	N	0	0	100	100	100	100	100	100
	F	100	34.6	0	0	0	0	0	0
	G	0	65.4	0	0	0	0	0	0
ADH	N	0	9.1	100	100	100	100	100	100
	F	100	90.9	0	0	0	0	0	0
LAP	F	0	0	0	0	0	69.2	36.8	0
	N	0	0	100	100	100	30.8	63.2	100
	S	46.2	92.3	0	0	0	0	0	0
	U	53.8	7.7	0	0	0	0	0	0
IDH	N	100	100	100	100	44.1	100	100	100
	F	0	0	0	0	55.9	0	0	0

1, 2, 3 and 4 : average of 8, 19, 19 and 10 populations, respectively

Discussion

Commercial, cultural and political contact between Tibet and the Himalayan hills are wellknown to have been historically extensive (see for example Bhatt 1977). Among crops, barley, Tibetan's major food as tsampa, is believed to have been transported across the Himalayas. Naked six-rowed barley cultivated in high mountain hills in Nepal and India comes from Tibet (Nakao 1956, Takahashi et al. 1968).

Buckwheat, both common and tartary, are extensively cultivated in the high hills of the Himalayas. They are commonly cultivated in eastern parts of Tibet (Wang 1989), Linzhi and Changdu districts, but cultivation is relatively rare in central and western parts of Tibet. Today, it is well recognized that common buckwheat originated in Yunnan province of China (Ohnishi 1991, Li and Yang 1992) and diffused along the southern slopes of the Himalayas in one direction (Ohnishi 1992b), while Tartary buckwheat was born in the western part of Sichuan province and travelled westward following the same route as common buckwheat (Ohnishi 1992a). Two wild weedy species, *F. cymosum* and *F. gracilipes* Hermsl. took the same route as cultivated buckwheat (see Ohnishi 1992b).

As can be seen in Figure 1, *F. cymosum* is distributed over the eastern parts of Tibet, but completely lacking in central and western parts of Tibet. In contrast, wild tartary buckwheat (*F. tataricum* ssp. *Potanini* Batalin) is widely distributed in Tibet, and further west up to Kashmir and northern Pakistan. Wild tartary has never been reported from the southern slopes of the Himalayas (see Hooker 1886, Hara 1966, Polunin and Stainton 1984). Among wild tartary populations, only the wild population from Sichuan province has genetic variability in isozymes(see Table 2, Ohnishi 1992a) that clearly indicates diffusion of wild tartary from east to west along the Yalutsangpu river, Nakao (1957)'s Tibetan arc. This is in sharp contrast with the diffusion of cultivated tartary mentioned above. Thus, in spite of a high possibility of transmittance of buckwheat species across the Himalayas, no buckwheat species seems to have crossed. The assertion that tartary buckwheat cultivated in the Himalayan hills came from Tibet, by Matsuoka (1956), may be misleading.

F. cymosum found at Zhangmu, in Tibet, located just on the border of Tibet and Nepal (see Fig. 1) is the only sample which crossed the Himalayas. Apparently, this sample came from Nepal. Wang(1989) described a notched wing tartary in Jilong xian in central Tibet. Contamination of notched wing tartary is a characteristic in the high hills of the Kaligandaki- Mustang region (Hirose et al. 1992); since Jilong is not so far from Kaligandaki, this tartary may also be a migrant from Nepal, rather than from eastern Tibet.

Why did buckwheat species not cross the Himalayas? As far as wild tartary in Tibet is concerned, climatic conditions at several passes over 4000 m a.s.l. between the Himalayas and the Yalutsangpu valley seems too severe for natural settlement. *F. cymosum* generally prefers a relatively warmer climate. It arrived at Zhangmu in Tibet from Nepal, but it could not proceed to the Tibetan plateau, probably for the same reason mentioned above.

Cultivated buckwheat may have overcome high passes or plateaus with human assistance, but actually it didn't. Migration of peoples and of the accompanying crops seem mainly from Tibet to the Himalayan hills; rice and sugarcane of Nepal and India were bartered for salt and wool from Tibet (see, for example, Hagen 1980).

Cultivation of buckwheat in central Tibet was rare, so buckwheat has no chance to migrate from Tibet to the Himalayan hills. Buckwheat cultivation in the Himalayan hills was widespread, and probably came from southern China or eastern Tibet as discussed in Ohnishi

(1992b). It never spread to the Tibetan plateau and the Yalutsangpu valley. At present, I have no reasonable explanation of this, but barren plateaus and high passes in Tibet rather than the Himalayan massif itself may have been a barrier against the diffusion of buckwheat.

References

Bhatt D. D. 1977. *Natural History and Economic Botany of Nepal.* Orient Longman, Calcutta

Hagen T. 1980. *Nepal-Kingdom in the Himalaya*, 3rd ed. Kumerly & Frey, Berne

Hara H. 1966. *The Flora of East Himalaya.* Tokyo Univ. Press Tokyo

Hirose T., Yoshida M., Matsuhira N., Ujihara A. and Minami M. 1992. Studies on the differentiation of ecotypes of genus *Fagopyrum* in Nepal. IV. Variation of grain type of tartary buckwheat in Mustang region. Jpn. J. Breed. 42. suppl. 1:280-281. (in Japanese)

Hooker J. D. 1866. *Flora of British India.* v. Polygonaceae pp.22-61. Reeve & Co., London

Li Q. Y. and Yang M. X. 1992. Preliminary investigation on buckwheat origin in Yunnan, China. Proc. 5th Intl. Symp. Buckwheat at Taiyuan(eds. Lin R., Zhou M., Tao Y., Li J. and Zhang Z.) pp.44-46. Agr. Publ. House, Beijin

Matsuoka M. 1956. Buckwheat. In *Land and Crops of Nepal Himalaya.* (ed. H. Kihara) pp.125-134. Fauna and Flora Res. Soc., Kyoto

Nakao S. 1956. Barley. In *Land and Crops of Nepal Himalaya.* (ed. H. Kihara) pp.313-343. Fauna and Flora Res. Soc., Kyoto

Nakao S. 1957. Transmittance of cultivated plants through Sino-Himalayan route. In *Peoples of Nepal Himalaya.* (ed. H. Kihara) pp.397-420. Fauna and Flora Res. Soc., Kyoto

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. 1990. Analyses of genetic variants in common buckwheat *Fagopyrum esculentum* Moench: A review. Fagopyrum 10:12-22.

Ohnishi O. 1991. Discovery of wild ancestor of common buckwheat. Fagopyrum 11:5-10.

Ohnishi O. 1992a. Search for the wild ancestor of tartary buckwheat. Jpn. J. Breed. 42. Suppl. 1:276-279. (in Japanese)

Ohnishi O. 1992b. Buckwheat in Bhutan. Fagopyrum 12:5-13.

Ohnishi O. and Nishimoto T. 1986. Population genetics of cultivated common buckwheat, *Fagopyrum esculentum* Moench. V. Further studies on allozyme variability in the Indian and Nepali Himalaya. Jpn. J. Genet. 62:397-414.

Polunin O. and Stainton A. 1984. *Flowers of the Himalaya.* Oxford Univ. Press, Delhi

Takahashi R., Hayashi J., Hiura U. and Yasuda S. 1968. A study of cultivated barleys from Nepal Himalaya and northern India, with special reference to their phylogeneti differentiation. Ber. Ohara Inst. Landw. Biol. Okayama Univ. 14:65-122.

Wang T. Y. 1989. Buckwheat germplasm resources in Tibet. pp. 49-51. In *Collection of Scientific Treatises on Buckwheat in China.* (ed. Chinese Buckwheat Research Assoc.) Academic Journal Publ. Co., Beijin (in Chinese)

Fagopyrum 13 (1993): 11 - 14

Zinc contents in various samples and products of buckwheat

Sayoko Ikeda and Yoshihisa Yamaguchi
Faculty of Nutrition, Kobe-Gakuin University Nishi-ku, Kobe 651-21, JAPAN

Abstract

The contents of zinc in various samples of buckwheat were analyzed. A variation in the zinc content of various cultivated buckwheat seeds, including tetraploid, was found. On the other hand, there was less or substantially no difference in zinc content between common buckwheat and tartary buckwheat. The content of zinc in commercial buckwheat flours available in Japan was in a range from 0.63 mg to 2.38 mg per 100 g flour; and the content of zinc in buckwheat flours available in Slovenia was relatively higher than those in Japan. The nutritional implications of these findings are discussed.

Vsebnost cinka v različnih vzorcih in izdelkih iz ajde

Avtorja sta raziskovala vsebnost cinka v različnih vzorcih ajde. Ugotovljene so različne vsebnosti v različnih vzorcih. Med vsebnostjo cinka navadne ajde in tatarske ajde praktično ni razlike. Vsebnost cinka mok ajde, ki so na prodaj na Japonskem je od 0,63 mg do 2,38 mg na 100 g moke; vsebnost cinka v mokah kupljenih v Sloveniji je razmeroma višja. Prediskutiran je prehranski pomen teh ugotovitev. (Prevod uredništva).

Introduction

Buckwheat is an important crop in the world. Although buckwheat is taxinomically distant from true cereals, the seed has many chemical characteristics in common with cereal grains and thus is usually classified with cereals. Cereals, including buckwheat, serve as an important source for supplying some essential nutrients due to their large daily consumption.

Zinc is an essential micronutrient for humans. Nutritional deficiency of zinc is fairly common throughout the world and is a major nutritional problem (Prasad 1982, Sandstead 1973, Moynahan and Barnes 1973). Interest in zinc in food and its nutritional function is growing rapidly. In this connection, buckwheat contains a relatively high amount of zinc (Ikeda et al. 1990, Pomeranz 1984). It appears that buckwheat may be a valuable source of dietary zinc. On the other hand, a wide variety of buckwheat species, including common and tartary species, is available for human consumption around the world. There are also many kinds of buckwheat products, both flour products and groats; but little information is available about the zinc content of these buckwheat species and products.

This study was designed to analyze the zinc content in various kinds of buckwheat species and products.

Materials and methods

MATERIALS. Twenty-seven samples of buckwheat were selected for this investigation: eight kinds of common buckwheat seed (F. esculentum); two kinds of tartary buckwheat seed (F. tataricum); twelve kinds of commercial common buckwheat flour; and five kinds of common buckwheat products

Two different kinds of buckwheat seeds,

i.e., Miyazakiootsubu (tetraploid), and a tartary species, which were cultivated in Miyazaki, Japan, were kindly provided by Dr. Takashi Nagatomo, Professor Emeritus of Miyazaki University. Four different species of buckwheat seeds, i e., Shinano No. 1, Kisozairai, Shinsyunatsusoba and Shinsyuoosoba (tetraploid), which were cultivated at the Nagano Chushin Agricultural Experiment Station, Japan were kindly provided by Dr. Hisayoshi Hayashi of the Station. Two different species of buckwheat seeds, i.e., Kitawase and Hokkai No 3 (tetraploid), grown in Hokkaido, were kindly provided by Japan Agricultural Cooperatives, Hokkaido Shikaoi Branch. Two different species of buckwheat seeds, i.e., Dolenjska Temna and a tartary species, grown in Slovenia, were kindly provided by Dr. Ivan Kreft, Professor at Ljubljana University, Slovenia. These buckwheat seeds were milled in an electric mill before analysis.

Nine different kinds of commercial buckwheat flour were purchased from local markets at various places in Japan: buckwheat flour A was purchased in Iwate prefecture; flours B and C, in Ehime pref.; flour D, in Gifu pref.; flour E, in Kouchi pref.; flour F, in Tokushima pref.; flour G, in Nagano pref.; and flours H and I, in Hyogo pref. Three different kinds of commercial buckwheat flour sold in Slovenia were kindly provided by Dr. Ivan Kreft, Professor at University of Ljubljana, Slovenia.

Five different kinds of commercial buckwheat products in Japan, i.e., four kinds of dried noodles and one kind of groat, were purchased in Japan: the four buckwheat noodles A, B, C and D were produced in factories in Nagano pref.; buckwheat groats, in a factory in Tokushima pref.

DETERMINATION OF ZINC. The zinc content of samples was determined with a Hitachi 208 atomic absorption spectrophotometer. In determining zinc in solid food samples, the samples were wet ashed with sulphuric acid and 30% hydrogen peroxide prior to atomic absorption spectrophotometry

STATISTICAL ANALYSIS. Data were subjected to analysis of variance and the significance of difference was determined by the t-test.

Results and discussion

Table 1 shows the contents of zinc in various samples of commercial buckwheat flour. The content of zinc of buckwheat flours available in Japan was in a range from 0.63 mg to 2.38 mg per 100 g flour, with an average of approximately 1.57 mg. On the other hand, the content of zinc of buckwheat flours available in Slovenia was in a range from 1.98 mg to 3.47 mg per 100 g flour, with an average of approximately 2.80 mg. The buckwheat flours of Slovenia contained a relatively higher level of zinc as compared with those of Japan (Table 1).

Table 1: Zinc contents in various commercial buckwheat flours.

Buckwheat samples	Zinc content in 100 g food (mg)
Buckwheat flour available in Japan	
Flour A	1.81 ± 0.01
B	1.36 ± 0.01
C	2.07 ± 0.02
D	0.63 ± 0.01
E	1.69 ± 0.01
F	1.92 ± 0.03
G	1.54 ± 0.02
H	2.38 ± 0.06
I	0.76 ± 0.02
Buckwheat flour available in Slovenia	
Flour A	2.96 ± 0.02
B	1.98 ± 0.04
C	3.47 ± 0.03

Values are means ± S.D. (n=4).

Table 2 shows the contents of zinc in commercial buckwheat products. The zinc content in the dried noodles was in a range from 1.32 mg to 1.84 mg per 100 g flour on a fresh weight basis, with an average of approximately 1.60 mg. Buckwheat groats contained a lower level of zinc as compared with the dried noodles. Although there are a variety of buckwheat products in Japan, buckwheat noodles are most popular. On the other hand, buckwheat groats, "Soba-gome" in Japanese, have been traditionally eaten in only a few regions of Japan, such as Tokushima and Yamagata. In Japan, buckwheat groats are usually prepared by boiling buckwheat seeds, followed by dehulling. One reason for a lower level of zinc in buckwheat groats (Table 2) may be, at least in part, ascribable to leakage of zinc into the boiling soak on preparing groats.

Table 2: Zinc contents in commercial buckwheat products.

Buckwheat samples		Zinc content in 100 g food (mg)
Buckwheat noodles	A	1.69 ± 0.02
	B	1.32 ± 0.02
	C	1.55 ± 0.05
	D	1.84 ± 0.04
Buckwheat groats		1.20 ± 0.08

Values are means ± S.D. (n=4).

Table 3 shows the contents of zinc in various species of buckwheat seeds cultivated. A variation in zinc content of cultivated buckwheat species, including tetraploid, was found (Table 3). The content of zinc in common species (F. esculentum) cultivated in Japan was in a range from 1.29 to 2.05 mg per 100 g flour. The content of zinc in a common buckwheat grown in Slovenia, Dolenjska temna, was significantly ($p<0.05$) higher than those grown in Japan. A similar finding appears in Table 1. These findings are interesting. Although the exact reason for the difference in zinc content in buckwheat samples (Tables 1 to 3) is still unclear, the content of zinc in buckwheat seeds may be generally influenced by some factors such as buckwheat species, soil conditions, milling conditions and so on.

Table 3: Zinc contents in various species of buckwheat seeds.

Buckwheat samples	Zinc content in 100 g food (mg)
Buckwheat seeds grown in Japan	
Shinano No.1	2.05 ± 0.06^{c}
Kisozairai	1.84 ± 0.18^{cd}
Shinsyunatsusoba	1.68 ± 0.01^{e}
Kitawase	1.60 ± 0.03^{f}
Hokkai No.3	1.78 ± 0.07^{d}
Miyazakiootsubu	1.29 ± 0.08^{g}
Shinsyuoosoba	1.77 ± 0.04^{d}
Tatary sp.	1.97 ± 0.19^{cd}
Buckwheat seeds grown in Slovenia	
Dolenjska Temna	4.88 ± 0.11^{a}
Tatary sp.	2.26 ± 0.13^{b}

Values are means ± S.D. (n=4)
Values that do not share a common superscript are significantly different at $p < 0.05$.

On the other hand, there was less or substantially no difference in zinc content between tartary species and common species (Table 3), although Dolenjska temna (F. esculentum) contained a significantly higher level of zinc than a tartary species grown in Slovenia (Table 3). There is a suggestion that tartary buckwheat has a beneficial effect on the therapy of diabetes mellitus in China (Lu Chun jing 1992), although the factors responsible for its effect have been not clarified. We have

shown that zinc may be closely associated with diabetes mellitus (Ikeda and Kotake 1984 and 1986). Research should be performed to reveal endogenous factors, perhaps including zinc as at least one factor, involved in such a beneficial effect.

Recommended dietary allowances for zinc were established in several countries during 1978 to 1991. The allowances of dietary zinc for adults ranges from 7 to 15 mg per day around the world. Recently, the Ministry of Health and Welfare of Japan has reported a suggested level of daily dietary intake of zinc as an additional item to the Recommended Dietary Allowances for Japanese (1984), although the adequacy of zinc intake for the Japanese people is still not established. In a report of the Ministry of Health and Welfare of Japan (1984), it is suggested that adults need about 10 mg zinc per day. We have estimated the contribution of dietary zinc from buckwheat noodles on a normal consumption level (ca. 80g noodle per one dish) based on both the data of the present study and the above report (1984); our estimate shows that one dish of buckwheat noodle may provide approximately 10% of the daily need for dietary zinc for adults. In conclusion, these findings suggests that buckwheat may be an important source of dietary zinc.

Acknowledgements

The authors wish to express their sincere gratitude to Dr. Takashi Nagatomo, Professor Emeritus of Miyazaki University, Japan, to Dr. Ivan Kreft, Professor of Ljubljana University, Slovenia and to Dr. Hisayoshi Hayashi, of the Nagano Chushin Agricultural Experiment Station, Japan for kindly providing buckwheat seeds. The authors are also sincerely grateful to Japan Agricultural Cooperatives, Hokkaido Shikaoi Branch for providing buckwheat seeds.

References

Ikeda S. and Kotake Y. 1984. Urinary excretion of xanthurenic acid and zinc in diabetes: 1) Separation of xanthurenic acid-Zn^{2+} complex by ion-exchange chromatography. Acta Vitaminol. Enzymol. 6: 23-28.

Ikeda S. and Kotake Y. 1986. Urinary excretion of xanthurenic acid and zinc in diabetes: 3) Occurrence of xanthurenic acid-Zn^{2+} complex in urine of diabetic patients and of experimentally-diabetic rats. Italian J. Biochem. 35: 232-241.

Ikeda S., Edotani M., Naito S. 1990. Zinc in buckwheat. Fagopyrum 10: 51-56.

Lu Chun-jing, Xu Jia-sheng, Zhao Ping, Ma Hui-jin, Tong Hui-jun, Jin Yan-zhen, Li Shu-qing 1992. Clinical application and therapeutic effect of composite tatary buckwheat flour on hyperglycemia and hyperlipidemia. Proc. 5th Intl.Symp. Buckwheat China, pp. 484-486.

Moynahan E.J. and Barnes P.M. 1973. Zinc deficiency and a synthetic diet for lactose intolerance. Lancet 1: 676-677.

Pomeranz Y. 1984. Buckwheat: structure, composition, and utilization. CRC Critical Reviews in Food Science and Nutrition 19: 213-258.

Prasad A.S. 1982. Clinical and biochemical spectrum of zinc deficiency in human subjects. in: Clinical, biological, and nutritional aspects of trace elements. Vol. 6, Current Topics in Nutrition and Disease, Prasad A.S. (Ed.) Alan R. Liss, Inc., New York, pp 3-62.

Sandstead, H.H. 1973. Zinc nutrition in the United States. Am. J. Clin. Nutr. 26: 1251-1260.

The Recommended Dietary Allowance for Japanese (in Japanese) 1984. (ed.) The Ministry of Health and Welfare, Dai-ichi Shuppan Pub.

Fagopyrum 13 (1993): 15 - 20

Changes in zinc of buckwheat on processing into noodles and cooking

Sayoko Ikeda and Takako Shimizu
Faculty of Nutrition, Kobe-Gakuin University Nishi-ku, Kobe 651-21, JAPAN

Key words: Zinc, buckwheat, chromatography, digestion, gel filtration, soluble zinc.

Abstract

Changes in zinc of buckwheat on processing into noodles and cooking were studied. When raw noodles prepared from buckwheat flour were cooked, approximately 20% of the zinc in the raw noodles was found to leak into the water soak during cooking. There was a significant difference in the proportion of zinc released on *in vitro* digestion, between raw noodles and cooked noodles. Chromatographic analysis indicated that most of the soluble zinc occurring on digestion of both raw and cooked noodles was found in substances with relatively low molecular weight. The nutritional implications of the present findings are discussed.

Spremembe vsebnosti cinka v ajdovem testu med izdelavo rezancev in kuhanjem

Avtorici sta raziskovali vsebnost cinka v ajdovem testu med izdelavo rezancev in kuhanjem. Med kuhanjem približno 20% cinka ajdovega testa rezancev preide v vodo. Značilna je razlika v deležu sproščenega iz testa med *in vitro* prebavljanjem surovih in kuhanih rezancev. Kromatografska analiza kaže, da večina cinka, dobljenega pri prebavljanju, izvira iz snovi z razmeroma majhno molekulsko težo. Avtorici sta prediskutirali prehranski pomen teh ugotovitev. (Prevod uredništva).

Introduction

Zinc is an essential micronutrient for humans. Nutritional deficiency of zinc is today fairly prevalent throughout the world and is one of the major nutritional problems (Prasad 1982). Evaluation of diets for zinc adequacy requires knowledge of both the amount and the availability of the zinc present. Although information on the zinc contents of foods has become reasonably adeguate (Murphy et al. 1975, Freeland and Cousins 1976) , knowledge of availability for the intestinal absorption of dietary zinc is largely incomplete. Current evidence suggests that the chemical form of dietary zinc prior to the absorption may have a profound influence on its availability, but the exact mechanism involved is still obscure.

Buckwheat (Fagopyrum esculentum Moench) is an important source for humans of some essential nutrients such as proteins and vitamins. Buckwheat contains a relatively high amount of zinc (Freeland and Cousins 1976, Pomeranz 1984, Ikeda et al. 1990a, Ikeda et al. 1991). The authors have recently shown that a high level of zinc was released on the *in vitro* digestion of buckwheat flour (Ikeda et al. 1990a). In this connection, processing and cooking may have any influence upon the chemical form of zinc in buckwheat. However, detailed information is not available.

The present study was undertaken to clarify changes in the chemical form of

zinc in buckwheat on processing and cooking.

Materials and analytical methods

MATERIALS. Fresh buckwheat flour was obtained locally and used immediately. Buckwheat noodles were prepared in our laboratory in a normal manner. Figure 1 provides a diagram of the preparation and cooking of buckwheat noodles. About 100 g of buckwheat flour was mixed with about 45 ml of hot distilled water, and buckwheat noodles were formed by hand. The prepared noodles were cooked in about 500 ml of boiling water for 3 min. Subsequently, additional water (about 100 ml) was added to the suspension of the noodles. The noodles were further cooked for 4 min. After cooking, the noodles were separated from the water soak. The noodles were fully washed in cold water. Figure 1 also shows changes in weight during the preparation of buckwheat noodles. Figure 2 shows buckwheat noodles prepared in this study.

Crystalline pepsin (EC 3.4.23.1, from porcine stomach mucosa, 2 x cryst.) was obtained from Sigma Chemicals Co.; and pancreatin NF, from Difco Laboratories. Sephadex G - 50 was a product of Pharmacia LKB Biotechnology. All other chemicals used were of analytical grade.

DETERMINATION OF ZINC. The zinc content of samples was determined with a Hitachi 208 atomic absorption spectro photometer. In determining zinc in solid food samples, the samples were wet-ashed with sulphuric acid and 30% hydrogen peroxide prior to atomic absorption spectrophotometry.

IN VITRO PROTEOLYTIC DIGESTION. Proteolytic digestion was performed according to the method of Akeson and Stahmann (1964) with a modification (Ikeda 1984). Peptic digestion was performed in 0.06N hydrochloric acid for 3hr at 37 °C. The enzyme-to-protein ratio was 1:100. Immediately after peptic digestion, the incubates were adjusted to pH 8.0 with 2M Tris - HCl buffer. Pancreatin solution was then added to the digestion mixtures (enzyme-to-protein ratio 1:20) and incubated for an additional 20hr at 37 °C under 0.2M Tris - HCl buffer (pH 8.0). Sodium azide was added to the digestion medium to a final concentration of 0.025% to prevent growth of microorganisms. Immediately after digestion, the suspensions were placed in an ice cold vessel to diminish enzymatic action and then clarified by centrifusion (10,000 x g, 20 min). Blank tests of the digestion were performed with the above two enzymes in the absence of the foods. The content of zinc in the soluble digesta obtained was determined by substraction of the food-free blank. Aliquots of the soluble digesta were applied to a Sephadex G - 50 column (1.6 x 95 cm) which had been pre-equilibrated against 0.1M Tris - HCl buffer (pH 8.0).

ANALYTICAL METHODS. Protein (N x 6.31) was assayed by the micro-Kjeldahl method (AOAC 1984). The distribution of protein in column effluents was determined by A_{280} measurements. The peptide content was assayed by the colorimetric procedure with 2,4,6-trinitrobenzene sulfonic acid (TNBS) (Goldharb 1966) and phosphorus was assayed by the method of Bartlett(1959). Data were subjected to analysis of variance and the significance of means was tested by the t- test.

Results and discussion

Table 1 shows the contents of zinc and protein in raw noodles, cooked noodles and water soak on cooking. The raw noodles examined contained approximately 1.51mg per 136g of noodles (Table 1). A decrease in the content of zinc in buckwheat noodles was observed on cooking (Table 1). A considerable amount of zinc was

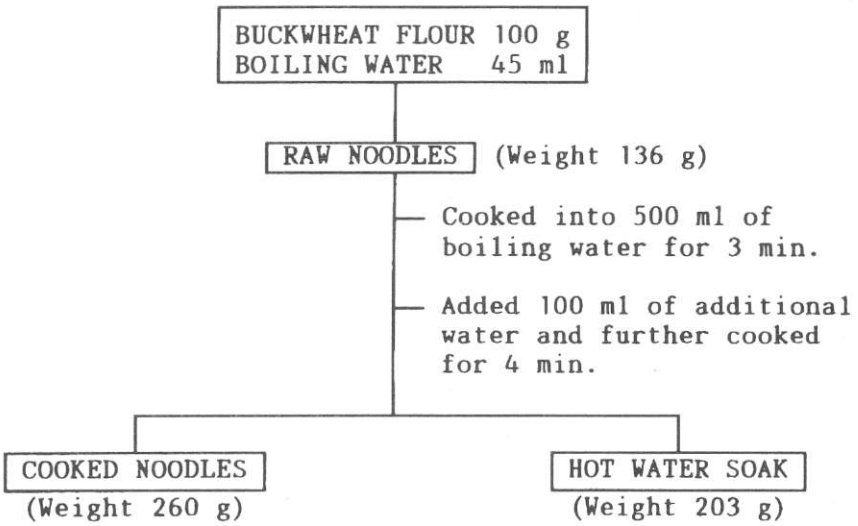


Fig. 1: Scheme on the preparation and cooking of buckwheat noodles.

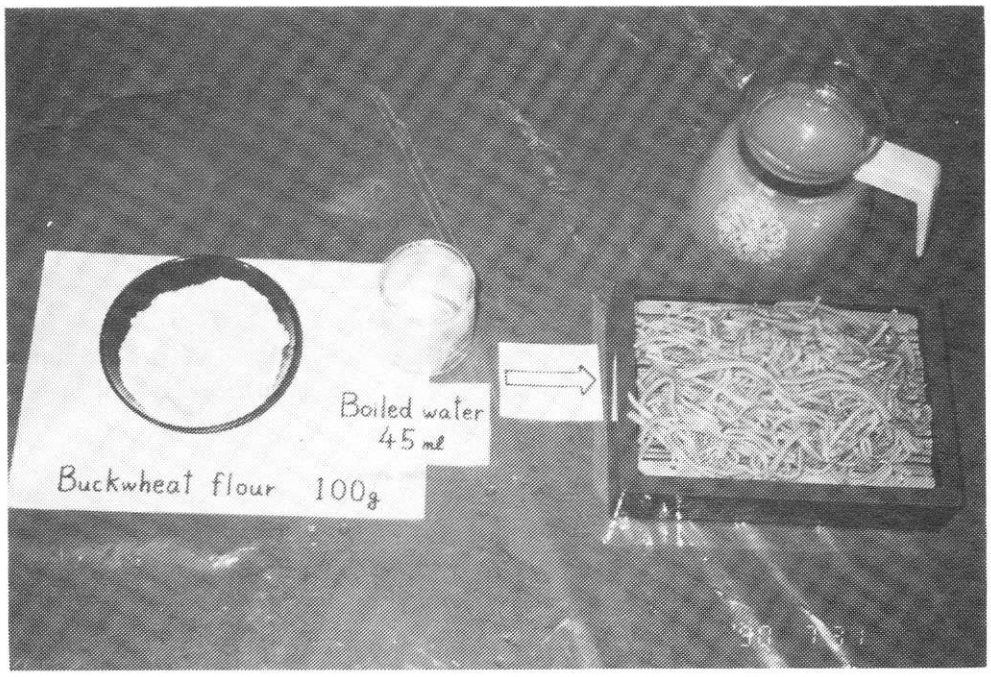


Fig. 2: Buckwheat noodles prepared

substantially found in the water soak: about 18% of zinc in raw buckwheat noodles leaked into the water soak on cooking. Japanese people often drink the hot water soak from the cooking of buckwheat noodles, "Soba-yu" in Japanese, after eating the noodles. It was believed that the water soak may contain some nutrients (Nagatomo 1984), although the scientific basis for such a habit has been yet not fully elucidated (Ikeda et al. 1990a). The water soak of noodles was shown to contain a considerable amount of zinc after cooking (Table 1).

Table 1: Zinc and protein contents in buckwheat noodles

Foods examineed	Weight[1] (g)	Zinc content (mg/each weight[1])	Protein content (g/each weight[1])
Raw noodles	136±3	1.51±0.29 (100%)	14.1±0.3
Cooked noodles	260±18	1.20±0.10 (80%)	11.5±0.7
Water soak	203±45	0.27±0.04 (18%)	2.1±0.1

Values are means S.D. (n=5)

A decrease in the content of protein in buckwheat noodles was also found on cooking (Table 1). It is known that buckwheat flour contains a high level of water- soluble protein (Javornik and Kreft 1984). The present finding indicates that a part of the water soluble protein in raw buckwheat noodles may also leak into the water soak.

Table 2 shows changes on cooking in the soluble zinc released on the peptic and pancreatic digestion of buckwheat noodles. The majority of zinc in the raw and cooked noodles was released in the digesta on peptic and pancreatic digestion (Table 2). Soluble zinc arising on digestion may emerge as a soluble complex bound with some component, such as peptide, in the digesta, whereas insoluble zinc may emerge on digestion as an insoluble complex bound with some component, such as phytic acid, in the digesta.

There was a significant ($P < 0.05$) difference in the proportion of zinc released on digestion between raw noodles and cooked noodles (Table 2). This may be ascribable to the fact that the cooked noodles contained less water-soluble zinc, since a considerable quantity of water-soluble zinc in the raw noodles leaked into the water soak on cooking (Table 1).

Table 2:Zinc released on *in vitro* digestion of buckwheat noodles

Foods examined	Per cent of zinc released on digestion to total zinc
Raw noodles	87.1±8.9[a]
Cooked noodles	71.5±10.1[b]

Values are means ± S.D. (n=5)
Values that do not share a common superscript are significantly different at $p<0.05$.

Figure 3 shows the chromatographic elution profiles on Sephadex G-50 of the soluble digesta from buckwheat flour, raw buckwheat noodles, cooked noodles and water soak. The soluble zinc in the digesta

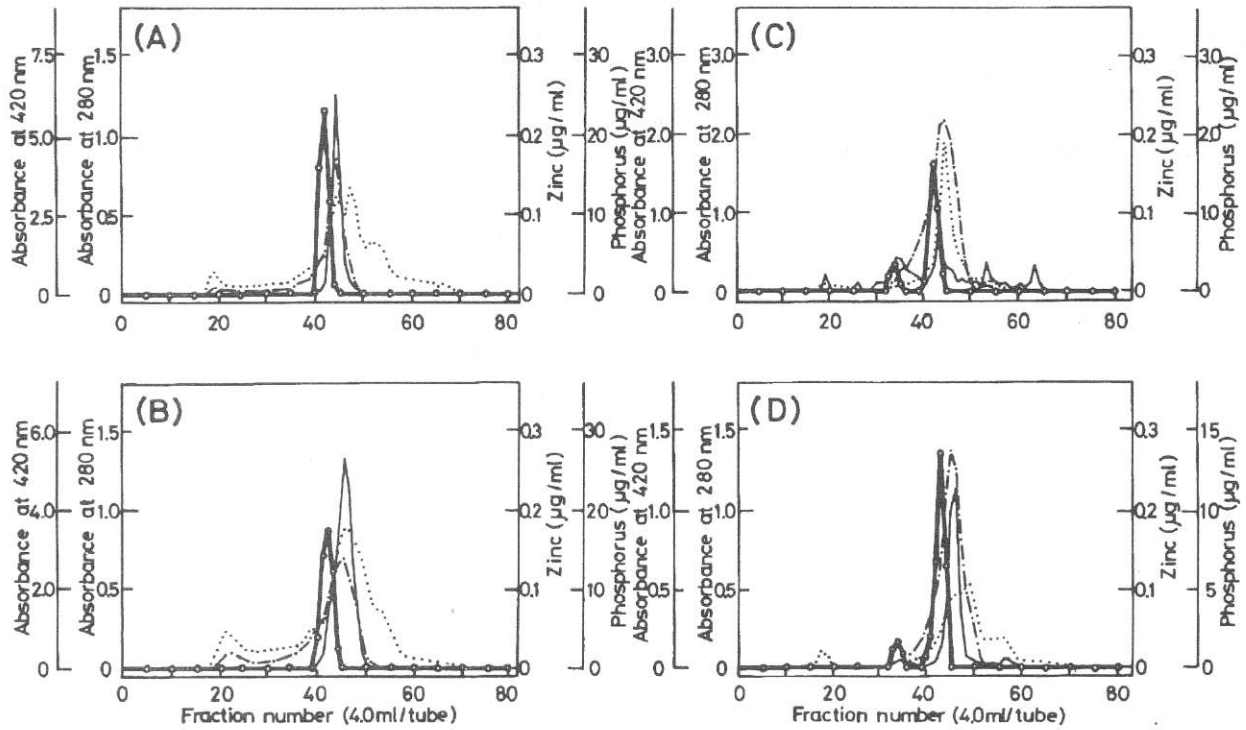


Fig. 3: Gel filtration chromatographic elution profiles of the soluble zinc components occurring on *in vitro* digestion of buckwheat flour, raw buckwheat noodles, cooked noodles and water soak. (A) Buckwheat flour; (B) raw buckwheat noodles; (C) cooked noodles; (D) water soak. -o—o-, Zinc; ————, phosphorus; ·····, absorbance at 280 nm; and -·-·-·, absorbance at 420 nm after incubation with TNBS.

consisted of one or two components. Zinc present in the soluble digesta of both buckwheat flour and raw noodles emerged as a single peak, with a molecular weight of around 1500 dalton. Zinc in cooked noodles and the water soak consisted of two components: a major component with molecular weight of around 1500 dalton; and a minor one with around 5000 dalton. Generally, soluble zinc formed on digestion is not necessarily easily available for absorption (Ikeda 1990b). Current evidence suggests that solubilization of dietary zinc, through its binding with some low-molecular-weight soluble-component on digestion, may be essential for the gastrointestinal ahsorption of dietary zinc (Ikeda 1990b). We have suggested that zinc in buckwheat may be available for intestinal absorption in view of the chemical form of zinc after digestion (Ikeda et al. 1990a). The present study further supports our suggestion. Research is currently underway in our laboratory further to characterize the zinc in buckwheat.

References

Akeson W.R. and Stahmann M.A. 1964. A pepsin pancreatin digest index of protein quality evaluation. J. Nutr. 83: 257-261.

AOAC 1984. In: Official Methods of Analysis, 14th Association of Official Analytical Chemists (Ed.), Washington, DC.

Bartlett G.R. 1959. Phosphorus assay in column chromatography. J. Biol. Chem. 234: 466-468.

Freeland J.H. and Cousins R.J. 1976. Zinc content of selected foods. J. Am. Dietet. Assoc. 68: 526-529.

Goldfarb A.R. 1966. A kinetic study of the reactions of amino acids and peptides with trinitrobenzenesulfonic acid. Biochem. 5: 2570-2574.

Ikeda S. 1984. Characterization of zinc components on *in vitro* enzymatic digestion of foods. J. Food Sci. 49: 1297-1300.

Ikeda S., Edotani M., Naito S. 1990a. Zinc in buckwheat. Fagopyrum 10: 51-55.

Ikeda S. 1990b. Dietary zinc and the zinc components in various foods subjected to *in-vitro* enzymatic digestion. J. Sci. Food Agric. 53: 229-234.

Ikeda S., Matsui N., Shimizu T., Murakami T. 1991. Zinc in cereals. In: Cereals Intl., Martin D.J. and Wrigley C.W. (Eds), pp.248-250.

Javornik B. and Kreft I. 1984. Characterization of buckwheat proteins. Fagopyrum 4: 30-38.

Murphy E.W., Willis B.W., Watt B.K. 1975. Provisional tables on the zinc content of foods. J. Am. Dietet. Assoc. 66: 345-355.

Nagatomo T. 1984. Soba no Kagaku, Nagatomo T. (Ed.), Shincho-shya, Japan.

Pomeranz Y. 1984. Buckwheat: structure, composition, and utilization. CRC Critical Reviews in Food Science and Nutrition. 19: 213-258.

Prasad A.S. 1982. Clinical and biochemical spectrum of zinc deficiency in human subjects. In: Clinical, Biological, and Nutritional Aspects of Trace Elements, Vol.6, Current Topics in Nutrition and Disease, Prasad A.S. (Ed.) Alan R. Liss, Inc., New York, pp 3-62.

Fagopyrum 13 (1993): 21 - 24

Digestibility of proteins in buckwheat seed

Kiyokazu Ikeda and Masayo Kishida
Faculty of Nutrition, Kobe-Gakuin University, Nishi-ku, Kobe 651-21, Japan

Abstract

The digestibility of proteins in buckwheat seed was studied. There was a difference in susceptibility to proteolytic action between buckwheat proteins and some other proteins, with buckwheat proteins being less digestible by pepsin than hemoglobin and ovalbumin. Electrophoretic analysis indicated that the major component of the albumin in buckwheat seed was less susceptible to pepsin action. The relationship between the digestibility of buckwheat proteins and their molecular structure is discussed.

Prebavljivost beljakovin semen ajde

Avtorja sta raziskovala prebavljivost beljakovin zrn ajde. Beljakovine ajde so slabše razgradljive s pepsinom kot hemoglobin in ovalbumin. Elektroforetska analiza nakazuje, da je glavni del albuminov ajde manj dovzeten za razgradnjo s pepsinom. Avtorja sta prediskutirala odnos med prebavljivostjo beljakovin ajde in njihovo molekulsko zgradbo. (Prevod uredništva).

Introduction

Buckwheat (Fagopyrum esculentum Moench) is an important crop in some areas of the world. Buckwheat seed contains a relatively high level of protein, and thus has potential value as a dietary source of protein. The protein in buckwheat seed consists of well-balanced amino acids (Pomeranz and Robbins 1972) with high biological value (Sure 1955). However, experiments with animals have shown that the availability of buckwheat protein for gastrointestinal absorption is low (Farrell 1978, Eggum et al. 1981, Thacker et al. 1983). The revised Japanese Standard Tables of Food Composition show the protein digestibility of buckwheat by humans to be lower than that of other edible seeds such as soybean and wheat (Resources Council, Science and Technology Agency, Japan 1981). Despite the importance of buckwheat as a dietary source of protein, factors responsible for the poor digestibility of the protein in buckwheat are still not fully clarified. We have suggested that the digestibility of buckwheat may be defined by two factors: the inhibitory potency of endogenous antinutrients, such as protease inhibitor and tannin, and the susceptibility of the protein to proteolytic action (Ikeda et al. 1991). However, detailed information about the digestibility of the protein per se is still obscure.

The present study was designed to clarify the digestibility of the protein in buckwheat seed.

Materials and analytical methods

Materials

Samples of fresh buckwheat seed were obtained from Takii Co. (Kyoto, Japan). The albumin and globulin from buckwheat were isolated from the seeds according to the procedure of Javornik and Kreft (1984). Hemoglobin, ovalbumin, bovine serum

albumin, β-lactoglobulin, trypsinogen, and lyzozyme were obtained from Sigma Chemical Co. All other chemicals used were analytical grade.

Analytical methods

The digestibility of proteins was assayed with pepsin: proteins examined (0-0.75 mg) were incubated at 37 °C with 0.3 mg of pepsin in 0.1M HCl-KCl buffer in a total volume of 2.5 ml. After incubation for 15 min, 1.0 ml of 15% trichloroacetic acid solution was added to each reaction mixture and allowed to stand for 10 min at room temperature. The suspensions were centrifuged at 3,000 rpm for 15 min, and the supernatants obtained were then assayed for peptide. Peptide and free amino group of protein were assayed by the colorimetric procedure with 2,4,6-trinitrobenzene sulfonate (Goldfarb 1966, Habeeb 1967), respectively. The free sulfhydryl group of proteins was determined by the procedure of Ellman (1959). The hydrophobicity of proteins was analyzed with 1-anilino-8-naphthalene sulfonate (Stryer 1965). Electrophoresis in the presence of sodium dodecylsulfate and mercaptoethanol was performed by the procedure of Laemmli (1970); and the marker proteins used were bovine serum albumin, trypsinogen, β lactoglobulin, and lysozyme.

Results and discussion

Figure 1 shows the susceptibility of buckwheat albumin, buckwheat globulin, hemoglobin, and ovalbumin to pepsin action. There was a striking difference in the susceptibility to pepsin action among the proteins examined; with hemoglobin being the most digestible by pepsin. Some reports (Kato et al 1985, Porter et al 1984) have shown that ovalbumin is relatively less digestible by proteases as compared with some other proteins. The two buckwheat proteins were less digestible than ovalbumin (Fig. 1). On the other hand, buckwheat globulin was more digestible by pepsin than buckwheat albumin (Fig. 1), agreeing with the previous report with pepsin and trypsin (Ikeda et al. 1991).

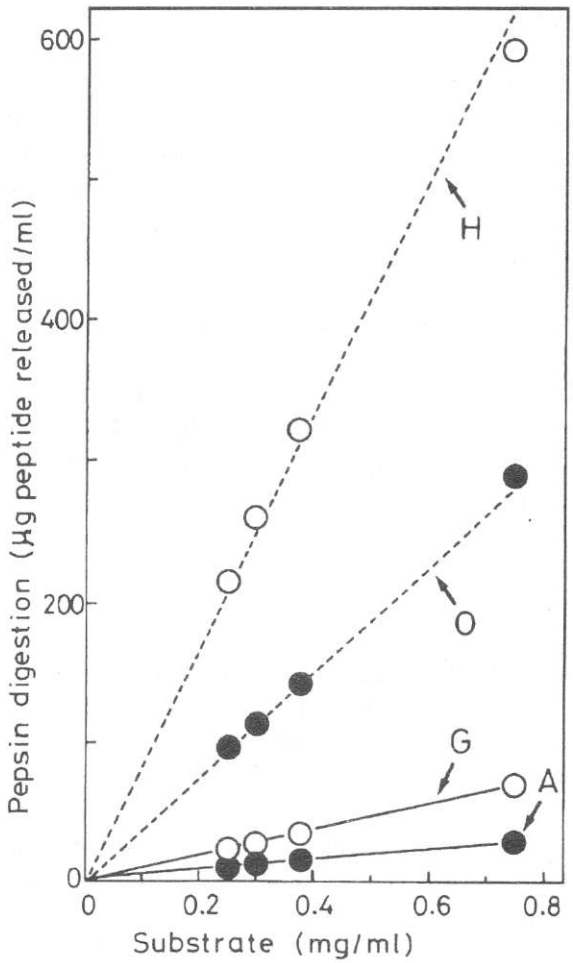


Fig 1: Susceptibility of various proteins to pepsin action. H denotes hemoglobin; O, ovalbumin; G, buckwheat globulin; and A, buckwheat albumin.

Figure 2 shows changes in buckwheat albumin during incubation with pepsin. Some minor components, which are distributed in the range of molecular weight among 24,000 dalton to 67,000

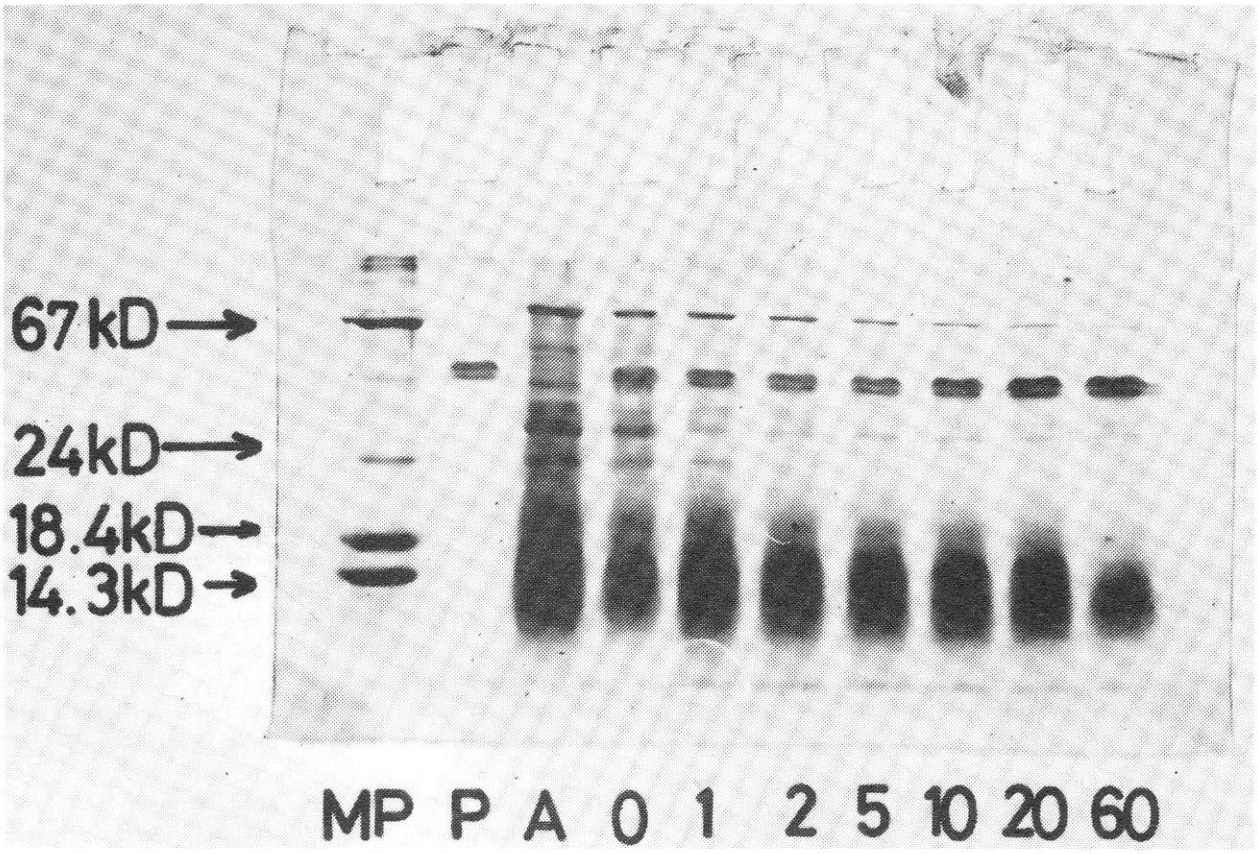


Fig 2: Changes in buckwheat albumin during incubation with pepsin. Pepsin digestion was performed with an enzyme-to-protein ratio of 1:5. MP denotes marker proteins; P, pepsin alone; A, buckwheat albumin alone; and 0-60, incubation periods (min) of buckwheat albumin with pepsin.

dalton (Fig. 2), disappeared rapidly as the enzymic reaction proceeded. On the other hand, a major component of the buckwheat albumin, which was located at a molecular weight of around 14,300 dalton, was less digestible with pepsin: a considerable amount of this albumin component persisted even after incubation with pepsin for 60 min (Fig. 2).

Table 1 shows the free amino groups and free sulfhydryl groups of the four proteins. The free SH group of hemoglobin could not be determined, since the intrinsic colour of this protein interfered with its determination. A higher level of free amino group characterized the two buckwheat proteins as compared with hemoglobin and ovalbumin. The correlation coefficient of the peptic digestibility of the four proteins (Fig. 1) to their free amino group was

Table 1 Free amino groups and free sulfhydryl (SH) groups of various proteins

Proteins examined	Free amino group (μmol/mg protein)	Free SH group (nmol/mg protein)
Buckwheat albumin	3.09	1.86
Buckwheat globulin	4.35	0.938
Ovalbumin	1.88	0.296
Hemoglobin	1.43	-

-0.822. In addition, the two buckwheat proteins had a relatively higher level of free SH group (Table 1). There was also a high correlation between the digestibility of the proteins and their free SH groups, although hemoglobin data was lacking. On the other hand, there was no correlation between the digestibility of these four proteins (Fig. 1) and their hydrophobicity (data not shown). These findings suggest that the surface state on the protein molecule in buckwheat seed may be closely associated with its digestibility.

Buckwheat seed serves as an important source of dietary protein around the world. The nutritional function of the proteins in buckwheat is a major concern in the utilization of buckwheat seed. Characterization of buckwheat protein in relation to bioavailability is in progress (Ikeda et al., unpublished data, 1993). In particular, our above suggestion on a relationship between digestibility and molecular structure should be confirmed.

Acknowledgement

The authors wish to thank Professor K. Yasumoto, Research Institute for Food Science, Kyoto University, for his suggestions during the course of this study.

References

Eggum, B.O., Kreft, I. and Javornik, B. 1981. Chemical composition and protein quality of buckwheat (Fagopyrum esculentum Moench). Qual. Plant. Plant Foods Hum. Nutr. 30:175-179.

Ellman, G.L. 1959. Tissue sulfhydryl groups. Arch. Biochem. Biophys. 82:70-77.

Farrell, D.J. 1978. A nutritive evaluation of buckwheat (Fagopyrum esculentum). Anim. Feed Sci. Technol. 3:95-108.

Goldfarb, A.R. 1966. A kinetic study of the reaction of amino acids and peptides with trinitrobenzenesulfonic acid. Biochem. 5:2570-2574.

Habeeb, A.F.S.A. 1966. Determination of free amino groups in proteins by trinitrobenzenesulfonic acid. Anal. Biochem. 14:328-336.

Ikeda, K., Sakaguchi, T., Kusano, T. and Yasumoto, K. 1991. Endogenous factors affecting protein digestibility in buckwheat. Cereal Chem. 68:424-427.

Javornik, B. and Kreft, I. 1984. Characterization of buckwheat proteins. Fagopyrum 4:30-38.

Kato, A., Komatsu, K., Fujimoto, K., and Kobayashi, K. 1985. Relationship between surface functional properties and flexibility of proteins detected by the protease susceptibility. J. Agric. Food Chem. 33:931-934.

Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680-685.

Pomeranz, Y. and Robbins,G.S. 1972. Amino acid composition of buckwheat. J. Agric. Food Chem. 20:270-274.

Porter, D., Swaisgood, H.E. and Catignani, G.L. 1984. Characterization of an immobilized digestive enzyme system for determination of protein digestibility. J. Agric. Food Chem. 32:334-339.

Resources Council. 1981. Data on the Revision of the 4th Standard Tables of Food Composition in Japan. Data No.92. Resources Council, Science and Technology Agency, Japan.

Stryer, L. 1965. The interaction of a naphthalene dye with apomyoglobin and apohemoglobin - A fluorescent probe of non-polar binding sites. J. Mol. Biol. 13:482-495.

Sure, B. 1955. Nutritive value of proteins in buckwheat and their role as supplements to proteins in cereal grains. J. Agric. Food Chem. 3:793-795.

Thacker, P.A., Anderson, D.M., and Bowland, J.P. 1983. Nutritive value of common buckwheat as a supplement to cereal grains when fed to laboratory rats. Can. J. Anim. Sci. 63:213-219.

Fagopyrum 13 (1993): 25 - 30

Morphology and identification by isozyme analysis of interspecific hybrids in buckwheats

Tamaki Hirose[1], Akio Ujihara[2], Hiromi Kitabayashi[1] and Mineo Minami[2]
[1] The United Graduate School of Agricultural Sciences, Shinshu University, Minamiminowa, Nagano 399-45, Japan
[2] Laboratory of Plant Breeding, Faculty of Agriculture, Shinshu University, Minamiminowa, Nagano 399-45, Japan

Key words: backcross, esterase, *Fagopyrum esculentum*, *Fagopyrum cymosum*, interspecific hybrid, isozyme analysis, morphology, ovule culture, tetraploid

Abstract

Fourteen interspecific hybrids were obtained through ovule culture between cultivated tetraploid common buckwheat (*Fagopyrum esculentum*, 2n=32) and the wild perennial species (*Fagopyrum cymosum*, 2n=32) originated in Nepal. The morphological characteristics of the hybrids and parent species are described, and the results of identification and estimation of genetic variation of the hybrids and backcrossing progenies by esterase isozyme analysis were reported. The hybrids exhibited polymorphism in their morphological characteristics. They had common band to either of their parents in zymograms. They had, at least, a common band to male parent and a common band to female parent.

Morfologija in identifikacija medvrstnih križancev ajde z esteraznimi izoencimi

S pomočjo kulture ovul so avtorji dobili štirinajst medvrstnih križancev med tetraploidno navadno ajdo (*Fagopyrum esculentum*, 2n=32) in divjo trpežno vrsto (*Fagopyrum cymosum*, 2n=32) iz Nepala. Opisane so morfološke lastnosti križancev in izhodiščnih vrst ter rezultati identifikacije. Z analizo esteraznih encimov je ocenjena genetska variabilnost križancev in povratnih križancev. Po morfoloških lastnostih so križanci polimorfni, cimogrami pa kažejo identičnost črt z vsaj enim od staršev. (Prevod uredništva).

Introduction

A large number of studies using the embryo or ovule culture system have been reported, in relation to the potential of interspecific hybrids to overcome cross incompatibility in many crops including their wild species (Hu *et al.* 1986). Accordingly, our research showed that the utilization of interspecific hybrids seems to be one of desirable methods for the promotion of breeding in common buckwheat, *Fagopyrum esculentum* Moench.

Through interspecific hybridization, it is possible to introduce into common buckwheat varieties, valuable characteristics of the wild species, such as tolerance to environmental stress and resistance to disease.

In addition to *Fagopyrum cymosum* Meissner, the typical and widespread wild species of the genus *Fagopyrum*, several other species have been identified in China (Wu *et al.* 1984, Ohnishi 1989).

Prior to the study, only two attempts at interspecific hybridization within the genus *Fagopyrum* had been made, by Nagatomo (1961) and Krotov (1975). Nagatomo's attempt with *F. esculentum* and *F. cymosum* was unsuccessful. In the USSR, Krotov was successful in producing *F. giganteum*, which is an interspecific hybrid

between *F. tataricum* and *F. cymosum*.

In 1987, we obtained fourteen interspecific hybrids through ovule culture between cultivated tetraploid common buckwheat (*F. esculentum*, 2n=32) and the wild perennial species (*F. cymosum*, 2n=32) originated in Nepal (Ujihara 1989, Ujihara *et al.* 1992).

In this paper, we will describe the morphological characteristics of the above hybrids and parent species, and also report on the results of identification and estimation of genetic variation of the hybrids by esterase isozyme analysis.

Materials and methods

Plant materials: Six clonal hybrid strains and two backcross progenies (hybrid No. 9 x Shinshu-Osoba) were used for the experiments. In order to compare the hybrids to their parents, Shinshu-Osoba (*F. esculentum*, tetraploid registered variety, 2n=32) and wild buckwheat introduced from north-west Nepal (*F. cymosum*, which has perennial growth habits and is also tetraploid, 2n=32) were also used.

In 1988-1989, all hybrid plants were grown in greenhouses after being transplanted from in vitro culture into soil pots.

Morphological observation: Morphological observation of the hybrids and parent species was carried out in 1989-1991. The nature of the hybrids was determined by comparing their morphological characteristics, such as the shape of leaves and flowers, and the degree of starch accumulation in the stem or rhizome, with those of the parents.

The leaf shape of the largest 2-3 leaves was measured at the fully flowering stage of each plant, and at the same time, the leaves were prepared for microscopic observation. Measurements of leaf parameters and floret shapes were taken as indicated in Fig. 1.

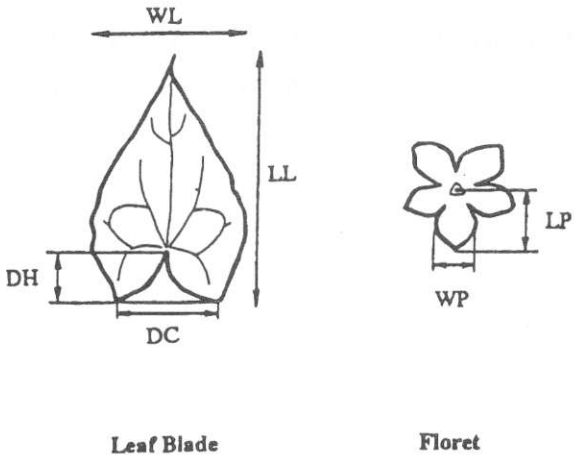


Fig. 1: Determination of the leaf and the floret characteristics.

Isozyme analysis: In order to identify the genetic variation of hybrid plants, we attempted to develop a simple analytical procedure by applying isoelectric electro focusing (IEF) for the analysis of esterase isozyme.

Florets for the analysis were collected from plants at the fully flowering stage. Samples from individual plants were treated separately throughout the analysis. Each sample was homogenized by a microhomogenizer with 40 µl of chilled extraction buffer (0.2% carrier ampholyte, 1% 2-mercaptoethanol) per floret. The IEF gel contained 5.82% (w/v) acrylamide, 0.18% (w/v) N,N'-methylene-bis-acrylamide, 4. 8% glyceroland 3.2% carrier ampholyte "LKB Ampholine pH 3.5-10". Isoelectric focusing was carried out under constant voltage conditions in a stepped-up fashion. Electrophoresis was run at 100V for 15 minutes, then at 200V for 15 minutes, and finally, at 450V for an additional 60 minutes. After each electrophoretic run, gels were immediately stained to detect isozyme bands according to the staining solution in Table 1. Gels were incubated at 30° C until the isozyme bands developed sufficient intensity to permit scoring.

Table 1. Staining solution for esterase

Stain ingredients	Quantity of ingredient
α -Naphthyl acetate	20mg
β -Naphthyl acetate	20mg
Aceton	1ml
Distilled water	1ml
1/15M Phosphate buffer,pH 7.0	50ml
Fast blue BB salt	50mg

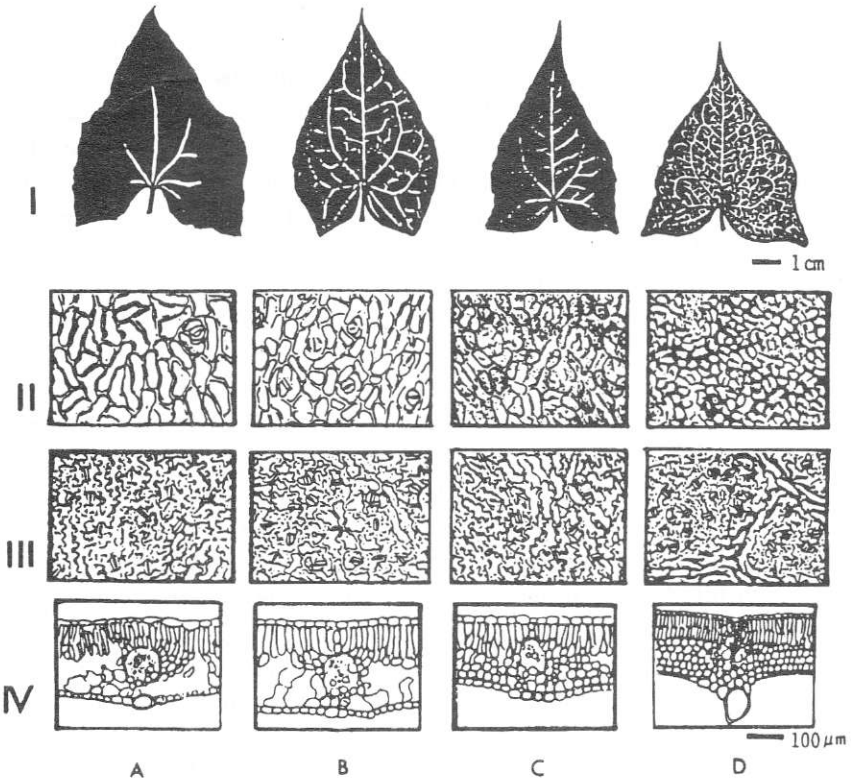
1/15M Phosphate buffer,pH 7.0: $Na_2HPO_4 \cdot 12H_2O$ 24g/l,KH_2PO_4 9g/l.

Results and discussion

Morphological characteristics: The growth of the hybrids was the same or less than that of the parent species. Comparisons of several traits were made between the hybrids and their parent species.

Generally, in flower shape, number of stomata, shape of epidermal cells and the development of the xylem and aerial roots of the stem, the hybrid plants exhibited intermediate characteristics of the parents.

However, leaf shape, development of veins, and density of hair were variable among hybrid strains.



I. leaf blade and vein

II. upper epidermis

III. lower epidermis

IV. cross section:
A. *F. esculentum*,
B. hybrid No. 9,
C. hybrid No. 18,
D. *F. cymosum*.

Fig. 2. Comparative representation of morphological characteristics of the leaf in interspecific hybrids (*Fagopyrum esculentum* x *F. cymosum*) and parent species.

Table 2. Morphological characteristics of interspecific hybrids *Fagopyrum esculentum* x *F. cymosum*) and parents.

Characteristics	*F.esculentum*	Hybrids						*F.cymosum*
		No.1	No.2	No.5	No.9	No.12	No.18	
Leaf index 1	1.33	0.96	1.01	1.01	1.71	1.34	1.27	0.91
Leaf index 2	0.19	0.12	0.09	0.16	0.24	0.18	0.16	0.13
Leaf index 3	0.8	0.98	1	0.91	0.49	1	0.9	1
Margin	undulate	entire	entire	entire	entire	entire	undulate	entire
Ocrea	truncate	triangular	triangular	truncate	triangular	triangular	triangular	acuminate
Ocrea index	1	1	2	2	2-3	2-3	2	3<
Leaf hair	+	-	-	+	+	-	++	++
Hair	+	-	-	-	++	+	+	+++
Bundle sheath	-	-	-	-	+	+	+	++
Palisade parenchyma	++	++	++	++	+	+	+	-
Starch	-	+	+	+	++	++	++	++
Xylem	-	+	+	+	+	+	+	++
Rhizome	-	+	+	+	+	+	+	++
Inflorescence index	1	2	2	2	3	3	3	4<
Flower index	1.07	*	*	*	1.13	1.13	1.18	1.56

Leaf index 1: LL/WL, Leaf index 2: DH/LL, Leaf index 3: DC/WL, Flower index: LP/WP; See Fig. 1. Ocrea index: length of ocrea/diameter of directly under internode, Inflorescence index: maximum number of raceme in inflorescence.

In the shape and size of the cotyledons and hypocotyls, the seedlings which emerged from ovules of the hybrids resembled the wild species, *F. cymosum*.

Furthermore, the hybrids exhibited root characteristics of the wild species, particularly in the substantial development of the rhizome, the accumulation of starch, and an elongated development of the tap root. These three root features are all present in perennials. It was not, therefore, surprising that all of the hybrids exhibited so many other perennial characteristics.

Fig. 2 and Table 2 show the morphological characteristics of interspecific hybrids and parent species.

Inheritance of flower type and backcrossing: The hybrids exhibited dimorphic flowers. The long-styled (pin) type and short-styled (thrum) type appeared at a ratio of 5:4 respectively. Table 3 shows hybrid combinations in the production of interspecific hybrids and each plant's flower type.

None of the hybrids with thrum type flowers grew as vigorously as the hybrids with pin type flowers.

When we attempted interspecific hybridization between common buckwheat (*F. esculentum*) and wild buckwheat (*F. cymosum*), we were able to observe fertilization, but we could not obtain mature seeds. We therefore rescued the ovules after the experiment and, through in vitro culture, we succeeded in obtaining fourteen hybrid plants.

Then we began backcrossing of the hybrids with common buckwheat for about two months, from August through September, 1989. We were quite successful in obtaining whole fertilized ovules, although full maturation was almost impossible to achieve. However, by again carrying out ovule culture, we succeeded in producing two whole progenies of backcrossed plants that achieved the flowering stage.

Through improving the seed fertility of the hybrids by this type of repeated backcrossing process, we believe there are great possibilities of successful results.

Isozyme analysis: We preliminarily examined five enzymes, namely peroxidase,

Table 3: Hybrid combinations and flower type of the interspecific hybrids in genus *Fagopyrum*

Hybrid combination	Strain	Flower type
F.esculentum (thrum) × *F.cymosum* (pin)	No.1	thrum
F.esculentum (thrum) × *F.cymosum* (pin)	No.2	thrum
F.esculentum (thrum) × *F.cymosum* (pin)	No.5	thrum
F.esculentum (pin) × *F.cymosum* (thrum)	No.6	(not flowering)
F.esculentum (thrum) × *F.cymosum* (pin)	No.8	pin
F.esculentum (pin) × *F.cymosum* (thrum)	No.9	pin
F.esculentum (pin) × *F.cymosum* (thrum)	No.10	(not flowering)
F.esculentum (thrum) × *F.cymosum* (pin)	No.12	pin
F.esculentum (thrum) × *F.cymosum* (pin)	No.13	(not flowering)
F.esculentum (thrum) × *F.cymosum* (pin)	No.17	(not flowering)
F.esculentum (pin) × *F.cymosum* (thrum)	No.18	pin
Hybrid No.9(pin) × *F.esculentum* (thrum)	No.9-1	thrum
Hybrid No.9(pin) × *F.esculentum* (thrum)	No.9-5	pin

acid phosphatase, malate dehydrogenase, 6-phosphogluconate dehydrogenase and esterase, and found that only the esterase zymogram gave distinct interspecific differences in every growth stage. Accordingly, we used esterase zymograms to identify interspecific hybrids. Magenta and deep-green bands were developed with staining solution containing alpha- and beta-naphthyl acetate in the range of pH 8-10.

The combination of magenta and deep-green bands showed interspecific differences. Two major isozyme bands were found in *F. esculentum*. In *F. cymosum*, two or three major bands and two minor bands were found. The esterase zymograms exhibited three to five bands in the hybrids, and each zymogram showed an identical pattern to at least one of its parents (Fig. 3).

The esterase zymograms revealed apparent interspecific differences. Hence, the esterase zymogram was considered to be a useful marker in the identification of interspecific hybrids.

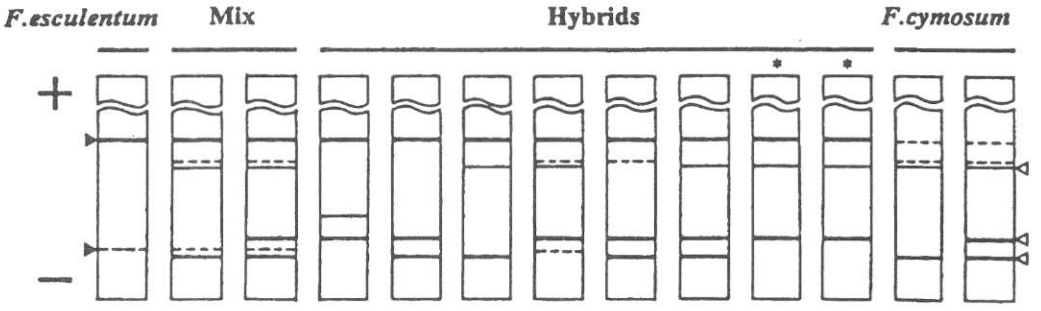


Fig. 3. Esterase zymograms of interspecific hybrids and parents.

*: Progeny of backcross.

References

Hirose T., Ujihara A., Iwasaki H. and Horiuchi A. 1991. Morphological characteristics of interspecific hybrids between tetraploid buckwheat (*Fagopyrum esculentum* Moench) and perennial buckwheat (*F. cymosum* Meissner). Jour. Fac. Agric. Shinshu Univ. 28: 53-62 (in Japanese with English summary).

Hu C. and Wang P. 1986. Embryo culture: Technique and applications. In "Handbook of plant cell culture", Evans D.A., Sharp W.R. and Ammirato P.V. (eds.), Vol. 4, pp. 43-96, Macmillan publishing company, New York.

Krotov A.S. 1975. *Fagopyrum* Mill. Flora of cultivated plants III. Groat crops. Kolos, Leningrad, pp. 7-118 (in Russian).

Nagatomo T. 1961. Studies on physiology of reproduction and some cases of inheritance in buckwheat. Miyazaki University, Japan (in Japanese with English summary).

Ohnishi O. 1989. Cultivated buckwheat species and their relatives in the Himalayas and southern China. Proc. 4th Intl. Symp. on Buckwheat, pp. 562-571.

Ujihara A. 1989. A preliminary report on the geographical distributiion and characteristics of cultivated buckwheat, genus *Fagopyrum* in Nepal. Exp. & collect. Plant genetic resources, Part I, pp. 161-170, JICA, Tokyo.

Ujihara A., Nakamura Y. and Minami M. 1992. Interspecific hybridization in genus *Fagopyrum* - Properties of hybrids (*F. esculentum* Moench x *F. cymosum* Meissner) through ovule culture. Gamma field symposia 29: 45-53, 1990, Institute of radiation breeding, NIAR, MAFF, Japan.

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. (eds.). 1984. Index Florae Yunnanensis. Tomus I, pp. 265-285. The People's Pub. House, Yunnan, China (in Chinese).

Fagopyrum 13 (1993): 31 - 34

Purification and characterization of superoxide dismutase from tartary buckwheat leaves

Wang Zhuanhua[1], Lin Rufa[2], Zhang Zheng[1], Zhou Mingde[3]

[1] Department of Biology, Shanxi University, Taiyuan, 030006, China

[2] Small Grain Programme, Shanxi Academy of Agricultural Sciences, Taiyuan, 030031, China

[3] IBPGR Office for East Asia c/o CAAS, Beijing, 100081, China

Key words: *Fagopyrum tataricum*, amino acid composition, buckwheat leaves, isozyme, purified, superoxide dismutase

Abstract

Superoxide dismutase (SOD) was isolated and purified from tartary buckwheat leaves by the modified method of Asada et al. (1974). The results of the isozyme zymogram of PAGE indicate that there are 3 bands. The SOD has a molecular weight of 31000 and, by SDS-PAGE determination, is composed of two subunits of equal size. The amino acid analysis of superoxide dismutase showed 308 residues in tartary buckwheat leaves.

Čiščenje in karakterizacija encima superoksidne dizmutaze iz listov ajde

Superoksidna dizmutaza (SOD) je izolirana iz listov tatarske ajde po modificirani metodi Asada et al. (1974). S PAGE so ugotovljene 3 elektroforetske črte. SOD ima molekulsko maso 31000 in ga sestavljata, kot je ugotovljeno z SDS-PAGE, dve podenoti. Z analizo amino kislin je ugotovljeno, da encim sestavlja 308 amino kislin. (Prevod uredništva).

Introduction

Superoxide dismutase (EC 1.15.1.1.) catalyzes the dismutation of superoxide radicals to oxygen and hydrogen peroxide. This enzyme has been isolated from a wide range of living organisms. With respect to the prosthetic metals, three types of SOD have been found: CuZn-, Mn- and Fe-containing enzymes. In prokaryotes, algae and protozoa, Fe- and Mn- SODS are the major enzymes. However, in animals and funghi, as well as plants, the major enzyme is CuZn-SOD.

The occurence of chloroplast and cytosol types of CuZn-SOD has been reported in pond, scum, horsetail (Kanematsu and Asada 1989b), rice (Kanematsu and Asada 1989a) and pea (Duck and Salin 1983). This report describes the purification and properties of SOD from tartary buckwheat leaves. The molecular weight, amino acid composition, isozyme, and inhibition by cyanide and by chloroform plus ethanol were also investigated.

Material and methods

Material. The experimental material was leaves of tartary buckwheat ('Wutai' tartary buckwheat) obtained from Shanxi Academy of Agricultural Sciences. Sephadex G-75, DEAE-32 and standard proteins for purification of SOD and determination of molecular weight were from Shanghai Chemicals.

Isolation and purification. The leaves (200g) from tartary buckwheat were homogenized in a Polytrom blender with 0.05 M

potassium phosphate buffer (pH 7.8)/0.1 mM EDTA for 1 min. After 1 h the homogenate was filtered through four layers of gauze and centrifuged at 17000 x g 15 min. The supernatant solution was treated with chloroform plus ethanol, and ammonium sulfate was added to the extract to 40% saturation, with the pH maintained at 7.8 by addition of ammonium hydroxide. After centrifugation, ammonium sulfate was added to the supernatant to 90% saturation. The precipitate obtained by centrifugation was dissolved in and dialyzed against 10 mM potassium phosphate (pH 7.8)/0.1 mM EDTA.

The dialyzed enzyme was clarified by centrifugation and applied to a column of DEAE-32 (2.3 i.d. x 20 cm), equilibrated with 10 mM potassium phosphate. The column was washed with the same buffer. A linear gradient of NaCl (0-200 mM, 600 ml) in 10 mM potassium phosphate (pH 7.8)/0.1 mM EDTA, was then applied and fractions were collected at a flow rate of 20 ml/h. Each fraction of SOD activity was pooled and concentrated by PEG-20000, and applied to a column of Sephadex G-75 (2.5 cm i.d. x 24 cm). The column was washed with the same buffer.

Assay for SOD. One unit of SOD activity was defined as the amount of enzyme required for the reduction of NBT by 50%. We used a reaction volume 3 ml (McCord and Fridovich 1969).

Methods for characterization of SOD. Native PAGE, identification of CuZn-, Mn-SOD by treatment of cyanide or chloroform plus ethanol were performed as described previously (Kanematsu and Asada 1989a). Molecular weight determination by SDS-PAGE, metals analysis, amino acid analysis and optical spectra were performed (Kanematsu and Asada 1989a).

Results and discussion

Purification of SOD. SOD was purified from tartary buckwheat leaves by a procedure similar to published methods (Asada et al. 1973, 1974). The results are summarized in Table 1.

Isozyme of SOD. Extracts of tartary buckwheat leaves generated three bands of SOD activity after native PAGE (Fig. 1A). Band a (beside cathode) was insensitive to cyanide, but it was sensitive to chloroform plus ethanol, being thus identifiable as Mn-SOD (Fig. 1B). The bottom two bands (b, c) were CuZn-SOD (Fig. 1C), which were sensitive to cyanide. By contrast, CuZn-SOD is the dominant isozyme in tartary buckwheat leaves.

Optical spectra. The absorption spectra of CuZn-SOD from tartary buckwheat leaves in the visible regions, is illustrated in Fig. 2. It had absorption maximum at 665 nm. The absorption spectra in the ultraviolet region is at 261 nm (Fig. 3). The optical properties of the buckwheat leaves enzyme are very similar to those already seen in the CuZn-SOD from other sources (Beauchamp et al. 1973, Steinman 1982).

Molecular weight and amino acid composition. Subunit molecular weight determination by SDS-PAGE was exhibited at 15500 (Fig. 4), calculated from the amino acid composition (Table 2), the molecular weight of CuZn-SOD was estimated to be 31000. Thus, it appears CuZn-SOD from buckwheat leaves is homodimer without disulfide bonds between the subunits, and it has the same properties as that from other sources (Kitagawa et al. 1986, 1987). Table 2 shows the amino acid composition of CuZn-SOD purified in buckwheat leaves. It did not contain tyrosine, as was deduced from the UV absorption spectra (Fig. 3).

Analysis indicated that the SOD in buckwheat leaves has a molecular weight in the range previously described for SOD from other sources though at the lower end of the range. Activity of the crude extract of SOD was inhibited by 90% in the presence of cyanide, but no inhibition was detected by the chloroform-ethanol

Table 1. Purification of CuZn-SOD from tartary buckwheat leaves

Purification step	Total protein (mg)	Total activity (units)	Specific activity (units/mg protein)	Yield %	Purification (fold)
Homogenate	1510	19250	12.7	100	1
Chloroform-ethanol	1100	18900	17.2	98.2	1.4
40% $(NH_4)_2SO_4$	600	14800	24.7	76.9	1.9
90% $(NH_4)_2SO_4$	120	7910	65.9	41	5.2
Sephadex G-75	11	2940	267.3	15.3	21
DEAE-32	0.7	1823	2604.3	9.5	205

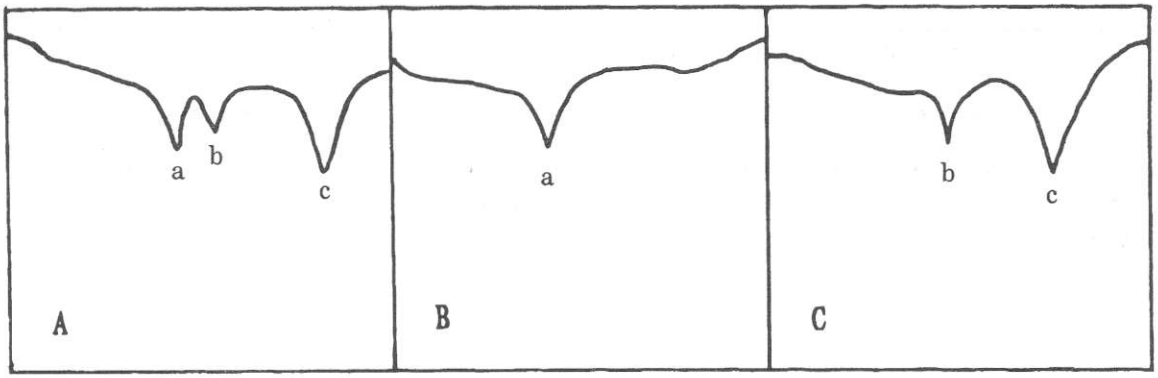


Fig. 1. Isozyme scanning zymogram of the SOD from tartary buckwheat leaves. After native polyacrylamide gel electrophoresis. A. extracts, B. extracts plus cyanide, C. extracts plus chloroform and ethanol.

Table 2. Amino acid composition of CuZn-SOD from buckwheat leaves

amino acid	residues per subunit	amino acid	residues per subunit
Lys	7	1/2 Cys	1
His	9	Val	16
Arg	4	Met	1
Asp	20	Ile	5
Thr	15	Leu	13
Ser	6	Tyr	0
Glu	12	Phe	2
Pro	8	Trp	0
Gly	24	Total	154
Ala	11		

treatment, indicating that SOD extracted from buckwheat leaves is a CuZn-SOD, and copper and zinc were found in the purified fraction in amounts corresponding to 2.01 g-atoms of Cu and 1.87 g-atoms of Zn per mol of enzyme.

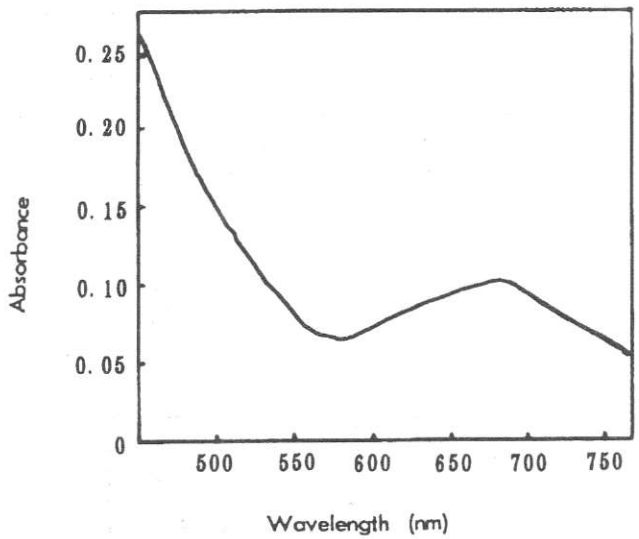


Fig. 2. Visible absorption spectrum at 1 mg/ml 10 mM potassium phosphate (pH 7.8)/0.1 mM EDTA.

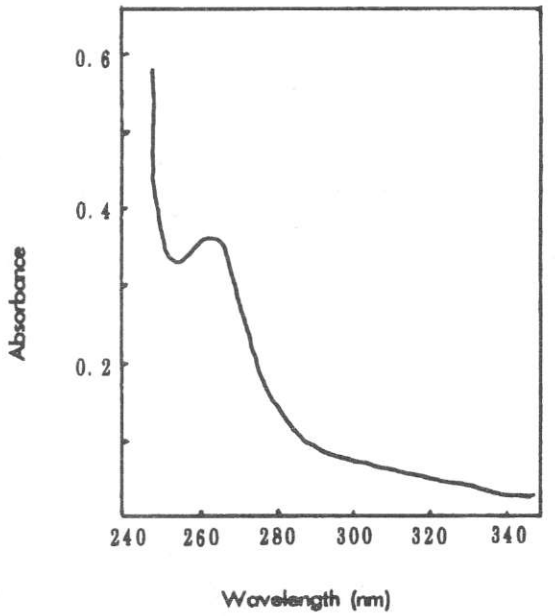


Fig. 3. Ultraviolet absorption spectrum at 0.5 mg/ml in 10 mM potassium phosphate (pH 7.8)/0.1 mM EDTA.

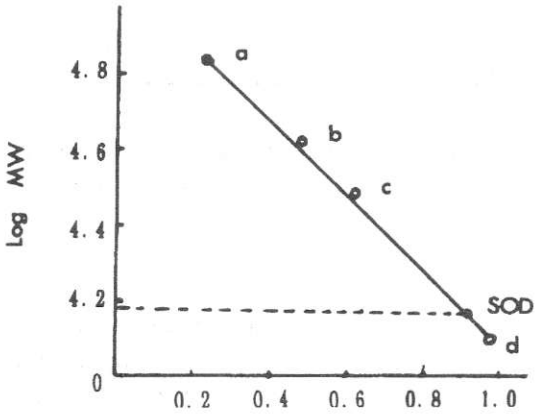


Fig. 4. Determination of the subunits molecular weight by SDS-PAGE. Reference proteins: a. BSA 867000); b. Egg albumin (43000); c. Carbonic anhydrase (30000); d. Cytochrome C (12000).

References

Asada K., Takahashi M., Nagate M. (1974): Assay and inhibitors of spinach superoxide dismutase. Agric. Biol. Chem. 38: 471-473.

Asada K., Urano M., Takahashi M. (1973): Subcellular locations of superoxide dismutase in spinach leaves and preparation and properties of crystalline spinach superoxide dismutase. Eur. J. Biochem. 36: 257-266.

Beauchamp C.O., Fridovich I. (1973): Isozymes of superoxide dismutase from wheat germ. Biochem. Biophys. Acta 317: 50-64.

Duke M.V., Salin M.L. (1983): Isoenzymes of cuprozinc superoxide dismutase from *Pisum sativum*. Phytochemistry 22: 2369-2373.

Kanematsu S., Asada K. (1989a): CuZn-superoxide dismutases in rice: occurrence of an active, monomeric enzyme and two types of isozyme in leaf and non-photosynthetic tissues. Plant Cell Physiol. 30: 381-391.

Kanematsu S., Asada K. (1989b): CuZn-superoxide dismutases from the fern *Equisetum arvense* and the green alga *Spirogyra* sp.: occurrence of chloroplast and cytosol types of enzyme. Plant Cell Physiol. 30: 717-727.

Kitagawa Y., Tsunasawa S., Tanaka N., Katsube Y., Sakiyama F., Asada K. (1986): Amino acid sequence of copper, zinc-superoxide dismutase from spinach leaves. J. Biochem 99: 1289-1298.

Kitagawa Y., Tsunasawa S., Tanaka N., Katsube Y., Sakiyama F., Asada K. (1987): Structure and molecular evolution of CuZn-superoxide dismutase from spinach. Proc. Int. Symp. Computer Anal. Life Sci pp. 65-72, Ohmusha, Kyoto.

McCord J.M., Fridovich I. (1969): Superoxide dismutase: an enzymatic function for erythrocuprein. J. Biol. Chem. 244: 6049-6055.

Steinman H.M. (1982): Copper-zinc superoxide dismutase from *Caulobacter crescentus* CB15. J. Biol. Chem. 257: 10283-10292.

Fagopyrum 13 (1993): 35 - 39

Random amplified polymorphic DNA (RAPD) markers in buckwheat

Branka Javornik and Bojka Kump
Center of Plant Biotechnology and Breeding, Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, Ljubljana 61111, Slovenia

Key words: buckwheat, DNA polymorphism, RAPD

Abstract

The recently developed approach to deriving genetic markers by amplification of random DNA segments (RAPD) with primers of arbitrary nucleotide sequences was tested in buckwheat. Analysis of a limited number of individuals of different cultivars and populations of *Fagopyrum esculentum* Moench and populations of *Fagopyrum tataricum* Gaertn. using three primers, showed high polymorphism within cultivars and populations and three cultivar specific RAPD were detected. RAPD analysis seems very promising in studying genetic variability in buckwheat.

Molekularni markerji (RAPD) v ajdi

V prispevku je opisana molekularna tehnika polimerazne verižne reakcije s poljubnimi primerji, katere rezultat so naključno amplificirani segmenti DNK (RAPD). Analiziranih je bilo omejeno število osebkov različnih kultivarjev in populacij *Fagopyrum esculentum* Moench in populacije *Fagopyrum tataricum* Gaertn s tremi primerji in prvi rezultati kažejo velik polimorfizem znotraj kultivarjev oz. populacij pri navadni ajdi. Kljub omejenemu številu analiz so bili najdeni specifični RAPD markerji. Opisana molekularna tehnika, ki je relativno enostavna, odpira možnosti za proučevanje genetske variabilnosti na molekularni ravni tudi pri ajdi.

Introduction

DNA polymorphism based on polymerase chain reaction (PCR) using single primers of an arbitrary nucleotide sequence and detected as amplified products of random DNA segments was described by Williams et al. (1990). Polymorphism, called random amplified polymorphic DNA (RAPD markers), between two individuals can arise either from chromosomal changes in the amplified regions or base changes that alter the primer binding. The RAPD assay is relatively simple, time and cost effective in comparison with well established RFLP (Restriction fragment length polymorphism) markers. This PCR based polymorphism assay does not require any prior information on the target genome and only minute quantities of DNA are needed. DNA sequences in the total genome DNA are amplified by arbitrary short primers to generate randomly amplified DNA segments. The amplification products are separated on agarose gel and visualized by ethidium bromide. Different DNA bands can be detected between individuals and polymorphisms serve as dominant genetic markers which are inherited in Mendelian fashion.

This type of polymorphism makes RAPD markers valuable in plant genotype fingerprinting (Welsh and McClelland 1990, Hu and Quiros 1991, Welsh et al. 1991, Wilde et al. 1992, Dweikat et al.

1993, Harada et al. 1993, Yang and Quiros 1993), estimation of genetic diversity (Chalmers et al. 1992, Kresovich et al. 1992) and genetic mapping (Williams et al. 1990, Martin et al. 1991, Sobral et al. 1993).

In this paper we report a preliminary survey of RAPD markers in buckwheat for further analysis of genetic diversity in buckwheat.

Materials and methods

Plant materials

The following buckwheats were included in the analysis: five cultivars (cvs. Siva, Darja, Darina and Rana 60 from Slovenia; cv. Majskaja from the Ukraine) and three populations (two from Nepal and one from Japan) of *Fagopyrum esculentum* Moench and four *F. tataricum* populations (one population from Slovenia and three from Nepal: accessions no.83-N-147, no.85-9093 and no.85-3044).

DNA isolation

The procedure for total genomic DNA isolation was based on the protocol of Saghai-Maroof et al. (1984) with modifications as described by Kump et al. (1992). 0.005 - 0.15 g of fresh leaves was homogenized in 1.5 ml microcentrifuge tubes. 600 µl of preheated (65°C) CTAB isolation buffer (2% (w/v) CTAB, cetyltriammonium bromide, Sigma), 1.4 M NaCl, 20 mM EDTA, 100 mM Tris-HCl (pH 8.0), 0.2% 2-mercaptoethanol) was added and incubated for 1.5 hour at 65°C. The mixture was extracted with chloroform : isoamylalcohol (24:1) and centrifuged at 10.000 rpm for 10 min. The DNA in the aqueous phase was precipitated by the addition of 0.1 vol of 3 M sodium accetate and 0.6 vol of isopropanol. The precipitates were washed in 70% ethanol and dissolved in a small volume of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). The DNA concentration was estimated by mini DNA fluorometer (Hoefer, TKO 100).

Polymerase chain reaction

The conditions described by Williams et al. (1990) for creating RAPD markers by PCR were first optimized for use, on a buckwheat template DNA. Tests of reaction components included variation of DNA concentration, magnesium concentration and enzyme concentration. The optimum polymerase chain reaction mixtures (25 µl final volume) contained approximately 40 ng genomic DNA, 50 mM each dATP, DGTP, dCTP and dTTP, 200 nM primer (Operon Technologies), 4 mM $MgCl_2$ and 0.5 units of Taq polymerase with 10x incubation buffer (Boehringer Mannheim). The reaction mixture was overlaid with approximately 30 µl of mineral oil to prevent evaporation. Amplifications were performed in a Perkin-Elmer/Cetus Thermal Cycler 480 with the following temperature conditions: preliminary 5 min denaturation of DNA at 95°C followed by the addition of the enzyme and then a total of 48 cycles of 1 min at 94°C, 1 min at 37°C and 1 min 30 sec at 72°C. The amplification finished with an incubation at 72°C for 10 min, followed by a 4°C soak until recovery.

Primers (Operon Technologies Inc., Alameda, CA, USA) :

OPA-01: 5'CAGGCCCTTC'3
OPA-13: 5'CAGCACCCAC'3
OPB-01: 5'GTTTCGCTCC'3

Agarose electrophoresis

10 µl of amplification products were loaded on 1,4% agarose (FMC) gels containing ethidium bromide (0,5 g/ml). Molecular DNA marker no. 4 of Boehringer Mannheim was used. DNA fragments were revealed by illumination with UV light and photographed with Polaroid MP-4. Only

intensive reproducable bands were scored.

Results and discussion

Three Operon primers were randomly selected to test RAPD markers produced in different buckwheat samples. The number of individual plants was limited to 4 - 7, except for cv. Rana 60 where 15 individuals were analysed.

Fagopyrum esculentum Moench:

Primer OPA-01 generated 14 scorable fragments ranging in length from 2300 to 500 base pairs (bp). High polymorphism was detected within cultivars or populations and cv. Darina and population from Nepal showed unique bands. Eleven fragments amplified by primer OPA-13 were scored in the range from 1600 to 500 bp in four cultivars. Three of the bands were monomorphic; others were polymorphic showing higher variabilty within cultivars than between them. Primer OPB-01 generated 8 fragments in the range from 2500 to 500 bp. Polymorphism was again higher within cultivars or populations than among them. To eliminate polymorphism within a sample, a bulk analysis was made pooling the DNA of five individuals of one sample and these combined DNAs served as a temple for amplification. RAPD's pattern (Fig. 1) revealed eight bands, of which six were monomorphic and only two were polymorphic. In addition, the population from Nepal and cv. Darina showed unique RAPD's.

Fagopyrum tatatricum Gaertn.:

Three primers generated 10 - 12 scorable fragments ranging in length from 2500 to 500 base pairs (Fig. 2). Tartatry buckwheat is a self-pollinated species, so much lower polymorphism was expected whithin accessions. As a test, we analysed only two individual plants of each accession and the same fragments were generated among samples with primers OPA-13 and OPB-01. However, with primer OPA-01 a unique fragment was detected in both samples of accession no.85-9093. Tartary buckwheat shares with common buckwheat one fragment amplified with OPA-01, one with OPB-01 and none with OPA-13.

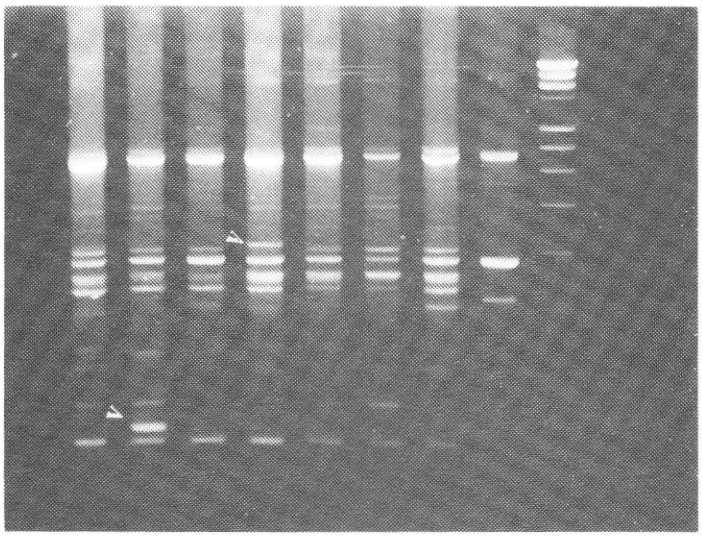


Figure 1: RAPD's of bulk DNA samples amplified by primer OPB-01. From left to right: a) cv. Rana 60, b) population from Nepal, c) cv. Majskaja, d) cv. Darina, e) population from Japan, d) population from Nepal, f) cv. Siva, g) tartary buckwheat, h) DNA marker. Specific RAPD's of Nepal population and cv. Darina are indicated by arrows.

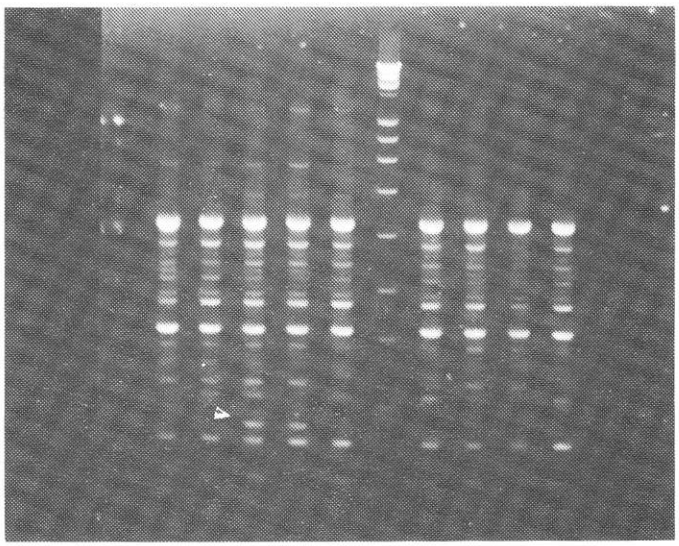


Figure 2: RAPDs of tartary buckwheat generated by primer OPA-01. From left to right: a-b) no.85-3044, c-d) no.85-9093, e), g) and j) population from Slovenia, f) DNA marker, h-i) no.83-N-147. Specific RAPD of accession no.85-9093 is indicated by an arrow.

A preliminary survey of RAPD's markers in F. esculentum Moench has shown a higher degree of polymorphism within cultivars or populations than between them. With bulk analysis and the application of a larger number of different primers, it would be possible to detect more specific RAPD's in genetically distant cultivars, which could be used for identification purposes. RAPD analysis can be used as a powerful tool because of the high number of markers for determining the extent of genetic diversity among buckwheat genotypes. RAPD analysis of buckwheat would contribute additional information to genetic variation of buckwheat studied by Ohnishi (1990).

References

Chalmers K.J., Waugh R., Sprent J.I., Simons A.J., Powell W. 1992. Detection of genetic variation between and within populations of *Gliricidia sepium* and *G. maculata* using RAPD markers. Heredity 69: 465-472.

Dweikat I., Mackenzie S., Levy M., Ohm H. 1993. Pedigree assessment using RAPD-DGGE in cereal crop species. Theor Appl Genet 85: 497-505.

Hu J. and Quiros C.F. 1991. Identification of broccoli and cauliflower cultivars with RAPD markers. Plant Cell Rep 10: 505-511.

Kresovich S., Williams J.G.K., McFerson J.R., Routman E.J., Schaal B.A. 1992. Characterization of genetic identities and relationships of *Brassica oleracea* L. via a random amplified polymorphic DNA assay. Theor Appl Genet 85: 190-196.

Kump B., Svetek S., Javornik B. 1992. Isolation of high molecular weight DNA from plant tissues. Zb. Biotehniške fak. UL 59: 63-66 (Sn).

Martin G.B., Williams J.G.K., Tanksley S.D. 1991. Rapid identification of markers linked to a *Pseudomonas* resistance gene in tomato by using random primers and near-isogenic lines. Proc Natl Acad Sci USA 88: 2336-2340.

Ohnishi O. 1990. Analysis of genetic variants in common buckwheat, *Fagopyrum eculentum* Moench: A review. Fagopyrum 10: 12-22.

Saghai-Maroof M.A., Soliman K.M., Jorgensen R.A., Allard R.W. 1984. Ribosomal DNA spacer lenght polymorphism in barley: Mendelian inheritance, chromosomal location and population dynamics. Proc Natl Acad Sci USA 81: 8014-8018.

Sobral B.W.S. and Honeycutt R.J. 1993. High output genetic mapping of polyploids using PCR-generated markers. Theor Appl Genet 86: 105-112.

Welsh, J. and McClelland M. 1990. Fingerprinting genome using PCR with arbitrary primers. Nucleic Acids Res 189: 7213-7218.

Welsh J., Honeycutt R.J., McClelland M., Sobral B.W.S. 1991. Parentage determination in maize hybrids using the arbitrarily primed polymerase chain reaction (AP-PCR). Theor Appl Genet 82: 473-476.

Wilde J., Waugh R., Powell W. 1992. Genetic fingerprinting of *Theobroma* clones using randomly amplified polymorphic DNA markers. Theor Appl Genet 83: 871-887.

Williams J.G.K., Kubelik A.R., Livak K.J., Rafalski J.A., Tingey S.V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acid Res 18: 6531-6535.

Yang X. and Quiros C. 1993. Identification and classification of celery cultivars with RAPD markers. Theor Appl Genet 86: 205-212.

FIRST ANNOUNCEMENT

SIXTH INTERNATIONAL SYMPOSIUM ON BUCKWHEAT

August 20-26, 1995

Shinshu University, Ina, Nagano
JAPAN

This is the first announcement of the sixth international symposium on buckwheat. The second announcement, including more detailed information on this symposium, will be sent in December, 1993 to those returning the preliminary registration form in advance.

INTRODUCTION

The Sixth International Symposium on Buckwheat will be held in Nagano, Japan, August 20-26, 1995. The first buckwheat symposium was held in Slovenia in 1980, and thereafter in each three years, the second being in Japan, the third in Poland, the fourth in Russia and the fifth in China in 1992 under the auspices of the International Buckwheat Research Association. More than one hundred buckwheat scientists participated in each symposium, which provided the impetus for international consultation among the participants. Nagano is a leading area of buckwheat production in Japan and is famous for its high mountains and summer resorts. We have many kinds of buckwheat dishes, but we have to import about 80% of our buckwheat consumption from other countries. There are many problems to be solved on buckwheat production. The organizing committee is pleased to invite you to this symposium which offers the opportunity to exchange the latest information on buckwheat research. The committee welcomes you to spend time enjoying beautiful buckwheat flowers, buckwheat noodles and other Japanese traditional dishes. We have a plan to publish a booklet titled "Buckwheat in Japan".

PROGRAM

The symposium will include special lectures, 20 minutes oral presentations, posters ($1x2m^2$), workshops, and an excursion, an exhibition and business meeting. The symposium will provide an opportunity for discussions on a wide range of topics:

Genetic resources
Ecology and physiology of productivity
Production technologies
Post-harvest technologies
Quality and nutrition
Processing technologies
Ethnobotany

EXHIBITION

An exhibition including harvesting machines, buckwheat products and books on buckwheat is planned. List and outlines of institutes for buckwheat study in Japan and many other countries will be also displayed.

SUBMISSION OF PAPER(S)/ POSTER(S)

Prospective authors are kindly requested to indicate the tentative title of their paper(s)/poster(s) in the attached preliminary registration form. Full text of paper should reach the secretary office by February 28, 1995. We recommend you to submit the paper written with a floppy disk, informing us of the software and the operating system of the disk you used.

LANGUAGE

The official language will be English.

SYMPOSIUM FEE

300 U.S. $

CORRESPONDENCE

All correspondence should be addressed to:

VI ISB Organizing Committee Faculty of Agriculture, SHINSHU UNIVERSITY Ina, Nagano 399-45, JAPAN

Tel: 0265-72-5255 ext. 311 or 312
Fax: 0265-72-5259 Tel&Fax: 0265-76-9331

Dr. Toshiko Matano

Chairman of the Organizing Committee
Sixth International Symposium on Buckwheat

Welsh J., Honeycutt R.J., McClelland M., Sobral B.W.S. 1991. Parentage determination in maize hybrids using the arbitrarily primed polymerase chain reaction (AP-PCR). Theor Appl Genet 82: 473-476.

Wilde J., Waugh R., Powell W. 1992. Genetic fingerprinting of *Theobroma* clones using randomly amplified polymorphic DNA markers. Theor Appl Genet 83: 871-887.

Williams J.G.K., Kubelik A.R., Livak K.J., Rafalski J.A., Tingey S.V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acid Res 18: 6531-6535.

Yang X. and Quiros C. 1993. Identification and classification of celery cultivars with RAPD markers. Theor Appl Genet 86: 205-212.

FIRST ANNOUNCEMENT

SIXTH INTERNATIONAL SYMPOSIUM ON BUCKWHEAT

August 20-26, 1995

Shinshu University, Ina, Nagano
JAPAN

This is the first announcement of the sixth international symposium on buckwheat. The second announcement, including more detailed information on this symposium, will be sent in December, 1993 to those returning the preliminary registration form in advance.

INTRODUCTION

The Sixth International Symposium on Buckwheat will be held in Nagano, Japan, August 20-26, 1995. The first buckwheat symposium was held in Slovenia in 1980, and thereafter in each three years, the second being in Japan, the third in Poland, the fourth in Russia and the fifth in China in 1992 under the auspices of the International Buckwheat Research Association. More than one hundred buckwheat scientists participated in each symposium, which provided the impetus for international consultation among the participants. Nagano is a leading area of buckwheat production in Japan and is famous for its high mountains and summer resorts. We have many kinds of buckwheat dishes, but we have to import about 80% of our buckwheat consumption from other countries. There are many problems to be solved on buckwheat production. The organizing committee is pleased to invite you to this symposium which offers the opportunity to exchange the latest information on buckwheat research. The committee welcomes you to spend time enjoying beautiful buckwheat flowers, buckwheat noodles and other Japanese traditional dishes. We have a plan to publish a booklet titled "Buckwheat in Japan".

PROGRAM

The symposium will include special lectures, 20 minutes oral presentations, posters ($1x2m^2$), workshops, and an excursion, an exhibition and business meeting. The symposium will provide an opportunity for discussions on a wide range of topics:

Genetic resources
Ecology and physiology of productivity
Production technologies
Post-harvest technologies
Quality and nutrition
Processing technologies
Ethnobotany

EXHIBITION

An exhibition including harvesting machines, buckwheat products and books on buckwheat is planned. List and outlines of institutes for buckwheat study in Japan and many other countries will be also displayed.

SUBMISSION OF PAPER(S)/ POSTER(S)

Prospective authors are kindly requested to indicate the tentative title of their paper(s)/poster(s) in the attached preliminary registration form. Full text of paper should reach the secretary office by February 28, 1995. We recommend you to submit the paper written with a floppy disk, informing us of the software and the operating system of the disk you used.

LANGUAGE

The official language will be English.

SYMPOSIUM FEE

300 U.S. $

CORRESPONDENCE

All correspondence should be addressed to:

VI ISB Organizing Committee Faculty of Agriculture, SHINSHU UNIVERSITY Ina, Nagano 399-45, JAPAN

Tel: 0265-72-5255 ext. 311 or 312
Fax: 0265-72-5259 Tel&Fax: 0265-76-9331

Dr. Toshiko Matano

Chairman of the Organizing Committee
Sixth International Symposium on Buckwheat

Fagopyrum 13 (1993): 41 - 44

PROFESSOR DR. MAREK RUSZKOWSKI
1925 - 1993

Professor Marek Ruszkowski passed away in March 1993, in Poland. He was a prominent scholar who, *inter alia*, spent many years investigating the biology and production technology of buckwheat. He was one of the founders of the International Buckwheat Research Association (IBRA), and was president of this organization for three years.

Prof. Ruszkowski died in Puławy, the oldest agricultural research center in Poland. Since the early 1950's he was a member of staff of the institute of Soil Science and Plant Cultivation, where he passed through all the stages of his scientific career, from PhD to the title of *professor ordinarius*. Due to his constant diligence, quick and inquisitive mind and organizational talents he obtained meaningful and important results mainly in the area of zoning and productivity of various cereal crops in different habitats and in cereal husbandry and production technology. Those achievements gained him the recognition of both the scientific and farming communities.

The life and work of Professor Ruszkowski were most closely linked with the turbulent fate of Poland and Polish science after World War II.

He was born in Torun, Poland, in 1925. By the time the war broke out, he had finished primary school and the first grade of high school. During the Nazi occupation he stayed in Lublin where he attended a commercial school. In the third year of the war he got a job of a workman in a seed-cleaning plant to earn a modest livelihood for himself and his family. In 1946, after completing high school, Professor Ruszkowski began to study agriculture at the newly-established Marie Curie-Sklodowska University in Lublin. As early as 1948, he gained an assistantship in the Department of Crop Husbandry headed by Professor S. Lewicki, his scientific and intellectual master for a few years to come. He worked at the university until 1952 and he published his first scientific paper there on plant vernalization.

By that time already a promising brilliant young scientist, Professor Ruszkowski joined the Institute of Plant Breeding and Acclimatization, Puławy, Poland. Within a few years, he was appointed to the Institute of Soil Science and Plant Cultivation, an institution regarded as the heir to the great tradition of agricultural research in Puławy. Soon after, in 1960, Professor Ruszkowski was appointed head of the Department of Cereal Crops and thus established his lifetime bond with the institute and with Puławy, a location that suited him both because of its tradition and its scenic landscape and climate. He felt good here and made Puławy his home, an ideal place where he could pursue his scientific and personal interests.

By no means an armchair scientist, Professor Ruszkowski conducted vigorous teaching, social and political activities alongside his research work.

He supervised more than 20 doctoral theses and associate professor's dissertations. Young scientists stuck by him, attracted by his kindness, willingness to help his junior colleagues and his broad intellectual and scientific horizons.

Professor Ruszkowski worked for many public and professional organizations and he also took an active part in the peasants movement. In the recent period he was a member of Solidarity, an organization he had much hope in. He was thus a scientist and an activist of no ordinary calibre. A recipient of many prizes and awards, he

was popular and esteemed by his fellow scientists and by the community.

His rather sudden and unexpected decease was received in those circles with great sorrow and with a profound sense of loss.

Professor Ruszkowski was remarkable for the broad scope of his interests and his receptiveness to new ideas, both scientific and social. His diligence being almost proverbial in the intellectual community, those interests bore fruit in the form of meaningful scientific, intellectual and organizational achievements.

Two wide fields of research, husbandry of cereals and development of up-to-date cereal production technologies, took up most of Professor Ruszkowski's time and energy during his long-time activity in the institute. He was the first to develop an integrated techology for cereal production in Poland.

Professor Ruszkowski was passionately devoted to research related to the biology, yield potential and production technology of buckwheat. His first paper on buckwheat production appeared as early as 1956. He followed it up with a number of publications written jointly with Professor S. Lewicki.

Professor Ruszkowski's output grew with the passing years. He probed more and more deeply into the principles of buckwheat's breeding and husbandry*. He became an unquestionable authority on buckwheat research in Poland, co-organizer of seminars and scientific sessions on buckwheat breeding and production technology. As already mentioned earlier, he was also active in the international forum. A member of IBRA, he gave presentations of his research results at various international symposia.

He had several hundred publications to his credit, mostly on broad-sense production technology of cereals and other crops, including 55 publications on buckwheat.

He kept introducing new topics to his buckwheat research and finding new issues to be solved.

In his last years, he studied a problem that had been seldom dealt with before - the relationship between buckwheat productivity and canopy architecture. Approached in an innovative way, the issue was the subject of his last papers published at home and abroad*.

Professor Ruszkowski was an able and recognized popularizer of buckwheat production in Poland. His output included a number of extension and dissemination instructions. He presented the results of his buckwheat investigations at many training seminars for farm advisers and farmers themselves. Publications by Professor Ruszkowski on buckwheat production under Poland's conditions have been re-edited several times.

On his initiative, a so called "buckwheat-growing commune" was organized on one of the communes of the Lublin province. The idea was to concentrate buckwheat production in one consolidated area, to be used as a test ground for improved buckwheat growing technologies and their dissemination among farmers, owners of small and medium sized farms.

Professor Ruszkowski had a gift of getting his ideas through to his audience. He was a welcome speaker at meetings and training courses in the field and he never tried to shun such occasions.

Sadly, Professor Ruszkowski passed away too early. The agricultural sciences have sustained a great loss. It will be particularly poignant in those communities and centers in Poland and abroad which conduct research on wheat and buckwheat.

---

*Professor Ruszkowski prepared four presentations on the subject to be delivered at the national seminar "Buckwheat husbandry and processing" held in June 1993 in Olsztyn; he did not live to deliver them.

Publications of Professor Dr. Marek Ruszkowski in the area of buckwheat research

[titles in brackets are English translations of original Polish titles]

1. **Ruszkowski M.**: [Buckwheat production techniques on farms in the Lublin and Rzeszowprovinces]. Nowe Rolnictwo 4, 1956.
2. **Lewicki S., Osińska K., Ruszkowski M.**: [Studies on buckwheat Part 1. Cultivation and fertilization problems]. Biuletyn IUNG. Prace Zakładu Roślin Zbożowych, 1957.
3. **Lewicki S., Ruszkowski M.**: [Studies on buckwheat Part 2. Some special practices that have potential to affect buckwheat yields]. Biuletyn IUNG. Prace Zakładu Roślin Zbożowych, 1957.
4. **Lewicki S., Ruszkowski M.**: [Studies on buckwheat Part 1. Biology of buckwheat germination]. Part 1, 1958, Hod.Rosl. Aklim. 2 (1): 67-68.
5. **Lewicki S., Ruszkowski M.**: [Studies on buckwheat Part 1. Biology of buckwheat germination]. Hodowla Roślin Aklimatyzacja i Nasiennictwo 2 (2),1958.
6. **Lewicki S., Ruszkowski M.**: [Studies on buckwheat Part 6. Variation of yields within different varieties and populations of buckwheat]. Hod.Rosl.Aklim., 1958, 2 (4):561-566.
7. **Lewicki S., Ruszkowski M., Ruszkowska M.**: [Causes of poor fruit set in buckwheat. Part 3. Effect of different cultural practices on fruit set in buckwheat]. Hod.Rosl.Aklim., 1958, 2 (5): 587-602.
8. **Ruszkowski M.**: [Studies on buckwheat. Part 7. Investigations on obtaining forms of buckwheat and millet less sensitive to spring chills]. Hod.Rosl.Aklim., 1958, 2 (6): 687-695.
9. **Ruszkowski M.**: [Studies on buckwheat. Part 8. Investigations of growth and development]. Hod.Rosl.Aklim., 1958.
10. **Lewicki S., Ruszkowski M., Kaszelej Z.**: [Studies on buckwheat. Part 9. Yields of grain and green matter from 10 different seeding dates]. Hod.Rosl.Aklim., 1959, 3 (2): 195-207.
11. **Ruszkowski M.**: [Studies on buckwheat. Part 10. Comparison of the growth and development of different populations]. Hod. Rosl.Aklim., 1959, 3 (2): 209-216.
12. **Lewicki S., Osińska K., Ruszkowski M.**: [Studies on buckwheat. Part 1. Problems of cultivation and fertilization] Doswiadczenia z roslinami zbozowymi. Warszawa 1960 no. 1: 175-228.
13. **Lewicki S., Ruszkowski M., Szklarz J.**: [Studies on buckwheat. Part 2. Suitability of certain special practices under this country's conditions]. Doswiadczenia z roslinami zbozowymi. Warszawa 1960, no. 1: 230-242.
14. **Mazurek J., Ruszkowski M.**: [Causes of poor fruit set in buckwheat. Part 5. Investigations on self pollination]. Pam.Puł., 1963, no. 10: 167-174.
15. **Ruszkowski M.**: [Studies on buckwheat. Relationship of growth and development to yields. Parts 1 and 2]. Pam.Puł. no. 19: 3-76.
16. **Duda G., Prochiak J., Ruszkowski M.**: [Results of buckwheat trials performed in 1955-1964]. PWRL 1967.
17. **Ruszkowscy B. i M.**: [Buckwheat] PWRL. Warszawa 1967.
18. **Ruszkowski M.**: [Buckwheat] PWRL. Warszawa 1974, 1977, 1981.
19. **Ruszkowski M.**: [Results of buckwheat variety trials conducted in 1965-1968]. PWRL 1970.
20. **Ruszkowska B., Ruszkowski M.**: [Buckwheat]. 2nd Edition. PWRL 1970.
21. **Ruszkowski M. et al.**: [Effects of some husbandry elements on the yields of tetraploid buckwheat cv. Emka]. R(120), 1977: 157-164.
22. **Ruszkowska B., Ruszkowski M.**: [Effect of nitrogen fertilization, seeding date and seeding rate on the yields of tetraploid buckwheat cv, Emka.]. Badania nad agrotechnika odmian zboż. IUNG, 1977, R(120).
23. **Ruszkowski M.**: The possibility of changing the yielding ability of buckwheat by breeding homostyle varieties. Buckwheat. Ljubljana 1980: 7-16.
24. **Ruszkowski M.**: [Buckwheat]. 3rd revised edition. PWRL 1981
25. **Ruszkowski M.**: The buckwheat productivity on different soils treated with soil conditioner Agromax (17-5-5). 1982. Fagopyrum 2.
26. **Ruszkowski M.**: The yield ability of buckwheat on various soils. 1982. Fagopyrum 2.
27. **Ruszkowski M.**: The cultivation and utilization of buckwheat in Poland. Proc. 2nd Intl.Symp.Buckwheat, Japan 1983: 13-23.
28. **Ruszkowski M.**: The productivity of homostyle homomorphic lines of buckwheat. Proc. 2nd Intl.Symp.Buckwheat, Japan 1983: 79-86.
29. **Ruszkowski M.**: Studies on the agrotechnical characteristics of buckwheat varieties in Europe and Japan. Proc. 2nd Intl.Symp.Buckwheat, Japan 1983: 127-129.
30. **Ruszkowski M.**: The influence of growth regulators on grain yield of buckwheat. 1984, Buckwheat Newsletter 1.
31. **Ruszkowski M.**: Studies on the agrotechnical characteristics of buckwheat varieties in Europe and Japan. 1986, Bull. of Environmental Conservation Shinshu University 10, 1986.
32. **Ruszkowski M.**: Studies on productivity of various buckwheat varieties in Japan and Europe. Japanese Journal of Crop Science 54: Extra Issue 1.

33. **Ruszkowski M.**: Productivity of buckwheat. Buckwheat Research 1986, IUNG Puławy: 78-98
34. **Ruszkowski M.**: The productivity of homomorphic and heteromorphic buckwheat forms in various agrotechnical conditions. Buckwheat Research 1986, IUNG Puławy: 105-109.
35. Edited by **M. Ruszkowski.** [Technology of buckwheat production (training handbook)]. IUNG Puławy 1986.
36. **Ruszkowski M.**: [Integrated buckwheat production technology. Extension paper no. 126/86]. IUNG Puławy, 1986.
37. **Ruszkowski M.**: [Results of investigations on buckwheat production technology]. IV Krajowe Sympozjum Gryki, 1987, Strzelno Puławy: 14-25.
38. **Ruszkowski M., Noworolnik K.**: [Effect of different husbandry practices and nitrogen fertilization on buckwheat yields]. IUNG ed., 1988, R(241): 5-18.
39. **Ruszkowski M., Noworolnik K.**: [Effect of nitrogen fertilization on the yields of two buckwheat cultivars]. IUNG ed., 1988, R(241): 19-28.
40. **Ruszkowski M.**: [Changes in yield components and canopy architecture as affected by husbandry factors]. IUNG ed., 1988, R(241): 41-66.
41. **Ruszkowski M.**: Structure of yield architecture and buckwheat productivity. Proc. of 4th Intl.Symp.Buckwheat, Orel, USSR, 1989, Buckwheat Research. Part 1: 7-34.
42. **Ruszkowski M., Hołubowicz-Kliza G.**: [Integrated buckwheat production technology. Extension instruction no 24/89] Ed. Puławy, 1989: 1-18.
43. **Ruszkowski M.**: [The phenomena of domination, competition and compensation in the production of buckwheat]. Niektore Zagadnienia z zakresu produkcji zbož. IUNG Puławy, 1990 R(279): 31-60.
44. **Ruszkowski M.**: The phenomenon of domination, competition, compensation and overcompensation in buckwheat productivity. 1990, Fagopyrum 10: 56.
45. **Ruszkowski M. et al.** [Improved buckwheat production technology. Extension training papers]. IUNG ed., 1990: 1-111.
46. **Ruszkowski M., Hołubowicz-Kliza G.**: [Improved buckwheat production technology. Extension instruction no. 162/90]. Puławy ed., 1990.
47. **Ruszkowski M.**: [Buckwheat productivity as affected by changes in the number of inflorescens in the 1st order branches]. VII Krajowe Sympozjum Gryki. IUNG Puławy, 1991: 15-22.
48. **Ruszkowski M.**: [Buckwheat productivity as affected by changes in leaf assimilation area of the main stalk]. VII Krajowe Sympozjum Gryki. IUNG Puławy, 1991: 22-30.
49. **Ruszkowski M.**: [Changes in the model and weight of buckwheat grain at different spacings of plants on a unit area] . VII Krajowe Sympozjum Gryki. IUNG Puławy, 1991: 30-35.
50. **Ruszkowski M. et al.**: [The phenomenon of domination, competition and compensation in the productivity of buckwheat]. IUNG Puławy, 1991, R(279): 5-30.
51. **Ruszkowski M.**: The study on buckwheat plant habitus and productivity in relation to assimilate leaf surface. Proc. 5th Intl.Symp.Buckwheat. China, Taiyuan, 1992: 357.
52. **Ruszkowski M.**: [Productivity of buckwheat in relation to changes in assimilation leaf area on 1st order branches]. ART Olsztyn, 1993.*
53. **Ruszkowski M.**: [Yield components and canopy architecture of buckwheat at different plant spacings on a unit area]. ART Olsztyn, 1993.*
54. **Ruszkowski M.**: [Yield components and canopy architecture of buckwheat at different leaf assimilation areas on the main stalk and 1st order branches]. ART Olsztyn, 1993.*
55. **Ruszkowski M.**: [Effect of removing leavs on the main stalk and the 1st order branch at different growth stages on the changes in habitus and productivity]. ART Olsztyn, 1993.*

*items 52 through 55 are presentations delivered at the 8th National Buckwheat Symposium

Biography and bibliography by IUNG, Puławy

INSTRUCTIONS FOR AUTHORS

"Fagopyrum" accepts
-scientific papers,
-information and bibliographies.

SCIENTIFIC PAPERS. Submitted papers are refereed by two reviewers. Papers submitted through a member of the advisory committee will be refereed by one additional reviewer. FAGOPYRUM will follow the system of ISI units (Systeme International d'Unitees).

In order to expedite publication and to reduce costs, the editor prefers "camera ready" manuscripts supplied by the authors. Your manuscript will be letterset or printed on a laser printer in a style similar to that in recent editions of Fagopyrum. In this case, corrections will not be possible after receipt of papers. Please make sure the text contains no typing mistakes. However, corrections will be needed if so proposed by a referee.

If it is not possible for you to send camera ready copy, the editor can arrange this but papers must then be submitted at least one month before the normal deadline, to allow time for setting type and mailing of proofs. More time should be allowed if the paper contains complicated tables or figures. For financial reasons, we are only able to accept materials in characters other than Latin if they are provided as camera ready copy.

The editors have no wish to impose a standard style, but they reserve the right to modify manuscripts which do not comform to accepted scientific standards, or which contain matter which is unnecessary, verbous or repetitive. Alterations of this nature will normally only be seen by the authors at the proof stage, unless a prior request is made.

Key words. Key words should contain words or phrases to enable indexing and retrieval by modern search techniques. Words which are part of the title of the paper should not be repeated in key words. Words should be in the following order: name of plant or animal species studied (Latin binomial); family; common name; and other typical words (within each mentioned group in alphabetical order).

Drawings. All drawings should be in the same style as in recent issues of "Fagopyrum", for reproduction by laser printer; with Indian ink or other black ink on white paper. We regret that we cannot accept brown drawings on yellow paper.

The name(s) of author(s) and page number and/or figure number should be clearly marked on the back of all diagrams, photographs etc. Illustrations should be planned to fit either a half or the full width of the page. Black and white photographs should be of good quality and contrast, lettering or marking should be of the same style and quality as in recent volumes of "Fagopyrum". If this is not possible for the author, photographs should be sent without lettering; but lettering and marks should be clearly positioned on a copy. Colour photographs for black and white printing. Colour photographs can exceptionaly be printed as black/white photographs, although the quality is inferior, especially if there are no good contrasts on the photograph, or if contrasts are in green/red tones. Avoid red backgrounds behind green objects and use white or other light background.

Colour photographs for colour printing. From a technical point of view, it is no problem to print colour photographs, if they are needed for ilustration of papers or other messages. However, in such a case, the printing procedure takes considerably longer, and costs are above normal costs of publication. So colour printing can be used only in cases in which information is clearly enclosed with the manuscript as to which institution will cover the additional costs.

INFORMATION AND BIBLIOGRAPHY. Information (including short preliminary reports) and bibliographical contributions, will be printed in a special section or, occasionally, in a special issue of "Fagopyrum". The maximum length of contributions in this section is two printed pages, and they will be published by direct photocopying and without being refereed.

We will try to do edit "Fagopyrum" to your best satisfaction, but please help us by critically reviewing your texts before sending them and by careful preparation of all illustrative material.

The Editor.